ORIGINAL ARTICLE

Effects of aerobic exercise on brain metabolism and grey matter volume in older adults: results of the randomised controlled SMART trial

S Matura[1,6], J Fleckenstein[2,6], R Deichmann[3], T Engeroff[2], E Füzéki[2], E Hattingen[4], R Hellweg[5], B Lienerth[3], U Pilatus[4], S Schwarz[2], VA Tesky[1], L Vogt[2], W Banzer[2] and J Pantel[1]

There is mounting evidence that aerobic exercise has a positive effect on cognitive functions in older adults. To date, little is known about the neurometabolic and molecular mechanisms underlying this positive effect. The present study used magnetic resonance spectroscopy and quantitative MRI to systematically explore the effects of physical activity on human brain metabolism and grey matter (GM) volume in healthy aging. This is a randomised controlled assessor-blinded two-armed trial ($n = 53$) to explore exercise-induced neuroprotective and metabolic effects on the brain in cognitively healthy older adults. Participants (age > 65) were allocated to a 12-week individualised aerobic exercise programme intervention ($n = 29$) or a 12-week waiting control group ($n = 24$). The main outcomes were the change in cerebral metabolism and its association to brain-derived neurotrophic factor (BDNF) levels as well as changes in GM volume. We found that cerebral choline concentrations remained stable after 12 weeks of aerobic exercise in the intervention group, whereas they increased in the waiting control group. No effect of training was seen on cerebral N-acetyl-aspartate concentrations, nor on markers of neuronal energy reserve or BDNF levels. Further, we observed no change in cortical GM volume in response to aerobic exercise. The finding of stable choline concentrations in the intervention group over the 3 month period might indicate a neuroprotective effect of aerobic exercise. Choline might constitute a valid marker for an effect of aerobic exercise on cerebral metabolism in healthy aging.

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INTRODUCTION

Physical activity seems beneficial in the prevention of cognitive impairment and dementia in the elderly.[1–3] The potential mechanisms behind the protective effects of physical activity on cognitive function are assumed to be multidimensional. It has been suggested that aerobic exercise renders the brain more efficient, plastic and adaptive, which leads to improved memory and executive function.[2,4] In brief, mechanisms comprise positive functional changes in hemodynamic activity, synaptic plasticity, neurogenesis and neural cell proliferation with newly formed neurons being integrated functionally into neural networks.[4–6]

The influence of physical activity on neural plasticity is closely linked or even dependent on various aspects of brain metabolism, for example, exercise upregulates multiple proteins in the hippocampus that have a defined role in energy metabolism, comprising enzymes involved in glucose catabolism, adenosin-tri-phosphate (ATP) synthesis and glutamate turnover.[7] Magnetic resonance spectroscopy (MRS) is a non-invasive, convenient and rapid magnetic resonance imaging (MRI) technique that is sensitive to metabolic changes in the brain.[8] Several biomolecules that are relevant to cerebral energy metabolism, neural plasticity and brain aging can be detected and quantified using proton MRS (^{1}H MRS) and/or phosphorus MRS (^{31}P MRS), including myo-inositol, glutamate, total choline (tCho), creatine (Cr),

phosphocreatine (PCr), ATP and N-acetylaspartate (NAA). Myo-inositol and glutamate are both astrocyte markers.[9] Total choline (the sum of phosphocholine and glycerophosphocholine) has been proposed as a marker of pathological membrane turnover and inflammation.[10] Neurodegeneration is commonly associated with decreased glutamate levels, while myo-inositol and total choline levels are typically elevated.[10] Creatine is commonly used as an internal standard to scale other metabolites, as it is generally constant in the normal brain spectrum.[11] Phosphocreatine has an important role in adenosine-tri-phosphate synthesis and is therefore considered as the central energy marker for neurons and astrocytes.[11] NAA in particular has been proposed as a marker of neuronal health, viability and number.[12] Prior studies have shown that levels of NAA and NAA/Cr ratios are lower in Alzheimer's disease (AD) compared to healthy aging, and that decreased NAA/Cr levels can predict future conversion from mild cognitive impairment (MCI) to AD.[13,14] Further, the NAA/Cr ratio is considered to be reflective of neuronal density, functional integrity, loss of synapses and higher pTau burden.[15]

Preservation or increase of regional grey matter (GM) volume is another non-invasive MRI marker that has been widely applied to study healthy or pathological brain aging. Accordingly, regional GM volume (for example, hippocampal volume) has been used as an outcome measure in studies on the favourable effects of

[1]Institute of General Practice, Goethe University, Frankfurt/Main, Germany; [2]Department of Sports Medicine, Institute of Sports Sciences, Goethe University, Frankfurt/Main, Germany; [3]Brain Imaging Center, Frankfurt/Main, Germany; [4]Institute of Neuroradiology, Goethe University Hospital Frankfurt, Frankfurt/Main, Germany and [5]Neurobiology and Neurotrophins Laboratory, Department of Psychiatry and Psychotherapy, Charité University Medicine, Berlin, Germany. Correspondence: Dr S Matura, Institut für Allgemeinmedizin, Goethe Universität, Theodor-Stern-Kai 7, 60590 Frankfurt/Main, Germany.
E-mail: Matura@allgemeinmedizin.uni-frankfurt.de
[6]These authors contributed equally to this work.

exercise on the brain structure in older people.[4,16] We know of only two previous studies that have investigated the effects of a controlled aerobic exercise intervention on brain metabolism and GM volume; one in healthy young men[17] and one in schizophrenic male patients.[18] To the best of our knowledge, no randomised controlled trial has been conducted so far to systematically investigate the effects of an individualised aerobic exercise programme on GM volume and brain metabolism in cognitively healthy older adults.

