Tesi di dottorato

Effectiveness of cognitive and physical training in slowing progression to dementia: a clinical and experimental study. Focus on relationship with cardiovascular fitness

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Alzheimer's disease (AD) is nowadays regarded as only one part of the whole dementia scenario, that encompasses primary neurodegenerative dementias, vascular dementia and mixed dementias. The number of patients affected by dementia has been growing steadily over the last decade, and neurodegenerative processes are a significant threat and health burden for the elderly – it is estimated that by 2030, there will be more than 65 million people with dementia worldwide (Prince, Bryce et al. 2013). It is still unclear what is the relative contribution of amyloid plaques (50 - 80%?) and by vascular pathology (20 - 30%?) to the dementia epidemic (Abbott 2011): they usually coexist, to some variable extent, in brains of people with dementia, although neuro-pathological criteria for AD can be found in 50% of the brains of aged individuals without cognitive impairment. Even in symptomatic individuals, there is poor correlation between severity of disease and anatomo-pathological burden (Wharton, Brayne et al. 2011). The link between vascular disease and AD is certain and probably strong (de la Torre 2012), but is neither simple nor entirely clear, with a blurred pathological divide between cognitive decline and normal brain and vessel aging (Picano, Bruno et al. 2014).

The vascular basis of brain aging and dementia

With normal aging, both cognitive function and cognitive reserve decline. Cognitive reserve refers to the brain's ability to cope with either increasing activities or damage. The concept of cognitive reserve is a predominantly functional one. It follows the concept that brain plasticity allows increasing brain connectivity after mental exercise, all throughout life, and this prolongs maintenance of function despite cell degeneration in older life. There is a threshold after which cognitive reserve finishes and cognitive impairment becomes evident (Snowdon and Nun 2003).

Normal aging causes brain atrophy, with MRI studies showing a 4% reduction in brain volume for every 10 years above age 65 (Fotenos, Mintun et al. 2008). This para-physiological descent is earlier and steeper in neurodegenerative diseases. Vascular reserve

refers, in its turn, to the vessels' ability to cope with increasing activity or damage, and is commonly measured as the ability to increase regional flow after a hyperemic stimulus, such as carbon dioxide in the brain, adenosine in the myocardium or ischemia in the forearm; the cerebral, coronary and systemic vascular flow reserve also decline with aging (Taddei, Virdis et al. 1995, Galderisi, Rigo et al. 2012). Since it is unable to store energy, the brain depends on continuous delivery of oxygen and glucose through blood flow. Under physiological conditions, essential for neuronal functional activity, there is a systematic coupling between activation of specific brain function and microcirculation flow activation, within the concept of the neurovascular unit with microvessels, endothelial cells, neurons and glial cells all closely integrated with continuous bidirectional signaling (Iadecola 2010, Del Zoppo 2013). The brain is a high-flow, low-impedance organ with a high metabolic rate, and typically receives 15% of total cardiac output and 25 to 50% of total body glucose utilization, despite accounting for only 2% of total body weight (Fehm, Kern et al. 2006, Wolff 2007). Blood flow is normally autoregulated, meaning that flow remains relatively constant over a wide range of mean perfusion pressures. If blood pressure falls below a certain point, termed the lower limit of autoregulation, cerebral blood flow decreases. Because of the high metabolic demand for oxygen in the brain, limited cerebral blood flow may lead to an energy crisis in the neurovascular unit (de la Torre 2012): cerebral hypoperfusion could be an early event in the cascade leading to cognitive decline.

For several decades the traditional classification of dementia have distinguished those forms of clinically relevant cognitive impairment in which the evidence of stroke or subclinical vascular brain injury can be identified (namely vascular cognitive impairment, VCI) from AD, describing different pathophysiological pathways in the two conditions. More recently, de la Torre hypothesized that AD can be viewed as a predominantly vascular disorder (de la Torre 2002). The hypothesis was based on several converging lines of evidence, including: 1) epidemiological studies showing that AD shares with cardiovascular disease a number of risk factors (from hypertension to diabetes) and virtually all risk factors for AD also lower cerebral perfusion (Kaffashian, Dugravot et al. 2013); 2) detection of regional cerebral hypoperfusion with the use of neuroimaging techniques is capable to identify AD candidates preclinically; 3) regional microvascular abnormalities in the brain appear before hypometabolism, cognitive decline and neurodegenerative changes; 4) clinical overlap

between AD and vascular dementia; 5) improvement of cerebral perfusion is obtained from drugs (such as cholinesterase inhibitors) used to reduce the symptoms of AD in patients who respond to treatment (Geaney, Soper et al. 1990, Van Beek and Claassen 2011). However, the interpretation of the cause-effect relationship between hypoperfusion and cognitive decline remains uncertain, since decreased flow could be interpreted as a mere consequence of reduced neuronal activity. The reverse could also be true, turning cerebral hypoperfusion from bystander to killer – and a potential target of therapeutic intervention. Evidence supporting the vascular contribution to AD has been reinforced and extended in the last decade. Brain and vessel premature aging share similar molecular, cellular and tissue mechanisms, such as abnormal intracellular calcium handling, epigenetic DNA damage, and low-level systemic and local inflammation with altered redox state (Pizza, Agresta et al. 2011), with imbalance between reduced trophic-protective factors and increased inhibitory factors and reduced endothelial nitric oxide production. AD and atherosclerosis show several common signaling pathways, with common genes, common miRNA family, and common molecular links. However, the clinical diagnostic and therapeutic implications of this improved understanding of the disease remain elusive, and usually no systematic cardiovascular workup is yet recommended in AD (Picano, Bruno et al. 2014).

Links between cardiovascular disease and cognitive impairment: a patho-physiological perspective

Changed cardiovascular conditions can induce changes in organs and above all in brain perfusion (Cipolla 2009), possibly one of the determinants of cognitive impairment. The system can be conceptualized as a chain: normal brain perfusion requires that all rings of the physiological chain be intact, since a chain can only lift the weight that its weakest link can bear. In this simplified description of cardiovascular hemodynamics, it is possible to identify distinct segments in series, contributing to normal perfusion with well-oxygenated blood in the healthy aging brain: the lungs, the heart, the large elastic vessels (aorta) (Muqtadar, Testai et al. 2012), the cerebrovascular arteries (carotid, middle cerebral and vertebral arteries) (Demarin and Morovic 2014), the small cerebral vessels (contributing to

50% of the overall cerebral resistance of the healthy brain) (Ostergaard, Aamand et al. 2013), and the cerebrospinal venous system. An anatomic or functional alteration of each segment can lead to impaired cerebrovascular perfusion and increased risk of dementia, although frequently these conditions are not isolated and are often mutually interrelated. The risk of critical cerebral hypoperfusion increases in a clinically relevant way when each of the rings of the adequate cardiovascular cerebral perfusion chain is weakened. No matter how brain hypoperfusion occurs, it may eventually enter a final common pathological pathway, provoking β-amyloid (Aβ) accumulation (Zlokovic 2011). Effective therapeutic interventions targeting cardiovascular disease might beneficially affect cognitive decline (Picano, Bruno et al. 2014).

Cerebrovascular disease

Cerebrovascular disease is recognized as the pathophysiological basis for VCI, ranging from mild impairment to full-blown dementia. Its clinical manifestations range from overt, repeated strokes to dementia accompanied by neuroimaging findings, showing brain small vessel disease (SVD) including dilated Virchow-Robin spaces, micro-infarcts, white matter degeneration and microbleeds. Indeed, neuroimaging abnormalities can be found in both AD and VCI, as well as in asymptomatic individuals (Gorelick, Scuteri et al. 2011). Both micro- and macrocirculation can be involved. At pathology verification, atherosclerotic changes in circle of Willis and intracranial arteries are more extensive in AD than in people without dementia (Roher, Tyas et al. 2011). Atherosclerosis of the extracranial carotid arteries is associated with increased prevalence of both vascular and AD dementia in the Rotterdam study population (Hofman, Ott et al. 1997). Arteriolosclerosis and, more in general, cerebral SVD, are also involved in cognitive impairment and they are thought to be a major component of degenerative brain disease. Age and hypertension appear to be the main factors inducing microvascular rarefaction and remodeling, but also AD, cerebral amyloid angiopathy and hereditary small-vessel syndromes are associated to SVD and contributors to VCI (Brown and Thore 2011, Gorelick, Scuteri et al. 2011, Picano, Bruno et al. 2014).

