A 3-Month Aerobic Training Program Improves Brain Energy Metabolism in Mild Alzheimer’s Disease: Preliminary Results from a Neuroimaging Study

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Abstract

Background: Aerobic training has some benefits for delaying the onset or progression of Alzheimer’s disease (AD). Little is known about the implication of the brain’s two main fuels, glucose and ketones (acetoacetate), associated with these benefits.

Objective: To determine whether aerobic exercise training modifies brain energy metabolism in mild AD.

Methods: In this uncontrolled study, ten patients with mild AD participated in a 3-month, individualized, moderate-intensity aerobic training on a treadmill (Walking). Quantitative measurement of brain uptake of glucose (CMRglu) and acetoacetate (CMRacac) using neuroimaging and cognitive testing were done before and after the Walking program.

Results: Four men and six women with an average global cognitive score (MMSE) of 26/30 and an average age of 73 y completed the Walking program. Average total distance and treadmill speed were 8 km/week and 4 km/h, respectively. Compared to the Baseline, after Walking, CMRacac was three-fold higher (0.6 ± 0.4 versus 0.2 ± 0.1 μmol/100 g/min; p = 0.01). Plasma acetoacetate concentration and the blood-to-brain acetoacetate influx rate constant were also increased by 2–3-fold (all p ≤ 0.01). CMRglu was unchanged after Walking (28.0 ± 0.1 μmol/100 g/min; p = 0.96). There was a tendency toward improvement in the Stroop–color naming test (−10% completion time, p = 0.06). Performance on the Trail Making A&B tests was also directly related to plasma acetoacetate and CMRacac (all p ≤ 0.01).
Conclusion: In mild AD, aerobic training improved brain energy metabolism by increasing ketone uptake and utilization while maintaining brain glucose uptake, and could potentially be associated with some cognitive improvement.

Keywords: Acetoacetate, aerobic training, Alzheimer's disease, brain energy, cognition, ketones, neuroimaging, PET

INTRODUCTION

Alzheimer’s disease (AD) is characterized by several well-established imaging biomarkers including glucose hypometabolism, synaptic loss, neurofibrillary tangles, and senile plaques [1]. With the limited efficacy of pharmacological treatments that are presently available for treating AD symptoms, non-pharmacological therapeutic approaches such as ketogenic supplements and physical exercise have been proposed [2, 3]. Physical exercise, specifically aerobic training (walking, jogging, indoor-cycling, swimming, etc.), may have several cognitive, mental health (anxiety, mood, and depression), and life quality benefits in the older population, as well as in prodromal AD (amnestic mild cognitive impairment, MCI) and AD dementia. Exercise may also reduce the risk of developing AD [4–8].

Extensive research in animals and humans has been undertaken to identify potential biological mechanisms by which cognition is enhanced through physical exercise. A reduction of neurofibrillary tangles [9] and amyloid-β deposition [10] have been reported, along with reduced brain atrophy [11], preservation of white matter integrity [12], increased cerebral blood flow [13], and increased brain energy metabolism [14]; the latter is of particular relevance to the present report.

Glucose is the brain’s primary fuel. When brain glucose supply decreases, ketones (acetoacetate, AcAc and β-hydroxybutyrate, βHB) are produced by the liver. During prolonged fasting, while on a very high fat ketogenic diet, or while undergoing strenuous exercise, blood glucose decreases and ketones can supply from 50–80% of brain energy requirements [15–17]. We and others have demonstrated that, unlike with glucose, brain ketone uptake is not disrupted in mild AD, so the brain’s energy deficit in AD appears to be specific to glucose [18–20]. Using positron emission tomography (PET), we and others have confirmed that brain ketone uptake is directly proportional to plasma ketone concentration [16, 21, 22]. Nevertheless, we are aware of no study that has investigated the effect of aerobic training on global and regional brain fuel (glucose and ketone) metabolism in AD.

The primary aim of the present study was to determine whether moderate aerobic training such as walking modifies brain glucose or ketone energy metabolism in mild AD. To address this question, a dynamic PET imaging procedure was used to quantify brain uptake of [11C]-acetoacetate ([11C-AcAc) and [18F]-fluorodeoxyglucose ([18F-FDG) in patients with mild AD before and after a 3-month aerobic training program on treadmill. A secondary aim was to monitor cognitive function before and after the 3-month Walking program.

