



## DRINKS WITH ALKALINE NEGATIVE OXIDATIVE REDUCTION POTENTIAL IMPROVE EXERCISE PERFORMANCE IN PHYSICALLY ACTIVE MEN AND WOMEN: DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED, CROSS-OVER TRIAL OF EFFICACY AND SAFETY

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**Abstract** In the current study we tested the hypothesis that an acute (7 days) intake of an alkaline negative oxidative reduction potential formulation (NORP) drink would reduce the rate of blood lactate accumulation during and after exercise, increase time to exhaustion, increase serum buffering capacity and not increase prevalence of adverse effects as compared to the control drink. Eleven participants (9 men and 2 women) met the criteria to take part in the study. Participants were randomized in a double-blind, cross-over design to receive the control and the NORP drinks within two single-week periods to study the efficacy of the NORP drink (at a dose of 1 L per day by oral administration). The NORP drink was supplied in bottles containing 2 g NORP, 6 g sucrose, 1-2 mg sodium per dose. The control drink was identically supplied and formulated except that it contained no NORP. Exercise testing was performed using a treadmill based ramp protocol. Blood glucose or total antioxidant capacity were not affected by supplementation ( $p > 0.05$ ) while serum bicarbonates were significantly higher after the NORP trial ( $p < 0.05$ ). Critical HR at the velocity of 8.1 mph during the test was significantly lower in NORP as compared to the control drink trial ( $p < 0.05$ ). Blood lactate sampled at velocity 8.1 mph during the test was significantly lower in the NORP group ( $p < 0.05$ ). No athletes reported any vexatious side effects of supplementation. It seems that NORP supplementation could have a beneficial effect on human performance during maximal exercise.

**Key words:** Running, antioxidant, bicarbonates, human subjects, alkalosis

## INTRODUCTION

The use of performance-enhancing aids has been documented since ancient times and such practices are not reserved for elite or Olympian-level athletes [15]. Since many athletes are looking for ergogenic aids that do not have side effects and cannot be detected during drug testing, nutritional ergogenic aids, including carbohydrates, bicarbonates and dietary antioxidants, are promising alternatives [1]. Athletes who engage in high-intensity exercise such as sprint cycling and swimming along with track events and team sports could be interested in ergogenic aids that buffer against lactic acid [14, 22]. On the other hand, intense physical exercise increases oxidative stress, which leads to enhanced production of free radicals, a factor related to prolonged recovery and exercise-induced fatigue [6] with antioxidant supplementation which can decrease biomarkers of oxidative stress and improve muscular performance in humans [13, 18, 19]. Therefore, a dietary supplement with both buffering and antioxidant effects, could be of particular interest to both recreational and top-level athletes as an effective ergogenic aid. Negative oxidation reduction potential (NORP) alkaline water is often promoted as an antioxidant and anti-aging agent, with clear health benefits yet to be determined. Several recent studies [8, 21] showed anti-microbial activity of electrolyzed oxidizing water against microorganisms relevant in medicine. As an ergogenic aid, NORP

alkaline water could be used by athletes in sports such as endurance running to combat the fatiguing effects of lactic acid. Moreover, the strong antioxidant potential of NORP water could be of particular interest for athletes to protect against the damaging effects of free radicals induced by exercise. However, to our best knowledge, no previous cross-sectional or longitudinal study examined the effects of NORP water on human performance. We investigated in a double-blind randomized cross-over trial, firstly, whether intake of the NORP drink improved running performance in young healthy active men and women, secondly, how many participants experienced adverse effects at follow up after this treatment. Therefore, in the current study we tested the hypothesis that an acute (7 days) intake of NORP would reduce the rate of blood lactate accumulation during and after exercise, increase the exhaustion time, increase serum buffering capacity and not increase prevalence of adverse effects.

