

THE EFFECT OF AN 8-WEEK ANAEROBIC GYMNASTICS TRAINING ON BDNF, VEGF, AND SOME PHYSIOLOGICAL CHARACTERISTICS IN CHILDREN

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Abstract

The purpose of the present study was to observe changes in levels of brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), resting metabolic rate (RMR) and maximum oxygen consumption (VO₂max) in the gymnast children after an anaerobic gymnastics training program. Thirty beginner gymnasts aged 8-12 years old were randomly assigned to control (n = 15) and experimental (n = 15) groups. The anaerobic gymnastics training was conducted for 8 weeks, 3 times per a week. Each session lasted 45 minutes: 10 min warm-up, 30 min core exercise, and 5 min cool down. The anthropometric and body composition of subjects were measured and growth factors were measured by using human BDNF and VEGF PicoKine™ ELISA Kit and analysis was performed using sandwich enzyme-linked immunosorbent assay (Morland et al.) before and after the intervention, and VO₂max, maximum heart rate and RMR were measured using a gas analyzer. At the baseline there were not any significant differences between both groups (p>0.05). But in the post-test, a significant difference was observed for BDNF(p=0.02) and VEGF(p=0.018) values between the two groups. Within-group there was a decrease in the value of the maximum heart rate indicator (P<0.05) and VO₂max and BDNF increased significantly after an intervention (P<0.05). In conclusion, the results of the present study suggest that anaerobic gymnastic training increases the level of salivary BDNF and VEGF in children. These types of exercises may also improve cardiorespiratory fitness in children.

Keywords: children, neurotropic factors, growth factors.

INTRODUCTION

Childhood is the best and the most important period of a lifetime for a lifetime warranty by prevention of diseases such as metabolic problems and improvement of health factors such as cardiorespiratory and growth indexes. Genetic factors, medical conditions, medications, and environmental factors are the most common causes of childhood disorders, which are usually managed through a diet regimen, exercise,

and surgical treatment. Individuals with lack of physical activities have a higher burden of vascular and neurological damage than healthy individuals, potentially explaining increased rates of cardiovascular diseases (Languren, Montiel, Julio-Amilpas, & Massieu, 2013). Increasing physical activities through exercise training programs has also been shown to improve vascular, peripheral

nerve, and cognitive function through vascular remodeling, angiogenesis, and neurogenesis (Leckie et al., 2014). Therefore, exercise interventions may be especially important for the prevention of complications of health and growth factors problems. Growth factors with angiogenic and neurotrophic properties such as vascular endothelial growth factor (VEGF) and brain-derived neurotrophic factor (BDNF) are implicated in vascular and neurological repair in both animal and human studies (El-Alameey, Ahmed, & Abushady, 2019; Kermani et al., 2005).

BDNF is well known to show an exercise-induced increase in expression and promote neuronal cell formation and angiogenesis. Also, various growth factors, such as VEGF is known to promote the production of new cells and angiogenesis. BDNF is the most abundant in the nerve growth factor family and is related to nerve growth factor, the first neurotrophic factor discovered and acts via protein tyrosine kinase receptor (TrkB) (Soppet et al., 1991). In the periphery, BDNF is found in the plasma, serum, and platelets and it is formed by vascular endothelial cells and by peripheral blood mononuclear cells (Sarchielli, Greco, Stipa, Floridi, & Gallai, 2002). A positive correlation between BDNF levels in the brain and serum was described therefore the peripheral levels of BDNF may reflect the brain levels and vice versa. It should be mentioned however, that some authors challenged the finding by Poduslo and Curran (Poduslo & Curran, 1996) and by Pan et al (Pan, Banks, Fasold, Bluth, & Kastin, 1998).

VEGF is a key cytokine known to increase vascular permeability and vasodilatation. Moreover, there is an evidence that VEGF is involved in the pathogenesis of cardiovascular risk factors, arteriosclerosis, obesity, and metabolism morbidity and mortality (Silha, Krsek, Sucharda, & Murphy, 2005). Angiogenesis is stimulated by VEGF, which also directly enhances neurogenesis and synaptic function; however, the initial molecular

signal that leads to increased cerebral VEGF in response to exercise has not been determined (De Rossi et al., 2016).

