**Full Title**

The Cognition and Flow Study: a Feasibility Randomised Controlled Trial of the Effects of Cognitive Training on Cerebral Blood Flow

**Short Running Title**

The Cognition and Flow Study (CogFlowS)

**Authors**

Lucy C Beishona, Ronney B Paneraia,b, Charley Budgeonc,d , Hari Subramaniame, Elizabeta Mukaetova-Ladinskae,f, Thompson G Robinsona,b, Victoria J Hauntona,b

**Affiliations**

a University of Leicester, Department of Cardiovascular Sciences, Leicester, UK

b NIHR Leicester Biomedical Research Centre, British Heart Foundation Cardiovascular Research Centre, Glenfield Hospital, Leicester, UK

c University of Leicester, Department of Cardiovascular Sciences, Leicester, UK

d School of Population and Global Health, University of Western Australia, Perth, Australia

e The Evington Centre, Leicestershire Partnership NHS Trust, Leicester, UK

e University of Leicester, Department of Neuroscience, Psychology and Behaviour, Leicester, UK

**Corresponding Author**

Lucy Beishon

Lb330@le.ac.uk

Room 228, Level 2 Clinical Sciences Building

Leicester Royal Infirmary

Leicester

LE2 7LX

Tel: 0116 252 3134

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**Abstract**

**Background**

Cognitive training (CT) has demonstrated benefits for healthy older adults (HG) and mild cognitive impairment (MCI), but the effects on vascular function are unknown.

**Objective**

This is a feasibility trial investigating the effects of CT on cerebral blood flow velocity (CBFv).

**Methods**

Twenty HG, 24 with Alzheimer’s disease (AD), and 12 with MCI were randomised to 12 weeks of multi-domain CT or control. Outcomes included: cognition (Addenbrooke’s Cognitive Examination III), mood, quality of life (QoL), physical, and neurovascular function (transcranial Doppler ultrasonography measured task activation of CBFv responses). Data are presented as mean difference (MD) and 95% confidence interval (CI).

**Results**

47 participants completed the trial. There were three drop-outs from the training arm in the AD group, and one in the HG group. The intervention was acceptable and feasible to the majority of participants with a high completion rate (89%). The drop-out rate was higher amongst participants with dementia. Few changes were identified on secondary analyses, but QoL was significantly improved in HG post-training (MD: 4.83 [95% CI: 1.13, 8.54]). CBFv response rate was not significantly different in HG (MD: 1.84 [95% CI: -4.81, 1.12]), but a significant increase was seen in the patient group (MD: 1.79 [95% CI: 0.005, 3.58]), requiring sample sizes of 56 and 84 participants respectively for a fully-powered trial.

**Conclusions**

A 12-week CT programme was acceptable and feasible in HG, AD and MCI. CT may be associated with alterations in vascular physiology which require further investigation in an appropriately powered randomised controlled trial.

**Key words**

Cognitive impairment

Cognition

Cerebral haemodynamics

Dementia

Cerebral blood flow

**Background**

The number of people living with dementia is expected to double by 2050 [1], but strategies to prevent and treat dementia remain limited [2]. Mild cognitive impairment (MCI) is characterised by subjective and objective impairments in cognition, but without impacting functional status [3]. Approximately 10-20% of people living with MCI will convert to dementia over five years, representing a high risk group for the development of dementia [3].

Cognitive training is a potential intervention which aims to improve cognitive function and processing by training individuals through a guided programme of repeated cognitive exercises that target specific cognitive domains [4]. Cognitive training is attractive owing to fewer side effects than drug therapies, and the ability to be delivered flexibly at home [5]. In healthy older adults, and those with mild cognitive impairment, cognitive training has promising effects on the improvement and maintenance of cognitive function [5, 6], but few studies have investigated the mechanisms by which these effects are mediated [7, 8]. Recent systematic reviews of neuroimaging outcomes have identified cognitive training induced changes in brain structure and function in healthy older adults, mild cognitive impairment and early dementia [7, 8].

Vascular dysfunction is increasingly recognised as a key pathological mechanism in the development of multiple dementia sub-types, including Alzheimer’s disease [9-11]. The Rotterdam study demonstrated that lower CBF in healthy older adults was predictive of future dementia risk [12], and higher resting CBF was associated with better cognitive performance [13, 14]. Furthermore, hypoperfusion has been shown to accelerate and compound Alzheimer-related disease pathology, resulting in hyperphosphorylation of tau, increased amyloid deposition and reduced amyloid clearance [9]. Given the importance of vascular pathology in the development and progression of cognitive disorders, understanding how vascular physiology and pathology is moderated by cognitive interventions is important. Cognitive processes are highly resource intensive for the brain and require a constant blood supply to meet these energy and oxygen demands [15]. Thus, in order to develop and sustain neuroplasticity, modifications to vascular structure and function will be a key process [16, 17]. This could be mediated through increasing the number of capillaries (angiogenesis), the release of vascular endothelial growth factor, and improvements in the efficiency of cerebral autoregulation and neurovascular coupling processes [17, 18]. However, few studies have specifically examined the effects of cognitive training on cerebrovascular function, and these are largely confined to healthy populations [19].

Transcranial Doppler ultrasonography (TCD) is a non-invasive, ultrasound-based technique to measure CBF velocity (CBFv) [20]. TCD has excellent temporal resolution, measuring rapid changes that occur in CBFv, and thus can be used to assess the integrity of cerebrovascular function [20]. No study has previously investigated the effects of cognitive training on TCD-measured changes in cerebrovascular function. Therefore, the primary aim of this study was to identify the feasibility of a randomised controlled trial of cognitive training in healthy older adults, mild cognitive impairment and Alzheimer’s disease. Secondary objectives were to investigate the effects of cognitive training on clinical outcomes and vascular physiology using TCD-measured cerebral haemodynamic outcomes.

## Methods

### *Recruitment*

The protocol for this study has been published previously [21]. In brief, 20 healthy older adults, 12 participants with mild cognitive impairment, and 24 with Alzheimer’s disease were recruited from poster advertisement, Join Dementia Research and outpatient memory clinics at the Leicestershire Partnership Trust in the United Kingdom. Recruitment and follow-up was conducted between January 2019 and April 2020. The trial closed early due to the COVID-19 pandemic. Mild cognitive impairment and Alzheimer’s disease were defined by the Petersen and NIA/AA 2011 criteria, respectively [22, 23]. Participants with mild cognitive impairment and Alzheimer’s disease were screened using the Montreal Cognitive Assessment (MoCA) to ensure participants with severe cognitive impairment (MoCA≤9) were not enrolled. Inclusion criteria were: age over 50 years, with access to the internet, and free of any medical co-morbidity or medication that could adversely affect cognition. Participants were suitable for inclusion irrespective of anti-dementia medication use. Volunteers with well-controlled co-morbidities (i.e. hypertension, diabetes) were considered for inclusion. Exclusion criteria were any medical condition or medication that could adversely affect cognition (healthy older adults), and poorly controlled medical conditions, specifically: severe heart failure (ejection fraction <20%), significant carotid artery stenosis (>50%), severe respiratory disease, major stroke, pregnancy or lactation, inadequate bilateral TCD windows, enrolled on an interventional study, and no access to the internet. The study was amended to provide the option of a laptop loan to improve access to the study for participants without suitable technology, and the upper limit of the MoCA was removed to enrol participants with mild to moderate dementia. All volunteers provided written, informed consent, or personal consultee declaration where participants lacked capacity. The study had ethics approval from the Bradford-Leeds Research Ethics Committee (ref: YH/18/0396), and all procedures were conducted in accordance with the Declaration of Helsinki 1975. The trial was registered prospectively on Clinicaltrials.gov, ref: NCT03656107.

### *Experimental procedures*

Participants were asked to refrain from caffeine, alcohol, heavy meals or strenuous exercise for at least four hours prior to the study. All TCD procedures were carried out in the Cerebral Haemodynamics in Ageing and Stroke Medicine research space, which is free from noise and distractions, and maintained at 24oC. Participants underwent continuous beat-to-beat monitoring of bilateral CBFv (DWL Doppler box) through insonation of the middle cerebral arteries, blood pressure (BP, Finometer, Finapres Medical Systems; Amsterdam, the Netherlands), heart rate (HR, three-lead ECG), and end-tidal CO2 (ETCO2, capnography, Capnocheck Plus). Initially, participants underwent a five-minute baseline recording at seated rest, followed by a task activation protocol lasting approximately ten to fifteen minutes. All measurements were made by the same investigator (LB) at baseline and follow-up. Participants were presented with five tasks in a standard order selected from the Addenbrooke’s Cognitive Examination III (ACE-III). Tasks covered five key cognitive domains: attention (serial subtraction), verbal fluency (naming words), language (repeating words and phrases), visuospatial (drawing a cube and infinity diagram), and memory (recalling three words learnt previously). The three words were presented at the beginning of the protocol and were recalled at the end of the tasks (approximately 10-15 minutes later).

