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Effect of exercise training on saliva brain derived neurotrophic factor, catalase and vitamin c

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Abstract

Background: The balance between production of Reactive Oxygen Species (ROS) and antioxidant defense in the body has important health implications. The aim of this study was to investigate the changes in salivary antioxidants: catalase, vitamin C and brain-derived neurotrophic factor (BDNF), in sedentary men at rest and after acute exhaustive exercise.

Methods: This randomized controlled clinical trial (The registry code IRCT2011053212431N1) recruited twenty-five sedentary men (age=21±3yrs; height=172±8cm; weight=66±9kg; VO2 max=37.6±7.4mL•kg^{-1}•min^{-1}) participated in a double-blind randomized experiment. Unstimulated whole saliva samples were collected before, immediately and 1 hour after exhaustive treadmill running. Catalase, vitamin C (Vit C) concentration, and BDNF concentrations were determined using biochemical assays and ELISA respectively. Repeated measures ANOVA and Bonferroni posthoc test were used to analyze data.

Results: The results of the present study showed that an acute intensive exercise causes a reduction in salivary catalase, Vit C and also BDNF concentration ($p<0.05$) compared with pre-exercise. Both catalase and Vit C showed a tendency to return to pre-exercise value after one hour. However, BDNF continued to reduction at least 1 hour after the ending of the training.

Conclusion: Reduction in antioxidants capacity of saliva might reflects disturbance in natural antioxidant defense mechanisms of the body after an acute intensive physical stress and possible further health threatening consequences.

Keywords: Saliva, Antioxidants, Catalase, Vitamin C, BDNF, Exercise.

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Introduction

The balance between production of Reactive Oxygen Species (ROS) and antioxidant defense in the body has important health implications. Reactive oxygen species, such as superoxide, hydrogen peroxide induce aging and apoptosis (1,2). Although it is assumed that most of these species are scavenged by endogenous antioxidant systems, such as: superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase, vitamins C and E (3,4). Physical activity despite all health benefits (5), leads to the generation of ROS due to the increase in metabolic rate and oxygen consumption (6) and causes detrimental consequences for the body (2,7). In our previous study similar to other studies (8,9), we showed that a short protocol of exercise causes an elevation in saliva uric acid and superoxide dismutase (10). However, there is one study showing a reduction in some of the salivary

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antioxidants after strenuous endurance training in humans (11). Considering the fact that human saliva is the first important body fluid to encounter the exogenous materials in the oral cavity, and also being easily accessible, (12) has got great importance in studying human health status in recent years. Saliva consists of proteins, ions and other organic compounds produced by salivary glands (parotid, submandibular and sublingual) and a small portion of the blood (13,14). Moreover, human saliva contains a complex antioxidant system including peroxidase, superoxide dismutase, catalase, glutathione reductase, vitamin C and uric acid (13,14). Vitamin C is a water-soluble keto lactone with two ionizable hydroxyl groups involving in enzymatic reactions that are catalyzed by members of the Fe^{2+}– 2-oxoglutarate-dependent families of dioxygenases. Vitamin C as a low-molecular-weight, water soluble antioxidant, (15,16) serve to protect cells and tissues from oxidative damage. Vitamin C contributes to the intracellular and extracellular redox by transferring electrons across the plasma membrane (16). Catalase; another salivary potent antioxidant is a heme protein enzyme, which catalysis the hydrogen peroxide into water and oxygen (17, 18).

In addition, Brain-derived neurotrophic factor (BDNF) as an important functional regulator of cell survival, (19) metabotropic and neurotrophic factor (20,21), antioxidative stress factor (22) plays an important role in homeostasis. There are great evidences showing protecting effect of chronic exercise against exercise-induced oxidative stress by up-regulating endogenous antioxidant defense systems and BDNF (23,24). However whether or not saliva could be used as a source reflecting the effects afforded by acute exhaustive exercise on antioxidants has not been addressed yet. Therefore, the aim of this study was to determine the change in antioxidant biomarkers of saliva in response to acute exercise in sedentary men.

Methods

Subject

A total of twenty–five healthy sedentary male students from the University of Guilan participated in this research voluntarily. This study was approved by the local ethics committee of the Guilan University of Medical Sciences and performed according to the principles of the Declaration of Helsinki. The study was started after signing an informed consent document by all the subjects (The registry code IRCT 2011053212 431N1).

