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Proceeding Paper

Antioxidant Supplementation Hinders the Role of Exercise Training as a Natural Activator of SIRT1 †

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Abstract: Exercise training (ET) is a natural activator of Sirtuin 1 (SIRT1), a stress-sensor able to increase the endogenous antioxidant system. SIRT1 activators, including polyphenols and vitamins, can enhance the antioxidant capacity. Antioxidant supplements are used to improve athletic performance. However, they might blunt ET-related benefits. Middle-distance runners (MDR) taking (MDR-S) or not taking antioxidant supplements (MDR-NoS) were compared with each other and with sedentary subjects (CTR) to evaluate the ET effects on SIRT1 levels and oxidative stress, and to investigate whether an exogenous source of antioxidants could interfere with such effects. Thirty-two MDR and 14 CTR were enrolled. SIRT1 mRNA and activity were measured in PBMCs. Total oxidative status (TOS) and total antioxidant capacity (TEAC) were determined in plasma. MDR showed higher levels of SIRT1 mRNA ($p = 0.0387$) and activity ($p = 0.0055$) than CTR. MDR-NoS also showed higher levels than MDR-S without reaching statistical significance. SIRT1 activity was higher ($p = 0.0012$) in MDR-NoS than in MDR-S. TOS did not differ among the groups, while MDR showed higher TEAC levels than CTR ($p = 0.0001$) as did MDR-S and MDR-NoS (MDR-S vs. CTR, $p = 0.0007$ and MDR-NoS vs. CTR, $p = 0.003$). TEAC ($\beta = 0.4488356$, 95% CI 0.2074645 0.6902067; $p = 0.0001$) and the MDR-NoS group ($\beta = 744.6433$, 95% CI 169.9954 1319.291; $p = 0.012$) predicted SIRT1 activity levels. Antioxidant supplementation seems to hinder the role of ET as a natural activator of SIRT1.

Keywords: antioxidant capacity; athletes; endurance training; sirtuins; vitamins

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1. Background

Exercise training (ET) is recommended by the International Health Authorities as it provides benefits to healthy individuals and patients belonging to several clinical settings [1–3]. ET contrasts oxidative stress, by decreasing radical oxygen species (ROS) and other oxidant molecules and/or increasing antioxidant ones [4]. On the other hand, because of increased oxygen consumption, during exercise the production of ROS may overcome the capacity of the endogenous antioxidant system to detoxify them, producing oxidative stress [5–7]. ET is a natural activator of Sirtuin 1 (SIRT1), which is a NAD+-dependent deacetylase acknowledged as a life-span- and health-span-prolonging agent [8–10]. SIRT1

activated during ET can contrast aging and age-associated diseases by increasing the cellular antioxidant capacity [10–12]. However, the ET-related effects, including SIRT1 activation, strongly depend on the type, intensity and duration of the training [13–16]. Other natural activators of SIRT1 include polyphenols, such as resveratrol, and several phenolic plants extracts whose antioxidant properties are widely acknowledged [17,18]. Supplementation with antioxidants can help prevent or contrast oxidative stress and its associated cellular damage. Indeed, supplements, especially those containing vitamins and other micronutrients, are commonly used to improve athletes' wellness and performance [19–21]. Despite this, the effects of antioxidant supplementation have not yet been elucidated, especially in athletes performing endurance training [20]. Therefore, in this study, we compared the effects on SIRT1 and antioxidant capacity in endurance athletes using or not using antioxidant supplements to investigate whether an exogenous source of antioxidants could interfere with ET-related effects.

