# **TEN MARATHONS IN TEN DAYS: EFFECTS ON BIOCHEMICAL** PARAMETERS AND REDOX BALANCE – CASE REPORT

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# DESET MARATONA ZA DESET DANA: UTICAJ NA BIOHEMIJSKE PARAMETRE I REDOKS RAVNOTEŽU – PRIKAZ SLUČAJA

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## ABSTRACT

Production of free radicals and oxidative damage during physical activity is a topic that is intensively studied and paid a lot of attention, first of all in professional sports. Marathon is categorized as extremely demanding sports discipline, as it induces high energy consumption and also requires special mental self-control. We presented cases of two athletes of different age, who have been on dissimilar level of sports readiness, and also had various approach to physical activity and exercise. During 10 days they ran out 10 marathons, partly on a flat terrain, and partly on hilly, which produced different level of effort in conquering the terrain. Also, both athletes had complex supplementation scheme in order to prevent electrolyte imbalance and excessive production of free radicals. Blood samples were taken in the morning and immediately after the end of the marathon. Measured oxidative stress biomarkers changed without a noticeable pattern, but these changes did not vary greatly among themselves. Catalase activity in both marathon runners was higher after marathon almost after every race for 10 days. On the other hand, amount of reduced glutathione was lower after marathon in both athletes in the same manner. Based on the obtained results we can conclude that adequate supplementation could have crucial role in prevention of oxidative damage.

Keywords: marathon; physical activity; oxidative stress; reactive oxygen species; supplementation

# SAŽETAK

Stvaranje slobodnih radikala i oksidaciono oštećenje je tema kojoj se posvećuje dosta pažnje i koja se intenzivno istražuje, pre svega kod profesionalnih sportista. Maraton se svrstava u izuzetno zahtevne sportske discipline koja podrazumeva veliku energetsku potrošnju i specifičnu mentalnu samokontrolu. Prikazali smo slučaj dvojice maratonaca različite starosne dobi, različite fizičke spremnosti i sa različitim prisupom fizičkoj aktivnosti i vežbanju. Tokom 10 dana istrčali su 10 maratona, delom na ravnom, a delom na brdovitom terenu, što je uzrokovalo različito opterećenje tokom savladavanja pojedinih deonica. Takođe, trkači su imali kompleksnu shemu suplementacije u cilju prevencije elektrolitnog disbalansa i prekomerne produkcije slobodnih radikala. Uzorci krvi su uzimani ujutru i odmah nakon završetka maratona. Promne vrednosti merenih biomarkera oksidacionog stresa nisu imale bilo kakav obrazac, ali ove promene nisu značajno varirale između maratonaca. Aktivnost katalaze kod oba maratonca je bila znatno viša nakon maratona gotovo tokom svih 10 dana. Sa druge strane, količina redukovanog glutationa je bila niža nakon maraton kod oba trkača. Na osnovu dobijenih rezultata može da se zaključi da adekvatna suplementacija može da ima presudnu ulogu u prevenciji oksidacionog oštećenja.

Ključne reči: maraton; fizička aktivnost; oksidacioni stres; reaktivne vrste kiseonika; suplementacija

## **ABBREVIATIONS**

ATP - adenosine triphosphate CoQ10 - Coenzyme Q10 **CARB** – protein carbonyls CAT – catalase **GSH** – reduced glutathione MUM – mountain ultra marathon NO – nitric oxide **RONS** – reactive oxygen and nitrogen species **ROS** – reactive oxygen species TAC - total antioxidant capacity TBARS - thiobarbituric acid reactive substances



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#### INTRODUCTION

Intense physical activity lasting over prolonged period of time presents great challenge even for well-trained athletes. Marathon is categorized as extremely demanding sports discipline, as it induces high energy consumption and also requires special mental self-control. However, as one of the oldest and most demanding sports disciplines, marathon is very popular both between professional athletes and for people who practice sports occasionally. Despite this fact, we do not have enough data on influence of trainings and marathon competitions on redox balance in athlete's body, its effect on production and elimination of free radicals and elements of antioxidative system, and as result of this possible tissue damage caused by free radicals.