Subjects' characteristics

Subjects' weight and height were measured using an electronic scale and vertical stadiometer, respectively. Skinfold measurement was also taken with a caliper from 3 points of abdominal, chest and thigh for men (25). Body densities were calculated using Aguirre and et al. equation, (26) and were converted to body fat percent by Fosbøl and et al. formula (27). All skin fold measures were taken by one examiner. Characteristics of participants are shown in Table 1. All of the subjects were lived in university dormitory and 7 days before exhaustive exercise, nutrition of all subjects were controlled. Average food intakes of them in 7 days were analyzed with software (Nutritionist 4) and shown in Table 2. Hydration status of subjects was standardized with consuming of 500 ml water, 90 min

Table 1. Physical and physiological characteristics of the subjects

Parameters	Value
Age (years)	21.1±3
Weight (kg)	67±2.2
Height (28)	172±8
Body fat (%)	17.5±4.4
Body mass index(kg/m^2)	22.5±2
VO_{2max} (ml.kg-1.min-1)	37.6±7.4

Values are given as mean ± SD

Table 2. Dietary intake assessed during the 3 days before each exercise session

Parameters	Value
Energy(kcal)	2444±211.2
Carbohydrate (gr)	357.2±10
Fat (gr)	97.7±3.9
Vitamin C(mgr)	96±7.3
Vitamin E(mgr)	17±3

Values are given as mean ± SD

Table 3. Changes in Salivary Flow Rate (ml/nm) after a Short Intense Exercise

Before	Immediately After Exercise	1 hr After Exercise
0.41± 0.09	0.39±0.12	0.42± 0.13

Values are given as mean ± SD

before exhaustive exercise.

Experimental design

All of the subjects brushed their teeth and rinsed their mouth with distilled water 3 h after lunch. After arrival to the laboratory, they rested for 15 min before starting the experimental trial. Then, they performed the protocol according to the Astrand treadmill test; that is a reliable test for acute aerobic training (29). Unstimulated whole saliva samples were collected three times: before, immediately and 1 h after treadmill run. Saliva sampling of subjects were collected into sterile Eppendorf tubes in seated position with their heads tilted down between the knees. Saliva samples were immediately centrifuged at 800 × g for 10 min and stored at -80^{0}C until analysis. During the exercise testing, the laboratory (Biochemistry lab, Faculty of Sciences, Guilan University) temperature and relative humidity were 22±1.4^0 C and 53±1.4% respectively. Saliva flow rate has been shown in Table 3.

Determination of VO_2 max

All subjects performed a continuous incremental treadmill run to exhaustion. The test began at a velocity of 8.5km/h, with an increase of 2.5% km/h every 2 min until exhaustion. Gas change parameters were analyzed during the run by a calibrated Sensormedics Horizon Metabolic Measurement Cart (Sensormedics, Anaheim, Calif). Heart rate was recorded by the monitor (30) with an interval of 2 minutes. Ratings of perceived exertion were measured every 2 minutes during exercise for exercise intensity prescription (30) (Table 4).

Determination of Catalase activity

The measurement of enzyme activity was done using hydrogen peroxide in phosphate buffer (pH 7.0). The absorption of the mixture was monitored at 240 nm at 10-second intervals during 2 minutes. Catalase activity was analyzed spectrophotometrically according to the ABE method. The obtained absorbencies were then divided by 39.4 to obtain catalase activity. One unit of catalase activity is defined as the amount of enzyme that decomposes one micromole of hydrogen peroxide in a minute at pH 7.0 (31).

Determination of vitamin C concentration

The concentration of saliva vitamin C was measured according to the colorimetric method of Roe and Kuether (32) using Trichloroacetic acid, Thiourea, 2.4-dinitrophenylhydrazine, H_2SO_4, and Norite.

Determination of BDNF concentration

Saliva BDNF was assayed in duplicate according to the manufacturer's instruc-

Table 4. Harte rate and Rate of perceived exertion of sedentary men

Acute intense exercise	Final minute before cessation	Total time of exercise
Heart rate (beat per minute)	193.9±3	165±5
Rate of perceived exertion	18.2±1	14.1±1

Values are given as mean ± SD

tions (R&D BDNF ELISA kit, USA) in Cellular & Molecular Research Center (Guilan University of Medical Sciences). The BDNF ELISA kit has a detection range from 7.8 to 500pg/mL; the intra and inter-assay variations were ±4.66% and ±9%, respectively.