2. Methods

Thirty-two endurance athletes, that are middle-distance runners (MDR), and fourteen age-matched sedentary volunteers (CTR) were enrolled. All participants signed informed consent and the study got approval from the local ethics committee (Observational Study n. 86/2020). MDR belonged to an amateur sports association called "Atletica Salerno". They were divided into two groups. One of them (MDR-S) consumed an antioxidant supplement every day (S) consisting of 240 mg vitamin C and 15 mg vitamin E, together with 861 mg sodium, 555 mg chlorine, 381 mg potassium and 66 mg magnesium. The other group did not use any antioxidant supplementation (MDR-noS). We recorded the athletes' data, including those regarding training regimen as well as information concerning alcohol consumption and tobacco use and dietary habit. Blood samples were collected in fasting conditions from each participant. Peripheral Blood Mononuclear Cells (PBMCs) were isolated by Ficoll-Paque density gradient. Serum samples were obtained by centrifugation at 1500× *g* for 10 min. Aliquots of serum and PBMCs were frozen at −80 °C until analysis. SIRT1 mRNA and activity were measured in PBMCs by Real-Time PCR and fluorimetric assay, respectively. Total oxidative status (TOS) and total antioxidant capacity (TEAC) were measured in plasma by colorimetric assay and oxidative stress index (OSI) was determined by TOS/TEAC ratio.

3. Results

The study population consisted of 14 CTR, 14 MDR-noS and 18 MDR-S. There were no differences in age, tobacco and alcohol use as well as in dietary habits between the two groups of athletes, and between athletes and sedentary controls. CTR had a BMI higher than MDR-S and MDR-noS (both, $p = 0.0001$), while no differences between MDR-S and MDR-noS were found. In addition, neither training time/week nor training frequency/week differed between MDR-S and MDR-noS. MDR demonstrated higher levels of SIRT1 mRNA compared to CTR ($p = 0.0387$). Notably, MDR-noS showed higher levels than CTR ($p = 0.0136$) while MDR-S did not differ from CTR. No differences between MDR-S and MDR-noS were found (Figure 1, panel A). MDR showed higher levels of SIRT1 activity compared to CTR ($p = 0.0055$). MDR-noS had the highest value, significantly higher compared to both CTR ($p = 0.0003$) and MDR-S ($p = 0.0012$) (Figure 1, panel B).

As shown in Figure 2 (panel A), no differences in TOS levels were found among the groups. MDR showed higher levels of TEAC compared to CTR ($p = 0.0001$). Notably, both the MDR-S and MDR-noS showed higher levels than CTR (MDR-noS vs. CTR, $p = 0.0003$ and MDR-S vs. CTR, $p = 0.0007$). No differences were found between MDR-S and MDR-noS (Figure 2, panel B). CTR demonstrated the highest levels of OSI (TOS/TEAC) compared to the other groups (CTR vs. MDR, $p = 0.0002$; CTR vs. MDR-noS, $p = 0.0015$ and CTR vs. MDR-S, $p = 0.0086$). No differences were found between MDR-S and MDR-noS (Figure 2, panel C).