The effects of exercise on brain metabolism and brain structure may be associated with an increase of the neurotrophin brain-derived neurotrophic factor (BDNF).[19] Exercise differentially regulates synaptic proteins associated with the function of BDNF.[15] Erickson et al.[4] have shown an association between BDNF levels and exercise-related changes in memory and hippocampal volumes in older adults.[4] However, other studies failed to show an association between hippocampal volume and an increase in BDNF following exercise.[20]

In sum, it can be hypothesised that an enhancement in neuronal plasticity following aerobic exercise might be associated with increased NAA levels and an increase of cortical GM volume, accompanied by increased serum BDNF levels. Furthermore, we assumed that aerobic exercise leads to an increase of markers of neuronal energy reserve, in particular, the ratios of phosphocreatine or adenosine-tri-phosphate to inorganic phosphate (Pi).

MATERIALS AND METHODS

Design of the study
This is a randomised controlled partially blinded two-armed trial, evaluating the effects of a 12-week individualised aerobic exercise programme on cerebral metabolism and GM volume in cognitively healthy older adults, in comparison to a waiting control group. The study had previously been approved by the Ethics Committee of the Goethe University of Frankfurt am Main, Germany (reference 107/13) and is in agreement with the Declaration of Helsinki (Version Fortaleza 2012). Trial registration is NCT02343029 (clinicaltrials.gov). The study protocol has been published previously.[21]

Setting
Participants (cognitive healthy, >65 years) were recruited in three assisted living facilities in Frankfurt am Main, Germany and via local print media informed by the university press agency.

Written informed consent was obtained from participants fulfilling the inclusion criteria and willing to participate: for details please refer to Fleckenstein et al.[21] At baseline participants passed three incoming visits to assess cognitive performance (visit 1a), movement-related parameters and cardiopulmonary fitness (visit 1b). Furthermore, brain scans comprising MRS and quantitative MRI (qMRI) (visit 1c) were conducted. Following the baseline assessments, participants were randomised to the intervention group or to the waiting control group (for details of the randomisation procedure see ref. 21). Participants in the intervention group started the individualised 12-week aerobic exercise intervention in the integrated gym hall of one of the participating assisted living facilities 6 days after the MRI scans. Participants in the waiting control group waited 12 weeks before starting the exercise intervention. All participants were asked not to change their habitual physical activity during the following 3 months, except for the intervention. Twelve weeks after allocation, all participants were scheduled for visits 2a–c adhering to fixed inter-assessment intervals. Exercise intervention started at this point for participants in the waiting control group. This is part of a secondary study protocol, which is beyond the scope of the present manuscript.

Type of participants
Only cognitive healthy participants (>65 years—no upper age limit) fulfilling the inclusion criteria (see ref. 21) were included in the study. Cognitive healthy was defined as presenting no signs of dementia or mild cognitive impairment in cognitive performance during neuropsychological assessment and no impairment in activities of daily living.

Intervention
The intervention group participated in a 12-week supervised cycle ergometer training (optibike med, ergoline, Bitz, Germany) with three sessions (each session 30 min) per week. Workload in watts (W) at first ventilatory threshold (VT1) (equivalent to $64 \pm 9\%$ VO$_2$max) was applied as individual aerobic exercise intensity.[22] After 4 weeks, all participants' physical performance was reassessed, and exercise intensity was readjusted, if necessary.

Outcome measures
Main outcome measures were the change in cerebral metabolism, as assessed by MRS and changes in GM volume, as assessed with qMRI. The metabolites investigated in this study included NAA, Cr, Cho, ATP, glycerophosphocholine (GPC), phosphocholine (PCho) and PCr.

The MRI scans took place at the Brain Imaging Center, Frankfurt am Main, Germany. Data were acquired using a 3 Tesla whole-body scanner (Magnetom Trio, Siemens Medical, Erlangen, Germany) optimised for examinations of the cranium. The MRI and MRS protocols have been reported elsewhere.[21] In contrast to conventional MRI techniques for the acquisition of data sets with different weightings, the goal of qMRI is the measurement of parameter maps, where the local intensity directly represents the value of a certain tissue parameter. Thus, these maps are not biased by any other parameters. Apart from the investigation of diffuse pathologies, parameter maps allow for the construction of synthetic anatomical data sets that are free from inhomogeneities of the radio frequency (RF) coils used and provide improved contrasts. In the present case, qMRI methods were employed to obtain maps of the longitudinal relaxation time (T1) and the proton density (PD) for the construction of T1-weighted, bias-free synthetic anatomies.

Venous blood samples for quantification of the neurotrophin BDNF were drawn between 0900 hours and 1300 hours. When possible, time of sampling was held consistent for each participant at baseline and follow-up. Quantification of serum BDNF was performed in the Laboratory for Clinical Pharmacology, Psychiatric University Hospital Charité in Berlin using a modified fluorometric ELISA method, as described elsewhere.[23] Secondary outcome measures included maximal and submaximal outcome measures from cardiopulmonary exercise testing (CPET), resting heart rate (HR rest) and measures of cognitive performance: verbal declarative memory, frontal executive control, working memory, semantic and phonematic fluency as well as nonverbal declarative memory (refer to ref. 21 for the respective assessment instruments that were used).

To determine the ventilatory thresholds, we adhered to the V-slope method. We strictly followed the instructions as given by Karlman Wassermann in regard of his 9-panels.

First ventilatory threshold (VT1) and respiratory compensation point (RCP) were independently determined by two investigators and differences were reviewed by a third person. First, ventilatory threshold was defined as (1) non-linear increase in VCO$_2$ vs VO$_2$, (2) first non-linear increase of ventilation vs workload (V_E/WL), (3) first increase of expiratory partial pressure of oxygen vs workload (P_{ET}O$_2$/WL), (4) first non-linear increase of ventilatory equivalent of oxygen (V_E/VO$_2$) vs workload with no concomitant increase of equivalent of carbon dioxide (V_E/VCO$_2$).[24] Respiratory compensation point was identified by (1) second non-linear increase in minute ventilation vs workload (V_E/WL), (2) first non-linear increase of V_E vs VCO$_2$ accompanied by a concomitant non-linear increase in V_E/VCO$_2$, (3) non-linear decrease of expiratory partial pressure of carbon dioxide vs workload (P_{ET}CO$_2$/WL).[24]

Further outcomes were physical performance parameters (for a detailed description please refer to ref. 21), but the respective results are beyond the scope of this manuscript.