There was one-minute rest between each task to allow CBFv levels to normalise prior to the next task. Each task was marked using an event recorder. A detailed description of the analysis and task activation protocol has been published previously [20]. Throughout, CBFv1 refers to the non-dominant hemisphere and CBFv2 to the dominant hemisphere. Hemispheric dominance was assessed by determining handedness using the Edinburgh Handedness Inventory [24]. Right-handed individuals were assigned as left hemisphere dominant, and left-handed individuals as right hemisphere dominant.

### *Outcome measures*

In addition to an assessment of neurovascular function, participants also underwent assessments of cognitive function (full ACE-III), mood (15-item Geriatric Depression Scale [GDS]), quality of life (dementia quality of life [DEMQOL]), and activities of daily living (Lawton instrumental activities of daily living [IADL]). Data were collected on key baseline demographic variables, including medical and medication history. Following collection of baseline data, participants were randomised by Sealed Envelope© to either a 12 week cognitive training programme or waiting list control. The randomisation procedure was a stratified, permuted block randomisation, with a block size of four. All participants were invited for a follow-up assessment at 12 weeks. The participants were randomised, enrolled, and assigned to the intervention by LB. Due to the nature of the intervention and outcome measures, participants and assessors could not be blinded to the intervention or outcomes. To mitigate this, the data collected on CBF was batch-blinded by an independent researcher prior to analysis.

### *Intervention – cognitive training programme*

The intervention was a multi-domain, online cognitive training programme provided by Lumosity©. The cognitive training programme covered the five key cognitive domains assessed in the CBFv task-activation protocol (i.e. attention, language, fluency, visuospatial and memory). Participants were requested to train for five, 30-minute sessions per week and adherence was monitored by Lumosity© as the number of sessions trained per week. The frequency and number of sessions were determined from research undertaken previously by Lumosity© suggesting that a minimum of 20 hours training time was required specifically for this programme. Training for five 30-minute sessions for 12 weeks would equate to a total of 30 hours training, therefore mitigating any missed sessions. In addition, the training dose and duration for this programme were informed by the results of a systematic review, which found that 30 minutes was determined to be the minimum effective duration for each training session, and that there is no benefit to training for more than three sessions per week [25]. We asked participants to train up to five times per week to mitigate for missed sessions and weeks where they would be unable to train (i.e. illness, holidays). Participants could train on additional sessions if they chose to. Given this is a feasibility study, understanding the adherence to the programme by participants in each group was an important aspect of feasibility in the evaluation of this study. Participants in the intervention arm were offered a weekly telephone call or email contact for support during the programme. The programme consisted of a total of twenty games (5 attention, 5 language and verbal fluency, 5 memory, 5 visuospatial exercises), the order of which varied for each session. Each session included at least one attention, memory, visuospatial, and language task, and the remaining task cycled through an additional domain each session. Each exercise took approximately 5-6 minutes to complete. A full description of exercises included in this programme is provided in Supplementary Table S.1.

### *Sample size calculation*

Given the primary aim of this study was to identify the feasibility of the protocol and intervention, sample size calculations were not performed *a priori*.

### *Statistical analysis*

Data are presented as mean (standard deviation) for normally distributed data, and median [inter-quartile range] for non-normally distributed continuous data. The difference between training and control groups for continuous outcomes was tested by independent t-test (normally distributed) or Mann Whitney-U (non-normally distributed). Nominal data are presented as number (percentage), with statistical testing by Chi-square.

Task activation was measured by percentage change in CBFv from a twenty-second baseline (T1) prior to task initiation. Two time points were taken to assess the change. Firstly, T2 which occurred at 5-10 seconds after task initiation, and T3 which occurred at 10-20 second after task initiation, where CBFv was averaged across these time intervals. These time intervals were determined by previous work from this group indicating that the peak response to task activation occurs at 5-10 seconds for the majority of tasks, indicating the initial response to stimulation [20, 26]. T3 therefore represents the sustained response after this initial peak, which was thought to be mediated more locally due to the metabolic gap [26].

In addition to the peak percentage change, a novel method to identify response to task activation was applied, which has been published recently [27]. In brief, response to task activation was classified as present or absent using two parameters: the cross correlation peak function (CCF) and the variance ratio (VR). The CCF is a measure of coherence between the individual response and the coherent average of the group response, and the VR is the ratio of the variance post-stimulus compared to the variance pre-stimulus. CCF values approaching one reflect a perfect response to task activation, and higher VR values correspond to larger task-activated response. Thresholds for the parameters are determined by calculating the upper 90th confidence limit based on randomly-marked baseline files, above which a response is determined as present. Disease-specific thresholds were determined to adjust for differences in baseline variability between healthy and disease groups which have been demonstrated in previous studies [28]. The group-specific thresholds are provided in Supplementary Table S.2. Rather than a percent change from baseline, a response is considered present where either the CCF or VR are above the pre-determined threshold. This provides an objective measure of the presence or absence of a response to a given cognitive task. The cumulative response rate (CRR) was then calculated from the sum of responses to each task (n=5) in each hemisphere (n=2), to give a score out of 10. Increasing CRR therefore reflects an increasing number of responses to task activation.

Independent t-tests between intervention groups and within diagnostic group were used to determine estimates of intervention effect size for each diagnostic group in CBFv at and T3 and in CRR. Correlations between percentage changes in CBFv or CRR and clinical outcome measures were assessed using Pearson correlations for normally distributed and continuous variable comparisons, or Spearman correlations for non-normally distributed and continuous and ordinal comparisons. Cohen’s *d* was calculated in Excel for each outcome measure to determine effect size [29]. Effect sizes were considered small where *d*=0.2, medium where *d*=0.5, or large where *d*=0.8 [29].

Whilst we acknowledge this feasibility study was not powered to test for intervention effects and sub group analyses, we have provided p-values for such tests with the intent of better informing future research. However, these should be interpreted with caution.

As a result of the loss to follow-up, data on Alzheimer’s disease and mild cognitive impairment participants were analysed as one cohort (cognitively impaired), and sub-group analyses were undertaken where possible.

*Sample Size Calculation Methodology for future studies*

Sample size calculations were performed using Statulator© [30], an online sample size calculator to determine the sample size required to detect the expected mean difference between training and control groups at follow-up (intervention effect size) that we found in this study. Sample sizes were calculated within diagnostic groups (healthy, control, AD and MCI combined, AD and MCI separately for CRR). This is a basic sample size calculation using a t-test. Given the sample size is powered to detect a difference within and not between groups using composite outcome measures, correction for multiple comparisons was not performed.

### *Data availability*

Additional data are provided in the supplement, including: CCF and VR thresholds, resting CBFv and peripheral parameters at baseline and follow-up, peak percentage change in CBFv and peripheral parameters in training and control groups at baseline and follow-up, significant correlations with cognitive test scores, and sample size calculations. Additional data are available on request from the corresponding author.

## Results

### *Baseline demographics*

Table 1 summarises the key baseline characteristics of the total sample, and between training and control groups. The mean age ± SD of participants in this sample was 65.2 ± 8.4 years (healthy cohort) and 70.2 ± 8.6 years (cognitively impaired cohort). In healthy older adults, 50% were female, compared to 30.6% in the cognitively impaired group. The majority of healthy older adults and disease cohort were Caucasian (95% and 100%, respectively) and right handed (90% and 91.7%, respectively). At baseline, there were no differences in the age of training or control groups, or the proportion of females in each group. At baseline, body mass index was significantly higher in the healthy control group (27.4 Vs. 24.2, p=0.001), and scores were lower on the ACE-III memory domain (25.5 Vs. 23.5, p=0.032) in the healthy control group. In the cognitively impaired cohort, antidepressant use was significantly higher in the control compared to training group (44.4 Vs. 11.1%, p=0.03). Median time to follow-up was longer in the healthy training group (98 Vs. 86 days, p=0.002), but comparable in the cognitively impaired cohort (90 Vs. 84.5 days, p=0.093). There were no other significant differences between control and training groups for either the healthy or cognitively impaired cohorts at baseline (Table 1).

### *Feasibility assessment*

Both the protocol and the intervention were well tolerated by healthy participants and those with MCI; there were no withdrawals from the study as a result of the study procedures, or TCD protocol. There were three drop-outs (25%) from the AD group as a result of the intervention, suggesting it was less well tolerated than in the healthy and MCI groups. Data on haemodynamic outcomes were lost on five participants in the AD group due to lack of TCD window (n=1), and the Covid-19 pandemic precluding face-to-face assessment. In healthy older adults, there was one loss to follow-up in the control group but this was unrelated to the study. There were no losses to follow-up in the mild cognitive impairment group. All participants were willing to be randomised to either intervention or waiting-list control.

In terms of adherence to the protocol, the median number of sessions trained by healthy older adults was 86.5 (Range: 51 -228 sessions, IQR: 75-102), but this was lower in the cognitively impaired cohort at 70 sessions (Range: 28-168, IQR: 47-83). Figure 1 summarises the recruitment process for the study.

### *Secondary outcome measures*

Table 2 summarises the clinical outcomes by training and control groups. There were no differences between groups in cognitive function (ACE-III), mood (GDS), or function (Lawton IADL). There were no differences in cognitive sub-domains of the ACE-III between groups. Quality of life was significantly improved in healthy training compared to healthy control (MD: 4.83 [95% CI: 1.13, 8.54]), but not cognitively impaired, cohort.

