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CAN AEROBIC TRAINING RESTORE THE LEVEL OF BDNF IN THE HIPPOCAMPUS OF RATS EXPOSED TO LEAD ACETATE?

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Abstract

The neuroprotective effects of prolonged exercise training on levels of lead acetate-induced brain-derived neurotrophic factor (BDNF) were investigated. Forty rats were randomly divided into 4 groups: (1) basic, (2) sham, (3) lead acetate + training and (4) lead acetate groups. The rats of groups 3 and 4 experienced treadmill running of 15 to 22 m/min for 25 to 64 minutes, 5 times a week for 8 weeks. Groups 1, 3 and 4 received lead acetate (20 mg/kg) and the sham group received solvent (ethyl oleat). The results showed that BDNF in lead acetate group decreased significantly, whereas in lead acetate + training group it increased. Malone dialdehyde (MDA) significantly increased in lead acetate group, while in lead acetate + training group it decreased. Finally, the levels of total antioxidant capacity (TAC) in lead acetate group significantly decreased and increased in lead acetate + training group. Data obtained suggest a lifestyle-induced protective potential in rehabilitation of lead-induced neurodegeneration.

Keywords: aerobic training, brain-derived neurotrophic factor (BDNF), hippocampus, lead acetate.

INTRODUCTION

Lead derived from industrial and town contamination, from agricultural runoff, as well as from natural geochemical processes enters the air, soil, and water, as well as the food chain. The neurotoxicity of lead has been extensively studied [*Cory-Slechta D., Schaumburg H., 2000*]. Moreover, lead levels are increasing in some cohorts of children (ATSDR, 1988, 1999) and in some species of birds [*Burger J. et al., 1994*], but not in others [*Burger J., Gochfeld M., 2003*]. There is increasing evidence that lead affects a number of cognitive and motor functions in a wide range of vertebrates, including humans. Animal models provide information on the effects of low- and medium level lead effects on neural and cognitive processes [*Lippman M., 1990; Alber S., Strupp J., 1996; Burger J., Gochfeld M., 2000*], and for the most part, the animal models and epidemiological data provide similar results [*Rice G., 1996; Burger J., Gochfeld M., 2004*]. The effects are pervasive and often subtle, with consequences ranging from cognitive impairment in children to peripheral neuropathy in adults. While occupational exposure among workers at smelters or battery recycling plants remains an occasional problem, the greatest current public health problem is exposure of young children to decaying fragments of lead paints. At higher blood levels, Pb^{++} disrupts the function of endothelial cells in the blood brain barrier. This may lead to hemorrhagic encephalopathy, characterized by seizures and coma. This disrupts long-term potentiating, which compromises the permanent retention of newly learned information [*Noureddine D. et al., 2004*]. It has been suggested that physical exercise modulates cognitive functions through various signaling mechanisms that lead to brain-derived neurotrophic factor (BDNF) up-regulation, especially in the hippocampus, a major hub for learning and memory formation [*Cotman C., Berchtold N., 2002; Gómez-Pinilla F. et al., 2002; Vaynman S. et al., 2004; Uysal N. et al., 2005*]. BDNF is needed for healthy brain functioning. Studies on BDNF have shown differences in brain region size, memory functioning and anxiety-related behavior when BDNF expression is altered [*Chen Z. et al., 2006*]. It is important for cell survival in neurogenesis studies and has been thought to play an important role in antidepres-

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sant action [*Sairanen M. et al., 2005*]. This has promoted research on BDNF levels in health and disease in the hope of better understanding the etiology and treatment effects [*Tang S. et al., 2008*]. The effect of short-term exercise (15 *min* step-exercise) on serum BDNF levels was evaluated in healthy human subjects [*Tang S. et al., 2008*]. The results showed a short-term, significant increase in serum BDNF levels after exercise. Intra-individual differences in serum BDNF levels were remarkably small on the rest day and when compared to other values on the day of the exercise test. Individual differences, on the other hand, were larger in comparison.

Studies found that the exercise-induced increase in BDNF is transient. The prolonged reduction in serum BDNF in affective disorder patients is most likely associated with other factors, in addition to whatever transient effect one sees after physical exertion. It should be noted that in recent study reduced serum BDNF levels were observed in both manic and depressed bipolar disorder patients [*Cunha A. et al., 2006*].