Exercise at high intensity, causing lactate from active skeletal muscles to accumulate in the blood, and lactate injections have been previously found to increase brain expression of VEGF (Lezi, Lu, Selfridge, Burns, & Swerdlow, 2013), but the mechanism is unknown. Moreover, in wounds, lactate is known to accumulate and stimulate angiogenesis, (Ruan & Kazlauskas, 2013). Lactate, released in situ from polymeric lactic acid microfibers, induces angiogenesis in the brain, again through unidentified mechanisms (Álvarez et al., 2014). Cerebral hypoxia, another condition known to increase lactate levels in the brain, also causes angiogenesis via VEGF (Shweiki, Itin, Soffer, & Keshet, 1992). However, as lactate or exercise does not increase hypoxia-inducible factor 1a (HIF-1a), hypoxia is unlikely to be part of the response (Poduslo & Curran, 1996). The mechanisms behind lactate-induced angiogenesis thus remain to be elucidated (Morland et al., 2017). The mechanisms by which exercise increases VEGF and BDNF are not clearly understood, but Increased density of capillaries due to angiogenesis, the sprouting of new capillaries from pre-existing vessels, is one mechanism (Ding et al., 2006) and Exercise induces cerebral VEGF and angiogenesis via the lactate receptor hydroxycarboxylic acid receptor1 (HCAR1) is another mechanism that is recognized by Cecilie Morland and et all (Morland et al., 2017).

Data from previous studies suggest an exercise intensity dependent effect on blood BDNF and VEGF concentrations. Most of these studies used blood lactate to determine the degree of the exercise intensity, suggesting that exercise with higher blood lactate concentrations results in elevated BDNF and plasma concentrations (Vega et al., 2006). However, it is not clear if lactate per se or other mechanisms are responsible for the described changes in blood BDNF

concentrations. To answer this question, Thorsten Schiffer and et al used the lactate clamp method at rest that is an established method to examine physiological and neurological responses of lactate in the human organism. After infusion of sodium-lactate, BDNF and lactate increased significantly and reached baseline values at the end of the experiment. They reported that blood lactate increases during high intensity exercise after the infusion of sodium-lactate but no metabolic acidosis is seen suggesting that the mechanism underlying blood BDNF augmentation is lactate per se (Schiffer et al., 2011). Based on the result of Thorsten Schiffer studies that Lactate infusion at rest increases BDNF blood concentration in humans (Schiffer et al., 2011), and according to the results of Cecilie Morland and et al that exercise induces cerebral VEGF and angiogenesis via the lactate receptor HCAR1 (Morland et al., 2017), this study aimed to investigate the effect of an anaerobic gymnastics training (AGT) on growth factors (BDNF and VEGF), vo₂max and resting metabolic rate in children.

METHODS

Thirty 8 to 12_year_old boys who enrolled in an elementary level of gymnastics participated in this study and were randomly divided into an experimental and control groups. Subjects were diagnosed based on the American Council on Exercise lists (Jackson and Pollock equation for three-point subcutaneous fat measurement considering the fat percentage of 6 to 13% as athletic category (normal weight group)) (Jackson & Pollock, 1978; Jackson, Pollock, & Ward, 1980) without concomitant diseases. Exclusion criteria included evidence of any disease, drug therapy, structural abnormality, and prohibition of exercise testing. The study protocol was approved by the Ethics Committee of Ardabil University of Medical Science (IR.ARUMS.REC.1397.290) and Iranian

Registry of Clinical Trials (IRCT20190917044807N1). This study was performed under the Declaration of Helsinki (Revised 2008). All subjects and their parents were informed about the study procedure and the possible risks involved, and both parents and subjects signed a written consent form. Baseline characteristics of both experimental and control groups are shown in table 1.