METHODS

Participants

This study was conducted with the informed written consent of all the participants and was approved by the appropriate ethics committees (Health and Social Services Center – Sherbrooke University Geriatrics Institute and the Centre hospitalier universitaire de Sherbrooke). This study is registered at ClinicalTrials.gov with identification number NCT02708485 under the title “Evaluating the impact of physical exercise on mild Alzheimer’s disease in a randomized clinical trial: quantification with 18F-FDG and 11C-AcAc PET imaging”. The original research protocol included two groups: a Walking group and a sedentary Control group. Most of the participants of the Control group discontinued the study because of the intense imaging protocol without the possibility of some level of benefit. There were no dropouts in the Walking group. Consequently, only results for the “Walking group” arm are reported here.

Patients were diagnosed as having probable or possible AD dementia using conventional NINCDS-ADRDA criteria. They were referred to this study by a geriatrician or a neurologist from the Memory Disorders Clinic at Health and Social Services Center – Sherbrooke University Geriatrics Institute or a physician from the Sherbrooke University Hospital Center (CIUSSS de l’Estrie – CHUS) between January 2010 and September 2015. All prospective participants were sedentary, e.g., not following a structured physical activity or training more than 30 min twice
a week. Exclusion criteria included an Mini-Mental State Examination (MMSE) score <20/30 [23], prescription drug addiction, alcohol abuse, depression, smoking, diabetes, overt evidence of heart, liver, or renal disease, and uncontrolled hypertension, dyslipidemia, or thyroid disease. All participants were taking an acetylcholinesterase inhibitor (donepezil, galantamine, or rivastigmine) for at least 3 months prior to study enrollment. Six were medicated for hypothyroid disorder (levothyroxine) and eight for dyslipidemia (pravastatin, simvastatin, rosvastatin, or atorvastatin).

Walking program

Participants were trained to walk on a motorized treadmill 3 days/week for 12 weeks. Most of the walking sessions were conducted at the exercise facility at the Research Centre on Aging, under the supervision of a kinesiologist. For 3 participants, some training sessions were conducted from home; in those cases, a Polar FT2 watch with T31 heart rate sensor strap (Polar Electro, Kempele, Finland) was used to monitor exercise intensity and duration. The Walking program was divided into two phases: phase one lasted 6 weeks and consisted of a gradual increase of the duration of the training from 15 min per session in Week 1, to 40 min per session in Week 6 (adding 5 min weekly); phase two also lasted 6 weeks and consisted of 40 min training sessions. The objective of each training session was to achieve 60% of maximum heart rate (pulse of 120 beats/min, bpm) and a perceived exertion at level 12–14 on the Borg scale, e.g., mild shortness of breath while still being able to speak during exercise. Heart rate reserve was determined during the pre-intervention visit. All the participants were advised to maintain their habitual diet and everyday activities during the intervention.

Neuroimaging protocol

To measure brain $^{18}$F-FDG and $^{11}$C-AcAc uptake, our previously described dynamic PET imaging protocol was chosen [18, 21]. Participants underwent a T1-weighted magnetic resonance image (MRI; scan duration = 9.14 min, TR = 16.00 ms, TE = 4.68 ms, field of view = 256 × 240 × 192 mm, matrix size = 256 × 256 × 164, flip angle = 20° and 1 mm isotropic voxels) on a 1.5 Tesla scanner (Sonata, Siemens Medical Solutions, Erlangen, Germany). All participants also underwent a brain dynamic acquisition (2 mm isotropic voxels, field of view = 25 cm and axial field = 18 cm) on a dual PET-CT Philips Gemini TF scanner (Philips Medical System, Eindhoven, The Netherlands). The PET scans were done twice, once at the beginning (Baseline) and once at the end of the 3-month aerobic training period (Walking). Briefly, for each scan, after a fasting period of 6–7 h after breakfast, the participant was positioned in the PET-CT scanner in the early afternoon in a dark quiet environment. After intravenous administration of 248 ± 89 MBq of $^{11}$C-AcAc via a forearm vein catheter, dynamic scans were obtained over a total duration of 10 min (time frames 12 × 10 s, 8 × 30 s, and 1 × 4 min). After a 60-min wash-out period, an i.v. dose of 189 ± 26 MBq of $^{18}$F-FDG was administered and PET images were acquired over 60 min (time frames = 12 × 10 s, 8 × 30 s, 6 × 4 min, and 3 × 10 min).

