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The Effect of 5-Week Exercise Program on Oxidative Stress and Response to Acute Exercise among Sedentary Subjects

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ABSTRACT

Physical training is associated with oxidative stress and improvement in blood antioxidant status. In this study, we investigated the effects of training on plasma malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) as markers of lipid peroxidation, superoxide dismutase (SOD) activity, and ascorbic acid (AA) after a single bout of acute exercise. Twelve healthy, untrained young adult men were recruited for 5 weeks of aerobic training period. They were subjected to a rope-skipping exercise for 20 minutes at the intensity of 65 – 80% of heart rate reserve, thrice weekly. They also had to perform a single, acute bout of the same exercise protocol prior to and after training period. Venous blood samples were collected at resting condition (BL), immediately (0 h) and 24 hours (24 h) post acute exercise on both single bout sessions. Results showed that the pattern changes of oxidative stress response are quite similar on both acute sessions. The acute bouts of rope-skipping is associated with a significant increased ($p = 0.001$) in lipid peroxidation markers immediately after cessation of exercise, with a concomitant increases in antioxidant levels, albeit higher when compared to pre-training values ($p = 0.001$). These changes were followed by a significant decreased ($p = 0.001$) in all parameters, toward resting values, 24 hours thereafter. The training program seemed to induce a significant increase in MDA and 4-HNE but also enhanced the antioxidant defense system namely SOD and AA among the untrained subjects.

Key words: aerobic exercise, training, oxidative stress, free radicals, antioxidants.

ABSTRAK

Latihan fizikal sering dikaitkan dengan tekanan oksidatif dan peningkatan status antioksidan darah. Kajian ini bertujuan untuk mengkaji kesan latihan senaman terhadap aras malondialdehid (MDA) dan 4-hidroksinonenal (4-HNE) plasma sebagai penanda peroksidasi lipid, aktiviti superoksid dismutase (SOD), dan asid askorbik (AA) selepas diberi sesi senaman akut. Dua belas

(n = 12) mahasiswa tidak terlatih, terlibat dalam program latihan senaman aerobik selama 5 minggu. Mereka menjalani senaman jenis lompat-tali, 3 kali seminggu selama 20 minit untuk setiap sesi pada intensiti 65-80% daripada kadar denyut jantung simpanan. Satu sesi senaman akut dengan protokol yang sama dilakukan sebelum bermula dan selepas berakhirnya program latihan senaman. Sampel darah vena diambil pada fasa rehat (BL), sejurus selepas (0 h) dan 24 jam (24 h) selepas setiap sesi senaman akut. Keputusan menunjukkan pola perubahan respons tekanan oksidatif adalah hampir sama pada kedua-dua sesi senaman akut iaitu sebelum dan latihan fizikal. Terdapat peningkatan yang signifikan (p = 0.001) pada aras penanda peroksidasi lipid sejurus selepas senaman akut, seiring dengan peningkatan aras antioksidan dan menunjukkan nilai yang lebih tinggi berbanding sebelum latihan senaman (p = 0.001). Perubahan tersebut disusuli dengan penurunan aras kesemua parameter (p = 0.001) menghampiri nilai normal selepas 24 jam. Secara kesimpulan, program latihan senaman yang dilaksanakan dalam kajian ini mengaruh peningkatan signifikan pada aras MDA dan 4-HNE dan pada masa yang sama juga meningkatkan sistem pertahanan antioksidan SOD dan AA dalam subjek tidak terlatih.

Kata kunci: senaman aerobik, latihan, tekanan oksidatif, radikal bebas, antioksidan.

