

Dietary nitrate enhances arterial oxygen saturation after dynamic apnea

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Breath-hold divers train to minimize their oxygen consumption to improve their apneic performance. Dietary nitrate has been shown to reduce the oxygen cost in a variety of situations, and our aim was to study its effect on arterial oxygen saturation (SaO₂) after dynamic apnea (DYN) performance. Fourteen healthy male apnea divers (aged 33 ± 11 years) received either 70 mL of concentrated nitrate-rich beetroot juice (BR) or placebo (PL) on different days. At 2.5 h after ingesting the juice, they were asked to perform 2 × 75 m DYN dives in a pool with 4.5-min recovery between dives. Each dive started

after 2-min countdown and without any warm-up apneas, hyperventilation, or lung packing. SaO₂ and heart rate were measured via pulse oximetry for 90 s before and after each dive. Mean SaO₂ nadir values after the dives were 83.4 ± 10.8% with BR and 78.3 ± 11.0% with PL (*P* < 0.05). At 20-s post-dive, mean SaO₂ was 86.3 ± 10.6% with BR and 79.4 ± 10.2% with PL (*P* < 0.05). In conclusion, BR juice was found to elevate SaO₂ after 75-m DYN. These results suggest an oxygen conserving effect of dietary nitrate supplementation, which likely has a positive effect on maximal apnea performance.

Interest in dietary nitrate (NO₃⁻) has increased over the past decade because acute ingestion has been shown to improve sports performance in a number of different conditions by reducing the oxygen (O₂) cost and enhancing tolerance to exercise of healthy subjects in normoxia (Larsen et al., 2007; Bailey et al., 2009; Lansley et al., 2011; Breese et al., 2013; Kelly et al., 2014), in hypoxia (Vanhatalo et al., 2011; Masschelein et al., 2012; Kelly et al., 2014) and in patients with peripheral artery disease (Kenjale et al., 2011).

The mechanism responsible for the O₂ conserving effect of NO₃⁻ is not clear. An improvement in mitochondrial efficiency is a potential explanation for a lower O₂ cost of submaximal exercise (Larsen et al., 2011). A lower adenosine triphosphate (ATP) requirement for the same power output is another explanation (Bailey et al., 2010) that could lower O₂ cost even if mitochondrial efficiency is unchanged (Whitfield et al., 2015).

Sports involving apneic diving rely completely on stored O₂, and therefore provide a unique model to study O₂ consumption. Competitive breath-hold diving is a growing sport, with disciplines that aim for maximal performance in underwater time (static

apnea), horizontal distance (dynamic apnea) with (DYN) or without fins, or depth. Performance depends upon a number of physiological factors, but achieving a minimal rate of O₂ consumption (VO_{2min}) is essential for success in all disciplines (Ferretti, 2001; Foster & Sheel, 2005; Schagatay, 2009, 2010, 2011).

During apnea, the results have however been conflicting whether NO₃⁻ ingestion enhances performance. Acute NO₃⁻ ingestion via beetroot juice was found to increase arterial O₂ saturation (SaO₂) after static apnea of a fixed duration, it caused greater driving bradycardia during maximal apneas, and it also prolonged maximal apnea duration by 11%, compared to placebo (Engan et al., 2012). However, a study by Schiffer et al. (2013) reported that NO₃⁻ supplementation via potassium NO₃⁻ reduced static apneic duration and enhanced desaturation during maximal duration static apneas. Yet in the same study, after simulated dynamic apnea by stationary cycling, a trend for increased SaO₂ after maximal duration apneas was reported with NO₃⁻ supplementation (Schiffer et al., 2013).

Dynamic apnea performance relies on factors also important during static apnea – total body storage of

usable O_2 , tolerance to asphyxia, and metabolic rate (Schagatay, 2009), but contains the added challenges of sustaining propulsive work despite the diving response (vasoconstriction and bradycardia) and central pooling of blood to high priority regions (Scholander et al., 1962; Gooden, 1994; Schagatay, 2010).