Oxidative stress is actually misbalance between production and elimination of free radicals. Numerous external and internal factors causes increased production of reactive type of oxygen and nitrogen (RONS), amongst others, physical activity is also accounted here. In early 80-ties causal relationship between physical activity and producing of RONS was noticed (1). Increased demand for ATP during aerobe physical activities can initially overwhelm electronic transport chain located on inner membrane of mitochondria and causes increased production of superoxide anion radicals (2). Taking into consideration that such increased production of free radicals is mainly of moderate extent, it is higher probability that physical activity and RONS produced in mitochondria's of skeletal muscles play role in signal paths, rather than directly inducing oxidative stress (3). Increased RONS production during moderate and repeated physical activity changes gene expression, which can cause genesis of mitochondria and increasing its capacity (4). However, increased oxygen consumption during intense oxidative phosphorylation occurring while practicing physical activity, as well as releasing catecholamine directly causes production of RONS, as well as increased phagocyte activity caused by muscle damage during physical activity (5). In addition to skeleton muscles, liver also plays very important role in body response to acute and repeated physical activity, as well as in the process of body adapting to increased production of free radicals and inflammation (6).

Sports training means body exposure to repeated periods of physical endeavor with aim to evolve mechanisms of adaptation to changes occurring in body during physical activity hormesis rule (7). However, physical endeavor of high intensity causes multiple changes which overstretch mechanisms of adaptation, eventually causing oxidative stress and inflammation and resulting in deterioration of physical parameters (8). Results of research on the topic of marathon effect on free radicals production and oxidative stress are contradictory and non-conclusive (9); while in other research has been shown that ultra-marathon does not cause significant changes in redox balance (10).

Taking into consideration all above facts, aim of this research is following of biochemical parameters and bio-

markers of oxidative stress and elements of anti-oxidative system in two marathon runners, of different age and different previous sports exposure and experience, who ran 10 marathons in 10 consecutive days, with previously established program for diet, supplements, recovery and rest.

#### **CASE REPORT**

We followed two athletes (S1 and S2) of different age (S1 – 22 years, S2 – 44 years), who have been on dissimilar level of sports readiness, and also had various approach to physical activity and exercise (S1 is professional triathlon sportsman, while S2 is non-professional marathon runner). Both participants were healthy, without any anatomic specifications of the body. During 10 days they ran out 10 marathons, partly on a flat terrain, and partly on hilly, where the altitude varied from 342 to 1161 meters above sea level, which produced different level of effort in conquering the terrain.

Both athletes were monitored daily for 10 days, in following areas: body weight, arterial tension, maximal and average heart rate, calories consumption, as well as supplements and recovery plans. Waking up occurred every day at 8:00 am, followed by morning measurement (body weight and arterial tension) and taking a blood sample at 8.30, breakfast at 9:00, with taking supplements during breakfast. Second blood sample was taken right after the end of the marathon. Marathon started at 12:00, and participants took rehydration during marathon. Immediately after finishing marathon second measurement of body weight and arterial tension was conducted, as well as second blood drawing. Recovery cocktail was taken around 17:00, and dinner and supplements were taken at 18:00. Massage and recovery was around 20:00, together with supplements. Going to bed was between 21:30 and 22:00.

Over the course of these 10 days of marathon races, runners on average imported 250 g of proteins, 500 g of carbohydrates, 60 g of fats and 7 g of fibers, which totals about 4000 kcal, while the average energy consumption per marathon amounted to 2289 kcal. Supplements before the start of marathon meant taking of 333.3 mg Ca<sup>2+</sup>, 133.3 mg of Mg<sup>2+</sup>, 8.3 mg of Zn<sup>2+</sup>, 1000 mg of Omega-3 fatty acids, 100 mg of CoQ10 and 400 IU of vitamin E. Solution

 Table 1. Reference values for observed laboratory parameters in the general population

Glucose	4.1-6.4 mmol/L
Urea	2-9 mmol/L
Creatinine	Female: 45-90 μmol/L Male: 50-105 μmol/L
Protein	66-83 g/L
Albumin	35-52 g/L
Globulin	25-33 g/L
Potassium	3.6-5.5 mmol/L
Sodium	135-155 mmol/L

