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INFLUENCE OF BEETROOT SUPPLEMENTATION ON OXYGEN MUSCLE SATURATION OF SEMI-PROFESSIONAL SOCCER PLAYERS FOLLOWING A REPEATED SPRINT TEST

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Abstract Dietary nitrates have hemodynamic and metabolic effects on the body. This study aimed to investigate nitrate intake's effect on quadricep muscle oxygen saturation (StO2) of semi-professional soccer players during a repeated speed test (RSA). In a randomized, crossover design, 10 semi-professional soccer men players (21.3 ±0.9yrs) performed two conditions: In one they consumed a nitrate-rich concentrated beetroot juice (250 mL/150 mg of NO3−) and in the other a placebo, 2h before a repeated sprint test. StO2 of the right vastus lateralis muscle was measured by near-infrared spectroscopy. A paired samples t-test was used to compute any differences in the subjects' performance on the StO2 and on RSA test. The level of significance was set at $p < 0.05$. The supplementation of nitrates limited the decrease in muscle oxygenation during the six sprints of RSA test ($p < 0.001$) and also, there seemed to be a tendency for limited the decrement of performance on RSA test ($t = -1.586$, $p = 0.157$, $\eta 2 = 0.218$). In conclusion, acute nitrate supplementation reduces the decrement of muscle oxygenation during exercise with repeated sprints and leads to a tendency of fatigue resistance during repeated sprints under controlled situations. Nitrate supplementation may have an ergogenic effect during exercise with repeated sprints via the higher muscle oxygenation.

Key words: nitrates, repeated sprint ability, NIRS, muscle oxygenation

Introduction

Soccer is an intermittent sport where both aerobic and anaerobic ability are important performance factors. Particularly important is the ability for repeated sprints in a short period of time (Glaister, 2005). This ability depends on neuromuscular as well as metabolic factors (Mendez-Villanueva et al., 2007), while the availability of O_2 seems to play an important role (Smith & Billaut, 2010).

Professional soccer players use a variety of dietary supplements to enhance performance. However, only some of them exhibit ergogenic activity (Burgess et al., 2006) like nitrates. In the last decade several studies have focused on the ergogenic action of nitrates (Jonvik et al., 2018). Nitrates in the human body are converted into nitric oxide (NO) which has hemodynamic and metabolic effects on the body. It is reported that they can cause vasodilation by lowering blood pressure and increasing the transfer of gases and nutrients to the exercised muscles (Jones et al., 2012). In the literature there are studies that report the ergogenic effect of nitrates (Domínguez et al., 2018; Jonvik et al., 2018; Karampelas et al., 2021) but also others who did not mention any influence (Jonvik et al., 2021; Pawlak-Chaouch et al., 2019; Poredos et al., 2022).

As mentioned above, the ability to repeat sprints is particularly important in soccer. During intense exercise the oxygen consumed by muscles exceeds oxygen supply and the oxygenation level in muscle decreased (Bhambhani, 2004; Grassi et al. 1999). Soon after the end of the exercise, the oxygen supply in muscles exceeds oxygen consumption and the muscle oxygenation level is recovered (McCully & Hamaoka, 2000). However, the time that is needed for reoxygenation after exercises depends on the ratio between oxygen supply and oxygen consumption by muscles. Nitrates during repeated sprints could enhance the transport of gases (like O_2) to the exercised muscles thus helping metabolic pathways like phosphocreatine resynthesis (PCr) (Burgess et al., 2006) enhancing the player's performance. Recent studies show positive effects of nitrates on repeated sprint test performance (Karampelas et al., 2021; Rojas-Valverde et al., 2021; Wylie et al., 2016)

Near-infrared spectroscopy (NIRS) is a non-invasive method used in examining muscle oxygenation which reflects the balance of O_2 delivery to working muscles and muscle O_2 consumption in capillary beds (Hamaoka et al., 1996). The NIRS is based on the differential absorption properties of the heme group of hemoglobins in the vascular bed and the heme group of myoglobin in muscle fibers (McCully & Hamaoka, 2000).

The effect of nitrates on muscle oxygenation during running exercise with repeated sprints has not been investigated. In the literature there are studies that report a change in muscle oxygenation without however a positive effect on the performance of endurance athletes. The aim of this study was to investigate the effect of nitrate intake on quadricep muscle oxygen saturation (StO_2) of semi-professional soccer players during a repeated speed test.

