Effects of treadmill training combined with Vitamin C or estradiol on Nitric Oxide metabolite, oxidative stress marker and liver enzymes levels in rat

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Original Article

Abstract

Introduction: The production of Reaction Oxygen Species (ROS), lowers cellular antioxidant levels, and enhances oxidative stress in many tissues, especially the liver. Efficient liver function is extremely important to the overall health. The key to helping prevent long-term damage is to decrease oxidative stress. The purpose of this study was to assess the effects of treadmill training with vitamin C or estradiol on nitric oxide metabolite, oxidative stress marker and liver enzymes levels in female rat.

Methods: Thirty two female rats were randomly divided into four groups of 8 rats each; consisting of control (Con), training (Tr), training + vitamin C (Tr+VitC), and training+estradiol (Tr+Es) groups. Vitamin C (250 mg/kg/day) was injected three times a week for 6 weeks, and estradiol (0.25 mg/kg/week) was injected for the two first weeks. Training groups performed aerobic exercise on a treadmill 5 days/week for 6 weeks. Serum and liver tissue levels of nitrite and malondialdehyde (MDA), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in serum were determined.

Results: The results showed significant reductions in the serum nitrite in Tr+VitC group (6.33±0.37μmole/l) compared with Con group (9.67± 1.39 μmole/l) and Tr group (9.91± 1.33 μmole/l) groups. While Tr group (0.22±0.02 μmole/l) exhibited lower liver nitrite compared with Con group (0.29±0.01 μmole/l) group. Serum MDA in Tr (6.68±0.31 μmole/l) and Tr+VitC (7.01±0.44 μmole/l) groups was significantly higher than in Con groups (5.20± 0.40 μmole/l).

Conclusion: Our findings indicate that the treadmill training program used in this study was able to attenuate the liver oxidative stress but administration of vitamin C or estradiol couldn't improve liver status.

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Introduction:

Reactive Oxygen Species (ROS) and lipid peroxidation have been implicated in hepatic injury (1). As a matter of fact, ROS are needed to maintain at a certain level in the body to perform its critical physiological functions such as defense against microorganisms (2). The balance between production and neutralize of ROS in the body is maintained by antioxidant defense system (3). Redox imbalance, known as oxidative stress (OS) (4). OS affects the major cellular components such as proteins, lipids and DNA. It play an important role in the pathogenesis of various degenerative diseases, such as diabetes, cancer, cardiovascular disorders or neurodegenerative diseases (5).

OS has also a vital role in chain of liver diseases. Liver is a major organ attacked by ROS. Furthermore, Liver variations may indicate anti-oxidative/oxidative status of the whole body (6).

Therefore administrations of various antioxidants are proposed to prevent oxidative stress induced liver diseases (7). It is documented that OS and antioxidant defense system is related to gender (8). In fact sexual hormones may play an important role in the progression of chronic liver diseases (9). Estrogen has anti-oxidative properties related to the airing phenolic hydroxyl group, which acts as an effective electron donor and a free radical scavenger and interrupts the lipoperoxidation reaction. Estrogen also protects females by up-regulating the expression of antioxidants such as glutathione peroxidase (GSH-Px) and manganese superoxide dismutase (MnSOD) (10). Vitamin C (ascorbic acid) is a potent, water-soluble antioxidant with a non-enzymatic structure. It prevents of cellular compounds oxidation. It is crucial to the regeneration of lipid-bound vitamin E (11).

There is evidence that regular physical activity induces up regulation of the antioxidant defense system and down-regulation of ROS production in the liver (12). Furthermore, regular moderate physical activity play an important role in the maintenance of optimal liver function and increase resistance to oxidant stress (13). So the purpose of the present study was to examine the effects of treadmill training combined with vitamin C or estradiol on nitric oxide metabolite, oxidative stress marker and liver enzymes levels in rat.

Methods:

Thirty two female Wistar rats (177.30±2.61 gr, Animal Centre, Isfahan University of Medical Sciences, Isfahan, Iran), were used. The animals had free access to water and standard rat chow, and were kept in collective cages (four rats per cage) under controlled temperature of 23-25°C with a 12-h, light/12-h dark cycle. All animal procedures were conducted in accordance with Isfahan University of Medical Sciences Ethics Committee.