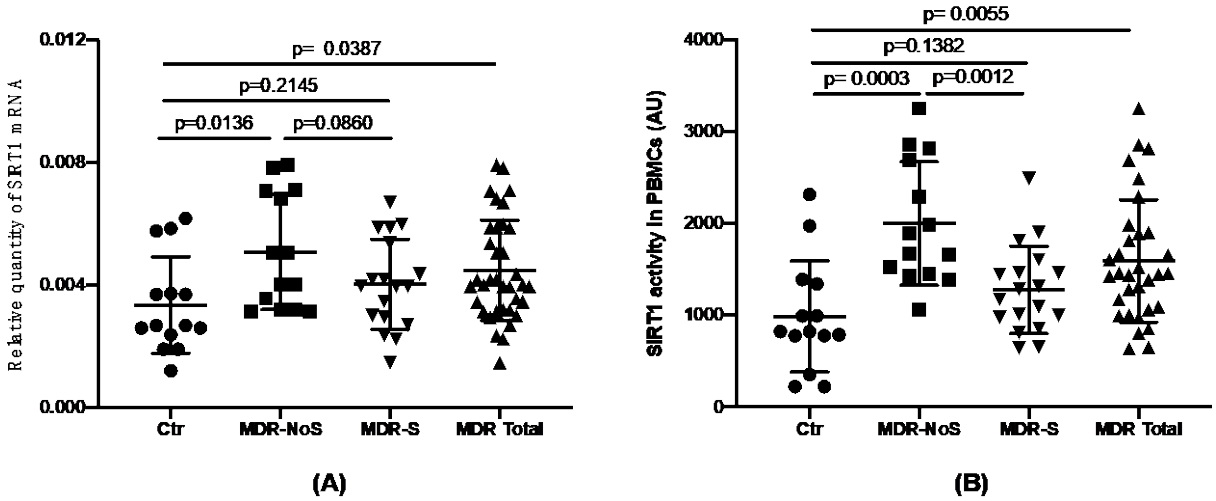


Figure 1. SIRTUIN 1 (SIRT1) mRNA expression (**A**) and activity (**B**) were measured in peripheral blood mononuclear cells (PBMCs) extracted from sedentary controls (CTR), middle distance runners not taking antioxidant supplements (MDR-NoS), and MDR taking antioxidant supplements (MDR-S). MDR indicates all the MDR, irrespective of antioxidants supplementation. CTR, MDR-NoS, MDR-S, and MDR are respectively indicated with a black circle, black square, black triangle with the vertex at the bottom, and black triangle with the vertex at the top. All data are expressed as mean ± SD.

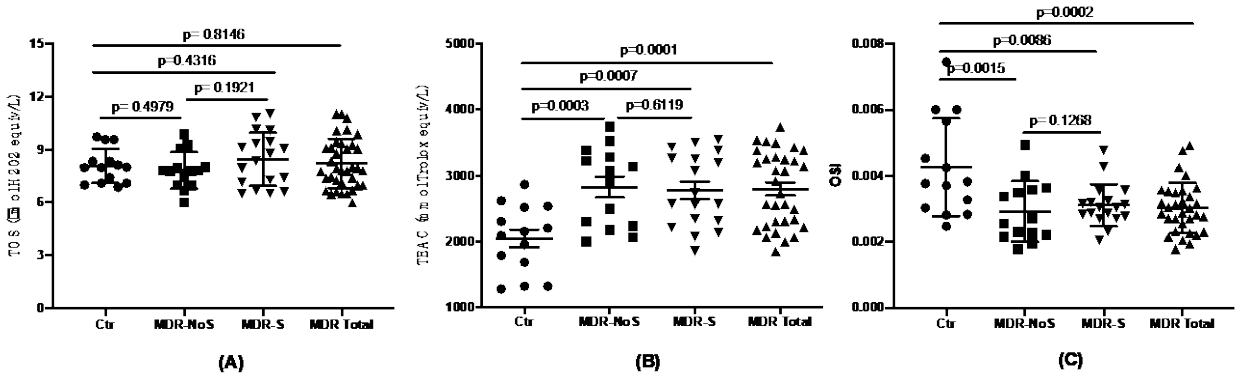
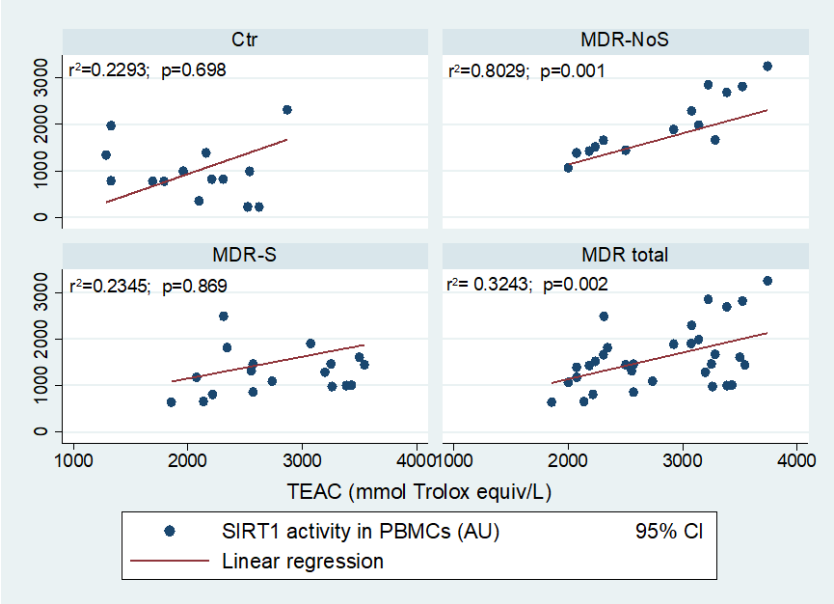
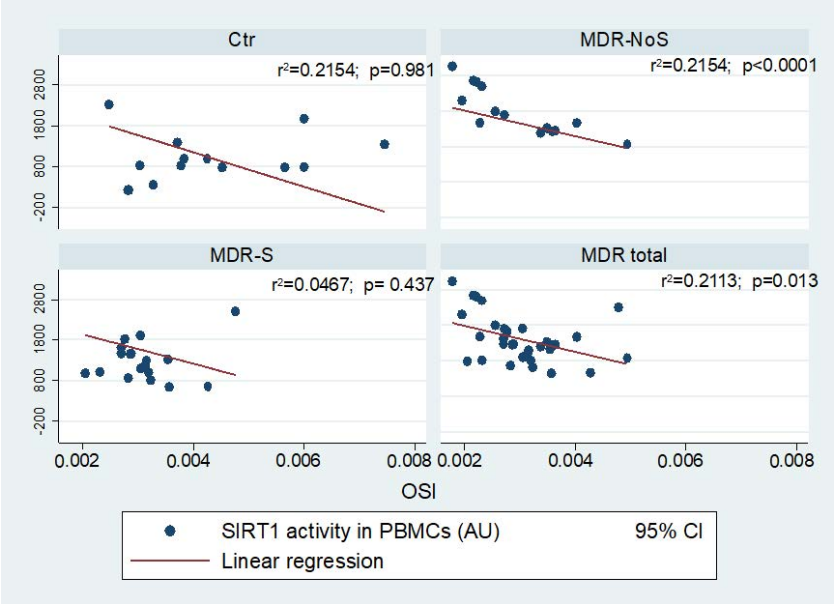


