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Anaerobic Gymnastics Exercises Evoke Systemic Brain-Derived Neurotrophic Factor in Obese and Normal-Weight Children

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Abstract

Background

Studies have shown that exercises that are anaerobic and produce lactate affect the level of Brain-derived neurotrophic family (BDNF). This study aimed to evaluate and compare the effects of anaerobic gymnastics exercise on salivary BDNF levels in obese and normal-weight boys.

Materials and Methods: In this semi-experimental study, sixty subjects with age range of 8 to 12 years old who enrolled in the elementary level of gymnastics participated in this study and were randomly divided into four groups [(obese experimental group, n=15), (obese control group, n= 15), (normal weight experimental group, n= 15), and (normal weight control group, n=15)]. Experimental groups performed 45 minutes anaerobic gymnastics exercise including 10-minute warm up, 30-minutes of main exercises, and 5 minutes cool down, 3 times per week for 8 weeks. Body composition characteristics and the levels of salivary BDNF were measured before and after 8 weeks of training. Significance was set at $p \leq 0.05$ for all analyses.

Results: The mean and standard deviations of the subjects' age in this study was 9.89±1.36 years. According to the results we found significant changes ($P<0.05$) following AGE in obese group (BDNF = +33.80%, p=0.002, weight= -8.09%, p=0.001, body fat%= -12.81, p=0.001, body fat weight= -19.38, p=0.001, lean body weight= -3.20, p=0.001), and in normal-weight group just (BDNF= +31.36%, p=0.003). Significant differences were found among obese and normal-weight groups in weight, body fat%, body fat weight, and BDNF ($p<0.05$).

Conclusion

Eight weeks anaerobic gymnastic training induces an increase in salivary BDNF levels in obese and normal-weight groups. Moreover, we demonstrated that weight decreased after our training protocol in obese children.

Key Words: Children, Growth factors, Obesity, Weight loss.

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1- INTRODUCTION

Neurotrophins are growth factors that are identified by their key role in neuronal survival and function. The family of neurotrophins consists of four proteins that are involved in the activity of the nervous system and affect the central and peripheral nervous systems. Brain-derived neurotrophic family (BDNF) and its receptor are expressed in the hippocampus and peripheral tissues such as smooth and skeletal muscle, endocrine organs, glands, and blood cells (1, 2). Notably, BDNF is a major component of the hypothalamic axis that controls body weight and plays an important role in regulating energy homeostasis, glucose metabolism, and eating behavior through the central nervous system (3). Inas et al. found that the one-year lifestyle intervention program increased serum BDNF concentration in obese children. They also suggest that the determination of BDNF concentration is a useful tool in the early diagnosis of obesity complications and early treatment of childhood obesity (4). Besides, BDNF has been implicated as part of repair mechanisms in injured muscles (5).

Various training methods including high-intensity interval training (HIIT), aerobic training, and resistance exercises (6-8) have been used to study the kinetics of BDNF. Renteria et al. showed that twelve sessions of HIIT increase circulating BDNF concentration in healthy young women (9). Another semi-experimental study was performed on twelve young healthy men. The circuit resistance exercise was performed at an intensity corresponding to 55% of 1-RM, consisted of three sets of 15 repetitions and two minutes rest between them. They showed that circuit resistance exercise led to a significant increase in levels of BDNF immediately after the exercise. Also, they suggested that acute circuit resistance exercise is a strong stimulus for the transient increase in plasma BDNF. Therefore, as a result of the beneficial effects of BDNF, this increase could be effective in brain health (10). Twenty-four immature 12 year old boys were divided into three groups of TRX suspension training (TRX), bodyweight training (BWT), and control (C). Training groups completed training programs, twice per week for eight weeks. They suggest that regular activity can provide sufficient stimulation to produce the BDNF (11). The effects of aerobic exercise on BDNF concentration in different age groups have been studied and the results showed that even a single training session can alter serum and plasma BDNF concentrations (12). In addition, other research has shown that BDNF levels do not change after 12 weeks of endurance and strength training (13). To our knowledge, no one has explored the effects of anaerobic gymnastics exercise as a basic exercise in childhood on BDNF saliva concentration. Therefore, the main purpose of this study was to determine the effect of anaerobic gymnastics exercise on saliva BDNF in obese and normal-weight children. The secondary objective was to compare the effects of AGE on BDNF concentration between two groups.

