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Combined Exercise and Insulin-Like Growth Factor-1 Supplementation Induces Neurogenesis in Old Rats, but Do Not Attenuate Age-Associated DNA Damage

Erika Koltai,[1] Zhongfu Zhao,[1,2] Zsombor Lacza,[3] Attila Cselenyak,[3] Gabriella Vacz,[3] Csaba Nyakas,[1] Istvan Boldogh,[4] Noriko Ichinoseki-Sekine,[5] and Zsolt Radak[1]

Abstract

We have investigated the effects of 2 weeks of insulin-like growth factor-1 (IGF-1) supplementation (5μg/kg per day) and 6 weeks of exercise training (60% of the maximal oxygen consumption [VO_2 max]) on neurogenesis, DNA damage/repair, and sirtuin content in the hippocampus of young (3 months old) and old (26 months old) rats. Exercise improved the spatial memory of the old group, but IGF-1 supplementation eliminated this effect. An age-associated decrease in neurogenesis was attenuated by exercise and IGF-1 treatment. Aging increased the levels of 8-oxo-7,8-dihydroguanine (8-oxoG) and the protein Ku70, indicating the role of DNA damage in age-related neuropathology. Acetylation of 8-oxoguanine DNA glycosylase (OGG1) was detected *in vivo*, and this decreased with aging. However, in young animals, exercise and IGF-1 treatment increased acetylated (ac) OGG1 levels. Sirtuin 1 (SIRT1) and SIRT3, as DNA damage–associated lysine deacetylases, were measured, and SIRT1 decreased with aging, resulting in a large increase in acetylated lysine residues in the hippocampus. On the other hand, SIRT3 increased with aging. Exercise-induced neurogenesis might not be a causative factor of increased spatial memory, because IGF-1 plus exercise can induce neurogenesis in the hippocampus of older rats. Data revealed that the age-associated increase in 8-oxoG levels is due to decreased acetylation of OGG1. Age-associated decreases in SIRT1 and the associated increase in lysine acetylation, in the hippocampus, could have significant impact on function and thus, could suggest a therapeutic target.

Introduction

Aging results in enhanced production of reactive oxygen species (ROS), leading to increased levels of oxidative modification in lipids, proteins, and DNA, which could be associated with decreased physiological function.[1–4] It is well documented that the interaction of ROS with lipids and proteins has a significant impact on cellular function in the brain.[5–8] However, recent investigations have revealed that DNA damage/repair can contribute to the age-associated neurodegeneration.[2–4] Indeed, significant damage to DNA causes activation of pro-apoptotic and DNA repair proteins. Depending on the size of DNA damage and the success of repair, cells either die or survive.[4,9,10] 8-Oxo-7,8-dihydroguanine (8-oxoG) is the most abundant ROS-related product of DNA oxidation and has been implicated in mutagenesis.[11–13] The mammalian homolog 8-oxoguanine DNA glycosylase (OGG1) is enzyme specific for incising 8-oxoG to avoid the transversion of GC → TA, or DNA damage–induced apoptosis. Liu et al. have recently (2010) shown that OGG1 can rescue neurons subjected to ischemic conditions.[3] It is known that acetylation of OGG1 can increase the activity of the enzyme nearly 10-fold in a cell culture,[14] but the presence of *in vivo* acetylation has yet to be reported.

Sirtuins are nicotinamide adenine dinucleotide (NAD)-dependent histone deacetylases, markedly influenced by the redox state. Generally, they play an antiapoptotic role.[15,16] Sirtuin 1 (SIRT1) and SIRT3 have wide-ranging roles in cell metabolism, inflammation, and differentiation.[17,18] They are implicated in DNA repair[19–25] because acetylation/deacetylation alters the activity of OGG1, the protein Ku70, and human apurinic/apyrimidinic endonuclease 1 (APE1).[26–29] It has

[1]Semmelweis University, Research Institute of Sport Science, Budapest, Hungary.
[2]Institute of Hepatology, Changzhi Medical College, Shanxi, China.
[3]Semmelweis University, Institute of Human Physiology and Clinical Experimental Research, Budapest, Hungary.
[4]Department of Microbiology and Immunology, University of Texas Medical Branch at Galveston, Galveston, Texas.
[5]Department of Exercise Physiology, School of Health and Sport Science, Juntendo University, Chiba, Japan.

further been shown that caloric restriction (CR) has an anti-apoptotic effect, with increases in the expression of SIRT1, which is attenuated by the administration of insulin-like growth factor-1 (IGF-1).[30] Moreover, it has been observed that SIRT1 mediates deacetylation of Ku70-depressed apoptosis and facilitates cell survival.[30] Indeed, it has been widely accepted that the CR-related downregulation of the insulin/IGF-1 pathway is one of the key factors by which CR increases maximal and mean life span in rodents.[31,32] Paradoxically, the brain tends to benefit from IGF-1 signaling, including neurogenesis.[33,34] However, the findings of a recent report suggest that IGF-1 could have beneficial as well as harmful effects on the developing brain.[35] This revelation supports the earlier observation that hyperactivated IGF-1 signaling shows accelerated aging and amyloid beta production in the brain.[36] Blockade of IGF-1 in nonexercising and exercising mice has revealed the complex role of IGF-1 in neurogenesis, dendritic spine density, CA1 pyramidal cells, and hippocampal structure.[37] In addition, it appears that insulin injection could partly eliminate the beneficial effects of regular exercise on brain function.[38]

Therefore, the possible interactions between exercise, IGF-1, and sirtuins on brain function with aging provide an exciting field of study. In spite of the complex relationship between SIRT1 and IGF-1, SIRT1 is closely involved with memory and plays a crucial role in brain function, at least as observed in SIRT1 knockout transgenic mice.[24,39] However, how the aging-associated decline in brain function is related to SIRT1 has yet to be clarified. Although, SIRT3 has been linked to human longevity, the available information about this sirtuin with aging and its relation to brain function are not known.

It is well documented that regular exercise benefits brain function via a number of mechanisms, including: Neurogenesis[40,41]; increases in brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and vascular endothelial growth factor (VEGF) production[42–45]; and decreases in oxidative stress.[46] Despite the differences in anxiety-like behavior, both voluntary and forced exercise regimes could result in improved brain function, induction of neurotrophins, and neurogenesis.[47,48]

The oxidative stress–related changes might be associated with sirtuins, because they can be modulated by oxidative challenges.[49–51] Aerobic exercise, which is often used to study the effects of physical activity on brain function, decreases the level of circulating IGF-1 with exercise of moderate intensity and long duration[52]; however, the findings of Trejo and co-workers[53] on exercising in wild-type and mutant mice with low levels of serum IGF-1 show the complexity of IGF-1 in exercise physiology.