## MATERIALS AND METHODS

### SUBJECTS

Both male and female athletes requesting a preparticipation medical examination at the TIMS Exercise Science Centre, Novi Sad, during November 2009, who were experienced in endurance training (> 2 years) and who were between 20 and 30 years of age ( $24.3 \pm 4.1$  years), were candidates for inclusion in the study. They were not admitted to the study if any of the following criteria were present: (1) a history of heart disease; (2) musculoskeletal dysfunction; (3) known metabolic disease; (4) smoking; (5) use of any performance-enhancing drugs or dietary supplements within the past 30 days; (6) an impaired response to exercise test; and (7) residence outside the city of Novi Sad, or unwillingness to return for follow-up. All participants were fully informed verbally and in writing about the nature and demands of the study as well as the known health risks. They completed a health history questionnaire, and gave their informed consent regarding their voluntary participation in the study. Upon initial recruitment, eleven ( $n = 11$ ) participants (9 men and 2 women) met the criteria to take part in the study. All procedures were performed in accordance with the Declaration of Helsinki and the study was approved by the local IRB. The study was carried out at the Exercise Physiology Laboratory, part of Faculty of Sport Sciences and Tourism, Novi Sad, University of Metropolitan, Serbia.

### INTERVENTIONS

Participants were randomized in a double-blind, cross-over design to receive the control drink and the NORP drink, with two single-week periods to study the efficacy of the NORP drink (at a dose of 1 L per day by oral administration) according to exercise rehydration guidelines [1]. The NORP drink was supplied in bottles containing water, 2 g NORP, 6 g sucrose, 1-2 mg sodium per dose. The control drink was identically supplied and formulated except that it contained no NORP (Gatorade Sport Drink, San Diego, CA, USA). Subjects self-administered the drink before (30 minutes), during (every 15 min) and after each training session (until 45 min of recovery). The primary endpoint with respect to the efficacy in human performance was the proportion of participants achieving a significant (5%) improvement in running exhaustion time from baseline to 1 week. Additional analyses were done on the blood lactate change during and after exercise and on prevalence of side effects. All testing was conducted at the end of the first and at the end of the second week and the subjects were assessed on the same day with the tests performed in the same order. Participants were instructed to report on adverse effects of supplementation through an open-ended questionnaire at the end of the first and the second weeks of supplementation.

### DIETARY CONTROL AND TRAINING

All subjects met a nutritionist who instructed them to maintain their normal dietary pattern throughout the study. During the supplementation regimen all subjects consumed similar standardized diet. Compliance was monitored by analyzing 3-d food records pre- and post-supplementation. Diet records were analyzed for daily caloric intake and composition using food analysis software package. During the trial (7+7 days) all subjects followed a similar training program. Subjects trained for 3 days per week on non-consecutive days. All subjects received a similar personalized training manual with prescribed exercise to be performed. All training sessions were performed at the Faculty's athletic training facility and monitored by a certified strength and conditioning coach.

### EXPERIMENTAL DESIGN

Subjects reported to the laboratory field at 10 a.m. after fast of between 10 and 12 h. Upon entering the laboratory, blood was drawn from the antecubital vein and analyzed for total antioxidant capacity (TAC) by the procedure of chemiluminescence (Boehringer Mannheim GmbH, Germany;  $cV\%=8.2$ ). Fasting blood also was obtained for measurement of glucose and bicarbonates and the sample was sent to the research laboratory, where glucose and bicarbonates were analyzed by standard enzymatic methods and an automated analyzer (Hitachi 704, Tokyo, Japan;  $cV\%=13.0$  and  $cV\%=8.5$ , respectively). For all values, the