2- MATERIALS AND METHODS

2-1. Subjects and groups

This study was a semi-experimental study and the number of subjects was selected based on the available samples. Sixty subjects with age range of 8 to 12 years old who enrolled in the elementary level of gymnastics participated in this study and were randomly divided into four groups [(obese experimental group, n=15), (obese control group, n= 15), (normal weight experimental group, n= 15) and (normal weight control group, n=15)]. 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 26 and above as obesity and fat percentage of 6 to 13% as athletic category (normal weight group) without concomitant diseases (14). Exclusion criteria included evidence of any disease, drug therapy, structural abnormality, and prohibition of exercise testing. The baseline characteristics of the four groups are shown in (**Table.1**).

2-2. Ethical Approval

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 in accordance with the Declaration of Helsinki 1975 (Revised 2013). Study procedures and any possible risks during the study were explained to the subject's parents and they signed a written consent form.

2-3. Experimental procedures and protocol

Experimental groups performed 8 weeks AGE training and control groups had no exercise activities. The experimental procedures consisted of a familiarization phase (including 3 sessions for familiarization of participants to the equipment and protocols), followed by pre-testing, then 8 weeks of AGE (3 days a week), and then post-testing. The total duration of each session was 45 minutes including 10-minute warm-up, 30-minutes main exercise (15), and 5 minutes cool down that was directed by an experienced trainer. During the warm-up, subjects performed fun gymnastics movements such as running, rabbit, cat, crab, bear, and kangaroo movements. AGE included 30-second Continuous Jumping (30-s CJ), 30seconds Vertical Continuous Jumping on Box (30 seconds VCJB), Specific Aerobic Gymnast Anaerobic Test (SAGAT), and Running Jumping Rolling (RJR) (16, 17) were used for the main part. We used 30 second CJ training, because, according to the 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 it has practical applications for coaches and athletes (17). Since, 30-s VCJB, has a close relationship to the standard laboratory 30-s Wingate test and this training is so common and prevalent in gymnastics physical training, we considered this training to be one part of the main exercise. The next training was SAGAT. We used this training protocol with a little change in the difficulty of movements according to age, body composition, and fitness level of subjects. SAGAT version used in the present study was as follows: The test is comprised of 2 sets; each set was 6 consecutive repetitions. Three minute recovery time was considered between the sets. Each repetition included anaerobic exercises performed in three parts (tuck jumps (**Figure.1A**), push-up (**Figure.1B**), and sit-ups (**Figure.1C**), and subjects were asked to complete the test as quickly as they can. As shown in (**Figure.1**), the test is executed in a 10×10m stage and the starting point is "A". 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 three mentioned 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.