Our objective was to determine if acute NO_3^- ingestion would enhance SaO_2 after a fixed distance dive. We therefore studied the effect of dietary NO_3^- on SaO_2 and heart rate after DYN in a pool. SaO_2 after the dive would reflect the O_2 consumption, which would likely be predictive also of maximal DYN performance. The heart rate recovery response could reflect the magnitude of vasoconstriction of the diving response occurring during the dive. We hypothesize that NO_3^- will decrease the O_2 cost and increase the diving response.

Methods

Subjects

Fourteen healthy male subjects (mean \pm SD age 33 ± 11 years, height 182 ± 8 cm and weight 82 ± 12 kg) volunteered to participate in this study. They were all experienced apneic divers with 5.0 ± 3.8 years of training in the sport with a DYN personal best of 119 ± 43 m. Most divers were close to their peak training level; eight were tested in connection with a major competition, two were safety divers for the competition who trained regularly, and four were tested after a training camp. Subjects gave their written informed consent after receiving written and oral information about the study procedures and potential risks. The protocol had been approved by the local research ethics committee and was conducted in accordance with the Helsinki declaration.

Methodological considerations

No maximal effort DYN dives were included, as they may lead to syncope, which is an unnecessary risk that would require a full-scale safety system with two safety divers in the water. In addition, the maximal performance varies from day-to-day due to both physiological and psychological factors, especially in trained athletes (Schagatay, 2010). The authors considered that the submaximal performance of 75 m would be more standardized and would produce an O_2 debt sufficient enough to conclude whether dietary NO_3^- had an effect on performance or not. The 75-m dives were performed as part of their training. Only trained apneists were included because of their ability (compared to non-divers) to manage the O_2 debt when performing 75-m DYN. A safety diver was present at the poolside during all events.

Experimental procedures

Subjects were assigned in a blinded, randomized, cross-over design to receive dietary supplementation with 70 mL of concentrated NO_3^- -rich beetroot (BR) or placebo (PL) juice on different days. The BR juice contained ~ 5.0 mmol NO_3^- and the PL contained ~ 0.003 mmol NO_3^- (James White Drinks Ltd., Ipswich, UK). BR is indistinguishable from PL in color, taste, smell, and texture (Lansley et al., 2011). Subjects were instructed to consume the beverage 2.5 h prior to each test, which were conducted on different days with at least 24 h between tests.

Each subject sat resting on a chair at the poolside for 5 min prior to entering the water (Fig. 1). In the water after an additional 2-min rest, baseline SaO_2 and heart rate were measured via finger pulse oximetry (Nonin Medical Inc., Plymouth, Minnesota, USA) every 10 s for a total of 90 s. Each dive was preceded by a 2-min countdown without any warm-up, hyperventilation, or lung packing, as these maneuvers may affect diving performance and are difficult to standardize. Warm-up apneas can pre-stimulate the spleen to release red blood cells that may prolong apneas (Schagatay et al., 2001), hyperventilation reduces the level of carbon dioxide (CO_2) and urge to breathe (Lin et al., 1974) and may cause hypocapnia-induced cerebral vasoconstriction (Brian, 1998), and lung packing increases the O_2 stores (Örnhaugen et al., 1998). Subjects were then asked to perform 2×75 m DYN in the pool with 4.5-min recovery between the dives. Starting within 10 s after surfacing after each 75 m DYN, SaO_2 and heart rate were measured continuously until 90 s after each dive. The SaO_2 nadir was defined as the lowest SaO_2 value recorded from each dive in the 90-s post-dive period. Water temperature was 26 ± 1 °C and air temperature was 32 ± 2 °C, inducing a thermal difference sufficient to enhance the diving response compared to apnea alone (Schagatay & Holm, 1996).

