Molecular Neurobiology (2019) 56:2022–2038
https://doi.org/10.1007/s12035-018-1210-x

Physical Exercise During Pregnancy Prevents Cognitive Impairment Induced by Amyloid-β in Adult Offspring Rats

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Received: 11 May 2018 / Accepted: 26 June 2018 / Published online: 7 July 2018

Abstract

Alzheimer's disease (AD) is the main aging-associated neurodegenerative disorder and is characterized by mitochondrial dysfunction, oxidative stress, synaptic failure, and cognitive decline. It has been a challenge to find disease course-modifying treatments. However, several studies demonstrated that regular physical activity and exercise are capable of promoting brain health by improving the cognitive function. Maternal lifestyle, including regular exercise during pregnancy, has also been shown to influence fetal development and disease susceptibility in adulthood through fetal metabolism programming. Here, we investigated the potential neuroprotective role of regular maternal swimming, before and during pregnancy, against amyloid-β neurotoxicity in the adult offspring. Behavioral and neurochemical analyses were performed 14 days after male offspring received a single, bilateral, intracerebroventricular (icv) injection of amyloid-β oligomers (AβOs). AβOs-injected rats of the sedentary maternal group exhibited learning and memory deficits, along with reduced synaptophysin, brain-derived neurotrophic factor (BDNF) levels, and alterations of mitochondrial function. Strikingly, the offspring of the sedentary maternal group had AβOs-induced behavioral alterations that were prevented by maternal exercise. This effect was accompanied by preventing the alteration of synaptophysin levels in the offspring of exercised dams. Additionally, offspring of the maternal exercise group exhibited an augmentation of functional mitochondria, as indicated by increases in mitochondrial mass and membrane potential, α-ketoglutarate dehydrogenase, and cytochrome c oxidase enzymes activities. Moreover, maternal exercise during pregnancy induced long-lasting modulation of fusion and fission proteins, Mfn1 and Drp1, respectively. Overall, our data demonstrates a potential protective effect of exercise during pregnancy against AβOs-induced neurotoxicity in the adult offspring brain, by mitigating the neurodegenerative process triggered by Alzheimer-associated AβOs through programming the brain metabolism.

Keywords Maternal swimming · Metabolic programming · Neuroprotection · Alzheimer's disease · Mitochondrial function

Introduction

Late-onset Alzheimer's disease (AD) is the main aging-associated neurodegenerative disorder and is the leading cause of dementia with progressive cognitive decline [1, 2]. The neuropathological hallmarks of AD are amyloid-β (Aβ) accumulation in the brain and intracellular neurofibrillary tangles that lead to neuronal damage [2]. Soluble Aβ oligomers (AβOs) are thought to exert the major neurotoxic effects found in AD [3, 4], due its ability to interact with central nervous system (CNS) cell surface receptors and intracellular proteins, and, thereby, trigger alterations in signaling pathways and loss of protein function [3, 5, 6]. As AβOs accumulate in mitochondria, they interact with

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key mitochondrial enzymes of the tricarboxylic acid (TCA) cycle and the electron transport system (ETS), induce reactive species formation, and consequently disrupt mitochondrial function and dynamics [6–8]. Mitochondria play an essential role in neuron energy metabolism and neurotransmission [9] because synapses have high energy requirements [10]. Mitochondrial dysfunction, bioenergetics failure, and synaptic failure are displayed during the course of AD [11]. In fact, synaptic dysfunction and the loss of synapses are the best correlates of cognitive decline in AD [12]. To date, finding disease-modifying treatments have been challenging, and there is a lack of effective treatments to halt or reverse the AD progress [13, 14]. Thus, several studies have suggested that therapeutic approaches aiming counteract AβO-induced neurotoxicity and enhance synaptic function by improving of mitochondrial function may be effective [15–18].

Health-promoting approaches, such as regular exercise, are generally recommended to improve the lifestyle of population and reduce the risk of developing brain-related diseases. Nearly two decades ago, it was proposed that physical activity and exercise are capable of enhancing brain function and plasticity and may improve cognition in patients with AD [19–23] by promoting neuroprotection through metabolic adaptation in the CNS [24]. However, benefits arising from exercise during mid-to-late life do not extinguish the risks factors accumulated by a sedentary lifestyle during an individual's youth [25].

Investigating the effects of maternal exercise, during pregnancy, on the metabolic programming of offspring throughout intrauterine and early postnatal development is a new, attractive research area. The updated guidelines from the American Congress of Obstetricians and Gynecologists (ACOG) recommend a moderate-intensity exercise program, lasting 20–30 min per day, for pregnant women without medical contraindications [26]. Clinical studies have shown that physical exercise during pregnancy enhances cerebral maturation in newborns [27] and childhood language development [28]. Similarly, in rodents, we, and others, have demonstrated that maternal exercise during pregnancy modulates the intrauterine environment to favor the offspring's health, and it can enhance brain function and cognition throughout life [29–35]. Moreover, the health-promoting effects of maternal exercise have emerged as an approach to reduce disease susceptibility in the offspring's adult life. This concept is also encompassed by the Developmental Origins of Health and Disease (DOHaD) paradigm, in which the intrauterine and early postnatal environments influence the offspring's development and induce permanent changes that dictate their health status later in life [36, 37]. This phenotype shaping is established during development, due the plastic processes that enables adaptation to the environment [38, 39]. Incipient studies have addressed the potential protective effect of maternal exercise during pregnancy against AD-like pathology in TgCRND8 mice [40], maternal high-fat-diet-induced glucose metabolism disturbance [41], obesity [42, 43], hepatic steatosis [44], and tumorigenesis [45]. Herein, we sought to investigate whether maternal swimming during pregnancy is able to prevent memory impairment, and to unveil some of the cellular mechanisms involved in the processes of brain damage in a rat model of Aβ neurotoxicity. Our findings demonstrated that maternal exercise during pregnancy has long-lasting metabolic effects on the offspring's brain, specifically on mitochondrial function, which are able to protect the offspring from Aβ neurotoxicity and cognitive impairment in adulthood. These data highlight maternal exercise as a promising approach to delay or even to prevent the development of AD. Moreover, it opens new avenues to investigate the prevention of other aging-related diseases through metabolic programming.

Experimental Procedures

Animals

Adult female (220–260 g, $n = 54$) and male (300–350 g, $n = 27$) Wistar rats (*Rattus norvegicus albinus*) were obtained from in-house breeding colonies at the Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil. The animal facility was under controlled light (12:12-h light/dark cycle), temperature (22 ± 1 °C), and humidity conditions (50–60%). Four adult animals were housed per cage, while the litters were maintained with their mothers until weaning. All animals had ad libitum access to a 20% (*w*/*w*) protein commercial chow and water.

Experimental Design

A timeline of experiments is depicted in Fig. 1. Adult female rats were randomly selected and divided into two groups: (1) sedentary control, rats exposed to aquatic environment stress, without exercising and (2) maternal exercise, rats subjected to the swimming protocol. The animals in the maternal exercise group underwent involuntary swimming for 4 weeks (without extra weight), with 1 week of involuntary swimming, so that the rats could adapt to the aquatic environment, prior to mating and swimming throughout the entire pregnancy period. At the end of the first week, two females were mated with one male, and the pregnancy was confirmed by the presence of a vaginal plug or sperm in the vaginal fluid. The pregnant rats underwent the exercise protocol during the entire pregnancy. Beginning on the 20th gestational day, the dams were housed individually and observed, twice a day (8 a.m. and 6 p.m.), to verify the litter's birth. The offspring's birth date was considered postnatal day (PND) 0. Within 24 h after delivery, randomly selected pups were culled to