Therefore, the present study was designed to test: (1) The effects of IGF-1 supplementation and exercise on brain function and neurogenesis and (2) the relationship between age-associated oxidative DNA damage and brain function, with a special focus on repair enzymes that are sensitive to acetylation/deacetylation, such as OGG1, APE-1, and Ku70; and (3) the possible contribution of the aging process on DNA repair- associated sirtuins.

Methods

Animals and training protocol

Twelve young (3 months old) and 12 old (26 months old) male Wistar rats were used in the study and grouped into young control (YC), young exercised (YE), young exercised IGF-1-treated (YEI), old control (OC), old exercised (OE), and old exercised IGF-1-treated (OEI). In the last 2 weeks, an Alzet pump was inserted subcutaneously in all animals, and IGF-1-treated animals received 5μg/kg per day, 0.5μL/hr,[54] whereas nontreated animals received saline via the pumps. With the help of the Alzet pumps, the 2-week supplementation of IGF-1 or saline could be maintained at constant flow, thus avoiding daily injections and their possible disturbance of behavioral and cognitive functions of the animals.

The investigation was carried out according to the requirements of The Guiding Principles for Care and Use of Animals of the European Union and approved by the local ethics committee. Exercised rats were introduced to treadmill running for 3 days; then for the next 2 weeks the running speed was set at 10 m/min, with a 5% incline for 30 min/day. The running speed and duration of the exercise were gradually increased to 60% of maximal oxygen consumption (VO_2 max) of the animals. Therefore, on the last week of the 6-week training program, young animals ran at 22 m/min, on a 10% incline, for 60 min, whereas old animals ran at 13 m/min, on a 10% incline for 60 min. To be able to monitor new cell formation, bromodeoxyuridine (BrdU) was injected into each animal for the last 4 weeks of the program. The animals were anesthetized with intraperitoneal injections of ketamine (50 mg/kg) and perfused by 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.4). This procedure was carried out 2 days after the last exercise session to avoid the metabolic effects of the final run.

Hippocampi were excised carefully and homogenized in buffer containing 137 mM NaCl, 20 mM Tris-HCl, pH 8.0, 2% NP-40, 10% glycerol, and protease inhibitors. The protein content was measured by the Bradford method using bovine serum albumin (BSA) as a standard. The samples were stored at -80°C.

Morris water maze test

Brain function was evaluated by the Morris water maze test on 4 consecutive days (four trials per day). A platform of 6 cm in diameter was placed 1 cm below the surface of the water in the center of the northwest quadrant of a circular pool of 60 cm in height and 100 cm in diameter. The water was held constant at 22–23°C throughout training and testing. During a given training trial, each rat was introduced into the pool at one of four possible starting points (north, south, west, or east) and allowed a period of 60 sec to find the platform. The order of starting points varied in a pseudo-random manner for each rat, each day.

Measurement of IGF-1 level

After sacrificing the animals, blood was collected, supercharged ethylenediaminetetraacetic acid (EDTA) was added, and the samples were centrifuged at 3000$\times g$, for 10 min at 4°C. Plasma was separated and kept at -80°C. A Quantikine Mouse/Rat IGF-1 Assay Kit (R&D Systems, cat. no. MG100) was used to detect IGF-1 levels according to the description of the supplier.

Immunohistochemistry for neuron generation detection

One-half of the brain tissue of the animals was embedded in paraffin followed by formalin fixation. The samples were

sectioned into 5-μm slices and, after deparaffination with xylene and ethanol, the sections were washed three times with PBS. Samples were washed in DNase buffer and 96% ethanol. Then, DNase I (Sigma Aldrich, cat. no. DN-25) was used to digest DNA. After the antigen retrieval with citrate buffer (pH 6.0), samples were heated to 95°C for 15 min and were left to cool slowly to room temperature. After washing with PBS three times for 5 min, sections were blocked in normal goat serum (Vector, S-1000) for 1 hr at room temperature. The primer mouse anti-BrdU antibody (BD Pharmingen, cat. no. 555627) was solubilized in blocking serum and absorbed onto the sections in a dilution of 1:200, overnight at 4°C, for scanning newly generated cells. After washing with 0.2% Triton X–PBS, Alexa Fluor 546 goat anti-mouse secondary antibody was used to visualize the labeling (30 min, room temperature, 1:200, Molecular Probes, cat. no. A11001). After washing, sections were incubated in anti-neuronal nuclei (NeuN) Alexa Fluor 488–conjugated monoclonal antibody at 4°C, overnight, to visualize the neurons on the slice. After washing in 0.2% Triton X–PBS, Hoechst 33342 (Molecular Probes, cat. no. H3570) was applied to the nuclei staining (10 min at room temperature). Before covering with Gel Mount (Sigma, cat. no. G0918), the sections were washed with distilled water.

To visualize the stained tissues on the sections, a Zeiss LSM 510 Meta confocal laser scanning microscope (Carl Zeiss, Jena, Germany) was used. The microscope was equipped with an inverted Axiovert 200M microscope, 63×Plan Apochromat oil immersion differential interference contrast (DIC) objectives (numerical aperture [NA] = 1.4). Hoechst staining was excited with a 405-30 diode laser (excitation 405 nm), and fluorescence was detected with a 420–480-nm bandpass (BP) filter. BrdU staining was excited with a helium–neon (HeNe) laser (excitation 543 nm), and the fluorescence was detected with a BP 560–615-nm filter. An argon laser (excitation 488 nm) was used for the NeuN visualization; the fluorescence was detected with A BP 505–570-nm filter.

Quantification

All morphological measurements were used with coded slides, and the experimenter was blind to the animal groupings. The number of BrdU- and NeuN-positive cells in the entire hippocampus was assessed by manual counting of those found in the granular cell layer of the dentate gyrus. Cells within the subgranular zone, defined as the area within two cell bodies of the inner edge of the granular cell layer, were combined for quantification. Three consecutive sections out of a total of 13 were used for counting BrdU/NeuN-positive cells, using a Zeiss microscope with a 63×objective lens, with the attached software.[55,56] The number of positive cells in the dentate gyrus was obtained by multiplying the value by 13/3. For the quantification of BrdU/NeuN-positive cells, unbiased stereology was used to determine the total area fraction of newly generated neurons in the region of interest. This method has the advantage of employing random selection techniques to properly estimate targeted components accepted in this tissue.[57]