Figure 2. Total oxidative status (TOS) (**A**), Trolox equivalent antioxidant capacity (TEAC) (**B**), and oxidative stress index (TOS/TEAC, OSI) (**C**) were determined in the serum extracted from sedentary controls (CTR), middle distance runners not taking antioxidant supplements (MDR-NoS) and MDR taking antioxidant supplements (MDR-S). MDR indicates all the MDR irrespective of antioxidant supplementation. CTR, MDR-NoS, MDR-S, and MDR are respectively indicated with a black circle, black square, black triangle with the vertex at the bottom, and black triangle with the vertex at the top. All data are expressed as mean ± SD.

A statistically significant correlation by linear regression analysis between SIRT1 activity and TEAC (p = 0.002, r^2 = 0.2345) was found. This correlation was determined by the results of MDR-noS (p = 0.001, r^2 = 0.8029) (Figure 3, panel A). Conversely, an inverse correlation between SIRT1 activity and OSI was found in MDR (p = 0.013, r^2 = 0.213). This finding was determined by the inverse correlation between the two considered parameters in MDR-noS (p < 0.0001, r^2 = 0.2154) (Figure 3, panel B).



(**A**)



(**B**)

Figure 3. (**A**) Linear regression analyses with SIRT1 (SIRT1) activity measured in peripheral blood mononuclear cells (PBMCs) and Trolox equivalent antioxidant capacity (TEAC) measured in serum of CTR, MDR-NoS, MDR-S, and MDR. (**B**) Linear regression analyses with SIRT1 (SIRT1) activity measured in peripheral blood mononuclear cells (PBMCs) and Oxidative Stress Index (OSI) calculated as the ratio of TOS on TEAC. The groups were: sedentary controls (CTR), middle distance runners (MDR) not taking antioxidant supplements (MDR-NoS), and MDR taking antioxidant supplements (MDR-S). All data are expressed as mean ± SD.

4. Conclusions

This study demonstrated that TEAC increased in MDR compared to CTR irrespective of an antioxidant supplementation intake. SIRT1 mRNA and activity increased in MDR-noS but not in MDR-S when compared to CTR. Notably, SIRT1 activity is strongly correlated with TEAC in MDR-noS but not in MDR-S. An exogenous source of antioxidants seems to hinder the role of endurance training as a natural activator of SIRT1.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent was obtained from all participants to publish this paper.

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Conflicts of Interest: The authors declare no conflict of interest.

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