### *Resting cerebral and peripheral physiological data*

There were no differences in resting cerebral and physiological parameters at baseline or follow-up between control and training groups in the healthy or cognitively impaired cohorts. Within group, there were no differences in resting values in the cognitively impaired or healthy training cohorts. In the healthy control group, resting values in CBFv1 and 2 were lower at follow-up. Supplementary Tables S.3 and S.4 summarise the resting cerebral and peripheral physiological data for baseline and follow-up visits by training and population group.

### *Task activated cerebral and peripheral physiological data*

In healthy older adults, peak percentage change in CBFv was significantly decreased at follow-up in the visuospatial task at T2 (right MD: -10.22% [95% CI: -19.87, -0.57]; left MD: -10.69% [95% CI: -20.93, -0.44]) in healthy training relative to healthy control (Figure 2). There were no significant differences in the task-activated response at T2 or T3 for either healthy or cognitively impaired cohorts for the other tasks. Data are provided for all task-activated responses in cerebral and peripheral parameters in Supplementary Tables S.5 and S.6. Figure 2 provides a representative trace of the temporal CBFv change to the visuospatial task in the control and training groups at follow-up in the healthy cohort.

At T2 the estimated difference between the training and control intervention for the healthy cohort was -5.35 (95% CI: -12.82, 2.11), and for the cognitively impaired cohort was **-**1.86 (95% CI: -5.60, 0.87) (Table 3). At T3, the estimated difference between the training and control intervention for the healthy cohort was 3.81 (95% CI: -10.03, 2.42), and for the cognitively impaired cohort was **-**0.27 (95% CI: -3.27, 2.73) (Table 3). Effect sizes were medium to large for the difference in peak percentage change between training and control groups at T2 for both cohorts (Cohen’s *d*= 0.59-0.69), and for the healthy cohort at T3 (Cohen’s *d*= 0.53), but small for the cognitively impaired cohort at T3 (Cohen’s *d*= 0.07) (Table 5).

### *CRR analysis*

The estimated difference in CRR between the training and control intervention for the healthy cohort was -1.84 (95% CI: -4.81, 1.12), and for the cognitively impaired group was 1.79 (95% CI: 0.005, 3.58) (Table 4, Figure 3). Effect sizes were medium to large for both cohorts in CRR difference between training and control (Cohen’s *d*= 0.41-1.11) (Table 5).

### *Correlations between clinical and CBFv outcomes*

There were a number of significant correlations in both the control and training groups between clinical outcome measures and change in task activated CBFv in healthy older adults. These are summarised in Supplementary Table S.7.

In the cognitively impaired cohort, there were no significant correlations between Δ peak percentage change in CBFv and change in clinical outcome measures (DEMQOL, GDS, IADL) or cognitive test scores for any tasks at T2 or T3.

*Sample sizes for a future study*

Table 5 provides estimates of sample sizes needed for future studies based on the estimates observed in this study. To detect the observed difference in CBFv at T2 and T3 would require a sample size of 102-200 participants in the healthy cohort, and 384-18140 in the cognitively impaired cohort. CRR required fewer participants to detect the observed changes: 84 participants in the healthy cohort, and 56 participants in the cognitively impaired cohort.

## Discussion

### *Main findings*

In summary, the study protocol was feasible and acceptable to both healthy older adults, and participants with a diagnosis of mild cognitive impairment or Alzheimer’s disease. Adherence to the training programme was better in the healthy compared to cognitively impaired cohort, with more training-related drop-outs in the Alzheimer’s disease group. Given that 89% of participants were able to complete the trial, and haemodynamic assessment was well tolerated, this warrants follow-up investigation in a larger trial. Drop-outs were more likely to occur in the AD group, indicating this group may need additional support, or alterations to the training programme to support completion. On secondary outcome analysis, preliminary results suggest there may be some evidence of training-related effects on cerebrovascular function, but we are unable to draw definitive conclusions from this study. Below we outline considerations for a future trial investigating the effects of CT on cerebral haemodynamics in the context of the current literature.

### *Context and possible mechanisms of vascular plasticity*

*Resting CBF*

A recent systematic review by Ten Brinke et al identified nine studies which examined functional imaging outcomes following cognitive training in healthy older adults and mild cognitive impairment [7]. The results of these studies were mixed, demonstrating both increased and decreased task-based fMRI responses [7]. The majority of studies have thus far investigated the effects of cognitive training on resting CBF in healthy populations rather than task activated responses [19, 31]. Mozolic et al found resting CBF increased in the prefrontal cortex following an eight week attention and distractibility programme in 66 healthy older adults, which correlated with improvements in cognitive outcome measures [19]. Similarly, Chapman et al investigated resting CBFv changes in 37 healthy older adults randomised to cognitive training or control for 12 weeks [31]. They also demonstrated training related rises in resting CBF which mirrored increases in functional connectivity in the default mode and central executive networks, preceded structural increases and were sustained across all time points of the study [31]. Given that both healthy ageing and dementia are associated with consistent declines in resting CBF [32-37], and that resting CBF can correlate with cognitive performance [13, 35, 38] and predict cognitive decline [12], increasing resting levels as seen in these studies may represent restitution of CBF to levels that are seen in younger adults or pre-pathological states [19, 31].

*Task-activated CBF*

In a recent systematic review, the majority of studies in mild cognitive impairment demonstrated an increase in task-activated responses after training [8]. There was only one study that investigated participants with established dementia and demonstrated a reduction in task-activated responses [39]. A meta-analysis of 2097 healthy older adults demonstrated both region-dependent increases and decreases in task-activated responses post-cognitive training [40]. Thus, it remains unclear whether training related increases or decreases in task-activation are beneficial. Certainly, this may depend on whether the focus is prevention of cognitive decline in healthy older adults, or restitution of normal brain function in those with mild cognitive impairment or dementia. Healthy ageing is associated with a number of key physiological changes when compared to younger adults, with lower resting CBF, loss of hemispheric lateralisation (HAROLD theory) [41, 42], prefrontal hyperactivation [16, 42], and increased cortical responses to task activation [34]. These changes could be compensatory [42], as the recruitment of additional neural circuits (CRUNCH theory) maintained cognitive performance in line with that of younger adults, but with a significant increase in resource requirement [43]. Thus, returning patterns of brain activity and activation to those seen in younger adults may indicate improvements in neural processing efficiency and reduction in resource requirement and compensation. In those with cognitive disorders, such as mild cognitive impairment and dementia, studies have demonstrated inconsistent findings in haemodynamic changes with both increases and decreases in resting and task-activated CBF [44-46]. One theory is the notion of a compensatory “hyperperfused” state in early mild cognitive impairment [46], progressing to hypoperfusion and reduced task activation in established dementia [36, 37, 47, 48]. Thus, in the early stages of cognitive decline, the goal could be similar to that in healthy older adults, but in later cognitive decline, this may shift to increasing resting CBF and task activated response. In this context, the trend towards reduced CBFv responses in the healthy cohort could represent a reduction in resource requirement by healthy older adults to execute a given task, which may be achieved through structural or functional vascular and neural remodelling [16]. In contrast, the increased response rate seen in participants with AD and MCI here, may represent an improvement in the blood flow response to cognitive stimulation, providing better coupling between brain activity and blood supply. However, these are cautious interpretations given the small sample size and need for further investigation.

### *Considerations for a future trial, limitations and future directions*

Only quality of life was significantly better in the healthy training cohort. Although the study was not powered to detect changes in secondary outcome measures, healthy older adults and participants with mild cognitive impairment performed well on the ACE-III and Lawton IADLs, suggesting participants were either close to or already at the ceiling of these scales. Thus, more sensitive scales may be needed for healthy adults and participants with mild cognitive impairment to detect clinically meaningful changes. In the absence of sensitive scales, physiological or functional brain imaging outcomes may provide a useful surrogate marker of cognitive training effects in studies of healthy older adults and participants with mild cognitive impairment.