The present study was semi-experimental and its design was pre-test and post-test with a control group. The control group was asked to stop training for eight weeks, and repeated phone calls prevented them from participating in the group's training programs. Depending on the age of the subjects, the subjects were in the pre-pubertal stage. The experimental procedures consisted of a familiarization phase (including 3 sessions for familiarization of participants to the equipment and protocols), followed by pre-testing (24 hours before starting training program), then 8 weeks of anaerobic gymnastics exercise (AGE) (3 days a week), and then post-testing (48 hours after the last session of training program). Each exercise session was guided by a trained instructor, and conducted for 45 minutes in three stages: A 10-minute warm-up, a 30-minute main exercise, and a 5 minutes cool down. During the warm-up, subjects performed fun gymnastics movements and fun animal movements, such as running, rabbit, cat, crab, bear, and kangaroo movements (Fediani, Dewi, Irfannuddin, Saleh, & Dhaini, 2014). AGE including 30-second Continues Jump (30-s CJ), 30-s Vertical Continues Jump on Box (30-s VCJB), Specific Aerobic Gymnast Anaerobic Test (SAGAT) and Running Jump Rolling (RJR) were used for the main part (Čular et al., 2018; Dal Pupo et al., 2014). Of course, we did a pilot study for evaluating the amount of Lactate level before and after doing designed training and showed that level of lactate increase up to 7-8 mmol/L compared to baseline level of 1- 1.6 mmol/L (figure 1).

We used 30-s CJ training, because, according to result and suggestion of previous studies, the continuous jump test seems to be more specific for sports that are acyclic such as gymnastics, basketball, volleyball, etc., all of which involve similar movement patterns and have practical application for coaches and athletes (Dal Pupo et al., 2014).

Since, 30-s VCJB, has a close relationship to the standard laboratory 30-s Wingate test and this training is so common and prevalence in gymnastics physical training, we considered this training to one part of the main exercise. The next training was SAGAT (figure 2). We used this training protocol with a little change in the difficulty of movements according to age, body composition, and fitness level of subjects.

RJR also was selected as one of the exercises performed in each session because of its anaerobic essence and it consisted of jumping over box and front-rolling (Figure 3). RJR test was performed in 2 sets; each set 5 repetitions with a 3-min recovery period between the sets.

Anthropometrical variables including height, weight, waist to hip ratio (WHR), body fat percentage (BF%), body fat weight (BFW), and lean body weight (LBW) were measured before and after eight weeks training. To measure waist and hip circumference, the subjects were asked to stand up straight and breathe out. The smallest circumference between the umbilicus and the xiphoid process was considered as waist and the largest circumference around the buttocks was considered as hip. These circumferences were measured by measure tape.

Three points skinfold test which is a reliable method for estimating body-fat percentage was used in the present study. Harpenden caliper was applied in tight (quadriceps), chest (pectoral), and belly (abdomen)) and Jackson/Pollock 3-Site equation was used to predict BF%. To obtain the best and most consistent measurements, all skin-fold measurements

were taken on the right and by the same person. Also, a minimum of two measurements was taken at each location. If the two measurements differed by more than 2 millimeters, a third measurement would be taken. The online body composition calculator then uses the average of the 2-3 measurements to make the calculations. BFW and LBW were calculated by the following formulas (Jackson et al., 1980):

$$BFW = \text{Body weight} \times BF\%,$$

$$LBW = \text{Body weight} - BFW$$

To measure VO₂max, Participants were instructed not to feed two hours before the test, abstain from caffeine, and not to perform any strenuous physical exercises 48 hours before the test. Participants were familiarized with the ergometer (automatic ergometer treadmill) a few days before the test. As subjects were children aged 8–12 years old, a modified Balke protocol was used for evaluation of their Vo₂max (Washington et al., 1994). Because this continuous protocol is well suited for the unfit, the obese, the very young child, or the chronically ill individual (Washington et al., 1994). After warming up, each subject performed modified Balke protocol, which progressively increases the grade from 2% to more than 10% at 2% increments anyone minutes until the subject could not maintain a constant speed of 3.5 mph and receive to exhaustion (Washington et al., 1994). Subjects continued test until exhaustion and they were verbally encouraged throughout the test. All subjects were assessed on a treadmill in two stages (pre-test and post-test) and data on respiration gas exchange were obtained breath-by-breath using a respiratory gas analyzer (QOSMED, Italy Part2001N.COO627-D2-91). Breath-by-breath data was transformed into 1 s data using KaleidaGraph software. We applied this method for data of VO₂, VCO₂ and HR in each subject and averages of all subjects' data were used for analyzing.

. The running time and distance were recorded and VO₂ and respiratory exchange ratio (García de la Torre et al.) VO₂max was considered the maximum value of VO₂ attained during the incremental test (Morinder, Larsson, Norgren, & Marcus, 2009). The subject's heart rate was measured by installing a polar on the subjects' chest and recorded every 1 second.