Assessment of intrahelical 8-oxoG

At the optimal cutting temperature, fixed paraffin-embedded samples were sliced into 5-μsections. Sections on microscope slides were deparaffinized and washed with PBS and air dried; the lipids were removed with acetone–methanol (ratio 1:1). Sections were rehydrated in PBS for 15 min and subjected to limited protease digestion (100 mg/mL pepsin; Sigma Biochemicals) in 0.1 N HCl (for 15 min at 37°C; determined in preliminary experiments).[58] These preparations were incubated with nonimmune immunoglobulin G (IgG) (0.1μg/mL) for 30 min and washed in PBS containing 0.5% BSA and 0.1% Tween-20 (PBS-T). Following incubation with the anti-8-desoxoguanine (Travigen Inc.) antibody (1:300 dilution in PBS-T) for 1 hr, the sections were washed in PBS-T three times (15 min each). Affinity purified fluorescein-conjugated (F[ab′]$_2$) secondary antibody (Santa Cruz, Biotechnology) was then applied for 60 min. After washing in PBS-T (three times), the DNA was stained with 10 ng/mL 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI). Sections were mounted in antifade medium (Dako Inc., Carpinteria, CA). Confocal microscopy was performed on a Zeiss LSM510 META System using the 488-nm laser for excitation of fluorescein. Images were captured at a magnification of 60×(oil immersion objective; NA 1.4). Fluorescent intensities of a minimum of 40 fields (approximately 300 cells) per section were determined using MetaMorph software Version 9.0r (Universal Imaging Corp.).[14,59]

Assessment of OGG1 and acetylated OGG1 levels

Sections on microscope slides were deparaffinized, washed with PBS, and air dried; lipids were removed with acetone–methanol (ratio 1:1). Sections were blocked with preimmune heterologous serum diluted 1:10 in PBS-T for 30 min and incubated with primary antibodies: (1) Affinity-purified mouse anti-OGG1 antibody (human OGG1 reactive) generated against a synthetic peptide, C-DLRQSRHA-QEPPAK, representing the carboxyl terminus of OGG1, was

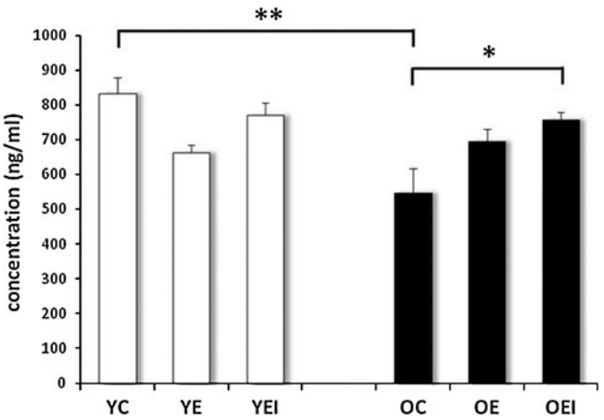
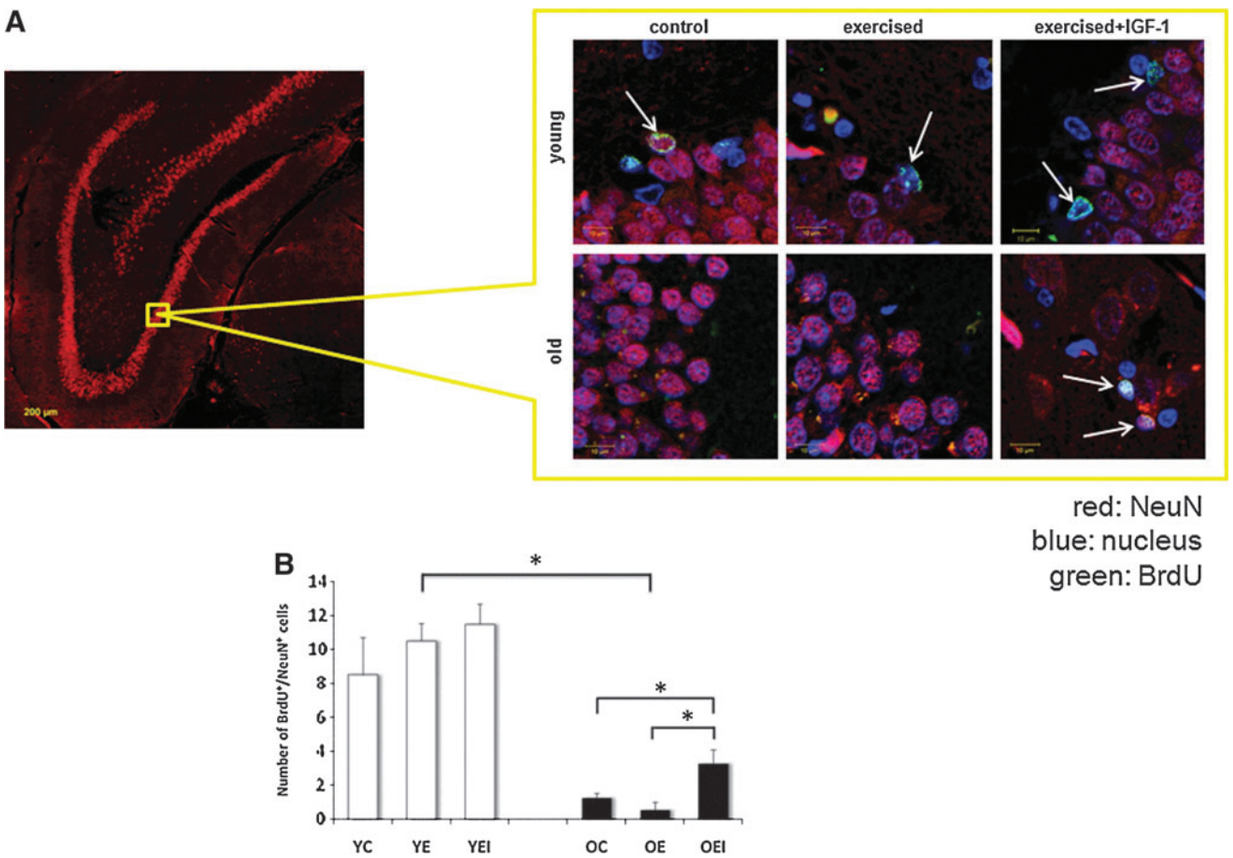


FIG. 1. The plasma insulin-like growth factor-1 (IGF-1) content was measured by use of an enzyme-linked immunosorbent assay (ELISA) kit. IGF-1 supplementation was completed by use of Alzet pumps in the last 2 weeks of the 6-week exercise program with a volume of 5μg/kg per day. IGF-1 levels decreased with aging, while the supplementation increased the levels in the aged exercise group. Groups: YC, young control; YE, young exercised; YEI, young exercised IGF-1 treated; OC, old control; OE, old exercised; OEI, old exercised IGF-1 treated. Values are means ± standard error (SE) for 6 animals per group. (*) $p < 0.05$; (**) $p < 0.01$.