Quantification of cerebral acetoacetate and glucose consumption

Cerebral $^{11}$C-AcAc and $^{18}$F-FDG PET images were analyzed using PMOD 3.7 (PMOD Technologies Ltd., Zurich, Switzerland) as previously described [18, 21]. Briefly, the cerebral metabolic rates (CMR; [µmoles/100 g/min]) of acetoacetate and glucose (CMR$_{acac}$ and CMR$_{glu}$, respectively) were quantified according to the graphical analysis method developed by Patlak et al. [24], based on the plasma time-activity curves determined from the blood samples obtained during the $^{11}$C-AcAc and $^{18}$F-FDG PET scans. The following equation was used: CMR = K*Cp/LC, where K is the rate constant for net uptake of the tracer, Cp is the plasma tracer, and LC is the lumped constant; the LC of CMR$_{acac}$ and CMR$_{glu}$ were set to 1.0 and 0.8, respectively [16, 25]. Brain segmentation was defined by Freesurfer parcellation labels (Freesurfer Suite 5.0). Brain 3D projections of parametric maps of CMR$_{acac}$ and CMR$_{glu}$ were visualized using MIM Neuro 6.4 (MIM Software Inc., Cleveland, OH, USA).

Estimation of derived cerebral ketone consumption

Brain ketone metabolism involves the utilization of both AcAc and βHB as fuels, but only AcAc utilization is actually measured using $^{11}$C-AcAc PET. Based on Blomqvist’s work [16], a ‘derived’ estimate of the cerebral metabolic rate of both ketones combined (dCMR$_{ket}$; AcAc plus βHB) can be calculated
from the measured CMR_{acac} as well as the plasma concentration of AcAc and βHB as follows:

\[
dCMR_{\text{ket}} = CMR_{\text{acac}} + CMR_{\text{βHB}}
\]

\[
CMR_{\text{acac}} = K_{\text{acac}} \times [\text{AcAc}]
\]

\[
CMR_{\text{βHB}} = K_{\text{acac}} \times \frac{[\text{AcAc}]}{r_k[\text{AcAc}]/[\text{βHB}]}
\]

with \( r_k \) (net extraction ratio) = 1.2 ± 1.1

Biochemical analysis

Most plasma metabolites were measured using an automated clinical chemistry analyzer (Dimension Xpand Plus; Siemens Healthcare Diagnostics, Deerfield, IL, USA). Plasma concentrations of homocysteine were analyzed by high performance liquid chromatography (Agilent technologies Santa Clara, CA, USA) according to a method described by Simard et al. [26]. Plasma insulin was analyzed by commercial enzyme-linked immunosorbent assay (Alpco, Salem, NH, USA) with a Victor X4 multilabel plate reader (Perkin Elmer, Woodbridge, ON, Canada). The homeostasis model assessment method was used to estimate insulin resistance (HOMA-IR) from fasting plasma glucose and insulin [27].

Cognitive tests

General cognitive status was estimated with the Modified Mini-Mental State Exam (3MS) [28]. The Hopkins Verbal Learning Test was used to measure episodic verbal memory [29]. Evaluation of working memory and attention was based on performance on Verbal Digit Span from the Wechsler Adult Intelligence Scale (WAIS-III) [30]. The Trail Making [31], Phonetic Verbal Fluency [32], Stroop Color and Word Test [33], and Digit Symbol Substitution Tests from the WAIS-III provided information on executive function and processing speed.

Statistical methods

We established from our previous work and from others [18, 21, 34, 35] that with an increase of blood ketones of 2-fold, a sample size of \( n = 10 \) would provide the required 80% power \( (p < 0.05) \) to detect a pre- to post-Walking difference in the primary outcome – global CMR_{acac}. Data are presented as mean ± SD. All statistical analyses were carried out using SPSS 24.0 software (SPSS Inc, Chicago, IL, USA). A Wilcoxon signed rank test was used to compare difference between the pre- and the post-Walking measurements with a statistical threshold of \( p \leq 0.05 \). Linear regression modelling was used to test whether a difference in plasma AcAc or global

![Fig. 1. A) Global cerebral metabolic rate of glucose (CMR_{glu}) and acetoacetate (CMR_{acac}), and (B) rate constant for net uptake of glucose (K_{glu}) and acetoacetate (K_{acac}) in AD before (Baseline) and after the 3-month aerobic training program (Walking). All values are mean ± SD (*p < 0.05). The Walking group had a higher global CMR_{acac} (+172%; p = 0.01) but no difference in CMR_{glu} (p = 0.96). The rate constant parameter, K, also increased after the walking program (K_{acac} +69%; p = 0.03) with no difference in K_{glu} (p = 0.48).](image-url)
CMR_{acac} from baseline to the end of the 3-month walking period was associated with a change in cognitive scores.

RESULTS

Walking program

Adherence to the Walking program for the 12 weeks was 91.4%. The weekly total distance covered by the participants (Week 7–12) was 9.9 ± 0.1 km. Treadmill average speed during this period was 4.7 ± 0.3 km/h which is considered to be an exercise of moderate intensity [36]. Heart rate monitored during each Walking session averaged 114 ± 2 bpm. Participant perception of their own exertion was rated 12 ± 1 on the Borg scale. Additional physical activity measures throughout the 3-months Walking period are reported in Supplementary Table 1.