The current study is limited by a small sample size. Whilst it was large enough to establish feasibility of the protocol and intervention, the sample size was not powered to detect changes in clinical and CBF outcome measures. In particular, the AD and MCI sub-groups were combined due to small sample sizes to improve the power of the analyses. AD and MCI may have different haemodynamic profiles depending on the stage of cognitive decline, and mechanisms at play (e.g. predominantly amnestic or not). However, in recent analyses [49], the haemodynamic profiles of these participants were found to be similar, which may be due to the relatively milder stage at which the AD participants were enrolled. However, the data from this study have provided the information to calculate the sample size needed for a future appropriately powered trial to investigate these findings further. Importantly, estimates of sample size varied considerably depending on the haemodynamic outcome measure used (between 20- 18000 participants). In particular, CRR and peak percentage change at T2 provided much smaller samples for future trials and this is an important consideration in the choice of outcome measure for future studies. The lack of blinding was a limitation in this study, as both patient and investigator could have introduced bias. Blinding of participants was problematic due to the lack of active control condition, and participants in the training arm may be more likely to report improvements or benefits, particularly on self-reported outcomes (quality of life, mood, and function), although few benefits were identified in these measures. Blinding of the investigator was, similarly, problematic due to limited operators with sufficient skills to undertake the physiological protocol. To mitigate this, haemodynamic and peripheral data were batch-blinded for analysis, but changes in operator technique cannot be excluded. In a future trial, blinding of both investigator and participants is important to minimise the effects of bias. In this study, the control condition was standard or usual care, with no active comparator. In studies investigating cognitive training, an active control or treatment comparator are preferred to eliminate other potential effects (i.e. computer use), and thus results may not be all directly attributable to the intervention. In addition, only participants with internet access were recruited, which may have entered selection bias by recruiting those with regular internet usage. The education level was lower amongst the cognitively impaired cohort compared to healthy older adults, although not significantly different between training and control groups. There were differences in some baseline co-variates between the intervention groups (e.g. BMI, antidepressant use). This may account for some of the differences in response to cognitive training between or within population groups. In future, a fully powered linear mixed effect model, taking into account significant co-variates, should be used to identify training related effects on cerebral haemodynamics. The use of TCD to measure changes in CBFv relies on the assumption that the vessel diameter remains constant despite fluctuations in BP and ETCO2. TCD measured-task activated has limited reproducibility [50] and this may affect the reliability of estimates of effect sizes. The tasks used in this protocol were not all selective for the MCA territory, but have been shown previously to produce robust responses in this region in healthy and cognitive impaired cohorts [20, 49]. Furthermore, TCD is limited by a lack of spatial resolution, even with selective tasks, and studies which use multiple neuroimaging techniques would be able to provide complementary information on spatial as well as temporal characteristics of the haemodynamic changes post-training. Peak percentage change in CBFv is susceptible to differences in resting CBFv baseline seen in cognitive impairment, and between baseline and follow-up measurements within groups as identified in the healthy control group in this study. However, CRR is susceptible to differences in resting variability of the baseline, which were corrected for using disease-specific thresholds from a larger data-set. CRR therefore may provide a better outcome measure due to reduced susceptibility to baseline fluctuations, effects of CO2 and blood pressure, and smaller sample sizes to power a future trial. Studies may also consider investigating the additive effects of multi-component interventions by investigating the effects of physical exercise, diet, education, and cognitive training on cerebral haemodynamic parameters. Future studies should investigate longer term outcomes of cognitive training and whether “booster” sessions are required to maintain functional brain changes. Furthermore, the optimal training dose (i.e. duration of programme and sessions, number and frequency of sessions) has not been fully determined in cognitively impaired populations. The training programme for this study was based on data from trials conducted with healthy participants [25], and further work is required to identify if a greater or lesser training dose is required to be effective in cognitively impaired populations.

# Summary

In summary, a protocol investigating the effects of cognitive training on cerebral haemodynamics in healthy older adults, and participants with cognitive impairment was feasible and acceptable. Training related changes were seen in cerebral haemodynamics, but the interpretations are limited by the small sample size. These results require further investigation in a larger appropriately powered trial of cognitive training with haemodynamic outcome measures to understand the role of cognitive training in modulating vascular plasticity and cognitive function in healthy older adults and those with dementia.

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**Conflicts of interest**

None to declare

**Author contributor statement**

All authors were involved in the design of the trial and drafting the manuscript. LB collected and analysed the data, drafted the manuscript. RBP assisted with data analyses and drafting the manuscript. EML, HS, TGR, and VJH contributed to the study design and drafting of the manuscript. CB contributed to the original statistical analysis plan for the trial, and reviewed and checked all the analyses in the current manuscript. All authors checked and approved the final version of the manuscript.

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|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Healthy older adults** | | | | **Cognitively impaired cohort** | | | |
| **Demographic** | **All (n=20)** | **Training (n=10)** | **Control (n=10)** | **P value** | **All (n=36)** | **Training (n=18)** | **Control (n=18)** | **P value** |
| Age (years) | 65.2 (8.4) | 63.0 (7.1) | 67.4 (9.3) | 0.25 | 70.19 (8.6) | 71.8 (9.0) | 70.3 (8.2) | 0.72 |
| Sex (n,% female) | 10 (50) | 6 (60) | 4 (40) | 0.37 | 11 (30.6) | 5 (27.8) | 6 (33.3) | 0.72 |
| Ethnicity (n,% Caucasian) | 19 (95) | 10 (100) | 9 (90) | 0.31 | 36 (100) | 18 (100) | 18 (100) | N/a |
| Diagnosis (n, %)  mild cognitive impairment  Alzheimer’s disease | - | - | - | - | 24 (66.7)  12 (33.3) | 6 (33.3)  12 (66.7) | 6 (33.3)  12 (66.7) | 1.00 |
| Education (years) | 17.1 (3.7) | 18.5 (3.6) | 15.6 (3.3) | 0.078 | 12 [11-17] | 15.5 [12-17] | 11 [11-12.75] | 0.10 |
| Handedness (n,%)  Left  Right | 2 (10)  18 (90) | 2 (20)  8 (80) | 0 (0)  10 (100) | 0.14 | 1 (2.8)  33 (91.7)  2 (5.6) | 0 (0)  17 (94.4)  1 (5.6) | 1 (5.6)  16 (88.9)  1 (5.6) | 0.60 |
| Smoker (n,%)  Current  Ex  Never | 0 (0)  10 (50)  10 (5) | 0 (0)  4 (40)  6 (60) | 0 (0)  6 (60)  4 (40) | 0.37 | 1 (2.8)  17 (47.2)  18 (50.0) | 0 (0)  8 (44.4)  10 (55.6) | 1 (5.6)  9 (50)  8 (44.4) | 0.53 |
| Alcohol (units/week) | 2 [0-10] | 0 [0-4.25] | 5 [0.5-13] | 0.19 | 0 [0-6.5] | 0 [0-7.5] | 0 [0-5] | 0.82 |
| BMI (kg/m2) | 25.8 (2.5) | 24.2 (1.8) | 27.4 (2.0) | **0.001** | 25.1 (3.8) | 24.3 (3.8) | 25.8 (3.8) | 0.23 |
| SBP (mmHg) | 139.3 (14.0) | 137.9 (15.4) | 140.6 (13.0) | 0.68 | 135.1 (18.5) | 129.8 (14.0) | 140.2 (21.0) | 0.35 |
| DBP (mmHg) | 81.0 (10.3) | 78.8 (10.2) | 83.1 (10.5) | 0.37 | 76.6 (10.9) | 75.8 (11.6) | 77.4 (10.5) | 0.86 |
| Diabetes (n,%) | 1 (5) | 1 (10) | 0 (0) | 0.31 | 5 (13.9) | 3 (16.7) | 2 (11.1) | 0.63 |
| Hypertension (n,%) | 8 (4) | 3 (30) | 5 (50) | 0.36 | 13 (36.1) | 6 (33.3) | 7 (38.9) | 0.73 |
| Hypercholesterolaemia (n,%) | 6 (30) | 3 (30) | 3 (30) | 1.00 | 19 (52.8) | 10 (55.6) | 9 (50) | 0.74 |
| Heart disease (n,%) | 1 (5) | 1 (10) | 0 (0) | 0.31 | 4 (11.1) | 1 (5.6) | 3 (16.7) | 0.29 |
| Previous stroke (n,%) | 0 (0) | 0 (0) | 0 (0) | - | 3 (8.3) | 1 (5.6) | 2 (11.1) | 0.55 |
| Depression (n,%) | 2 (10) | 0 (0) | 2 (20) | 0.14 | 7 (19.4) | 2 (11.1) | 5 (27.8) | 0.21 |
| Heart failure (n,%) | 1 (5) | 0 (0) | 1 (10) | 0.31 | 0 (0) | 0 (0) | 0 (0) | N/A |
| Total number of medications | 2 [0.75-3.5] | 2 (2.1) | 2.5 (2.1) | 0.60 | 5 [3-6] | 3 [1.25-5.75] | 5 [4-7] | 0.053 |
| ACHeI (n, %) | 0 (0) | 0 (0) | 0 (0) | - | 16 (44.4) | 8 (44.4) | 8 (44.4) | 1.00 |
| NMDA (N, %) | 0 (0) | 0 (0) | 0 (0) | - | 7 (19.4) | 3 (16.7) | 4 (22.2) | 0.67 |
| Antidepressant (n,%) | 2 (10) | 0 (0) | 2 (20) | 0.14 | 10 (27.8) | 2 (11.1) | 8 (44.4) | **0.03** |
| Antihypertensive (n,%) | 7 (35) | 3 (30) | 4 (40) | 0.64 | 15 (41.7) | 6 (33.3) | 9 (50) | 0.31 |
| ACE-III  Total  Attention  Fluency  Memory  Language  Visuospatial | 97.5 [94.8-99]  18 [17.5-18]  13.5 [11.8-14]  25 [23-26]  26 [26-26]  16 [15.8-16] | 97.5 [95.5-99.8]  18 [18-18]  13 [12-14]  25.5 [25-26]  26 [26-26]  16 [16-16] | 97.5 [93.5-98]  18 [17.3-18]  14 [11.3-14]  23.5 [22.3-25]  26 [26-26]  16 [15.3-16] | 0.48  0.74  0.91  **0.035**  0.48  0.74 | 82 [74-92]16 [12-17]  10 [8-12]  18 [12.75-23]  25 [24-26]  15 [14.8-16] | 84 [74-86.8]  16 [14.3-17.8]  9 [7.3-11]  18 [12.3-23]  25 [24.3-26]  16 [15-16] | 82 [75-93]  15 [12-17]  10 [9.3-12]  18 [13.5-19]  25 [24-26]  15 [14-16] | 0.58  0.17  0.29  0.67  0.74  0.21 |
| DEMQOL | 105 [100.3-109.3] | 106.4 (5.9) | 101.7 (9.2) | 0.19 | 93 [85-102] | 96 [86.8-101.5] | 91 [85-101.3] | 0.54 |
| GDS | 1 [0.75-2] | 1 [0.25-1] | 1.5 [1-2.8] | 0.14 | 2 [1-4] | 2.5 [1-3] | 2 [2-4] | 0.72 |
| Lawton IADL | 8 [8-8] | 8 [8-8] | 8 [8-8] | 1 | 6 [5-8] | 7 [5-8] | 5 [5-7.8] | 0.52 |
| Time to follow up (days) | 89 [85.5-98] | 98 [90.8-117.5] | 86 [84-89] | **0.002** | 86 [84-91] | 90 [83.5-101] | 84.5 [84-88] | 0.093 |

