The Effect of Resistance Training on Nerve Growth Factor (NGF) and Apoptosis in Rat Hippocampus

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ABSTRACT
The positive effects of regular physical exercise on cognitive functions in human and animals is well known, but details on the effect of different type of exercises are still unknown. In this study the effects of regularly performed resistance exercise (RE), for eight weeks, was investigated on the nerve growth factor (NGF), caspase-3 activity and TUNEL positive cells in the hippocampus of rats. 90 male wistar rats were divided to RE group and control group. Results show that amount of NGF increased significantly in RE group in forth week. Further, caspase-3 activity was decreased considerably in exercise group five and three days after last session in eighth week. TUNEL positive cells decreased notably in forth week in RE group but this decrease was not significant in eighth week. This study indicated that low intensity resistance exercise enhanced NGF level in hippocampus and suppresses apoptosis.

INTRODUCTION
Brain health is one of the most important issues of public health. A growing body of evidence indicates that participation in regular physical exercise induces neurobiological adaptations that result in a reduced severity of depression and improved cognitive function [6,10]. Physical exercise is considered to improve cognitive function by promoting neurogenesis and cell survival that could take place in hippocampus [16,17] which is a part of the brain with important roles in learning and memory. Animals subjected to physical activity developed more hippocampal neurons than those living under standard laboratory conditions [8]. The improvement in cell survival may result from increase in neurogenesis or decrease in apoptosis or both.

It has been demonstrated that these neuroprotective effects can be observed following aerobic exercise [1,9,11]. However, it remains unknown whether resistance exercise could improve hippocampal neurogenesis or not.

It has been shown that resistance exercise has a lot of benefits for body, like improvement in bone mineral density, power, improved body composition, muscle strength, and functional abilities [2,14]. In addition, a number of studies performed in human have suggested that resistance training improves several aspects of cognition [2,14,15].

Apoptosis or programmed cell death occurs in normal aging process. Many studies have presented that aerobic exercise prevented apoptotic cell death in the hippocampus [10,13]. However, the effect of resistance exercise on hippocampal apoptosis is unknown. Therefore the purpose of this study is to investigate the effect of resistance exercise on hippocampal neurotrophin NGF and to evaluate level of apoptosis in hippocampus.

Methods:
Animals:
Male wistar rats, weighing 180±10g (eight weeks in age) were obtained from Pasteur institute of Iran. Animals were housed under laboratory conditions for one week prior to experiment under standard condition.
with a 12h light: 12h dark cycle (light on 07:00h) and constant room temperature (23±2°C), humidity (60%) and ad libitum food and water throughout the experiments.

**Familiarization protocol:**
Before any manipulations or resistance training, all animals were submitted to a familiarization, climbing the ladder from the bottom to the top cage for 3 days, after which the resistance-training regimen started.

**Resistance exercise protocol:**
The rats in exercise group were subjected to one training period per day every third day for eight week. Training was carried out utilizing a 1-m ladder with 2-cm grid steps and inclined at 85°. A weight was attached to the base of the tail with hypoallergenic tape. The initial weight attached to each animal was 50% of its body weight. Rats were positioned at the bottom of ladder and motivated to climb by touching the tail. When the rats reached the top of the ladder, they were allowed to rest for 2 min. After the rest period, additional weight attached, and the rats were returned to the bottom of the ladder for subsequent climbs. Rats climbed the ladder with 50, 75, 90, and 100% of maximal load from the previous exercise session. For determination of maximal load, additional weights were placed in 30-g increments for each subsequent climb. This procedure was repeated until rat failed to climb the entire length of the ladder.

**Tissue preparation:**
The animals were sacrificed at the 24 h after last session in fourth, eighth week and three day and five day after last session in eighth week. The animals were decapitating under ether anesthesia and the brain were quickly removed. The hippocampus excised and immediately frozen on liquid nitrogen. Samples were stored at -80°C until processing.

**NGF level and caspase-3 activities:**
Rat hippocampal homogenerate Caspase-3 and NGF levels were determined using Enzyme Linked Immuno Sorbent Assay method (Rat Caspase-3 ELISA kit, Cusabio Biotech, Wuhan, China, and Rat NGF ELISA kit, Cusabio Biotech, Wuhan, China). The assays sensitivity for Caspase-3 and NGF were 0.08ng/ml and 0.2pg/ml respectively. The assays coefficient of variation for both assays was less than 10%.