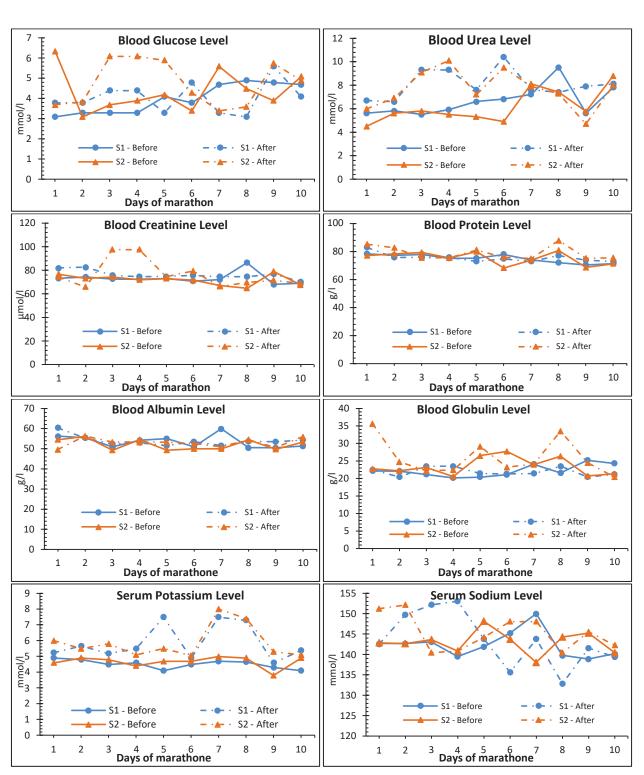


Figure 1

for rehydration during marathon race contained amino acid solution (20 ml), 5 g of glutabolic, 20 g of dextrose, 5 g of potassium chloride, 1000 mg of vitamin C dissolved in 1000 ml of water. Recovery cocktail contained 45 g of whey proteins, 44 g of carbohydrates and complex of vitamin B. Supplements at the end of the day meant 1000 mg of Omega-3 fatty acids, 44 g of carbohydrates, 333.3 mg of Ca<sup>2+</sup>, 133.3 mg of Mg<sup>2+</sup>, 8.3 mg of Zn<sup>2+</sup> and 3 tablets of vitamin B complex.

Values of blood glucose, as well as urea and creatinine, varied mainly within physiological range (Figure 1, Table 1). Similarly, blood protein, albumin and globulin levels were maintained within normal values (Figure 1, Table 1). In both runners serum potassium levels were higher after marathon compared to values before, but in the 7<sup>th</sup> and 8th day these values were considerably above the upper limit value (Figure 1, Table 1). Serum sodium levels were changed without a certain rule, but mostly within the

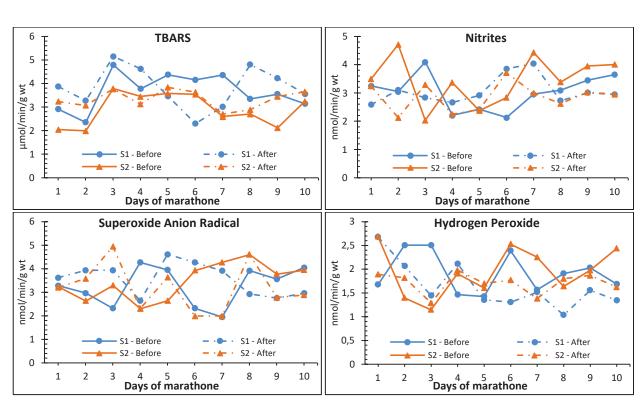
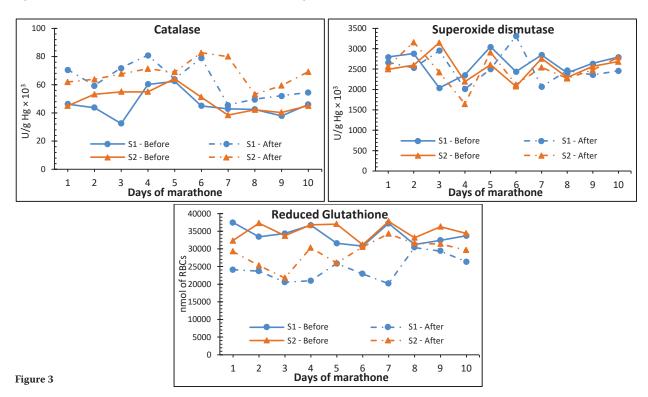


Figure 2

physiological range (Figure 1, Table 1). All mentioned biochemical parameters were measured using standard biochemical procedures.

Measured oxidative stress biomarkers (Level of lipid peroxidation measured by thiobarbituric acid assay – TBARS, Nitrites - as a measure of released nitric oxide (NO), Superoxide anion radical, and Hydrogen peroxide) changed without a noticeable pattern, but these changes did not vary greatly among themselves (Figure 2). All above mentioned oxidative stress biomarkers were determined spectrophotometrically using the previously described methods (11-14).