acquired from Antibodies-Online GmbH (Atlanta, GA); (2) the immunogen affinity-purified human reactive rabbit polyclonal to Ogg1 acetyl K338 + K341 (cat no. ab93670) was obtained from AbCam. After 1 hr of incubation at 37°C, sections were washed three times for 15 min in PBS-T. Affinity-purified secondary antibodies (anti goat-F[ab′]$_2$-conjugated to fluorescein and anti-rabbit F[ab′]$_2$-rhodamine-conjugated; Santa Cruz Biotechnology, Santa Cruz, CA) were incubated for 1 hr with the sections and washed in PBS-T (three times). After washing, the DNA was stained with 10 ng/mL DAPI. Sections were mounted in antifade mounting solution (DAKO Inc., Carpinteria, CA). Confocal microscopy was performed on a Zeiss LSM510 META system (Carl Zeiss Microimaging, Inc., Thornwood, NY) using the 488-nm line of the argon laser for excitation of fluorescein and HeNe 543-nm line excitation of rhodamine, combined with appropriate dichroic mirrors and emission band filters to discriminate between green and red fluorescence. Images were captured at a magnification of 60×. Colocalization was visualized by superimposition of green and red images using MetaMorph software version 9.0r (Universal Imaging Corp., Sunnyvale, CA).[14,59]

Western blots

Ten to 50μg of protein were electrophoresed on 8–12% vol/vol polyacrylamide sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels. Proteins were electrotransferred on to polyvinylidene fluoride (PVDF) membranes. The membranes were subsequently blocked, and after blocking the PVDF membranes were incubated at room temperature with antibodies (1:500 Upstate SIRT1, cat no. 07-131, Millipore; 1:500 Cell Signaling Acetylated-Lysine, cat. no. 9441; 1:1,000 Ku70, cat no. K4763, Sigma; 1:2,000 SIRT3, cat. no. ab40006, Abcam; 1:1,000 SIRT4, cat. no. ab10140, Abcam; 1:500 PGC-1 [H-300], cat. no. sc-13067, Santa Cruz; 1:15,000 alpha-tubulin, cat. no. T6199, Sigma). After incubation with primary antibodies, membranes were washed in TBS-Tween-20 and incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies. After incubation with a secondary antibody, membranes were washed repeatedly. Membranes were incubated with chemiluminescent substrate (Thermo Scientific, SuperSignal West Pico Chemiluminescent Substrate, cat. no. 34080), and protein bands were visualized on X-ray films. The bands



4C ▶

FIG. 2. Neurogenesis was measured by the incorporation of bromodeoxyuridine (BrdU; detected by BD Pharmingen, cat. no. 555627 antibody) into DNA, and neurons were identified by an anti-neuronal nuclei antibody (NeuN; Millipore cat. no. AB337X) and merged samples were counted. Aging decreased, and exercise plus IGF-1 treatment increased new neuron formation. (**A**) Samples from histochemical measurements; (**B**) quantified data. Values are means ± standard error (SE) for 6 animals per group. (*) $p < 0.05$. Groups: YC, young control; YE, young exercised; YEI, young exercised IGF-1 treated; OC, old control; OE, old exercised; OEI, old exercised IGF-1 treated.

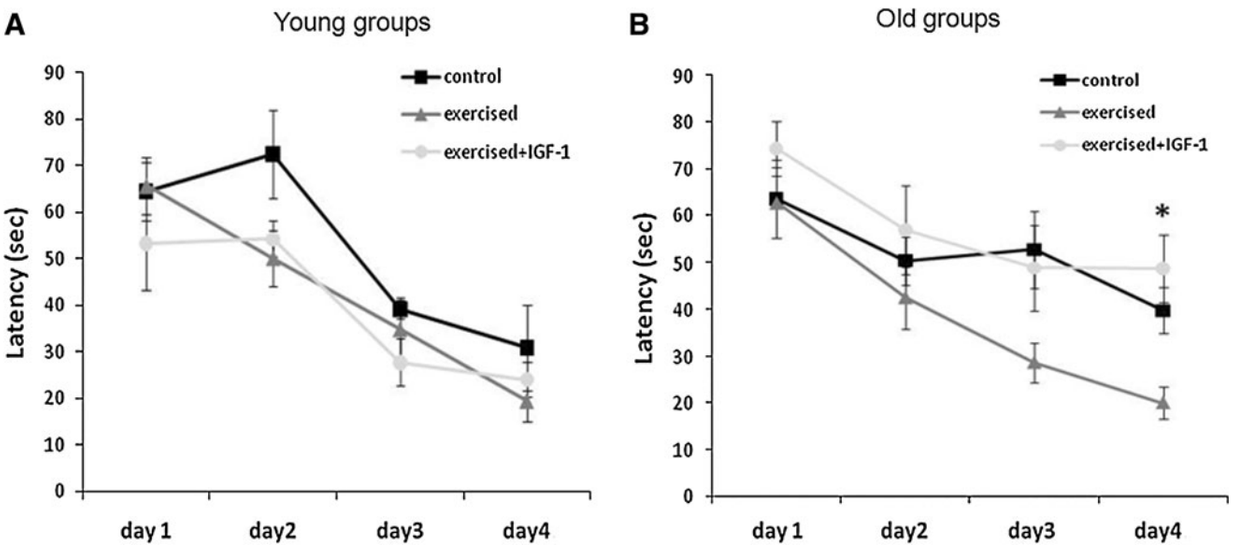


FIG. 3. Spatial memory was measured by the Morris water maze test with a 4-day trial. Results for young (**A**) and for old (**B**) animals. Insulin-like growth factor-1 (IGF-1) treatment eliminated the beneficial effects of exercise training on spatial memory of old rats. Values are means ± standard error (SE) for 6 animals per group. (*) $p < 0.05$.

were quantified by ImageJ software and normalized to tubulin, which served as an internal control.

Statistical analyses

Data are presented as means ± standard error (SE). Statistical significance was assessed by one-way analysis of variance (ANOVA), followed by the Tukey *post hoc* test. Nonparametric Kruskal–Wallis ANOVA was applied to evaluate the differences in the levels of 8-oxoG, OGG1 content, and the acetylation of OGG1. The significance level was set at $p < 0.05$.

Results

Results of aging

F1 ▶ The levels of circulating IGF-1 decreased with aging (Fig. 1). The presence of neurogenesis was checked by the incorporation of BrdU into the newly formed DNA of neurons. Data revealed that aging significantly reduced the level of F2 ▶ neurogenesis ($p < 0.05$) (Fig. 2). The latency time to find the platform on day 4 was significantly longer for OC than YC F3 ▶ rats ($p < 0.05$) (Fig. 3).

