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Original Article

Effect of anaerobic gymnastics exercise on vascular endothelial growth factor in obese boys

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Abstract

Background Vascular endothelial growth factor (VEGF) is the most important growth factor involved in angiogenesis and appears to be mediated through exercise training, lead***ing to increased blood lactate.***

Objective To evaluate and compare the effects of anaerobic gymnastics exercise (AGE) on systemic VEGF in obese and normoweight boys.

Methods Sixty boys aged 8 to 12 years who enrolled in elementary level of gymnastics participated in this study and were randomly divided into four groups of 15 subjects each: obese AGE, obese control, normoweight AGE, and normoweight control. The control group didn't have any exercise during the study. The experimental groups performed 45 minutes of AGE 3 times per week for 8 weeks, which included a 10-minute warm-up, 30-minute main exercises, and 5-minute cool down. Body composition characteristics and VEGF levels in saliva were measured before and after 8 weeks of training.

Results Significant changes following AGE were found in the obese group in terms of weight (-8.09%; P=0.001), body fat% (BF%) (-12.81; P=0.001), body fat weight (BFW) (-19.38; P=0.001), and lean body weight (LBW) (-3.20; P=0.001). Saliva levels of VEGF increased post-AGE in the obese (+21.64%; P=0.79) and normoweight groups (+28.22; P=0.06), but the differences were not significant. Significant differences in weight, BF%, and BFW were found between obese AGE group with obese control, normoweight control and normoweight AGE (P<0.05).

Conclusion Circulating VEGF concentrations slightly increase after 8 weeks of AGE in obese and normal-weight groups. Moreover, we demonstrate that weight significantly decreased in obese children after they engaged in AGE training. **[Paediatr Indones. 2020;60:293-202 ; DOI: 10.14238/pi60.4.2020.293-302**].

Keywords: *pediatric obesity; endothelial growth factors; anaerobic gymnastics; weight loss*

Increasing physical activity through exercise training programs has been shown to improve vascular remodeling and angiogenesis.[1] Therefore, exercise interventions may be especially important to prevent health complications and growth factor problems. Many growth factors are involved in the angiogenesis process, but vascular endothelial growth factor (VEGF) is reportedly the most important growth factor involved in this process.[2] The VEGF is a 45-kDa glycoprotein secreted from tumor cells and endothelial cells.[3] This glycoprotein delivers its signaling through binding to vascular endothelial growth factor receptor-1 (VEGFR-1) and vascular endothelial growth factor receptor-2 (VEGFR-2) in endothelial cells. The VEGF promotes endothelial cell survival, proliferation, migration, and permeability through increased regulation of anti-apoptotic components, DNA synthesis,

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basement membrane destruction, phosphorylation of intercellular adhesion components and tight junctions.[4,5] Angiogenesis causes vessels to develop by two processes, sprouting and intussusception. Many factors affect VEGF expression, including hypoxia, intensity and duration of exercise training, as well as shear stress.[6]

A study hypothesized that the lactate receptor, hydroxyl carboxylic acid receptor 1 (HCAR1; also known as HCA1 or GPR81), can be considered as the initial molecular signal that leads to increased cerebral VEGF-A in response to exercise.[7] They measured VEGF-A levels in wild-type and Hcar1 knockout mice after exercise or L-lactate treatment, and found increased VEGF-A levels in the hippocampus of wild-type mice after exercise or L-lactate treatment compared to wild-type controls. In knockout mice, neither of the treatments increased VEGF levels above baseline. This observation indicates a direct link between HCAR1 activation and VEGF-A signaling, resulting in angiogenesis. No similar changes in VEGF-A were observed in the cerebellum.[7] Angiogenesis in the developing nervous system as well as in response to exercise is regulated by VEGF-A.[8] A previous study showed that serum VEGF levels did not change in obese 50 to 60 years men after 6 months of regular exercise.[9] However, another study showed that exercise training increased VEGF gene expression in adipose tissue and was an important angiogenesis factor in this tissue.[10] Also a study demonstrated that extra adipose tissue increases VEGF levels in obese individuals, while weight loss and decreased adipose tissue caused a decrease in VEGF in obese individuals.[11] Another study showed that serum VEGF levels were affected by weight changes, but was not significantly reduced in the weight loss group. Moreover, weight loss was generally associated with a decrease in angiogenesis activity in circulation.[12]