Fig. 2. Voxel-wise three-dimensional view of the brain surfaces showing the cerebral metabolic rate of acetoacetate (CMR_{acac}) and glucose (CMR_{glu}) in μmol/100 g/min before (Baseline) and after the 3-month aerobic training program (Walking).
Fig. 3. Regional derived cerebral metabolic rate of ketone (dCMRket) in mild AD before (Baseline) and after the 3-month aerobic training program (Walking). All values are mean ± SD (*p < 0.05). The Walking group had a higher global dCMRket in all brain regions assessed (+143–163%; all p ≤ 0.01). Front., frontal; Par., parietal; Temp., temporal; Occ., occipital; Cing., cingulate; Subcort., subcortical.

Fig. 4. Scatter plots of (A) performance at the Trail making A test (s) and global cerebral metabolic rate of acetoacetate (CMRacac; µmol/100 g/min); (B) performance at the Trail making B test (s) and plasma acetoacetate levels ([AcAc]; mmol/L).

Body weight and blood parameters

At Baseline, all plasma values were within our reference range (Table 1). Participants had 88–140% higher fasting plasma ketones after Walking (0.62 ± 0.53 mM versus 0.31 ± 0.21 mM; p = 0.04). Plasma homocysteine was near the upper reference limit fixed for an older population (normal cut-off ≤ 12 µmol/L [37]). Walking did not decrease plasma homocysteine (Walking versus Baseline, 10.4 ± 2.8 versus 11.4 ± 4.6 µmol/L, respectively; p = 0.15). Body weight as well as glycemic parameters (fasting glucose and insulin, HOMA-IR) and lipid profile (total cholesterol, triglycerides, and free fatty acids) did not change after Walking (data not shown; all p ≥ 0.14).

Brain energy metabolism

Walking increased global CMRacac in gray matter as a whole by 2.6 fold (Walking versus Baseline, 0.64 ± 0.40 versus 0.24 ± 0.12 µmol/100 g/min; p = 0.01), and increased global Kacac by 1.7 fold (0.05 ± 0.05 versus 0.03 ± 0.01 min⁻¹; p = 0.03). CMRacac and Kacac increased to broadly the same extent (1.5- to 3.0-fold) in the majority of brain regions studied (all p ≤ 0.05; Fig. 2). Delta CMRacac (Walking minus Baseline) was positively related to delta AcAc in plasma (β = 1.8 ± 0.6; p = 0.03). Globally, dCMRket was 2.5-fold higher after walking (1.77 ± 1.25 versus 0.70 ± 0.46 µmol/100 g/min at Baseline; p = 0.01); this increase was generally of the same amplitude across brain regions (+1.0 ± 0.2 µmol/100 g/min in average, all p ≤ 0.01; Fig. 3). Compared to baseline, a 3-month Walking program did not significantly change global or regional CMRglu or Kglu (all p ≥ 0.17; Fig. 1).

Detailed regional analysis of brain glucose and acetoacetate metabolism are reported in Supplementary Tables 2–5.

Cognitive function

Walking did not induce a significant increase in global cognitive performance (Table 2). However, participants showed a tendency toward improvement on condition 2 (colour naming) of the Stroop test (−10% time to complete; p = 0.06). Also, shorter completion time on the Trail making A test was related to higher global CMRacac (β = −60 ± 17; p = 0.01; Fig. 4). The same inverse relation was observed.
between completion time on the Trail making B and plasma AcAc ($\beta = -511 \pm 93, p = 0.01; \text{Fig. 4}$).

**DISCUSSION**

Our main observation is that after a 3-month aerobic training program involving supervised walking, participants with mild AD had two-fold higher brain ketone uptake (CMR_{acac}, $K_{acac}$, and dCMR_{ket}) while maintaining brain glucose uptake. We estimate that the post-Walking ketone levels in blood would sustain about 6% of total brain energy requirements, up from 2% at baseline. This three-fold increase in brain energy supply contributed by ketones would counteract about one third the global brain glucose deficit early in AD [3]. The increase in plasma and brain ketones observed in this study was also associated with some indication of cognitive improvement, i.e., a tendency toward faster processing speed as well as a positive relationship between better executive function and higher plasma/brain ketones.