In agreement with the accepted oxidative stress theory of aging, the concentration of 8-oxoG increased significantly in F4 ▶ the aged groups (Fig. 4). Aging tended to increase the levels of OGG1 (OC vs. YC, $p = 0.08$) and decreased the level of acet- F5 ▶ ylation (Fig. 5). The total and acetylated level of apurinic en- AU1 ▶ donuclease-1 (APE-1) did not change in any groups by aging, training, or IGF-1 treatment (data are not shown). On the other hand, the levels of the DNA double strand break–repairing protein Ku70, increased with aging in the hippocampus of old F6 ▶ rats (Fig. 6). Because OGG1 and Ku70 activity can be modified by acetylation, the levels of NAD–dependent deacetylasers, such as SIRT1 and SIRT3, were checked. SIRT1 content decreased with aging and was associated with an increased level F7 ▶ of acetylation of the lysine residues of cytosolic proteins (Fig. F8 ▶ 7), whereas SIRT3 content increased with aging (Fig. 8).

Finally, one of the main targets of the SIRT1 protein, which plays an important role in mitochondrial biogenesis, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), showed no significant alteration in the hippocampus (Fig. 9). ◀ F9

Results of aerobic exercise training

Exercise did not cause significant changes in the circulating IGF-1 levels. However, these levels did tend to decrease in the young groups ($p < 0.076$) (Fig. 1). Large differences in the levels of neurogenesis between young and old rats were

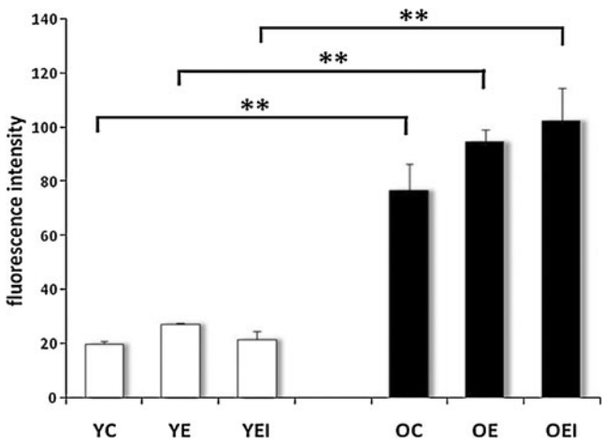
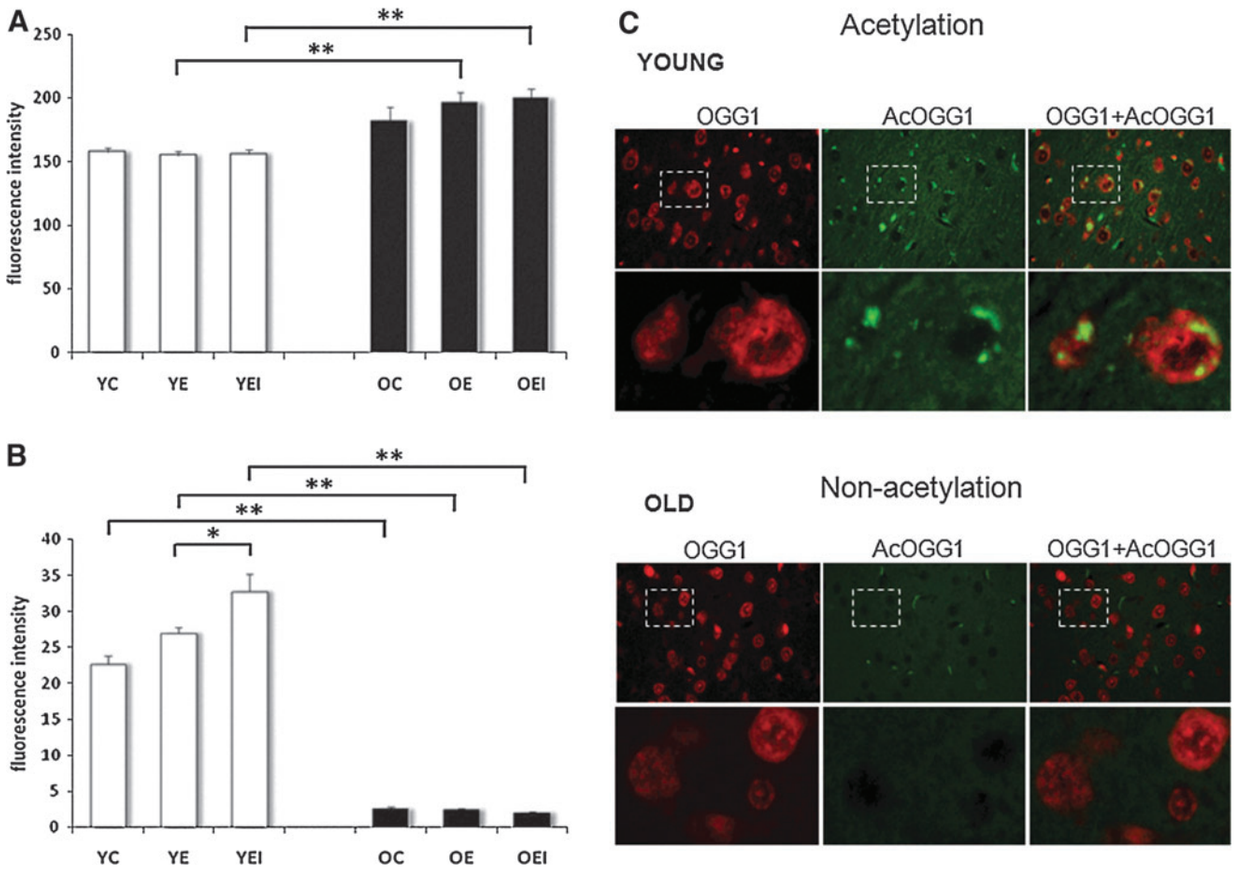


FIG. 4. The 8-oxoguanine (8-oxoG) levels were measured from the hippocampus using anti-8-desoxoguanine antibody (Trevigen Inc.) with histochemistry. Quantified data revealed age-associated significant increases in 8-oxoG levels. Values are means ± standard error (SE) for 6 animals per group. (**) $p < 0.01$. Groups: YC, young control; YE, young exercised; YEI, young exercised IGF-1 treated; OC, old control; OE, old exercised; OEI, old exercised IGF-1 treated.