Data analysis and power calculations
Power calculations had been estimated on data published by Pajonk et al.[18] A group size of 30 participants was deemed to be adequate. The risks of data losses due to poor data quality or subject drop-out were estimated to be about 25% in each case. Thus, the total sample size was 60. For full power calculations please refer to the study protocol.[21]

Data analysis was performed by blinded evaluators. Statistical analysis adhered to current standards in reporting clinical trials, differentiating for parametric and non-parametric data, first using the Kolgomorov–Smirnov test and applying the respective tests thereafter. Changes over time were analysed by applying repeated-measures methods. Mulitvariate analyses of variance with a repeated-measures design (MANOVAs$_{RM}$) with group

(target vs control intervention) as between-subject factor and time (pre, post) as within subject factor were performed for MRS data analysis and qMRI data analysis. Repeated measure ANOVAS with group as between-subject factor and time as within subject factor were performed for BDNF concentrations and cognitive performance scores. Furthermore, correlational analyses were performed for changes in fitness levels and changes in those variables that showed a significant time × group interaction to analyse whether changes in the outcome measures could be attributed to the effects of aerobic exercise. For all analyses, SPSS 22 (IBM SPSS Statistics for Windows, Version 22, IBM, Armonk, NY, USA) was used.

MRS data analysis
The ^{1}H MRS spectra were fitted with the software tool LCModel, using the downloadable test version at: http://s-provencher.com;[25] which simulates the spectra with a linear combination of model spectra and is considered to be the most suitable tool for the analysis of short-TE spectra.[26] Baseline correction included macromolecules. The ^{31}P MRS data were analysed with the tool jMRUI,[27] which was found to be more appropriate for these types of spectra. Concentration changes of ^{1}H MRS metabolites were quantified as ratios to either total creatine (tCr: the sum of PCr and Cr) or total choline (tCho: the sum of GPC and PC), whereas concentrations of ^{31}P MRS metabolites were quantified as ratios to either PCr or Pi. Metabolite signal intensities depend on coil loading and local B1, thus, absolute quantification of metabolite concentrations requires corrections, which take into account the interindividual changes of these parameters. As those parameters affect all metabolites evenly, their effect is cancelled when using metabolite ratios.

Figure 1 depicts the target regions and representative spectra.

qMRI data analysis
From the quantitative maps of PD and T1, synthetic MPRAGE[28] data sets were derived, using a mathematical formalism described in the literature,[29,30] assuming the parameters: TR = 2420 ms, TI = 960 ms, excitation angle 9°, echo spacing 10.2 ms and isotropic spatial resolution: 1 mm.

Changes in GM volume following the intervention were analysed with voxel-based morphometry[31] in SPM8 (Wellcome Department of Cognitive Neurology, London, UK) running under Matlab 8 (Mathworks, Sherborn, MA, USA). Data preprocessing and statistical analyses were performed by applying a longitudinal data processing batch implemented in the vbm8 toolbox (http://dbm.neuro.uni-jena.de/vbm). For a detailed description of the processing chain see ref. 17. Smoothing of GM segments was performed using a 6 mm Gaussian Kernel. A two way ANOVA with group (target vs control intervention) as first factor and time (pre, post) as second factor was calculated for the smoothed GM segments. The whole-brain analysis was followed by a region of Interest (ROI) analysis. For the ROI analysis, a hippocampus ROI in MNI space was used that was provided by the Wake Forest University (WFU) PickAtlas Toolbox (http://fmri.wfubmc.edu/software/pickatlas). All analyses were thresholded with a family-wise error (FWE)-corrected voxel significance level of $P < 0.05$. In addition, volumes of bilateral hippocampi (in cm^{3}) were determined for each subject and time point and then included in the repeated-measures MANOVAs in SPSS 22.

RESULTS

Population characteristics
Of the 74 subjects screened, 62 subjects meeting the criteria of eligibility were scheduled for visit 1 and 60 participants were randomised into the study. At 3 month, seven participants had dropped out (6 in the control group, reasons: health issues $n = 3$, group allocation $n = 1$, non-compliance with schedule $n = 1$, non-compliance with MRI scan $n = 2$). Of these 53 participants (25 female and 28 male, age 75.3 ± 7.2 years, 74.2 ± 13.5 kg, 168.3 ± 10 cm), 31 participants were recruited in three assisted living facilities and 22 participants were recruited by local print media. Twenty-nine of the participants were part of the intervention group, receiving individualised training sessions of 40 min. On average, they performed 34.6 ± 3.3 (range: 27–44) training sessions with a total of 1384 ± 133.7 min. All 53 participants completed the assessments at baseline and after 3 month, and were subject of

the statistical analyses (see Table 1 for demographic data of the sample).

The study flow chart is depicted in Figure 2.

Physical performance
Repeated measures ANOVA (group × time) revealed significant effects ($P < 0.05$) of ergometer training on resting heart rate (HR rest) and maximal heart rate during CPET (HR max) (Table 2). Furthermore, there was a trend towards an effect of training on workload at respiratory compensation point (RCP), but no effects on maximal oxygen uptake (VO$_2$max), first ventilatory threshold (VT1) or maximal workload.