All subjects had 8 hours of sleep, did not perform any exercises for 48 hours before each session, and did not eat or consume any liquids, except water, for 12 h before testing. Each subject was transported by motor vehicle to the testing site to ensure minimal activity before rest metabolic rate (RMR) determination. All RMR measurements were performed between 09:00 and 11:00 hours. RMR was determined by a gas analyzer system by use of the open-circuit technique while the subject was sitting (Consolazio, 1963). After entering the laboratory, subjects rested in a chair for 15 min in an isolated temperature controlled room (21-24 C). After the first 15 min rest, the second 15 minutes started and subjects were fitted with a Hans Rudolf face mask which was connected to the gas analyzer system for the determination of breath by breath oxygen analysis. Analyzers were calibrated before each test according to the specifications of the manufacturer. During the test, the room was darkened, and the noise was kept to a minimum. The subjects were instructed to remain awake, quiet, and motionless before and throughout the entire 15-min period. The average of the last 10 min of the measurement period was used to obtain resting metabolism by the following formula (Gilliat-Wimberly, Manore, Woolf, D SWAN, & Carroll, 2001):

$$\text{RMR} = 3.941 \left[\text{VO}_2 \left(\frac{\text{L}}{\text{min}} \right) \right] + 1.106 \left[\text{VCO}_2 \left(\frac{\text{L}}{\text{min}} \right) \right]$$

$$= \text{Kcal/min}$$

Saliva samples were collected between 09:00 and 11:00 hours. The parents/guardians and children were

requested to adhere as closely as possible to the following standardized saliva collection instructions (Chiappin, Antonelli, Gatti, & Elio, 2007): The children should 1) not eat anything 60 minutes prior to sample collection, 2) not brush their teeth before the sample collection (this may cause the gums to bleed causing blood contamination of the saliva), 3) rinse their mouths with water to remove food residue before the sample collection, and swallow to increase hydration, and 4) wait at least 10 minutes after rinsing before collecting saliva to avoid sample dilution (Chiappin et al., 2007). Saliva samples were collected via unstimulated passive drool over five minutes. The seated children were asked to lean slightly forward and tilt their heads down and accumulate saliva in the floor of the mouth for a minute, and saliva was subsequently swallowed. Then there was a four-minute collection where the children dribbled saliva through a 5 cm plastic straw into a pre-weighed polypropylene cryovial tube (5 ml capacity). Care was taken to allow saliva to dribble into the collecting tubes with minimal orofacial movement. After collection the samples were analyzed in the laboratory (Chiappin et al., 2007).

Human BDNF PicoKine™ ELISA Kit (Catalog No. EK0307; R&D Systems, Austria) and Human VEGF PicoKine™ ELISA Kit (Catalog No. EK0539; R&D Systems, Austria) were used for measurement of BDNF and VEGF respectively. Collected saliva samples were centrifuged for 15 min at 4000 rpm. The evaluation was performed according to the manufacturer's instructions for the use of buffers, diluents, and materials. The analysis of BDNF and VEGF were performed using a sandwich enzyme-linked immunosorbent assay (ELISA). Fluorescence was measured at 450 nm with a microplate reader.

Data are expressed as mean and standard deviations (SD). All analyses were performed using SPSS version 23.0. The Kolmogorov-Smirnov test was used for the normality of distribution and Levin's test

was used for the homogeneity of variance. Also, the homogeneity of the regression slope test was used for the test of homogeneity of the regression slope. To compare the mean post-test scores in two

groups, analysis of covariance test (ANCOVA) was used. Paired t-tests were used to examine significant differences within groups. A value of $p < 0.05$ was regarded as statistically significant.

Table 1
Characteristics of the subjects at baseline.