first reading was discarded and the mean of the next three consecutive readings with a coefficient of variation below 15% was used in the study. A week before the study, the subjects performed a familiarization trial on the treadmill. In the 24 hours before the experiment, the subjects did not participate in any prolonged exercise or drink alcoholic and/or caffeine beverages. Before experimental sessions body mass, height, percentage of body fat, muscle mass and total body hydration from bioimpedance analysis were determined for each subject. Then, the subjects were instrumented for maximal oxygen consumption ( $VO_{2MAX}$ ) and telemetric heart rate (HR) assessment. Exercise test was performed according to the ramp protocol up to the maximal symptom-tolerated level using a treadmill system (Trackmaster TMX425C, Newton, USA). Gas-exchange data were collected throughout the exercise test using a breath-by-breath metabolic system (Vacu-Med CPX, Ventura, USA) with  $VO_{2MAX}$  defined as the highest  $VO_2$  achieved during the test with data smoothed before calculating  $VO_{2MAX}$ . The heart rate was continuously recorded with a heart rate monitor (Polar S810, Kempele, Finland). The modified rates of perceived exertion (RPE) [17] were monitored during the test (at 3-min intervals), at the end of the test ( $RPE_{max}$ ) and after 3 minutes of recovery. During the test (at 8.1 mph running speed) and after the test was completed, the blood was drawn from the fingertip and analyzed immediately for lactate by the procedures of reflectance photometry (Accutrend, USA;  $cV\% = 12.4$ ). The accuracy of the lactate analyzer was checked before each test using standards. The level of blood lactate measured in the third minute after the test was recorded as  $Lact_{rec3}$ , and the level of lactate measured 5 minutes after the test was recorded as  $Lact_{rec5}$ .

### STATISTICAL ANALYSIS

The data are expressed as Means  $\pm$  SD. Statistical significance was assessed using Student's t test for paired samples to evaluate the significance of differences between the values obtained. P values of less than 0.05 were considered to be statistically significant. The data were analyzed using the statistical package SPSS, PC program, version 16.0 (SPSS Inc., USA).

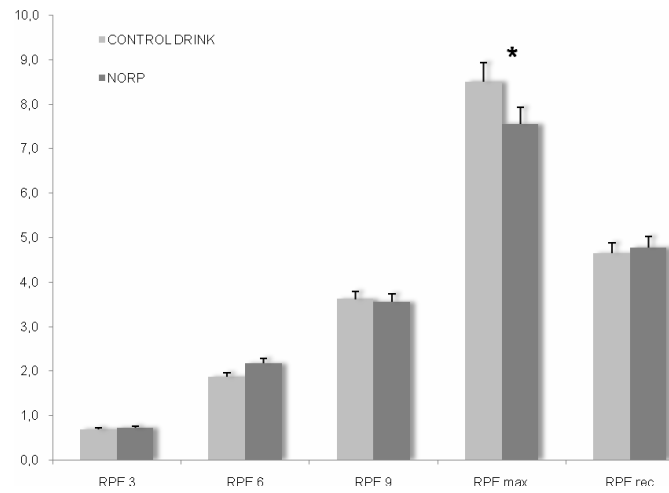
### RESULTS

There were no differences in weight, body fat, lean body mass, time to exhaustion, maximal oxygen uptake or ventilatory threshold between the trials (Table 1) (all  $p > 0.05$ ). Blood glucose and TAC were not affected by supplementation ( $p > 0.05$ ), while serum bicarbonates were significantly higher after the NORP trial ( $26.5 \pm 2.0$  vs.  $31.2 \pm 1.7$  mmol/L;  $p < 0.05$ ). During the test at 3-min intervals and 3-min post-exercise RPE were similar between both trials ( $p > 0.05$ ) (Figure 1). Yet, treatment with NORP resulted in significant decrease of RPE at the end of the running test as compared to placebo ( $p < 0.05$ ). Peak HR, HR at the anaerobic threshold and HRR were not affected by either placebo or NORP ( $p > 0.05$ ) (Figure 2). Critical HR at the velocity of 8.1 mph during the test was significantly lower in NORP as compared to the placebo trial ( $p < 0.05$ ). The level of blood lactate sampled at velocity 8.1 mph during the test was significantly lower in the NORP group ( $p < 0.05$ ) (Figure 3). Post-exercise blood lactates were similar between the trials ( $p > 0.05$ ). No athletes reported any vexatious side effects of supplementation.