**Table 1. Baseline demographics of the healthy participants and cognitively impaired cohort (Alzheimer’s disease and mild cognitive impairment) by training or control. Data are number (%) for nominal data, mean (standard deviation) for continuous parametric data, and median [inter-quartile range] for continuous non-parametric data. Significance testing was by Chi-square for nominal data, independent t-test for continuous parametric data, and Mann-Whitney-U for non-parametric, continuous data. Significance level was set at p<0.05.** Abbreviations: BMI= body mass index, SBP= systolic blood pressure, DBP= diastolic blood pressure, ACE-III= Addenbrooke’s Cognitive Examination, DEMQOL= Dementia Quality of Life, GDS= Geriatric Depression Scale, IADL= Instrumental Activities of Daily Living.

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Healthy older adults** | | | | **Cognitively impaired cohort** | | | |
| **Outcome** | **Δ Training (n=10)** | **Δ Control (n=9)** | **MD (95% CI)** | **P value** | **Δ Training (n=15)** | **Δ Control (n=18)** | **MD (95% CI)** | **P value** |
| ACE-III total | 0.20 (1.61) | -0.11 (3.95) | 0.31 (-2.55, 3.18) | 0.83 | 1.33 (4.45) | -0.53 (4.00) | 1.86 (-1.19, 4.91) | 0.22 |
| Attention | -0.1 (0.88) | -0.56 (1.01) | 0.46 (-0.46, 1.37) | 0.46 | -0.13 (1.30) | 0.00 (2.20) | -0.13 (-1.45, 1.18) | 0.84 |
| Fluency | 0.40 (1.26) | 0.56 (1.13) | -0.16 (-1.32, 1.01) | 0.78 | 0.40 (2.03) | -0.06 (0.94) | 0.46 (-0.64, 1.55) | 0.43 |
| Visuospatial | 0.20 (0.42) | 0.00 (0.50) | 0.20 (-0.25, 0.65) | 0.36 | -0.20 (0.94) | 0.18 (1.33) | -0.38 (-1.22, 0.47) | 0.37 |
| Language | -0.1 (0.32) | -0.22 (0.67) | 0.12 (-0.37, 0.62) | 0.12 | 0.27 (1.49) | -0.24 (1.30) | 0.50 (-0.50, 1.51) | 0.32 |
| Memory | -0.2 (1.13) | 0.78 (2.28) | -0.98 (-2.82, 0.86) | 0.25 | 0.93 (2.89) | -0.22 (2.32) | 1.16 (-0.69, 3.00) | 0.21 |
| DEMQOL | **1.50 (2.72)** | **-3.33 (4.77)** | **4.83 (1.13, 8.54)** | **0.014** | 2.53 (6.97) | -0.94 (10.10) | 3.48 (-2.81, 9.77) | 0.27 |
| GDS | -0.1 (0.74) | 0.33 (1.00) | -0.43 (-1.28, 0.41) | 0.29 | -0.20 (1.70) | 0.44 (2.62) | -0.64 (-2.25, 0.96) | 0.42 |
| Lawton IADL | 0.00 (0.00) | -0.11 (0.33) | 0.11 (-0.15, 0.37) | 0.35 | 0.07 (1.10) | 0.11 (0.90) | -0.04 (-0.75, 0.67) | 0.90 |

**Table 2. Secondary outcome measures. Data are mean Δ baseline to follow-up (standard deviation), and mean difference (95% confidence interval). Significance testing was by independent t-test and the significance level was set at p<0.05.** Abbreviations: ACE-III= Addenbrooke’s Cognitive Examination-III, DEMQOL= Dementia Quality of Life, GDS= Geriatric Depression Scale, IADL= Instrumental Activities of Daily Living.

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | **T2** |  |  |  | **T3** |  |
| **Diagnosis** | **Δ Training** | **Δ Control** | **Mean difference (95% CI)** | **P value** | **Δ Training** | **Δ Control** | **Mean difference (95% CI)** | **P value** |
| **Healthy** | **-2.87 (7.57)** | **2.48 (7.85)** | **-5.35 (-12.82, 2.11)** | **0.15** | **-3.18 (6.06)** | **0.62 (6.81)** | **-3.81 (-10.03, 2.42)** | **0.22** |
| **Cognitively impaired** | **-0.73 (4.01)** | **1.13 (3.04)** | **-1.86 (-5.60, 0.87)** | **0.17** | **-0.01 (3.12)** | **0.25 (4.26)** | **-0.27 (-3.27, 2.73)** | **0.86** |
| **Mild cognitive impairment** | **-3.39 (1.67)** | **0.40 (1.64)** | **-3.79 (-5.92, -1.65)** | **0.003** | **-0.34 (1.51)** | **1.32 (3.50)** | **-1.66 (-5.12, 1.60)** | **0.31** |
| **Alzheimer’s disease** | **1.92 (3.95)** | **1.57 (3.64)** | **0.35 (-3.81, 4.51)** | **0.86** | **0.31 (4.35)** | **-0.39 (4.72)** | **0.70 (-4.38, 5.78)** | **0.77** |

**Table 3. Mean and 95% confidence intervals for the average peak % change in CBFv response to 5 tasks by training group, diagnosis, and time-point. Statistical testing by independent t-test.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Diagnosis** | **Δ Training** | **Δ Control** | **Mean difference** | **P value** |
| Healthy control | -2.40 (3.57) | -0.56 (2.35) | -1.84 (-4.81, 1.12) | 0.21 |
| Alzheimer’s disease and mild cognitive impairment | 0.92 (1.73) | -0.88 (2.60) | 1.79 (0.005, 3.58) | **0.049** |
| Mild cognitive impairment | -0.17 (0.75) | -1.00 (2.37) | 0.83 (-1.43, 3.09) | 0.43 |
| Alzheimer’s disease | 2.00 (1.79) | -0.80 (2.86) | 2.80 (-0.00, 5.60) | **0.050** |

**Table 4. Mean (standard deviation), mean difference and 95% confidence interval for change in cumulative response rate in training and control groups. Significance testing by independent t-test.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Diagnosis** | **Expected mean difference** | **Cohen’s *d*** | **Standard deviation** | **Sample size** |
| **Δ Peak % change in CBFv** | | | | |
| **Healthy control** |  |  |  |  |
| T2 | 5.35 | 0.69 | 9.59 | 102 |
| T3 | 3.81 | 0.59 | 9.59 | 200 |
| **AD and MCI** |  |  |  |  |
| T2 | 1.86 | 0.53 | 6.49 | 384 |
| T3 | 0.27 | 0.07 | 6.49 | 18140 |
| **ΔCRR** | | | | |
| Healthy control | -1.84 | 0.60 | 3.00 | 84 |
| AD and MCI | 1.79 | 0.79 | 2.39 | 56 |
| MCI | 0.83 | 0.47 | 2.66 | 334 |
| AD | 2.80 | 1.11 | 2.14 | 20 |

**Table 5. Sample sizes required to detect the mean difference in percentage change in CBFv or CRR between training and control groups, with standard deviation pooled from the follow-up data. Cohen’s *d* provided as a measure of effect size. Alpha was set at 0.05 and power at 80%.**

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**Figure 1. Recruitment flow chart.**

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**Figure 2. Changes in the visuospatial task in the healthy cohort. (a) Δ Percentage change in CBFv with mean and 95% confidence intervals at T2 (left) and T3 (right) from baseline to follow-up in control, and training groups for CBFv1 (black circles) and CBFv2 (clear circles). (b) Change in peak percentage at T2 for CBFv1 (left), and CBFv2 (right) in control and training groups from baseline to follow-up. (c) Δ Percentage change in MAP (left) at T2 (black circles) and T3 (clear circles), and ETCO2 (right) at T2 (black circles), and T3 (clear circles). (d) Representative trace of the temporal CBFv response (solid line) with standard deviation (dashed line) to the visuospatial task at follow-up in the training (grey) and control (black) groups for CBFv1 (right) and CBFv2 (left) hemispheres. The arrow denotes task initiation.** **\*significantly different between training and control (p<0.05).**