Clinical implications: diagnosis and therapy of the vascular component of cognitive impairment

A thorough evaluation of patients with cognitive impairment should systematically include the assessment of the cardiovascular rings of the chain linking blood perfusion to brain function: this systematic screening may also lead to identification of potentially treatable factors enhancing cognitive decay. In middle-age subjects, an abnormally high pressure is a risk factor for long-term development of dementia, and antihypertensive therapy may protect from hypertension-associated dementia (Levi Marpillat, Macquin-Mavier et al. 2013). However, in elderly patients the risk of mental deterioration may be enhanced also when diastolic pressure becomes too low, since the relationship between blood pressure and cognitive function appears to be curvilinear, so that a low diastolic and mean blood pressure are associated with more progression of sub-cortical brain atrophy in patients with manifest arterial disease (Jochemsen, Muller et al. 2013). Cerebrovascular disease can involve both large and small vessels. Elderly symptomatic patients with severe carotid lesions, easily diagnosed by ultrasound, had significant improvement in cognitive performance scores after carotid endarterectomy (Baracchini, Mazzalai et al. 2012). Cerebral small vessel disease can be indirectly detected through its functional consequences via transcranial Doppler sonography, which, although not as accurate as computed tomography or MRI imaging, is a non-invasive imaging technique assessing mean arterial blood flow velocity (an accepted proxy for cerebral blood flow) and the pulsatility index (a surrogate for cerebrovascular resistance and intracranial compliance). Patients with AD have a reduced cerebral blood flow velocity and increased pulsatility index, and these changes are even more obvious in vascular dementia (Sabayan, Jansen et al. 2012).

Endothelial cells seem to play a major role in maintaining the integrity and homeostasis of the blood-brain barrier. Endothelial nitric oxide (NO) is a known major vasodilator playing a key role in regulating local blood flow, and is altered in many vascular conditions. However, endothelial NO has also shown the ability to affect synaptic plasticity, mitochondrial biogenesis, and function of neural progenitor cells, also modulating processing of amyloid precursor protein. It has been suggested that endothelial NO could be the key molecule linking cerebrovascular and neuronal function and the “protector of a

healthy mind" (and also of a healthy vessel, probably a prerequisite for the preservation of a healthy mind) (Katusic and Austin 2014). In particular, reduced bioavailability of NO promotes the formation of amyloid plaques: in the neural cells, it increases the production of both amyloid precursor protein (APP) and its cleavage enzyme; in the microglia, it favors a pro-inflammatory phenotype. In its turn, Aβ deposition causes endothelial dysfunction in the brain circulation through an increase in reactive oxygen species as byproduct of NADPH oxidase (Zlokovic 2011). The maintenance of cerebrovascular endothelial function through regular exercise and healthy lifestyle is a key element for successful brain aging (Bolduc, Thorin-Trescases et al. 2013, Picano, Bruno et al. 2014).

Arterial stiffness and cognitive decline

Stiffening of large arteries occurs with aging. It reduces the buffering capacity of the arterial tree; thus, peripheral circulation and small vessels are subject to increased flow and pressure pulsatility. This is especially dangerous for organs such as the brain and the kidney, which are characterized by torrential flow and very low resistances, with lower defense against large, and possibly deleterious, oscillations in pressure with the cardiac cycle. Arterial stiffening is, indeed, associated with brain damage (Waldstein, Rice et al. 2008); high pulse pressure (particularly at the central level), whose arterial stiffness is a major determinant, is also associated with brain damage (Waldstein, Rice et al. 2008). It is known that an increase in arterial stiffness is a major risk factor for dementia (de la Torre 2002, Luchsinger, Reitz et al. 2005, Waldstein, Rice et al. 2008, Zlokovic 2011). Indeed, a higher aortic stiffness is associated to a steeper decline of cognitive function over time (Waldstein, Rice et al. 2008, Mitchell 2011, Mitchell, van Buchem et al. 2011, Pase, Herbert et al. 2012), although neutral results were found for carotid stiffness (Poels, van Oijen et al. 2007). Carotid plaques and intima-media thickness are associated with cognitive decline (Wendell, Zonderman et al. 2009), and reduced carotid distensibility might cause cerebral vascular damage by increasing the transmission of flow and pressure pulsatility to the cerebral microcirculation (O'Rourke and Safar 2005) and by favoring plaque rupture and embolization (Paini, Boutouyrie et al. 2007). Improved carotid distensibility might therefore contribute to reduce vascular contribution to dementia pathogenesis. In a recent meta-

analysis a significant improvement in aortic stiffness was observed in response to aerobic exercise training (Ashor, Lara et al. 2014), while results regarding carotid stiffness are few and less consistent (Kitzman, Brubaker et al. 2013, Tanahashi, Akazawa et al. 2014).
In this framework, the use of biomarkers such as arterial stiffness and endothelial function, sensitive to the integrated effect of the sum of a number of cardiovascular risk factors over time directly on their main target, the arterial tree, is appealing, since it would allow identifying individuals at risk not only for cardiovascular events, but also for cognitive decline. Improved arterial function and distensibility might contribute, on its turn, to reduce vascular contribution to dementia pathogenesis.
Driven by a better understanding of underlying mechanisms, the core of therapeutic intervention can shift from individual treatment to aggressive prevention of environmental causes, with both individual and social benefits.

Environmental nurturing of brain and vessel plasticity

Environment can both protect and damage the aging brain and modulate vessel plasticity. Many factors exert their influence in a similar direction for both brain and vessel function. Premature brain and vessel aging can be promoted by environmental factors such as sedentary lifestyle, heavy ecosystem pressure (high urban and industrial pollution, agriculture, military and medical toxicants), "brain drain" (stress, isolation, depression), and eating habits (overeating, junk food).
Environmental enrichment (EE) profoundly affects the central nervous system at the functional, anatomical and molecular levels, during the critical early growth period, during adulthood, in the aging brain and even in neurodegenerative states, such as early AD. The efficacy of this therapeutic approach has been demonstrated in the experimental setting (Sale, Berardi et al. 2014), although more clinical data are needed, and a recent US National Institute of Health Consensus panel on AD concluded that it is too soon to tell whether lifestyle changes including physical, cognitive, sensory, and social training or any other prevention strategy can affect the development or course of AD (Deweerdt 2011).

Lifestyle changes are likely to have only limited benefit if overt dementia is enrolled, without exclusion of patients with important comorbidities. On the contrary, benefits might be magnified in early stages of disease without critical comorbidities.

Even small gains in cognitive function or slight delays in the development of symptoms could greatly reduce the burden of disease. Obviously, an aging brain in the pre-dementia stage cannot have the same synaptic and vascular plasticity as a healthy adult brain, which even in normal conditions will suffer a 20% reduction in functional capacity by about 68 years of age. However, assuming that environmental enrichment might produce a reduction of the rate of decline of only 0.1% per year from young adulthood onward, such a functional decline would then be delayed until age 88. Negative environmental nurturing could, on its turn, advance the 20% cognitive decay to the age of 58. In addition, the same lifestyle and environmental factors modulating cognitive function also modulate vascular function.

This model explains why even trivial improvements can add up over time to exert significant consequences for the individual. The recommendations of neurobiologists to prevent cognitive decay by lifestyle and environmental intervention closely mirror those of cardiologists to prevent progression of vascular disease: quitting smoking, achievement and maintenance of ideal body weight, regular exercise, reduced intake of saturated fats and sugars, and decreasing the level of stress, which is best achieved by improving one's family, community, and society relationships (Fihn, Blankenship et al. 2015). An integrated physical, cognitive, and dietary program of environmental enrichment allows the neurobiologist and the cardiologist to kill but two birds (brain and vessel protection) with one stone (lifestyle changes).

To date, research on nonpharmacological treatment for AD and VCI is limited, despite preliminary evidence that these approaches are no less efficacious than the antidementia drugs (Pitkala, Poysti et al. 2013): exercise programs (Forbes, Forbes et al. 2015), cognitive intervention (Reijnders, van Heugten et al. 2013) and sensorial enrichment (Kverno, Black et al. 2009), either alone or combined (Fiatarone Singh, Gates et al. 2014) can slow the progression of MCI and AD, with a beneficial impact on health care costs (Pitkala, Poysti et al. 2013).

