Am J Physiol Regul Integr Comp Physiol 315: R453–R460, 2018.
First published April 25, 2018; doi:10.1152/ajpregu.00017.2018.

RESEARCH ARTICLE | *Obesity, Diabetes and Energy Homeostasis*

Ergogenic effects of beetroot juice supplementation during severe-intensity exercise in obese adolescents

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Submitted 22 January 2018; accepted in final form 22 April 2018

Rasica L, Porcelli S, Marzorati M, Salvadego D, Vezzoli A, Agosti F, De Col A, Tringali G, Jones AM, Sartorio A, Grassi B. Ergogenic effects of beetroot juice supplementation during severe-intensity exercise in obese adolescents. *Am J Physiol Regul Integr Comp Physiol* 315: R453–R460, 2018. First published April 25, 2018; doi:10.1152/ajpregu.00017.2018.—Previous studies showed a higher O_2 cost of exercise, and therefore, a reduced exercise tolerance in patients with obesity during constant work rate (CWR) exercise compared with healthy subjects. Among the ergogenic effects of dietary nitrate (NO_3^-) supplementation in sedentary healthy subjects, a reduced O_2 cost and enhanced exercise tolerance have often been demonstrated. The aim of this study was to evaluate the effects of beetroot juice (BR) supplementation, rich in NO_3^-, on physiological variables associated with exercise tolerance in adolescents with obesity. In a double-blind, randomized crossover study, 10 adolescents with obesity (8 girls, 2 boys; age = 16 ± 1 yr; body mass index = 35.2 ± 5.0 kg/m^2) were tested after 6 days of supplementation with BR (5 mmol NO_3^- per day) or placebo (PLA). Following each supplementation period, patients carried out two repetitions of 6-min moderate-intensity CWR exercise and one severe-intensity CWR exercise until exhaustion. Plasma NO_3^- concentration was significantly higher in BR versus PLA (108 ± 37 vs. 15 ± 5 μM, $P < 0.0001$). The O_2 cost of moderate-intensity exercise was not different in BR versus PLA (13.3 ± 1.7 vs. 12.9 ± 1.1 ml·min^{-1}·W^{-1}, $P = 0.517$). During severe-intensity exercise, signs of a reduced amplitude of the O_2 uptake slow component were observed in BR, in association with a significantly longer time to exhaustion (561 ± 198 s in BR vs. 457 ± 101 s in PLA, $P = 0.0143$). In obese adolescents, short-term dietary NO_3^- supplementation is effective in improving exercise tolerance during severe-intensity exercise. This may prove to be useful in counteracting early fatigue and reduced physical activity in this at-risk population.

endurance exercise; nitric oxide; obesity; oxidative metabolism; O_2 uptake kinetics

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INTRODUCTION

Obesity is one of the main public health challenges in industrialized countries and the number of people affected by this condition is growing from year to year. Taking into consideration children and adolescents (2–19 yr), the percentage of people with obesity in the United States is an alarming 46.9%. Obesity during childhood is associated with an excess in body mass (BM) in adulthood, and among adults obesity is a significant risk factor for several chronic diseases and, conditions, including cardiovascular diseases, diabetes, and cancer (26).

Patients with obesity show a significant exercise intolerance and an impairment in physical activities of daily living because of a generalized sense of fatigue, which negatively affects their quality of life and may lead to severe deconditioning. The reduced exercise tolerance is associated with a higher O_2 cost for submaximal exercise (30, 33, 37), a lower gas exchange threshold, slower adjustment of the fundamental component of oxygen consumption ($\dot{V}O_2$) kinetics (30), and a more pronounced amplitude of the slow component of $\dot{V}O_2$ kinetics during heavy intensity exercise (30). These impairments are attributable to cardiovascular (33), respiratory (31, 32), and skeletal muscle factors (23, 30). In a vicious circle, the impaired exercise tolerance and the associated reduced level of physical activity worsen obesity and interfere with interventions (such as exercise prescription) aimed at its control.

Patients with obesity show an impaired endogenous nitric oxide (NO) synthesis, in particular in the presence of the metabolic syndrome (34). The mechanisms seem to be related to an increased generation of reactive oxygen species, an impaired function of NO synthase (NOS), and to decreased insulin signaling (34). More specifically, the inhibition of NOS enzyme activity in obesity is prevalent in the endothelium (20), but it can be also found in skeletal muscle (36), suggesting a possible shortage of NO bioavailability at the skeletal muscle level.

NO is produced endogenously from the oxidation of L-arginine in a reaction catalyzed by a family of NOS enzymes (L-arginine-NOS-NO pathway) (19). An alternative pathway of NO synthesis is, however, present. The ingestion of foods rich in nitrate (NO_3^-), like green leafy vegetables and beetroot, can increase NO bioavailability through the conversion of NO_3^- to nitrite (NO_2^-) in the entero-salivary circulation, and the subsequent conversion of NO_2^- to NO by peripheral enzymes or proteins (such as deoxygenated hemoglobin and myoglobin) after NO_2^- has entered the systemic circulation (19).

Recent evidence suggests that an enhanced NO bioavailability can positively affect exercise tolerance through a variety of mechanisms (14). For example, an increased NO bioavailability may improve the efficiency of mitochondrial respiration, as evaluated by the amount of oxygen consumed per ATP produced (P/O ratio) (22); ameliorate muscle metabolic perturbations found during exercise, which may decrease the ATP cost of force production (1); and increase skeletal muscle microvascular blood flow, at least toward type 2 fibers, thereby improving the intramuscular matching between O_2 delivery and O_2 utilization and increasing microvascular P_{O_2} and peripheral O_2 diffusion (9). Thus, an increased oxidative efficiency (i.e., a lower O_2 requirement leading to a lower pulmonary $\dot{V}_{O_2}$ for the same work rate) could be of utmost interest in patients with obesity who typically show an increased O_2 cost of exercise ($\Delta\dot{V}_{O_2}/\Delta$ work rate, where Δ represents change in) (33, 37), which is partly attributable to an increased O_2 cost of breathing (31, 32).

The aim of the present study was to evaluate the effects of short-term (6 days) dietary NO_3^- supplementation on physiological parameters associated with exercise tolerance in a group of obese adolescents. We hypothesized that in this population NO_3^- supplementation would lead to an enhanced exercise tolerance by reducing the O_2 cost of exercise in different intensity domains and by increasing the time to exhaustion during severe-intensity constant work rate (CWR) exercise. These results would extend the ergogenic effects of dietary NO_3^- supplementations previously demonstrated in healthy subjects (2, 29) and in patients with peripheral arterial disease (18), chronic obstructive pulmonary disease (8), and chronic heart failure (42, 43) to patients with obesity.