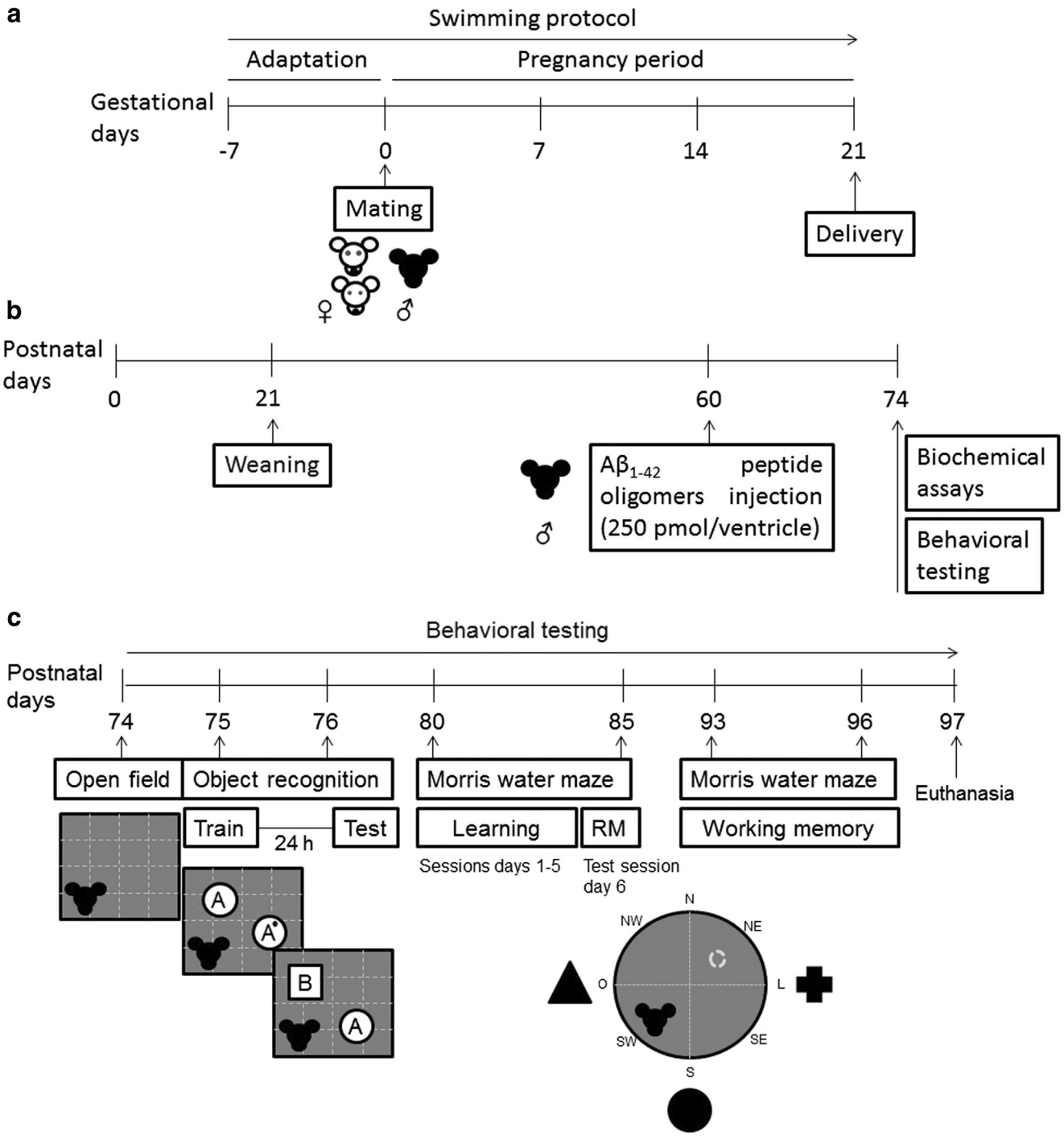

Fig. 1 Experimental design. **a** Maternal swimming protocol, **b** offspring timeline, and **c** offspring's behavioral testing schedule

maintain litters of eight pups per dam. From delivery (PND 0) until weaning (PND 21), each dam was housed with its litter. On PND 21, male and female offspring from each dam were separated by sex, female littermates were euthanized, and male littermates were housed, four per cage, until the PND 60. On PND 60, male offspring were subjected to a surgical procedure in order to bilaterally microinject Aβ peptide oligomers (AβO) or vehicle into the brain ventricles. Male offspring was selected in order to avoid interference of the hormonal and estrous cycle on the results. Thus, the offspring of maternal groups was subdivided into two groups, yielding four offspring groups: (1) sedentary control + vehicle, (2) maternal exercise + vehicle, (3) sedentary + AβOs, and (4) maternal exercise + AβOs. On the 14th day post-surgery, vehicle- and AβOs-injected adult male offspring born to sedentary

or exercised rats were randomly designated for behavioral testing or euthanized by decapitation without anesthesia, to collect samples for analysis of biochemical parameters.

Swimming Protocol

The exercise type, duration, frequency, and intensity are important components of exercise that influence the conferred benefits from mother and fetus [46]; swimming exercise is a highly recommended form of physical exercise for pregnant females [47]. According to the protocol initially described by Lee et al. [48] and modified by Marcelino et al. [34], adult female rats underwent individual swimming in a pool (30 cm wide × 30 cm long × 90 cm deep) filled with water at 32 ± 1 °C, without additional weight. Swimming sessions were performed from 9 to 12 a.m., 5 days/week and lasted 30 min, daily, for 4 weeks. The animals were left free to swim and were gently stimulated to swim when necessary. Following the same schedule of swimming group, the sedentary/control rats were exposed to aquatic environment, without exercising, in order to avoid any bias of water contact. Control rats were immersed in water, carefully dried, and returned to the housing boxes.

$A\beta_{1-42}$ Peptide Oligomers Preparation

Soluble AβOs were prepared according to the protocol published by Klein [49]. The Aβ (sequence 1–42) peptide (American Peptide Co., Sunnyvale, CA, USA) was dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) (Sigma Chemical Co., St. Louis, MO, USA) to stabilize monomers of the Aβ peptide. Then, the monomers were incubated for 1 h at room temperature, followed by a 10-min incubation on ice. After being aliquoted, the tubes containing the Aβ peptide were maintained in the hood overnight to allow the complete removal of HFIP. Following this step, samples were centrifuged in a SpeedVac system for 10 min to completely remove the HFIP and result in a clear film of monomeric Aβ peptide at the bottom of the tubes. The tubes were stored at − 80 °C. At the time of use, aliquots were solubilized in DMSO, diluted in phosphate saline buffer pH 7.4 (PBS), and incubated at 4 °C for 24 h. After incubation, the tubes were centrifuged at 14,000*g* for 10 min at 4 °C, and the AβO-containing supernatants were transferred to a new tube. Protein concentration was measured using the Pierce BCA Protein Assay Kit (Thermo Fischer), and structural characterization was performed through western blot analysis using the specific Aβ 1–16 antibody, 6E10 (Covance).

Surgical Procedure for $A\beta O_{1-42}$ Infusion

On the PND 60, male offspring were subjected to surgical procedures previously described by Hoppe et al. [50]. Animals were anesthetized with ketamine and xylazine (100 and 15 mg/kg, respectively) through the intraperitoneal (i.p.) route and then placed in a stereotaxic frame. Using sterile surgical instruments, a middle sagittal incision was made in the scalp and bilateral holes were drilled in the skull, using a dental drill over the lateral ventricles, according to Paxinos and Watson atlas coordinates: 0.8 mm posterior to the bregma and 1.5 mm lateral to the sagittal suture [51]. The depth of the microinjection, 3.5 mm beneath the surface of brain, was also chosen according to Paxinos and Watson coordinates. Rats received a single, bilateral, icv injection of 5 μL $A\beta_{1-42}$ peptide oligomers (500 pmol/rat), and control rats received injections of an equal volume of PBS and 2% DMSO into each lateral ventricle. The dose of $A\beta O_{1-42}$ for icv injection was chosen according previous works [5, 52]. Microinjections were performed using a 10-μL Hamilton syringe fitted with a 26-gauge needle, and an injection rate of 1 μL/min over a period of 5 min. At the end of infusion, the needle was left in place for an additional 3 to 5 min before being slowly withdrawn, to allow diffusion from the tip and prevent reflux of the solution. After the injection, the scalp was sutured, and the animals were allowed to recover from the anesthesia on a heating pad, to maintain body temperature at 37.5 ± 0.5 °C.

Behavioral Analyses

Behavioral tests were performed from PND 74 to 96 (Fig. 1c). The offspring underwent the open field test on PND 74, the object recognition test from PND 75 to 76, and the Morris water maze tests from PND 80 to 85 (reference memory) and from PND 93 to 96 (working memory). All behavioral tasks were conducted from 8 a.m. to 1 p.m. in a room with low light intensity (up to 60 lx) and attenuated sound. Before starting each test, animals were allowed to habituate to the testing room for 1 h. Behavior data were collected and analyzed automatically using a video-tracking system (Anymaze, Stoelting, Woods Dale, IL), with the camera positioned above the center of the apparatus.

Open Field Task

The open field task was used to assess spontaneous locomotor behavior in a novel environment [53]. The open field arena consisted of a 60 × 60 × 60 cm (length × width × height) black wood square chamber. Rats were placed individually in the left corner of the arena facing the wall and their behavior was tracked for 5 min. Through the automated video-tracking software, the ground floor of the arena was segmented into a grid with equal sized squares, and then further divided into two zones, the periphery and the center. For analysis of locomotion, total distance traveled, average speed, and number of squares crossed were measured. For analysis of anxiety-like

behavior, the excretion of fecal bolus, and the overall time spent in the central and peripheral zones were measured. The arena was cleaned with 10% (*v*/*v*) ethanol and dried after each animal had completed the task. The apparatus was also used for the novel object recognition task, so the open field task was considered as the habituation phase.

Novel Object Recognition Task

The novel object recognition task is based on the tendency of rodents to interact with a novel object over a familiar object in order to study learning and memory. The task was conducted according the method initially described by Ennaceur and Delacour [54], with modifications. The novel object recognition task consisted of three phases to assess the effect of AβOs on recognition memory. Fourteen days after AβOs (500 pmol/rat), animals underwent a habituation phase in the open field arena. On the next day, the training phase, the rats were placed in the arena with two identical objects (A and A') and allowed to freely explore them for 5 min. On a 24-h retention interval, the test phase to evaluate long-term memory, the rats were placed in the arena with a familiar (A') and a novel object (B) and allowed to freely explore them for 5 min. Object exploration was defined when the animal directed the nose to the object at a maximal distance of 2 cm, sniffed or touched the object. Climbing onto the object, unless the rat sniffed it, was not considered exploration. The total interaction time with both objects in training and testing phases was recorded. The discrimination ratio, a memory index, was calculated according the following formula: (exploration of novel object)/(exploration of novel + exploration of familiar object), where a higher time exploring the novel object was assigned to an enhanced cognitive performance [50]. After performing the task, the rats returned to their home cage. The arena and the objects were cleaned with 10% (*v*/*v*) ethanol and dried to minimize olfactory cues to the next use.