Considering that depressed patients have been reported to be more inactive physically than non-depressed controls, while manic patients have the reverse activity profile [*Wolff E. et al., 1985; Kerschensteiner M. et al., 1999*]. However, while most studies that correlated physical activity with BDNF expression used the "voluntary wheel running" model, a major critical issue is that the exercise parameters, i.e. intensity, duration, and frequency, are highly variable and dependent on the motor activity of the animal. To resolve this, in this study, we used a special treadmill running protocol in order to examine hippocampus activation and BDNF expression and present evidence that treadmill running at highly controlled mild intensities differentially affects the time-course of BDNF induction in the rat hippocampus.

MATERIAL AND METHODS

Animals: Forty male Wistar rats (age: 50 days; weight: 256-290 *g*) were maintained under standard laboratory conditions (12-*h* light/12-*h* dark) at room temperature of 22±2°C and food and water *ad lib*. Animals were acclimatized for a week before treadmill exercise began.

Classification of animals and exercise training: We replicated a previously-reported lead-dosing regimen that caused oxidative stress so that the dose of lead acetate was 20 *mg/kg* [*Daniel S. et al., 2004; Asali M. et al., 2011; Dabidi R. et al., 2011*]. The experimental protocol was approved by L. Orbeli Institute of Physiology, National Academy of Sciences of Armenia. All the procedures utilizing rats were performed according to the "principles of laboratory animal care" (NIH publication № 85-23 revised 1985), as well as the specific rules provided by the animal care and use committee of national medical and health service.

Rats were randomly assigned in tens to the following groups:

1) basic group (Base or B),
2) control group (Sham or S),
3) group with lead injection (Lead or L) and
4) treadmill exercise with lead injection group (Training + Lead or TL).

All rats were weighed on a daily basis during the exercise training phase. Animals were acclimatized to ambient rearing conditions for 4-5 days in group housing conditions (four rats per cage) and then habituated to run on a treadmill (KN-73, Natsume Ltd., Japan) for a total of seven sessions over 8 weeks. The running speed and distance were gradually increased from 15 to 22 *m/min* and from 25 to 64 *m/min*. Belt speed was 10 *m/min*, a walking rate for adult rats and a speed that improves Morris maze performance in the adolescent rat. At the end of the belt there were electrified stationary wire loops. A mild shock (0.75 *mA*, 500 *ms* duration, 0.5 *Hz* rate) was delivered through these loops to motivate the rats to continuously walk on the moving belt and thus avoid foot shock. The wire loops were activated during all exercise sessions, and an experimenter monitored all treadmill sessions. Rats quickly learned to stay on the belt and avoid shock, except for one rat that would not stay on the moving belt, and thus was quickly removed from the exercise group. Rats of groups L and TL were run 5 days a week, with primary time of 25 up to 64 *min* at the end of the 8th week (Table 1).

Injections: Group S received physiological solution, while L and LT groups got 2% lead acetate (20 *mg/kg, i/p*) 3 days in a week during all 8 training weeks. Until the sacrifice, all the rats were kept in their own cages. Rats were sacrificed by decapitation approximately 16 *h* after last exercises. After an intraperitoneal injection of 1% ketamine (30 *mg/kg*) and zalayzine hydrochloride (4 *mg/kg*) rats were rapidly decapitated and the brains were quickly removed. The brain region of hippocampus was quickly dissected out. Transverse sections of hippocampus were prepared using a McIlwain tissue chop-

Table 1.

The scheme of 8-week training

Training days	Speed & time of trainings \ Number of week	1	2	3	4	5	6	7	8
1	Treadmill speed (*m/min*)	15	16	17	18	19	20	21	22
	Time (*min*)	25	30	35	40	45	50	55	60
2	Treadmill speed (*m/min*)	15	16	17	18	19	20	21	22
	Time (*min*)	26	31	36	41	46	51	56	61
3	Treadmill speed (*m/min*)	15	16	17	18	19	20	21	22
	Time (*min*)	27	32	37	42	47	52	57	62
4	Treadmill speed (*m/min*)	15	16	17	18	19	20	21	22
	Time (*min*)	28	32	38	43	48	53	58	63
5	Treadmill speed (*m/min*)	15	16	17	18	19	20	21	22
	Time (*min*)	29	34	39	44	49	54	59	64

per. Then they were frozen with dry ice and cryopreserved at -80ºC for inset hybridization and BDNF ELISA assay. Blood samples were mixed with 100 *mg/mL* of EDTA to suppress coagulation and cooled with ice for analysis.