MATERIALS AND METHODS

Subjects. Ten adolescents with obesity, 2 boys and 8 girls [age, 16 ± 1 yr (means ± SD); BM, 95.5 ± 16.8 kg; height, 1.64 ± 0.08 m], body mass index (BMI), 35.2 ± 5.0 kg/m^2 (above the age-specific 97th percentile) (7) participated in the present study. The standard deviation score of BMI, calculated by applying the LMS method (7) to Italian reference values for adolescents, was 2.8 ± 0.5.

Bioelectric impedance analysis was used to assess fat-free mass (FFM). Whole-body resistance to an applied current (50 kHz, 0.8 mA) was measured with a tetrapolar device (Human IM, Dietosystem, Milan, Italy). FFM, calculated with equations derived with a 2-compartment model (12), was 60.4 ± 5.5 kg. Fat mass (FM) was calculated as the difference between total BM and FFM and resulted in 36.4 ± 6.6 kg. Patients were recruited from those involved in a multidisciplinary body weight reduction program at the Division of Auxology, San Giuseppe Hospital, Istituto di Ricovero e Cura a Carattere Scientifico, Istituto Auxologico Italiano, Piancavallo, Italy. All patients had no signs or symptoms related to cardiovascular, pulmonary, or orthopedic diseases contraindicating the possibility to perform exercise. The patients and their parents were fully informed of the procedures and possible risks associated with the experiments before giving their written consent to participate to the study. The protocol was approved by the local ethics committee (reference code: 01C407–2014; acronym: SUPNITESEROB). All procedures were in accordance with the Declaration of Helsinki (2000) of the World Medical Association.

Study design. The patients were involved in a placebo (PLA)-controlled, randomized, double-blind crossover study. Following a preevaluation test (see below for details), each patient completed two periods of 6 days of supplementation with beetroot juice (BR) [70 ml/day containing 5 mmol of NO_3^-, (Beet It, James White Drinks, Ipswich, UK)] or nitrate-depleted PLA, matched in flavor, appearance, and packaging [70 ml/day with negligible content of NO_3^-, (Beet It)]. A washout period lasting 7 days was interspersed between the two supplementation periods. A similar intervention in terms of dose and duration of supplementation has been demonstrated effective in improving exercise tolerance in healthy subjects (e.g., 2). On *day 6*, at the end of each supplementation period, the patients performed three CWR exercises at two different intensities (*Exercise tests*). Patients were required to abstain during the study period from using antibacterial mouthwashes and chewing gums, which are known to alter the oral bacteria responsible for the reduction of NO_3^- to NO_2^- (39). All participants were hospitalized for a 3-wk multidisciplinary body weight reduction program, entailing constant and monitored physical activity, psychological counseling, nutritional education, and moderate energy restriction (~500 kcal lower than the measured resting energy expenditure), provided and monitored daily by expert nutritionists. The amount of ingested NO_3^- (~150 mg/day, 2.5 mmol/day) was in accordance with dietary guidelines, estimated considering the food intake and kept constant along the intervention period.

Exercise tests. At the beginning of the study, each patient performed an incremental exercise on a cycle-ergometer (Monark Ergomedic 839E, Vansbro, Sweden) until voluntary exhaustion. The work rate started with 40 watt (W) for 2 min and then was increased by 20 W every minute. Exhaustion was defined when at least three of the following criteria were reached: *1*) inability to maintain the pedaling frequency (60–80 revolutions/min) despite vigorous verbal encouragement by the operators; *2*) maximal levels (higher than 7.5) of self-perceived exertion, using the Borg's modified CR10 scale (5); *3*) heart rate (HR) values higher than 85% of the age-predicted maximum; and *4*) gas exchange ratio (R) value equal or above 1.1. No validation test for the determination of maximal oxygen consumption (27) was carried out in the recovery following the incremental test.

Data collected during the incremental test were utilized to identify individual gas exchange threshold (GET) and peak oxygen consumption ($\dot{V}_{O_{2peak}}$), and to set the intensity of the CWR exercises to be carried out at the end of each supplementation period.

Both in BR and in PLA, 3 h after the last supplementation each patient performed two repetitions of 6-min moderate-intensity CWR exercise (approximately at 80% of GET) separated by 10 min of passive recovery. Thereafter, a severe-intensity CWR exercise (at a work rate representing GET plus 65% of the difference between the work rate at GET and at $\dot{V}_{O_{2peak}}$, ~80% of peak work rate) was performed until voluntary exhaustion or until the maximal duration of 15 min was reached. CWR exercises were carried out at the same absolute work rate after the two supplementation periods.

Measurements

Blood samples. Resting blood samples were collected to determine plasma levels of NO_3^- before the incremental test and on *day 6* of both supplementation periods; blood samples were taken at least 2.5 h after the last PLA or BR intake. Venous blood was drawn from the antecubital vein into a 5-ml EDTA vacutainer tube (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). Plasma was immediately separated by centrifuge (5702-R; Eppendorf, Germany) at 1,000 *g* for 10 min at 4°C. Plasma samples were then ultrafiltrated through a 10-kDa molecular mass cut-off (AmiconUltra; Millipore, EMD Millipore, Billerica, MA) utilizing an ultracentrifuge (4237-R, ALC) at 14,000 *g* for 60 min at 4°C, to reduce background absorbance because of the presence of hemoglobin. The ultrafiltrate was recovered and used to measure NO_3^- concentration by the Griess method using a commercial kit (Cayman Chemical, Ann Arbor, MI). Samples were read by the addition of Griess reagents at 545 nm by a microplate reader spectrophotometer (Infinite M-200, Tecan, Mannedorf, Switzerland). A linear calibration curve was computed from pure NO_3^- standard. All samples were determined in duplicate and the interassay coefficient of variation was in the range indicated by the manufacturer.

Physiological response to exercise. Tidal volume, breathing frequency, pulmonary ventilation ($\dot{V}_E$), $\dot{V}O_2$, and carbon dioxide output ($\dot{V}CO_2$) were measured breath-by-breath by a computerized metabolic cart (Quark b2; COSMED, Rome, Italy). The gas exchange ratio (R) was calculated as $\dot{V}CO_2/\dot{V}O_2$. GET was determined by standard methods (3), and peak values of the main cardiovascular, respiratory, and metabolic parameters were taken as the highest 20-s mean values reached during the incremental test. During moderate CWR, breath-by-breath $\dot{V}O_2$ data in the two repetitions were initially examined to exclude errant breaths caused by coughing, swallowing, sighing, etc., and those values lying more than 4 SDs from the local mean were removed. These data were time aligned and then superimposed for each subject. Oxygen cost was calculated as $\Delta\dot{V}O_2$ ($\dot{V}O_2$ during last 30 s of CWR exercise minus resting $\dot{V}O_2$, defined as the mean $\dot{V}O_2$ measured over the final 60 s of resting period) divided by work rate. HR was determined continuously by a chest band (Polar Electro, Oulu, Finland); mean values were calculated every 5 s. Ratings of perceived exertion were obtained at rest and every minute during exercise using the Borg's modified CR10 scale (5). Considering the low number of exercise repetitions, a formal $\dot{V}O_2$ kinetics analysis was not performed (21).