MRS results
Repeated measures MANOVA with group as between-subject factor and time (pre, post) as within subject factor revealed a trend towards an effect of training on NAA/tCr and NAA/tCho concentrations (group × time: $F_{(2, 50)} = 2.2$, $P = 0.12$, $\eta_p^2 = 0.091$). Contrast analysis revealed a significant effect ($P = 0.04$, $\eta_p^2 = 0.083$) for cerebral NAA/tCho for the training group as compared to the control group, whereas there was no significant effect of training on cerebral NAA/tCr concentrations ($P = 0.40$, $\eta_p^2 = 0.004$) (Table 3; Figure 3). The significant group × time interaction of cerebral NAA/tCho concentrations was mainly driven by a significant increase in tCho/Cr in the waiting control group ($P = 0.017$, paired *t*-test), whereas changes in the training group were not significant ($P = 0.52$). However, no significant effect was observed for the group × time interaction for the ratio of ^{31}P MRS detectable choline metabolites (GPC and PCho) to phosphocreatine (PCr) and no increase of these ratios was observed for both groups.

Potential changes in energy metabolism may lead to concentration changes in the high-energy phosphate compounds PCr and ATP. To detect these changes, we calculated the signal intensity ratio of both compounds to the Pi signal intensity. No significant changes ($P < 0.05$) were observed, either for the group × time interaction nor for the concentration in any of the groups.

qMRI results
Repeated measures ANOVA with time as within subject factor and group as between-subject factor did not reveal an effect of training on GM volume (group × time interaction, $P > 0.05$, FWE-corrected). The longitudinal ROI analysis of left and right hippocampus did not reveal an effect of training on grey matter volume at the significance level of $P < 0.05$, FWE-corrected either. A subsequent repeated-measures MANOVA in SPSS 22 of the extracted hippocampi volumes with group as between-subject factor and time as within subject factor did not show an effect of training on left and right hippocampal GM volume (group × time: $F = 1.33$, $P = 0.27$). Follow-up univariate analysis (repeated-measures ANOVAs) neither showed an effect of training on left hippocampal GM volume (group × time: $F_{(1, 52)} = 0.01$, $P = 0.92$), nor on right hippocampal GM volume (group × time: $F_{(1, 52)} = 1.75$, $P = 0.192$) (Table 3).

BDNF
Repeated measures ANOVA (group × time) did not show a significant effect ($P > 0.05$) of aerobic exercise on serum BDNF concentrations (Table 3).

Cognitive performance
Repeated measures ANOVA (group × time) did not reveal a significant effect ($P > 0.05$) of aerobic exercise on any of the cognitive domains that were assessed in this study (Table 2).
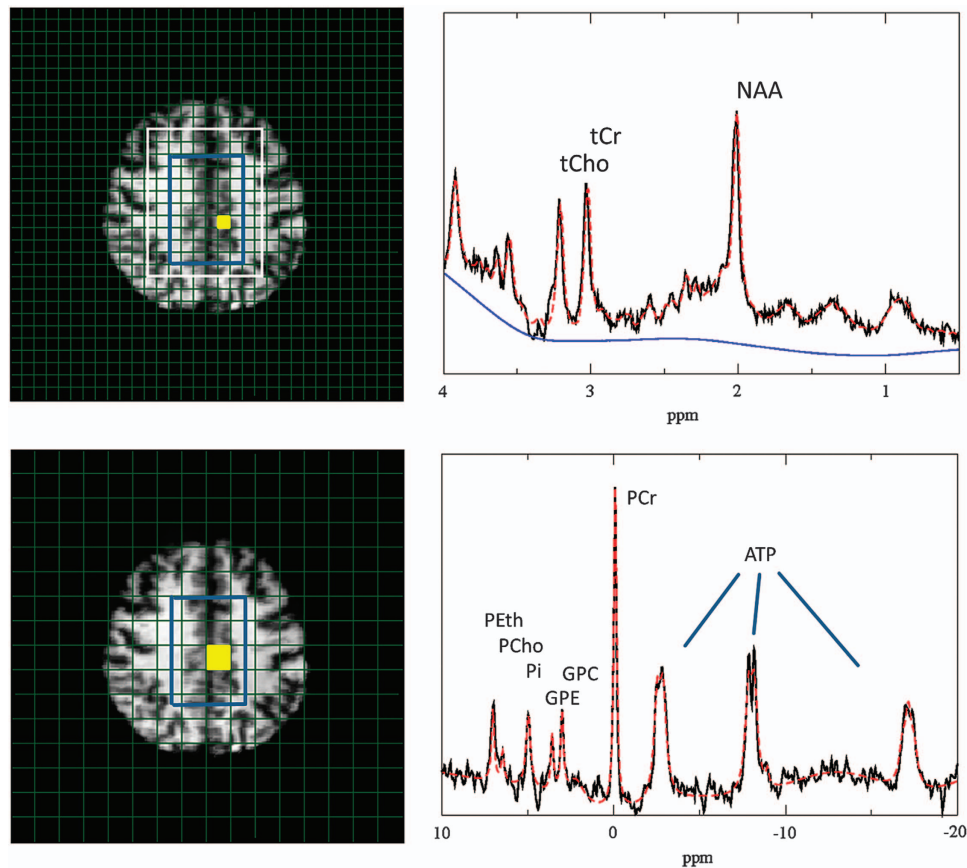
Figure 1. Target region and representative spectra. The upper row shows 1H data with the target region indicated by the blue box in the image. The spectrum on the right can be assigned to the yellow marked grey matter voxel. Main metabolites are labelled with abbreviations for total choline: tCho (the sum of glycerophosphocholine and phosphocholine), total creatine: tCr (the sum of phosphocreatine and creatine), and N-acetylaspartate (NAA). The broken red line shows the result of the LCModel fit, the blue line the baseline obtained by LCModel. The lower row shows 31P data with the target region indicated by the blue box in the image. Values for voxels incorporated only partially into the target area were weighted according to their partial contribution. The spectrum on the right can be assigned to the yellow marked predominantly grey matter voxel. Metabolites are labelled with abbreviations for phosphoethanolamine (PEth), phosphocholine (PCho), glycerophosphoethanolamine (GPE) glycerophosphocholine (GPC), phosphocreatine (PCr) and the three signals of adenosine-tri-phosphate (ATP). The broken red line shows the result of the jMRUI fit.