4C ▶

FIG. 5. Protein concentration of 8-oxoguanine DNA glycosylase (OGG1) (**A**) and acetylated (ac) OGG1 (**B**), and histochemical samples of fluorescence detection (**C**) from rat hippocampus. Data were obtained from either incubation with purified mouse anti-OGG1 antibody (human OGG1 reactive) generated against a synthetic peptide, C-DLRQSRHAQEPPAK, representing the carboxylterminus of OGG1 (Antibodies-Online GmbH, Atlanta, GA) or immunogen affinity-purified human reactive rabbit polyclonal to Ogg1 acetyl K338 + K341, obtained from AbCam) primary antibodies. The quantification of the data is described in the Methods section. Values are means ± standard error (SE) for 6 animals per group. (*) $p < 0.05$; (**) $p < 0.01$. Groups: YC, young control; YE, young exercised; YEI, young exercised IGF-1 treated; OC, old control; OE, old exercised; OEI, old exercised IGF-1 treated.

noted; young animals had greater levels of new neuron formation (Fig. 2). The difference in BrdU/NeuN–positive cells between the YC and YE groups was $p = 0.057$, suggesting that the exercise almost significantly increased the neurogenesis in young groups. Treadmill running improved the spatial memory of old rats, whereas significant improvement was not observed in the young groups (Fig. 3). The 8-oxoG, OGG1, acetylated (ac) OGG1, and Ku70 levels were not significantly induced by exercise (Figs. 4–6). The SIRT1, SIRT3, and acetylated lysine levels were not altered with exercise or IGF-1 treatment in the hippocampus (Figs. 7 and 8). Similarly, the content of PGC-1α remained unmodified by exercise and IGF-1 intervention (Fig. 9).

Results of IGF-1 supplementation

The 2-week supplementation of IGF-1 increased the levels of circulating IGF-1 in the aged exercise group (Fig. 1). On the other hand, IGF-1 treatment with exercise increased the levels of neurogenesis for the old group (Fig. 2**).** Surprisingly, the beneficial effects of exercise on spatial memory were eliminated by IGF-1 supplementation, and the control and exercise trained IGF-1–treated performances were identical for the Morris water maze test (Fig. 3). IGF-1 tended to increase 8-oxoG levels in the aged exercised group ($p = 0.114993$) and decreased them in the young animals ($p = 0.988458$), but the differences did not reach statistically significant levels (Fig. 4). The total OGG1 levels were not altered by IGF-1 supplementation. However, for the young animals, the supplementation increased the levels of OGG1 acetylation (Fig. 5).

The protein levels of Ku70 tended to decrease with exercise training in the old groups, and the IGF-1 treatment further increased the differences between the old controls and OEI rats, reaching statistical significance (Fig. 6). The IGF-treatment did not significantly influence the levels of SIRT1, SIRT3, lysine acetylation, or PGC-1α (Figs. 7–9).

Discussion

The landmark paper of van Praag and co-workers [60] showed that exercise not only improves spatial memory, but it also results in neurogenesis. These findings have been

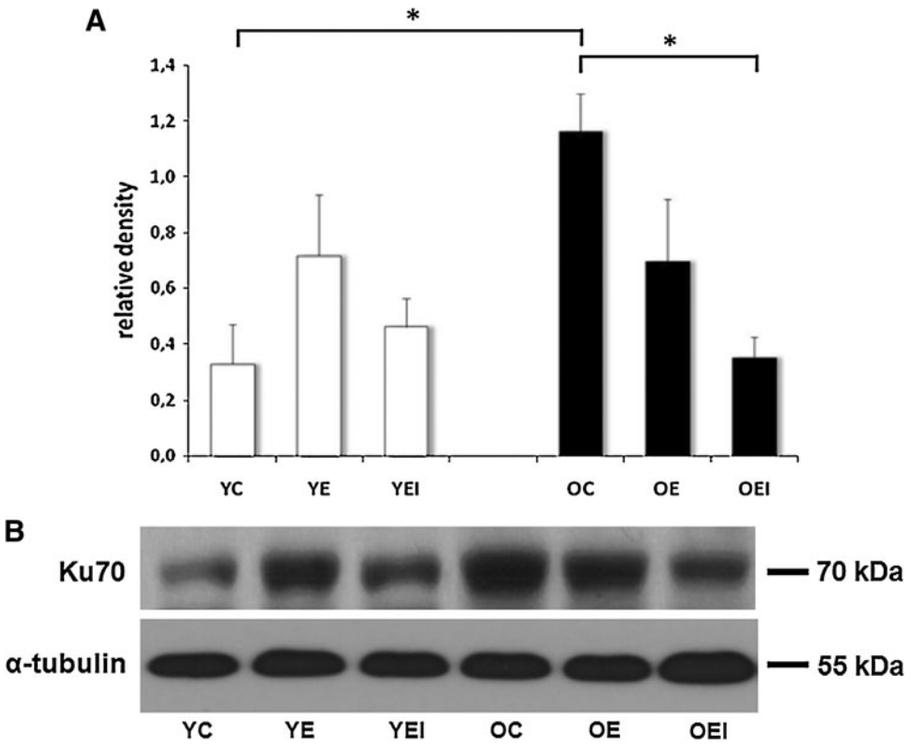


FIG. 6. Ku70 is an important protein for DNA repair; Ku70 content increased with aging while exercise attenuated this increase. (**A**) Western blot results; (**B**) Densitometric results. Values are means ± standard error (SE) for 6 animals per group. (*) $p < 0.05$. Groups: YC, young control; YE, young exercised; YEI, young exercised IGF-1 treated; OC, old control; OE, old exercised; OEI, old exercised IGF-1 treated.

confirmed by others.[61] Moreover, van Praag et al.[62] also showed that the newly formed neurons were functional. Hence, a link was established between newly formed neurons and the functional benefits of exercise (see the recent review of Lazarov et al.[63]). However, a recent report has challenged this finding, because the data from this study showed that exercise was able to improve results on the Morris water maze test, even with inhibition of neurogenesis.[64]

Most studies on neurogenesis have used voluntary running,[65,66] but studies using enforced running[37,67] have shown similar results. However, the data from these studies further suggest that voluntary and treadmill running have different effects on brain plasticity in different regions of the brain.[68] Furthermore, the nature of exercise-induced neurogenesis has been shown to be different in mice and rats.[69] In the present study, treadmill running failed to increase the number of BrdU/NeuN-positive cells in young and old exercise groups, a finding that differs from most earlier observations (see review by Fabel and Kempermann[41]). Few data exist on the effects of treadmill running on neurogenesis in healthy rats, and only one study was found that reported unchanged neurogenesis after high-intensity enforced exercise,[70] as observed in the present study. This paucity of available data makes comparisons of treadmill-trained rats and aging difficult. Thus it could be suggested that exercise can promote brain function via complex mechanisms, including enhanced vascularization and metabolism, up-regulation of neurotrophins and housekeeping enzymes that result in increased neuroprotection, and better synaptic plasticity.[42–46,55,71]