In the end, it remains uncertain whether AD is a vascular disorder with neurodegenerative consequences or a neurodegenerative disorder with vascular consequences. It is certain that

the cardiovascular system and the central nervous system are two complex systems whose functions are deeply intertwined, with similar processes participating in the phase of involution, the same markers indicating vulnerability to decay, and possibly similar factors slowing progression and promoting resilience to disease. The concepts of pure vascular and pure neuro-degenerative dementia have been replaced with the recognition that most patients exhibit a spectrum of neurodegenerative and vascular processes (Viswanathan, Rocca et al. 2009, Zlokovic 2011), suggesting that the disorder might, at least in some instances, be secondary to cerebrovascular disease (Picano, Bruno et al. 2014).

The Train the Brain study

Within this framework, the Italian National Research Council (CNR) launched a prospective, randomized, parallel-group, open-label clinical trial called "Train the Brain", and enrolled (between March 2012 and January 2014) 118 patients (aged 69 - 89 years) with Mild Cognitive Impairment, who were then randomized to standard vs. environmental enrichment therapy. In the enrollment phase, a thorough cardiovascular characterization (including resting echocardiography, flow-mediated dilation of brachial artery and aortic stiffness) was performed, in order to better define the study cohort from the cardiovascular viewpoint. A complete clinical, neuropsychological and brain (anatomic, perfusion and functional) MRI assessment was performed at study entry, then following the 7-month period. The study was aimed at providing insight into the neuropsychological, cerebral microvascular and cardiovascular alterations present in mild cognitive impairment, as well as their modification by an environmental enrichment program.
The intervention involved a 3-h environmental enrichment session 3 times a week for 7 months, consisting of tailored, stepwise physical, cognitive and social training, including music therapy and dance, under the close supervision of highly specialized personnel in an ad-hoc facility built within the Pisa CNR Research campus.

A clinical and experimental study of the efficacy of cognitive training and physical exercise in dementia

Age-dependent cognitive decline is a highly impacting problem at the clinical, economical and assistance level. Age, indeed, is the major risk factor for dementia. In Italy there are around 700.000 patients diagnosed with dementia and around 100.000 new cases every year.
At present, there are no effective therapeutic strategies for dementia, a pathology practically orphan of treatment. It is therefore more and more evident the need of strategies aimed at preventing or slowing down cognitive decline, starting from the early stages of the disease. The Train the Brain project assesses the efficacy of a combination of physical exercise and cognitive training in slowing down the progression of symptoms in subjects at risk of dementia.

Several studies in humans have demonstrated that the exposure to a cognitively and socially stimulating environment and physical exercise have beneficial effects on brain functioning, especially in the elderly, and reduce the risk of developing dementia (Fratiglioni, Paillard-Borg et al. 2004, Marx 2005, Kramer and Erickson 2007). The estimated reduction in the risk of developing dementia varies from study to study but generally ranges between 20 and 50%.
In parallel, a large number of studies performed in animal models has demonstrated that EE – physical exercise and exposition to a cognitive and socially stimulating environment – ameliorates cognitive performance, slows down the decline associated with aging, is neuroprotective, and enhances neural plasticity (Mainardi, Di Garbo et al. 2014). EE ameliorates cognitive deficits in animal models of AD (Jankowsky, Melnikova et al. 2005). These results show the EE potential as non-pharmacological therapeutic strategy to prevent the onset of cognitive deficits, but studies on the efficacy of a combined physical and cognitive intervention in subjects with dementia are few and present methodological limits. An important point that has recently emerged both from the human and animal research, is that the combination of physical and cognitive activity seems to have additive effects on brain plasticity and age related cognitive decline (Fabel, Wolf et al. 2009, Paillard-Borg, Fratiglioni et al. 2012) (Fiatarone Singh, Gates et al. 2014). In this line, a very important

study is that by Ngandu et al., 2015 (Ngandu, Lehtisalo et al. 2015). More than 1250 elders, with cognition in the mean level for age, were randomized to a two-year multidomain intervention of diet, exercise, cognitive training, and vascular risk monitoring versus control. Findings show that global cognitive status, executive functions and processing speed improved more in the intervention than in the control group, suggesting that a multidomain intervention could indeed improve or maintain cognitive functioning in elders.

Evidences from the experimental and clinical literature indicate that mild cognitive impairments and the presence of subtle cerebral alterations, detectable with techniques such as structural and functional neuroimaging, precede of several years the clinical onset of dementia (Garrett, Browndyke et al. 2004, Jones, Laukka et al. 2004, 2008). This early stage of the disease is referred to as Mild Cognitive Impairment (MCI). In the majority of cases, MCI heralds overt dementia, with a rate of yearly progression to dementia of MCI subjects being much higher (up to 20 times more frequent) than in the non-MCI elderly, so much that about 70% of MCI cases develop overt dementia within a period of five years. It is believed that in the preclinical phase of dementia a progressive loss in the number, efficacy and modifiability of synaptic connections. The progression of these alterations and the manifestation of this loss in terms of clinical signs would be contrasted by plastic reorganizations within the brain that tend to compensate for the reduced density and functionality of synapses and that increase neural plasticity, favoring the establishment and recall of memory traces. A precocious identification of MCI would allow initiating a therapeutical / rehabilitative intervention precociously, making it possible to maximally exploit the remaining potential for plasticity in the brain. This would markedly strengthen the efficacy of interventions aimed at prevent the progression of these subjects toward a condition of severe dementia.

The scientific hypothesis underlying this project is that interventions including physical exercise and cognitive stimulation can significantly affect the cognitive decline in subjects at risk for developing dementia. This goal, if achieved, would determine a delay in the loss of self-sufficiency and, therefore, an increase in the quality of life of the patients and caregivers, resulting in a net reduction of the direct and indirect costs associated with continuous assistance, and without risks of collateral effects.

The primary aim of the Train the Brain study was therefore to assess the efficacy of a protocol of physical exercise and cognitive stimulation on the progression of the disease in subjects at risk of dementia, identified through an advanced battery of diagnostic tests, with the aim to develop a nonpharmacological preventive/therapeutic strategy easily applicable to humans and exploitable by the National and Regional Health Service.

Methods

Study protocol

The eligible population for the study included elderly subjects (65-89 years) with Mild Cognitive Impairment confirmed at the neurological examination; severe neurological pathologies such as brain lesions or conditions barring participation to the cognitive or physical training program were the only substantial exclusion criteria.

Phase 1 - Patient recruitment and baseline cognitive evaluation: General Practitioners of Pisa Municipality were instructed to select subjects aged 65 to 89 years, in good health, with no significant cerebral vascular disease, psychoses, depression, dementia or mental retardation. A preliminary questionnaire regarding memory troubles (MAC-Q) was self-administered. These potentially eligible subjects underwent a clinical confirmation with a set of neuropsychological tests, which also provide the baseline cognitive status, and a comprehensive collection of medical history and physical, cognitive, and affective examination. Subjects with a confirmed diagnosis of MCI and matching inclusion criteria were enrolled in the study and randomly assigned either to intervention or control group; all enrolled subjects underwent baseline instrumental evaluation.

Recruitment was performed using a two-phase scheme consisting of a screening phase, to identify subjects with possible cognitive impairment, and a neuropsychological evaluation of subjects screened as positive to confirm or reject the diagnosis of Mild Cognitive Impairment. The screening evaluation, phase 1, includes:

• Mini Mental State Examination (MMSE), largely used to screen for cognitive impairment and to estimate severity;

• Clock Drawing Test (CDT), as a valid screening method for Mild Cognitive Impairment;

• Clinical Dementia Rating Scale (CDR), a structured interview with the patient and an informant, used to quantify the severity of symptoms of dementia;

• Geriatric Depression Scale (GDS) a self-report assessment used to identify depression in the elderly.

To be eligible for phase II, all participants had to meet the following operational criteria:

1) CDR of 0.5 or 1, with at least a 0.5 in the memory domain and with MMSE score greater

than or equal to 24 and CDT score greater than 8;

2) a CDT score lower or equal than 8, even if CDR equal 0 and MMSE score greater than or equal to 24;

3) Geriatric Depression Scale (GDS) score lower than 9.

Subjects sufficiently impaired, cognitively and functionally, to meet criteria for dementia were excluded.

Phase 2 - Baseline instrumental evaluation: evaluation of brain volumetry and function (MRI, fMRI); cardiovascular assessment;

Phase 3 - Intervention, 7 months: the physical training protocol consists of aerobic physical activity undertaken for 1 hour per session, three times per week, under the supervision of trained and experienced personnel, including physiotherapists and personal trainers. The protocol includes a combination of aerobic exercises and of callisthenic exercises for muscular strengthening, balance and joint flexibility improvement. In broad terms, each session was structured with a first session of callisthenic exercise, a second session of aerobic exercise on exercise bicycle and a third session of cool-down.