Morris Water Maze Task

The Morris water maze task was used to assess spatial learning and long-term and working memories [55]. Using distal cues to escape the water, the rats must learn to navigate toward to a hidden platform, since they start from random locations in the water tank. The water maze consisted of a black circular tank, 200 cm in diameter and 100 cm in height. The tank was filled with a 50-cm depth of water (22 ± 1 °C) and the transparent acrylic platform was submerged 2 cm beneath the water surface at the center of a quadrant. The tank was conceptually segmented by two perpendicular lines (+) demarcating North (N), South (S), East (E), and West (W) points, creating four equal sized quadrants designated as Southeast (SE), Northeast (NE), Southwest (SW), and Northwest (NW). On the walls of the testing room, distal visual cues were available. A random start position for each trial was established and no sequence was repeated, and the rat was placed in water facing the tank wall. The Morris water maze task consisted in three phases: acquisition (learning), retention (reference memory), and working memory [56].

Learning and Reference Memory The acquisition phase is required as training prior the retention test [55]. To this end, learning trials were conducted across 5 days and consisted of four trials/day. Inter-trial intervals lasted 20 min. Rats were allowed 60 s to search for the platform, which was located in the SE quadrant during all acquisition phases. The escape latency was measured in each trial, and if the rat failed to find the platform, it was gently guided to it. At the end of each trial, the rats were allowed to remain on the platform for 15 s, and then they were removed, dried, and returned to their home cages. The retention phase refers to the probe trial and assesses spatial reference memory. To this end, on the sixth day, each rat was placed into the water in the opposite quadrant, and the platform was removed to measure the latency to the first target-site crossover, the number of platform-site crossovers, and the time spent in each quadrant.

Working Memory The working memory task was conducted 1 week after the reference memory probe trial to assess trial-dependent learning and memory [55]. The platform was reallocated daily and the rats were subjected to four consecutive trials/day, with the inter-trial interval lasting 30 s, during four testing days. Mean latencies to find the platform in each trial was calculated for all testing days [57].

Mature BDNF Assay

Mature BDNF protein content was measured in the hippocampi and prefrontal cortices of rats that underwent behavioral tasks. Rats were euthanized 24 h after the last working memory test session. Mature BDNF was measured through the E-Max ELISA kit (Promega), according to the manufacturer's recommendations. Briefly, the hippocampus and prefrontal cortex of each rat were individually homogenized (1:10 *w*:*v*) in lysis buffer containing 137 mM NaCl, 20 mM Tris-HCl (pH 8.0), Igepal (1%), glycerol (10%), 1 mM phenylmethanesulfonyl fluoride (PMSF), 0.5 mM sodium vanadate, 0.1 mM EDTA, and 0.1 mM EGTA, and lysates were centrifuged for 3 min at 14,000 rpm at 4 °C. Supernatant was diluted (1:5 *v*/*v*) in sample buffer and incubated in 96-well flat-bottom plates previously coated with anti-BDNF monoclonal antibody, and blocked with Block & Sample buffer. After sample

incubation, plates were incubated with a polyclonal anti-human antibody for 2 h and horseradish peroxidase for 1 h. Colorimetric reaction with tetramethylbenzidine was quantified using a plate reader at 450 nm. The standard BDNF curve, ranging from 0 to 500 pg/mL was assayed in each plate in parallel with the samples.

Western Blot Assay

Prefrontal cortices and hippocampi were homogenized in ice-cold lysis buffer containing 4% sodium dodecyl sulfate (SDS), 2 mM EDTA, 50 mM Tris, and 1% protease inhibitor cocktail. The homogenates were denatured at 100 °C for 5 min and then centrifuged at 10,000*g* for 30 min. After this, the supernatant containing the cytosolic fraction was collected, β-mercaptoethanol was added to a final concentration of 5%, and the samples were stored at − 80 °C until use. Equal concentration of protein (50 μg) was loaded and immunodetected, as previously described [50]. Membranes were incubated for 60 min at 4 °C in blocking solution (Tris-buffered saline containing 5% non-fat milk and 0.1% Tween-20, pH 7.4) prior to the incubation with the primary antibody. Membranes were incubated, overnight at 4 °C, in blocking solution containing one of the following primary antibodies: rabbit monoclonal anti-synaptophysin (1:2000, Millipore, catalog number #AB9272), anti-mitofusin 1 (1:1000, Abcam, catalog number # ab104274), anti-dinamin-related protein 1 (1:1000, Abcam, catalog number #ab154879), or rabbit monoclonal anti-β-actin (1:2000, Cell Signaling, catalog number #4967). After washing, the membranes were incubated with a secondary antibody containing peroxidase-conjugated anti-rabbit IgG (1:2000, GE Healthcare Life Sciences, catalog number #NA934V) for 1 h. The chemiluminescence was detected using a digital imaging system (Image Quant LAS 4000, GE Healthcare Life Sciences) and analyzed using the Image J Software. The average optical density for the control group was designated as 100%.

Tricarboxylic Acid Cycle (TCA) α-Ketoglutarate Dehydrogenase (α-KGDH) Enzyme Activity

For the tricarboxylic acid cycle enzymes, the hippocampus and prefrontal cortex were dissected, weighed, and put into ice-cold isolation buffer for the mitochondria-enriched fraction. Mitochondrial fraction was isolated as described by Rosenthal et al. [58], with slight modifications [59] using a buffer containing 225 mM mannitol, 75 mM sucrose, 1 mM EGTA, 0.1% bovine serum albumin (free of fatty acids), and 10 mM HEPES (pH 7.2). The homogenate was centrifuged at 2000*g* for 3 min at 4 °C. After centrifugation, the supernatant was again centrifuged at 12000*g* for 8 min at 4 °C. The pellet was suspended in isolation buffer containing 4 μL of 10% digitonin and centrifuged at 12000*g* for 10 min at 4 °C. The supernatant was discarded, and the final pellet was gently washed and suspended in isolation buffer devoid of EGTA, at an approximate protein concentration of 2.5 mg mL^{-1}. The activity of α-KGDH (EC 1.2.4.2) complex was assayed according to Lai, Cooper [60] and Tretter, Adam-Vizi [61], with some modifications. The incubation medium contained mitochondrial preparations, 1 mM $MgCl_2$, 0.2 mM thiamine pyrophosphate, 0.4 mM ADP, 10 μM rotenone, 0.2 mM EGTA, 0.12 mM coenzyme A-SH, 1 mM α-ketoglutarate, 2 mM NAD^+, 0.1% Triton X-100, and 50 mM potassium phosphate, pH 7.4. The reduction of NAD^+ was recorded at wavelengths of excitation and emission of 366 and 450 nm, respectively. α-KGDH activity was calculated and expressed as nmol $NADH.H^+$ min^{-1} mg $protein^{-1}$.

Respiratory System Complex IV Activity

Animals were euthanized 14 days after the AβOs injection by decapitation, without anesthesia, in order to avoid tissue chemical contamination. Regarding the respiratory system complex IV (cytochrome c oxidase) activity assessment, the prefrontal cortex and hippocampus were rapidly dissected, weighed, and immediately frozen (− 80 °C) until homogenization. The encephalic areas were homogenized (1:20 *w*/*v*) in SETH buffer, pH 7.4, that contained 250 mM sucrose, 2.0 mM EDTA, 10 mM Trizma base, and 50 IU mL^{-1} heparin, and were centrifuged at 800*g* for 10 min at 4 °C. The pellet was discarded, and the supernatant was collected and subjected to three subsequent freeze-thaw procedures before performing the experiments [59]. Mitochondrial respiratory system enzyme activity was measured in the homogenates with a protein concentration varying from 1.5 to 5.0 mg protein mL^{-1}. The activity of cytochrome c oxidase (EC 1.9.3.1) was measured according to Rustin et al. [62]. The incubation medium consisted in 10 mM potassium phosphate buffer, pH 7.0, 125 mM n-dodecil-β-D-maltoside, and 1% cytochrome c. Complex IV activity was determined, following the decrease in absorbance, due to reduced cytochrome c oxidation at 550 nm. The activity of complex IV was calculated as nmol min^{-1} mg $protein^{-1}$.

Protein Determination

Protein concentration was measured according the method described by Lowry et al. [63], which was adapted for the microplate, using bovine serum albumin as standard. The absorbance was measured at 750 nm. The results were presented as milligrams of protein/mL.

Flow Cytometry Assay

Flow cytometric analysis was conducted according to the protocol described by Marcelino et al. [34]. Briefly, the tissue

samples (approximately 100 mg) were dissociated in PBS containing 1 mg% of colagenase IV and 0.5 mg% of DNase, filtered using a 40-μm pore cell strainer (SPL Lifesciences Co., Naechon-Myeon Pocheon, South Korea), and were then incubated for 45 min at 37 °C with the molecular probes. Mitochondrial mass and membrane potential were measured using 100 nM MitoTracker® Green and 100 nM MitoTracker® Red (Invitrogen, Molecular Probes, Eugene, OR, USA), respectively. Cells were gated based on the FSC and SSC pattern of the sample cells and 30,000 events were acquired per sample in a FACSCalibur flow cytometer (BD Biosciences); a non-labeled sample was used as negative fluorescent control. Data were analyzed using the FlowJo software.