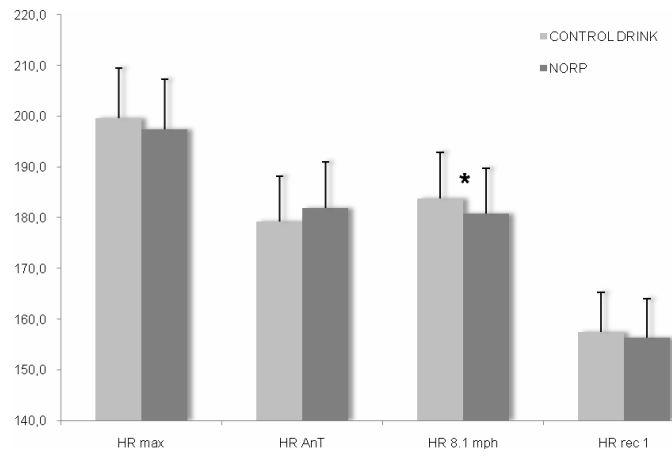
**Table 1.** Physical and physiological characteristics of the subjects (Mean  $\pm$  SD)

	Control drink (n = 9)	NORP (n = 9)
Weight (kg)	80.0 $\pm$ 13.1	80.2 $\pm$ 13.1
Body fat (%)	20.2 $\pm$ 5.4	20.4 $\pm$ 5.3
Lean body mass (kg)	60.7 $\pm$ 10.0	60.8 $\pm$ 9.6
Total body water (%)	54.7 $\pm$ 3.3	55.0 $\pm$ 3.4
Time to exhaustion (sec)	787.8 $\pm$ 76.6	801.1 $\pm$ 82.7
Maximal oxygen uptake (ml/kg/min)	48.4 $\pm$ 7.6	50.2 $\pm$ 6.8
Ventilatory threshold (% $VO_{2MAX}$ )	76.6 $\pm$ 7.2	71.3 $\pm$ 10.2
Glucose (mmol/L)	5.1 $\pm$ 0.7	5.1 $\pm$ 0.9
Bicarbonates (mmol/L)	26.5 $\pm$ 2.0	31.2 $\pm$ 1.7*
Total antioxidant capacity (mmol/L)	1.1 $\pm$ 0.4	1.3 $\pm$ 0.6

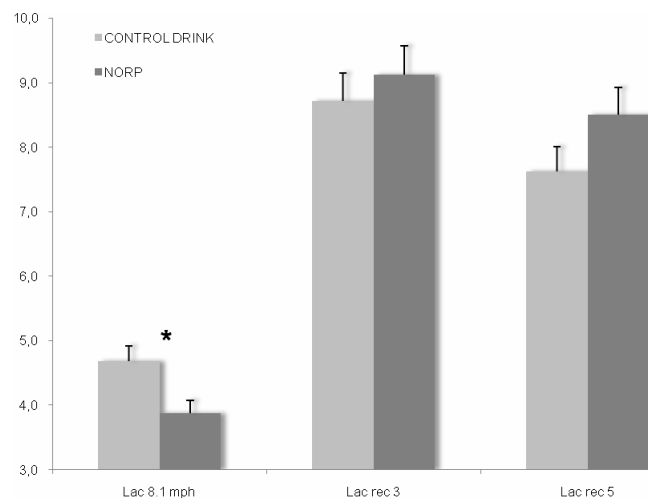
Note: \* indicates significant difference ( $p < 0.05$ ) between the trials



**Figure 1.** Rates of perceived exertion (RPE) during the study.  
 Note: \* indicates significant difference ( $p < 0.05$ ) between the trials



**Figure 2.** Heart rate (HR) responses during and after the exercise test  
 Note: \* indicates significant difference ( $p < 0.05$ ) between the trials