For the cognitive training, patients participated to the selected activities two hours per session, three times per week. The adopted cognitive training program was based on a mixed strategy which provides alternation between single-modality training sessions and sessions of recreational and leisure cognitive activities. Single-modality training activities are designed specifically to stimulate cognitive functions such as memory, attention, verbal fluency and executive functions. The recreational and leisure activities are intended to promote a context of multimodality training in a context of increased socialization and interpersonal exchange, in order to arouse curiosity and participation and to reduce the anxiety level linked to an excessive repetition of single modality activities. In addition, one hour music therapy session was performed once a week. The program also included lessons aimed at implementing compensatory strategies for maintaining a good performance in cognitive processes such as memory, attention, metacognition and thinking.

Phase 4 - T7, End of intervention: both groups, intervention and control, underwent a complete baseline-like (neuropsychological and instrumental) re-evaluation after 7 months of training/usual care.

Neuropsychological assessment

The protocol investigation of phase II consisted of a neuropsychological assessment of cognitive functions in order to identify MCI. The diagnostic criteria for MCI proposed by European Consortium on Alzheimer's Disease Working Group on MCI (Winblad, Palmer et al. 2004, Portet, Ousset et al. 2006) were applied:

(1) Cognitive complaints corroborated by an informant;

(2) The reporting of a decline in cognitive functioning relative to previous abilities during the past year by the patient or informant;

(3) Cognitive disorders as evidenced by clinical evaluation (impairment in memory or in another cognitive domain);

(4) no or minimal impairment in activities of daily living as determined by a clinical interview with the patient and informant (ADCS MCI-ADL Scale), a modification of the ADCS-ADL Scale designed to increase sensitivity to impairments in instrumental activities that may occur in MCI.

A final clinical diagnosis had to be confirmed by the results of both the neuropsychological examination and the clinical evaluation. Patients diagnosed as MCI underwent a psycho-behavioural evaluation, which included the following scales:

• Alzheimer's Disease Assessment Scale-cognitive (ADAS-Cog) (Fioravanti, Nacca et al. 1994) to measure the severity of the most important symptoms of AD;

• Neuropsychiatric Inventory (NPI), a scale administered to the informant, to evaluate severity and frequency of 12 neuropsychiatric disturbances common in dementia;

• Memory Functioning Questionnaire, a questionnaire of 18 items with a score ranging from 1 to 7 for each item, the lowest score meaning the higher use of mnemonics strategies.

The complete neuropsychological evaluation and ADAS-Cog administration has been performed at the start of training (T0) and at the end of training (T7).

Note that the ADAS-Cog score is computed so that the more severe the cognitive deficit, the higher the score, with low values meaning better cognitive function; i.e., the lower the better.

Cognitive training

Enrolled subjects were assigned to mixed-gender classes of 10 subjects each and given two sessions of cognitive training of 60 min each per day, three times a week, every other day from Monday to Friday (for a total of six hours per week). The cognitive training programme was based on eight cycles; each cycle was composed of 18 sessions of cognitive stimulation, with exercises and activities aimed at stimulating multiple cognitive functions. Each cycle lasted three weeks, after which the same kind of cognitive stimulation sessions were re-started, with exercises and activities of increased complexity compared to the previous cycle. Each cycle was structured in sessions aimed at stimulating the following main functions: acoustic attention, visual attention, visual memory, imagination, orientation and spatial memory, personal and temporal orientation, verbal memory, lexical abilities, memory for terms and meanings, affective memory, memory for texts, memory for faces and names, logic. Each cycle lasted with a theoretic-strategic lesson given by the cognitive trainers and focused on specific cognitive processes, such as memory, learning, attention, meta-cognition.

During the various sessions, there was an alternation of paper and pencil tests, social games, and multimedia-computer exercises, and the cognitive training was based on a balanced alternation of sessions for single cognitive modality and sessions focused on multimodal activities. Single modality sessions were aimed at intensively stimulating specific cognitive domains, such as memory, attention and executive functions. Multimodal activities were aimed at reconstructing a more ecological environment for the enrolled subjects, in order to favour socialisation and interpersonal exchanges in an enriching context. Alternation between the two types of sessions allowed maintaining an active participation in the enrolled subjects, stimulating their curiosity and attention and reducing levels of anxiety and potentially negative stress factors. Moreover, an alternation between sessions for single cognitive modality and multimodal activities was also aimed at avoiding habituation processes and at favouring diffuse and generalised brain stimulation, in order to maximise the chance to positively impact on the subject everyday life. In order to monitor performance improvement during the seven months of training, subjects were given frequent examination tests aimed at assessing their abilities in key cognitive function domains. Once a month, a session of movie watching and discussion was given to all classes together.

Music therapy

Once a week, subjects of each class attended together a session of one-hour music therapy, during which they were involved both in listening activities and also directly engaged in singing, playing musical instrument and rhythmic movements.

Physical training

Supervised seven-month aerobic exercise training was performed in a Fitness Centre built on purpose within the National Research Council Area of Research in Pisa. The participants attended one-hour lessons three times per week in small groups with a maximum number of 10. Each lesson was planned according to the American College of Sports Medicine guidelines and included aerobic exercise on an ergometer cycle, whose duration was increased gradually from 10 to 20 minutes as the participants progressed through the exercise intervention, followed by exercises targeted to improve muscle strength, physical function (static and dynamic), neuromuscular control and flexibility.

Endothelium-dependent and -independent vasodilation in the brachial artery

Endothelium-dependent response was assessed by flow mediated dilation (FMD) (Ghiadoni, Salvetti et al. 2015). Briefly, a pediatric cuff was positioned around the right forearm and the right brachial artery was located and scanned using a 10 MHz linear array transducer (MyLab 25, ESAOTE Florence, Italy). After one minute of baseline recording, the cuff was inflated at 250 mmHg for five minutes, and then deflated to induce reactive hyperemia. Endothelium-independent vasodilation was obtained by the sublingual administration of 25 µg GTN. Brachial artery diameter and flow velocity were continuously monitored by computerized edge detection system (Cardiovascular Suite; Quipu srl, Italy). FMD and response to GTN were calculated as the maximal percent increase in diameter above baseline.

Aortic and carotid stiffness

Arterial tonometry was performed according to international recommendations (Van Bortel, Laurent et al. 2012). Carotid-femoral pulse wave velocity (PWV) was assessed by SphygmoCor device (AtCor Medical: Sidney, Australia), recording waveforms at the two recording sites, sequentially. PWV was calculated as the ratio between the subtracted distance between the two recording sites and wave transit time. Applanation tonometry was also performed on the radial artery in order to obtain central blood pressure values, by using a validated transfer function (SphygmoCor, AtCor Medical: Sidney, Australia), and augmentation index (AIx). Three consecutive measurements were recorded and the median value was considered. Carotid systolic BP and pulse pressure (PP) were then obtained from carotid pressure waveform, using central diastolic and mean BP for calibration.
Scans of the common carotid artery were obtained by high-resolution ultrasound with a 10 MHz linear array transducer (MyLab25; ESAOTE, Florence, Italy). Two 10'-clips were acquired from each common carotid artery (1 cm proximal to the carotid bulb in a region 1 cm-wide and free of plaques) and then analyzed offline by means of Carotid Studio (Quipu srl, Pisa, Italy), an algorithm for the automatic evaluation of the instantaneous carotid diameter. The method is based on a well-validated contour tracking technique, allowing automatic evaluation of diameter stroke changes during the heart cycle, and was validated for accuracy against the gold standard measurement by radiofrequency (Bianchini, Bozec et al. 2010). Carotid lumen area was derived from the diameter values, assuming the cross-section of the artery to be circular. Common carotid intima-media thickness (IMT) was automatically measured on the same image sequences as the mean of the relative values of the last 10 seconds. Wall cross-sectional area (WCSA) was computed combining the values of diameter and IMT. The following parameters were calculated:
- carotid distension (D), that is the stroke change in diameter, calculated as the difference between the systolic and diastolic values;
- cross-sectional distensibility coefficient (DC), calculated as $DC = \Delta A / (A * \text{carotid PP})$, where A is the diastolic lumen area, and ΔA is the stroke change in lumen area;
- carotid stiffness (CS), calculated by using the Moens-Korteweg equation, $(\rho * DC)^{-1/2}$, where ρ is the blood density;

- cross-sectional compliance coefficient (CC), i.e. the absolute change in lumen area during systole for a given pressure change (CC = ΔA / carotid PP);
- Young's elastic modulus or incremental elastic modulus (Einc), a measurement of intrinsic stiffness of the vessel wall components, calculated as Einc = [3 (1 + A / WCSA)] / DC.
All parameters were considered as the mean of the right and left common carotid, except when the analysis at one time point was viable for one vessel only: in this case, that side was the only one considered in subsequent analyses.