Statistical analysis. Data are expressed as mean values ± SD. Normal distribution of values was assessed with the Kolmogorov-Smirnov normality test. Time to exhaustion during severe-intensity CWR was considered the primary outcome of this study for power analysis. Considering the mean difference in this parameter previously observed in healthy subjects supplemented with BR, a sample size of eight was requested to achieve a power of 80% with a significance level of 0.05.

To check the statistical significance of differences between BR and PLA, paired Student's *t*-tests were performed. Linear regression and correlation analyses were carried out by the least-squared residual method. $\dot{V}O_2$ values obtained during CWR at moderate intensity were analyzed using a two-way ANOVA for repeated measures (treatment × time). Post hoc analysis was completed using Bonferroni multiple comparisons. The significant level was set at $P < 0.05$. Statistical analysis was performed using a software package (Prism 6.0, GraphPad Software, La Jolla, CA).

RESULTS

Adherence and safety. All participants completed the intervention and follow-up testing. Adherence to BR supplementation, estimated by returned bottle count, and consumption log was 100%. There were no adverse events related to the supplementation, except temporarily red urine and red stool.

Plasma nitrate levels. Plasma [NO_3^-] was significantly higher in BR (108.4 ± 37.2 μM) compared with PLA (15.4 ± 5.0 μM) ($P < 0.0001$) (Fig. 1). Higher values in BR were observed in all patients. The increase in plasma [NO_3^-] was inversely related to the patients' BM ($r^2 = 0.63$; $P = 0.0072$).

Incremental exercise. Peak values of the main respiratory, cardiovascular, and metabolic variables obtained at exhaustion during incremental exercise are shown in Table 1. HR values reached ~85% of maximal predicted HR. GET occurred at 59 ± 9% of maximal work rate and 63 ± 9% of $\dot{V}O_{2peak}$.

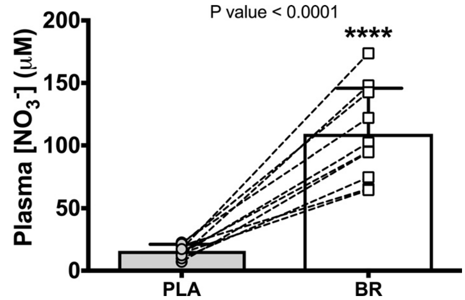

Fig. 1. Mean values (± SD) of plasma nitrate (NO_3^-) concentration after placebo (PLA) and beetroot juice (BR) supplementation (light gray and white columns, respectively). Dotted lines show individual changes. *P* value of paired Student's *t*-test is also shown. ****$P < 0.0001$, significantly different from PLA.

Moderate-intensity CWR exercise. All patients completed the imposed 6 min of exercise. Breath-by-breath values obtained in the two repetitions of the exercise were time aligned and then superimposed for each subject. Mean values of the main physiological variables determined in both BR and PLA during the last 30 s of moderate-intensity CWR exercises are presented in Table 2. No significant differences were found for any of the variables between the two conditions. Work rate was set to be identical in each patient in the two conditions, and the value corresponded to ~45% of peak work rate. HR, expressed as percentage of maximal HR reached during the incremental exercise, was 73 ± 8% and 73 ± 9% in PLA and BR, respectively (no significant difference). Mean values of $\dot{V}O_2$ calculated at different time points (last 3 min and last 1 min) were not different between BR (1.35 ± 0.23 and 1.35 ± 0.22 l/min, respectively) and PLA (1.32 ± 0.25 and 1.32 ± 0.25, l/min, respectively), suggesting that steady state was reached. The O_2 cost of cycling was 13.3 ± 1.7 $ml \cdot min^{-1} \cdot W^{-1}$ in BR and 12.9 ± 1.1 $ml \cdot min^{-1} \cdot W^{-1}$ in PLA.

Figure 2 shows the time courses of $\dot{V}O_2$ obtained every 30 s during the moderate-intensity CWR exercise in both PLA and BR. The time-courses of the variable in the two conditions were comparable and no statistical differences were observed between the values obtained in the two groups at any given time.

Severe-intensity CWR exercise. Mean values of the main physiological variables determined in BR and PLA during the last 30 s of CWR severe-intensity CWR exercises are shown in Table 2. Work rate was designed to be the same in each patient in the two conditions and corresponded to 83 ± 4% of peak work rate. HR was 96 ± 9% of peak HR in PLA and 103 ± 4% of peak HR in BR. No patients in PLA and only one patient in BR completed the 15-min exercise. Time to exhaustion was significantly increased (by 23%) by NO_3^- supplementation (561 ± 198 s and 457 ± 101 s, in BR and PLA, respectively, $P = 0.0143$) (Fig. 3). In three of the patients, time to exhaustion did not increase following NO_3^- supplementation. No correlation was observed between the increase in plasma (NO_3^-) and time to exhaustion ($r^2 = 0.13$; $P = 0.30$). The O_2 cost of cycling (14.1 ± 0.9 $ml \cdot min^{-1} \cdot W^{-1}$ in BR vs. 14.9 ± 1.3 $ml \cdot min^{-1} \cdot W^{-1}$ in PLA) was not different following NO_3^- administration ($P = 0.38$). No correlation was observed between the increase in plasma (NO_3^-) and O_2 cost of cycling ($r^2 = 0.38$; $P = 0.10$). Figure 4 (*left*) shows the time courses of

Table 1. *Peak values of the main respiratory, cardiovascular, and metabolic variables determined at exhaustion during incremental exercise*

Work Rate, W	$\dot{V}O_2$, l/min	$\dot{V}O_2$, ml·kg^{-1}·min^{-1}	$\dot{V}CO_2$, l/min	R	V_T, liters	fR, breaths/min	$\dot{V}E$, l/min	PET_{O_2}, mmHg	PET_{CO_2}, mmHg	HR, beats/min	GET, W	GET, l/min	RPE, (0–10)
174 ± 44	2.33 ± 0.68	24.4 ± 5.5	2.71 ± 0.81	1.16 ± 0.07	1.71 ± 0.41	39 ± 6	66.7 ± 17.0	103.1 ± 5.1	45.8 ± 5.2	174 ± 10	100 ± 21	1.46 ± 0.36	7.9 ± 2.4

Values are mean (± SD). fR, breathing frequency; GET, gas exchange threshold; HR, heart rate; PET_{CO_2}, end-tidal CO_2 partial pressure; PET_{O_2}, end-tidal O_2 partial pressure; R, gas exchange ratio; RPE, rating of perceived exertion; $\dot{V}E$, pulmonary ventilation; $\dot{V}CO_2$, carbon dioxide output; $\dot{V}O_2$, oxygen consumption; V_T, tidal volume.