Material and Methods

Subjects

Some of the measurements in this study are part of a larger study (Karampelas et al., 2021). Ten healthy semi-professional male soccer players took part in the study voluntarily (age: 21.3 ±0.9 yrs, weight: 73.49 ±4.44 kg, body fat %: 12.68 ±3.09). The study was approved by the Ethics Committee of the School of Physical Education and Sport Science at Thessaloniki (66/2021) and conformed to the spirit of the Helsinki Declaration. All of the participants were members of soccer teams, trained five days a week and participated in one match a week. The participants were informed about the details of the study and signed the corresponding written consent form. In addition, they completed questionnaires about medical history and nitrate consumption (Karampelas et al., 2021).

Design

Participants participated in two conditions that were at least five days away from each other. In a randomized crossover design, subjects were then assigned to receive placebo (PG) or nitrate (NG) (250 mL/150 mg of NO_3^-). The juice was prepared in the laboratory by mixing beetroot powder with juice.

Each day of measurement was preceded by two days of wash-out. On these days, participants were given a list of foods and beverages that they should not consume as they contain nitrates and may have influenced the study. Participants were asked to record their diet on these days in detail which was checked not to include the above foods. During the participants' first visit anthropometric and height, weight and body fat measurements were made. The design of the study is presented in Figure 1. After a standard warm-up lasting 15 min, the repeated sprint test (RSA) was carried out (Rampinini et al., 2007). The measurements were made on an open soccer field with synthetic turf. The measurements were carried out in the morning and the only meal that preceded it was in the morning which included food without nitrates. The study was carried out during the season by modifying the contents of the participants' training sessions.

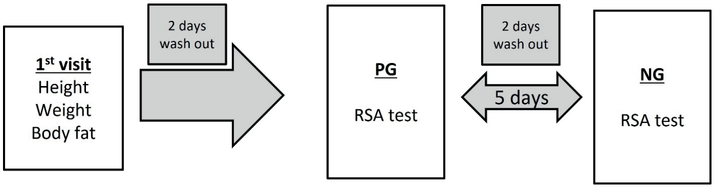

Figure 1. Study design.

Anthropometric Measurements

The following procedure for measuring anthropometric characteristics is described as performed in the study by Karampelas et al. (2021). An electronic digital weight scale and a height scale (Seca 2020e, Seca, Hamburg, Germany) were used to measure the body mass and height of the players. These two measurements had an accuracy of 0.1 kg and 0.1 cm in the respective evaluations. The participants, during the measurements, were barefoot and wearing only underwear. To assess body fat, a Lafayette skinfold caliber (Sagamore, Lafayette, Ins. Co., Indiana) was used to measure the thickness of the soccer players' hypodermic fat in four of their skinfolds (biceps, triceps, suprailiac, subscapular). All skinfold measurements were taken on the right side of the body, and body fat percentage was calculated with the use of Siri equation (1956).

RSA Test (Repeated Sprint Ability Test)

The RSA test consists of 6 × 40 m sprints (20 + 20 m) with a 20 s break in between sprints (Rampinini et al., 2007). The athletes started from the starting line sprinting for 20 m, stepping on a line and returning to the starting point as quickly as possible. They followed with 20 s of recovery before starting the next sprint. In the last 5 s of the recovery time there was a countdown to the athlete being ready to start at the end of the 20 s. In the starting line there were photoelectric gates (photocells) (Microgate, Bolzano, Italy) to record the time of each sprint. Photocells were placed 0.6m above the ground (approximately at the hip level) to capture the movement of the trunk rather than a false signal due to a limb motion. The coefficient of variation for test–retest trials was 4.1%. Three indicators were used from the test: a) RSAbest: the fastest sprint of six, b) RSAmean: the average time of six sprints and c) RSAdec: the rate of decline in performance during the six sprints (was used the time in the fastest sprint and the slowest).

Near-Infrared Spectroscopy

Moxy (Fortiori Design LLC, Hutchinson, MN, USA) a portable NIRS apparatus was used in this study. Moxy is a compact (61 × 44 × 21 mm) and lightweight (48 g) system which employs four wave lengths of NIR light at 680, 720, 760 and 800 nm (Schmitz, 2015). The sensor contains a single LED and two detectors were placed 12.5 and 25.0 mm from the source. Moxy measures the ratio of the oxyhaemoglobin concentration in the muscle in real time and reports it as a percentage, which is indicated as muscle oxygen saturation (StO_2) or muscle oxygenation (SmO_2). Data acquisition (2Hz) was obtained from the internal memory sensors and extracted from the device as a csv file. The CV values for Moxy when resting were 5.7% to 6.2% (McManus et al., 2018). Moxy was positioned on the right vastus lateralis muscle belly (approximately 10–12 cm above the patella) before exercise using a black plastic spacer. The probe was held in place via double sided, stick discs and black bandages to reduce the intrusion of extraneous light (Michailidis et al., 2020).