**Figure 3. Δ CRR from baseline to follow-up in control and training groups. Left panel= mean and 95% confidence interval for ΔCRR in control (black circles), and training (clear circles). Right panel= change per participant in baseline CRR (back circles), to follow-up CRR (clear circles) by training and control groups. (a) Healthy older adults, (b) Alzheimer’s disease, (c) mild cognitive impairment, and (d) Alzheimer’s disease and mild cognitive impairment combined. \*significantly different between training and control (p<0.05).**

**Supplementary Information**

|  |  |
| --- | --- |
| **Task** | **Description** |
| **Attention** | |
| **Lost in migration** | **Selective attention: participants must correctly identify the direction of the central bird (target) amongst a flock (distractors).** |
| **Trouble brewing** | **Divided attention: participants must fill simultaneous coffee orders with the correct ingredients and ensuring cups do not overfill, requires multi-tasking and sustained attention.** |
| **Playing koi** | **Selective attention: Participants must keep track of moving fish, and feed each fish only once. Requires multiple object tracking and sustained attention.** |
| **Star search** | **Selective attention: Participants must identify the unique object amongst a series of similar objects.** |
| **Train of thought** | **Divided attention: Participant direct trains to the correct station by adjusting the tracks. Requires distributed and sustained attention, multiple object tracking and planning.** |
| **Language and verbal fluency** | |
| **Editors choice** | **Vocabulary and semantics: Participants select words which are most related to a given word.** |
| **Contextual** | **Vocabulary and reading comprehension: Participants must read a given text passage and identify the incorrect words in the text.** |
| **Continuum** | **Vocabulary and semantics: participants are given a series of words and must order them based on their meaning (e.g. fast, medium, slow).** |
| **Taking root** | **Vocabulary and semantics: Participants are provided with a root word and must derive word meanings based on this root.** |
| **Word bubbles** | **Participants list as many words as possible starting with a given stem.** |
| **Memory** | |
| **Memory serves** | **Working memory: participants have to deliver bags to hotel guests. They need to remember how many bags they have and deliver the correct number when requested.** |
| **Tidal treasures** | **Working memory: participants are presented with a series of objects and each time they must select items they have not done so before.** |
| **Follow that frog** | **Working memory: participants must remember and recall the number and direction of jumps a frog makes between lily pads.** |
| **Memory matrix** | **Spatial recall: remember and recall the location of blocks on a grid (without rotation).** |
| **Memory match** | **Working memory: participants indicate whether a card matches one shown (n) times previously** |
| **Visuospatial** | |
| **Masterpiece** | **Spatial reasoning and mental rotation: participants fit shapes together into a larger outline.** |
| **Penguin pursuit** | **Spatial orientation: Participants must direct a penguin through a maze against an opponent to reach the fish first. The maze rotates during the task and participants have to re-orientate and alter the direction of the keys accordingly.** |
| **Rotation matrix** | **Spatial orientation: Participants have to remember and recall location of blocks on a grid after it has rotated. Requires mental rotation and memory.** |
| **Speed pack** | **Visualisation: Participants must move objects within a suitcase so that when it closes the objects do not overlap.** |
| **Spatial speed match** | **Matching: participants must determine whether the current symbol matches the previous one presented to them.** |

**Supplementary Table 1. A description of the cognitive exercises included in the CT programme and their respective cognitive domains.**

|  |  |  |
| --- | --- | --- |
| **Diagnosis** | **CCF90** | **VR90** |
| Healthy older adult | 2.66 | 0.56 |
| MCI | 2.60 | 0.65 |
| AD | 3.28 | 0.56 |

**Supplementary Table 2. Group-specific CCF90 and VR90 thresholds used to determine responses to task-activation in healthy older adults, MCI and AD groups.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Healthy older adults** | | | | **AD and MCI** | | | |
| **Parameter** | **Training (n=10)** | **Control (n=9)** | **MD (95% CI)** | **P value** | **Training (n=12)** | **Control (n=16)** | **MD (95% CI)** | **P value** |
| Baseline CBFv1 (cm/s) | 52.8 (8.8) | 56.4 (8.5) | -3.6 (-11.7, 4.5) | 0.36 | 47.12 (11.29) | 44.99 (7.10) | 2.14 (-4.30, 8.57) | 0.50 |
| Follow-up CBFv1 (cm/s) | 52.8 (8.0) | 48.5 (6.7) | 4.3 (-2.9, 11.5) | 0.22 | 48.57 (10.94) | 44.32 (8.03) | 4.25 (-3.11, 11.60) | 0.25 |
| Baseline CBFv2 (cm/s) | 52.9 (7.7) | 56.2 (6.1) | -3.3 (-9.8, 3.2) | 0.30 | 48.61 (8.88) | 44.36 (7.21) | 4.26 (-1.23, 9.74) | 0.12 |
| Follow-up CBFv2 (cm/s) | 54.6 (8.8) | 47.8 (6.9) | 6.8 (-0.9, 14.6) | 0.08 | 46.77 (8.07) | 43.71 (9.01) | 3.06 (-3.71, 9.83) | 0.36 |
| Baseline MAP (mmHg) | 98.7 (8.9) | 99.9 (12.9) | -1.3 (-11.7, 9.1) | 0.80 | 93.01 (19.99) | 98.49 (15.93) | -5.48 (-17.72, 6.77) | 0.37 |
| Follow-up MAP (mmHg) | 91.5 (10.2) | 94.9 (3.6) | -3.4 (-11.0, 4.1) | 0.35 | 91.55 (12.91) | 99.50 (18.43) | -7.95 (-20.76, 4.86) | 0.21 |
| Baseline HR (bpm) | 68.2 (9.3) | 68.1 (19.6) | 0.1 (-14.4, 14.5) | 0.99 | 65.53 (11.36) | 58.39 (9.65) | 7.14 (-0.00, 14.28) | 0.05 |
| Follow-up HR (bpm) | 73.4 (8.9) | 71.8 (17.0) | 1.6 (-11.3, 14.5) | 0.17 | 67.34 (10.94) | 63.49 (10.34) | 3.85 (-4.46, 12.17) | 0.35 |
| Baseline ETCO2 (mmHg) | 37.4 (3.2) | 36.6 (2.8) | 0.9 (-2.0, 3.7) | 0.53 | 35.73 (2.33) | 33.53 (4.37) | 2.20 (-0.17, 4.58) | 0.068 |
| Follow-up ETCO2 (mmHg) | 37.3 (1.4) | 36.7 (2.3) | 0.6 (-1.2, 2.5) | 0.47 | 33.29 (5.11) | 36.26 (4.23) | -0.98 (-4.60, 2.65) | 0.59 |

**Supplementary Table 3. Resting cerebral blood flow velocity (CBFv) and peripheral parameters at baseline and follow-up assessments by training group. Data are mean (standard deviation), and mean difference (MD) (95% confidence interval).** Abbreviations: MAP= mean arterial blood pressure, HR= heart rate, and ETCO2= end-tidal CO2. Significance testing was by independent t-test between training groups and paired t-test within training group. P values were considered significant at <0.05.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Healthy older adults** | | | | **AD and MCI** | | | |
| **Parameter** | **Baseline** | **Follow-up** | **MD (95% CI)** | **P value** | **Baseline** | **Follow-up** | **MD (95% CI)** | **P value** |
| Training | N=10 | N=10 |  |  | N=12 | N=12 |  |  |
| CBFv1 (cm/s) | 52.83 (8.80) | 52.84 (7.98) | -0.01 (-3.44, 3.42) | 0.99 | 48.54 (11.83) | 48.57 (10.94) | -0.03 (-5.43, 5.36) | 0.99 |
| CBFv2 (cm/s) | 52.87 (7.66) | 54.65 (8.84) | -1.78 (-4.77, 1.22) | 0.21 | 48.62 (9.05) | 46.77 (8.07) | 1.85 (-1.10, 4.80) | 0.19 |
| MAP (mmHg) | 98.66 (8.89) | 91.47 (10.16) | 7.19 (-0.57, 14.94) | 0.07 | 90.63 (21.76) | 91.55 (12.91) | -0.91 (-14.79, 12.96) | 0.89 |
| HR (bpm) | 68.2 (9.32) | 73.41 (8.87) | -5.21 (-10.89, 0.47) | 0.07 | 67.16 (12.30) | 67.34 (10.94) | -0.19 (-3.08, 2.70) | 0.89 |
| ETCO2 (mmHg) | 37.42 (3.17) | 37.31 (1.42) | 0.12 (-1.27, 1.50) | 0.85 | 36.06 (2.68) | 33.29 (5.11) | 2.77 (-0.06, 5.60) | 0.054 |
| Control | N=9 | N=9 |  |  | N=16 | N=16 |  |  |
| CBFv1 (cm/s) | 54.95 (7.47) | 48.53 (6.72) | 6.42 (3.10, 9.73) | 0.002 | 46.30 (6.37) | 44.32 (8.03) | 1.97 (-2.29, 6.24) | 0.34 |
| CBFv2 (cm/s) | 55.75 (6.33) | 47.83 (6.94) | 7.91 (2.76, 13.07) | 0.008 | 44.84 (7.40) | 43.71 (9.01) | 1.13 (-4.26, 6.53) | 0.66 |
| MAP (mmHg) | 99.59 (13.66) | 94.89 (3.57) | 4.70 (-4.87, 14.26) | 0.29 | 98.44 (16.77) | 99.50 (18.43) | -1.06 (-9.03, 6.91) | 0.78 |
| HR (bpm) | 67.8 (20.81) | 71.79 (16.98) | -3.99 (-8.87, 0.89) | 0.10 | 59.55 (9.41) | 63.49 (10.34) | -3.94 (-10.44, 2.55) | 0.22 |
| ETCO2 (mmHg) | 36.44 (2.98) | 36.66 (2.32) | -0.22 (-1.48, 1.03) | 0.69 | 33.58 (4.52) | 34.26 (4.23) | -0.68 (-1.97, 0.60) | 0.27 |