$\dot{V}O_2$ in the two conditions. To obtain this figure, individual $\dot{V}O_2$ values were grouped for discrete time intervals, which were determined at discrete percentages of the individual total exercise time. Mean absolute individual values were calculated for the *x* and *y* variables for each time interval and these data were utilized to obtain the mean group values shown in Figure 4. Until 180 s of exercise, the exponential time courses of $\dot{V}O_2$ in the two conditions were substantially superimposed. After 180 s, the time courses of $\dot{V}O_2$ followed a linear increase without reaching a steady state. The slopes of the regression lines, calculated from the 3rd minute to the end of exercise were significantly different from zero ($P < 0.0001$). The rate of $\dot{V}O_2$ increase, calculated as $\Delta\dot{V}O_2$ ($\dot{V}O_2$ at end-exercise minus $\dot{V}O_2$ at 3 min) divided by time (time at end-exercise minus 3 min), was significantly lower in BR versus PLA (29.3 ± 18.1 vs. 60.9 ± 18.1 ml/min^2, $P = 0.0002$) (Fig. 4, *right*).

DISCUSSION

The main result of the present study was that in adolescents with obesity, short-term (6 days) dietary NO_3^- supplementation enhanced exercise tolerance by significantly increasing the time to exhaustion during severe-intensity CWR exercise. The increased exercise tolerance was associated with a less pronounced slow component of pulmonary $\dot{V}O_2$ kinetics. However, NO_3^- supplementation had no effects on other physiological variables determined during moderate-intensity CWR exercise.

Obesity, also in adolescence, is usually associated with reduced cardiorespiratory fitness and exercise intolerance (30, 33). Whereas $\dot{V}O_{2peak}$, expressed in absolute units (e.g., l/min), is usually normal compared with normal-weight controls, when it is normalized per unit of BM (ml·kg^{-1}·min^{-1}) values in obese patients are ~40% lower than in controls (23, 30). Other factors contributing to the impaired exercise tolerance in obesity include a higher O_2 cost for submaximal exercise (30, 33, 37), which is at least in part attributable to an increased O_2 cost of breathing (31, 32), a lower GET, slower adjustment of the fundamental component of $\dot{V}O_2$ kinetics (30), and a more pronounced amplitude of the slow component of $\dot{V}O_2$ kinetics during heavy-intensity exercise (30). At least in part, these impairments can be overcome by exercise training (24), including training specifically directed toward respiratory muscles (31).

In the present study, we evaluated the effects of a 6-day dietary NO_3^- supplementation on a group of adolescents with obesity and we observed an improved exercise tolerance after the intervention. More specifically, time to exhaustion during severe-intensity CWR exercise, which represents a classic parameter for the evaluation of exercise tolerance, was significantly increased (by ~25%) after dietary NO_3^- supplementation. Figure 4 shows that the linear increase in $\dot{V}O_2$ from 200 s to the end of the exercise was characterized by a significantly lower slope in BR than in PLA. This represents clear evidence that the "excess $\dot{V}O_2$" (11, 16), was reduced by NO_3^- supplementation. The term excess $\dot{V}O_2$ is usually utilized to indicate the progressive increase in $\dot{V}O_2$ associated with the "slow component" of the $\dot{V}O_2$ kinetics, that is the $\dot{V}O_2$ in excess above that determined as the asymptote of the fundamental component. Because work rate is constant, this excess $\dot{V}O_2$ indicates a reduced efficiency of muscle contraction. Considering the

Table 2. *Main respiratory, cardiovascular, and metabolic variables determined at the end of the moderate- and severe-intensity CWR exercises after PLA and BR supplementation*

	Work Rate	$\dot{V}O_2$, l/min	$\dot{V}O_2$, ml·kg^{-1}·min^{-1}	$\dot{V}O_2$, %peak	$\dot{V}CO_2$, l/min	R	$\dot{V}E$, l/min	HR, beats/min	HR, %peak
Moderate									
PLA	77 ± 13	1.32 ± 0.21	14.2 ± 1.3	59 ± 12	1.29 ± 0.17	0.98 ± 0.06	34.3 ± 6.2	124 ± 12	73 ± 8
BR	77 ± 13	1.34 ± 0.21	14.6 ± 2.2	60 ± 12	1.30 ± 0.15	0.97 ± 0.06	34.1 ± 4.6	124 ± 12	73 ± 9
Severe									
PLA	142 ± 38	2.33 ± 0.54	24.7 ± 4.8	100 ± 24	2.57 ± 0.56	1.10 ± 0.07	73.8 ± 18.0	169 ± 16	96 ± 9
BR	142 ± 38	2.22 ± 0.56	23.1 ± 4.5	95 ± 25	2.45 ± 0.58	1.10 ± 0.04	69.9 ± 16.6	172 ± 10	103 ± 4

Values are mean (± SD). BR, beetroot juice; CWR, constant work rate; HR, heart rate; PLA, placebo; R, gas exchange ratio; $\dot{V}E$, pulmonary ventilation; $\dot{V}CO_2$, carbon dioxide output; $\dot{V}O_2$, oxygen consumption.

close interconnections between the loss of efficiency and fatigue, and ultimately exercise tolerance, during severe-intensity exercise (11, 16), it is not surprising that the lower slope of $\dot{V}O_2$ as a function of time in BR was associated with (or was responsible for) a longer time to fatigue and an enhanced exercise tolerance, as demonstrated in previous works on healthy subjects (6, 29). Interestingly, in BR the time to exhaustion did not improve in 3 of 10 subjects, whereas it was remarkably higher in 5 of 10 subjects. This heterogeneity of results has been already reported by other authors both in healthy subjects (28) and in patients (43), and it has been shown to be correlated with changes of plasma [NO_3^-] or [NO_2^-] after supplementation. In our subjects, we did not observe any correlation between time to exhaustion and plasma [NO_3^-]. However, in BR the rate of $\dot{V}O_2$ increase during severe-intensity exercise was reduced, suggesting less oxidative inefficiency, in all subjects.

