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The effect of a dietary nitrate supplementation in the form of a single shot of beetroot juice on static and dynamic apnoea performance

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Running Title: Dietary Nitrate Supplementation and Apnoea Performance

Abstract

Introduction: The purpose of the present study was to assess the effects of acute nitrate (NO_3^-)-rich beetroot juice supplementation on peripheral oxygen saturation (S_pO_2), heart rate (HR), and pulmonary gas exchange during submaximal static and dynamic apnoea. *Methods:* Nine (six male, three female) trained apneists (age: 39.6 ± 8.2 years, stature: 170.4 ± 11.5cm, body mass: 72.0 ± 11.5 kg) performed three submaximal static apnoeas at 60%, 70% and 80% of the participant's current reported personal best time, followed by three submaximal (~ 75% or personal best distance) dynamic apnoeas following the consumption of either a 140 ml concentrated NO_3^--rich beetroot juice (BRJ; 7.7 mmol NO_3^-) or a NO_3^--depleted placebo (PLA; 0.1 mmol NO_3^-) in double-blind randomised manner. HR and S_pO_2 were measured via fingertip pulse oximetry at the nadir, and online gas analysis was used to assess pulmonary oxygen uptake ($\dot{V}O_2$) during recovery following breath-holds. *Results:* There were no differences (P <0.05) between conditions for HR (PLA = 59 ± 11 bpm and BRJ = 61 ± 12 bpm), S_pO_2 (PLA = 83 ± 14% and BRJ = 84 ± 9%) or $\dot{V}O_2$ (PLA = 1.00 ± 0.22 L.min^{-1} and BRJ = 0.97 ± 0.27 L.min^{-1}). *Conclusion:* The consumption of 7.7mmol of beetroot juice supplementation prior to a series of submaximal static and dynamic apnoeas did not induce a significant change in S_pO_2, HR and $\dot{V}O_2$, when compared to placebo. Therefore there is no apparent physiological response that may benefit free-divers as a result of the supplementation.

Keywords: Freediving, Breath-hold, Ergogenic aid.

Introduction

Competitive apnoea also known as Free Diving or Breath-hold diving is an increasingly popular sport, in which individuals attempt to achieve the greatest possible stationary breath-hold duration (i.e. static apnoea) or maximal underwater distance or depth (i.e. dynamic apnoea). During apnoea, oxygen exchange with the environment is restricted, such that oxygen stored in the lungs and blood, and other tissue is drawn upon to meet the metabolic demands of the body (Schagatay et al., 2009). As the apnoea continues, stored oxygen is gradually depleted, whilst carbon dioxide, lactate, and hydrogen (H^+) ions accumulate (Schagatay, 2010). A low oxygen consumption is hence viewed as key to successful apnoea performance (for review see Schagatay, 2009; 2010; 2011), and strategies which minimise oxygen consumption are highly desired.

Recently, dietary nitrate (NO_3^-) supplementation has attracted considerable interest, following discovery of the NO_3^--nitrite (NO_2^-)-nitric oxide (NO) pathway – a means by which ingested or endogenously derived NO_3^- can be converted into the pleiotropic signalling molecule NO (Lundberg et al., 2008). NO_3^- supplementation has been reported to enhance the efficiency of muscle contraction (Bailey et al., 2010) and mitochondrial respiration (Larsen et al., 2011), concomitantly reducing the oxygen cost of submaximal exercise (Larsen et al., 2007; Bailey et al., 2009; Pawlak-Chaouch et al., 2016). Interestingly, generation of NO via the NO_3^--NO_2^--NO pathway (Castello et al., 2006), and thus the physiological effects of NO_3^- supplementation (Kelly et al., 2014), appear to be enhanced when oxygen availability is limited. It is therefore possible that NO_3^- supplementation might be beneficial for apnoea performance.

It is logical to assume that NO_3^- supplementation should only be used during maximal competitive efforts to allow for maximal stimulus during training; however it is suggested that

NO_3^- may be a beneficial training aid allowing for increased training load due to reduced training fatigue (Mahoney et al, 2012).

Engan et al. (2012) reported an increase in arterial oxygen saturation (S_pO_2), indicative of reduced tissue oxygen consumption, during a sub-maximal 'dry' (i.e. not submersed in water) apnoea after acute NO_3^--rich (~5 mmol NO_3^-) beetroot juice supplementation. Maximal apnoea duration was also extended by 11 %. Patrician and Schagatay (2016) also recently reported elevated post-exercise S_pO_2 values following sub-maximal horizontal underwater dives in a swimming pool subsequent to NO_3^- -rich (~5 mmol) beetroot juice supplementation. In contrast, potassium NO_3^- supplementation decreased S_pO_2 and reduced maximal static apnoea duration (Schiffer et al. 2013). In the same investigation, NO_3^- supplementation had no effect on S_pO_2 following maximal dynamic apnoea on a cycle ergometer (50 Watts at 60 rpm for 60 and 90 sec) although maximal apnoea duration was no different between conditions (Placebo 119 ± 5.17 s vs. NO_3^- 123 ± 6.4 s, P = 0.50) (Schiffer et al., 2013). These conflicting findings may be related to differences in the NO_3^- supplementation protocol, apnoea protocol, participant training status, and other methodological factors. Consequently, further research is warranted to elucidate the potential efficacy of NO_3^- supplementation on apnoea performance.