**Figure 3.** Blood lactate (Lac) responses during and after the exercise test  
 Note: \* indicates significant difference ( $p < 0.05$ ) between the trials

## DISCUSSION

This study has provided the first direct analysis of influence of NORP supplementation on human performance indicators and serum antioxidant and buffering capacity in young college athletes. The results of the present study suggest a beneficial effect of NORP supplementation on serum bicarbonates, maximal rate of perceived exertion, heart rate and blood lactate levels at critical running speed (8.1 mph) during the maximal exercise. Treatment with a 2 g oral second-day dose of NORP for 7 days had no significant effect on body mass, body fat, total body hydration and lean body mass nor maximal oxygen uptake in young college athletes, and levels of glucose and total antioxidant capacity were not significantly changed after supplementation of NORP as compared to placebo.

During near-maximal exercise efforts lasting more than 60 s approximately, muscles rely on the anaerobic breakdown of glucose to lactic acid and this metabolic by-product, however, increases muscular  $[H^+]$  [12]. The drop of pH as a result of lactic acid accumulation is thought to inhibit the resynthesis of ATP as well as inhibit muscle contraction [5, 20]. Different buffering agents (i.e. bicarbonates, phosphates, citrates) could increase the body's capacity to neutralize lactic acid, thereby delaying fatigue during high-intensity exercise [3, 20]. According to the results of the present study, the NORP drink seems to be an effective ergogenic aid since the levels of blood lactates at critical running speed during the maximal running test were lower after NORP intake along with increased bicarbonate levels as compared to the control drink. Similar but non-significant alkalinizing effect of NORP has been seen after the exercise test, with blood lactate levels slightly lower at all sampling points for NORP as compared to the control trial. Lower blood lactates at critical speed and improved buffering capacity after NORP intake might indicate better environment for repeated muscle contraction. It could be postulated that the NORP drink buffers  $[H^+]$  generated by exercise by its alkalinity potential [10]. Yet, since we did not assess exercise performance indicators (e.g. power output) it is not clear if NORP is effective sports ergogenic. However, future studies should further evaluate the pharmacokinetics of NORP along with bioavailability issues, because nowadays there is no scientific evidence about the potential ergogenic effect of NORP. According to the results of the present study, orally administered NORP did increase blood bicarbonates, while other indicators of acid-base balance in arterial blood (e.g. pH,  $pCO_2$ , hemoglobin) were not assessed for the present study. Due to the fact that NORP exhibits high pH, low dissolved oxygen and extremely high dissolved molecular hydrogen, it seems that increased non-volatile base indicators in the plasma for NORP group arose from ingestion of the alkaline drink. Due to the fact that the intestine is directly involved in acid and/or base generation [21], it appears that NORP has a strong alkalinizing effect as a result of absorption of inorganic cations, while protective mechanism of NORP results from active atomic hydrogen with high reductive ability. Although we did not assess pH and  $pCO_2$  in the present study, it could be hypothesized that oral intake of alkaline NORP could induce respiratory compensation as the high plasma pH depresses respiration. In consequence, the  $pCO_2$  rose and the plasma pH tended to fall towards normal. Yet, since the final correction of alkalosis due to ingestion of base is corrected by renal excretion of the excess base, the analysis of effects of NORP on blood buffering capacity will require assessment of kidney functions (e.g. urine pH, total renal net acid excretion) in prospective studies. Although we examined healthy subjects during the present study, the fact that the appropriate treatment of acute metabolic acidosis (in particular its organic form such as exercise-induced acidosis) has been rather controversial enhances further studies with NORP as a potential anti-acidic treatment strategy and its safe application in clinical patients.