Rats were randomly divided in four groups consisting of control (Con), training (Tr), training+ vitamin C (Tr+VitC), training+estradiol (Tr+Es) that containing 8 rats each. The control group did not receive any intervention. Experimental protocol was performed for 6 weeks. Vitamin C was purchased from Sigma (St. Louis, MO, USA) and was injected intraperitoneally with dos of 250 mg/kg/day three times a week in Ex+VitC group and the other groups received saline. Estradiol valerate was obtained from Aburaihan Co. (Tehran, Iran). 0.25 mg/kg/week estradiol dissolved in sesame oil and was injected intramuscular in to Tr+Es group for two first weeks, and the other groups received only sesame oil. Two rats died during the experimental protocol.

Training was performed on treadmill. The training protocol consisted of a modification protocol used previously for female rats (14,15). The animals were adapted to the treadmill for one week (10 min/day; 0.3 Km/h). From the second week on, training duration was constant (60 min/day). The training intensity was gradually increased in speed from 0.6 to 1.2 km/h, and performed 5 times per week, with two days of rest during the 6 weeks period. This training was performed at low-moderate intensity (50-70% maximal running speed). The training intensity was about 55% VO2max (16,17).

By the end of the experimental protocol, the rats were anesthetized with chloral hydrate injection (450 mg/kg; ip), blood samples were obtained via heart puncture and centrifuged at 6000 rpm, and then serum was collected and frozen at -80°C until analysis.

Immediately postmortem, the livers were removed, weighted and frozen at -80°C until analysis. The uterus was also removed and weighed. The liver tissue was homogenized and

centrifuged. The supernatant was used for the measurement of malondialdehyde (MDA).

MDA levels in serum and tissue were also measured manually. Liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) in serum were determined using quantitative diagnostic kits (Pars Azmoon, Iran) by automatic analyzer (Technicon, RA1000 model).

The data are presented as mean±SEM. Differences among groups were assessed in terms of the serum and tissue levels of nitrite and MDA, the serum level of ALT and AST by a one way analysis of variance (ANOVA), followed by a post hoc analysis using the least significant difference (LSD) test. The body weight was analyzed by repeated measures. The sphericity hypothesis was rejected, therefore, we use of Greenhouse–Geisser. A mean difference was significant at the 0.05 level.

Results:

The result showed serum nitrite in Tr+VitC group was significantly lower than Con (P= 0.046) and Tr groups (P= 0.033), while liver nitrite in Tr group was significantly lower than Con (P=0.038) group (Figure 1).

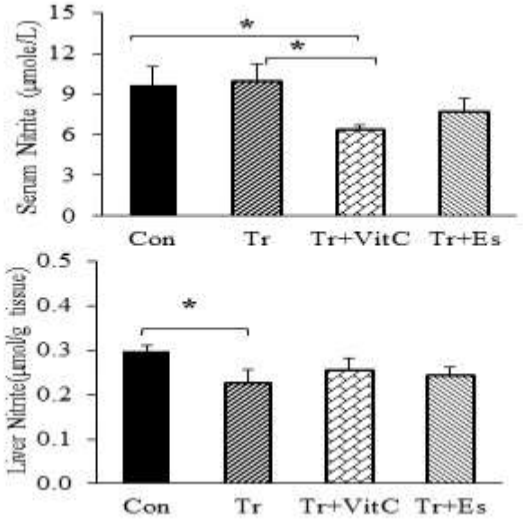

Figure 1. The levels of serum and liver nitrite (nitric oxide metabolite) in experimental groups. The data were reported as mean±SEM. *Indicate significant difference (P<0.05). Abbreviations of Con, Tr, Tr+VitC and Tr+Es were used stand of control, exercise and the combination of exercise with Vitamin C or Estradiol.

But serum MDA in Con group was significantly lower than Tr (P=0.016), Tr+VitC (P=0.004) and Tr+Es (P=0.049) groups. No significant differences were observed in MDA liver between the groups (Figure 2).

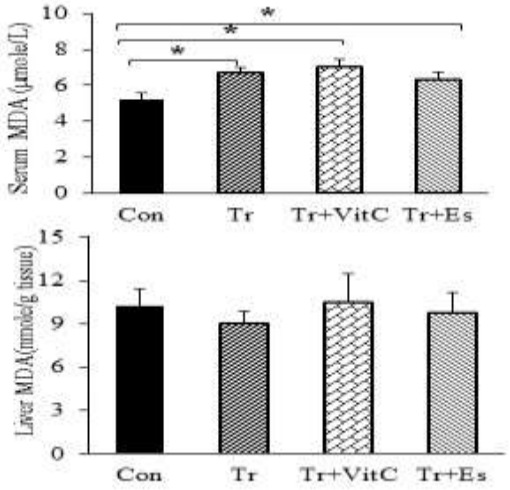

Figure 2. The levels of serum and liver malondialdehyde (MDA) in experimental groups. The data were reported as mean±SEM. *Indicate significant difference (P<0.05). Abbreviations of Con, Tr, Tr+VitC and Tr+Es were used stand of control, exercise and the combination of exercise with Vitamin C or Estradiol.

