

Full Length Research Paper

Oxidative stress and altered levels of antioxidants in adolescents with Down syndrome during pre-exercise and post-exercise

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The aim of this study was to determine the influence of exercise on activity of erythrocyte superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and plasma malondialdehyde (MDA), β -carotene, retinol levels in adolescents with Down syndrome (DS). Twenty adolescents with DS (14.86 \pm 7.07 years) performed a 12-week training program. Body composition was measured by bioelectric impedance method. SOD, GPx, CAT, MDA, retinol, β -carotene levels was determined spectrophotometrically. When compared to baseline, MDA level was increased ($P < 0.05$). SOD, GPx activities and β -carotene level were decreased ($P < 0.05$). There was no difference during pre-post exercise in terms of CAT activity and retinol level ($P > 0.05$). The mean value of percentage of fat mass was reduced. Regular exercise decreased SOD, GPx activities and β -carotene level and did not affect CAT activity and retinol level in individuals with DS. Exercise increases oxidative stress depending on level of training.

Key words: Down syndrome, exercise, body composition, antioxidant enzyme, obesity, fat mass percentage.

INTRODUCTION

It is well-documented in the literature that persons with mental retardation consistently display inferior health-related physical fitness components when compared to their nondisabled counterparts. During the past 25 years, investigations have been conducted primarily on physical fitness levels of adolescents with mental retardation (Pitetti et al., 1992; Eichstaedt and Lavay, 1992).

Mental retardation has been associated with metabolic syndrome, especially in individuals living in community settings (Jansson and Johansson, 1995). This tendency, which might be due to lifestyle, increases the risk of

cardiovascular disease (Draheim et al., 2002). Moreover, insulin resistance (IR), a feature of metabolic syndrome, has been associated with a decrease in the ability of muscles to oxidize fat (Perez-Martin et al., 2001; Venables et al., 2005). In Down syndrome (DS), metabolic syndrome might be even more prevalent, as it has been frequently associated with an alteration in lipid profile (Brattstrom et al., 1987; Flore et al., 2005) and of the clinical variables, testes volume and body height were significantly lower in patients with Down's syndrome than in control patients (Hestnes et al., 1991).

Down syndrome (DS) is a genetic condition owing to complete or at least partial trisomy of chromosome 21 (that is, three instead of two 21st chromosomes) (Perez-Martin et al., 2001). Down syndrome is caused by the presence of the whole or part of an extra copy of

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chromosome 21. This disorder can be diagnosed in utero by screening or karyotyping, or early after birth by muscle hypotonia (poor muscle tone) and other symptoms and confirmed by karyotype analysis of a blood sample (Andriolo et al., 2010). As a result of improvements in health and social care, the average life expectancy has increased dramatically. As a result of improvements in health and social care, the average life expectancy has increased dramatically (Day et al., 2005).

Oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage. In addition, a high level of oxidative stress has been clearly demonstrated in participants with DS as a result of an over expression of Cu-Zn superoxide dismutase (SOD1), the activity of which might be increased by 50% (De Haan et al., 1997; Pallardo et al., 2006). Since, it has been widely accepted that reactive oxygen species (ROS) can activate signaling processes and induce cytotoxicity in many disorders; there has been intense ongoing research on this topic, mainly regarding the role of the antioxidant system.

In recent years, it has been claimed that trisomic cells are more sensitive to oxidative stress since there is an imbalance in hydrogen peroxide metabolism (Carratelli et al., 2001). Thus, individuals with DS present an increase in SOD1 catalytic activity that produces an excess of H₂O₂ that reacts with superoxide anion (O²⁻), producing hydroxyl radical (OH⁻), which is one of the most active radical oxygen species (Chance et al., 1979). This fact is of particular interest since oxidative stress has been proposed as a pathogenic mechanism of atherosclerosis, cell aging, carcinogenic events, immunological default, cataract formation and neurologic disorders in individuals with DS (Cengiz et al., 2002; Iannello et al., 1999; Pastore et al., 2003). Erythrocytes are susceptible to oxidative damage as a result of the high polyunsaturated free fatty acid content of their membrane and the high cellular concentrations of oxygen and hemoglobin, a potentially powerful promoter of oxidative processes. To date, major antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) have been found to be increased in erythrocytes of individuals with Down syndrome (Groner et al., 1994; Muchova et al., 2001).