Echocardiography

Echocardiography was performed using a commercially available portable echo machine (Vivid I, General Electric Healthcare Clinical System) with a 2.5-3.5 MHz phased-array transducer, second harmonic technology. Linear internal measurements of the left ventricle (LV) and its walls were performed in the parasternal long-axis view, perpendicular to the LV long axis and measured at or immediately below the level of the mitral valve leaflet tips, according to current guidelines, and used to calculated LV mass. LV end-systolic and end-diastolic volumes were measured from the apical four- and two-chamber views and ejection fraction (EF) was calculated by the modified biplane Simpson's rule. Mitral regurgitation was assessed semi-quantitatively, from 0 to 3. Each parameter was measured as the average of at least three consecutive beats. All measurements were performed according to the recommendations of the European Association of Echocardiography / American Society of Echocardiography (Lang, Badano et al. 2015).

Results

MCI vs. non-MCI

One hundred thirty-eight elder subjects completed the cardiovascular evaluation at baseline. 98 were defined as having MCI at the neurological examination; 40 were not.
Results are shown in Table 1. The two groups showed no significant difference in terms of age, BMI or blood pressure. No difference was found in carotid-femoral pulse wave velocity, central pressures or parameters from pulse wave analysis.
Flow-mediated dilation was not different between groups, neither was endothelium-independent dilation. The difference did not become significant neither introducing baseline arterial diameter as a covariate, nor introducing GTN-induced dilation as a covariate in FMD analysis (p=ns).
All data from ultrasound evaluation of the common carotid show no difference in vessel size, structure, or elasticity.
With echocardiography, the two groups were comparable as for left ventricular mass and ejection fraction. (Note that echocardiographic parameters are at the time being available for baseline evaluation only.)

Table 1. Non-MCI vs. MCI.

	Non-MCI (n=40)	MCI (n=98)	p
Sex, M/F	21/19	46/52	0.6
Age, years	73.5 (4.7)	75.3 (4.9)	0.053
BMI, Kg/m^2	28.0 (5.8)	26.7 (4.0)	0.141
Systolic blood pressure, mmHg	138.5 (16.6)	141.1 (17.4)	0.4
Diastolic blood pressure, mmHg	73.8 (8.1)	72.9 (8.8)	0.7
Pulse pressure, mmHg	64.8 (14.2)	68.2 (15.9)	0.2
Heart rate, bpm	64.1 (8.2)	65.0 (10.8)	0.6
Central systolic pressure, mmHg	128.1 (14.0)	127.1 (14.5)	0.7
Central diastolic pressure, mmHg	74.7 (8.3)	71.9 (8.6)	0.089
Central pulse pressure, mmHg	53.3 (11.1)	55.2 (13.6)	0.4
Mean pressure, mmHg	96.7 (10.1)	94.4 (9.5)	0.2
Augmentation pressure, mmHg	18.3 (6.0)	17.7 (7.6)	0.7
Augmentation index, %	34.3 (9.7)	31.3 (8.4)	0.078
Pulse wave velocity, m/s	10.23 (2.33)	10.74 (2.63)	0.3
Brachial artery diameter, mm	4.12 (0.79)	4.31 (0.85)	0.3
Flow mediated dilation, %	3.78 (2.12)	3.01 (2.08)	0.064
Dilation to nitroglycerin, %	7.60 (4.46)	6.54 (3.63)	0.175
Carotid diameter, mm	7.88 (0.84)	8.02 (0.85)	0.4
Intima-media thickness, mm	0.757 (0.131)	0.776 (0.160)	0.5
Wall cross sectional area, mm^2	16.34 (3.60)	17.17 (4.20)	0.3
Distension, mm	0.518 (0.127)	0.546 (0.155)	0.3
Distensibility, Pa^{-1}	19.66 (6.33)	20.28 (7.96)	0.7
Compliance, mm^2KPa^{-1}	0.882 (0.253)	0.950 (0.363)	0.3
Stiffness, m/s	7.48 (1.10)	7.47 (1.22)	1.0
Elastic modulus, KPa	504.6 (181.6)	512.4 (229.5)	0.9
Left ventricular mass index, g/m^2	92.6 (18.0)	95.4 (18.5)	0.5
Ejection fraction, %	60.9 (3.4)	61.2 (4.3)	0.7

MCI: training vs. usual care

Ninety patients had a viable cardiovascular evaluation both at baseline and after the intervention period: 54 in the training group, 36 in the non-training group (Table 2). There were no differences at baseline in age, BMI, peripheral or central pressure, pulse wave analysis, flow mediated dilation, or pulse wave velocity (all p=ns).

The training group had higher cognitive impairment, measured as ADAS-Cog score, at baseline. ADAS-Cog had no significant correlation with endothelium-dependent or -independent dilation, neither with pulse wave velocity or any parameter derived from carotid imaging (all p's $\geq$0.137).

The two groups were similar in carotid diameter and intima-media thickness, resulting in similar wall cross-sectional area. The non-training group happened to have higher net vessel distension: this was reflected in a significantly higher distensibility, while the differences in other parameters of elasticity such as compliance, stiffness, and Young's modulus did not reach statistical significance (p's between 0.073 and 0.106).

Table 2. Non-training vs. Training.