Catalase activity in both marathon runners was higher after marathon almost after every race for 10 days (Figure 3). On the other hand, amount of reduced glutathione was lower after marathon in both athletes in the same manner





(Figure 3). Activity of superoxide dismutase did not exhibit any regular pattern of changes (Figure 3). Similarly to oxidative stress biomarkers, values of described antioxidative elements were determined by spectrophotometry (15-18).

### DISCUSSION

Kratz and coworkers in their study followed 37 marathon runners during the 2001 Boston Marathon and showed the statistically significant increase of in the values for glucose, albumin, total protein, creatinine and some other biochemical parameters, which were not the subject of this investigation, 4 hours after marathon (19). Namely, authors of mentioned study took blood samples day before marathon, and 4 hours and 24 hours after marathon. 24 hours after marathon values of creatinine were still higher compared to values before marathon. On the other hand, there were statistically insignificant increase in values of sodium and potassium. Traiperm and colleagues followed fifty adolescent marathon runners, and blood samples were taken before, at the end and 24 h after a marathon to investigate parameters of metabolism, liver and kidney function (20). There was a statistically significant increase in the levels of blood glucose and creatinine immediately after the race. Bearing in mind that in this investigation glucose and creatinine level were generally maintained within the physiological values, these discrepancies could be the consequence of different amount of carbohydrate in rehydration beverages. Gratze and coworkers in their study showed unchanged values of serum potassium before and after marathon (21).

In a study performed by Mrakic-Sposta and colleagues it was assessed the effect of mountain ultra marathon (MUM) on production of ROS and oxidative damage in forty-six experienced ultra-marathon runners (10). It was shown that this extreme physical effort has induced increase in production of free radicals, antioxidative defense and oxidative damage. These results differ from the results of this research, and this difference could be the consequence of various race regimes, as well as probably different supplementation protocols. Namely, MUM represents an extreme physical effort, much more demanding than the regime in this research, while there was no specific supplementation protocol and any control of use of vitamin/minerals supplements, herbs and medications. Samaras and coauthors showed results regarding the effects of different supplementation regimes on redox balance and endothelial function in ultra-marathon runners for a two-months-period (22). In group of athletes with supplementation enriched with whey protein bar containing carbohydrates and protein in a specific ratio (1:1) and tomato juice, measured oxidative stress biomarkers (total antioxidant capacity - TAC, GSH, TBARS and protein carbonyls - CARB) were significantly lower. Similarly to the results of this study, Ypatios and colleagues pointed out the reduction of GSH during MUM race of 103 km (23), while TBARS, TAC, CARB, CAT activity in erythrocytes, as other measured parameters of oxidative stress, were widely varied in the respondents, so there were no statistically significant differences in the changes. Bearing in mind the results of this study and other mentioned investigations it is indicative that it is not possible to define a redox status change pattern during physical activity, above all extreme physical efforts. One of the reasons for the nonconformity of research results that deal with this topic is a large number of factors that can affect redox balance, but supplementation with antioxidants is probably one of the key issues (24).

## CONCLUSION

Taking into account the results of this case presentation, and other studies dealing with similar topics, it can be concluded that redox balance during physical activity depends on many factors. Namely, increased production of free radicals during physical activity is undisputed, but oxidative damage could be prevented. One of the most important links in extreme physical efforts is adequate supplementation, among other things, the use of appropriate antioxidants at the right time. Some future investigations should reveal what are the most appropriate supplements in certain types of sports, and how to adapt them to each athlete individually in accordance with their constitution and needs, in order to achieve as good results as possible with the least damage.

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## REFERENCES

- 1. Quintanilha AT, Packer L. Vitamin E, physical exercise and tissue oxidative damage. Ciba Found Symp. 1983; 101: 56-69.
- Vasilaki A, Mansouri A, Van Remmen H, van der Meulen JH, Larkin L, Richardson AG, McArdle A, Faulkner JA, Jackson MJ. Free radical generation by skeletal muscle of adult and old mice: effect of contractile activity. Aging Cell. 2006; 5(2): 109-17.
- Jackson MJ. Control of reactive oxygen species production in contracting skeletal muscle. Antioxid Redox Signal. 2011; 15(9): 2477-86.
- 4. Webb R, Hughes MG, Thomas AW, Morris K. The Ability of Exercise-Associated Oxidative Stress to Trigger Redox-Sensitive Signalling Responses. Antioxidants (Basel). 2017; 6(3): E63.
- 5. Russell AP, Foletta VC, Snow RJ, Wadley GD. Skeletal muscle mitochondria: a major player in exercise, health and disease. Biochim Biophys Acta. 2014; 1840(4): 1276-84.