INTRODUCTION

Oxidative stress occurs when the generation of reactive oxygen species (ROS) in a system exceeds the system's ability to neutralize and eliminate them. The imbalance can result from a lack of antioxidant capacity caused by disturbance in production, distribution, or by an over-abundance of ROS from an environmental or physical stressor (Urso & Clarkson 2003). Of late, much attention has been paid to the role of ROS and antioxidant system in exercise and physical training. Exercise enhances the hemodynamic and metabolic response of the body, particularly during acute or extenuating conditions (Thompson et al. 2001; Viguie et al. 1993). An immediate effect of exercise is the 100-fold increase in the maximal oxygen consumption (VO_2max) and metabolic activity due to increased muscle contraction when performing the physical activity (Alessio 2000). This condition can lead to an imbalance between free radicals and antioxidants, as the increased consumption of oxygen for respiration may generate increased amounts of ROS, mainly through leakage of electrons from the mitochondrial electron transport chain and the oxidation of xanthine by xanthine oxidase (Vi˙a et al. 2000). This phenomenon is predominantly occured in skeletal muscles because their antioxidant defenses is poor (Sen 1995) and may occur under a variety of exercise regimens and intensities (Villa-Caballero et al. 2007).

According to the current understanding of adaptation to physical exercise, until an over-training syndrome appears, regular exercise has beneficial effects. There is irrefutable evidence of the effectiveness of regular physical activity in the primary and secondary prevention of several chronic diseases e.g. cardiovascular disease, diabetes, cancer, hypertension, obesity, depression, and osteoporosis (Warburton et al. 2006). Animals and humans clearly undergo significant adaptive responses to regular, moderate endurance exercise that involve greatly increased endurance capacity, permitted by dramatic mitochondrial biogenesis and significantly increased number of muscle mitochondria (Packer et al. 2007). Interestingly, mild oxidative stress induced by repeated bouts of aerobic exercise have been reported to stimulate the expression and increases the *de novo* protein synthesis of antioxidant enzymes, DNA repair molecules and protein degrading enzymes, resulting in decreases in the incidence of oxidative stress-related diseases and retardation of the aging process (Ji et al. 2006; Radak et al. 2005). The basis for this phenomenon may be explained by the concept of hormesis. The hormesis theory purports that biological systems respond with a bell-shaped curve to exposure to chemicals, toxins, and radiation (Minois 2000), which can be explained as a particular dose – response relationship in which a low dose of a substance is stimulatory and a high dose is inhibitory (Calabrese & Baldwin 2003). Recently the hormesis theory has been extended to the ROS-generating effects of exercise. There is now increasing evidence and recognition that ROS are not merely damaging agents inflicting random destruction to the cell structure and function, but useful signaling molecules to regulate growth, differentiation, proliferation, and apoptosis, at least within the physiological concentration (Ji 2007). In this context, free radicals may be seen as beneficial, as they act as signals to enhance defenses rather than as deleterious agents.

Thus, exercise provides an excellent opportunity to study the dynamic balance between oxidant challenge and antioxidant defense in a biological system. Despite numerous data on the influence of exercise on oxidant status in humans, the results vary from increased (Brites et al. 1999; Aslan et al. 1998), to decreased (Clarkson & Thompson 2000) or unchanged values (Polidori et al. 2000). The same is true for antioxidant enzymes; reports of exercise-induced increase (Brites et al. 1999; Ortenblad et al. 1997), decrease (Akova et al. 2001) or no change in activity (Selamoglu et al. 2000) have been reported. However, the changes reported in antioxidant capacity and in oxidative stress are related to differences in the studied population, namely in dietary and lifestyle habits, and/or in the experimental protocols. Although abundant data on the influence of exercise training on oxidant/antioxidant status in men and women exist, most of the experimental protocols consisted of cycling (Jammes et al. 2004), swimming (Reddy et al. 1995), walking (Villa-Caballero et al. 2007), and running (Child et al. 1998; Ortenblad et al. 1997). Since different forms of exercise may result in different levels of oxidative stress, the influence of exercise on oxidative stress was still

controversial. Additionally, there are scarce reports on rope-skipping exercise and its relation to training effects on oxidative stress. The present study was undertaken in healthy sedentary subjects to assess whether aerobic exercise training induce adaptations to reduce the extent of oxidative stress by looking at the kinetics of oxidative stress markers over 24-hour epoch in response to a single bout of exercise, before and after training period.

MATERIALS AND METHODS

SUBJECTS

Twelve (n = 12) untrained and healthy, young males from Faculty of Allied Health Sciences, Universiti Kebangsaan Malaysia, aged 19-22 years, were recruited for this study. Only sedentary subjects involved with no more than an hour per week of regular physical activity for the preceding 3 months were selected. Excluded from this study were smokers, regular alcohol users, subjects with a body mass index (BMI) more than 26 kg/m^2, subjects with physical disabilities or other chronic diseases and subjects receiving prescription medication or using antioxidants or multivitamins and trace element supplements for at least 3 months prior to the study. During recruitment, the participants were given a brief description of the study and those who expressed interest were asked to read and sign the informed consent documents.