Data analysis

All subjects served as their own controls and all but one subject completed all tests; the one subject interrupted the second swim before 75 m. Another three subjects had incomplete data from one of their 75-m swims. For these four subjects, calculations were based on one dive from each condition, in the same dive order (dive 1 or 2) to avoid an order effect. Therefore, 10-s group mean values were calculated using the means of two dives in nine subjects and one dive in four subjects in each of the BR and PL conditions. One subject was excluded from the 10-s group mean value analysis, but not the SaO_2 nadir value comparison, because a complete data from each dive were not in the same dive order. Data are presented as mean \pm SD, in 14 subjects for the SaO_2 nadir values, and in 13 subjects for the continuous SaO_2 and heart rate values. The lowest individual post-dive SaO_2 nadir value was used for calculating mean SaO_2 nadir values. SaO_2 rates of recovery after surfacing were calculated from SaO_2 nadir to 97% SaO_2 .

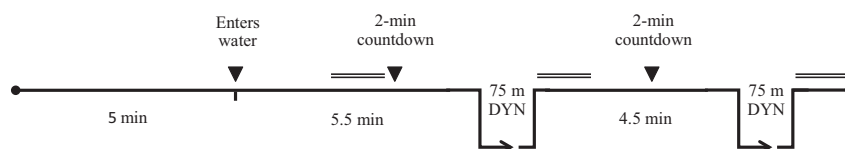


Fig. 1. The experimental procedure consisting of 2×75 m dynamic apnea with fins (DYN) with 4.5-min recovery between the dives. == Line signifies the 90-s measurement of SaO_2 and heart rate.

Statistics

Wilcoxon matched-pairs signed rank test was used to compare SaO₂ and heart rate between BR and PL conditions. Bonferroni correction was used for multiple comparisons of up to 40 s after surfacing. Statistical significance was accepted at $P \leq 0.05$.

Results

Arterial O₂ saturation

The resting control SaO₂ was $98.2 \pm 0.7\%$ with BR and it was $98.1 \pm 0.5\%$ with PL ($P = 0.481$). Mean of individual SaO₂ nadir values were $83.4 \pm 10.8\%$ with BR and $78.3 \pm 11.0\%$ with PL ($P = 0.023$). The reductions from resting control values to SaO₂ nadir were $14.8 \pm 11.0\%$ with BR and $19.8 \pm 11.1\%$ with PL ($P = 0.027$; Fig. 2). At 20-s post-dive, mean SaO₂ was $86.3 \pm 10.6\%$ with BR and $79.4 \pm 10.2\%$ with PL ($P = 0.019$; Figs 3 and 4). There were no differences between BR and PL in the ensuing 10-s intervals across recovery. Mean rate of SaO₂ recovery from SaO₂ nadir to 97% was

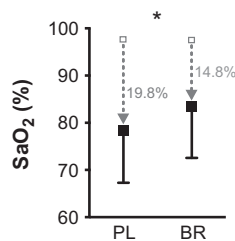


Fig. 2. Mean \pm SD ($n = 14$) for SaO₂ nadir with beetroot (BR) and placebo (PL) juice after 75-m dynamic apnea (DYN). The “open” and “closed” squares, respectively, represent the mean SaO₂ for baseline and after the DYN. Values inserted are the percent reduction from baseline SaO₂ before the DYN to SaO₂ nadir values ($*P < 0.05$).

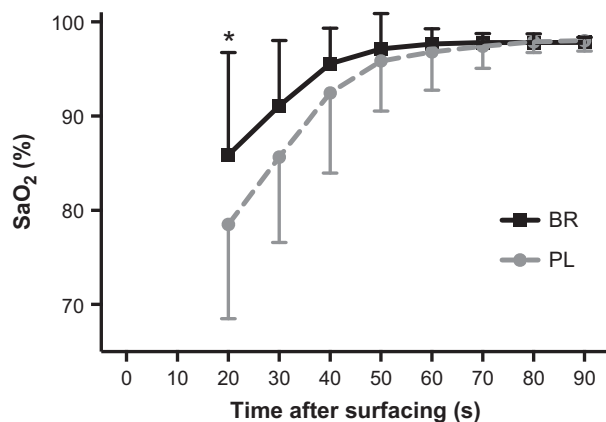


Fig. 3. Mean \pm SD ($n = 13$) arterial oxygen saturation (SaO₂) with beetroot (BR) and placebo (PL) juice during 90 s of recovery after 75 m dynamic apnea (DYN). Zero (“0”) indicates the moment the diver surfaces ($*P < 0.05$).