Plasma VEGF level is associated with body composition, obesity, and weight loss.[8,13] The VEGF increases vascular permeability and vasodilatation. In addition, pathogenesis of cardiovascular risk factors, arteriosclerosis, obesity, and metabolism morbidity and mortality are related to VEGF.[14] Also adipose tissue produces VEGF in a considerable amount as an endocrine organ.[15] Overweight and obese individuals have been shown to have elevated serum VEGF levels.[16,17] In a study of subjects who lost at least 1.85% of their baseline fat mass in a 12-month exercise intervention, significant reductions in some biomarkers of angiogenesis were observed, compared to sedentary controls.[18] To build on this study, we hypothesized that the increased presence of secretory adipose tissue in obese subjects would result in systemic elevations of VEGF, and that AGE would affect body composition and VEGF levels in trained groups compared to control groups.

Past study suggests that angiogenesis in response to lactate-producing exercise is regulated by VEGF, yet some results were contradictory. To our knowledge, no one has explored the effects of anaerobic gymnastics exercise on VEGF concentration in children. Thus, we aimed to assess for an effect of AGE on salivary VEGF in obese and normoweight boys. Our secondary objective was to compare VEGF concentration before and after AGE intervention in obese and normoweight boys.

Methods

Sixty boys aged 8 to 12 years who enrolled in elementary level gymnastics participated in this study. At first obese and normoweight boys separated from each other, then were randomly divided into four groups of 15 subjects each: obese AGE, obese control, normoweight AGE, and normoweight control. Subjects were diagnosed based on the American Council on Exercise Chart using the Jackson and Pollock equation for three-point subcutaneous fat measurements.[19] Children with fat percentages of 26% or above were considered to be obese, and 6 to 13% as athletic (normoweight), without concomitant diseases.[19] Exclusion criteria were evidence of any disease, drug therapy, structural abnormality, or prohibition of exercise testing. The study protocol was approved by the Ethics Committee of Ardabil University of Medical Science and the Iranian Registry of Clinical Trials. This study was performed in accordance with the 1975 Declaration of Helsinki (revised 2013). Study procedures and any possible risks were explained to the subjects' parents, who provided written informed consent.

The experimental procedures consisted of a familiarization phase (including 3 sessions to familiarize the participants with the equipment and

protocols and to reduce any learning effect), followed by pre-testing, 8 weeks of AGE, and post-testing. The total duration of each session was 45 minutes, including a 10-minute warm-up, 30-minute AGE, and 5-minute cool down, directed by an experienced trainer. During the warm-up, subjects performed fun gymnastics movements such as running, rabbit, cat, crab, bear, and kangaroo movements. The AGE main exercise included 30-second continuous jump (30-s CJ), 30-s vertical continuous jump on box (30-s VCJB), specific aerobic gymnast anaerobic test (SAGAT), and running jumping rolling (RJR) (**Figure 1**).[20,21]

We used 30-s CJ training, because, according to previous studies, the continuous jump test was more specific for acyclic sports such as gymnastics, basketball, and volleyball. These sports involved a similar movement pattern; hence, CJ had a practical application for coaches and athletes.[21]

Since 30-s VCJB has a close relationship to the standard laboratory 30-s Wingate test[21] and this training was common in gymnastics physical training, we included it as part of the main exercise. In addition, SAGAT was used in the training protocol with small changes in the level of difficulty, according to subjects' age, body composition, and fitness level. The SAGAT version used was comprised of 2 sets of 6 consecutive repetitions. Recovery time was 3 minutes between sets. Each repetition included anaerobic exercises performed in three parts [tuck jumps (**Figure 2A**), push-ups (**Figure 2B**), and sit-ups (**Figure 2C**)]; subjects were asked to complete the test as quickly as they could. As shown in (**Figure 2**), a test was executed in a 10×10m stage, with starting point "A". After the start command, the subject tapped the floor and ran seven meters to "point B". At that point, the subject tapped the floor again and returned two meters towards "point A" (Line 1). At that point, the subject performed the three aforementioned exercises, one time each, then he returned to "point B" and tapped the floor. This was the end of the first repetition and the start of the second repetition. The subject again ran seven meters to "point A", tapped the floor, returned two meters towards "point B" (Line 2), performed the exercises described above, returned to "point A" and tapped the floor, indicating the end of the second repetition and start of the third repetition. This pattern continued until a total of 6 repetitions were completed.