EXERCISE INTERVENTION

1. *Training Protocol*

Subjects underwent a supervised, aerobic group exercise program for 5 weeks. The type of aerobic exercise chosen for this study was rope-skipping. Frequency of training sessions was three, 20-minute sessions per week at the intensity corresponding to the individually recommended training heart rate zones. The prescription for moderate intensity exercise was according to published guidelines by the American College of Sports Medicine (ACSM 2000). The intensity of training was individualized and adapted to the basal physical fitness of each subject and was set at 65-75% of heart rate reserve, which was estimated by using Karvonen's method (Robergs & Roberts 1997). Exercise heart rates were monitored using chest heart rate monitors (Polar, USA), a small chest belt that records heart rates in time sequence. Subjects can observe their exercise heart rates on a wristwatch digital screen that receives signals from the chest-belt monitor while exercising to ensure adherence to their prescribed target heart rate zone. Each subject was supplied a diary in which they were instructed to record daily physical activity patterns, exercise session dates, exercise heart rates at 7, 14, and 20 minutes as well as weekly resting heart rates. The latter were monitored

in order to adjust subjects' individualized target heart rates accordingly when necessary.

2. *Acute Exercise Protocol*

Subjects were required to perform an acute bout of the same exercise protocol, i.e. rope-skipping for 20 minutes at 65-75% of heart rate reserve, 48 hours before the commencement and after the completion of training period.

3. *Cardiorespiratory Fitness Assessment*

Cardiorespiratory fitness was measured by the multistage 20-meter shuttle run test (Ransbottom et al. 1988). This test was validated for estimating maximal oxygen consumption on field (Léger & Lambert 1982). The test required subjects to run back and forth between a distance set at 20 meters apart. Running pace was determined by audio signals, emitted from a pre-recorded cassette tape, the initial pace being 8.5 km/h, and increased by 0.5 km/h each stage (shuttle). The cassette tape was calibrated over 1 minute duration prior to each test. Subjects were instructed to run in a straight line, pivot upon reaching the other end of the line and to pace themselves in accordance with the time intervals. The test was concluded when the subject failed to reach the end lines concurrent with the audio signals on two consecutive occasions. The final score was determined as the number of shuttles and levels completed and converted to a VO_2max equivalent. A constant level of encouragement was given to participants throughout the test. Subjects were required to perform this test prior to and after the completion of training period.

BLOOD SAMPLING

Blood sampling sessions were performed in the morning, during the acute protocol sessions; before and after training period. The latter session was carried out at least 36 hours after the last session of training day to eliminate possible effects of the last bout of exercise on blood variables. All procedures involving human subjects were in accordance with the ethical standards of the Hospital Universiti Kebangsaan Malaysia Research Ethics Committee (No. 5/2003) and with the Helsinki Declaration of 1975 as revised in 1983. Venous blood samples via venipuncture in the forearm according to standard phlebotomy procedures were drawn at rest, which was prior to the acute bout of exercise (BL), immediately after cessation of exercise (0 h) and 24 hours thereafter (24 h) on both sessions. Oxidative stress markers in plasma were estimated by the measurement of malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) by using the lipid peroxidation colorimetric assay kit (Calbiochem, USA). The activities of two antioxidants in plasma (superoxide dismutase and ascorbic acid) were determined by using methods of Bayer & Fridovich (1987) and Wei et al. (1996) respectively.

STATISTICAL ANALYSIS

Data were verified for normality of distribution. Paired Student's *t*-test was used to test difference in physiological measurements between pre-training and post-training. To test intervention and time main effects as well as intervention by time interaction, a 2-way intervention (pre-training vs post-training) by sampling time (BL vs 0 h vs 24 h) analysis of variance with repeated measures was used (SPSS 12.0, SPSS, Chicago). The limit of significance was set at $p < 0.05$ for all analyses and values are expressed as means ± SEM.