Statistical Analysis

Descriptive statistics (mean ± standard deviation, SD) were calculated for each parameter. Data normality was verified with the Kolmogorov–Smirnoff test. A paired samples t-test was used to compute any differences in the subjects' performance on the muscle saturation and on RSA test. The level of significance was set at $p < 0.05$. Effect sizes (ES) were estimated by calculating partial eta squared $\eta^2 = t^2 / (t^2 + N\text{-}1)$ and were classified as small (0.01 to 0.058), medium (0.059 to 0.137) or large (0.138 or higher) according to Cohen (1988). The SPSS version 25.0 was used for all analyses (SPSS Inc., Chicago, IL, USA).

Results

In Figure 2 the typical kinetic of oxygen saturation during RSA test for the two conditions (PG & NG) is presented. It is obvious that the supplement of nitrates limited the decrease in muscle oxygenation during repeated sprints.

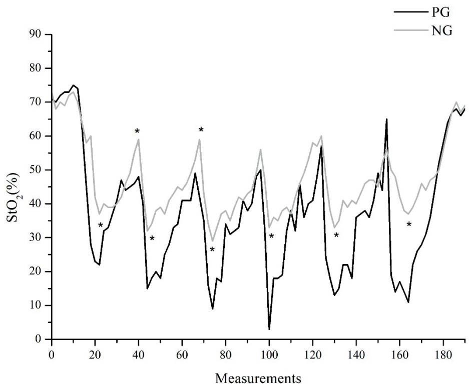

Figure 2. Typical kinetic of oxygen saturation during RSA test for the two conditions (NG & PG). * Denotes significant difference between the conditions

In Table 1 the baseline values, the lowest value after each sprint and the maximum value of muscle oxygenation during recovery of each sprint as well as the statistical indicators are presented.

Table 1. The lowest values of StO2 after each of the six sprint, the max values of StO2 during recovery after each of the six sprint and statistical indicators (mean ± standard deviation, SD)

Measurement	NG	PG	t	p
Pre	73 ±10	71 ±12	1.296	0.114
Lowest after 1st sprint	39 ±12	22 ±15	6.547	<0.001
Recovery after 1st sprint	59 ±16	48 ±18	5.810	<0.001
Lowest after 2nd sprint	32 ±14	15 ±16	16.588	<0.001
Recovery after 2nd sprint	59 ±18	49 ±19	5.361	<0.001
Lowest after 3rd sprint	29 ±17	9 ±20	42.458	<0.001
Recovery after 3rd sprint	48 ±18	50 ±17	1.686	0.063
Lowest after 4th sprint	33 ±18	3 ±25	15.595	<0.001
Recovery after 4th sprint	60 ±16	57 ±18	1.885	0.086
Lowest after 5th sprint	33 ±18	13 ±16	23.066	<0.001
Recovery after 5th sprint	56 ±20	65 ±19	1.745	0.112
Lowest after 6th sprint	38 ±17	11 ±16	23.209	<0.001
Recovery after 6th sprint	69 ±18	55 ±17	1.325	0.418

The results of the statistics for the RSA test did not show any significant differences between the two conditions for each indicator of the test: RSAbest ($t = 1.382$, $p = 0.210$, $\eta^2 = 0.175$), RSAmean ($t = –0.339$, $p = 0.745$, $\eta^2 = 0.013$), RSAdec ($t = –1.586$, $p = 0.157$, $\eta^2 = 0.218$).

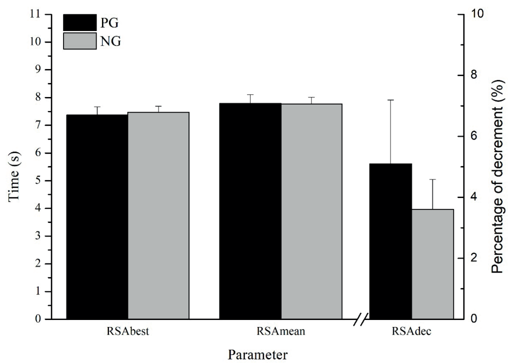

Figure 3. RSA best sprint; RSA mean time; RSA percentage of performance decrement

Discussion

The results showed that nitrate supplementation can affect the StO_2 of the muscles during exercise. However, this effect did not improve the performance of the players in the RSA test. We have to mention that in RSAdec there seemed to be a tendency for limited the decrement of performance, but this was not statistically significant.