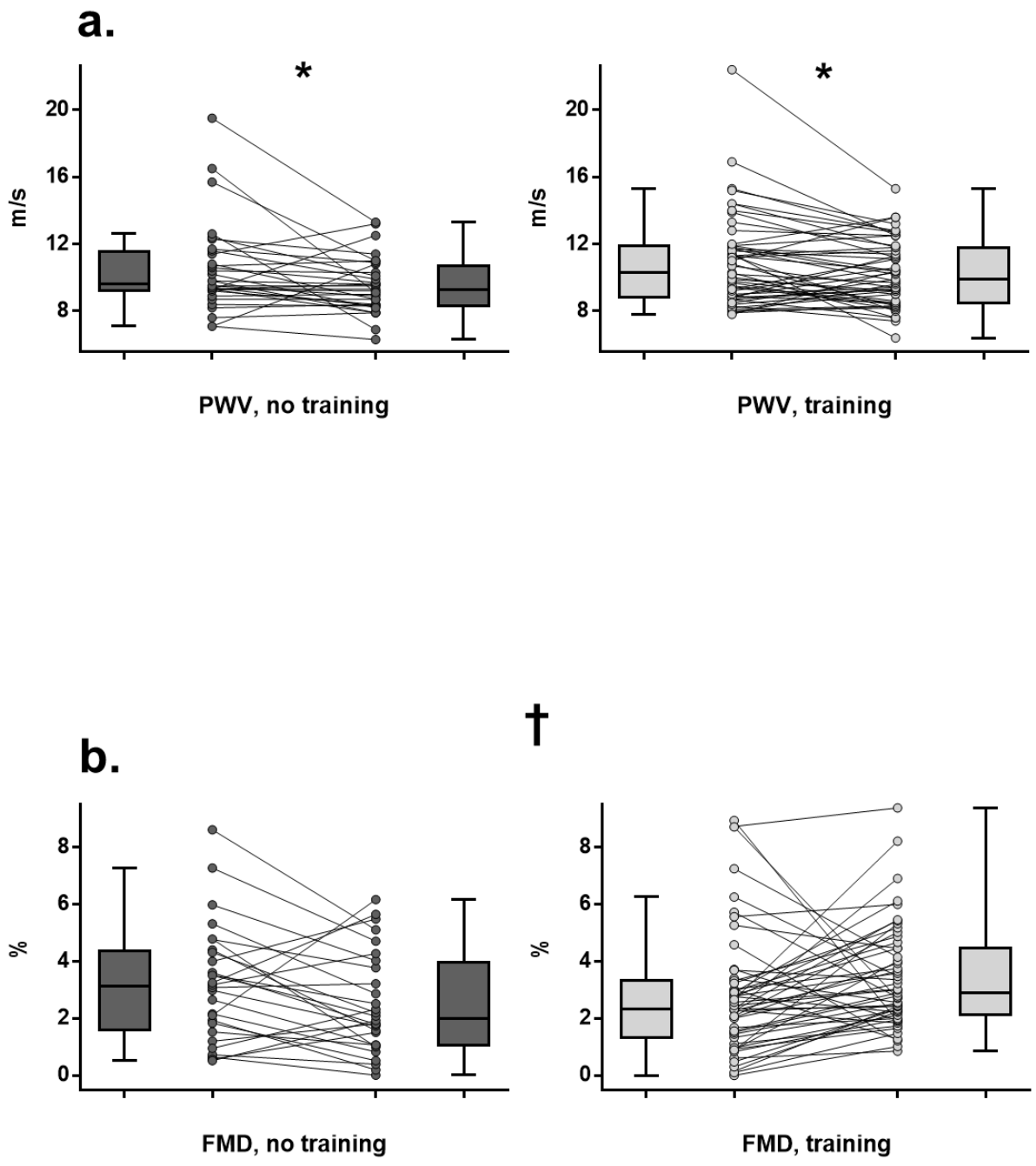
	Non-training (n=36)	Training (n=54)	p
Sex, M/F	16/20	25/29	0.9
Age, years	76.2 (5.6)	75.6 (4.8)	0.6
BMI, Kg/m^2	27.2 (4.3)	26.6 (3.7)	0.5
ADAS-Cog score	11.95 (3.86)	14.32 (4.27)	0.010
Systolic blood pressure, mmHg	140.4 (18.1)	141.3 (15.7)	0.8
Diastolic blood pressure, mmHg	72.6 (10.0)	73.2 (8.1)	0.7
Pulse pressure, mmHg	67.8 (17.3)	68.1 (13.4)	0.9
Heart rate, bpm	67.6 (12.2)	64.0 (10.8)	0.156
Central systolic pressure, mmHg	126.7 (11.5)	126.1 (13.4)	0.8
Central diastolic pressure, mmHg	71.5 (8.7)	71.9 (8.6)	0.8
Central pulse pressure, mmHg	55.2 (13.7)	54.2 (11.2)	0.7
Mean pressure, mmHg	93.7 (8.1)	94.2 (9.8)	0.8
Augmentation pressure, mmHg	17.1 (7.3)	17.6 (6.6)	0.7
Augmentation index, %	30.8 (9.3)	31.6 (7.6)	0.6
Pulse wave velocity, m/s	10.50 (2.66)	10.91 (2.76)	0.5
Brachial artery diameter, mm	4.23 (0.91)	4.37 (0.84)	0.5
Flow mediated dilation, %	3.20 (2.03)	2.82 (2.19)	0.5
Dilation to nitroglycerin, %	7.07 (3.27)	6.19 (3.65)	0.5
Carotid diameter, mm	8.00 (0.93)	8.07 (0.78)	0.7
Intima-media thickness, mm	0.764 (0.176)	0.776 (0.153)	0.8
Wall cross sectional area, mm^2	16.74 (5.22)	17.22 (3.60)	0.6
Distension, mm	0.63 (0.19)	0.52 (0.13)	0.009
Distensibility, Pa^{-1}	23.65 (11.39)	18.83 (5.38)	0.046
Compliance, mm^2KPa^{-1}	1.973 (0.488)	0.889 (0.272)	0.073
Stiffness, m/s	7.10 (1.51)	7.63 (0.99)	0.106
Elastic modulus, KPa	445.2 (176.2)	538.3 (242.3)	0.087
Left ventricular mass index, g/m^2	90.6 (17.7)	98.2 (19.0)	0.084
Ejection fraction, %	61.3 (3.9)	61.5 (4.3)	0.9

After the 7-months period, ADAS-Cog increased in the non-training and decreased in the training group, with statistical significance for both the time/treatment interaction and the intra-group comparisons, thus showing a positive effect of the intervention: 11.95 ± 3.86 to 13.00 ± 4.73 for no training, p=0.030; 14.32 ± 4.27 to 12.85 ± 4.03 for training, p=0.003; time x treatment p<0.001. The net variation in ADAS-Cog between the end and the beginning of the study period had no significant correlation with variations in vascular parameters, except with that in carotid diameter (r=0.28, p=0.016; all other p's ≥0.09). Patients were also dichotomized to improved or non-improved according to a change in time above or below zero: there was no significant correlation at the chi-square test between improvement in ADAS-Cog and those in FMD, carotid distension, or elastic modulus (p's ≥0.34)

There was no significant effect of the combination of time and treatment on systolic, diastolic, or pulse pressure (all p's =.38 or more). At the intra-group comparison, the non-training group had a significant decrease in diastolic pressure only (72.6 ± 10.0 to 68.3 ± 9.7 mmHg, p=0.048) while the training group showed a decrease in time of both systolic and diastolic (SBP: 141.3 ± 15.7 to 134.1 ± 14.6 mmHg, p=0.001; DBP: 73.2 ± 8.1 to 69.0 ± 9.1, p<0.001).

There was no difference of behavior in time of carotid-femoral pulse wave velocity (p=0.5; Figure 1a); it decreased significantly in both groups (p=0.022 for no training, p=0.015 for training).

Figure 1. PWV and FMD.



a., carotid-femoral pulse wave velocity. No training: from 10.50 (2.66) to 9.51 (1.65) m/s; training: from 10.91 (2.76) to 10.23 (1.94).
b., brachial flow-mediated dilation. No training: from 3.20 (2.03) to 2.50 (1.77) %; training: from 2.82 (2.19) to 3.42 (1.82).
† = $p<0.05$ for interaction time x treatment; * = $p<0.05$ for intra-group variation.

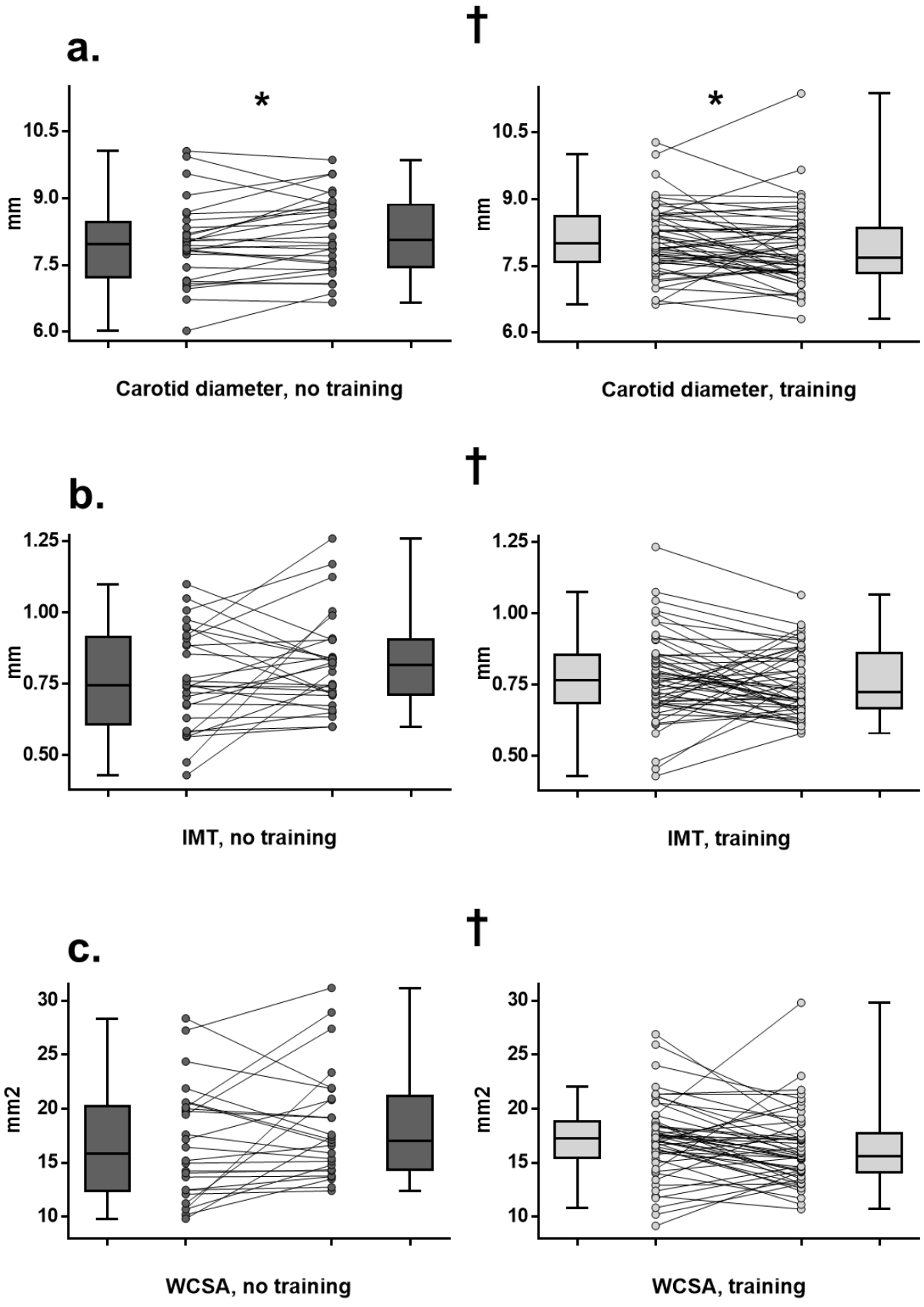
There was a significant diverging behavior in FMD between the two groups (p=0.014, Figure 1b). The decreasing trend in the non-training group and the increasing trend in the training group did not reach statistical significance (p=0.054 and p=0.075, respectively). GTN or brachial diameter showed no difference or change (all p's =0.2 or more).