RESULTS

Anthropometric and physiological measurements before and after the exercise training program are shown in Table 1. Body weight was measured to the nearest 100 g and height to the nearest centimeter using standard anthropometric methods. Compliance rate for exercise show-up for the 5-week intervention was 93%. From the subjects' diary inputs, their daily physical activity pattern did not differ considerably from one another, perhaps due to the fact they were residing in the same campus and were not physically-active individuals. An increase in cardiorespiratory fitness by 9.64% ($p = 0.03$) as evaluated by multistage shuttle-run test and a decrease in mean arterial pressure by 2.72% ($p = 0.04$) were achieved at the end of the 5-week aerobic training program. Lipid peroxidation markers and antioxidant status in response to acute bout sessions before and after the 5-week training program are presented in Table 2.

In both sampling sessions, MDA levels increased significantly immediately after cessation of exercise before declining towards baseline values 24 hours later ($F = 120.76$, $p = 0.001$, $eta^2 = 0.85$). These values also differed significantly between pre-training and post-training ($F = 16.92$, $p = 0.001$, $eta^2 = 0.44$). Similar pattern of change was also observed in 4-HNE levels where the levels increased

TABLE 1. Anthropometric and physiological measurements before and after training period (mean ± SD)

Parameters (n = 12)	Pre-training	Post-training
Age (years)	20.25 ± 0.70	-
Height (m)	1.72 ± 5.39	-
Weight (kg)	64.21 ± 10.23	63.06 ± 9.65
Body Mass Index (kg/m^2)	21.70 ± 3.31	21.31 ± 2.56
Mean Arterial Pressure (mmHg)	94.16 ± 4.92	91.66 ± 4.49*
Predicted VO_2max ($mL \times min^{-1} \times kg^{-1}$)	37.31 ± 4.25	40.91 ± 3.68*

* Significantly different ($p < 0.05$) from pre-training using paired Student's *t*-test.

TABLE 2. Oxidative stress and antioxidant parameters across sampling times before and after training period (mean ± SEM)

Parameters (n = 10)	Time	Pre-training acute bout	Post-training acute bout	Main Effects Intv	Time	(*p*-value) Intr
MDA (μM)	BL	0.86 ± 0.03	1.04 ± 0.03	0.001	0.001	0.32
	0 h	1.14 ± 0.05	1.32 ± 0.05			
	24 h	1.01 ± 0.03	1.15 ± 0.04			
4-HNE (μM)	BL	0.89 ± 0.03	1.07 ± 0.03	0.001	0.001	0.54
	0 h	1.18 ± 0.05	1.28 ± 0.05			
	24 h	1.02 ± 0.04	1.12 ± 0.04			
SOD (u.e/min/gprot)	BL	70.53 ± 0.96	81.45 ± 1.63	0.001	0.001	0.001
	0 h	75.63 ± 1.34	98.75 ± 1.53			
	24 h	73.06 ± 1.16	90.91 ± 1.43			
AA (mg/dl)	BL	0.54 ± 0.02	0.63 ± 0.01	0.001	0.001	0.001
	0 h	0.63 ± 0.02	0.76 ± 0.01			
	24 h	0.59 ± 0.02	0.68 ± 0.01			

MDA, malondialdehyde; 4-HNE, 4-hydroxynonenal; SOD, superoxide dismutase; AA, ascorbic acid
BL, baseline; 0 h, post exercise 0 hour; 24 h, post exercise 24 hours
Intv, intervention; Intr, intervention x time interaction

significantly at 0 h and saw a reduction 24 hours later ($F = 119.06$, $p = 0.001$, $eta^2 = 0.85$). The pattern of change in 4-HNE also differed significantly between pre-training and post-training ($F = 15.79$, $p = 0.001$, $eta^2 = 0.43$). Immediately after and 24 hours post-exercise, SOD enzyme activities increased significantly with regard to baseline values ($F = 128.82$, $p = 0.001$, $eta^2 = 0.85$) and varied significantly between pre-training and post-training ($F = 97.46$, $p = 0.001$, $eta^2 = 0.81$). Significant interaction effects occurred in pre-training compared to post-training activities and across all sampling times when adjusted for Bonferonni correction. Similar pattern of change over time was also noted in AA levels ($F = 209.49$, $p = 0.001$, $eta^2 = 0.91$) and differed significantly between pre-training and post-training ($F = 29.18$ $p = 0.001$, $eta^2 = 0.58$). Significant interaction effects occurred in pre-training compared to post-training levels and across all sampling times when adjusted for Bonferonni correction.