Variables/Group	Control (n = 15) XA±SD	Experimental (n = 15) XA±SD	P-value *
Age (years)	9.60 ± 1.05	9.80 ± 1.47	0.672
Height (cm)	133.37 ± 5.03	133.60 ± 5.36	0.903
Weight (kg)	29.43 ± 3.66	29.40 ± 3.56	0.980

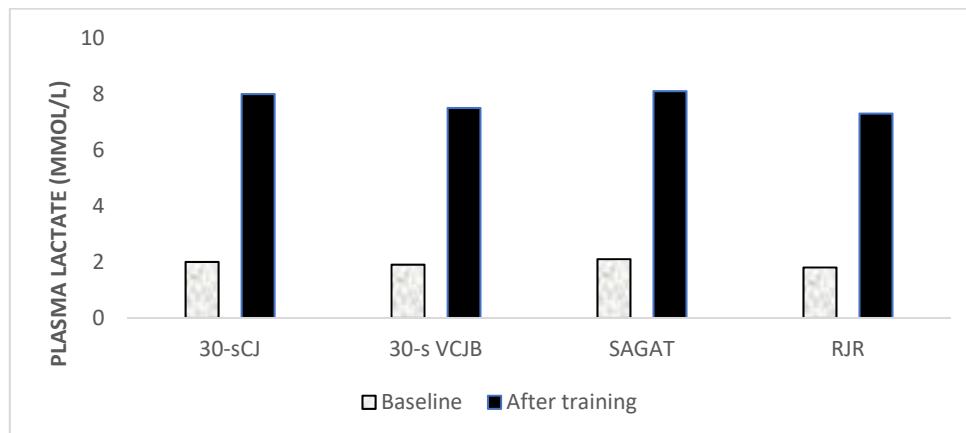


Figure 1. Plasma lactate response to AGE (30-second Continues Jump (30-s CJ), 30-s Vertical Continues Jump on Box (30-s VCJB), Specific Aerobic Gymnast Anaerobic Test (SAGAT) and Running jump rolling (RJR) in a pilot study.

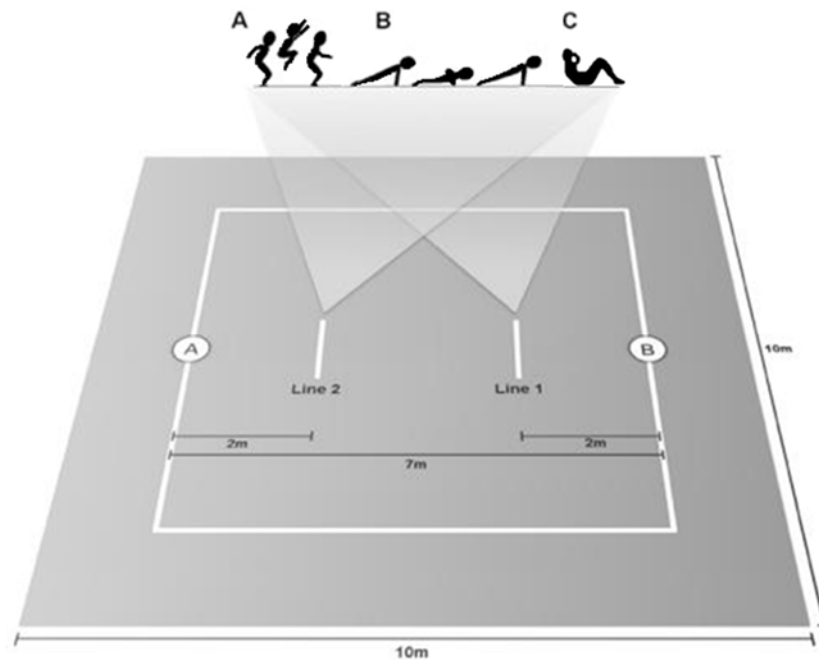


Figure 2. Illustration of SAGAT test: After the start command, the subject taps the floor and runs seven meters to “point B”. At this point, the subject taps the floor again and returns two meters towards “point A” (Line 1). At this point the subject performs tuck jumps, push-ups, and sit-ups exercises, each one time, and then returns to “point B” and taps the floor. This is the end of the first repetition and the start of the second repetition and subject runs seven meters to “point A”, taps the floor, returns two meters towards “point B” (Line 2), performs exercises described above, and then returns to “Point A” and taps the floor that means the end of the second repetition and the start of the third repetition. This pattern continues until a total of 6 repetitions are completed.