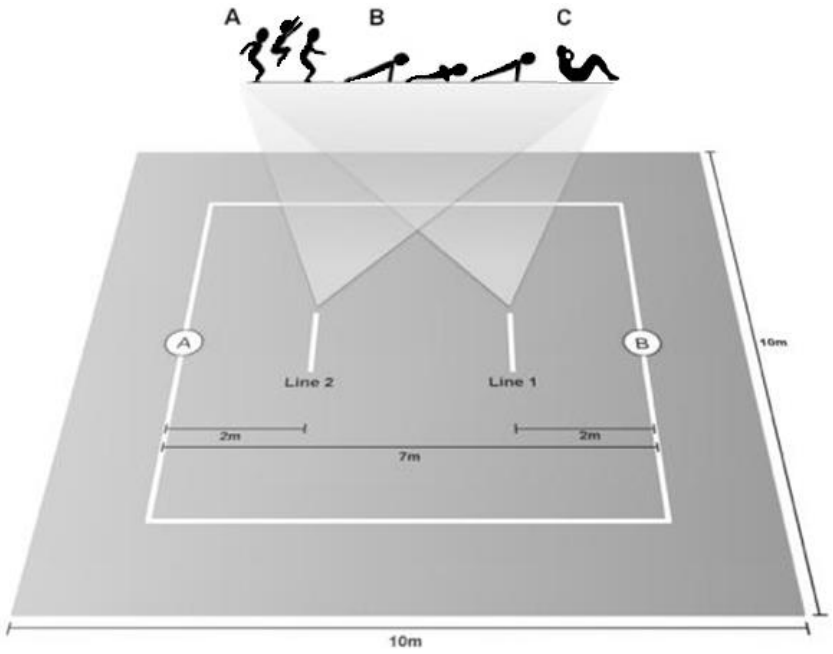

Fig.1: Illustration of SAGAT test: After the start command, the subject taps the floor and runs seven meters to "point B". At this point, the subject taps the floor again and returns two meters towards "point A" (**Line 1**). At this point the subject performs tuck jumps, push-up, and sit-ups exercises, 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.

RJR was also 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.2**). RJR test was performed in 2 sets; each set 5 repetitions with a 3-min recovery period between the sets. Each repetition was as follows: As shown in (**Figure.2**), 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 running towards "point C" to perform front-rolling. Following rolling, the subject must change the direction and run fast to reach "point D" for doing jumping over the box, then run to "point E" for performing front-rolling and at the end run to start point (point A). After completion of 5 repetitions (first set) subject recovers for three minutes and then starts the second set.

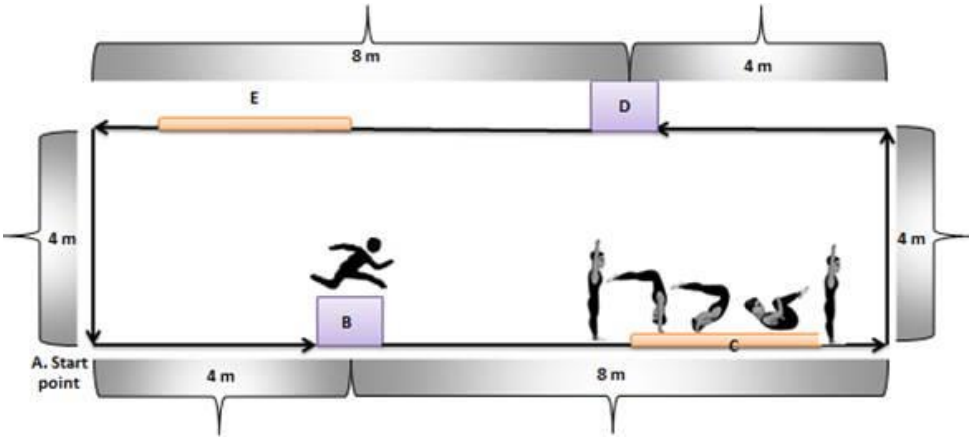

Fig.2: Illustration of RJR test. Each repetition was as follows: after the start command, the subject runs four meters toward "point B" to perform jumping over a box with a height of 50 cm, then continues running towards "point c" to perform front- rolling. Following rolling, the subject must change the direction and run fast to reach "point D" for doing jumping over the box, then run to "point E" for performing front-rolling and at the end run to start point (point A). After completion of 5 repetitions (first set) subject recovers for three minutes and then starts the second set.

2-4. Anthropometrical and body composition measurements

Anthropometrical variables including height, weight, body fat percentage (BF %), body fat weight (BFW), and lean body weight (LBW) were measured before and after eight weeks of training. Height was measured using a stadiometer with an error coefficient of 1% cm (SECA213; SECA, Hamburg, Germany). The subjects were instructed to remove their footwear and to stand in an upright position with their feet together. Weight was measured using a portable scale with an error coefficient of 1% kg (H20B; Biospace, Seoul, Korea). Subjects were requested to remove heavy clothing and to stand up straight. Three points skinfold test is a reliable method for estimating body-fat percentage that 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%. An online body composition calculator was used to obtain BF%. BFW and LBW were calculated by the following formulas (14):

BF % =495 / (1.10938- (0.0008267×s) + (0.0000016×s×s)-(0.0002574×a)) - 450
s = sum of 3 skin-fold mm a = age