DISCUSSION

Rope-skipping, or jumping rope is perhaps the single most underrated but comprehensive and beneficial exercise a person can do. It is considered to be one of the most efficient method to increase cardiovascular fitness while improving bone density and muscular strength. It also optimizes both aerobic

and anaerobic endurance while maximizing athletic skills combining agility, coordination, power, and speed (Lee 2003). Rope-skipping training according to the specified protocol in the present study managed to improve subjects' cardiorespiratory fitness after 5 weeks, thereby supporting the facts that rope-skipping is just as effective as any other recognized forms of exercise.

This experimental study showed that a single bout of rope-skipping exercise of 20 minutes at the selected intensity induced oxidative stress and increased the antioxidant system in our untrained subjects. A number of studies have assessed the effect of exercise training or a single exercise bout on plasma lipid peroxidation by-products, the most common being the MDA (Margonis et al. 2007). Since free radicals are highly reactive and short-lived, most research has focused on determination of markers derived from radical-mediated oxidative damage to cellular macromolecules, e.g. proteins, lipids, and DNA, but there is as yet no consensus as to which may be the most useful biomarker (Mally et al. 2007). Kadiiska and colleagues (2005) proposed that determination of plasma MDA may serve as a reliable indicator of oxidative damage in their report of Biomarkers of Oxidative Stress Study. In addition, radical attack of lipids not only leads to the formation of MDA but also 4-hydroxy-2(E)-nonenal (4-HNE), an α, β-unsaturated aldehyde, a diffusable product of membrane lipid peroxidation, suggested to be a key mediator to attenuation of cellular glutathione (GSH) pools, ROS, nitric oxide (NO) and $ONOO^-$ generation leading to redox imbalances (Raza & John 2006; Poli & Schaur 2000). By some means, many exercise-induced oxidative stress investigations neglect to measure this particular by-product when it clearly plays an important role in redox imbalance. In our investigation, acute aerobic exercise resulted in an increase in oxidative stress markers, indicated by MDA and 4-HNE, above resting values. Single bouts of exercise of moderate intensity and long duration or of relatively short duration have been shown to increase lipid peroxidation in blood (Jammes et al. 2004; Koska et al. 2000; Davies et al. 1982).The observed increases in MDA and 4-HNE concentrations in the current study indicate that subjects underwent significant oxidative stress consequent to the given exercise. Sano et al. (1998) demonstrated there was a strong correlation exist between MDA and ROS production, as measured *in vivo* by electron paramagnetic resonance (ESR). The physical stress brought upon by rope-skipping had likely resulted in increased ROS production and lipid peroxidation, leading to an increase in both MDA and 4-HNE concentrations immediately post exercise. However, both markers declined possibly toward resting values 24 hours thereafter, suggesting that MDA and 4-NHE may increase only transiently after an acute application of a physiological stressor such as exercise. Also, this is probably as a result of clearance by blood antioxidants which neutralize ROS effectively over the 24-hour period.

Despite the paradox that exercise might induce ROS production, regular exercise is known to bring about significant benefits to health and to improve quality of life. In parallel with previous findings, we also found higher SOD

activities and AA levels in response to the single bout of exercise, a finding that may well have important beneficial consequences because it signifies the role of exercise in the up-regulation of antioxidant system. In human and animal studies, changes in the antioxidant enzymes occurring after exercise are believed to be a physiologic response to oxidative stress induced by physical exercise (Gago-Dominguez et al. 2007; Gomez-Cabrera et al. 2006; Hollander et al. 2000; Ortenblad et al. 1997). Several studies have also reported exercise-induced oxidative stress and the changes in plasma AA and SOD where they found that the concentration of plasma AA increased after exercise (Rokitzski et al. 1994; Viguie et al. 1993; Gleeson et al. 1987). The significant rises in AA concentration and SOD enzyme activity in response to acute exercise may have been as a protective response against the enhanced ROS production; demonstrated by the raised MDA and 4-HNE concentrations. Similarly, SOD enzyme activity and AA concentration also declined but remained significantly higher than basal values 24 hours post exercise. These observations indicate that it is possible that the levels of plasma antioxidants remained increased in the span of 24 hours in order to eliminate the accumulated ROS generated in response to the given exercise.