$0.8 \pm 0.6\%/s$ with BR and $0.9 \pm 0.5\%/s$ with PL ($P = 0.340$).

Heart rate

The resting control heart rate was 72 ± 12 beats/min with BR and 76 ± 15 beats/min with PL ($P = 0.727$). There were no significant differences in heart rate between BR and PL conditions after the dives (Fig. 5).

Discussion

The principal finding was that SaO₂ after 75-m DYN was higher after BR ingestion compared to PL. This was true both for the mean nadir SaO₂ and the SaO₂ at the point 20 s after the dive, suggesting that BR reduces the O₂ cost of exercise during dynamic apnea.

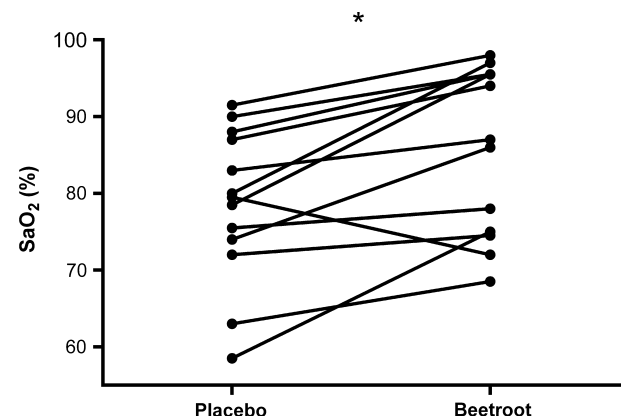


Fig. 4. Individual mean arterial oxygen saturation (SaO₂; $n = 13$) between placebo and beetroot juice 20 s after surfacing from 75-m dynamic apnea (DYN; $*P < 0.05$).

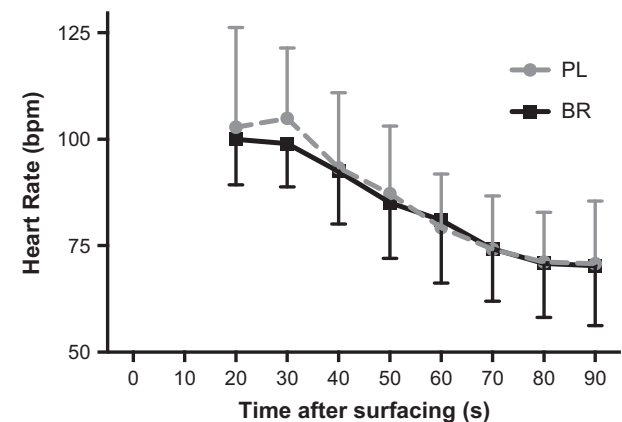


Fig. 5. Mean \pm SD ($n = 13$) heart rate with beetroot (BR) and placebo (PL) juice during 90 s of recovery after 75-m dynamic apnea (DYN). Zero (“0”) indicates the moment the diver surfaces.

This finding aligns with results from an apneic model without exercise (Engan et al., 2012) and with the tendency for O_2 conservation observed in another exercise-apnea model (Schiffer et al., 2013). It also aligns with several studies involving eupneic exercise in normoxia (Larsen et al., 2007; Bailey et al., 2009; Lansley et al., 2011; Breese et al., 2013; Kelly et al., 2014) and in hypoxia (Vanhatalo et al., 2011; Masschelein et al., 2012; Kelly et al., 2014). The result suggests that the total apneic duration and the total distance covered in DYN would likely be increased during a maximal attempt, due to increased remaining O_2 stores (Schagatay, 2010).