Statistical Analyses

Data are expressed as mean ± standard error of the mean (SEM), and statistical analyses were performed using the GraphPad Prism 6.0 software. All data were tested for normality. Two-way ANOVA was used to analyze the effect of the two independent variables, maternal exercise and AβOs injection. All tests involving multiples observations per group were analyzed with repeated measures. Differences between the groups concerning repeated measures analysis were demonstrated by Tukey post hoc test. Data were considered statistically significant when $p < 0.05$.

Results

AβOs Injection Did Not Alter Motor and Exploratory Activities in Adult Offspring Born to Sedentary or Exercised Rats

The adult offspring rats were tested in the open field for 5 min to habituate to the new space and to assess both exploratory activity and spontaneous locomotor behavior (Table 1). AβOs injection (500 pmol/rat) did not cause any change in the exploratory and motor activities in the offspring born to exercised or sedentary rats, as measured by total distance traveled [$F(1,53) = 0.009$, $p = 0.924$], average speed [$F(1,53) = 0.123$, $p = 0.727$], and crossings [$F(1,52) = 0.269$, $p = 0.605$]. Moreover, signs of anxiety-like behavior such as number of excreted fecal bolus [$F(1,52) = 0.009$, $p = 0.924$], time spent in the periphery [$F(1,53) = 0.309$, $p = 0.580$] or in the center [$F(1,53) = 0.446$, $p = 0.507$] of the arena were not altered. These results showed that neither maternal exercise during pregnancy nor AβOs injection altered exploratory, locomotor, and anxiety-like behavior in the adult offspring.

AβOs Impaired Object Recognition Memory in the Adult Offspring, Which Was Prevented by Maternal Exercise During Pregnancy

Recognition memory was assessed through the novel object recognition test, a non-spatial memory task that relies mainly on hippocampus processing [64]. The more time spent exploring the novel object depicts intact object recognition memory. In the training phase, all animals explored both objects equally (Fig. 2a), showing no preference for any object [$F(1,47) = 0.138$, $p = 0.711$]. In the test phase, matching analysis for objects showed that AβOs-injected rats spent similar time exploring the novel object and the familiar object, whereas the other groups showed preference for the novel object (Fig. 2b; $p < 0.001$ Tukey post hoc test). In addition, discrimination ratio in sedentary + AβO group was significantly reduced compared to control (Fig. 2c) [$F(1,47) = 4.446$, $p = 0.040$], suggesting an impaired recognition memory in these animals. Strikingly, maternal exercise prevents the recognition memory deficit in the AβOs-injected adult offspring in comparison to AβOs-injected sedentary offspring (Fig. 2c) [$F(1,47) = 8.950$, $p = 0.004$]. These data suggest that maternal exercise during pregnancy is able to prevent recognition memory impairment elicited by AβOs injection into cerebral ventricles.

Table 1 Open filed test parameters assessed in adult offspring injected with vehicle or $A\beta O_{1–42}$

	Sedentary Mean ± SEM	Maternal exercise Mean ± SEM	Sedentary + $A\beta O_{1–42}$ Mean ± SEM	Maternal exercise + $A\beta O_{1–42}$ Mean ± SEM	*F* value	*p* value*
Distance traveled (m)	15.4 ± ±.28	15.8 ± 0.62	15.9 ± 0.92	15.2 ± 1.08	(1,53) = 0.009	0.924
Average speed (cm/s)	4.90 ± 0.41	5.24 ± 0.30	5.18 ± 0.20	5.02 ± 0.36	(1,53) = 0.123	0.727
Crossing	276 ± 13.0	293 ± 11.0	286 ± 17.0	267 ± 17.0	(1,52) = 0.269	0.605
Fecal bolus	5.55 ± 0.44	6.06 ± 0.84	5.64 ± 0.91	5.81 ± 0.71	(1,52) = 0.009	0.924
Time in periphery (s)	281 ± 2.68	282 ± 2.87	280 ± 3.14	283 ± 2.75	(1,53) = 0.309	0.580
Time in center (s)	18.6 ± 2.68	17.5 ± 3.09	20.2 ± 3.14	17.2 ± 2.75	(1,53) = 0.446	0.507

*A two-way ANOVA showed no significant differences ($p > 0.05$; $n = 10–17$/group)

Maternal Exercise Benefits on Learning and Reference Memory in the Adult Offspring Prevailed in the AβOs Injection Detrimental Effects

Spatial learning in the Morris water maze task depends on distal cues for rats to navigate and to locate a hidden platform [55]. The escape latency during the acquisition phase decreased from days one to five for all groups (Fig. 3a) [$F(4,167) = 16.59$, $p < 0.0001$]. On the fifth day, the sedentary + AβO adult offspring group spent more time to find the platform (higher mean escape latency) compared to the sedentary/control group [$F(3,42) = 2.995$, $p = 0.032$]. Further analysis showed an interaction between maternal exercise and AβOs injection on day 5 [$F(1,42) = 4.122$, $p = 0.048$]. Assessing reference memory on the retention phase is a measure of long-term memory. Latency to first target-site crossover, number of platform-site crossovers (platform entries), and time spent in the target quadrant compared with the other quadrants (Fig. 3b–d, respectively) were used to assess reference memory. On the sixth day, an interaction between maternal exercise and AβOs injection was observed in the escape latency to first target-site crossover [$F(1,46) = 4.883$, $p = 0.032$]. In addition, sedentary + AβO animals took more time to first target-site crossover compared to the sedentary/control group (Fig. 3b) [$F(1,46) = 8.556$, $p = 0.005$]. No difference was observed in the number of platform-site crossovers among the experimental groups (Fig. 3c); two-way ANOVA showed no effect of maternal exercise [$F(1,46) = 0.033$, $p = 0.857$] and AβOs injection [$F(1,46) = 1.949$, $p = 0.169$]. Analysis of the time spent in each quadrant (Fig. 3d) in the water maze showed that the sedentary + AβO group spent less time in the target quadrant (SE) compared to sedentary/control group, as indicated by significant interaction between the factors [$F(1,46) = 4.738$, $p = 0.035$]. These results suggest that maternal exercise during pregnancy abrogates the impairment in learning and long-term spatial memory elicited by AβOs injection on adulthood, pointing to a neuroprotective role of maternal exercise on the hippocampal-dependent memory acquisition and consolidation in the offspring.

Intact Working Memory Was Observed in AβOs-Injected Adult Offspring Born to Exercised Rats

The working memory task in the Morris water maze is also called the reversal phase; therefore, it depends on the rat's knowledge of the location of the hidden platform before this phase begins [55]. The platform is relocated daily, and the animals should learn the new location as they perform the successive trials (four trials/day) in order to travel the shortest path to find the hidden platform. AβOs injection

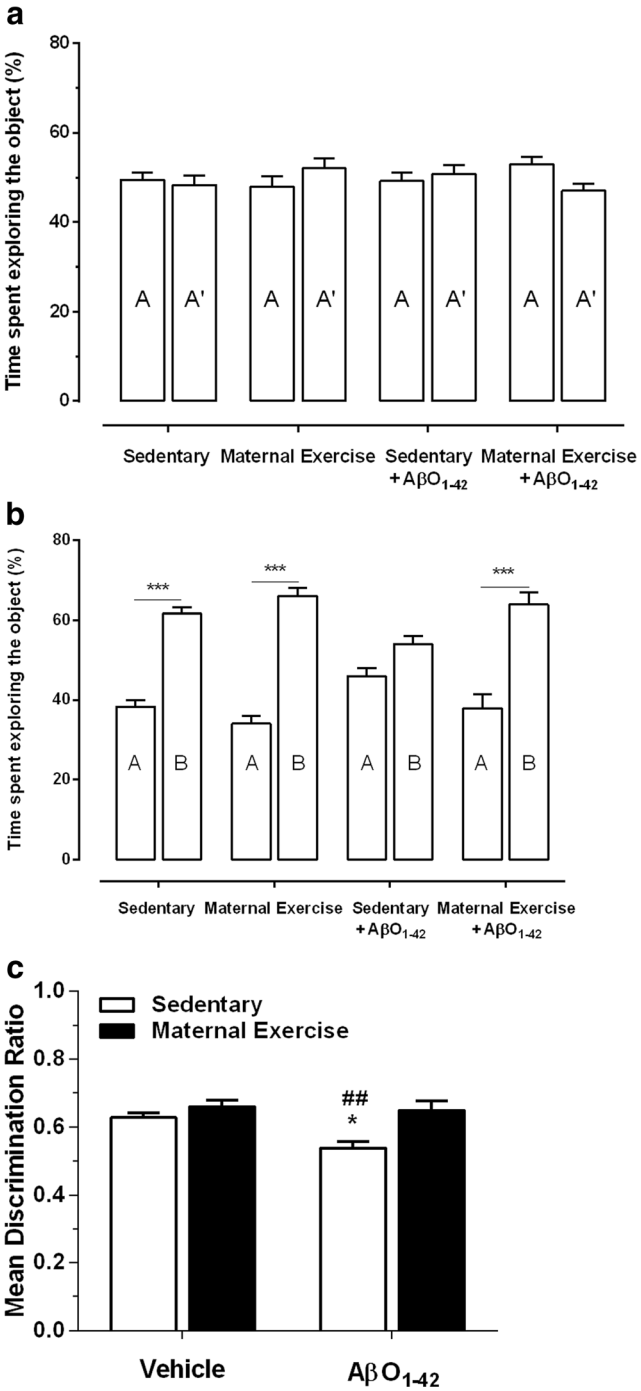

Fig. 2 Maternal exercise prevents $A\beta O_{1–42}$-elicited object recognition memory impairment in the adult offspring. **a**, **b** Time spent exploring each object in the training and test sessions, respectively, in the novel object recognition task in the adult offspring, after injection of vehicle or $A\beta O_{1–42}$ (500 pmol/rat), born to exercised or sedentary dams. **c** Discrimination ratio between familiar and novel object. In the bars of graphs, A and A' denote the familiar object and B denotes the novel object. A two-way ANOVA showed an effect of maternal exercise (n = 10–16/group). Data are expressed as mean + SEM. * $p < 0.05$ compared to sedentary/control; *** $p < 0.001$ object B compared to object A (Tukey post hoc test repeated measures); ## $p < 0.01$ compared to maternal exercise + $A\beta O_{1–42}$ (Tukey post hoc test)