Table 1. Characteristics of the training and control group

Variables	Exercise group	Waiting control group
N	29	24
Age (years)	73.3 (5.5)	77 (8.1)
Gender (% female)	42%	52%
BMI	26.4 (4.5)	25.8 (2.9)
MMSE	29.3 (0.8)	28.8 (1.2)
Height (cm)	166.8 (10)	170.1 (9.2)
Weight	72.3 (13)	76.4 (14)

Abbreviations: BMI, body mass index; MMSE, Mini Mental State Examination. Means ($\pm$ s.d.) shown for all participants that completed baseline and follow-up assessments ($n = 53$).

Correlation of changes

Changes in VT1 were positively associated with changes in NAA/tCho concentrations ($r = 0.43$, $P = 0.021$) in the exercise group, but not in the waiting control group ($P > 0.05$).

DISCUSSION

Extending the findings of previous studies on young healthy subjects,[17] and a mixed population of healthy subjects and schizophrenic patients,[18] the present study shows effects of aerobic exercise on markers of brain metabolism in healthy older adults. We focused on a moderate-to-vigorous ergometer training to clearly differentiate physical activity from other brain-stimulating factors and therefore chose an intervention that was not very high on coordinative or cognitive demands. The significant effect of aerobic exercise on physical fitness, shown by increased cardiac efficiency in the intervention group during exercise and at rest underlines the cardiovascular benefit of the applied intervention. Our training regime aimed at high compliance and feasibility for elderly subjects. Similar to other studies, investigating the effect of a comparable amount and intensity of aerobic exercise on cerebral metabolism,[17,18] the change in physical fitness in the exercise group was not reflected in significantly increased VO_2max (which is often used as a primary outcome for exercise effects on fitness), but rather in physiological parameters measuring cardiac efficiency. Our results therefore suggest that exercise effects seem to be mediated not only by increased maximal aerobic capacity. The lack of an increase in VO_2max in the training group indicate, that 12 weeks of threshold-based endurance training is not sufficient to increase VO_2max in elderly subjects.

Although there was no significant increase in VO_2max, there was an overall change in fitness level reflected in improved cardiac efficiency in the training group. Moreover, changes in
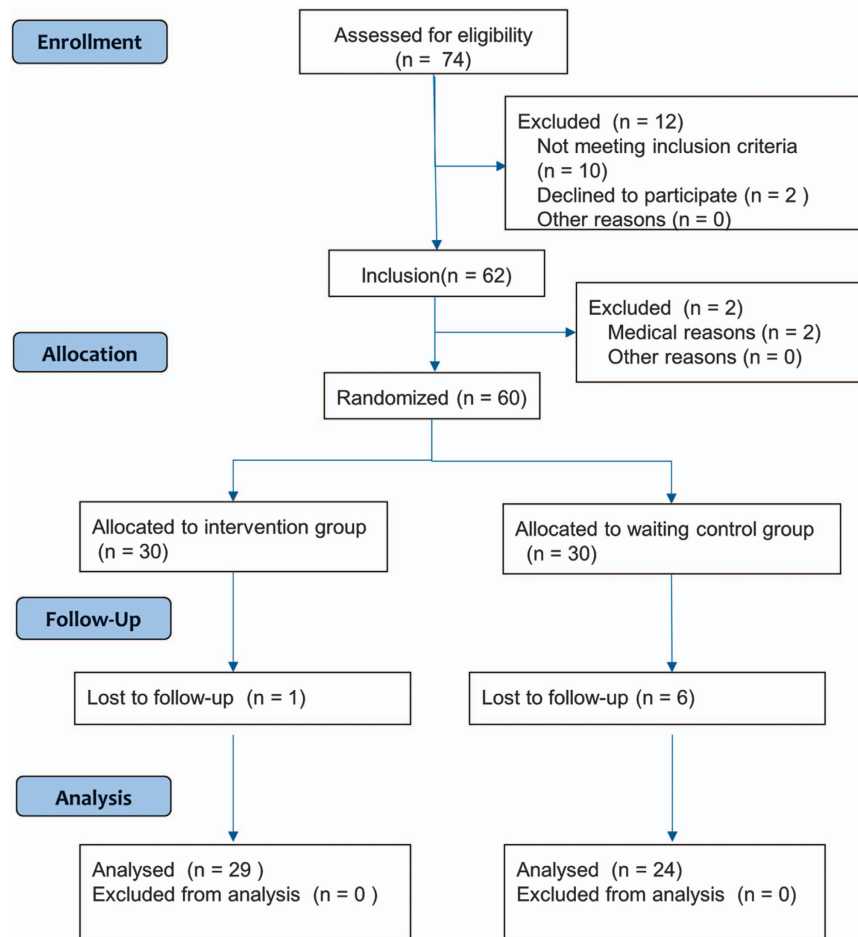
Figure 2. Study flow chart.

fitness level (VT1) were positively associated with changes in metabolite concentrations (NAA/tCho) in the training group, thus suggesting that fitness is closely linked to cerebral brain metabolism. Overall, the currently available data indicate that aerobic training interventions with moderate intensity may improve both, brain metabolism and cardiopulmonary function.