Analysis of BDNF concentration: BDNF protein was assessed using the ELISA kit (Demeditec Diagnostics GmbH, Kiel, Germany) according to manufacturer's recommendations. The hippocampus was individually homogenized in lysis buffer containing (in *mM*): 137 NaCl, 20 Tris–HCl (pH 8.0), Igepal (1%), glycerol (10%), 1 PMSF, 0.5 sodium vanadate, 0.1 EDTA and 0.1 EGTA. Then it was centrifuged 10 *min* at 14000 *rpm* under 4°C. Supernatant was diluted in sample buffer and incubated on 96-well flat-bottom plates previously coated with anti-BDNF monoclonal antibody. Standards, controls and samples were added, and then plates were incubated with biotinated secondary monoclonal antibody for 2 *h* and streptavidin horseradish peroxides for 1 *h*. Then color reaction with substrate reagent (tetraethyl benzedrine) was quantified in a plate reader at 450 *nm*. The mean absorbance value for each set of duplicate standards, controls and samples were calculated and the average zero standard optical density subtracted. Standard curve was plotted using Elisa Reader Plot Software. The standard BDNF curve ranged from 0 to16 *ng/mL*.

Measurement of Total Antioxidant Capacity (TAC): The Total Antioxidant Capacity (TAC) was determined using the FRAP method [*Benzie I., Strain J., 1996*]. This method was modified from I.F.F. Benzie and J.J. Strain [*Benzie I., Strain J., 1999*]. The working FRAP reagent was produced by mixing 300 *mM* acetate buffer (pH 3.6), 10 *mM* 2,4,6-tripyridyl-*s*-triazine (TPTZ) solution and 20 *mM* $FeCl_3 \cdot 6H_2O$ in a 10:1:1 ratio just before use and heated to 37°C. The 300 *mM* acetate buffer was prepared by mixing 3.1 *g* of sodium acetate trihydrate ($C_2H_3NaO_2 \cdot 3H_2O$) with 16 *mL* glacial acetic acid and brought to 1 *L* with distilled water. The TPTZ solution was prepared by making a solution of 10 *mM* TPTZ in 40 *mM* HCl.

A total of 1.5 *mL* of working FRAP reagent was added to each tube. After incubation tubes were kept in oven at 37°C for 5 *min*. A total of 25 *μL* of sample and 25 *μL* of deionized water added to each well. Each sample was run in triplicate. After addition of sample to the FRAP reagent, readings of the colored product (ferrous tripyridyltriazine complex) were then taken at 593 *nm*. The change in absorbance after 8 *min* from the initial blank reading was then compared to that of a standard that was run simultaneously. Standards of known Fe(II) concentrations ($FeSO_4 \cdot 7H_2O$) were run in triplicate using several concentrations between 50 and 1000 *μM* (25, 50, 75, 100, 150, 200, 500 and 1000 *μM*). A standard curve was then prepared by plotting the average FRAP value for each standard *versus* its concen-

Table 2.

Hippocampus BDNF values in different groups at the 8th week

Groups	Number of rats	Mean	Standard Deviation	Standard Error	Minimum	Maximum
Base (B)	8	1.8375	0.14772	0.05223	1.65	2.11
Sham (S)	8	1.7988	0.65221	0.23059	0.36	2.32
Lead + Training (L+T)	7	2.7057	1.66047	0.62760	1.43	5.14
Lead (L)	7	1.5400	0.21602	0.08165	1.24	1.87

tration. The FRAP values for the samples were then determined using this standard curve.

Measurement of lipid peroxidation: The level of thiobarbituric acid reactive substance (TBRAS) was measured as an index of lipid peroxidation [*Wills E., 1969*] in the specimens using the method of T. Asakawa and S. Matsushita [*Asakawa T., Matsushita S., 1985*]. Malone dialdehyde (MDA) is the by-product of lipid peroxidation that forms adduct with thiobarbituric acid (TBA). On boiling, it produces pink colored complex, which absorbs maximally at 532 *nm*. Two *mL* of TBA-TCA-HCl reagent were added to 1 *mL* of a sample. The mixture was boiled in a boiling water-bath for 15 *min*. After cooling and centrifugation at 1000 × *g* for 10 *min*, the absorbance of the supernatant was determined at 532 *nm* using UV/VIS spectrophotometer (Jenway 6505, UK). A blank control consisting of 1 *mL* of saline and 2 *mL* of TBA-TCA-HCL reagent was always carried out and any absorbance due to reagents was subtracted from the corresponding experimental sample. The standard curve was prepared using serial concentrations of 1,1,3,3-tetraethoxypropane (Sigma, St. Louis, MO, USA). The malone dialdehyde – thiobarbituric acid (MDA-TBA) adduct was shown at 532 *nm* and quantified by reference to a standard curve of 1,1,3,3-tetraethoxypropane submitted to the TBA colorimetric procedure [*Mahjoub S. et al., 2007*].