Engan et al. (2012), who concluded there was an O_2 conserving effect during static apnea, used similar NO_3^- administration procedures as in this study, and did not incorporate warm-up apneas or allow subjects to hyperventilate (Schagatay et al., 2001; Rasmussen et al., 2006). In Schiffer et al. (2013), who reported the contrary effect, a different NO_3^- administration was used, and warm-up apneas and hyperventilation were not controlled for, which could have contributed to their different results. Varying warm-up procedures likely play important roles in the outcome of physiological responses during static apnea performance (Schagatay, 2009) after consuming dietary NO_3^- . Interesting, however, is the effect during exercise of NO_3^- supplementation in the study by Schiffer et al. (2013), where their data show a tendency for the same O_2 -conserving effect as seen in the current study.

There was no effect of BR on heart rate in neither the current study nor in Schiffer et al. (2013), while Engan et al. (2012) found that the diving bradycardia was more pronounced in maximal static apneas. NO_3^- supplementation has been shown to reduce the O_2 cost during submaximal exercise (Larsen et al., 2007; Bailey et al., 2009), and during the submaximal effort in the current study, but it did not appear from the heart rate response that an accumulated O_2 debt occurred with BR. An aspect unique to the current study is the inclusion of immersion, particularly facial immersion (de Bruijn et al., 2009) which has been shown to be an important contributor to apneic performance by augmenting the diving response (Schuitema & Holm, 1988). A fully activated diving response due to a thermal stimulus, could provide beneficial conditions during exercise, when an O_2 conserving effect is even more essential than compared to rest. Therefore, it would be interesting to further investigate the influence of BR on the cardiovascular diving response during submaximal exercise.

From the moment the diver surfaces, time is required to dry the hand and allow the pulse oximeters to obtain a reliable reading, resulting in a first

usable value about 10–15 s after surfacing. Consequently, the focus of the analysis was set on the period from 15 s and onwards. Measuring SaO_2 from the hand is favorable in this case, compared to the ear for example, due to the longer circulation time between the lungs, heart, and the finger. A delay of approximately 15–20 s occurs before the SaO_2 nadir corresponding to the end of apnea appears in the finger (Andersson & Evaggelidis, 2009), thus we could likely detect the nadir values.

In conclusion, BR juice was found to result in a higher SaO_2 after 75-m DYN. These results suggest an O_2 -conserving effect of dietary NO_3^- supplementation, which increases the safety margins of submaximal dives and potentially has a positive effect on maximal apnea performance.

NO_3^- supplementation could also be applied to other sporting activities with limited oxygen availability, e.g., swimming where breathing is restricted to optimize hydrodynamics, competition apnea, spearfishing, synchronized swimming, and underwater team sports such as underwater rugby and hockey. Short-term supplementation with beetroot juice has also been shown to improve the walking time performance of patients with peripheral arterial disease (Kenjale et al., 2011), counteract the decline in endothelial function occurring during ascent to high altitude environments (Bakker et al., 2015) and preserve muscle oxygenation during exercise in acute normobaric hypoxia (Masschelein et al., 2012).

Perspectives

The O_2 -conserving effect of dietary NO_3^- could likely be beneficial in various sports involving apnea, e.g., apnea diving, spearfishing, swimming, synchronized swimming and underwater rugby, and hockey. Effects in apneic-related sports would be expected on several levels; by increasing safety margins in submaximal dives, potentially increasing maximal performance in competition, and by reducing recovery time between short, repeated dives. Other applications of dietary NO_3^- supplementation could possibly be found in exercise at high altitude where the O_2 supply is also limited.

Key words: Apneic diving, breath-hold, hypoxia, immersion, arterial desaturation, anaerobic exercise, nitrate, sport performance.

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