**Supplementary Table 4.** **Resting cerebral blood flow velocity (CBFv) and peripheral parameters at baseline and follow-up assessments within intervention groups. Data are mean (standard deviation), and mean difference (MD) (95% confidence interval).** Abbreviations: MAP= mean arterial blood pressure, HR= heart rate, and ETCO2= end-tidal CO2. Significance testing was by independent t-test between training groups and paired t-test within training group. P values were considered significant at <0.05.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Healthy older adults** | | | | **AD and MCI** | | | |
| **Domain** | **Δ Training (n=10)** | **Δ Control (n=9)** | **MD (95% CI)** | **P value** | **Δ Training (n=12)** | **Δ Control (n=16)** | **MD (95% CI)** | **P value** |
| Attention |  |  |  |  |  |  |  |  |
| CBFv1 (%) | -5.13 (8.61) | 2.00 (14.27) | -7.13 (-18.4, 4.13) | 0.20 | 1.11 (7.04) | 1.16 (6.80) | -0.05 (-5.47, 5.36) | 0.98 |
| CBFv2 (%) | -4.47 (12.46) | 0.72 (9.75) | -5.19 (-16.11, 5.74) | 0.33 | -0.55 (8.28) | 0.82 (4.85) | -1.37 (-6.49, 3.76) | 0.59 |
| MAP (%) | -2.31 (7.65) | -1.49 (16.09) | -0.83 (-12.81, 11.16) | 0.89 | 0.11 (17.4) | -0.09 (13.81) | 0.19 (-11.92, 12.31) | 0.97 |
| HR (bpm) | 0.67 (37.67) | 21.46 (46.49) | -20.79 (-61.56, 19.98) | 0.30 | -2.71 (40.22) | 2.21 (35.57) | -4.92 (-34.44, 24.60) | 0.74 |
| ETCO2 (mmHg) | 0.16 (1.83) | 0.79 (1.82) | -0.63 (-2.40, 1.14) | 0.46 | -2.35 (4.18) | 0.58 (2.12) | -2.93 (-5.74, -0.13) | **0.041** |
| Fluency |  |  |  |  |  |  |  |  |
| CBFv1 (%) | 1.25 (9.73) | 0.70 (13.51) | 0.55 (-10.76, 11.86) | 0.92 | -0.69 (3.64) | 2.92 (10.76) | -3.61 (-10.29, 3.07) | 0.28 |
| CBFv2 (%) | 0.96 (10.30) | 0.86 (12.43) | 0.10 (-10.91, 11.11) | 0.99 | -1.21 (6.76) | 2.11 (11.59) | -3.32 (-11.04, 4.41) | 0.39 |
| MAP (%) | -3.93 (16.28) | -0.20 (8.12) | -3.74 (-16.43, 8.95) | 0.54 | -5.09 (11.56) | 2.07 (13.70) | -7.16 (-17.24, 2.91) | 0.16 |
| HR (bpm) | -2.44 (38.75) | 14.91 (43.38) | -17.35 (-57.09, 22.39) | 0.37 | 1.93 (44.61) | 3.09 (35.73) | -1.17 (-32.36, 30.02) | 0.94 |
| ETCO2 (mmHg) | 0.56 (2.71) | -0.10 (2.63) | 0.66 (-1.92, 3.25) | 0.60 | -3.31 (3.92) | 0.28 (3.00) | -3.59 (-6.27, -0.90) | **0.011** |
| Visuospatial |  |  |  |  |  |  |  |  |
| **CBFv1 (%)** | **-3.36 (7.41)** | **6.86 (12.21)** | **-10.22 (-19.87, -0.57)** | **0.039** | -1.52 (9.04) | 2.76 (6.42) | -4.28 (-10.27, 1.72) | 0.16 |
| **CBFv2 (%)** | **-4.49 (10.04)** | **6.20 (11.13)** | **-10.69 (-20.93, -0.44)** | **0.042** | -0.99 (9.96) | 3.83 (8.02) | -4.82 (-11.80, 2.16) | 0.17 |
| MAP (%) | -6.48 (9.03) | -1.58 (6.45) | -4.90 (-12.58, 2.78) | 0.20 | -4.39 (14.98) | 1.79 (9.36) | -6.18 (-16.54, 3.29) | 0.19 |
| HR (bpm) | -1.94 (47.52) | 14.98 (39.07) | -16.92 (-59.32, 25.49) | 0.41 | -5.93 (47.20) | 6.09 (33.21) | -12.03 (-43.22, 19.17) | 0.44 |
| ETCO2 (mmHg) | 1.96 (4.58) | 0.70 (2.90) | 1.25 (-2.51, 5.02) | 0.49 | -5.20 (4.73) | 0.20 (2.22) | -5.40 (-8.55, -2.25) | **0.002** |
| Language |  |  |  |  |  |  |  |  |
| CBFv1 (%) | -3.85 (15.36) | 3.75 (9.95) | -7.60 (-20.29, 5.10) | 0.22 | -2.05 (9.61) | -3.28 (7.26) | 1.23 (-5.31, 7.78) | 0.70 |
| CBFv2 (%) | -4.47 (11.34) | 2.99 (8.70) | -7.46 (-17.34, 2.41) | 0.13 | -3.47 (8.66) | 1.32 (9.54) | -4.79 (-11.99, 2.42) | 0.18 |
| MAP (%) | -1.95 (12.46) | -3.44 (10.05) | 1.50 (-9.54, 12.54) | 0.78 | -1.02 (16.71) | -3.63 (13.53) | 2.61 (-9.13, 14.35) | 0.65 |
| HR (npm) | -2.09 (36.71) | 12.31 (42.67) | -14.39 (-52.80, 24.02) | 0.44 | -2.52 (41.28) | 7.73 (31.29) | -10.25 (-38.40, 17.90) | 0.46 |
| ETCO2 (mmHg) | 1.04 (3.85) | 0.57 (2.01) | 0.46 (-2.56, 3.48) | 0.75 | -3.20 (4.10 | 0.24 (2.33) | -3.44 (-6.23, -0.64) | **0.019** |
| Memory |  |  |  |  |  |  |  |  |
| CBFv1 (%) | -2.28 (8.82) | 0.82 (12.04) | -3.11 (-13.24, 7.03) | 0.53 | 0.17 (7.11) | -0.47 (8.97) | 0.63 (-5.83, 7.10) | 0.84 |
| CBFv2 (%) | -0.38 (10.93) | -0.11 (11.69) | -0.26 (-11.21, 10.68) | 0.96 | -1.14 (7.58) | 0.13 (6.68) | -1.27 (-6.82, 4.29) | 0.64 |
| MAP (%) | -1.71 (8.55) | 1.45 (12.54) | -3.17 (-13.46, 7.13) | 0.53 | -5.64 (12.77) | -0.10 (10.90) | -5.53 (-14.75, 3.67) | 0.23 |
| HR (bpm) | -0.51 (43.54) | 27.19 (48.22) | -27.70 (-72.10, 16.70) | 0.21 | -2.65 (38.66) | 5.37 (28.44) | -8.02 (-34.04, 18.00) | 0.53 |
| ETCO2 (mmHg) | 2.33 (5.06) | -0.40 (3.39) | 2.73 (-1.50, 6.95) | 0.19 | -3.74 (5.12) | -0.83 (2.23) | -2.91 (-6.30, 0.47) | 0.086 |