Systematic programs of physical exercise can benefit persons with mental retardation in areas of physical growth and development, movement proficiency, and overall health (Eichstaedt and Lavay, 1992). Also, regular physical activity may increase the antioxidant defense system in healthy young men and women (Elosua et al., 2003). Therefore, this study was designed to determine the influence of training program on the activity of erythrocyte GPx, CAT, SOD and plasma malondialdehyde (MDA), β -carotene, retinol levels in adolescents with DS.

MATERIALS AND METHODS

Participants

This study comprised of a total of 20 adolescents with DS (20 male, mean age: 14.86 \pm 7.07 years) admitted to Firat University Mentally-Disabled Children Research and Education Centre, Elazig. DS was confirmed by chromosomal analysis at the Medical Genetics Division. IQ assessments of the children with DS were performed by methods, namely the Wechsler Intelligence Scales for Children (WISC) for those older than six years old. IQ levels in children with DS ranged between 40 and 60. None of them suffered acute medical problems at that moment and had not taken part in any physical activity program in the last one year. It is also important to identify medical conditions. Persons with Down syndrome may have congenital heart disorders or respiratory problems. Before the investigation, informed consent was given each participant's parents. Venous blood samples were taken from the antecubital vein with suitable vacutainers with EDTA as anticoagulant. The basal venous blood samples were obtained from all the participants in this study on the morning after 12 h of overnight fasting. In all cases blood samples were taken according to the principles of the Helsinki declaration.

Tests

The blood samples were drawn from the patients. Whole blood was separated for GSH-Px assay. Then remaining blood was immediately centrifuged at 1500 g for 5 min. The plasma (for MDA, β -carotene, vitamin A assays) and erythrocytes (for CAT assay) were collected separately. All of the samples were kept at -20°C until assays. The variables were measured within 15 days after sampling. Among all participants, information on demographic characteristics and risk factors was collected using a structured questionnaire.

The Erythrocyte SOD enzyme activity measurement is based on the nitroblue tetrazolium (NBT) degradation by the superoxide radical, which was produced with the xanthine-xanthine oxidase system. The form obtained at the end of the reactions exhibits a blue colour and maximally absorbs at 560 nm (Sun et al., 1988). The SOD enzyme activity was calculated as U/g haemoglobin.

Erythrocyte GPx activity was measured by the method of Beutler (1975) in which cumene hydroperoxide was used as substrate. Oxidized glutathione (GSSG) produced by the action of erythrocyte GPx and cumene hydroperoxide was reduced by glutathione reductase and NADPH. The decrease of the NADPH concentration was measured at 340 nm. The enzyme activity in erythrocytes was expressed as units per gr of Hb (U/g Hb).

The end-product of polyunsaturated fatty acid peroxidation, MDA, which reacts with thiobarbituric acid in plasma samples, was determined by the methods of Placer et al. (1966) with slight modifications. The quantification of thiobarbituric acid (TBA) reactive substances was determined by comparing the absorption to the standard curve of MDA equivalents generated by acid catalyzed hydrolysis of 1, 1, 3, 3-tetraethoxypropane. The values of MDA reactive material was expressed as MDA quantities for plasma volume (nmol/ml plasma).

Erythrocyte CAT activity were determined according to the method of Aebi (1984) and CAT activity was calculated as katal/g Hb. The decomposition of H₂O₂ can be directly followed by the decrease of absorbance at 240 nm wavelength. The difference in absorbance at 240 nm per time unit allows determining the CAT activity.

Vitamin A and β -carotene in plasma samples were determined according to the method of Suzuki and Katoh (1990). The plasma (1 ml) was placed in a dark brown test tube. To the plasma, 1 ml of

Table 1. Identifying information's of patients with DS.

Parameter	Pre-training	Post-training	t	P
Age (years)	14.86 ± 7.07	14.86 ± 7.07	-	-
Height (cm)	129.00 ± 7.28	129.00 ± 7.28	-	-
Weight (kg)	46.39 ± 16.79	44.10 ± 16.91	6.149	0.000*
Body mass index-BMI (kg/m ²)	31.92 ± 6.83	28.80 ± 4.94	3.269	0.007*
Waist Circumference-WC (cm)	87.12 ± 3.5	86.7 ± 3.9	6.346	0.000*
Waist/hip-WHR (N< 1)	0.87 ± 0.15	0.84 ± 0.17	2.394	0.040*

*, Significance was found at $p < 0.05$ and results was expressed as mean ±SD. N=20.