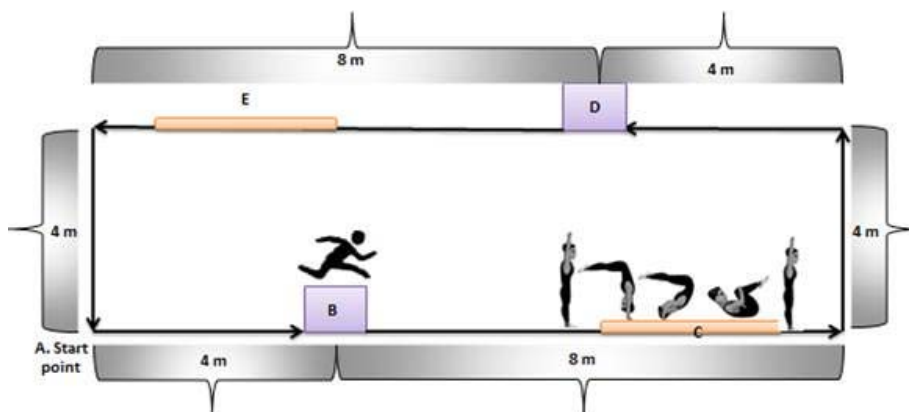


Figure 3. Illustration of RJR test. Each repetition was as follows: after the start command, the subject runs four meters toward “point B” to perform jumping over a box with a height of 50 cm, then continues to running towards “point c” to perform front-rolling. Following rolling, the subject must change the direction and runs fast to reach “point D” for doing jumping over the box, then runs to “point E” for performing front-rolling and at the end runs to start point (point A). After completion of 5 repetitions (first set) subject recovers for three minutes and then starts the second set.

RESULTS

Subject's characteristics including anthropometrical, body composition and physiological factors, and the level of salivary BDNF, and VEGF in experimental and control groups were not significantly different at baseline and are presented at table 2 ($p > 0.05$). Before the test, its defaults were checked. The results of the Kolmogorov-Smirnov test showed that the data distribution was normal ($P > 0.05$). The results of the Leven test were not significant for any of the variables and indicated the

homogeneity of variance ($P > 0.05$). Also, the study of homogeneity of regression slope showed that statistical F-ratio is not significant for any of the variables and indicates the assumption of homogeneity of regression slope ($P > 0.05$). To compare the mean post-test scores after controlling the pre-test effect in two groups, analysis of covariance test (ANCOVA) was used. These results have been shown in table 3. According to the results, only in BDNF ($p = 0.03$) and VEGF ($p = 0.03$) variables, a significant difference was observed between the two groups.

Table 2

Characteristics of the subjects (anthropometric, body composition, physiological, BDNF, and VEGF) at baseline.

Variable	Groups (n=15 for any group)		P-value
	Control	Experimental	
Weight (kg)	29.43 ± 3.66	29.40 ± 3.56	0.98
WHR (cm/cm)	0.87 ± 0.02	0.86 ± 0.02	0.58
BF (%)	6.79 ± 1.78	6.74 ± 1.76	0.94
BFW (kg)	2.09 ± 0.81	2.07 ± 0.81	0.94
LBW (kg)	27.80 ± 3.96	27.85 ± 3.98	0.97
VO ₂ max (mL/kg/min)	35.78 ± 4.49	37.63 ± 5.56	0.32
RMR (Kcal/day)	1008.46 ± 145.38	1023.06 ± 187.71	0.81
MHR (beat/ minutes)	185 ± 18	188 ± 14	0.69
BDNF (pg/ml)	0.061 ± 0.005	0.061 ± 0.003	0.87
VEGF (pg/ml)	1.593 ± 0.401	1.631 ± 0.437	0.80

Note: Data are presented as mean ± standard deviation. WHR= Waist hip ratio, BF (%) = Body fat percentage, BFW= Body fat weight, LBW= Lean body weight, VO₂max =Maximum oxygen consumption, RMR= Resting metabolic rate, MHR= Maximum heart rate, BDNF= Brain derived neurotrophin factor, VEGF= Vascular endothelial growth factor.

Table 3

Results of analysis of covariance to compare posttest scores of variables in two groups.