The RJR was included in each session because of its anaerobic essence; it consisted of jumping over a box and front-rolling. Two sets of 5 repetitions each were performed with a 3-min recovery period between sets. Each repetition was as follows: after the start command, the subject ran four meters to "point B", jumped over a box (height of 50 cm), ran to "point C," and performed a front roll. Following the front roll, the subject changed direction, ran to "point D," jumped over the box, ran to "point E" to perform a front roll, then ran back to the start point (point A). After completing 5 repetitions (first set), the subject recovered for three minutes then started the second set (**Figure 3**).

Anthropometric variables including height, weight, body fat percentage (BF%), body fat weight (BFW), and lean body weight (LBW) were measured

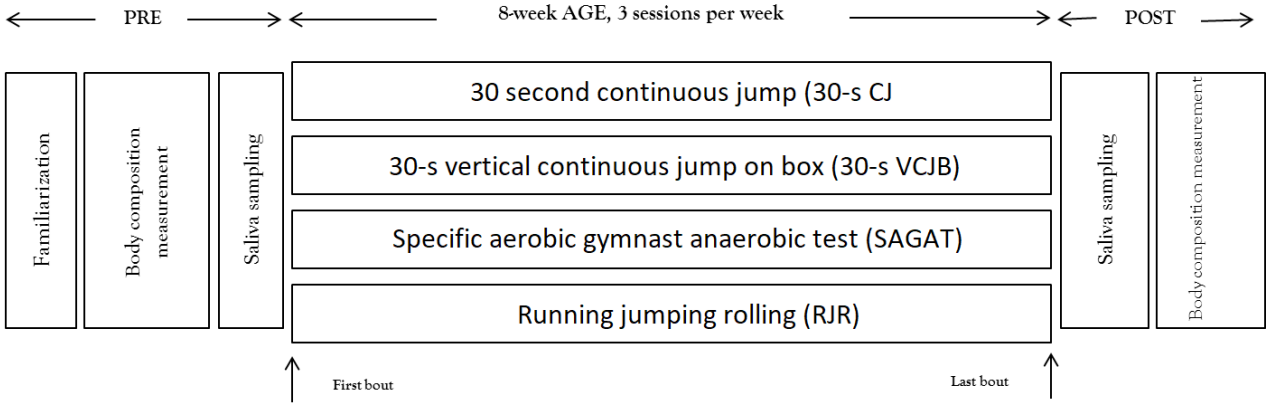

Figure 1. Illustration of experimental procedures

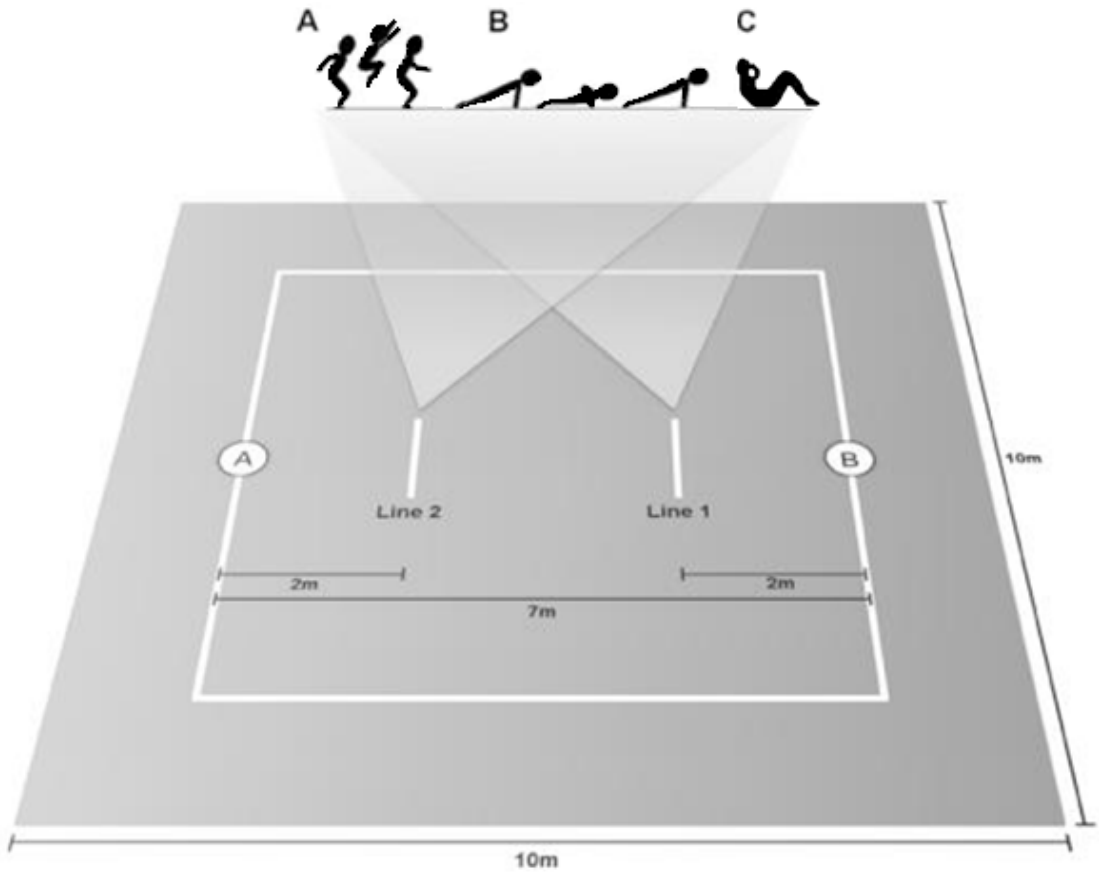

Figure 2. Illustration of SAGAT test

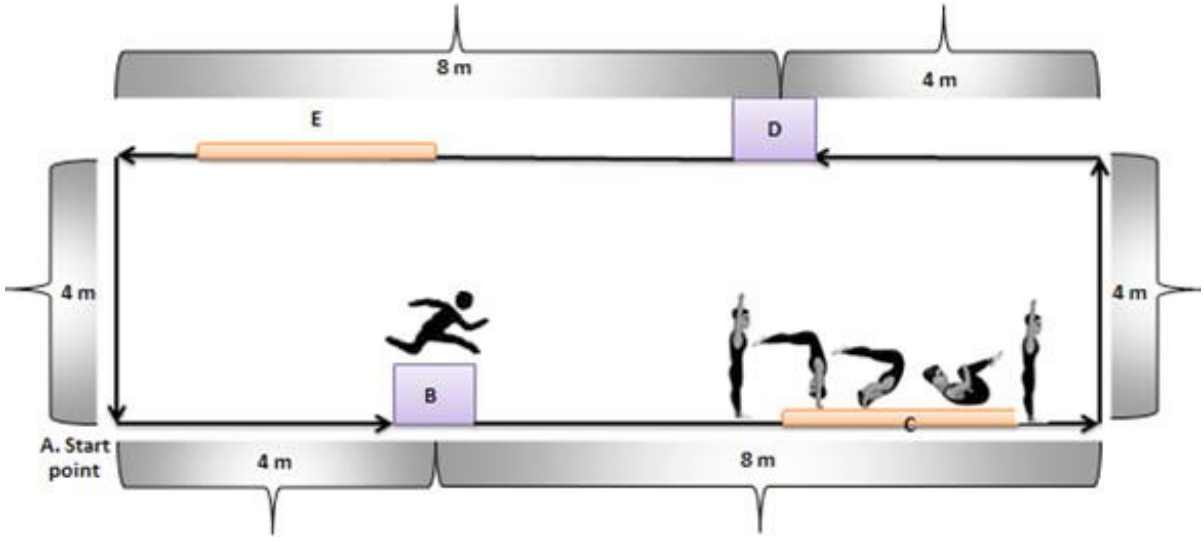

Figure 3. Illustration of the RJR test

before and after eight weeks of training. Height was measured using a stadiometer with an error coefficient of 1cm (*SECA213*; SECA, Hamburg, Germany), with subjects standing in an upright position, feet together, and no footwear. Weight was measured using a portable scale with an error coefficient of 1kg (*H20B*; Biospace, Seoul, Korea), with subjects wearing light clothing and standing upright. Body-fat percentage was estimated by the three-point skinfold test. A Harpenden caliper was used to measure three sites: (quadriceps), chest (pectoral), and belly (abdomen). The Jackson/Pollock 3-site equation (online body composition calculator) was used to predict BF%.[19]