Supplementation of IGF-1 increased the levels of new neuron formation in aged groups, but unexpectedly eliminated the beneficial effects of exercise on spatial learning. A recent finding suggests that the administration of anti-IGF-1 antibody to block the function of IGF-1 is not influenced by the time it takes mice to find a hidden platform in the Morris water maze test.[72] IGF-1 affects exercise-mediated neurogenesis, but brain plasticity could be an IGF-1-dependent and/or- independent process.[72] Indeed, it has been suggested that the beneficial effects of exercise on brain function are partly dependent upon IGF-1.[53] IGF-1 and insulin act through the insulin/IR signaling pathway, the activation of ◀AU2 which supports neuronal survival and brain plasticity.[73] The neuroprotective effects of the IR pathway are well documented,[34,74] but it has also been shown that insulin injection could impair brain function.[75,76] Also, a recent paper has reportd findings similar to ours, namely, that insulin injection eliminates the beneficial effects of exercise as shown on the Morris water maze test, and it was suggested that this could be a result of the IR signaling on *N*-methyl-D-aspartate receptors.[38] Therefore, the available data suggest that activation of IGF-1/insulin signaling could be both beneficial and harmful, thus, stress the importance of the very delicate IR signaling in the brain. This finding could also suggest that, whereas certain IGF-1/insulin signaling has been shown to

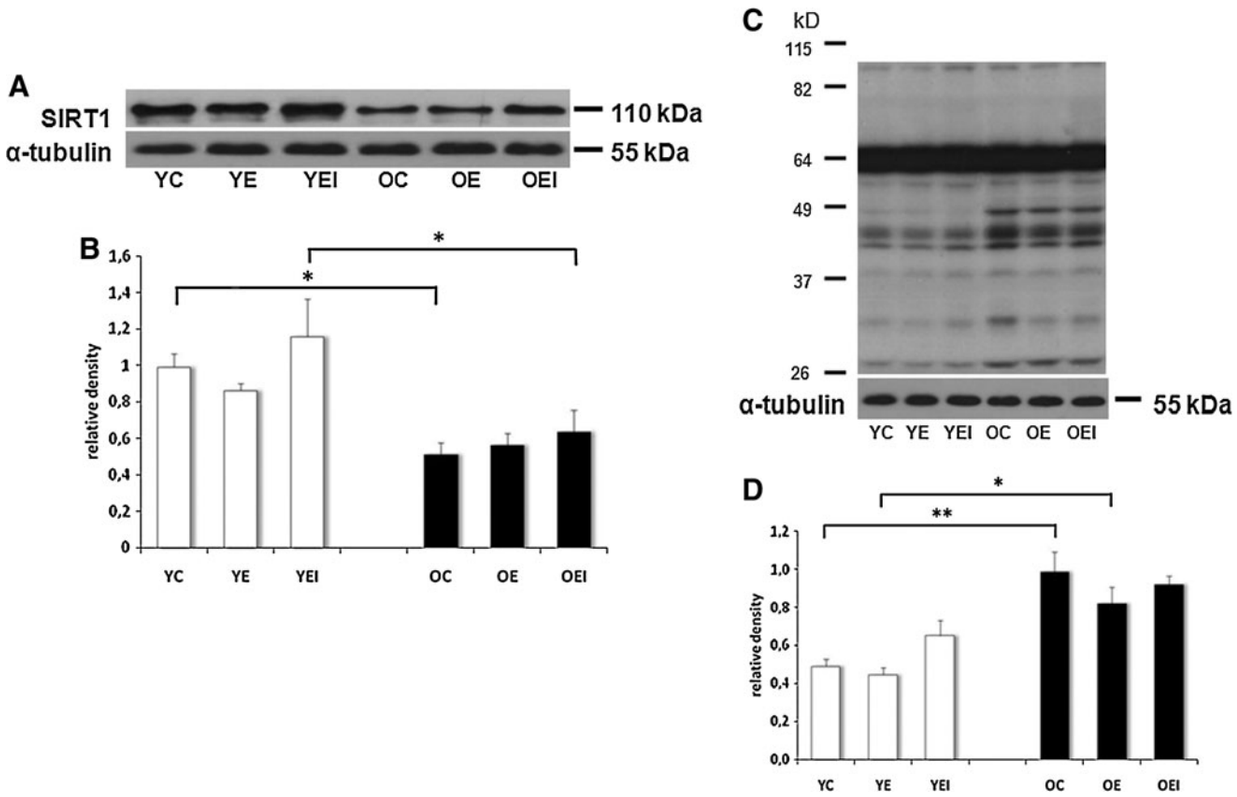


FIG. 7. Sirtuin-1 (SIRT1) content was measured by western blotting (**A**) and quantified by densitometry (**B**); it showed age-related decreases. The decreased levels of SIRT1 were associated with increased levels of lysine acetylation as measured by western blotting (**C**), which was confirmed by quantification (**D**). Values are means ± standard error (SE) for 6 animals per group. (*) $p < 0.05$; (**) $p < 0.01$. Groups: YC, young control; YE, young exercised; YEI, young exercised IGF-1 treated; OC, old control; OE, old exercised; OEI, old exercised IGF-1 treated.

benefit brain function, insulin resistance is closely related to the etiology of neurodegenerative diseases. However, it has to be mentioned that we just monitored spatial memory by the Morris water maze test, and it could not be excluded that IGF-1–induced neurogenesis could result in better results for other brain functions.

Some of the noticeable effects of exercise on the brain are the modulation of ROS generation, redox signaling, and oxidative damage, all of which can readily interfere with function.[77–79] Oxidative modification of DNA could lead to increased apoptosis. Impaired function and accumulation of DNA damage in neurons have been suggested to be major factors related to brain aging and neurodegenerative diseases.[80,81] In the present study, it was observed that aging increases the levels of 8-oxoG in hippocampus of rats, which potentially could jeopardize brain function.[82,83] Indeed, the repair of 8-oxoG is a high priority of cells for survival. The total protein content of OGG1 was increased in aged rats, which could be a cellular attempt to combat the enhanced levels of 8-oxoG, but, in this case, without significant success. Similar phenomena, increased levels of 8-oxoG and OGG1 protein, were reported in the aging lens of rats after exposure to hyperoxia,[84] indicating that an increased level of OGG1 is not always sufficient to decrease the level of 8-oxoG. This was also confirmed in our recent finding from aged human skeletal muscle (Radak et al., in press).