Dependent Variables	Mean Differences	95% Confidence Interval for Difference		P-value
		Lower Bound	Upper Bound	
Weight (kg)	-0.202	-0.773	0.370	0.475
WHR (cm)	-0.002	-0.007	0.003	0.466
BF (%)	-0.011	-0.366	0.344	0.951
BFW (kg)	-0.021	-0.214	0.172	0.827
LBW (kg)	0.011	-1/88	1.902	0.991
VO ₂ max (ml/Kg/min)	2.311	-0.235	4.858	0.073
RMR (Kcal/day)	-33.145	-73.50	7.212	0.103
MHR (beat/minutes)	-4.716	-9.451	0.02	0.051
BDNF (pg/ml)	0.008	0.001	0.015	0.02*
VEGF (pg/ml)	0.294	0.054	0.534	0.018*

Note: WHR= Waist hip ratio, BF (%) = Body fat percentage, BFW= Body fat weight, LBW= Lean body weight, VO₂max =Maximum oxygen consumption, RMR= Resting metabolic rate, MHR= Maximum heart rate, BDNF= Brain derived nerotrphin factor, VEGF= Vascular endothelial growth factor.

Table 4

Pre-training vs. post-training values for anthropometric, body composition, physiological, BDNF, and VEGF variables in the two groups.

Variables		Groups	
		control	Experimental
Weight (kg)	Pre-test	29.43 ± 3.66	29.40 ± 3.56
	Post-test	29.96 ± 3.58	29.73 ± 3.42
	P Value	0.06	0.06
WHR (cm)	Pre-test	0.87 ± 0.021	0.86 ± 0.023
	Post-test	0.87 ± 0.025	0.86 ± 0.022
	P Value	0.17	0.16
BF (%)	Pre-test	6.79 ± 1.78	6.74 ± 1.76
	Post-test	6.88 ± 1.25	6.84 ± 1.21
	P Value	0.66	0.65
BFW (kg)	Pre-test	2.09 ± 0.81	2.07 ± 0.81
	Post-test	2.10 ± 0.58	2.07 ± 0.55
	P Value	0.89	1.00
LBW (kg)	Pre-test	27.80 ± 3.96	27.85 ± 3.98
	Post-test	27.28 ± 3.98	27.34 ± 3.99
	P Value	0.45	0.45
VO ₂ max (mL/kg/min)	Pre-test	35.78 ± 4.49	37.63 ± 5.56
	Post-test	36.48 ± 4.88	40.14 ± 4.88
	P Value	0.47	0.03†
RMR (kcal/day)	Pre-test	1008.46±145.38	1023.06±187.71
	Post-test	1006.80±147.76	984.86 ± 121.91
	P Value	0.23	0.22
MHR (beat/minutes)	Pre-test	185 ± 18	188± 14
	Post-test	184.00 ± 15.07	181± 10
	P Value	0.27	0.04†
BDNF (pg/ml)	Pre-test	0.061±0.005	0.061±0.003
	Post-test	0.062±0.004	0.070±0.013
	P Value	0.33	0.04†
VEGF (pg/ml)	Pre-test	1.593±0.401	1.631±0.437
	Post-test	1.614±0.406	1.928±0.428
	P Value	0.39	0.06

Note. N = 15 in each group. Data Shown are mean ±S.D.

†: significant difference between pre-test and post-test identified by paired t-test

WHR= Waist hip ratio, BF (%) = Body fat percentage, BFW= Body fat weight, LBW= Lean body weight, VO₂max =Maximum oxygen consumption, RMR= Resting metabolic rate, MHR= Maximum heart rate, BDNF= Brain derived nerotrphin factor, VEGF= Vascular endothelial growth factor.

DISCUSSION

In the present study our results suggest that regular AGE increases the level of salivary BDNF and VEGF, and improves $\dot{V}O_{2\max}$ and maximal heart rate (MHR) in the trained children. The important point for us to the utilization of AGE in this research was the studies in humans that used an infusion of sodium-lactate to determine the level of exercise intensity, suggesting that exercise with increased blood lactate concentrations results in increased BDNF plasma level concentrations. Some studies used the lactate clamp method at rest that is an established method to examine physiological and neurological responses of lactate in the human organism. After the infusion of sodium-lactate, BDNF and lactate increased significantly and reached baseline values at the end of the experiment. They found that the infusion of sodium-lactate provides an increase in blood lactate without metabolic acidosis, which is accompanied during high intensity and lactate exercise (Schiffer et al., 2011). Previous studies have reported that also aerobic, anaerobic, high intensity interval training (HIIT) and resistance exercises increase the expression of growth factors such as BDNF and VEGF and these factors promote production of neurons and have an effect on health, cardiorespiratory indexes, body composition and performance variables (Gillen & Gibala, 2013; Roth, Elfers, Gebhardt, Müller, & Reinehr, 2013), but there is a contradiction in the results (Roth et al., 2013).