To the best of our knowledge, we are the first to show that regular aerobic exercise over 3 months leads to increased cerebral NAA/tCho concentrations compared to a waiting control condition. The significant time × group interaction regarding the NAA/tCho concentration can be mainly accounted for by the significant increase in total choline (tCho/Cr) in the waiting control group. Elevated tCho/Cr levels have been commonly found in patients suffering from dementia, especially Alzheimer's dementia and dementia with Lewy bodies, which are characterised by cholinergic dysfunction.[32–35] The elevation of tCho/Cr has been interpreted as a sign of increased membrane turnover due to degeneration and a marker of cholinergic dysfunction.[33,35] Thus, our finding of increased tCho/Cr in the waiting control group but not in the training group could be interpreted as a neuroprotective effect of regular aerobic exercise by possibly slowing down neurodegeneration. Contrary to our hypothesis, the increase of NAA/tCho in the training group was not paralleled by plasma BDNF levels. Also, there was no training-associated increase in NAA/Cr levels. Cross-sectional studies have shown that higher aerobic fitness is associated with higher NAA/Cr levels.[5,36] However, longitudinal studies on the effect of training on cerebral NAA/Cr levels have produced different results. Pajonk *et al.*[18] found an increase of NAA/Cr in schizophrenic patients after 3 months of aerobic exercise, but not in healthy subjects. Similarly,

and in line with our results, Wagner *et al.*[17] found no increase of NAA/Cr in young healthy adults after 6 weeks of aerobic exercise. Overall, the lack of increase in NAA/Cr levels does not suggest enhanced neuronal viability following aerobic exercise.

Contrary to our hypothesis, markers of neuronal energy reserve did not increase. This may be due to the fact that potential concentration changes are too small for detection with ^{31}P MRS, especially, as an improved energy metabolism may rather increase turnover rate of the respective metabolites, whereas concentrations are kept at the regular values.

Similar to the study by Erickson *et al.*[4] and Mass *et al.*,[20] we did not find an increase in BDNF in the exercise group compared to the control group. Overall, studies have shown heterogeneous results with regard to the effect of exercise on serum BDNF levels.[37] A recent meta-analysis of Dinoff *et al.*[37] examined the effect of exercise training on resting concentrations of peripheral BDNF. Of the 29 studies that were included into the meta-analysis, only 9 studies reported a significant exercise-induced increase in resting peripheral BDNF concentrations, whereas 20 studies reported no significant change. Thus, there are no consistent findings regarding the effect of exercise on resting peripheral BDNF concentrations.

Structural results

In the present study, aerobic exercise did not lead to increased total grey matter volume, nor increases in grey matter volume in left or right hippocampus. In contrast to our study, a number of trials have reported increases in hippocampal grey matter volume following aerobic exercise (for example, refs 4,38). A possible

Table 2. Measures of physical fitness and cognitive performance at Baseline and after 3 months of aerobic exercise or no exercise

	Control group		Exercise group		Group × time		
	Pre (baseline) N = 24	Post (3 months) N = 24	Pre (baseline) N = 29	Post (3 months) N = 29	$F_{(1, 52)}$	P-value	η_p^2
VT1	14.5 (2.9) [13.3, 15.7]	13.7 (2.6) [12.6, 14.8]	14.1 (3.6) [12.6, 15.6]	14.3 (3) [13.1, 15.5]	1.80	0.19	0.01
VO₂max, ml kg⁻¹ min⁻¹	23.3 (5.5) [20.9, 25.6]	22.7 (4.8) [20.63, 24.7]	21.4 (6.5) [18.9, 23.8]	21.7 (6.8) [19.1, 24.2]	1.85	0.18	0.09
HR max, bpm	137 (22.3) [127.6, 146.4]	140.5 (23.5) [130.5, 150.4]	148.1 (23.3) [139.4, 157.2]	143 (23) [134.6, 151.9]	5.61	0.02	0.10
HR rest, b.p.m.	67.9 (8.8) [64.1, 71.6]	69 (10.6) [64.5, 73.4]	76 (10) [72.1, 79.5]	71 (12) [66.9, 75.2]	6.48	0.01	0.11
Workload, W kg⁻¹	1.5 (0.4) [1.3, 1.7]	1.3 (0.4) [1.1, 1.4]	1.3 (0.5) [1.1, 1.5]	1.5 (0.5) [1.3, 1.7]	0.19	0.67	0.00
Workload RCP, W kg⁻¹	1.3 (0.4) [1.1, 1.5]	1.2 (0.4) [1, 1.3]	1.2 (0.4) [1, 1.4]	1.4 (0.3) [1.3, 1.6]	3.56	0.07	0.06
Memory							
VLMT early recall	5.3 (1.7) [4.61, 6.06]	5.8 (1.8) [5.1, 6.6]	5.3 (1.9) [4.6, 6]	6.1 (1.7) [5.5, 6.8]	0.46	0.50	0.21
VLMT late recall	8.8 (3.2) [7.4, 10.1]	10 (3.2) [8.7, 11.4]	8.8 (3.4) [7.5, 10.1]	9.2 (3.54) [7.9, 10.6]	1.06	0.31	0.07
VLMT recognition	10.3 (3.9) [8.9, 11.9]	10.6 (4.7) [8.6, 12.6]	10.8 (5.1) [9, 12.1]	10.6 (3.8) [8.7, 12.7]	0.5	0.83	0.00
CERAD figure recall	8.9 (1.93) [8.1, 9.7]	8.7 (2.14) [7.8, 9.6]	8.3 (2.4) [7.42, 9.27]	8.8 (2.3) [7.9, 9.7]	0.78	0.38	0.00
Executive control							
Stroop interference (s)	88 (26.7) [76.8, 99.3]	87 (20.5) [78, 3, 96]	91.3 (18.1) [84.1, 98.5]	84.6 (14.4) [78.6, 90.2]	3.01	0.09	0.08
Working memory							
Digit span forwards	8.5 (2.2) [7.6, 9.5]	8 (2) [7.1, 8.7]	8.6 (1.94) [7.81, 9.3]	8.1 (1.8) [7.4, 8.8]	0.15	0.71	0.05
Digit span backwards	6.3 (2.3) [5.4, 7.3]	6.8 (1.9) [5.9, 7.6]	6.7 (2.5) [5.8, 7.7]	7.2 (1.9) [6.42, 7.95]	0.25	0.62	0.05
Verbal fluency							
Semantic fluency	21.2 (5.09) [19.1, 23.4]	21.2 (4.1) [19.5, 23]	21.7 (4.8) [19.8, 23.5]	21.9 (5.34) [19.7, 23.9]	0.23	0.63	0.01
Phonematic fluency	15 (6.45) [12.1, 17.7]	13.92 (4.9) [11.9, 16]	16.1 (4.7) [14.3, 17.9]	16.2 (4.37) [14.52, 17.91]	0.96	0.33	0.05