The improved exercise tolerance of the present study confirms similar findings obtained in healthy subjects (2, 29) and in disease populations (8, 18, 42), such as patients with peripheral arterial disease (18), patients with chronic obstructive pulmonary diseases (8), and heart failure patients (43). Similar results have also been shown by different interventions aimed to increase NO bioavailability. For example, Sperandio et al. (35) demonstrated that sildenafil consumption in chronic heart failure patients improved muscle oxygenation and increased exercise capacity.

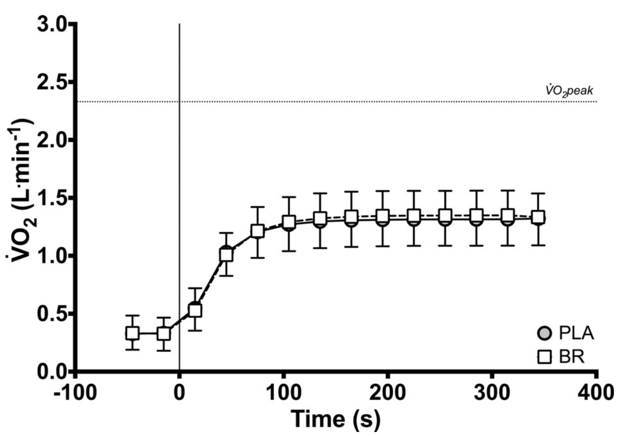

Fig. 2. Group mean (± SD) of oxygen consumption ($\dot{V}O_2$) every 30 s during moderate-intensity exercise following placebo (PLA, circles) and beetroot juice (BR) supplementation (squares). Horizontal dotted line indicates mean peak $\dot{V}O_2$ values reached during the incremental exercise. Vertical line: exercise started at *time 0.*

In the present study, dietary NO_3^- supplementation did not affect physiological responses to moderate-intensity CWR exercise. In this exercise domain the O_2 cost of cycling was not significantly different in BR (12.9 ± 1.1 ml·min^{-1}·W^{-1}) versus PLA (13.3 ± 1.7 ml·min^{-1}·W^{-1}), being in both conditions substantially higher than the values usually observed in subjects with normal weight (~9–10 ml·min^{-1}·W^{-1}) (37). This confirms our previous data (30–32). Thus, in this exercise domain, NO_3^- did not have any effect on the $\dot{V}O_2$ kinetics. These results are in accordance to the results of some other studies (4, 6).

The lack of ergogenic effects of BR supplementation in the moderate intensity domain does not limit the relevance of the results of this study. The effects of BR supplementation during high-intensity exercise is indeed noteworthy, considering that the latter is considered a very effective intervention for weight reduction and improved health status in obesity (38).

The question could be raised if the NO_3^- dose utilized in the present study was enough to induce significant changes in NO_2^- and NO bioavailability. Whereas NO is too labile to be determined (13), in the $NO_3^- \rightarrow NO_2^- \rightarrow$ NO pathway NO_2^- would obviously be "closer" to the physiological relevant molecule (NO). Plasma [NO_2^-] could not be determined in the present study. Ample previous experimental evidence, however, demonstrates that following dietary NO_2^- supplementation the increases in plasma [NO_2^-] and [NO_2^-] are closely correlated (13). In the present study, the daily NO_3^- dose (5 mmol) was toward the low end of the efficacy spectrum (40, 41), particularly considering that we were treating patients with obesity

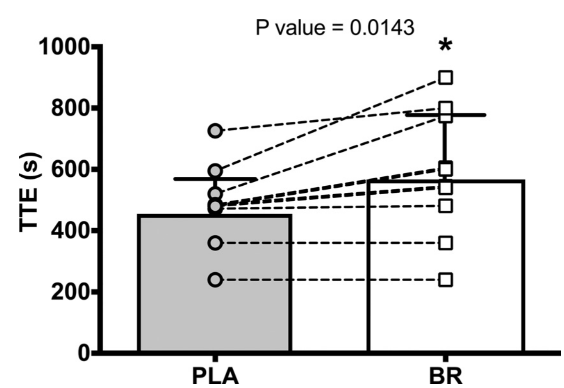

Fig. 3. Time to exhaustion (TTE) reached during severe-intensity exercise after placebo (PLA) and beetroot juice (BR) supplementation (mean ± SD). Dotted lines show individual changes. *P* value of paired Student's *t*-test is also shown. *$P < 0.05$, significantly different from PLA.

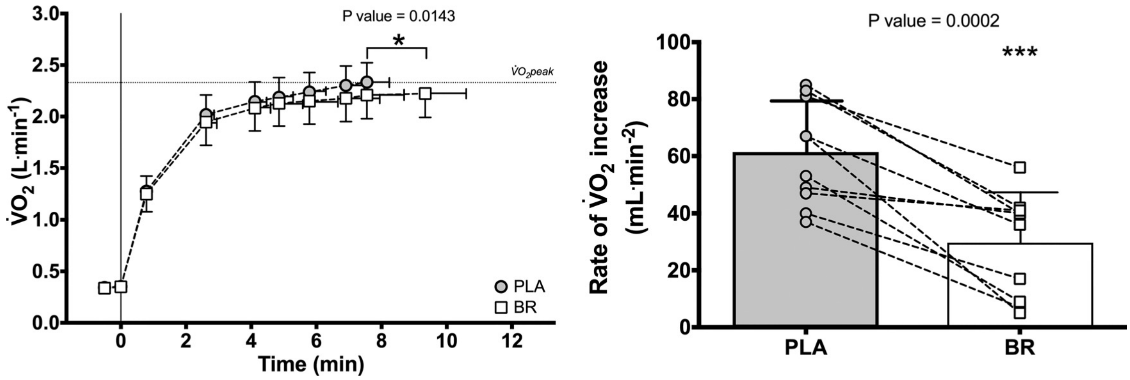

Fig. 4. Group mean values of oxygen consumption ($\dot{V}O_2$), grouped for discrete time intervals, during severe-intensity exercise following placebo (PLA, circles) and beetroot juice (BR, squares) supplementation (*left*). Vertical line: exercise started at *time 0*. Horizontal dot-point line indicates mean peak $\dot{V}O_2$ reached during incremental exercise. *P* value of paired Student's *t*-test is also shown. Time to exhaustion was significantly higher after BR compared with PLA ($*P < 0.05$). Mean values (± SD) of rate of $\dot{V}O_2$ increase calculated from the 3rd minute to the end of exercise in both PLA and BR (*right*). Dotted lines show individual changes. *P* value of paired Student's *t*-test is also shown. $***P < 0.001$, significantly different from PLA.

with an elevated BM. The treatment, however, was continued for 6 days, i.e. for a period substantially longer than that adopted in previous "acute" supplementation studies (18, 25). The end result was that in all patients of the present study plasma [NO_3^-] substantially increased after the supplementation. On average the plasma [NO_3^-] increase was approximately sevenfold, reaching values quite similar to or even higher than those obtained in previous studies (13, 17) in which dietary NO_3^- supplementation proved to be effective. Moreover, although in the present study plasma [NO_3^-] was inversely correlated with BM, no correlation was observed between plasma [NO_3^-] and the main outcome measure, the time to exhaustion during severe-intensity exercise.