BFW=Body weight× BF%, LBM=Body weight-BFW

2-5. Saliva BDNF measurement

Saliva samples were collected between 09:00 and 11:00 am. The parents/guardians and children were requested to adhere as closely as possible to the following standardized saliva collection instructions: Subjects should not consume anything and brush their teeth before sample collection because brushing may cause bleeding of the gums and blood contamination of the saliva (18). They also should rinse their mouths with water and then swallow to increase hydration. After that they should wait at least 10 minutes to avoid sample dilution. Saliva samples were collected via unstimulated passive drool over five minutes. The subjects leaned slightly

forward and tilted their heads down and filled saliva in the floor of the mouth for one minute, and saliva was subsequently swallowed. Then there was a four-minute collection where the children dripped saliva through a 5 cm plastic straw into a pre-weighed polypropylene cryovial tube (5 ml capacity). Saliva was dripped into the collecting tubes with minimal orofacial movement carefully. After collection, the samples were analyzed in the laboratory (18). Human BDNF PicoKine™ ELISA Kit (Catalog No. EK0307; R&D Systems, Austria) was used for the measurement of BDNF. 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 was performed using a sandwich enzyme-linked immunosorbent assay (19). Fluorescence was measured at 450 nm with a microplate reader. Saliva BDNF was also measured before and after 8 weeks in all groups.

2-6. Statistical Analysis

All analyses were performed using SPSS version 23.0. Data are expressed as means ± SD. The Kolmogorov–Smirnov test was used to test the normality of the distribution. Levene's test was used to determine variance differences between groups. A 2 (pre, post) by 4 (groups) repeated-measures ANOVA compared changes in the dependent measures over time and between groups. A Fisher's least significant difference (LSD) post hoc test compared differences between groups when a significant *F*-ratio was observed. Significance was set at $p \leq 0.05$ for all analyses. Change scores (Δ %) were calculated for each group on all variables as follows: Δ%= ([Post-test – Pre-test]/Pre-test) x 100.

3- RESULTS

The baseline characteristics of the participants are summarized in (**Table.1**). As can be seen in **Table.1** there was no significant difference between subjects' age in four groups ($p>0.05$). But there were significant differences in weight, BF%, BFW, and LBW ($p<0.05$).

Table-1: Baseline characteristics of study participants.

Variables	Group (n=15 for each)				
	Obese control	Obese experimental	Normal weight control	Normal weight experimental	P-value
Age (yr.)	9.80±1.32	10.13±1.35	9.86±1.30	9.80±1.47	0.89
Weight (kg)	50.60±5.76	50.73±5.04	29.43±3.66	29.40±3.56	0.001*
BF (%)	27.03±0.69	27.33±0.67	6.79±0.45	6.74±0.35	0.001*
BFW (kg)	13.52±0.71	13.81±1.89	2.09±0.25	2.06±0.17	0.001*
LBW (kg)	37.20±4.07	36.58±3.62	27.80±3.96	27.85±3.96	0.001*
BDNF (pg/ml)	0.058±0.005	0.059±0.006	0.062±0.015	0.061±0.014	0.80

Note. All values are means (± SD). BF (%) = percentage of body fat; BFW = body fat weight; LBW = lean body. weight; BDNF = brain-derived neurotropic factor. ∗the significant difference between groups ($p<0.05$).

Changes in body composition variables and BDNF after 8 weeks are presented in **Table.2**. Following 8 weeks of training, weight, BF%, BFW, and LBW significantly decreased (p=0.001), and BDNF increased (p=0.002) in the obese group. BDNF level also increased significantly in the normal-weight group (p=0.003), but changes in all variables were not significant in both control groups (p>0.05) (**Table.2**). Comparing the percentage of changes in variables between the four groups, we found that there were significant changes in the variables of weight, p= 0.001, fat percentage p= 0.005, body fat weight p=0.005, and salivary BDNF p=0.005. Fisher's least significant difference (LSD) post hoc test was used to explain differences of Δ% between each pair of groups. The differences in Δ% weight, Δ% BF%, and Δ% BFW were significant between obese experimental and obese control group, obese experimental and normal weight experimental group, and obese experimental and normal-weight control group (p<0.05). The differences in Δ% BDNF were significant between obese experimental and obese control group, the obese experimental and normal-weight control group, normal weight experimental and obese control group, normal weight experimental and normal-weight control group (p<0.05) (**Table.3**).

Table-2: Pre-training vs. post-training values for body composition variables and BDNF in the different groups.