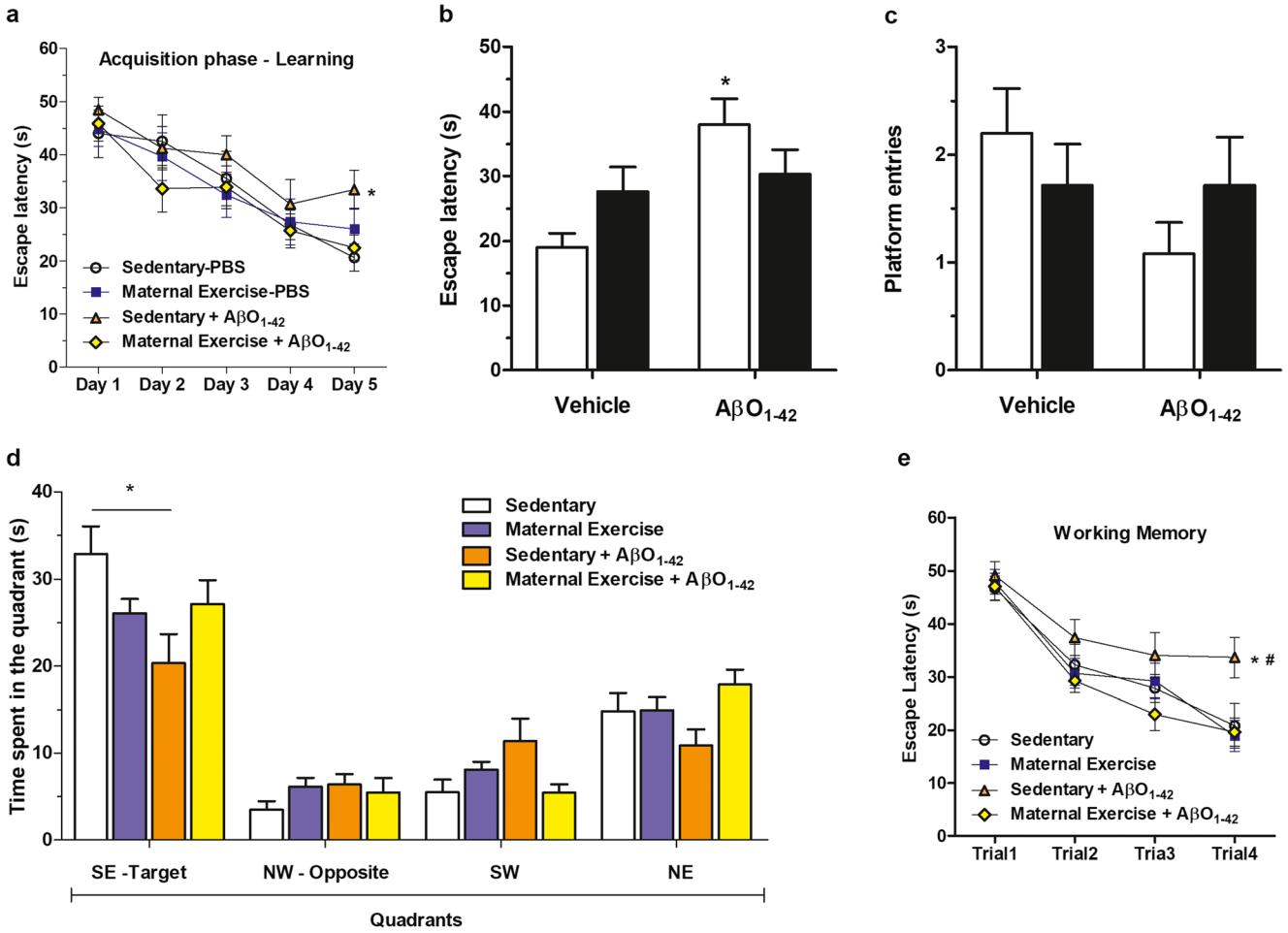

Fig. 3 Maternal exercise prevents memory deficits caused by $A\beta O_{1–42}$ injection in the adult offspring in the Morris water maze task. **a** Escape latency to find the platform during learning phase across the days 1 to 5; two-way ANOVA showed an interaction between maternal exercise and $A\beta O_{1–42}$ injection ($p < 0.05$). **b** Platform latency during reference memory on day 6; two-way ANOVA showed an interaction between maternal exercise and $A\beta O_{1–42}$ injection. **c** Platform entries during reference memory on day 6; two-way ANOVA showed no significance. **d** Time spent in each quadrant during reference memory on day 6; two-way ANOVA matching by group showed an effect of $A\beta O_{1–42}$ injection on the time spent in the target quadrant (SE). **e** Escape latency to find the platform in a new location during the working memory task on trials 1 to 4 in each day; two-way ANOVA showed an effect for maternal exercise and for $A\beta O_{1–42}$ injection. Data are expressed as mean + SEM; $n = 10–14$/group. $^*p < 0.05$ compared to sedentary/control group; $\#p < 0.05$ compared to maternal exercise + $A\beta O_{1–42}$ group

significantly affected the working memory in the adult offspring of sedentary rats (Fig. 3e), which was demonstrated by the high escape latency, to find the platform new location at the fourth trial compared to sedentary/control group [$F(3,46) = 3.634$, $p = 0.019$]. Strikingly, rats from the maternal exercise + AβO group displayed a high performance in the escape latency, observed by reduced escape latency that differed significantly from sedentary + AβO group [$F(3,46) = 3.634$, $p = 0.019$], and similar to that observed in the control group. These results indicate the spatial working memory that depends on the hippocampal-prefrontal interaction is disrupted by AβOs injection in the offspring of sedentary rats but remains unaffected in maternally exercised offspring, supporting a preventive potential of maternal exercise against the challenged-promoted cognitive decline in adulthood.

AβOs Injection Induced a Reduction in Hippocampal but Not in Prefrontal Cortical BDNF Levels

Mature BDNF levels were measured in the offsprings' hippocampus and prefrontal cortex 24 h after the end of behavioral tests. In accordance with other authors [50, 65, 66], mature hippocampal BDNF levels were reduced by AβOs injection (Fig. 4, right) [$F(1,27) = 6.261$, $p = 0.019$]; however, maternal exercise during pregnancy does not prevented such reduction

[$F(1,27) = 0.017$, $p = 0.897$]. Conversely, mature BDNF levels in the prefrontal cortex (Fig. 4, left) were not altered by either maternal exercise or AβOs injection [$F(1,26) = 0.822$, $p = 0.373$; $F(1,26) = 0.130$, $p = 0.722$, respectively].

Reduced Hippocampal Synaptophysin by $A\beta O_{1–42}$ Injection in Adult Offspring Was Prevented by Maternal Exercise During Pregnancy

Synaptophysin is a presynaptic protein that is considered to be a synaptic marker, and it is known to be downregulated in AD [67, 68]. Here, we measure the immunocontent of the synaptophysin through western blot analysis (Fig. 5). There was no statistical difference in the synaptophysin content measured in the offsprings' prefrontal cortex after AβOs injection [$F(1,26) = 0.539$, $p = 0.469$] and maternal exercise during pregnancy [$F(1,26) = 0.606$, $p = 0.443$]. Furthermore, there was a significant reduction in the hippocampal synaptophysin content after AβOs injection, in comparison to control/sedentary group [$F(1,27) = 4.636$, $p = 0.040$]. Strikingly, maternal exercise during pregnancy was able to prevent such effect on the offsprings' hippocampus [$F(1,27) = 4.811$, $p = 0.037$].