Another issue we should take into consideration relates to the recruitment pattern of muscle fibers. It is known that additional fibers, mainly type 2 fibers, are recruited with time during heavy- and severe-intensity exercise in temporal association with the slow component of the $\dot{V}O_2$ kinetics (16). Although it has been previously demonstrated that additional recruitment of fibers is not necessary for the development of the slow component (44), in several experimental conditions the slow component may be linked to a shift in fiber-type recruitment (15). BR supplementation seems to be more effective in type 2 compared with type 1 fibers (16). Indeed, the reduction of NO_2^- to NO, mainly responsible of the ergogenic effects of NO_3^- supplementation, seems to be augmented in conditions of low pH and P_{O_2}, typical conditions associated with heavy- and severe-intensity exercise, in which type 2 fibers are predominantly activated. In rats running at 70% of $\dot{V}O_{2peak}$ blood flow was predominantly elevated in fast twitch muscle after BR ingestion (9). Thus, it is plausible that in presence of lower concentrations of NO, heavy- and severe-intensity domains of exercise could exacerbate tissue hypoxia within type 2 muscle fibers, resulting in a lower "metabolic stability" and fatigue development (10). Good metabolic stability during exercise is associated with smaller perturbations of the levels of metabolites related to muscle inefficiency and fatigue (10), such as decreases (compared with rest) in phosphocreatine, and increases in P_i, ADP-free, AMP-free, and IMP-free, etc. (10). Therefore, good metabolic stability is directly associated with a greater exercise tolerance. However, when intramuscular NO bioavailability is increased (as after NO_3^- supplementation) the improved matching between O_2 delivery and O_2 uptake could improve "metabolic stability," reduce metabolic inefficiency and fatigue, and improve enhance exercise tolerance (10).

Physical training of patients with obesity usually involves activities on cycle-ergometers because of the reduced load on hip, ankle, and knee joints compared with walking or running. However, cycling requires a lower metabolic demand compared with the other exercises, and this may limit the weight-loss process. Thus, future interventional studies to be carried out in patients with obesity should evaluate the ergogenic aid of dietary NO_3^- supplementation during walking and running. The effects of dietary NO_3^- supplementation on voluntary physical activities would also be an interesting topic of investigation. In this study, we did not evaluate the effects of NO_3^- supplementation on blood pressure. Inorganic NO_3^- and BR consumption are associated with a significant reduction in systolic and diastolic blood pressure (e.g., 2, 17); this effect would be particularly useful in subjects at high risk for cardiovascular diseases, such as and overweight patients or patients with obesity. Finally, in this study, we increased NO bioavailability by BR supplementation providing an amount of 100 kcal per day (proteins 4 g, carbohydrates 21 g, fats 0.1 g, fibers 0.6 g, and sodium 0.3 g). Although relatively small, this amount could become a problem during long-term supplementation in the obese population. Future studies should therefore consider other sources of NO_3^- for chronic supplementations, such as nitrate-rich vegetables, as previously utilized in healthy subjects (28).

Perspectives and Significance

The results of the present study suggest that short-term (6 days) dietary NO_3^- supplementation in adolescents with obesity is safe and effective in improving exercise tolerance-during severe-intensity exercises. The effect may be related to improved efficiency of oxidative metabolism (i.e., reduction of slow component), and it needs to be confirmed by longer-term studies. By contrasting the vicious circle of early fatigue → reduced exercise tolerance → reduced physical activity → obesity → early fatigue, the intervention could represent a useful adjunct (similar to respiratory muscle endurance training, see our recent work) (31) in the control of obesity in adolescents and possibly also in adult obese patients.

ACKNOWLEDGMENTS

We thank all the participants for their contributions to this research project. We acknowledge the head nurse, A. Seddone, and the nursing staff at the Division of Auxology for their professional collaboration in blood sampling and monitoring during the execution of the tests. We also thank Dr. Teresa Lorenzon for valuable help.

GRANTS

The work was partially supported by Progetti di Ricerca Corrente, Istituto Auxologico Italiano, Istituto Auxologico Italiano, Istituto di Ricovero e Cura a Carattere Scientifico, Milan and Piancavallo (Project Reference Code: 01C407–2014; acronym: SUPNITESEROB) and by Progetto di interesse Invecchiamento CNR WP3.7.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

L.R., S.P., M.M., D.S., F.A., A.D.C., G.T., A.M.J., A.S., and B.G. conceived and designed research; L.R., S.P., D.S., A.V., F.A., A.D.C., and G.T. performed experiments; L.R., S.P., D.S., and A.V. analyzed data; L.R., S.P., M.M., D.S., A.V., A.M.J., A.S., and B.G. interpreted results of experiments; L.R. and S.P. prepared figures; L.R., S.P., and B.G. drafted manuscript; L.R., S.P., M.M., D.S., A.V., F.A., A.D.C., G.T., A.M.J., A.S., and B.G. edited and revised manuscript; L.R., S.P., M.M., D.S., A.V., F.A., A.D.C., G.T., A.M.J., A.S., and B.G. approved final version of manuscript.