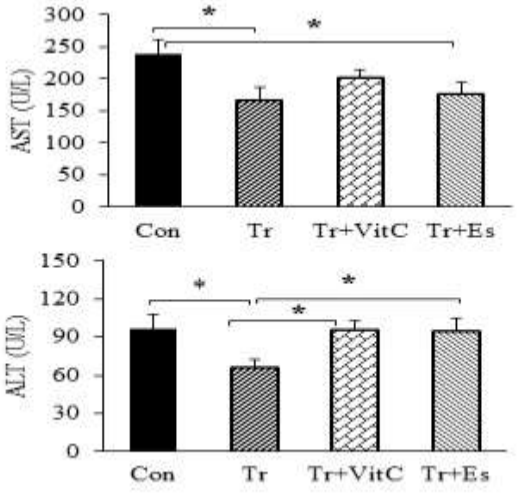

Figure 3. The levels of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in experimental groups. The data were reported as mean±SEM. *Indicate significant difference (P<0.05). Abbreviations of Con, Tr, Tr+VitC and Tr+Es were used stand of control, exercise and the combination of exercise with Vitamin C or Estradiol.

There was a significant decrease in the serum level of ALT in Tr group compared with Con (P=0.033), Tr+Vit C (P=0.041) and Tr+Es (P=0.046) groups. While the serum level of AST in Con group was significantly higher than Tr (P=0.012) and Tr+Es (P=0.023) groups (Figure 3).

Conclusion:

In our study, treadmill training alone decreased liver nitrite as well as with vitamin C decreased serum nitrite. There is some evidence that the NO releasing could be gender-related. The half-life of NO in blood circulation is very short, but its metabolites; nitrite or nitrate are stable and measureable. Variation of serum nitrite level is related to kind of exercise (18). Among the NO metabolites, nitrite is a major oxidative metabolite (19). Abbasi et al (2017) showed that training increased liver nitrite in ovariectomized rats but, exercise with vitamin C and estradiol decreased it (20).

The results showed that training alone or with vitamin C and estradiol increased serum MDA Therefore we can conclude that the increased level of MDA is due to exercise. Furthermore, vitamin C or estradiol couldn't attenuate the hepatic enzymes. Lipid peroxidation is a well-known tissue damage mechanism in humans. MDA is a product of lipid peroxidation and is often used to express OS (21,22). It has been clearly shown that exhausting exercise causes lipid peroxidation and OS in the liver (23,24). OS is dependent on multiple factors such as type, intensity and duration of exercise (25). Most studies of rats have shown moderate aerobic exercise leads to decrease OS, and consequently OS markers are decreased (26). Therefore hepatic antioxidant enzymes activities increased (27,28). In our study the exercise intensity was moderate and probably the exercise duration might be excessive.

Nevertheless, in our study treadmill training led to decrease serum ALT and AST level. So, it had partly positive effect on liver status. The liver plays a major role in exertion of toxin from body. When the liver cells injured, the liver enzymes spilled to the blood stream. It has been found that increased level of these enzymes in the blood is an early symptom of liver disease. Among the liver enzymes, aminotransferases such as AST and ALT are usually used (29). It has been shown markers of hepatic function such as ALT correlated negatively with habitual physical activity (30) and a sedentary lifestyle induced steatosis (31). On the other hand there is a direct relationship between physical activity, fitness and serum aminotransferase levels (13). Acute exercise increases the production of ROS due to the increased volume of O_2 inhaled, alterations in intracellular Ca^{++} homeostasis, vasomotor variations, and ischemia–reperfusion. But in long-term, ROS generated in exercise led to an adaptive response of the antioxidant system (32). On the other hand, ROS act in important intracellular signaling pathways that are sensitive to OS such as NF-Kβ and mitogen-activated protein kinase (MAPK) pathways. These pathways cause to promoting the genes expression of antioxidant enzyme such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) and thereby maintain intracellular redox equilibrium (33).