A diverging trend was statistically significant for diameter of the common carotid (p=0.014, Figure 2a). It increased in the non-training group and decreased in the training group (p=0.047 and p=0.049, respectively; Figure 2a).

The effect of the combination of time and treatment was significant also for intima-media thickness (p=0.049, Figure 2b), without statistical significance for intra-group comparison (p=0.121 or more).

These variations in vessel diameter and thickness determined a significant difference in behavior of wall cross-sectional area (p=0.012, Figure 2b; p=0.074 or more for intra-group comparisons).

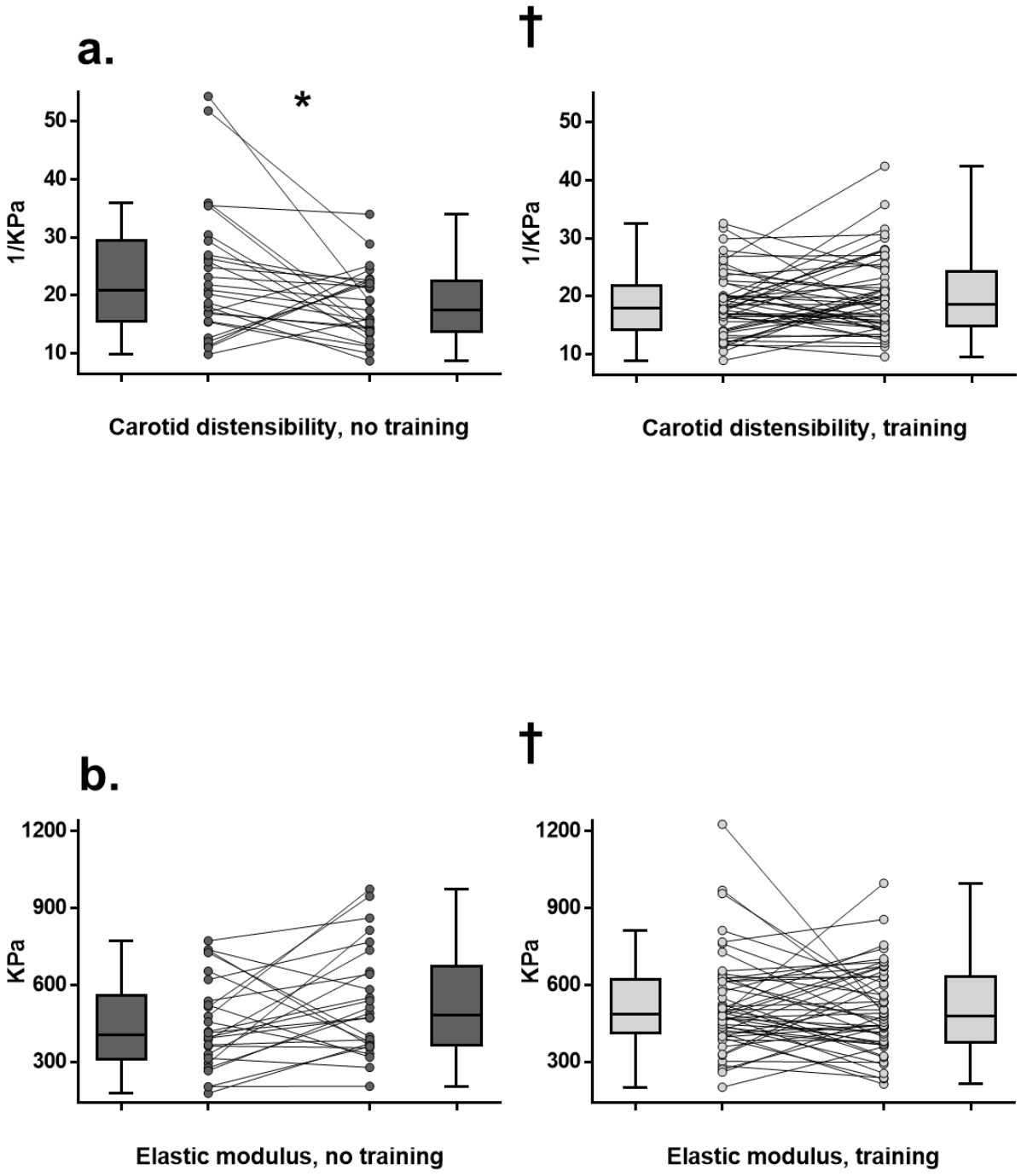
Figure 2. Carotid diameter, intima-media thickness and wall cross-sectional area.



a., carotid diameter. No training: from 8.00 (0.93) to 8.18 (0.87) mm; training: from 8.07 (0.78) to 7.88 (0.86).
b., intima-media thickness. No training: from 0.764 (0.176) to 0.821 (0.165) mm; training: from 0.776 (0.153) to 0.756 (0.115) .
c., wall cross-sectional area. No training: from 16.74 (5.22) to 18.31 (5.04) mm2; training: from 17.22 (3.60) to 16.30 (3.41) .
† = $p<0.05$ for interaction time x treatment; * = $p<0.05$ for intra-group variation.

The analysis of distension showed a significant divergence with time ($p=0.005$), with a significant decrease in time of the non-training group (no training: 0.63 ± 0.19 to 0.51 ± 0.13 mm, $p=0.013$; training: 0.52 ± 0.13 to 0.52 ± 0.16, $p=1.0$). It has to be pointed out that there was no difference or change in carotid pulse pressure (no training: from 60.5 ± 17.9 to 55.9 ± 14.1 mmHg, $p=0.140$; training: from 57.4 ± 13.8 to 54.5 ± 11.8, $p=0.124$; baseline difference: $p=0.5$; time x treatment: $p=0.6$).
Time x treatment had a significant effect on distensibility ($p=0.002$; Figure 3a). Distensibility decreased at the intra-group comparison in the standard care group ($p=0.024$).
The analyses of compliance also showed a significant effect of time and treatment ($p=0.029$; no training: from 1.07 ± 0.48 to 0.87 ± 0.27 mm^2kPa^{-1}, $p=0.086$; training: from 0.89 ± 0.27 to 0.91 ± 0.32, $p=0.7$), as it was the case for stiffness ($p=0.013$; no training: from 7.10 ± 1.51 to 7.75 ± 1.32 m/s, $p=0.092$; training: from 7.63 ± 0.99 to 7.45 ± 1.10, $p=0.3$).
Young's elastic modulus was subject to the combined effect of time and treatment ($p=0.023$, Figure 3b), without a significant change at the intra-group comparison (no training: from 445.2 ± 176.2 to 529.8 ± 212.0 KPa, $p=0.080$; training: from 538.3 ± 242.3 to 500.2 ± 166.8, $p=0.3$).

Figure 3. Carotid distensibility and elastic modulus.



a., carotid distensibility coefficient. No training: from 23.65 (11.39) to 18.27 (6.07) KPa-1 ; training: from 18.83 (5.38) to 20.14 (6.71).
b., Young's elastic modulus. No training: from 445.2 (176.2) to 529.8 (212.0) KPa; training: from 538.3 (242.3) to 500.2 (166.8) (one data point is outside the axis limits at baseline).
† = $p<0.05$ for interaction time x treatment; * = $p<0.05$ for intra-group variation.