REFERENCES

1. **Bailey SJ, Fulford J, Vanhatalo A, Winyard PG, Blackwell JR, DiMenna FJ, Wilkerson DP, Benjamin N, Jones AM.** Dietary nitrate supplementation enhances muscle contractile efficiency during knee-extensor exercise in humans. *J Appl Physiol (1985)* 109: 135–148, 2010. doi:10.1152/japplphysiol.00046.2010.
2. **Bailey SJ, Winyard P, Vanhatalo A, Blackwell JR, Dimenna FJ, Wilkerson DP, Tarr J, Benjamin N, Jones AM.** Dietary nitrate supplementation reduces the O_2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. *J Appl Physiol (1985)* 107: 1144–1155, 2009. doi:10.1152/japplphysiol.00722.2009.
3. **Beaver WL, Wasserman K, Whipp BJ.** A new method for detecting anaerobic threshold by gas exchange. *J Appl Physiol (1985)* 60: 2020–2027, 1986. doi:10.1152/jappl.1986.60.6.2020.
4. **Bescós R, Rodríguez FA, Iglesias X, Ferrer MD, Iborra E, Pons A.** Acute administration of inorganic nitrate reduces $\dot{V}O_{2peak}$ in endurance athletes. *Med Sci Sports Exerc* 43: 1979–1986, 2011. doi:10.1249/MSS.0b013e318217d439.
5. **Borg GA.** Perceived exertion: a note on "history" and methods. *Med Sci Sports* 5: 90–93, 1973.
6. **Breese BC, McNarry MA, Marwood S, Blackwell JR, Bailey SJ, Jones AM.** Beetroot juice supplementation speeds O_2 uptake kinetics and improves exercise tolerance during severe-intensity exercise initiated from an elevated metabolic rate. *Am J Physiol Regul Integr Comp Physiol* 305: R1441–R1450, 2013. doi:10.1152/ajpregu.00295.2013.
7. **Cacciari E, Milani S, Balsamo A, Spada E, Bona G, Cavallo L, Cerutti F, Gargantini L, Greggio N, Tonini G, Cicognani A.** Italian cross-sectional growth charts for height, weight and BMI (2 to 20 yr). *J Endocrinol Invest* 29: 581–593, 2006. doi:10.1007/BF03344156.
8. **Curtis KJ, O'Brien KA, Tanner RJ, Polkey JI, Minnion M, Feelisch M, Polkey MI, Edwards LM, Hopkinson NS.** Acute dietary nitrate supplementation and exercise performance in COPD: a double-blind, placebo-controlled, randomised controlled pilot study. *PLoS One* 10: e0144504, 2015. doi:10.1371/journal.pone.0144504.
9. **Ferguson SK, Hirai DM, Copp SW, Holdsworth CT, Allen JD, Jones AM, Musch TI, Poole DC.** Impact of dietary nitrate supplementation via beetroot juice on exercising muscle vascular control in rats. *J Physiol* 591: 547–557, 2013. doi:10.1113/jphysiol.2012.243121.
10. **Grassi B, Porcelli S, Salvadego D, Zoladz JA.** Slow VO_2 kinetics during moderate-intensity exercise as markers of lower metabolic stability and lower exercise tolerance. *Eur J Appl Physiol* 111: 345–355, 2011. doi:10.1007/s00421-010-1609-1.
11. **Grassi B, Rossiter HB, Zoladz JA.** Skeletal muscle fatigue and decreased efficiency: two sides of the same coin? *Exerc Sport Sci Rev* 43: 75–83, 2015. doi:10.1249/JES.0000000000000043.
12. **Gray DS, Bray GA, Gemayel N, Kaplan K.** Effect of obesity on bioelectrical impedance. *Am J Clin Nutr* 50: 255–260, 1989. doi:10.1093/ajcn/50.2.255.
13. **James PE, Willis GR, Allen JD, Winyard PG, Jones AM.** Nitrate pharmacokinetics: Taking note of the difference. *Nitric Oxide* 48: 44–50, 2015. doi:10.1016/j.niox.2015.04.006.
14. **Jones AM.** Dietary nitrate supplementation and exercise performance. *Sports Med* 44, *Suppl* 1: 35–45, 2014. doi:10.1007/s40279-014-0149-y.
15. **Jones AM, Ferguson SK, Bailey SJ, Vanhatalo A, Poole DC.** Fiber type-specific effects of dietary nitrate. *Exerc Sport Sci Rev* 44: 53–60, 2016. doi:10.1249/JES.0000000000000074.
16. **Jones AM, Grassi B, Christensen PM, Krustrup P, Bangsbo J, Poole DC.** Slow component of VO2 kinetics: mechanistic bases and practical applications. *Med Sci Sports Exerc* 43: 2046–2062, 2011. doi:10.1249/MSS.0b013e31821fcfc1.
17. **Kapil V, Milsom AB, Okorie M, Maleki-Toyserkani S, Akram F, Rehman F, Arghandawi S, Pearl V, Benjamin N, Loukogeorgakis S, Macallister R, Hobbs AJ, Webb AJ, Ahluwalia A.** Inorganic nitrate supplementation lowers blood pressure in humans: role for nitrite-derived NO. *Hypertension* 56: 274–281, 2010. [Erratum in *Hypertension* 56: e39–e39, 2010.] doi:10.1161/HYPERTENSIONAHA.110.153536.
18. **Kenjale AA, Ham KL, Stabler T, Robbins JL, Johnson JL, Vanbruggen M, Privette G, Yim E, Kraus WE, Allen JD.** Dietary nitrate supplementation enhances exercise performance in peripheral arterial disease. *J Appl Physiol (1985)* 110: 1582–1591, 2011. doi:10.1152/japplphysiol.00071.2011.
19. **Kobayashi J, Ohtake K, Uchida H.** NO-rich diet for lifestyle-related diseases. *Nutrients* 7: 4911–4937, 2015. doi:10.3390/nu7064911.
20. **Kraus RM, Houmard JA, Kraus WE, Tanner CJ, Pierce JR, Choi MD, Hickner RC.** Obesity, insulin resistance, and skeletal muscle nitric oxide synthase. *J Appl Physiol (1985)* 113: 758–765, 2012. doi:10.1152/japplphysiol.01018.2011.
21. **Lamarra N, Whipp BJ, Ward SA, Wasserman K.** Effect of interbreath fluctuations on characterizing exercise gas exchange kinetics. *J Appl Physiol (1985)* 62: 2003–2012, 1987. doi:10.1152/jappl.1987.62.5.2003.
22. **Larsen FJ, Schiffer TA, Borniquel S, Sahlin K, Ekblom B, Lundberg JO, Weitzberg E.** Dietary inorganic nitrate improves mitochondrial efficiency in humans. *Cell Metab* 13: 149–159, 2011. doi:10.1016/j.cmet.2011.01.004.
23. **Lazzer S, Salvadego D, Porcelli S, Rejc E, Agosti F, Sartorio A, Grassi B.** Skeletal muscle oxygen uptake in obese patients: functional evaluation by knee-extension exercise. *Eur J Appl Physiol* 113: 2125–2132, 2013. doi:10.1007/s00421-013-2647-2.
24. **McNarry MA, Lambrick D, Westrupp N, Faulkner J.** The influence of a six-week, high-intensity games intervention on the pulmonary oxygen uptake kinetics in prepubertal obese and normal-weight children. *Appl Physiol Nutr Metab* 40: 1012–1018, 2015. doi:10.1139/apnm-2015-0051.
25. **Muggeridge DJ, Howe CC, Spendiff O, Pedlar C, James PE, Easton C.** The effects of a single dose of concentrated beetroot juice on performance in trained flatwater kayakers. *Int J Sport Nutr Exerc Metab* 23: 498–506, 2013. doi:10.1123/ijsnem.23.5.498.
26. **National Center for Health Statistics.** *Health, United States, 2015: With Special Feature on Racial and Ethnic Health Disparities*. Hyattsville, MD: National Center for Health Statistics, 2016.
27. **Poole DC, Jones AM.** Measurement of the maximum oxygen uptake $\dot{V}O_{2max}$: $\dot{V}O_{2peak}$ is no longer acceptable. *J Appl Physiol (1985)* 122: 997–1002, 2017. doi:10.1152/japplphysiol.01063.2016.
28. **Porcelli S, Pugliese L, Rejc E, Pavei G, Bonato M, Montorsi M, La Torre A, Rasica L, Marzorati M.** Effects of short-term high-nitrate diet on exercise performance. *Nutrients* 8: E534, 2016. doi:10.3390/nu8090534.
29. **Porcelli S, Ramaglia M, Bellistri G, Pavei G, Pugliese L, Montorsi M, Rasica L, Marzorati M.** Aerobic fitness affects the exercise performance responses to nitrate supplementation. *Med Sci Sports Exerc* 47: 1643–1651, 2015. doi:10.1249/MSS.0000000000000577.
30. **Salvadego D, Lazzer S, Busti C, Galli R, Agosti F, Lafortuna C, Sartorio A, Grassi B.** Gas exchange kinetics in obese adolescents. Inferences on exercise tolerance and prescription. *Am J Physiol Regul*