$$BF\% = \frac{495}{[(1.10938 - (0.0008267*s) + (0.0000016*s*s) - (0.0002574*a)]} - 450$$

Notes: s=sum of 3 skin-fold mm, a=age

Afterward the BFW and LBW were calculated by following formulas: BFW=body weight x BF% and LBW = body weight - BFW.[19]

Saliva specimens 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: subjects should not consume food or drink nor brush their teeth before specimen collection, because brushing might cause bleeding of the gums and blood contamination of the saliva.[22] They also rinsed their mouths with water then swallowed to increase oral hydration. After that they waited at least 10 minutes to avoid specimen dilution. Saliva specimens were collected via unstimulated passive drool for five minutes. The subject would lean slightly forward, tilt the head down, let saliva filled the floor of the mouth for one minute, then swallowed that saliva. Subsequently, the child dripped saliva through a 5-cm plastic straw into a pre-weighed polypropylene cryovial tube (5 mL capacity) for 4 minutes. Saliva was carefully dripped into the collecting tubes with minimal orofacial movement. After collection, the specimens were analyzed in a laboratory.[22] *Human VEGF PicoKine™ ELISA Kit* (Catalog No. EK0539; R&D Systems, Austria) was used to measure VEGF, according to manufacturer's instructions. Subjects in all groups underwent saliva VEGF measurements before and after 8 weeks.