Gymnastics training is becoming very popular, fun, and basic exercise among children. Thanks to its short-time, varied, and attractive functional exercises, it has become an interesting alternative for all those who do not like long, low-intensity exercise sessions aimed at improvement in physical performance. Several factors affect the production of BDNF and VEGF such as age, physical activity, body weight, nutritional status, gender, and genetics (Fediani et al., 2014). To reduce bias,

authors conducted the equivalency of age, sex, nutritional status, and physical activity levels between two groups by exclusion criteria. The age of less than 8 or more than 12, extreme nutritional status, and a child with a history of champion and professional exercise were excluded. Recent studies showed that an exercise modifies both the synthesis and secretion of BDNF in the brain (Seifert et al., 2010). In humans, these adaptations commonly result in higher circulating BDNF concentrations (Seifert et al., 2010). Others demonstrated higher systemic BDNF concentrations after three months of "Cross-Fit", a mode of intense exercise that is in line with our research results (Murawska-Cialowicz, Wojna, & Zuwała-Jagiello, 2015). In the current study, we demonstrated that an AGE provided enough stimuli to increase salivary BDNF in healthy children. The higher systemic BDNF concentrations could be the consequence of an increase in BDNF synthesis in the brain or peripheral storage and release system.

Gymnastics is an anaerobic physical activity. During anaerobic exercise, the body including the brain needs greater oxygen and calorie supplies, results in intermittent hypoxia and hypoglycemia. Intermittent hypoxia and hypoglycemia trigger the production of Hypoxia-inducible factor 1-alpha (HIF-1a) and sirtuin proteins. These gene transcription proteins stimulate the production of factors, such as BDNF and Nerve growth factor (NGF), the synthesis of VEGF for improving blood flow, and increase the production of various antioxidants to reduce inflammation (Jones, Lee, Brown, Jarrott, & Beart, 2006; Satoh et al., 2010). Furthermore, in our study the level of VEGF in post-test was significantly different between two experimental and control groups. This suggests that AGE can induce an increase in this factor and increases both skeletal muscle mass and circulation and both processes require the up-regulation of angiogenesis.

The MHR in the progressive modified Balk test was also less than the pre-test in

the post-test. The HR is under the control of the autonomic nervous system (Almeida & Araújo, 2003) and although we did not assess parasympathetic activity, vagal activity might increase after 8-weeks of AGE in the experimental group. Vo2max increased significantly after training in the trained group. These changes indicate an improvement in cardiovascular fitness, which meant the heart, handled the workload as a relatively lighter physiological demand. A similar outcome has been reported in previous studies (Gutin et al., 2002).

Multiple possible mechanisms are involved in the effects of exercise in increasing trophic factor expression. For example, BDNF is an important mediator of the beneficial effects of exercise on brain health and plays a vital role in the function of the peripheral organs in both the central metabolic pathway and the modulation of energy metabolism (Pedersen et al., 2009). It has also been observed that increased levels of BDNF after exercise lead to increased oxidation of glucose and triglycerides, resulting in increased body temperature, energy, and oxygen consumption (Huang, Larsen, Ried-Larsen, Møller, & Andersen, 2014). Although there is no evidence in humans regarding the effects of exercise on BDNF receptor expression in peripheral tissues, studies in rodents have demonstrated that exercise increases BDNF receptor expression in skeletal muscle (Ogborn & Gardiner, 2010). Accordingly, other authors suggest that regular exercise increases BDNF sensitivity in peripheral and central organs in humans (Currie, Ramsbottom, Ludlow, Nevill, & Gilder, 2009).

Our study had some limitation such as lack of control over the excitements and other mental factors in participants, as well as their sleep and resting conditions, the small sample size and not using girls as subjects. To explore practical usage and the mechanisms that appear to increase serum BDNF and VEGF in children, it is

suggested to the next studies to use a large sample size and girl subjects in their studies.

CONCLUSIONS

In conclusion, the results of the present study suggest that anaerobic gymnastics training increases the level of salivary BDNF and VEGF in children. These types of exercises may also improve cardiorespiratory fitness in children.

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