Integr Comp Physiol 299: R1298–R1305, 2010. doi:10.1152/ajpregu.00038.2010.

31. **Salvadego D, Sartorio A, Agosti F, Tringali G, Patrizi A, Isola M, LoMauro A, Aliverti A, Grassi B.** Respiratory muscle endurance training reduces the O_2 cost of cycling and perceived exertion in obese adolescents. *Am J Physiol Regul Integr Comp Physiol* 313: R487–R495, 2017. doi:10.1152/ajpregu.00396.2016.
32. **Salvadego D, Sartorio A, Agosti F, Tringali G, Patrizi A, Mauro AL, Aliverti A, Grassi B.** Acute respiratory muscle unloading by normoxic helium-O_2 breathing reduces the O_2 cost of cycling and perceived exertion in obese adolescents. *Eur J Appl Physiol* 115: 99–109, 2015. doi:10.1007/s00421-014-2993-8.
33. **Salvadori A, Fanari P, Fontana M, Buontempi L, Saezza A, Baudo S, Miserocchi G, Longhini E.** Oxygen uptake and cardiac performance in obese and normal subjects during exercise. *Respiration* 66: 25–33, 1999. doi:10.1159/000029333.
34. **Siervo M, Jackson SJ, Bluck LJC.** In-vivo nitric oxide synthesis is reduced in obese patients with metabolic syndrome: application of a novel stable isotopic method. *J Hypertens* 29: 1515–1527, 2011. doi:10.1097/HJH.0b013e3283487806.
35. **Sperandio PA, Oliveira MF, Rodrigues MK, Berton DC, Treptow E, Nery LE, Almeida DR, Neder JA.** Sildenafil improves microvascular O_2 delivery-to-utilization matching and accelerates exercise O_2 uptake kinetics in chronic heart failure. *Am J Physiol Heart Circ Physiol* 303: H1474–H1480, 2012. doi:10.1152/ajpheart.00435.2012.
36. **Tengan CH, Rodrigues GS, Godinho RO.** Nitric oxide in skeletal muscle: role on mitochondrial biogenesis and function. *Int J Mol Sci* 13: 17160–17184, 2012. doi:10.3390/ijms131217160.
37. **Wasserman K, Whipp BJ.** Excercise physiology in health and disease. *Am Rev Respir Dis* 112: 219–249, 1975. doi:10.1164/arrd.1975.112.2.219.
38. **Wewege M, van den Berg R, Ward RE, Keech A.** The effects of high-intensity interval training vs. moderate-intensity continuous training on body composition in overweight and obese adults: a systematic review and meta-analysis. *Obes Rev* 18: 635–646, 2017. doi:10.1111/obr.12532.
39. **Woessner M, Smoliga JM, Tarzia B, Stabler T, Van Bruggen M, Allen JD.** A stepwise reduction in plasma and salivary nitrite with increasing strengths of mouthwash following a dietary nitrate load. *Nitric Oxide* 54: 1–7, 2016. doi:10.1016/j.niox.2016.01.002.
40. **Wylie LJ, Ortiz de Zevallos J, Isidore T, Nyman L, Vanhatalo A, Bailey SJ, Jones AM.** Dose-dependent effects of dietary nitrate on the oxygen cost of moderate-intensity exercise: Acute vs. chronic supplementation. *Nitric Oxide* 57: 30–39, 2016. doi:10.1016/j.niox.2016.04.004.
41. **Wylie LJ, Kelly J, Bailey SJ, Blackwell JR, Skiba PF, Winyard PG, Jeukendrup AE, Vanhatalo A, Jones AM.** Beetroot juice and exercise: pharmacodynamic and dose-response relationships. *J Appl Physiol (1985)* 115: 325–336, 2013. doi:10.1152/japplphysiol.00372.2013.
42. **Zamani P, Rawat D, Shiva-Kumar P, Geraci S, Bhuva R, Konda P, Doulias PT, Ischiropoulos H, Townsend RR, Margulies KB, Cappola TP, Poole DC, Chirinos JA.** Effect of inorganic nitrate on exercise capacity in heart failure with preserved ejection fraction. *Circulation* 131: 371–380, 2015. doi:10.1161/CIRCULATIONAHA.114.012957.
43. **Zamani P, Tan V, Soto-Calderon H, Beraun M, Brandimarto JA, Trieu L, Varakantam S, Doulias PT, Townsend RR, Chittams J, Margulies KB, Cappola TP, Poole DC, Ischiropoulos H, Chirinos JA.** Pharmacokinetics and pharmacodynamics of inorganic nitrate in heart failure with preserved ejection fraction. *Circ Res* 120: 1151–1161, 2017. doi:10.1161/CIRCRESAHA.116.309832.
44. **Zoladz JA, Gladden LB, Hogan MC, Nieckarz Z, Grassi B.** Progressive recruitment of muscle fibers is not necessary for the slow component of VO2 kinetics. *J Appl Physiol (1985)* 105: 575–580, 2008. doi:10.1152/japplphysiol.01129.2007.