Statistical analysis

All data were analyzed using SPSS software 19 and were reported as mean±SD. The normality of the data was assured before statistical analysis by Kolmogorov–Smirnov test. Repeated measures ANOVA and Bonferroni post-hoc test were used to analyze data. Statistical significance was set at $p<0.05$.

Results

Table 3 shows the results of flow rate before and after exercise. There was a slight insignificant alteration in saliva flow rate before and after exercise. It was found that the saliva flow rate ranged 0.7 to 1.2 ml/·min before exercise, was reduced to (0.6-1.0ml/·min) after intense training, and

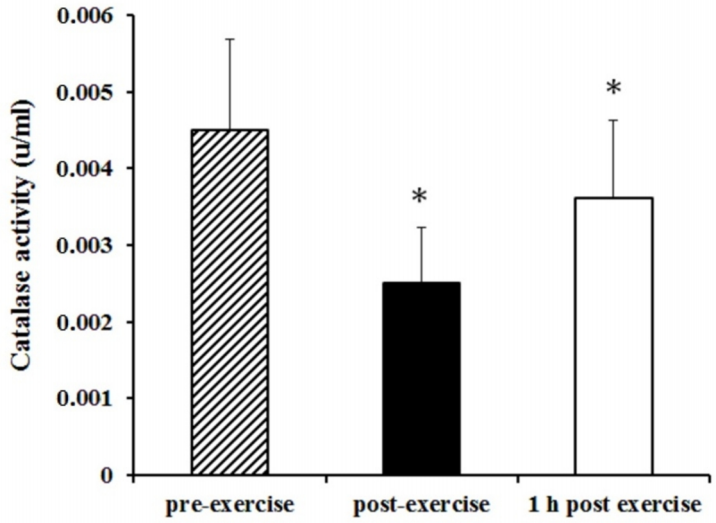

Fig. 1. Effect of acute intense exercise on salivary catalase activity periods at before, immediately, and 1 h after
* Significantly different in comparison with pre-exercise ($p\leq0.05$)

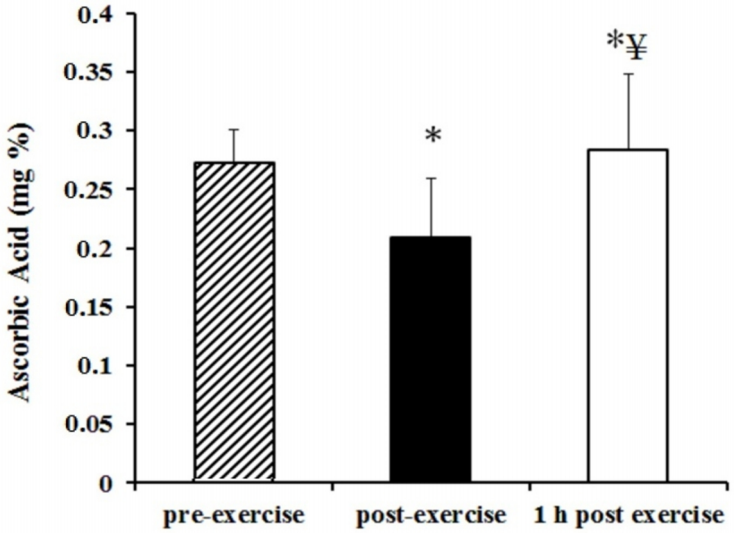

Fig. 2. Effect of acute intense exercise on salivary ascorbic acid concentration periods at before, immediately, and 1 h after
* Significantly different in comparison with pre-exercise ($p\leq0.05$). ¥ Significantly different in comparison with post-exercise ($p\leq0.05$)

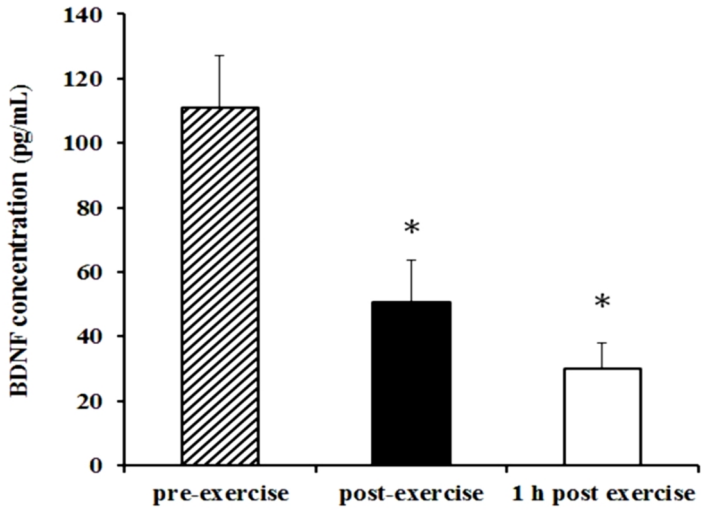

Fig. 3. Effect of acute intense exercise on salivary BDNF concentration periods at before, immediately, and 1 h after
* Significantly different in comparison with pre-exercise ($p \leq 0.05$)

then returned to the normal value after 1 hr.