All analyses were performed using *SPSS version 23.0*. Data were expressed as means (SD). The Kolmogorov-Smirnov test was used to test the distribution normality. A 2 (pre, post) by 4 (groups) repeated-measures ANOVA test 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. Change scores (Δ %) were calculated for each group on all variables as follows: Δ%= [(post-test – pre-test)/pre-test] x 100.

Results

The baseline characteristics of participants are summarized in **Table 1**. There were significant differences in baseline height, weight, BF%, BFW and LBW variables between all obese vs. normoweight.

Changes in body composition variables and VEGF are presented in **Table 2**. Following 8 weeks of training, the weight, BF%, BFW, and LBW significantly decreased only in the obese AGE group (P=0.001). Although VEGF increased by +21.64% in the obese AGE group and +28.22% in the normoweight AGE

Table 1. Baseline characteristics of study participants

Characteristics	Obese control (n=15)	Obese AGE (n=15)	Normoweight control (n=15)	Normoweight AGE (n=15)	P value
Mean age, years (SD)	9.80 (1.32)	10.13 (1.35)	9.86 (1.30)	9.80 (1.47)	0.89
Mean height, cm (SD)	141.02 (9.88)	141.64 (6.55)	133.37 (5.03)	133.60 (5.36)	0.001*
Mean weight, kg (SD)	50.60 (5.76)	50.73 (5.04)	29.43 (3.66)	29.40 (3.56)	0.001*
Mean body fat %, (SD)	27.03 (0.69)	27.33 (0.67)	6.79 (0.45)	6.74 (0.35)	0.001*
Mean body fat weight, kg (SD)	13.52 (0.71)	13.81 (1.89)	2.09 (0.25)	2.06 (0.17)	0.001*
Mean lean body weight, kg (SD)	37.20 (4.07)	36.58 (3.62)	27.80 (3.96)	27.85 (3.96)	0.001*
Mean VEGF, pg/mL (SD)	1.551 (0.433)	1.367 (0.368)	1.593 (0.401)	1.631 (0.437)	0.31

*between groups comparison value (P□0.05)

group, these changes were not significant (P>0.05) (**Table 2**).