As shown in our study estradiol and vitamin C couldn't attenuate liver enzymes. Cutler (34,35) described "the OS compensation model" that explains most people are able to maintain their set point of OS. Therefor no matter how much additional antioxidant supplements they consumed in their diet. This model is consistent with our result about vitamin C and estradiol.

It is concluded that moderate training reduces hepatic OS. In addition, estradiol and vitamin C couldn't improve liver status. However, need to perform more studies in this field.

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Conflict of Interest:

The authors declare no conflict of interest.

Ethical approval:

The experimental procedures were in advance approved by the Isfahan University of Medical Sciences Ethics Committee.

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تأثیر تمرین تردمیل همراه با مصرف ویتامین C یا استرادیول بر سطوح متابولیت نیتریک اکسید، شاخص استرس اکسیداتیو و آنزیم‌های کبدی در رت

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مجله پزشکی هرمزگان سال بیست و یکم شماره پنجم ۹۶ صفحات ۲۴-۱۸

چکیده

مقدمه: *تولید گونه‌های فعال اکسیژن در بدن منجر به کاهش سطوح آنتی‌اکسیدان‌ها و افزایش استرس اکسیداتیو در تعدادی بافت‌ها به ویژه کبد می‌شود. عملکرد کبدی کارآمد برای سلامت کلی بسیار مهم است. کلید کمک به پیشگیری از آسیب‌های درازمدت کبدی، کاهش استرس اکسیداتیو است. مصرف آنتی‌اکسیدان‌ها و ورزش منظم از جمله راه‌کارهای رسیدن به این امر مهم می‌باشد؛ اما اثر همزمان آنها کمتر مورد مطالعه قرار گرفته است. هدف از این مطالعه، تأثیر تمرین تردمیل همراه با ویتامین C یا استرادیول بر سطوح متابولیت نیتریک اکسید، شاخص استرس اکسیداتیو و آنزیم‌های کبدی در رت‌های ماده بود.*

روش کار: *۳۲ سر رت ماده به چهار گروه شامل کنترل، تمرین، تمرین + ویتامین C و تمرین + استرادیول تقسیم شدند. ویتامین C (۲۵۰میلی‌گرم/کیلوگرم در روز) به مدت ۶ هفته و هر هفته ۳ بار تزریق شد. استرادیول (۰/۲۵ میلی‌گرم/کیلوگرم در هفته) در دو هفته اول و هفته‌ای یک بار تزریق شد. گروه‌های تمرین، تمرین تردمیل به صورت دویدن بر روی تردمیل را به مدت ۶ هفته، هر هفته ۵ روز و هر روز یک ساعت اجرا کردند. سطوح نیتریت و مالون دی آلدئید (MDA) در بافت کبد و سرم و سطح آسپارتات آمینوترانسفراز (AST) و آلانین آمینوترانسفراز (ALT) در سرم مورد اندازه‌گیری قرار گرفتند.*

نتایج: *نتایج کاهش معنی‌داری را در سطوح نیتریت سرم در گروه تمرین + ویتامین C (۰/۳۷± ۶/۳۲ میکرومول در لیتر) نسبت به گروه تمرین (۱/۳۳± ۹/۹۱ میکرومول در لیتر) و کنترل (۱/۳۹± ۹/۶۷ننخ میکرومول در لیتر) به طور معنی‌داری کمتر بود. در حالی که سطح نیتریت کبد در گروه تمرین (۰/۰۲± ۰/۲۲ میکرومول در لیتر) نسبت به گروه کنترل (۰/۰۱± ۰/۲۹ میکرومول در لیتر) به طور معنی‌داری کمتر بود. سطح سرمی MDA در گروه‌های تمرین (۰/۳۱± ۶/۶۸ میکرومول در لیتر) و تمرین + ویتامین C (۰/۴۴± ۷/۰۱ میکرومول در لیتر) نسبت به گروه کنترل (۰/۴۰± ۵/۲۰ میکرومول در لیتر) به طور معنی‌داری بیشتر بود.*

نتیجه‌گیری: *نتایج نشان داد که این برنامه تمرین تردمیل (هوازی) مورد استفاده در این مطالعه منجر به کاهش آمینوترانسفرازها شد، اما مصرف ویتامین C و استرادیول نتوانست وضعیت کبد را بهبود ببخشد.*

کلیدواژه‌ها: *استرادیول، استرس اکسیداتیو، نیتریت، کبد، رت*

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