Increased Number of Functional Mitochondria, Induced by Maternal Exercise During Pregnancy, Was Not Abolished by AβOs Injection in the Adult Offsprings' Prefrontal Cortex and Hippocampus

Mitochondrial dysfunction is an event that accompanies AD pathology, underlying the major metabolic changes [7]. We examined the number of functional mitochondria through flow cytometric analysis of simultaneous Mitotracker Green

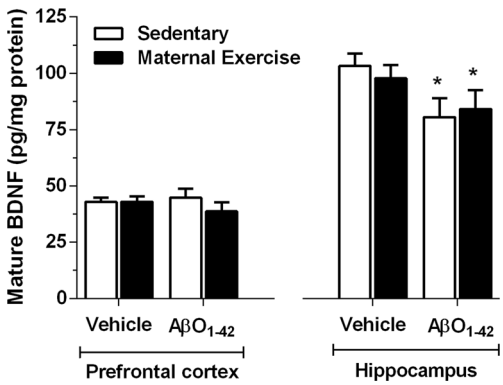

Fig. 4 Effect of maternal exercise during pregnancy on mature BDNF levels in the hippocampus and prefrontal cortex of the adult offspring. Mature BDNF levels in the prefrontal cortex (left panel) and hippocampus (right panel) of adult offspring, after injection of vehicle or $A\beta O_{1–42}$ (500 pmol/rat), born to exercised or sedentary dams. A two-way ANOVA showed an effect of $A\beta O_{1–42}$ on hippocampal mature BDNF levels ($p < 0.05$; $n = 6–9$/group). Data are expressed as mean + SEM

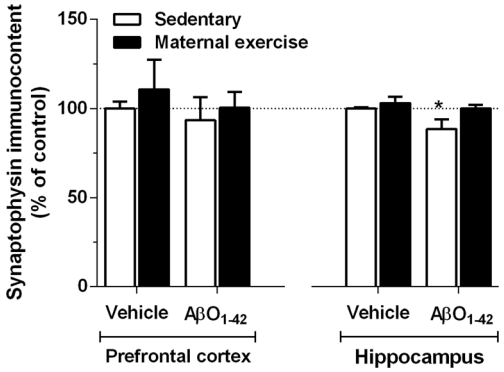

Fig. 5 Maternal exercise prevents hippocampal synaptophysin reduction induced by $A\beta O_{1–42}$ injection. Synaptophysin immunocontent in the prefrontal cortex (left panel) and hippocampus (right panel) of adult offspring, after injection of vehicle or $A\beta O_{1–42}$ (500 pmol/rat), born to exercised or sedentary dams. Synaptophysin level is expressed as the average percentage of control. Representative quantification of synaptophysin immunocontent normalized to b-actin protein (loading control) is shown below the graphs. A two-way ANOVA showed an effect of both, $A\beta O_{1–42}$ and maternal exercise, on hippocampal synaptophysin levels ($p < 0.05$; $n = 7–9$/group). Data are expressed as mean + SEM

and Mitotracker Red labeled events, which indicate mitochondrial mass and membrane potential, respectively. Double positive labeling indicates functional respiring mitochondria, and an increase in fluorescence is indicative of mitochondrial biogenesis [69]. It was observed that maternal exercise during pregnancy induces an increase in the number of functional mitochondria in the offsprings' prefrontal cortex [$F(1,26) = 4.375$, $p = 0.046$] and hippocampus [$F(1,37) = 18.15$, $p < 0.0001$], evidenced by the increased percentage of Mitotracker Green and Mitotracker Red double positive events compared to sedentary/control group (Fig. 6a, b). Furthermore, AβOs injection did not alter the beneficial role elicited by maternal exercise on mitochondrial functionality in both brain areas, the prefrontal cortex [$F(1,26) = 0.348$, $p = 0.560$] and hippocampus [$F(1,37) = 0.674$, $p = 0.417$].

AβOs-Induced Reduction in the Adult offspring's Hippocampal α-KGDH Enzyme Activity Was Prevented by Maternal Exercise During Pregnancy

Long-term reduced brain energy metabolism is observed in AD and is highly correlated to cognitive decline. This shift in energy metabolism involves alterations in mitochondrial enzymes [70]. To examine the mitochondrial metabolism, we measured the activity of an important enzyme of the tricarboxylic acid cycle that is involved in mitochondrial bioenergetics in the prefrontal cortex and hippocampus of offspring born to exercised and sedentary rats. The activity of α-KGDH in the prefrontal cortex (Fig. 7a, left panel) showed that maternal exercise during pregnancy increased α-KGDH activity [$F(1,19) =$

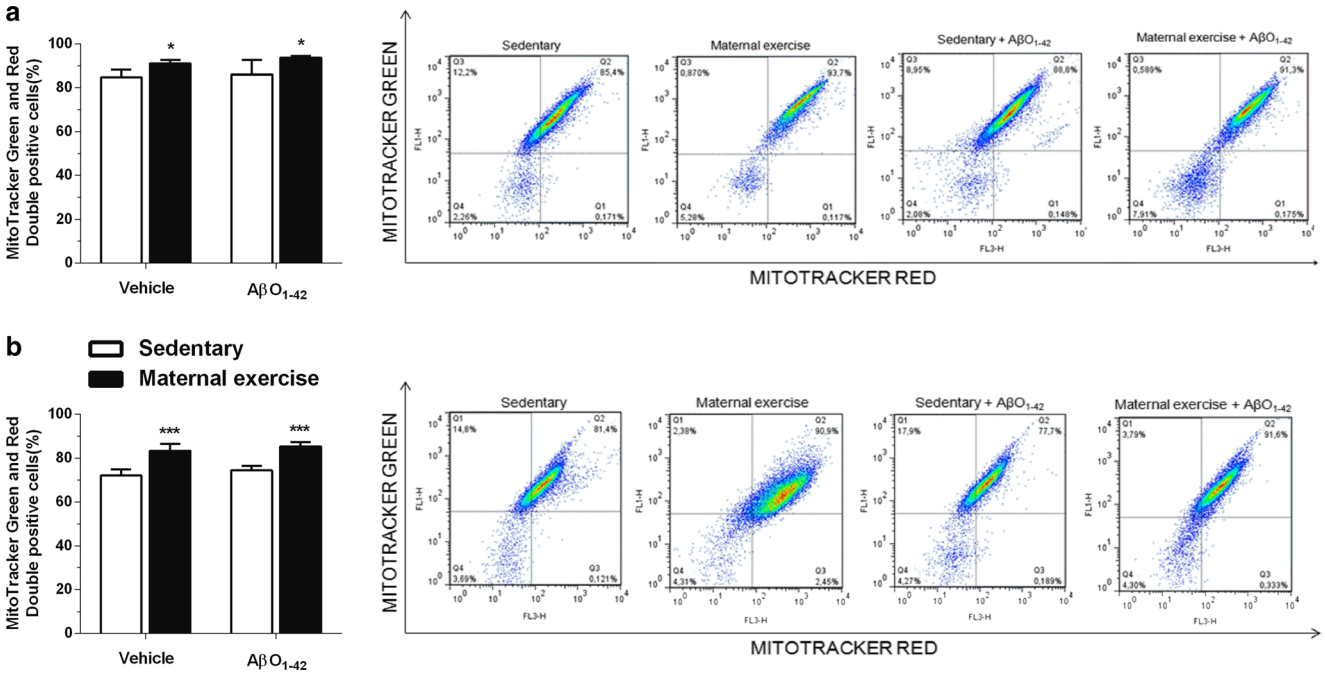

Fig. 6 Maternal exercise induces an increment in mitochondrial functionality in the adult offspring's prefrontal cortex and hippocampus. Percentage of double positive Mitotracker Green and Mitotracker Red labeled cells were measured in the **a** prefrontal cortex and **b** hippocampus of adult offspring, after injection of vehicle or $A\beta O_{1-42}$ (500 pmol/rat), born to exercised or sedentary dams. Representative dot plot analyses of Mitotracker Green and Mitotracker Red by flow cytometer are depicted in right panels (**a**, **b**). Two-way ANOVA showed an effect of maternal exercise on mitochondrial mass and membrane potential double positive cells number ($p < 0.05$; $n = 9$–11/group). Data are expressed as mean + SEM. *$p < 0.05$ compared to sedentary/control group; ***$p < 0.0001$ compared to sedentary/control

6.861, $p = 0.017$], while AβOs injection did not exert any effect on enzyme activity [$F(1,19) = 0.013$, $p = 0.908$]. Similarly, α-KGDH activity in the hippocampus (Fig. 7a, right panel) was significantly reduced by AβOs injection compared to control/sedentary [$F(1,21) = 6.928$, $p = 0.015$] offspring. Strikingly, maternal exercise during pregnancy prevented the decrease of α-KGDH activity [$F(1,21) = 8.100$, $p = 0.001$] elicited by AβOs infusion, which was evidenced by a statistical difference in hippocampal α-KGDH activity of offspring born to sedentary + AβO and maternal exercise + AβO groups. These data indicate that AβOs promote the impairment of TCA function and that maternal exercise during pregnancy increases TCA α-KGDH enzyme activity, preventing AβOs effects.

Increased Respiratory System Complex IV Activity Induced by Maternal Exercise During Pregnancy Was Not Abolished by AβOs Injection in the Adult Offspring's Prefrontal Cortex and Hippocampus

Aβ peptide has been shown to impair mitochondrial respiration by affecting the activity of the electron transport system [71]. To investigate whether Aβ peptide could alter the transfer of electrons, we then assessed the effect of maternal exercise and AβOs injection on respiratory system complex IV. Strikingly, as shown in Fig. 7b, maternal exercise increased cytochrome c oxidase activity (CIV) in the prefrontal cortex [$F(1,24) = 5.042$, $p = 0.034$] and hippocampus [$F(1,25) = 5.635$, $p = 0.026$]. AβOs injection did not significantly effect CIV in the prefrontal cortex [$F(1,24) = 0.691$, $p = 0.414$] or hippocampus [$F(1,25) = 2.634$, $p = 0.118$]. These data indicate that maternal exercise augments activity of the mitochondrial electric transport system and that AβOs injection did not affect mitochondrial respiration.