The comparison of percentage changes in body composition and VEGF after 8 weeks of AGE are presented in **Table 3**. After 8-weeks AGE there was a significant difference between groups in weight, BF%, and BFW variables (P<0.05).

Fisher's least significant difference (LSD) post-hoc test was used to explain the Δ% between-group differences (**Table 4**). There were significant differences in Δ% weight, Δ% BF%, and Δ% BFW in the following comparisons: control for obese AGE vs.

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

Variables	Group (N=15 for each)			
	Obese control	Obese AGE	Normoweight control	Normoweight AGE
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
VEGF, pg/ml				
Pre	1.551 (0.433)	1.367 (0.368)	1.593 (0.401)	1.631 (0.437)
Post	1.670 (0.420)	1.561 (0.472)	1.614 (0.406)	1.928 (0.428)
%Δ	+13.86	+21.64	+1.51	+28.22
P value	0.34	0.79	0.39	0.06

Notes: All values are mean (SD); Δ%=percentage change, BF%=percentage of body fat; BFW=body fat weight; LBW=lean body weight; VEGF=vascular endothelial growth factor. †Significantly greater than pre-training value (P< 0.05).

Table 3. Comparison of percentage changes in body composition and VEGF variables after 8 weeks AGE.

Variables	Obese control (n=15)	Obese AGE (n=15)	Normoweight control (n=15)	NormoweightAGE (n=15)	P value
Weight, kg	50.70 (5.75)	46.46 (3.97)	29.96 (3.58)	29.73 (3.42)	0.001*
Δ%	+0.13	-8.09	+1.89	+1.09	
BF%	27.17 (1.06)	23.84 (1.53)	6.88 (1.25)	6.84 (1.21)	0.005*
Δ%	+0.52	-12.81	+1.37	+1.27	
BFW, kg	13.50 (1.74)	11.15 (1.73)	2.10 (0.58)	2.07 (0.55)	0.005*
Δ%	-0.18	-19.38	+0.16	+0.009	
LBW, kg	37.26 (4.05)	35.38 (3.22)	27.28 (3.98)	27.34 (3.99)	0.517
Δ%	+0.16	-3.20	-1.47	-1.46	
VEGF,pg/mL	1.670 (0.420)	1.561 (0.472)	1.614 (0.406)	1.928 (0.428)	0.272
Δ%	+13.86	+21.64	+1.51	+28.22	

Notes: All values are mean (SD); Δ%=percentage change, BF%=percentage of body fat; BFW=body fat weight; LBW=lean body weight; VEGF=vascular endothelial growth factor. *between groups comparison value (P<0.05).

Table 4. LSD post-hoc analysis of Δ% between-group comparisons of body composition variables

Variables	Group		Mean difference	P value
Weight	Obese control	Obese AGE	8.23	0.001*
		Normoweight control	-1.76	0.06
		Normoweight AGE	-0.95	0.31
	Obese AGE	Normoweight control	-9.99	0.001*
		Normoweight AGE	-9.18	0.001*
	Normoweight control	Normoweight AGE	0.80	0.39
Body fat %	Obese control	Obese AGE	+13.34	0.004*
		Normoweight control	-0.84	0.85
		Normoweight AGE	-0.74	0.86
	Obese AGE	Normoweight control	-14.18	0.003*
		Normoweight AGE	-14.08	0.003*
	Normoweight control	Normoweight AGE	0.10	0.98
Body fat weight	Obese control	Obese AGE	+19.20	0.004*
		Normoweight control	-0.34	0.95
		Normoweight AGE	-0.19	0.97
	Obese AGE	Normoweight control	-19.54	0.003*
		Normoweight AGE	-19.39	0.003*
	Normoweight control	Normal weight AGE	0.15	0.98

Note: all values are mean (SD). *between groups comparison value (P<0.05)

obese control, obese AGE vs. normoweight control, and obese AGE *vs.* normoweight AGE (P<0.05) (**Table 4**).