As Figure 1 and 2 shows, acute intense exercise caused a reduction in salivary catalase activity and also Vit C or ascorbic acid concentration ($p \leq 0.05$) compared with pre-exercise. Both catalase and ascorbic acid show tendency to return to pre-exercise value after one hour. Although the concentration of BDNF showed significant reduction immediately after exercise (50±28) compared to pre-exercise (111±30) ($p<0.05$), surprisingly, it continued to reduce even 1 hour after the ending of the exercise (29.88±24.35) (Fig. 3).

Discussion

The present study showed that exhaustive acute aerobic exercise causes a significant reduction in the activity of salivary catalase, vitamin C, and BDNF levels immediately after the ending of the exercise. In agreement with our results, some studies on plasma have shown a reduction in glutathione (33,34), total antioxidant capacity (35) vitamin C (36) and BDNF (37). Catalase is an enzyme capable of changing the hydrogen peroxide into water and oxygen (38, 39). On the other hand, ascorbic acid or vitamin C is involved in a number of biochemical pathways that are important to exercise metabolism and the health of exercising individuals; for example protecting the biochemical machinery of cells (16) and integrity of tissues (40,41). Exercise generally causes a transient increase in circulating ascorbic acid in the 1 hour following exercise, but a decline below pre-exercise levels occurs in the days after prolonged exercise. These changes could be associated with increased exercise-induced oxidative stress (42). It is assumed that the exercise protocol used in the present study probably induced ROS and consequently increased demand for utilization of the antioxidant system of saliva. Regarding the secretion of Vit C from adrenal gland during the physical activity (43,44); the reduction in saliva Vit C possibly reflects overriding of consumption to production (16).

In addition, BDNF reduced significantly in saliva after physical stress, and it might be due to sublingual nerve absorption of protein for the brain. It has been well known that brain is the most vulnerable organ due to high oxygen utilization and ROS generation produces BDNF (45), as a potential antioxidant (37), and neurotrophic factor (45). However, BDNF is synthesized as a 32 kD N-glycosylated and glycosylated perform (45,46) from the salivary glands too (46,47). In addition to above-mentioned explanation, hydrogen peroxide (H2O2),

per se is capable of reducing to BDNF in a dose-dependent manner (48).

Previous studies showed that serum BDNF is increased after an acute exercise (49,50). It has been well addressed that BDNF signaling mediates up-regulation of several proteins including the chaperone proteins, antioxidant enzymes, the cell survival proteins, and the DNA repair enzyme (23). Also, BDNF mediates exercise -induced cognitive improvement in healthy people (51); Therefore Insufficient BDNF production resulting in the vulnerability of the brain to injury and neurodegenerative disorders.

Finding that the saliva flow rate returned to the normal value after 1 hr of exercise indicates that dehydration during exercise does not seriously influence the normal flow rate of saliva.

Therefore, no significant change in saliva volume, and flow rate, shows that reduction in saliva antioxidants are probably due to consumption or redistribution rather than less production. Moreover, reduction in saliva, but not serum BDNF is in contradictory with previous finding that cAMP-responsive element-binding protein which is one of the transcription factors of BDNF is redox sensitive (52) indicating elevation in BDNF.

Considering previous findings and current results, reduction in antioxidants in response to physical stress encounter physiological systems to more stress oxidative (53, 54) and molecular damages (55,56). ROS alterations in saliva are recommended for future studies to clarify precise mechanisms underlying saliva antioxidants response to acute exercise training. From a clinical point of view, using saliva as an easy noninvasive method could be considered in experimental and clinical trials.

Conclusion

In conclusion, an exhaustive short exercise leads to a reduction in salivary antioxidants, more notably, BDNF, Vit C and catalase. Supplementations of certain antioxidant nutrients are suggested before single exhaustive exercise in order to prevent further health threatening consequences.

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Conflicts of interest

All authors have none to declare.

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