Maternal Exercise During Pregnancy Upregulated Mitochondrial Fusion Protein in the Offspring's Prefrontal Cortex

Mitochondria continuously undergo fusion and fission cycles to allow proliferation, distribution, and cellular adaptation to energy shifts [9]. To assess mitochondrial dynamics, we measured the immunocontent of the fusion protein mitofusin 1 (Mfn1) and the fission protein dynamin-related protein (Drp1) (Fig. 8). Maternal exercise during pregnancy increased Mfn1 immunocontent in the offspring's prefrontal cortex [$F(1,36) = 5.347$, $p = 0.027$], which was not abolished by AβOs injection [$F(1,36) = 0.026$, $p = 0.871$] (Fig. 8a, left panel). Maternal exercise during pregnancy did not affect Drp1 immunocontent in the prefrontal cortex [$F(1,31) = 1.453$, $p = 0.237$] and neither did the AβOs injection [$F(1,31) = 0.003$, $p = 0.955$] (Fig. 8b, left panel).

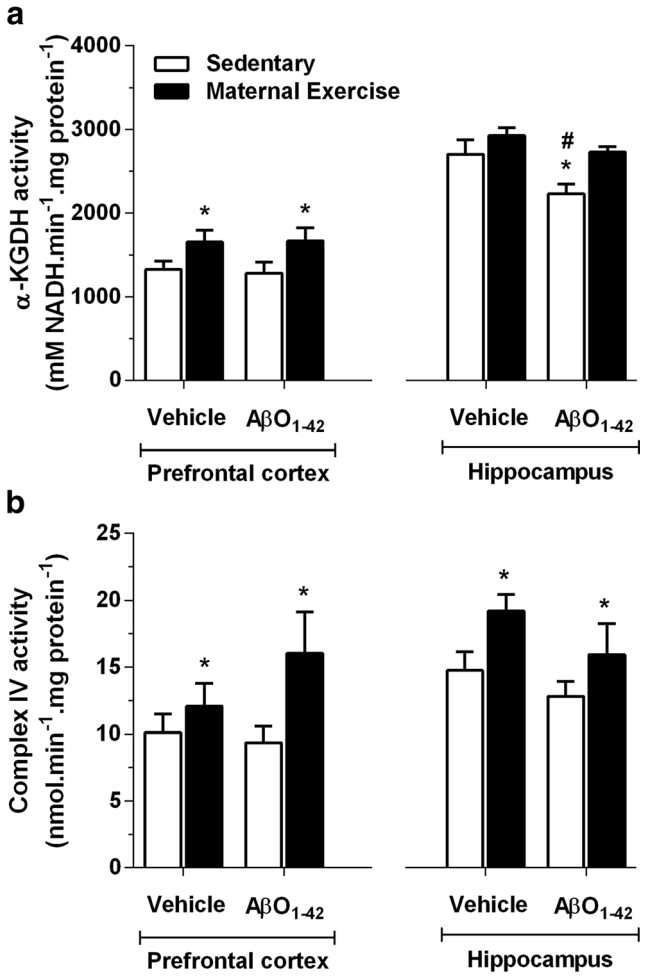

Fig. 7 Maternal exercise increases mitochondrial function of the tricarboxylic acid cycle enzymes in the adult offspring's prefrontal cortex and hippocampus. The activity of the enzymes **a** α-ketoglutarate dehydrogenase (α-KGHD) and **b** cytochrome c oxidase (CIV) was measured in the prefrontal cortex (left panels) and hippocampus (right panels) of adult offspring born to exercised or sedentary dams, after injection of vehicle or $A\beta O_{1–42}$ (500 pmol/rat). Two-way ANOVA showed an effect of maternal exercise and $A\beta O_{1–42}$ injection on α-KGHD enzyme activity in the prefrontal cortex, and an effect of maternal exercise on hippocampal α-KGHD enzyme activity ($p < 0.05$; $n = 5–7$/group). Two-way ANOVA showed an effect of maternal exercise on CIV enzyme activity in the prefrontal cortex and hippocampus ($p < 0.05$; $n = 6–9$/group). Data are expressed as mean + SEM. $^*p < 0.05$ compared to sedentary/control group; $\#p < 0.05$ compared to maternal exercise + $A\beta O_{1–42}$ group

Altered Immunocontent of Mitochondrial Fission Protein in the Hippocampus of AβOs-Injected Offspring Is Prevented by Maternal Exercise During Pregnancy

To assess mitochondrial dynamics in the hippocampus, we measured the immunocontent of the Mfn1 and Drp1 (Fig. 8). Maternal exercise during pregnancy did not affect hippocampal Mfn1 immunocontent [$F(1,20) = 0.017$, $p = 0.897$] and neither did the AβOs injection [$F(1,20) = 0.059$, $p = 0.811$]. However, AβOs injection did increase Drp1 levels [$F(1,29) = 6.908$, $p = 0.013$]. In contrast, Drp1 immunocontent in the hippocampi of offspring from the maternal exercised group injected with AβOs was similar to the control group [$F(1,29) = 4.226$, $p = 0.049$]. These results indicate that AβOs injection increased the mitochondrial fission protein, which was prevented by maternal exercise during pregnancy.

Discussion

This study examined the potential role of maternal exercise during pregnancy on the in utero programming of the

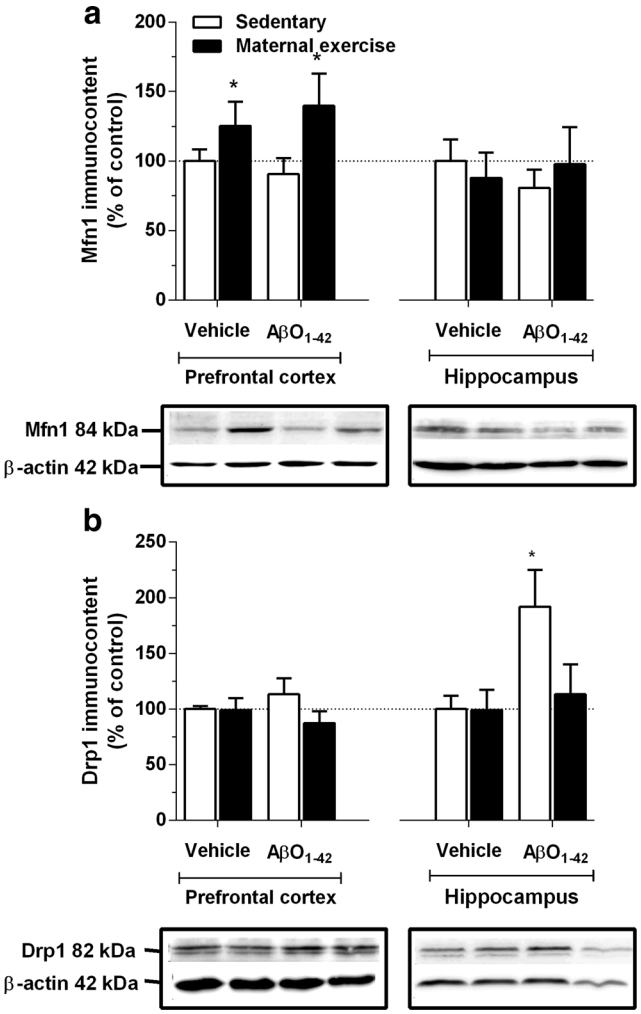

Fig. 8 Effects of $A\beta O_{1–42}$ injection and maternal exercise during pregnancy on mitochondrial dynamic-related proteins levels. **a** Immunocontent of mitofusin and **b** dynamin-related protein in the prefrontal cortex (left panel) and hippocampus (right panel) of adult offspring born to exercised or sedentary dams, after injection of vehicle or $A\beta O_{1–42}$ (500 pmol/rat). Two-way ANOVA showed an effect of maternal exercise on mitofusin immunocontent in the prefrontal cortex, and an effect of maternal exercise and $A\beta O_{1–42}$ injection on dynamin-related protein immunocontent in the hippocampus ($p < 0.05$; $n = 8–10$/group). Data are expressed as mean + SEM. $^*p < 0.05$ compared to sedentary/control group

offsprings' brain metabolism, and if this programming could modify the course of diseases later in life. Here, we report that a single, bilateral injection of AβOs into the brain ventricles can elicit memory deficits and dysregulation of energy metabolism in specific brain regions of the offspring, when assessed 14 days after the induction of AD-associated AβOs pathology. In addition, the intrauterine environment, triggered by maternal exercise, can influence the long-term health of the offspring by modifying the response to hazardous stimulus of AβOs infusion. This effect occurred through the modulation of cellular metabolism, particularly mitochondrial function, and prevented the mnemonic deficits induced by AβOs.