Variables	Group (n=15 for each)			
	Obese control	Obese experimental	Normal weight control	Normal weight experimental
weight (kg)				
Pre	50.60±5.76	50.73±5.04	29.43±3.66	29.40±3.56
Post	50.70±5.75	46.46±3.97	29.96±3.58	29.73±3.42
Δ%	+0.13	-8.09	+1.89	+1.09
P-value	0.08	0.001†	0.06	0.06
BF (%)				
Pre	27.03±0.69	27.33±0.67	6.79±0.45	6.74±0.35
Post	27.17±1.06	23.84±1.53	6.88±1.25	6.84±1.21
Δ%	+0.52	-12.81	+1.37	+1.27
P-value	0.44	0.001†	0.78	0.75
BFW (kg)				
Pre	13.52±0.71	13.81±1.89	2.09±0.25	2.06±0.17
Post	13.50±1.74	11.15±1.73	2.10±0.58	2.07±0.55
Δ%	− 0.18	-19.38	+ 0.16	+ 0.009
P-value	0.94	0.001†	0.91	0.96
LBW (kg)				
Pre	37.20±4.07	36.58±3.62	27.80±3.96	27.85±3.96
Post	37.26±4.05	35.38±3.22	27.28±3.98	27.34±3.99
Δ%	+0.16	-3.20	-1.47	− 1.46
P-value	0.35	0.001†	0.45	0.45
BDNF (pg/ml)				
Pre	0.058±0.005	0.059±0.006	0.062±0.015	0.061±0.014
Post	0.061±0.002	0.078±0.019	0.062±0.004	0.078±0.022
Δ%	+5.11	+33.80	+8.82	+31.36
P-value	0.13	0.002†	1.00	0.003†

Note. All values are means (± SD). BF (%) = percentage of body fat; BFW = body fat weight; LBW = lean body weight; BDNF = brain-derived neurotropic factor. †Significantly changes than pre-training value ($p < 0.05$).

Table-3: Results of LSD post hoc test for comparison of body composition and BDNF variables Δ% between each pair of the groups.

Variables		Group	Mean difference (Δ %)	P-value
Weight	Obese control	Obese experimental	8.23	0.001*
		Normal weight control	-1.76	0.06
		Normal weight experimental	-0.95	0.31
	Obese experimental	Normal weight control	-9.99	0.001*
		Normal weight experimental	-9.18	0.001*
	Normal weight control	Normal weight experimental	0.80	0.39
Body fat %	Obese control	Obese experimental	+13.34	0.004*
		Normal weight control	-0.84	0.85
		Normal weight experimental	-0.74	0.86
	Obese experimental	Normal weight control	-14.18	0.003*
		Normal weight experimental	-14.08	0.003*
	Normal weight control	Normal weight experimental	0.10	0.98
Body fat weight	Obese control	Obese experimental	+19.20	0.004*
		Normal weight control	-0.34	0.95
		Normal weight experimental	-0.19	0.97
	Obese experimental	Normal weight control	-19.54	0.003*
		Normal weight experimental	-19.39	0.003*
	Normal weight control	Normal weight experimental	0.15	0.98
BDNF	Obese control	Obese experimental	-28.69	0.004*
		Normal weight control	-3.71	0.70
		Normal weight experimental	-26.25	0.009*
	Obese experimental	Normal weight control	+24.98	0.01*
		Normal weight experimental	+2.44	0.80
	Normal weight control	Normal weight experimental	-22.54	0.02*

Note. All values are means (± SD). ∗Between groups comparison value ($p<0.05$), BDNF = brain-derived neurotropic factor.

4- DISCUSSION

According to the literature that we studied, this is the first study to examine the effect of AGE on salivary BDNF and weight control in children. The main findings of the present study were that AGE increased salivary BDNF levels in both obese and normal-weight children. It reduced weight in the obese group but not in the normal-weight group. The important point for us for the utilization of AGE in this research was the studies in humans that used an infusion of sodium-lactate and reported the increased BDNF plasma level after infusion (20). Schiffer et al. showed that lactate infusion at rest led to a significant increase in blood BDNF concentration in young and healthy men (20). All types of exercise and even more intense exercise emphasize anaerobic metabolic pathways resulting in the production of lactic acid (21). Higher blood lactate indicates a higher intensity of exercise. Ferris et al. suggested that the amount of increase in BDNF level is dependent on the intensity of exercise and accordingly, higher blood BDNF concentrations in higher intensities of exercises can be related to higher blood lactate concentrations (22). However, it is not known whether lactate itself or other exercise-related factors such as pH or blood gases increase BDNF. But lactate can act as a pseudo-hormone. The brain can utilize an active shuttle of astrocyte neurons and neurons composed of muscles