- 6. Pillon Barcelos R, Freire Royes LF, Gonzalez-Gallego J, Bresciani G. Oxidative stress and inflammation: liver responses and adaptations to acute and regular exercise. Free Radic Res. 2017 Feb;51(2):222-236.
- Radak Z, Ishihara K, Tekus E, Varga C, Posa A, Balogh L, Boldogh I, Koltai E. Exercise, oxidants, and antioxidants change the shape of the bell-shaped hormesis curve. Redox Biol. 2017 Aug;12:285-290.
- de Lucas RD, Caputo F, Mendes de Souza K, Sigwalt AR, Ghisoni K, Lock Silveira PC, Remor AP, da Luz Scheffer D, Guglielmo LG, Latini A. Increased platelet oxidative metabolism, blood oxidative stress and neopterin levels after ultra-endurance exercise. J Sports Sci. 2014;32(1):22-30.
- 9. Seifi-Skishahr F, Damirchi A, Farjaminezhad M, Babaei P. Physical Training Status Determines Oxidative Stress and Redox Changes in Response to an Acute Aerobic Exercise. Biochem Res Int. 2016;2016:3757623.
- Mrakic-Sposta S, Gussoni M, Moretti S, Pratali L, Giardini G, Tacchini P, Dellanoce C, Tonacci A, Mastorci F, Borghini A, Montorsi M, Vezzoli A. Effects of Mountain Ultra-Marathon Running on ROS Production and Oxidative Damage by Micro-Invasive Analytic Techniques. PLoS One. 2015 Nov 5;10(11):e0141780.
- 11. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95: 351–358
- Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Anal Biochem. 1982; 126(1): 131-8.
- Pick E, Keisari Y. A simple colorimetric method for the measurement of hydrogen peroxide produced by cells in culture. J Immunol Methods. 1980; 38(1-2): 161-70.
- 14. Auclair C, Voisin E. Nitroblue tetrazolium reduction. In: Greenvvald RA (ed) Handbook of methods for oxygen radical research. CRC Press, Boka Raton. 1985. 123–132.
- 15. McCord JM, Fridovich I. The utility of superoxide dismutase in studying free radical reactions. I. Radicals

generated by the interaction of sulfite, dimethyl sulfoxide, and oxygen. J Biol Chem. 1969; 244(22): 6056-63.

- Catalase BE. In: Beutler E (ed) Red cell metabolism, a manual of biochemical methods. Grune and Stratton, New York. 1982. 105–106.
- 17. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972; 247(10): 3170-5.
- 18. Beutler E. Reduced glutathione GSH. In: Beutler E (ed) Red cell metabolism: a manual of biochemical methods. Grane and Straton, New York. 1975. 112–114.
- 19. Kratz A, Lewandrowski KB, Siegel AJ, Chun KY, Flood JG, Van Cott EM, Lee-Lewandrowski E. Effect of marathon running on hematologic and biochemical laboratory parameters, including cardiac markers. Am J Clin Pathol. 2002; 118(6): 856-63.
- 20. Traiperm N, Gatterer H, Pariwat P, Burtscher M. Energy metabolism, liver and kidney function in adolescent marathon runners. Eur J Clin Invest. 2016; 46(1): 27-33.
- 21. Gratze G, Mayer H, Skrabal F. Sympathetic reserve, serum potassium, and orthostatic intolerance after endurance exercise and implications for neurocardiogenic syncope. Eur Heart J. 2008; 29(12): 1531-41.
- 22. Samaras A, Tsarouhas K, Paschalidis E, Giamouzis G, Triposkiadis F, Tsitsimpikou C, Becker AT, Goutzourelas N, Kouretas D. Effect of a special carbohydrate-protein bar and tomato juice supplementation on oxidative stress markers and vascular endothelial dynamics in ultra-marathon runners. Food Chem Toxicol. 2014; 69: 231-6.
- 23. Ypatios S, Dimitrios S, Marina O, Nikolaos G, David BO, Demetrios S, Demetrios K. Variations In Oxidative Stress Levels In Three Days Follow-Up In Ultra-Marathon Mountain Race Athletes. J Strength Cond Res. 2016; doi: 10.1519/JSC.000000000001584
- 24. Neubauer O, Yfanti C. Antioxidants in Athlete's Basic Nutrition: Considerations towards a Guideline for the Intake of Vitamin C and Vitamin E. In: Lamprecht M, editor. Antioxidants in Sport Nutrition. Boca Raton (FL): CRC Press/Taylor & Francis; 2015. Chapter 3.