Intracerebroventricular administration of the Aβ peptide is a well-described model of the early phase of AD-like pathology, and it has been used extensively to characterize Aβ neurotoxicity in rodents [3, 5, 72]. Aβ peptides accumulate primarily in the prefrontal cortex and hippocampus [73], triggering molecular and cellular alterations that lead to neurodegeneration and, finally, culminate in cognitive deficits characteristically observed in AD patients [5, 6]. In AD pathology, episodic and spatial memory impairments are common features [74, 75]; the former is widely recognized to be assessed in rodents through the novel object recognition task [35, 54, 64], and the later through the spatial navigation in the mazes tasks [76–78]. Here, we demonstrated that icv injection of AβOs caused a marked decline in non-spatial object recognition memory, and it impaired the acquisition of new information for spatial reference and the working memories of adult male offspring born to sedentary rats. Cognitive deficits caused by AβO are well correlated with synaptic dysfunction and loss [11]. We demonstrated that AβOs injection significantly reduced the synaptic marker, synaptophysin, in the hippocampus. Decreased levels of synaptophysin have long been associated with Aβ toxicity in human post-mortem brains [67, 68], in animal models [79], and in in vitro studies [80]. In view of AβOs toxicity on synaptic function, therapeutic approaches aiming to prevent synaptic deficits, with potential to prevent or decelerate cognitive decline, are desired [10]. In addition, the benefits on cognition promoted by maternal exercise seem to be modulated by mitochondrial function and are not dependent on BDNF, because mature BDNF levels in offspring were not affected by maternal swimming exercise. Accordingly, our previous publication demonstrated that maternal swimming before and during pregnancy improved object recognition memory in adult male offspring in a BDNF-independent manner [32]. Moreover, the high performance observed in the object recognition test by offspring of the maternal exercise group might be explained by c-FOS expression-associated enhanced object recognition memory and increased neural activity in these brain regions, as demonstrated by Robinson and Bucci [35].

The regulation of synaptic density and plasticity rely on proper mitochondrial energy metabolism [81]. The distribution of mitochondria in the dendrites is critical to meet the high metabolic requirement of neurons for vesicular neurotransmitter release [82] and to support learning and memory [83], which are impaired in AD [84]. Several studies have already shown that physical exercise promotes metabolic adaptation in the CNS, such as an increase of glucose uptake [85], activity of the electron transport system [86], endogenous antioxidant defense [87], and induction of mitochondrial biogenesis [17]. Interestingly, there has been an increase in the publication of clinical papers [27, 28] and experimental studies [29–34] that have evaluated the benefits of maternal exercise on offspring metabolism. Among the neurometabolic benefits promoted by maternal exercise during pregnancy, there is an increased antioxidant defense system, induction of mitochondriogenesis in different encephalic areas [34], neurogenesis [29, 48], and memory improvement [29, 32]. Moreover, maternal exercise during pregnancy has the potential to protect the offspring against disease susceptibility to either peripheral or CNS disorders [40–42, 44, 45]. Based on these data, maternal exercise during pregnancy may represent a remarkable way to mitigate amyloid-β-induced synaptic loss and mitochondrial dysfunction. Thus, we employed the maternal swimming exercise protocol, before and during pregnancy, aiming to show that metabolic adaptations can confer neuroprotection to the offspring against Aβ-induced neurotoxicity in adulthood. We demonstrate here that maternal swimming during pregnancy protected the AβOs-induced cognitive impairment of adult offspring in the novel object recognition and water maze tests. These observations might be due to an adaptive response of fetal brain metabolism to the maternal environment that is triggered by swimming exercise during pregnancy. As already suggested in a previous work, Herring et al. [40] have demonstrated that running exercise during pregnancy mitigates Alzheimer-like pathology in the offspring of TgCRND8 mice, through promotion of long-lasting protection, i.e., reduced Aβ plaque burden, inflammation, and oxidative stress, against neurodegeneration [34].

One remarkable feature of AβOs injection in experimental models of AD is their accumulation in mitochondria. Thereby, AβOs target essential metabolic enzymes, impairing their function and altering mitochondrial dynamics [6–8]. The disruption of these processes promotes the dysfunction of mitochondrial bioenergetics [3, 70], leading to reduced energy metabolism that negatively affects the axonal transport, and contributes to cognitive deficits [88]. Our findings of reduced α-KGDH activity in the hippocampus of offspring from the sedentary maternal group injected with AβOs are in accord with previous works, in which α-KGDH enzyme activity is found to be decreased in the brain of AD patients [89]. The neuroenergetic failure hypothesis posits that a compensatory increase in oxidative phosphorylation, in healthy neurons, occurs to maintain adequate energy production and to ensure neuronal viability and counterbalance energy failure of

damaged neurons [90]. The lack of an AβOs injection-induced alteration of the activity of ETS enzymes might agree with the neuroenergetic failure hypothesis, and, therefore, there was no difference in enzyme activities between groups. As proposed by Klupp et al. [91], these processes are thought to be due to the AβO toxicity-induced decrease in functional connectivity between brain regions. Strikingly, AβOs-induced downregulation of α-KGDH was prevented by maternal exercise during pregnancy.

Mitochondrial dynamics is important to maintain bioenergetic functionality of each mitochondrion [9]. Continuous mitochondrial fusion and fission cycles control the morphology, number, and bioenergetics functionality of the mitochondria, and both play important role in physiology and development of CNS and synapses [92]. Exercise has been associated with improved mitochondrial function and it can modulate the expression of fusion and fission proteins. We demonstrated that maternal exercise during pregnancy was able to increase the immunocontent of the Mfn1, an important protein for mitochondrial fusion, in the prefrontal cortex of the adult offspring, while this long-lasting effect was not observed at the Drp1 levels. It has been demonstrated that the protein Drp1, which is related to mitochondrial fission, is altered in AD brain in response to metabolic changes [93]. Persistent fission negatively affects mitochondrial function, leading to deleterious effects on synaptic function and bioenergetics, and, consequently, contributes to neurodegeneration [9]. Together with the other findings observed in the present study, increased Drp1 levels elicited by AβOs injection suggest that a bioenergetics failure stimulates mitochondrial fission in the sedentary group, which seems to be prevented in the maternal exercise group. Moreover, these results support the hypothesis for a mitochondrial compensatory mechanism. As described elsewhere, increased Drp1 levels are responsible for excessive fragmentation of mitochondria, which in turn are unable to move to synapses and supply the necessary ATP at nerve terminals [80]. Further, by attempting to deal with the energy failure, the defective mitochondria undergo excessive fission; thus, they may not be able to support the energy demand necessary to sustain neurotransmission, leading to synaptic damage and neurodegeneration [94].

Mechanisms underlying cellular and metabolic adaptive responses are influenced by intrauterine and early postnatal environment and rely on epigenetic modifications that modulate gene expression and the redox state throughout offsprings' life [95]. Exploiting the benefits of maternal swimming on the brains of adult offspring, we highlight some points: (1) the ability to prevent the AβO-induced loss of synaptophysin in the hippocampus; (2) the increased number of functional mitochondria in the prefrontal cortex and hippocampus, as indicated by increases in the mitochondrial mass and membrane potential; (3) the increased activity of α-KGDH enzyme of tricarboxylic acid cycle in the prefrontal cortex and hippocampus; (4) the increased activity of the ETS enzyme cytochrome c oxidase in the prefrontal cortex and hippocampus; (5) the increased Mfn1, in the prefrontal cortex; and (6) the capacity to prevent the increase of Drp1 immunocontent in the hippocampus, which was induced by icv AβO injection. Moreover, our group previously reported that maternal swimming induced mitochondrial biogenesis in the hippocampus, parietal cortex, and cerebellum of 7-day-old pups [34]. Taken together, these data suggest that maternal swimming exercise before and during pregnancy might induce long-lasting mitochondriogenesis in the prefrontal cortex and hippocampus of offspring. The present work highlights the important role of maternal exercise in determining long-term health, as stated by the concept of DOHaD. To the best of our knowledge, this is the first report to demonstrate that moderate-intensity exercise during pregnancy, involuntary swimming, promotes long-lasting neurometabolic adaptations in the offspring and protects against Aβ peptide-induced cognitive deficits.

In summary, our data reinforce and extend the notion that intrauterine environment, provided by maternal exercise to the fetus, improve the offsprings' brain metabolism and confers resistance against metabolic changes triggered by AD-associated AβO. Therefore, successfully extrapolating the health-promoting findings of maternal exercise to the clinic might open new insights into biomedical area; this lifestyle-based approach, allied to low costs to public health, has the advantage of preventing the development of chronic diseases and may reduce the global burden of dementia-causing neurodegeneration, such as AD.

Acknowledgements The authors thank Mariana Maier Gaelzer and Fabrício Figueiró for their valuable assistance in flow cytometry analyses.

Funding Information This study was supported by the Pró-Reitoria de Pesquisa/Universidade Federal do Rio Grande do Sul (PROPESQ/UFRGS). CPK is a PhD Postgraduate student in Biological Sciences – Biochemistry receiving grants from the Brazilian agency Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). CM received grants from CNPq (Universal 442406/2014-2 and INCT 465671/2014-4).

Compliance with Ethical Standards

All experimental procedures were approved by the local Ethics Commission on the Use of Animals (Comissão de Ética no Uso de Animais - CEUA/UFRGS) under the protocol number 27349 and were performed in accordance with the National Animal Rights Regulations (Law 11.794/2008), the American National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication No. 80-23, revised 1996), and the Directive 2010/63/EU.

Conflict of Interest The authors declare that they have no conflict of interest.

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