Table 2. Impact of a 12-weeks moderate aerobic training program on body composition of male adolescents with Down syndrome.

Parameter	Pre-training	Post-training	t	P
Percent body fat (%)	34.17 ± 7.28	27.96 ± 8.16	3.130	0.016*
Body fat-free Mass (kg)	30.10 ± 10.51	35.27 ± 11.31	-11.558	0.000*
Mass body fat (kg)	16.29 ± 7.25	12.22 ± 4.89	4.505	0.004*
Soft lean mass (kg)	27.48 ± 9.60	32.63 ± 10.40	-11.881	0.000*

*, Significance was found at $P < 0.05$ and results was expressed as mean ±SD. N=20.

ascorbic acid solution (in ethanol w/v) was added by 3 ml of hexane and the mixture shaken mechanically for 10 min. After centrifugation, 2 ml of hexane were removed from all tubes and measured at 453 (for β -carotene) and 325 nm (for vitamin A) in a spectrophotometer.

Three days after the end of treatment, anthropometrical parameters were assessed. Anthropometrical measurements such as body mass index (BMI), waist circumference (WC) and waist hip rate (WHR) were performed by a single observer. Body composition was measured by bioelectric impedance method (BIA). Body weight and percentage body fat (PBF) were measured using a portable Tanita body fat scale (Model TBF-410 M). Waist circumference (WC) was measured to the nearest 0.1 cm midway between the lowest rib and the superior border of the iliac crest with a measuring tape and determined during minimal inspiration. The WHR was evaluated from the minimal waist and the maximal hip girth in the standing position.

Training for adolescents with Down syndrome was approximately an hour in length, for 3 days per week, adhering to the following format: Warm-up, 10 to 15 min of slow walking and simple stretching exercise (for example, trunk twisters); also strengthening exercises such as sit-ups for 1 min. Once participants had learned the warm-up and strengthening exercise routine, each was periodically chosen to lead the group. Run/walk, each participant ran or walked for 30 min around a 220-yard indoor track at the athletic complex on the university campus in the community. Cool down, 10 to 15 min of easy walking and slow stretching. Once again, each participant was periodically responsible for leading the group through the cool-down session (Eichstaedt and Lavay, 1992).

Statistical analysis

Results were expressed as mean ± SD. The statistical analysis of data was performed using Student's t-test for unpaired data. The significance of the changes observed was ascertained at $P < 0.05$. The Statistical Package for the Social Sciences (SPSS) 15.0 (SPSS

Inc, Chicago, IL, USA) was used for statistical analysis.

RESULTS

Regarding anthropometrical parameters, BMI was 31.92 ± 6.83 (28.80 ± 4.94) kg/m², WC 87.12 ± 3.5 (86.7 ± 3.9) cm and WHR 0.87 ± 0.15 (0.84 ± 0.17) during pre and post training period in adolescents with DS (Table 1).

At baseline, the mean fat mass percentage was 16.29 ± 7.25 in the male adolescents with Down syndrome. After the 12-week aerobic training program, it was reduced to 12.22 ± 4.89 . The body fat-PBF percentage was 34.17 ± 7.28 in the male adolescents with Down syndrome. After the 12-week aerobic training program, it was reduced to $27.96 \pm 8.16\%$. The Lean body fat-LBM (kg) was 30.10 ± 10.51 in the male adolescents with DS. After the 12-week aerobic training program, it was increased to $35.27 \pm 11.31\%$. The Soft lean mass-SLM (kg) was 27.48 ± 9.60 in the male adolescents with DS. After the 12-week aerobic training program, it was increased to $32.63 \pm 10.40\%$.

The paired t-test demonstrated significant differences between them ($P < 0.05$), indicating the validity of our program. It should also be mentioned that none of participants left the program. These results are summarized in Table 2.

SOD, GPx, CAT activities and MDA, retinol, β -carotene levels in adolescents with Down syndrome during pre-post exercise are listed in Table 3. After a 12-week training program, plasma MDA level was increased significantly ($P < 0.05$). SOD, GPx activities ($P < 0.05$) and

Table 3. Superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) activities and MDA, retinol, β -carotene levels in adolescents with Down syndrome during pre and post training period.