Discussion

To our knowledge, this study is the first to examine AGE in children. Our main findings did not support our hypothesis that AGE affects body composition and VEGF in trained groups compared to control groups who did not exercise, with the exception of body variables in the obese AGE group *vs.* obese control group. Mean body weight of obese children decreased after AGE. In addition, salivary VEGF concentration in experimental obese and normoweight groups increased after 8 weeks of AGE, although the percentage changes were not significant. In addition to the intensity and duration of physical activity, other factors influence the increase in VEGF concentration, such as mechanical, metabolic, and hormonal stimuli, as well as volume of physical activity.[23] Therefore, the angiogenesis may increase in the experimental groups compared to the control groups, probably due to mechanisms of shear stress, metabolite accumulation, and muscle tension, or the interaction of these factors to increase systemic VEGF concentration and growth of new capillaries.[24] A study reported increased serum VEGF expression after 6 weeks of exercise and concluded that exercise stimulated VEGF production in skeletal muscle.[12] Another study showed that a two-week combined training program VEGF increased in obese subjects and that exercise could improve metabolism and VEGF regulation by increasing blood flow velocity.[25] Nevertheless, our results were not in line with previous studies indicating that overweight and obese individuals display elevated serum VEGF levels.[11,16,17] Adipose tissue may act as an endocrine organ that produces considerable amounts of VEGF. Also, adipose tissue is highly plastic, and requires vascularization to expand.[15] Extra adipose tissue causes an increase in the VEGF levels in obese individuals; weight loss and decreased adipose tissue may decrease their VEGF levels.[11] In a study of obese subjects randomized to either a diet or combination of diet and exercise groups, reduced VEGF and weight loss were observed in only the combined exercise and diet group. Hence, their findings indicated that exercise alone did not affect VEGF in post-menopausal women.[11] Another study showed that serum VEGF-A levels were significantly higher in obese patients than in lean controls; VEGF-A decreased after weight loss with bariatric surgery.[16] Although in this study the

weight of obese children decreased after 8 weeks of AGE, their systemic VEGF levels did not decrease, but slightly increased. This observation might have been related to the effect of exercise on VEGF. Exercise increases both skeletal muscle mass and blood circulation and both processes require up-regulation of angiogenesis.[26]

Exercise improves blood flow velocity and increases blood flow capacity.[26] This increase in blood flow capacity is one of the factors that causes the release of higher serum VEGF levels in trained compared to untrained individuals. A previous study showed that high exercise intensity increases vasodilators and vasodilation.[27] In our study, one reason for the slightly increased systemic VEGF levels in the trained groups may have been the intensity of exercise. During exercise, several stimuli combine to provide vascularization of skeletal muscle tissue, including the ischemic and hypoxic conditions of the skeletal muscle, increased blood flow (shear stress), vasodilators caused by increased shear stress, mechanical stretching of the tissue, skeletal muscle contraction, and the resulting metabolites. The rate of secretion of angiogenesis agents varies depending on the type of stimulus applied. On the other hand, stimulation of VEGF-mRNA release and VEGF protein may be different. Because of the specific exercise intervention in this study, all of the above factors could have been the cause of increased VEGF in the trained groups. To answer which of these mechanisms contributed the most was not in the scope and purpose of this study, so further research is needed.

In our study, weight, BF%, and BFW significantly decreased in obese children after eight weeks of AGE, similar to results of previous studies.[28,29] A previous study has shown that physical fitness in obese individuals is significantly lower than in normoweight individuals.30 Another study found that lower levels of physical fitness in obese individuals are indicative of inactivity compared to normoweight individuals. Inactivity is one of the causes of insufficient physical fitness in obese people.[31] We noted that AGE was more effective in the obese group than in the normoweight group, in terms of greater weight loss. The omission of girls in our study and the small sample size limits the areas of application and emphasizes the need for extra studies to explore practical usage and mechanisms that appear to increase saliva VEGF in children. In conclusion, saliva VEGF concentrations increase not significantly after 8 weeks of AGE in both obese and normoweight boys. We suggest that if anaerobic exercise is used for a longer period of time instead of 8 weeks, it is likely that the increase in salivary VEGF levels will be significant. Moreover, obese boys had significant weight decrease after the training protocol.

Conflict of Interest

None declared.

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