Discussion

The main result from the vascular analysis in the Train the Brain study is that a 7-month comprehensive program of cognitive and physical training is able to produce detectable changes in the vasculature of elder subjects with mild cognitive impairment, compared to peers assigned to standard care. These variations oppose the usual patho-physiological effects of aging on vessels, i.e. progressive endothelial dysfunction, dilation, wall thickening, and loss of elasticity (Lakatta 2002).

The initial evaluation, aimed at characterizing the cardiovascular status of the population to detect differences between MCI and normal subjects in the same age span, did not show any significant difference. In particular, there was no difference in endothelial function, as measured through flow-mediated dilation.

Nitric oxide plays an important role in the regulation of the cerebral circulation. Pharmacological blockade of NO synthesis reduces cerebral flow (White, Vallance et al. 2000); in healthy subjects, cerebral activity during a verbal memory test, measured by fMRI, is correlated with endothelial function in the brachial artery (Gonzales, Tarumi et al. 2010). As for cognitive impairment, the reactivity of skin microcirculation – another method to estimate endothelial function – is reduced in AD subjects, in comparison to healthy peers, and altered also in initial cognitive impairment (Khalil, LoGiudice et al. 2007); adhesion molecules, a marker of endothelial activation, are altered both in overt cerebrovascular disease and in dementia, to further suggest a common patho-physiology (Zuliani, Cavalieri et al. 2008). In general, however, the relationship between endothelial function and cognitive losses seems to have been underinvestigated and mostly speculative.

We found no specific study linking flow mediated dilation of the brachial artery and the development of dementia: the analysis in the Train the Brain study is the first of this kind. The group with mild cognitive impairment did not differ in FMD from that without. The finding seems in contrast with the hints from the above-mentioned previous studies; it has to be noticed, however, that the FMD values found in the present population are low in average – as expected in elders, since age is the main contributor to endothelial dysfunction – with some subjects even having a barely detectable response. With low values, higher is the relative contribution of intrinsic variability and errors of the methodology, so that the

analysis is performed around its resolution limits. This could have concurred in finding a difference between groups that just misses statistical significance (p=0.064, Table 1): a few more subjects could perhaps have changed the result.

Much more investigated and solid is the relationship between arterial stiffness and cognitive decline – although different districts, measurements and biomarkers have been studied (de la Torre 2002, Luchsinger, Reitz et al. 2005, Waldstein, Rice et al. 2008, Mitchell 2011, Mitchell, van Buchem et al. 2011, Zlokovic 2011, Pase, Herbert et al. 2012) – and between cognitive decline and atherosclerotic burden in the carotid artery (Hofman, Ott et al. 1997, Haley, Forman et al. 2007, Wendell, Zonderman et al. 2009, Baracchini, Mazzalai et al. 2012). In the Train the Brain study, when the two groups with and without MCI were compared, no difference was apparent in carotid-femoral pulse wave velocity, neither in common carotid size, structure and function. A possible explanation is that in this population the effect of age – the main contributor to arterial stiffening in all populations and all studies – on large vessels is predominant at such an extent that it obscures the differences and any other contributor. It must be also noticed that other studies enrolled patients with more advanced cognitive deficits, thus comparing two well distinct groups (normal and dementia), while the present study enrolled an elder population and divided it in two according to a diagnosis of Mild Cognitive Impairment: patho-physiological differences are plausibly less pronounced (subjects with overt dementia were expressly excluded).

It is known that cognitive and physical stimulation help in maintaining cognitive function. At the same time, the positive effect of physical exercise on the vasculature has been known for decades, and the link between cardiovascular health, vessel damage and cognitive fitness is by now beyond doubt, even if a lot has still to be elucidated. While the negative prognostic value of endothelial dysfunction, arterial stiffness and alterations in the carotid wall is established, their susceptibility to interventions is less widely known, and even less is a possible clinical meaning of their changes in time.
We therefore hypothesized that the proposed program of physical and cognitive training could exert beneficial effects on the vessels, in addition to those (directly evident) on

cognition. An improvement in arterial health could indeed be one of the mechanisms through cognitive health is maintained.

Noteworthy, the present study demonstrated for the first time that a combined cognitive-exercise training is able to increase carotid distensibility, and in general to counteract physiological aging in large vessels, including arterial dilation and thickening, in individuals with MCI. The design allowed detecting differences of behavior in time between the two groups, thus overcoming the need for the power to detect significant intra-group variations (which were, anyway, significant for some parameters).
Carotid-femoral pulse wave velocity decreased in both groups, without differences in behavior. This result could be secondary to the lower pressure load, due to the similar decrease in blood pressure in the two groups. This parameters measures PWV mostly along the aorta, an elastic vessel with a low muscular component: possible changes in vessel composition could have needed a fiber rearrangement that needs more than seven months to show as a significant difference in PWV, especially if there is already a major change due to pressure decrease.

An improvement was evident for endothelial function. The conclusion that the increase in percent FMD is due to the endothelium is supported by the fact that neither brachial artery diameter – a known determinant of percent dilation – neither GTN-induced dilation, used to estimate reactivity of smooth muscle cells, showed any change, and that by introducing these parameters as covariates in the analysis, variation was still significant for FMD.

A change was apparent in all parameters calculated from ultrasound scanning of the common carotid, in the absence of a significant change in local distending pressure. The vessel kept progressively dilating in the non-training group, while the increase in size was reversed in the training group. Intima-media thickness showed a significant diverging behavior, with a trend to an increase in no-training, a decrease in the training group. The combination of the two led to another statistically significant finding, i.e. a trend to an increase in wall cross-sectional area without training, and to a decrease with training.

As for elasticity, all parameters considered to measure it in the common carotid were consistent in showing a significant divergence, with a tendency towards stiffening with standard care, and towards a de-stiffening with training. This happens with the parameters considering elasticity of the vessel as a whole, but also with Young's elastic modulus, that is a measure of intrinsic stiffness of the vessel components. Taken altogether, these results suggest that the intervention produced anatomical and functional changes in the common carotid, both quantitative (as seen with WCSA) and qualitative (as seen with Young's modulus), and the changes in elasticity derive from a combination of the two. Given also the data for endothelial function, the known role of the endothelium in modulating vessel function and composition can be hypothesized to have played a role.

This is the first study to have performed a comprehensive vascular characterization at two distinct time points in such a population. Findings from the carotid artery are a novelty: they constitute the evidence of the effectiveness, in terms of changes in vessels, of a rather short-term and low-intensity intervention. It has to be kept in mind that elders do not have the same vessel plasticity as it can be expected in younger adults – especially not completely healthy elders, such as these. On the other hand, the intervention program was not particularly intense, neither time consuming, as it had to be appropriate for all of the elder population.

Since the program was a combination of physical and cognitive training, the relative contribution of the two activities to the observed changes can not be discriminated. The importance of physical activity for vessel health can never be overrated, but also the interplay between cerebral activity and blood flow in the afferent vessels has been extensively discussed. Additional study arms with only one intervention component would have been a practical problem, and, since the main aim and outcome of the study was cognitive function, the Train the Brain study tried to implement the combined strategies that proved to be the most effective in giving benefits (Fabel, Wolf et al. 2009, Paillard-Borg, Fratiglioni et al. 2012). It can not be excluded that some additive effect of the multi-modality approach could have been reflected in the changes observed at the vascular analysis.

In this context, it is a rather disappointing finding that, even if the intervention clearly improved both cognitive function (measured as ADAS-Cog) and vessel health, there seems to be no correlation between the two changes at the individual level. The study does not help in establishing and clarifying the link between vascular and cognitive health, and does not support the hypothesis that an intervention successful in improving vessel structure and function will automatically translate in slowing cognitive decay. A follow-up aimed at assessing the persistence of benefits would perhaps help, and it would be interesting to know if the changes have translated at least into a reduction in cardiovascular risk.

In conclusion, a non-pharmacological, combined physical and cognitive training in a social setting slows, and partly reverses, the decline in cognitive abilities, endothelial function, and carotid anatomy and elasticity in a population of elders with mild cognitive impairment. Further analysis will be necessary to unravel the molecular factors underlying vascular changes in response to intervention and will also be necessary to understand whether and to which extent vascular stiffness is (causally?) related to cognitive function, what is the impact of its change and if it translates in a real prognostic benefit.

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