Aerobic training has the potential to improve cognition in AD (see review Ströhle et al. [2]). Some neuroimaging reports using PET or magnetic resonance spectroscopy demonstrate that aerobic training enhances brain carbohydrate metabolism [38] and mitochondrial metabolic efficiency [39]. However, little is known about the implication of the brain ketone fuel in the underlying neurobiological mechanisms associated with the cognitive benefits of aerobic training in AD. Ketones are produced by the beta-oxidation of fatty acids. Fasting or physical exercise stimulates adipose tissue lipolysis and some of the resulting free fatty acids are converted to ketones by the liver [40]. Aerobic training stimulates ketogenesis [41]. The first evidence of the importance of ketones as an alternate fuel to glucose was reported almost fifty years ago in humans undergoing medically-supervised prolonged fasting [15]. Since then, research using different animal models including the dog [42], rat [43], and baboon [44], has confirmed this initial observation in humans. Similarly, studies using either ketone arterio-venous difference across the brain or brain ketone PET have reported an increase in brain ketone metabolism during ketone infusion [45], and while on a very high fat ketogenic diet [21]. The present study extends these reports by demonstrating that walking also increases both blood ketones and brain ketone metabolism in mild AD. The present results are in good agreement with our previous work and that of others in which a direct relationship between plasma [AcAc] and CMR_{acac} has been demonstrated [21, 46]. Significantly, the present study is the first to demonstrate that walking increased the capacity of the brain to take up ketones (influx rate constant – $K_{acac}$) more than simply increasing plasma [AcAc]. An increase in $K_{acac}$ could potentially be explained in part by increased cerebral blood flow [47] and/or increased expression of the monocarboxylic acid transporter in the blood-brain barrier [48], but these parameters were not measured in the present study.

Explanations for most of the effect of aerobic training on brain energy metabolism tend to focus on brain glucose and/or lactate uptake. Ide and collaborators [14] reported that whole brain metabolic
activity (glucose plus lactate) was increased by 30% during light aerobic exercise (cycling 20 min at 30 to 60% VO2max). Based on PET with 18F-FDG, higher brain glucose consumption in the parieto-occipital cerebral regions was observed following a 4–5 km run [38] in young adults. In the present study, neither global nor regional CMRglu changed after the 3-month period of Walking. This discrepancy may be explained by the fact that previous studies were done in younger population (20–30 y versus 70 y with mild AD in the present study), with a different intensity and duration of physical exercise. One study used PET with 18F-FDG to evaluate the effect of aerobic exercise on brain glucose uptake in 70-year-olds with MCI; Porto et al. [49] showed that 30 to 50 min on a treadmill, twice/week for 24 weeks, modified glucose uptake in the cingulate cortex. The inconsistency with our present study could be related to the duration of the training; twice/week is under the minimum usually recommended to induce sustained physiological adaptations [50]. Different methods are used to report brain glucose metabolism – Standard Uptake Value (SUV) which is a static index of glucose metabolism used in Porto study [49], versus quantification of CMRglu based on a dynamic 18F-FDG PET in the present study. In the absence of a non-exercise control group, we cannot exclude the possibility that aerobic training may potentially stabilize or slow down the decrease in brain glucose metabolism in AD [51].

Improved cognition following aerobic training has previously been reported in cognitively normal older persons [5], in frail older adults [6], in patients with MCI [52], and in Parkinson’s disease [53]. The participants in the present study did not show any significant improvement of global cognitive function after the aerobic training program. However, there was an indication of a potential improvement in processing speed after the walking intervention. A positive correlation was also found between plasma AcAc and CMRacac versus performance Trail making A and B, which confirms a recent report [54].

Our dynamic PET/MRI neuroimaging protocol permitted accurate quantification of brain ketone and glucose metabolism. However, the present study had several limitations, notably a small sample size for cognitive outcomes and short intervention period. A sedentary Control group was planned for and recruited but finally excluded from the data analysis due to a high drop-out rate caused mainly by the intensive neuroimaging protocol and the absence of potential benefit by not being included in the exercise (Walking) group. Future studies should consider a Control group with stretching and balance exercises. Regarding the blood parameters, homocysteine was measured because of its association with brain hypoperfusion, atrophy, and faster cognitive decline in AD [55]. Aerobic exercise has been reported to reduce total plasma homocysteine in individuals with hyperhomocysteinemia [56]. Blood homocysteine level may also be modulated by the intensity of an exercise intervention [57]. The duration of our aerobic training program (a 6-week progression phase follow by a 6-week period at the targeted low-moderate intensity) may be part of the explanation as to why the present intervention did not significantly change plasma homocysteine.

In conclusion, this pilot study shows for the first time that aerobic training improves brain energy metabolism in mild AD, specifically by increasing both ketone availability to the brain and the brain’s capacity to metabolize ketones while maintaining brain glucose metabolism.

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SUPPLEMENTARY MATERIAL

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