The 5 weeks of training used in this study brought about similar pattern changes in oxidative stress and antioxidant activities as has been previously described in a similar study by Elosua et al. (2003). Training has been found to increase basal SOD activity and AA levels significantly after 5 weeks as well as lipid peroxidation markers. In spite of the maintenance of AA and SOD activities after cessation of training period, a slight increase in basal MDA and 4-HNE was observed in the subjects. Similar findings have previously reported on the increase of lipid peroxidation by-products in both animals and humans after training (Lambertucci et al. 2007; Aslan et al. 1998). A recent study indicates that, as in the present investigation, moderate to strenuous exercise elicits a delayed (> 48 h) thiobarbituric acid reactive substances (TBARS) elevation probably triggered by leukocyte and macrophage infiltration or xanthine oxidase activation due to the ischemia-reperfusion process (Margonis et al. 2007). The raised MDA and 4-HNE concentration in blood could also be caused by lipid peroxidation of low-density lipoproteins and oxygen-mediated injury of muscle cell membranes (Hulbert 2005). Yagi (1992) had previously reported that lipid peroxidation markers decreased steadily during a nine-month exercise training. Therefore, we strongly believe that if the subjects were to carry on training for an extended period, MDA and 4-HNE levels would return to baseline values, or perhaps lower. Furthermore, with the absence of a control group, we were not able make comparison between trained and sedentary individuals, for which we expect would be a marked difference in oxidative stress status. Animals and humans frequently exposed to chronic exercise training have shown less oxidative damage after exhaustive exercise than untrained counterparts (Pinho et al. 2006; Oztasan et al. 2004; Sen et al. 1992; Jenkins 1988). There is a growing evidence that the continued presence of low concentrations of ROS is able to induce the expression of antioxidant

enzymes and other defense mechanisms in exercising individuals (Gomez-Cabrera et al. 2006). ROS generated during muscle contraction either from mitochondrial respiratory chain or other sources play a critical role in muscle adaptation to exercise-induced oxidative stress by regulating the mRNA levels through activation of signaling pathways (Fulle et al. 2004) and activating specific redox-sensitive transcription factors such as AP-1 and NF-κB (Lambertucci et al. 2007). It is believed that the adaptive response in the present investigation results from the cumulative effects of repeated exercise bouts, which provided the initial signals for the stimulation leading to the long-term modulation that occur after each individual exercise bout (Hollander et al. 2001). It is interesting to note that the protective effect of antioxidants on the generation of exercise-induced lipid peroxidation seem to occur after the single bouts of exercise in our study, which lends support to general hypothesis of lipid peroxidation as a potential important mechanism behind the physical exercise-antioxidant relationship (Radak et al. 2007).

There are several potential limitations to be acknowledged to the present study. Diet during the training period was not controlled. Future research may warrant the need to utilize food diaries, as well as extend the length of training period, employ larger number of study subjects ($n > 30$) and wider range of oxidative stress markers to further substantiate our findings. Also, it is logical to assume that different forms of exercise may lead to formation of different oxidation products, depending on the chemistry of the oxidative insult (Mally et al. 2007). Therefore, additional models need to be studied to further examine the relationship between exercise and oxidative stress. It is also necessary to recruit a control group of which the effect of training on subjects could be truly determined.

CONCLUSION

In conclusion, the rope-skipping exercise training did not seem to modify the time response pattern of lipid peroxidation and antioxidant system after both acute bouts of rope skipping. Nonetheless, training induced marked changes in the oxidative state and antioxidant status in our subjects. It is likely that regular exercise induces adaptive responses in antioxidant and repair systems and the combined effects of these changes may results in enhanced protection against ROS and a decrease in the accumulation of further exercise-induced oxidative damage.

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