Acetylation of OGG1 is a posttranslational activation of the incision activity of this enzyme.[14,29] Thus, it cannot be excluded that the age-associated increase in 8-oxoG levels could be due to the large decrease in acetylation of OGG1. On the other hand, exercise with IGF-1 supplementation increased the levels of OGG1 acetylation. We show here, for the first time, that acetylation of OGG1 takes place *in vivo* and exercise increases the rate of acetylation. This finding could suggest that pharmacological manipulations, which induce OGG1 acetylation, might be beneficial in the aging process and could affect specific diseases where 8-oxoG-mediated apoptosis and mutations are markedly enhanced. However, it has to be noted that other processes besides acetylation might also effect the accumulation of 8-oxoG with aging.

Ku70 is an important DNA repair protein which, upon acetylation, interacts with Bax and mediates apoptosis.[85] Both SIRT1 and SIRT3 have been shown to deacetylate Ku70[21,26] and increase cell survival. However, the present study did not allow us to test the interaction between sirtuins and Ku70.

Sirtuins have been suggested to play a causative role in the aging process,[86,87] and SIRT1 knockout mice suffer from impaired brain function.[24,39] The data from the present study demonstrate that aging results in decreased levels of SIRT1 and increased levels of SIRT3, suggesting different control-

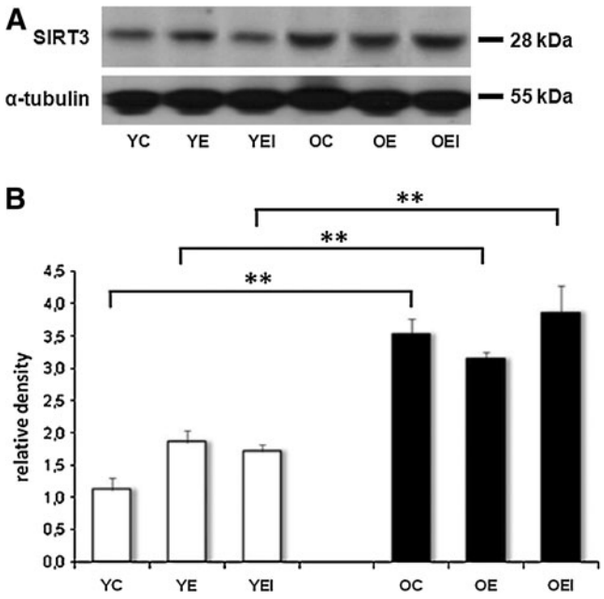


FIG. 8. Sirtuin-3 (SIRT3) was involved in deacetylation of Ku70 and showed an age-associated increase. Western blot (**A**) densitometric data (**B**) are shown. Values are means ± standard error (SE) for 6 animals per group. (**) $p < 0.01$. Groups: YC, young control; YE, young exercised; YEI, young exercised IGF-1 treated; OC, old control; OE, old exercised; OEI, old exercised IGF-1 treated.

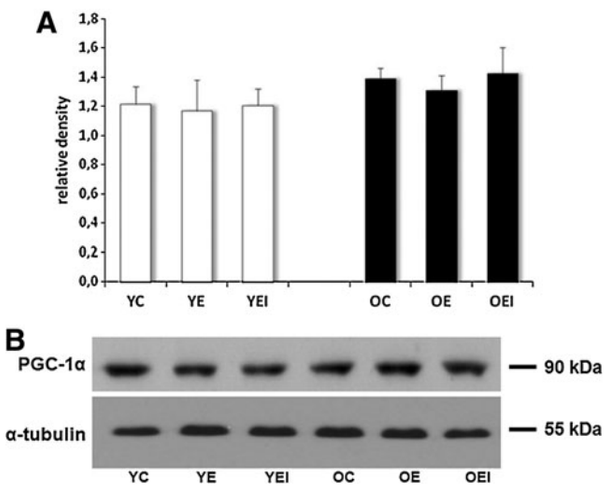


FIG. 9. To appraise the level of mitochondrial biogenesis, the protein concentration of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α was measured by western blotting (**A**). Densitometric analyses (**B**) revealed that biogenesis was not altered by aging, exercise or insulin-like growth factor-1 (IGF-1) treatment. Values are means ± standard error (SE) for 6 animals per group. Groups: YC, young control; YE, young exercised; YEI, young exercised IGF-1 treated; OC, old control; OE, old exercised; OEI, old exercised IGF-1 treated.

ling mechanisms for these sirtuins. The significant decrease of SIRT1 content was associated with a large change in lysine acetylation in the hippocampus, which could affect protein stability.[88] Acetylation of lysine residues could prevent the ubiquitination of the same residue, which would be important for the substrate recognition of proteasome-to-protein degradation. Therefore, overacetylation could impact protein stability and the half-life of proteins.[89–91] The large age-induced protein acetylation of the hippocampus might be one of the causative factors of impaired brain function, as demonstrated by the observation that knocking out SIRT1 causes significant loss in brain function.[24,39] Besides sirtuins, there are three classes of histone deacetylases, the functions of which could also account for the increased level of acetylation, as could a number of histone acetyltransferase proteins. The fact that SIRT ablation impairs, while histone deacetylases inhibitors generally improve, brain function points out the very delicate role of acetylation/deacetylation on brain function.[92,93]

With the decrease in SIRT1 content, similar tendencies were expected for PGC-1α levels, because SIRT1 is a well-known stimulator of PGC-1α.[94] However, the present data suggest that PGC-1α is not the master regulator of metabolic processes and mitochondrial biogenesis in the brain, but is important for the expression of antioxidant enzymes.[95] Therefore, it can be speculated that, despite the decreased SIRT1 content, neurons successfully attempted to maintain the levels of PGC-1α to cope with age-induced oxidative stress.

In summary, we have observed that exercise-induced neurogenesis is independent of the spatial learning–enhancing capacity of exercise. We have shown that the combined effects of IGF-1 supplementation and exercise could result in new neuron generation in the hippocampus of aged rats. The data indicate that the age-associated increase in 8-oxoG levels in the hippocampus is due to the decreased acetylation levels of OGG1, which can be induced by exercise. We have shown that aging increases the acetylation levels of proteins in the hippocampus, and this is probably due to the decreased levels of SIRT1 and supports the observation that SIRT1 is important in brain function.

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Address correspondence to:
Zsolt Radak, Ph.D
Institute of Sport Science
Faculty of Physical Education and Sport Science
Semmelweis University
Alkotas u. 44, TF
Budapest, Hungary

E-mail: radak@mail.hupe.hu

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AU1: Is this the correct identification of abbreviation?
AU2: IR = insulin resistance?; if yes, please identify in text