Indications from the bibliography for the possible ergogenic activity of nitrates led the International Olympic Committee (IOC) classify them as a dietary supplement that can improve performance (Mowbray et al., 2009). Nitrates in the human body are converted into nitric oxide (NO) (Maréchal & Gailly, 1999) which is important for several signaling pathways of the body related to exercise, such as vasodilation, mitochondrial respiration, blood flow and muscle contraction (Arazi & Eghbali, 2021; Haider & Folland, 2014). More specifically, NO increases blood flow during exercise (Menard et al., 2008) which is considered to improve athletic performance as the muscles are supplied with O_2 and glucose (Bailey et al., 2010) and may help re-synthesize phosphocreatine (PCr) between the sets (Trump et al., 1996; Vanhatalo et al., 2011). In addition, better blood flow accelerates the removal and metabolism of lactic acid which can be a limiting factor in performance in exercise with repeated sprints. It is also mentioned that nitrates reduce the cost of high-energy phosphates in the generation of skeletal muscle contraction (Bailey et al., 2010; Fulford et al., 2013) and the intramuscular accumulation of adenosine diphosphate (ADP) and phosphate, factors that are expected to reduce the expansion of fatigue in skeletal muscle (Allen et al., 2008).

Nitrate supplementation did not affect any of the RSAmean, RSAbest and RSAdec indicators. However, in the NG condition the decrement in performance was more limited. This observation may be based on the fact that nitrates can limit the required energy to produce ATP and the use of phosphocreatine (Larsen et al., 2007).

It was recently reported that in order to have a positive effect on performance, the nitrate supplement should be >5 mmol/serving (Gallardo & Coggan, 2019; Peeling et al., 2015). A recent review article states that there are differences between acute and chronic supplementations (Rojas-Valverde et al., 2020). The authors state that chronic administration should be given 2 times a day in concentrations of ~5–6.5 mmol of NO3 (70 ml in the morning and 70 ml in the evening) (Kent et al. 2019; Reynolds et al. 2020). On the other hand, acute intake should be given 2–3 hours before exercise with 70 ml of a concentration of ~5-6.5 mmol of NO3 (Kokkinoplitis & Chester 2014; Thompson et al. 2015). A dose above the 5 mmol/l threshold was used in the present study, however no significant effect on performance was observed

This is the first study to investigate the effect of nitrate supplementation on the muscle saturation StO_2 of vastus lateralis in semi-professional soccer players during a repeated sprint ability test. Bailey et al., (2009) showed that deoxyhemoglobin peak amplitude was significantly smaller after nitrate supplementation for 4–6 days. In contrast, in a more recent study no differences were shown in StO_2 with nitrate supplementation during exercise (Husmann et al., 2019) although, the researchers mentioned that a subsample analysis of those who have improved their time to exhaustion with dietary nitrate has revealed a significant condition effect. In another study Haider and Folland (2014) mentioned that nitrate supplementation increased peak force at low frequencies of electrical stimulation (1–20 Hz) and explosive force production at low (1 Hz) and high (300 Hz) frequencies of stimulation during unilateral isometric contractions of the knee extensors in untrained individuals. However, these moderate effects on the contractile properties did not translate into any significant changes in maximum or explosive voluntary force production. In the above studies, nitrates were taken for a period (e.g. 1 week) before the test in contrast to the present study which used a single dose of nitrates before the test.

However, there are also some limitations to the study, such as the small sample used, which does not allow us to generalize the results. The changes in plasma concentrations of nitrates (NO_2 and NO_3) after taking the supplements were also not measured to confirm the effect of supplements.

Conclusions

In conclusion, acute nitrate supplementation reduces the decrement of muscle oxygenation during exercise with repeated sprints and leads to a tendency of a fatigue resistance during repeated sprints under controlled situations. Factors such as dosage and the chronic or immediate intake of supplement can affect their ergogenic effect. Additional studies are needed on the role that high flow of oxygen can play in the exercised muscle for both performance and rehabilitation.

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