Heart rate (HR) seems to act as a clear indicator of cardiovascular stress induced by exercise. The ease and low-cost of monitoring HR has led to the widespread use of HR as a gauge of relative exercise intensity. Lower HR at similar exercise intensity, induced by training, supplementation or genetic factors, indicates lesser cardiovascular stress and could enhance performance [2]. Due to the fact that HR at the critical speed (8.1 mph) was lower in the NORP trial, it could be postulated that NORP decreases the level of cardiovascular stress response to exercise but the clear mechanism is not known yet. The influence of the NORP drink on blood volume, myocardium contractility and/or stroke volume, and autonomic control of HR should be investigated in the future.

Several research studies suggest that supplemental dietary antioxidants, singularly and in combination, reduce indices of oxidative stress, such as lipid and protein peroxidation [7, 18, 19]. While further studies are needed for specific recommendations for levels of dietary antioxidant intake in athletes, evidence thus far supports that supplemental intake of antioxidants protects against oxidative stress due to exercise and perhaps enhances recovery and minimizes muscle soreness. Although studies in the past showed antioxidant properties of NORP in non-athletic environment [11, 16], during the present study we did not find measurable effects of NORP on the subjects' antioxidant status assessed by total antioxidant capacity. Measurements of antioxidative status and/or oxidative stress are very complex but generally involve by-products of lipid peroxidation (conjugated dienes, thiobarbituric acid-reactive substances, malondialdehyde, or lipid peroxides). Future studies examining the antioxidant properties of NORP in physically active subjects should be focused on different biomarkers of oxidative stress related to exercise.

Furthermore, to connect free radicals with acidosis, some of the biomarkers of oxidative damage need to be measured (LOOH, MDA or F2-isoprostanes).

Perceived exertion is based on the physical sensations a person experiences during physical activity, including increased heart rate, increased respiration or breathing rate, increased sweating, and muscle fatigue [17]. The Borg's perceived exertion scale is a simple method of rating perceived exertion (RPE) and can be used by sport scientists to gauge an athlete's level of intensity in exercise and training [4]. Although RPEs were similar between the trials in the present study during the test and at 3-min post-exercise, the treatment with NORP resulted in significant decrease of RPE at the end of the running test as compared to placebo. The subjects rated the endpoint of exercise as "very hard" ( $7.8 \pm 1.0$ ) after 7-days of NORP administration, while average RPE after administration of the control drink was  $8.6 \pm 0.9$  and the subjects described the test endpoint as "very, very hard". Due to the fact that RPE illustrates both peripheral and central mechanisms of fatigue [4], it could be hypothesized that the NORP drink decreases the feeling of maximal physical stress affecting both adaptivity of musculoskeletal and reticular activating system of the lower brain to physical exercise [9], which requires further investigation. Assessment of motor cortex excitation, contractile mechanism control and/or sarcolemma excitability after NORP administration is needed to further explore the effects of the NORP drink on fatigue decrement.

Since we had controlled and comparable conditions for all subjects during the study and a double-blind, placebo-controlled design, it is apparent that NORP ingestion had a significant buffering effect for the sample of individuals in the present study. It is noteworthy that in this study we evaluated healthy physically active participants following a regular training program. Yet, it would be premature to conclude that NORP has a performance enhancing effect in all individuals, since no other published studies exist on NORP in the field of biochemistry or nutrition. Dosage and duration of ingestion, purity of the intervention, or the health status of individual may affect the efficacy of NORP administration. A longer supplementation protocol and a higher dosage of the formulation, along with proven bioavailability of the formulation, coupled with monitoring other buffering indicators may be necessary to determine if NORP has a considerable ergogenic effect.

## CONCLUSION AND PRACTICAL APPLICATION

This is the first approach to understand the NORP effect in athletes. Intake of a NORP formulation for one week seems to increase serum bicarbonates and reduce the rate of blood lactate accumulation during exercise, maximal rate of perceived exertion and cardiovascular stress at critical running velocity with no significant adverse effects. Future studies should be undertaken in order to fully understand ergogenic potential of negative ionized water.

### ACKNOWLEDGEMENTS

The authors declare no conflict of interest.

Study was partially supported by the Serbian Ministry of Science (Grant No. 145082).

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