Parameter	Pre-training	Post-training	t	P
SOD (U/g Hb)	679.0 \pm 82	502.0 \pm 70	3.187	0.019*
GPx (U/g Hb)	74.31 \pm 40.88	38.53 \pm 15.09	2.361	0.050*
CAT (k/g Hb)	23.53 \pm 8.94	27.53 \pm 5.59	0.953	0.373
MDA (nmol/ml)	2.39 \pm 1.28	3.83 \pm 0.86	2.992	0.020*
Retinol (μ g/dl)	16.40 \pm 10.89	11.47 \pm 3.79	1.726	0.128
β -carotene (μ g/dl)	37.53 \pm 10.38	16.66 \pm 4.34	5.512	0.001*

*Significance was found at $P < 0.05$ and results was expressed as mean \pm SD. N=20.

β -carotene level ($P < 0.001$) were decreased significantly. There was no difference during pre-post exercise in terms of CAT activity and retinol level ($P < 0.05$).

DISCUSSION

Several disorders have been related to oxidative stress in people with Down syndrome (DS) and consequently it is of interest to examine the role of antioxidant defenses in general and enzymatic systems in particular. In this respect, the importance of blood cells is increasing, since they are cells rich in antioxidant enzymes and they may reflect changes in the catalytic activity of these enzymes in other less accessible tissues (Richards et al., 1998).

A significant decreased of SOD, GPx activity and β -carotene level in adolescents with DS was observed after a 12 week training program. In contrast, Ordonez et al. (2006) stated that a 12-week training program significantly increased the total antioxidant status and glutathione peroxidase activity in adolescents with Down syndrome (Ordonez et al., 2006). Also, it was reported that the levels of reduced glutathione (GSH) were increased after a 16-week training program in adults with Down syndrome (Monteiro et al., 1997). In our results, there was a decrease in the levels of antioxidants (such as SOD, GPx and β -carotene) after a 12-week training of adolescents with DS. Consequently, these results suggested that regular exercise may not contribute to improve redox balance in this population. In any case, it should be emphasized that our protocol lasted only 12 weeks.

Previous studies had successfully explored the relationship of anthropometrical parameters, such as body mass index, and diet, exercise, disability status and degree of social integration in individuals with DS (Fujiura et al., 1997). However, far less information is available on person with disabilities such as Down syndrome, regarding association between anthropometrical parameters and antioxidant system. In this respect, we found a negative but significant association between GPx activity and WC. Regarding BMI and WHR, they were neither strongly nor significantly correlated to GPx.

Similarly, BMI was not associated with antioxidant enzyme, SOD, in obese diabetics type II (Skrha et al., 2005). On the other hand, anthropometrical parameters have been widely associated with biomarkers of oxidative stress. Similarly, BMI and WHR were associated to lipid peroxidation expressed as MDA content in obese prepubertal children (Mohn et al., 2005; Urakawa et al., 2003; Weinbrenner et al., 2006). These findings may be explained, at least in part, since obesity has been associated with accelerated oxidative stress.

In the present study and in some past research, a significantly higher catalytic activity levels for SOD in erythrocytes from adolescents with Down syndrome compared with controls were observed (Groner et al., 1994; Muchova et al., 2001). The increase in SOD enzyme activity was due to an excess of genetic information given that SOD gene is localized to chromosome 21q22. As a consequence of gene dosage excess, SOD activity has shown an increased by about 50% in all tissues of patient DS (De La Torre et al., 1996; Gulesserian et al., 2001). SOD overexpression in people with Down syndrome unbalances this equation, which may ultimately lead to increased H_2O_2 production. This indicates that trisomic cells are more sensitive to oxidative stress (Pastore et al., 2003). We found that a 12 week training program did not significantly increase erythrocyte SOD activity in adolescents with Down syndrome.

SOD catalyses the dismutation of the superoxide anion (O_2^-) to H_2O_2 and then, in a second step, GPx and CAT converts hydrogen peroxide (H_2O_2) to water. Accordingly, it is widely accepted that in the activity of the first-step SOD and second-step GPx, CAT antioxidant enzymes must be balanced to prevent cell damage by oxidative stress (Crosti et al., 1989). Consequently and in agreement with Pastor et al. (2003), it is conceivable that any excess H_2O_2 produced by the action of SOD would induce a significant increase in the catalytic activity of GPx. In this study, we observed reduced SOD and GPx activity at the same time and catalase activity was not increased significantly after a 12-week training program. On the other hand, no significant changes were observed in the activity of CAT as was reported previously (Brugge

et al., 1999; Muchova et al., 2001). In conclusion, regular exercise did not significantly increase catalase activity. Further studies are required in order to highlight potential benefits of regular exercise in redox metabolism of Down syndrome individuals.