**Supplementary Table 5. Mean Δ from baseline to follow-up (standard deviation) in task activated cerebral blood flow velocity (CBFv) and peripheral measures by training group at T2 (5-10 seconds), and mean difference (95% confidence interval) between training and control groups.** Abbreviations: CBFv1= cerebral blood flow velocity non-dominant, CBFv2 = cerebral blood flow velocity dominant, MAP= mean arterial blood pressure, HR= heart rate, and ETCO2= end-tidal CO2. Statistical significance was by Independent t-test. Statistical significance was set at P<0.05.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Healthy older adults** | | | | **AD and MCI** | | | |
| **Domain** | **Δ Training (n=10)** | **Δ Control (n=9)** | **MD (95% CI)** | **P value** | **Δ Training (n=12)** | **Δ Control (n=16)** | **MD (95% CI)** | **P value** |
| Attention |  |  |  |  |  |  |  |  |
| CBFv1 (%) | -5.19 (4.58) | 0.85 (17.71) | -6.05 (-18.26, 6.17) | 0.31 | 0.79 (9.96) | 0.98 (7.85) | -0.19 (-7.10, 6.72) | 0.96 |
| CBFv2 (%) | -6.62 (13.17) | -0.85 (10.59) | -5.77 (-17.43, 5.89) | 0.31 | -0.66 (9.97) | 1.60 (5.98) | -2.26 (-8.48, 3.95) | 0.46 |
| MAP (%) | -1.69 (4.83) | 0.71 (14.43) | -2.39 (-12.58, 7.79) | 0.63 | 3.15 (15.30) | -1.67 (9.86) | 4.82 (-5.82, 15.46) | 0.35 |
| HR (bpm) | 2.56 (32.25) | 20.48 (43.93) | -17.93 (-54.95, 19.10) | 0.32 | 0.78 (41.63) | 3.17 (32.82) | -2.39 (-32.71, 27.93) | 0.87 |
| ETCO2 (mmHg) | 1.55 (1.80) | 0.78 (1.67) | 0.77 (-0.92, 2.45) | 0.35 | -2.12 (4.30) | 0.51 (2.80) | -2.63 (-5.62, 0.37) | 0.08 |
| Fluency |  |  |  |  |  |  |  |  |
| CBFv1 (%) | 0.72 (9.05) | -1.36 (11.07) | 2.08 (-7.67, 11.83) | 0.66 | 1.32 (5.15) | -0.73 (10.65) | 2.06 (-4.82, 8.93) | 0.51 |
| CBFv2 (%) | -0.15 (9.88) | -1.29 (10.46) | 1.14 (-8.70, 11.04) | 0.81 | 0.36 (6.45) | 0.32 (10.85) | 0.04 (-6.74, 6.81) | 0.99 |
| MAP (%) | -3.99 (11.26) | -2.07 (4.63) | -1.92 (-10.43, 6.60) | 0.64 | -2.94 (12.12) | 1.35 (12.23) | -4.30 (-13.86, 5.27) | 0.36 |
| HR (bpm) | -4.56 (42.15) | 12.44 (47.40) | -17.00 (-60.32, 26.33) | 0.42 | 0.67 (44.07) | 2.18 (37.54) | -1.52 (-33.25, 30.22) | 0.92 |
| ETCO2 (mmHg) | 0.16 (3.32) | -0.35 (2.39) | 0.51 (-2.28, 3.30) | 0.71 | -3.53 (4.03) | 0.28 (3.42) | -3.81 (-6.71, -0.92) | 0.012 |
| Visuospatial |  |  |  |  |  |  |  |  |
| CBFv1 (%) | -1.81 (5.48) | 2.82 (14.37) | -4.63 (-14.94, 5.67) | 0.38 | 0.17 (6.47) | 0.08 (9.35) | 0.10 (-6.38, 6.58) | 0.98 |
| CBFv2 (%) | -4.67 (9.29) | 2.61 (11.34) | -7.28 (-17.27, 2.71) | 0.14 | -1.10 (8.48) | 1.06 (11.33) | -2.17 (-10.19, 5.86) | 0.58 |
| MAP (%) | -1.94 (13.12) | 4.47 (8.06) | -6.41 (-16.94, 4.12) | 0.22 | -2.43 (9.56) | 0.72 (8.53) | -3.16 (-10.21, 3.89) | 0.37 |
| HR (bpm) | -5.98 (40.03) | 17.08 (41.34) | -23.06 (-62.47, 16.48) | 0.23 | -3.07 (41.76) | 6.80 (29.76) | -9.87 (-37.61, 17.87) | 0.47 |
| ETCO2 (mmHg) | 1.76 (4.23) | 0.95 (1.85) | 0.81 (1.53, -2.42) | 0.61 | -4.57 (5.54) | -0.27 (4.09) | -4.29 (-8.26, -0.33) | 0.035 |
| Language |  |  |  |  |  |  |  |  |
| CBFv1 (%) | -4.72 (15.51) | 3.25 (8.89) | -7.97 (-20.21, 4.27) | 0.19 | 2.64 (10.55) | -3.19 (10.06) | 5.83 (-2.23, 13.89) | 0.15 |
| CBFv2 (%) | -6.10 (13.26) | 3.97 (7.62) | -10.07 (-20.54, 0.41) | 0.058 | 0.42 (9.80) | 3.48 (10.96) | -3.07 (-11.30, 5.16) | 0.45 |
| MAP (%) | -3.03 (10.56) | -0.83 (5.72) | -2.19 (-10.56, 6.17) | 0.59 | 9.28 (22.79) | -8.03 (21.13) | 17.31 (0.16, 34.46) | 0.048 |
| HR (bpm) | -3.09 (35.76) | 15.86 (39.02) | -18.95 (-55.42, 17.53) | 0.29 | -4.16 (41.37) | 5.00 (29.69) | -9.16 (-36.72, 18.40) | 0.50 |
| ETCO2 (mmHg) | 1.65 (2.98) | 0.35 (2.91) | 1.30 (-1.56, 4.16) | 0.35 | -2.63 (4.16) | 0.98 (2.96) | -3.61 (-6.37, -0.84) | 0.019 |
| Memory |  |  |  |  |  |  |  |  |
| CBFv1 (%) | -2.85 (5.16) | -2.87 (10.84) | 0.021 (-8.05, 8.10) | 1.00 | -1.03 (4.62) | -1.20 (10.86) | 1.69 (-6.11, 6.45) | 0.96 |
| CBFv2 (%) | -2.93 (6.97) | -0.91 (9.37) | -2.02 (-10.18, 6.14) | 0.60 | -0.06 (7.71) | 0.12 (9.04) | -0.18 (-6.85, 6.50 | 0.96 |
| MAP (%) | -2.02 (8.97) | 0.15 (10.25) | -2.16 (-11.46, 7.14) | 0.63 | 1.37 (13.33) | -2.25 (12.46) | 3.62 (-6.46, 13.69) | 0.47 |
| HR (bpm) | 0.17 (38.35) | 25.43 (43.71) | -25.26 (-64.97, 14.44) | 0.20 | 0.18 (39.11) | 2.07 (28.80) | -1.89 (-28.23, 24.44) | 0.88 |
| ETCO2 (mmHg) | 2.20 (4.84) | 0.36 (3.01) | 1.85 (-2.11, 5.81) | 0.34 | -3.44 (5.31) | -1.01 (2.45) | -2.44 (-5.52, 0.65) | 0.12 |

**Supplementary Table 6. Mean Δ from baseline to follow-up (standard deviation) in task activated cerebral blood flow velocity (CBFv) and peripheral by training group at T3 (10-20 seconds), and mean difference (95% confidence interval) between training and control groups.** Abbreviations: CBFv1= cerebral blood flow velocity non-dominant, CBFv2 = cerebral blood flow velocity dominant. MAP= arterial blood pressure, HR= heart rate, and ETCO2= end-tidal CO2. Statistical significance was by Independent t-test. Statistical significance was set at P<0.05.

|  |  |  |
| --- | --- | --- |
| **Domain and correlation** | **Control** | **Training** |
| **Healthy older adults** | | |
| Attention task Δ CBFv at T2 | | |
| Δ GDS score | NS | Non-dominant: r=0.70, p=0.026 |
| Attention task Δ CBFv at T3 | | |
| Δ ACE-III score | NS | Dominant: r=-0.72, p=0.019 |
| Δ Attention score | Dominant: r=-0.72, p=0.029; non-dominant: r=-0.72, p=0.027 | NS |
| Δ DEMQOL score | NS | Dominant: r= 0.64, p=0.047 |
| Visuospatial task Δ CBFv at T2 | | |
| Δ ACE-III score | Non-dominant: r=-0.82, 0.007 | NS |
| Visuospatial task Δ CBFv at T3 | | |
| Δ ACE-III score | Non-dominant: r=-0.76, p=0.017 | NS |
| Δ DEMQOL score | Non-dominant: r=-0.78, p=0.014 | NS |
| Language task Δ CBFv at T3 | | |
| Δ ACE-III score | Non-dominant: r=-0.71, p=0.032 | NS |
| Δ GDS score | NS | Dominant r=0.70, p=0.024; non-dominant r=0.77, p=0.01 |
| Memory task Δ CBFv at T2 | | |
| Δ ACE-III score | Dominant: r=-0.80, p=0.009; non-dominant r=-0.92, p=0.001 | NS |
| Δ Memory score | Dominant: r=-0.71, p=0.032; non-dominant: r=-0.90, p=0.001 | NS |
| Δ CRR | | |
| Δ Memory score | NS | R=-0.71, p=0.022 |

**Supplementary Table 7. Correlations between Δ task-activated CBFv at T2 or T3 with change in clinical outcome measures.** Abbreviations: CBFv= cerebral blood flow velocity, ACE-III= Addenbrooke’s Cognitive Examination III, DEMQOL= dementia quality of life, NS= non-significant. Correlations were performed using Pearson or Spearman correlation. R= correlation coefficient.