**TUNEL assay:**
**Statistical analyses:**
The results from experiment group and control group were compared by paired t-test. Statistical significance was set at p<0.05 and analysis were done by the SPSS13.

**Results:**

**NGF:**
Resistance exercise group exhibited markedly higher hippocampal NGF level in fourth week (p=0.027). No significant differences were observed in other times between two groups.

**Fig.1:** Hippocampal NGF during eight week and two days follows up in RE and control groups.

**Caspase-3:**
There was no significant difference between groups in fourth and eighth weeks, but three days and five days after last session of resistance exercise significant decrease in caspase-3 activity was observed in exercise group(p=0.007),(p=0.022).
Fig. 2: Hippocampal caspase-3 activities during eight week and two days follow up in RE and control groups. TUNEL positive cells.

There was significant differences between RE group and control group in forth week (p=0.036) but not in other times. From this result it appears that resistance exercise affects the level of apoptosis in hippocampus of rats and decreased it in forth week that level of resistance exercise is low.

Fig. 3: Number of TUNEL positive cells in hippocampus during eight week and two days follow up in RE and control groups.

Discussion:

The hippocampus plays an important role in learning and memory. Several studies have demonstrated that environmental factors and exercise training could affect neurogenesis [9,5]. The mechanism behind the regulation of neurogenesis has not been fully clarified. A number of studies have shown that neurogenesis in hippocampus may be integrally linked to the increase in neurotrophins [1,6]. In addition close relationship between neurogenesis and apoptosis has been suggested [9].

The major finding of this study is that resistance exercise resulted in elevated levels of NGF in hippocampus and also reduced apoptosis. Resistance exercise is one of the most recommended exercises and is applied as a primary intervention for improvement of physical function, and prevention of functional limitations and muscle weakness in elderly persons [12]. Also, there is growing evidence that resistance exercise may be a positive factor for cognitive functions [2,15]. Suijo et al. investigate the effect of voluntary progressive-resistance wheel exercise on hippocampal synaptic plasticity and Morris water maze was also performed to estimate learning and memory. Their results showed that resistance exercise enhanced hippocampal synaptic plasticity-related molecules and cognitive function [15].

To the best of our knowledge, ours is the first study that shows the positive impact of progressive resistance exercise on NGF level in hippocampus.

The mechanism that may play a role in the increase of NGF is neuronal activity which reported to have regulating role to neurotrophicmDNA level [1]. Increasing neuronal activity by physical exercise may therefore increase neurotrophic factors leading to neurogenesis and specific patterns of neural impulses regulate genes controlling nervous system development and plasticity [4].

After the intervention period, the resistance exercise group shows higher level of NGF after 4 week. There was no significant differences in eighth week between exercise and control groups. This result suggest that low level resistance exercises could have better effect on neurotrophins like NGF. On the other hand, significant difference was observed in the number of TUNEL-positive cells in forth week. This result indicates that resistance exercise intensity is important element to affect apoptosis in hippocampus and increasing in intensity could suppress beneficial effects. Exercise might even increase the susceptibility to glucocorticoid-induced...
-suppression of neurogenesis [5]. Glucocorticoid could cross blood brain barrier and excess glucocorticoid results in a decrease in some neurotrophins function [7].

NGF has antiapoptotic effects and activates various signaling cascades that stimulates cell survival. One of them is MAPK cascade, which functions through Erk phosphorylation and another system is PI3-K/Akt signaling pathway. However, these two different pathways have mutual crosstalk [3]. The PI3-K signaling pathway could prevents apoptosis by suppressing caspases apoptotic signal. This pathway also suppresses BAD protein function and apoptotic gene expression [3]. Therefore NGF protective roles against cellular death, could be mediated by this pathways. Thus NGF increasement is one of the mechanism for beneficial effects of exercise on brain. Although further studies should have done to explore the mechanism of exercise improving hippocampal neurogenesis. In conclusion, resistance exercise could decrease apoptosis in hippocampus and increase NGF level which has beneficial effects on cells survival.

REFERENCES