In addition, it has been recently published that regular exercise may enhance the blood antioxidant system in general and GPx activity in particular both in human and animal experimental research (Elosua et al., 2003; Oztasan et al., 2004). On the contrary, acute exercise does not affect the activity of serum GPx in healthy young people (Rush and Sandiford, 2003). In our study, significantly decreased erythrocyte GPx activity was found in adolescents with Down syndrome after a 12 week training program.

Measures of lipid peroxidation include expired pentane, MDA, lipid hydroperoxides, isoprostanes, and conjugated dienes. Most studies have used MDA as a measure of oxidative stress imposed by exercise. MDA, a major end-product of oxidation of polyunsaturated fatty acids, has been frequently measured as an indicator of lipid peroxidation and oxidative stress *in vivo*. We observed that regular exercise increased significantly plasmatic MDA levels in male adolescents with Down syndrome. Adverse results found in healthy children (Gonenc et al., 2000). Consequently these results suggested that regular exercise may cause to decrease antioxidant defense in this population that may finally enhance lipid oxidation. The main finding of the study was that a 12-week exercise program significantly increased lipid peroxidation in terms of plasmatic MDA content in male adolescents with Down syndrome. Further studies concerning the influence of regular exercise on redox metabolism in this population are required.

In previous reports, deficiencies in serum vitamin A were considered a frequent finding in persons with DS. Based on this assumption, the regular determination of both serum carotene and vitamin A has been recommended for the preventive care of this population (Storm, 1990). The most important provitamin A, carotenoid is β -carotene. Absorption of carotenoids in man is limited. The capacity for absorption of unchanged β -carotene into the blood was reported to be only 10% of the ingested labeled dose. The carotenoids are concentrated in body tissues, including the liver. Some β -carotene may be converted to vitamin A in the liver. The blood carotenoid levels in normal children have been documented by several authors. Recent research found that children aged 4 to 13 years had a mean serum carotenoid level of 140 $\mu\text{g}/100\text{ ml}$ and the range was 40 to 304 $\mu\text{g}/100\text{ ml}$. The peak level was at the age of 1 year and adult levels were reached in early adolescence.

Fasting serum carotenoid and vitamin A levels were measured in 77 profoundly mentally retarded children aged 3 to 19 years. 22.1% of them had carotenemia. This agrees with the observations of other authors who found elevated serum carotenoid levels in adolescents with

Down syndrome (Patel et al., 1973). In adolescents with Down syndrome, the serum vitamin A levels were higher when compared with post training. In our study, the level of β -carotene level were found significantly different in adolescents with Down syndrome when compared with post training ($P=0.001$). This study demonstrates that regular exercise significantly decreases β -carotene level in mentally retarded children.

For adults with Down syndrome, both life expectancy and potential for active functioning in society are increasing (Coppus et al., 2008). However, there is a lack of consistent information about the safety and effectiveness of aerobic exercise training for this population. A systematic review of the evidence that addresses psychosocial and physiological outcomes is required.

In spite of the fact that moderate and regular physical exercise is popular and established as a sound lifestyle decision both for health and as an intervention to improve quality of life in the general population, current evidence from published studies suggest that in adolescents with Down syndrome work performance outcomes may be improved by aerobic exercise training programmes, regardless of age range within adulthood. However, cardiorespiratory measures may not be improved. Overall, the effectiveness of aerobic exercise training programmes in improving the health of adolescents adults with Down syndrome remains uncertain.

We conclude that, regular exercise in adolescents with Down syndrome decreased significantly GPx, SOD activity and β -carotene levels. However, it was found to increase in MDA level. Adolescents with Down syndrome subjected to exercise may cause oxidative stress and may lead to oxidative damage in adolescents with Down syndrome. Finally, attention to long-term maintenance of any improvements caused by aerobic exercise training programmes, adherence of participants to an active lifestyle, and issues of dose-response are all important to the design of future research.

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