Abbreviations: CERAD, Consortium to Establish a Registry for Alzheimer's Disease; HR max, maximal heart rate during CPET; HR rest, resting heart rate; VLMT, verbal learning and memory test; VO₂max, maximal oxygen uptake during cardiopulmonary exercise testing (CPET); VT1, ventilatory threshold; workload RCP, workload at respiratory compensation point during CPET; workload, maximal workload in watt during CPET. Means (± s.d.) and 95% confidence interval shown for all participants that completed baseline and follow-up assessments (n = 53).

Table 3. Measures of magnetic resonance spectroscopy, brain structure and BDNF levels at baseline and after 3 months of exercise or no exercise

	Control group		Exercise group		Group×time		
	Pre (baseline) N=24	Post (3 months) N=24	Pre (baseline) N=29	Post (3 months) N=29	$F_{(1, 52)}$	P-value	η_p^2
Metabolites (ratios)							
NAA:tCho ratio	4 (0.334) [3.9, 4.1]	3.9 (0.33) [3.8, 4]	4.1 (0.5) [3.9, 4.3]	4.2 (0.6) [3.9, 4.4]	4.56	0.04	0.08
NAA:Cre ratio	1.4 (0.1) [1.3, 1.4]	1.4 (0.1) [1.3, 1.4]	1.4 (0.09) [1.3, 1.4]	1.4 (0.1) [1.3, 1.4]	0.23	0.40	0.00
PCr:Pi ratio	3.4 (0.5) [3.2, 3.6]	3.5 (0.45) [3.3, 3.6]	3.45 (0.43) [3.3, 3.6]	3.5 (0.48) [3.3, 3.6]	0.48	0.49	0.01
ATP:Pi ratio	1.5 (0.3) [1.3, 1.6]	1.5 (0.2) [1.4, 1.6]	1.4 (0.18) [1.4, 1.5]	1.52 (0.30) [1.4, 1.6]	0.02	0.89	0.00
GPC:PCr ratio	0.27 (0.038) [0.25, 0.28]	0.26 (0.03) [0.25, 0.27]	0.25 (0.03) [0.24, 0.26]	0.24 (0.04) [0.22, 0.25]	0.06	0.81	0.00
PCho:PCr ratio	0.09 (0.03) [0.83, 0.26]	0.10 (0.02) [0.09, 0.11]	0.09 (0.03) [0.08, 0.10]	0.09 (0.02) [0.08, 0.10]	0.02	0.89	0.00
Volume (cm³)							
Total brain vol.	1432.7 (135.7) [1375.4, 1490]	1439 (141.1) [1380, 1499.9]	1412.9 (105.1) [1372.8, 1453]	1413.5 (106.4) [1373, 1454]	1.36	0.25	0.03
Grey matter vol.	661.2 (65.6) [633.5, 688.9]	665.1 (68.7) [636.1, 694.2]	657.3 (51.2) [637.7, 676.9]	656.4 (52.4) [636.5, 676.3]	1.98	0.17	0.04
Right hippocampus vol.	3.3 (0.3) [3.2, 3.4]	3.32 (0.30) [3.2, 3.4]	3.2 (0.4) [3.1, 3.3]	3.2 (0.37) [3.1, 3.4]	1.75	0.19	0.03
Left hippocampus vol.	3.37 (0.33) [3.2, 3.5]	3.38 (0.34) [3.2, 3.5]	3.33 (0.4) [3.2, 3.5]	3.3 (0.40) [3.2, 3.5]	0.01	0.92	0.00
BDNF (pg ml⁻¹)	3903.1 (1412.2) [3307.3, 4500]	3647.9 (1040.8) [3208.4, 4087.4]	3718.2 (1242.8) [3245.4, 4190.9]	3807 (1091.7) [3391.8, 4222.3]	2.32	0.13	0.04

Abbreviations: BDNF, brain-derived neurotrophic factor; GPC, glycerophosphocholine; NAA, N-acetylaspartate; PCr, phosphocreatine; Pi, inorganic Phosphate; tCho, total choline; tCr, total creatine. Means (± s.d.) and 95% confidence interval shown for all participants that completed baseline and follow-up assessments (n = 53).
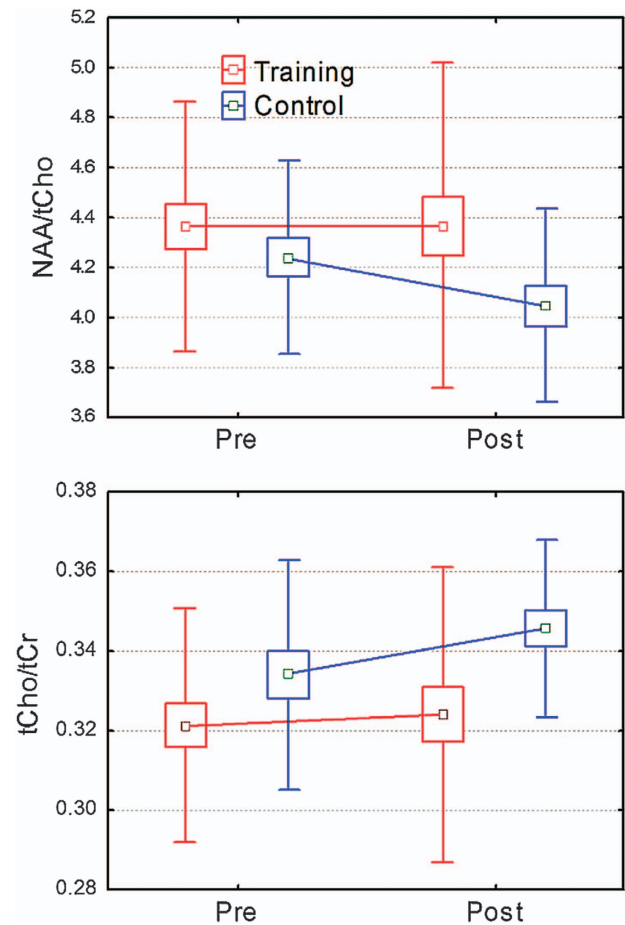