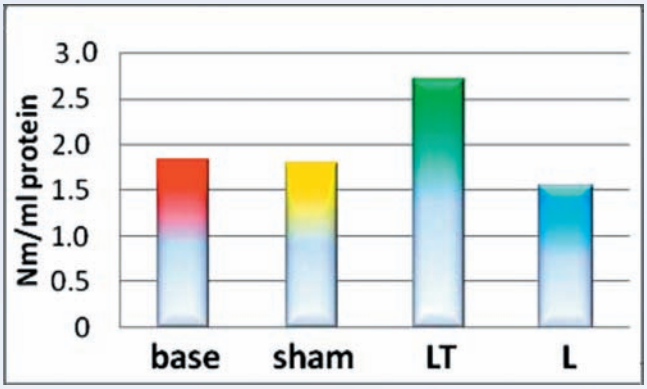

Figure. *BDNF levels in rat hippocampus.*

Data analysis: Statistical analyses were conducted by two-tailed Student's *t*-test or one-way ANOVA followed by Tukey's test, when indicated. All data are presented as mean (±SEM).

RESULTS

Experiments were performed in 40 Wistar male rats. The rats of the L and TL groups were run 5 days a week, with primary time of treadmill speed 15 *m/min* for 25 *min* during a day and at the end of the first week the same speed but with 29 *min* duration. Finally at the end of trainings (8th week) treadmill speed was 22 *m/min* with training duration of 64 *min*.

Results of the experiments showed that at the end of tests (8th week) the value of BDNF in Lead (L) group in comparison with those of Base (B) group (1.837±0.148 SD; n= 8) reduced to (1.540 ± 0.216 SD; n=7). After trainings (see the scheme of training presented in Table 2 and Figure) in Lead + Training (LT) group at the 8th week BDNF value increased up to 2.7 (2.706± 1.660 SD; n=7) (Table 2; Figure).

As obvious from Table 2 and the Figure below, the value of BDNF in group L (1.54 *Nm/mg* protein) is the lowest and in group LT (2.70 *Nm/mg* protein) is the highest, which might be due to the effect of increasing prolonged exercises. The same we can see for the value of BDNF in Sham (S) group (1.79 *Nm/mg* protein) and Base (B) group (1.83 *Nm/mL* protein). In Table 2 the standard deviations and significations are shown for all groups.

Can the high-intensity endurance training alleviate exercise-induced oxidative stress? Results indicate that high-intensity endurance training can elevate antioxidant enzyme activities in erythrocytes, and decrease neutrophil O_2 production in response to exhausting exercise. Furthermore, this up-regulation in antioxidant defenses was accompanied by a reduction in exercise-induced lipid peroxidation in

erythrocyte membrane [Miyazaki *H. et al., 2001*]. Changes in antioxidant systems due to free radicals were investigated in short distance (100 *m*) and long-distance (800 *m*) swimmers, within whom the anaerobic and aerobic metabolisms dominate, respectively. It was concluded that both long-distance and particularly short-distance (100 *m*) swimming increased the activities of antioxidant defense enzymes [*Inal M. et al., 2001*]. High intensity resistance exercise increases free radicals production, and vitamin E supplementation may decrease muscle membrane disruption [*McBride J. et al., 1998*]. Furthermore, the consumption of strawberries, spinach or red wine, which are rich in antioxidant phenolic compounds, can increase the serum antioxidant capacity in humans [Cao *G. et al., 1998*].

Results obtained by measuring the TAC show that its value for LT group (411.75 *Nm/mg* protein) is significantly higher than in groups B (392.85 *Nm/mg* protein), S (385.75 *Nm/mg* protein) and L (279.85 *Nm/mL* protein), respectively (Table 3). Thus, the exposure to lead acetate and training cause the increase of free radicals and reduction of TAC of the blood serum in rats.

In contrast, MDA value in group L (46.086 *Nm/mg* protein) is significantly higher than in groups B (24.986 *Nm/mg* protein), S (26.825) and LT (18.412 *Nm/mL* protein) (Table 4). The lowest value of MDA in LT group probably testifies to the role of training.

Table 3.

Total antioxidant capacity (TAC))

Groups	Mean	Standard Deviation	Min	Max
Base	392.8571	11.40802	376.00	410.00
Sham	385.7500	8.89221	369.00	396.00
LT	411.7500	13.87444	389.00	435.00
L	279,8571	18,33420	256,00	305,00

Table 4.