(23). Nike et al. suggested that lactate may be a peripheral exercise signal that regulates skeletal muscle mass used by motoneurons during exercise (23, 24). Nike et al. suggested that lactate may be a peripheral exercise signal that regulates skeletal muscle mass used by motoneurons during exercise (25). However, the lactate promoter functions as a potential hormone enabling lactates to support BDNF production at its major secretion site during exercise in the central nervous system (1, 26). BDNF is a neurotrophin that is expressed in different regions of the human brain (27). Studies reported higher concentrations of systemic BDNF after a period of resistance training including TRX, and CrossFit which is a high-intensity exercise mode (10, 11, 28).

Renteria et al. showed that twelve sessions of HIIT increase circulating BDNF concentrations in healthy women (9). Past research has shown that aerobic exercise also stimulates the synthesis and secretion of BDNF in the brain (26, 29). In the present study, we showed that an AGE provided sufficient stimulus to increase salivary BDNF in obese and normal-weight children. Higher concentrations of systemic BDNF can result from increased synthesis of BDNF in the brain or peripheral organs. Other exercise models have been used to study the effects of BDNF. For instance, Cho et al. suggested that high-intensity Taekwondo training may help to improve children's cognitive function, and an increase in the levels of BDNF (30). Hwang et al. employed an exercise model of 20-min running on a treadmill (2 minutes warm-up, 5 minutes to reach a target heart rate (HR) relative to the subject's 85-90% VO2 max (maximal oxygen consumption), 10 minutes running at the target HR, 3 minutes recovery) on young healthy women. Their findings provide support to increased BDNF in response to acute exercise. (31). Similarly, new research showed higher circulating BDNF levels after high-intensity exercise in men (32). Although there is no evidence in humans about the effects of physical activity on BDNF receptor expression in peripheral tissues, studies in animal models have shown that physical activity increases BDNF receptor expression in skeletal muscle (33). For that reason, other authors offered that regular physical activity increases BDNF sensitivity in peripheral and central members in humans (34). However some studies are in contrast with other recent work and find lower circulating BDNF levels after physical activity (35). Interestingly BDNF is considered the main constituent of the hypothalamic axis which controls body weight and plays an important role in regulating energy homeostasis, glucose metabolism, and eating manner through the central nervous system (7). It has been demonstrated that serum BDNF increases during physical activity and muscle-derived BDNF increases fatty acid oxidation in skeletal muscle and induces weight loss in obese children (7, 25).

Gymnastics is a high energy demanding sport. Accordingly the body requires high energy expenditure and oxygen during gymnastics training, leading to serial hypoxia and hypoglycemia. Resulted hypoxia and hypoglycemia stimulates the synthesis of hypoxia-inducible factor 1-alpha (HIF-1a), and sirtuin proteins and BDNF factor (30). Recent research demonstrated that increased levels of BDNF after exercise lead to increased oxidation of glucose and triglycerides, resulting in increased body temperature, energy, and oxygen consumption (36). In our study weight, BF%, and BFW decreased significantly after eight weeks AGE that is in line with the results of previous studies (36, 37). It seems that the overweight in the obese group has made our AGE more effective than the normal-weight group and has enough stimulation to weight loss in the obese group.

4-1. Study Limitations

Our work had its limitations, such as the lack of girls and the small sample size used. To explore practical usage and the mechanisms that appear to increase serum BDNF in children, it is suggested that future studies use a large sample size, and female subjects in their studies.

5- CONCLUSION

In summary, the results of the present study revealed that salivary BDNF concentrations increase after 8 weeks of AGE in both obese and normal-weight children. Moreover, we demonstrate that weight decreased after our training protocol in obese children.

6- CONFLICT OF INTEREST: None.

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