Figure 3. Effect of aerobic exercise on cerebral NAA/Cho and Cho/Cr concentrations. The figure depicts box-and-whisker plots with maximum, minimum and median. There was a significant group × time interaction (P = 0.04) for cerebral NAA/tCho. The significant interaction of cerebral NAA/tCho concentrations was mainly driven by an increase of tCho/Cr in the waiting control group (P = 0.017, paired t-test), whereas changes in the training group were not significant (P = 0.52).

explanation for the contradictory findings might be the overall duration of the intervention period. Although the training load per week was almost comparable, participants in the Erickson[4] study exercised for 1 year. Therefore, the slight changes in hippocampal volume (~1.4% decrease in the control group, and ~ 2% increase in the intervention group) found by Erickson et al.[4] might be attributable to the longer training interval. In other words, a 3 month intervention as in our study might not be sufficient to enhance neurogenesis and promote angiogenesis in the hippocampus to an extent that can be measured with common analyses methods such as voxel-based morphometry. This assumption is supported by studies with shorter intervention periods (for example, 6 weeks) that also failed to show effects of aerobic exercise on hippocampal morphology.[17]

Another difference of our study compared to the study by Erickson et al. lies in the age of the participants: 67.6 vs 73.3 years in our study. Increasing age seems to be negatively related to perfusion changes in the hippocampus following exercise.[20] More specifically, a study by Mass et al.[38] could show that individuals between 60 and 70 years tended towards perfusion increases as a result of exercise, whereas older participants tended toward decreases. As participants in our study were on average older than 70 years, they might have responded with decreased perfusion in the hippocampus following exercise. As hippocampal perfusion is

8

closely linked to hippocampal volume,[20] decreased perfusion rates might explain why exercise did not lead to an increase in hippocampal grey matter volume. Lastly, the method used in our study to quantify hippocampal volume might not have been refined enough to detect subtle alterations. Manual segmentation (instead of automated segmentation as in our study) of the hippocampus combined with a high resolution (7 Tesla in the Maass et al.[38] study as compared to 3 Tesla in our study) is more likely to allow the detection of subtle, region-specific changes in hippocampal volume. This assumption is supported by the findings of Erickson et al.[4] and Mass et al.[38] that have shown most pronounced changes of hippocampal volume in anterior hippocampus and hippocampal head. As we did not analyse specific subregions of the hippocampus, we might have missed region-specific subtle changes.

Cognitive performance

There was no significant effect of the aerobic exercise intervention on any of the cognitive domains (verbal declarative memory, frontal executive control, working memory, semantic and phonematic fluency as well as nonverbal declarative memory) assessed in this study. This finding is in line with other studies that did not find an immediate specific effect of aerobic exercise on cognitive performance.[4,38] Thus, it appears that the protective effects of physical activity on cognitive health that have been shown by numerous studies[39,40] are rather explained by a slowdown of age-related cognitive decline than by an improvement of cognition after a relatively short intervention period.

Limitations

Limitations of our study include the relatively short intervention period of only 3 months and the rather small sample size. Negative findings for cerebral energy metabolism, brain structure and cognitive performance could relate to the duration of the training period. Furthermore, the sample size might have been too small to reach sufficient statistical power, in particular, to detect significant group × time interactions. Future studies involving larger sample sizes and long-term interventions are needed to reassess the effect of exercise on cerebral energy metabolism, brain structure and cognitive performance.

Finally, one can question the rationale of an incremental step CPET protocol instead of a ramp protocol to assess the cardiopulmonary endpoints. Both protocol types adequately determine the peak physiologic responses, so that the choice of protocol for the measurement of maximal exercise capacity remains one of laboratory preference.[41] We used 25 W steps to attenuate sudden alterations in metabolism and motor unit recruitment, and to strengthen the relationship between VO_2 and work rate.[42] There is considerable inter- and intraobserver variability in the visual detection of the onset of anaerobic metabolism from the breath-by-breath data.[43] Therefore, visual detection was performed by two researchers independently and differences were reviewed by a third person.

CONCLUSION

A 12-week aerobic exercise training in cognitively healthy older adults resulted in stable concentrations of cerebral tCho, whereas tCho levels increased in waiting controls. As choline is a marker of neurodegeneration, this finding suggests a neuroprotective effect of aerobic exercise. Overall, our findings indicate that cerebral tCho might constitute a valid marker for an effect of aerobic exercise on the brain in healthy aging.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors substantially contributed to the conception and design of the study. JF and SM coordinated and conducted the study and wrote this manuscript. TE, SS, EF, VT, UP, EH, RD, LV, WB and JP participated in the conduction of the study and critically revised the manuscript for important intellectual content. RH contributed the BDNF data. WB and JP conceived of the study. All authors read and approved the final manuscript.

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