Value of MDA serum

Groups	Mean	Standard Deviation	Min	Max
Base (B)	24.9857	4.13982	19.80	32.30
Sham (S)	26.8250	2.85394	21.20	30.00
Lead + Training (L+T)	18.4125	3.28479	14.60	23.70
Lead (L)	46.0857	9.22143	34.40	59.00

DISCUSSION

In our experiments the neuroprotective effects of prolonged exercise training on lead acetate-induced BDNF levels were investigated. Forty rats were randomly divided into 4 groups: (1) Base, (2) Sham, (3) Lead acetate + Training and (4) Lead acetate groups. The rats in groups 3 and 4 experienced the treadmill running of 15 to 22 *m/min* for 25 to 64 *min*, 5 times a week for 8 weeks. Groups 1, 3 and 4 received lead acetate (20 *mg/kg*) and the Sham group received solvent (ethyl oleat). The results showed that BDNF in Lead acetate group decreased significantly, whereas in Lead acetate + Training group it was increased. MDA in Lead acetate group significantly increased, while in Lead acetate + Training group it was decreased. Finally, the levels of TAC in Lead acetate group significantly decreased and increased in Lead acetate + Training group.

Lead, a metal commonly-used worldwide, has been used since ancient times. Studies have shown that lead has harmful effects on several tissues including the nervous system, blood, the cardiovascular system, and the reproductive and urinary systems. Recent studies suggest that one of the mechanisms, by which lead can exert some of its toxic effects, is through the disruption of the delicate prooxidant/antioxidant balance that exists within mammalian cells. *In vivo* studies have suggested that lead exposure is capable of generating reactive oxygen species (ROS) and thus alters antioxidant defense systems in animals [*Daniel S. et al., 2004*]. Thus, potential exists for biological mechanisms linking pollutants such as lead with cardiovascular disease through inflammation and oxidative stress [*Chuang K. et al., 2007*]. In the current study, TAC was assayed as marker of inflammation and tissue damage in brain degenerative cells of rats treated with lead. Results of our investigation revealed a significant increase in brains' BDNF levels of rats exposed to lead with training. This suggests that lead acetate had damaging effects on brain tissue during chronic exposure, which may heighten neurodegenerative diseases [*Gholamhosseini B. et al., 2009; Dabidi R. et al., 2011*].

Exercise appears to increase reactive oxygen species, which can result in damage to cells. Exercise results in increased amounts of malone dialdehyde in blood and pentane in breath; both serve as indirect indicators of lipid peroxidation. However, not all studies report increases; these equivocal results may

be due to the large inter-subject variability in response to the non-specificity of the assays. Some studies have reported that supplementation with vitamins C and E, other antioxidants, or antioxidant mixtures can reduce symptoms or indicators of oxidative stress as a result of exercise. However, these supplements appear to have no beneficial effect on performance. Exercise training seems to reduce the oxidative stress of exercise, such that trained athletes show less evidence of lipid peroxidation for a given bout of exercise and an enhanced defense system in relation to untrained subjects. Whether the body's natural antioxidant defense system is sufficient to counteract the increase in reactive oxygen species with exercise or whether additional exogenous supplements are needed is not known, although trained athletes, who received antioxidant supplements, show evidence of reduced oxidative stress. Until research, it was fully substantiated that the long-term use of antioxidants is safe and effective, the prudent recommendation for physically active individuals is to ingest a diet rich in antioxidants [*Clarkson P., Thompson H., 2000*].

Available results obtained by isolated studies suggest that exercise alters BDNF levels and oxidative status. It has been described that physical activity induces members of the neurotrophins family, especially BDNF, that modulate neuronal survival and plasticity [*Oliff H. et al., 1998*], maturation and outgrowth in the developing brain and exert neuroprotective actions in the mature brain submitted to metabolic insults [*Lee I., Paffenbarger R., 1998*]. In addition, exercise induces BDNF mRNA in the hippocampus [*Neeper S. et al., 1996; Vaynman S. et al., 2004; Cechetti F. et al., 2008*]. There is evidence indicating that physical activity may reduce age-induced cognitive decline and it is recommended as a therapeutic strategy to prevent, or recover from, neurodegenerative disease [*Kramer A. et al., 1999]*. Although the exact molecular mechanisms, by which physical exercise affects the brain function, are unclear, the effect is considered as neuroprotection.

Thus, the obtained data indicate the significant role of exercises in the gain of BDNF level. On the other hand, the increased level of BDNF may act as neuroprotector for recovery of numerous disturbances, such as reduction of the brain weight, memory loss, and different degenerative processes. It may be concluded that the increase of BDNF in hippocampus may cause positive plastic changes and provide the prevention of the mentioned disturbances.

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