To date, no study has evaluated the effects of NO_3^- supplementation on the physiological response to underwater static apnoea, nor compared the effects of NO_3^- supplementation on underwater static and dynamic apnoea. This provides a particularly interesting model for further exploration given the majority of apnoea competitions are conducted underwater, where the effects of NO_3^- supplementation might interact with the diving response, and potential differences in the effects of NO_3^- supplementation during static and dynamic apnoea. Therefore, the purpose of the present study was to assess the effects of acute NO_3^--rich beetroot juice supplementation on S_pO_2, heart rate (HR), and pulmonary gas exchange during

submaximal static and dynamic apnoea. In accordance with the recent investigation of Patrician & Schagatay (2016), we decided to explore the effects of NO_3^- supplementation on sub-maximal rather than maximal responses, which are predictive of maximal apnoea performance yet do not pose unnecessary health risks, including syncope, to participants.

Methods

Participants

Nine (six male, three female) trained apneists (age: 39.62 ± 8.18 years, stature: 170.4 ± 11.5cm, body mass: 72.0 ± 11.5 kg) volunteered and provided fully informed written consent to take part in this study, approved by the institutional ethics committee at Leeds Beckett University, UK. All participants were identified as 'low risk' according to the ACSM 2014 guidelines (Heyward & Gibson, 2014) prior to participation. All of the participants were qualified as a minimum as 2* according to the qualifications of the International Association for the Development of Apnoea (AIDA) and were training specifically for free diving a minimum of three times per week and competing at a national level. Testing was conducted in accordance with the Declaration of Helsinki.

Overview

Participants visited the laboratory on three separate occasions over a three week period. The first visit included pre-screening and familiarisation, which replicated the experimental testing procedures but without any intervention. Subsequent visits comprised the experimental and placebo trials, and were conducted in a randomised order (randomisation was achieved using https://www.randomizer.org/), and preceded by the consumption of 140 ml concentrated NO_3^- -rich beetroot juice (BRJ; 7.7 mmol NO_3^-) or a NO_3^- -depleted placebo (PLA; 0.1 mmol NO_3^-) (Beet It, James White Drinks Ltd., Ipswich, UK). Supplements were administered double-

blind, two and a half hours before testing. The NO_3^- concentration of supplements was determined prior to testing via ozone based chemiluminescence, as previously described (Shannon et al. 2016). Participants were asked to abstain from intense exercise, alcohol, and caffeine on the day of testing, and consumption of food in the four hour period prior to each trial. However, water was permitted *ad libitum* during this time to ensure adequate hydration. Testing was conducted in either a 21 m or 25 m swimming pool depending on participant residency and availability. Participants wore either a nose-enclosed facemask or goggles and nose-clip, and a wetsuit, for the dive.

Methodological considerations

The present study included only submaximal apnoeas, given the unnecessary safety risks, including syncope, associated with maximal breath-holds. The authors reasoned the submaximal apnoeas employed would be sufficient to invoke significant physiological changes, sufficient to detect difference between BRJ and PLA. Only trained apneists were included (AIDA 2* and above), given the enhanced ability of this population to safely tolerate oxygen debt. Additionally, the findings of this study are of greater relevance to this cohort, relative to non-divers. An AIDA qualified safety diver was present at all trials, as a further safety precaution. All apnoeas were performed at durations or distances relative to each diver's recent personal best (within the month previous). This was to ensure a relative physiological challenge across participants of sufficient intensity to make worthwhile observations whilst limiting the risk of syncope.

Experimental trials

Static apnoea protocol

Each experimental trial commenced with three submaximal static apnoeas (STA) at 60%, 70% and 80% (140 ± 25 sec, 164 ± 29 sec, 187 ± 33 sec respectively) of the participant's current reported personal best time (maximal duration) (mean personal best 234 ± 41 sec), separated with four minute recovery periods. Participants entered the water two minutes prior to the first apnoea and a countdown was provided one and a half minutes before each breath-hold commenced. A final inhalation was made, and a stopwatch was started at the moment the participant's airway was closed. Participants assumed a face-down floating position in the water, and were given regular time cues via taps throughout the apnoea. Ten seconds before the end of the apnoea the participant withdrew their face from the water, and removed their facemask whilst still holding their breath until the end of the dive.

Dynamic apnoea protocol

Participants rested for 15 minutes following the static apnoea protocol. They then performed three submaximal, dynamic apnoeas (DYN) separated by four minute recovery periods. Participants were required to swim underwater, covering a distance ~ 75 % of their current reported personal best (mean personal best 110 ± 22m actual distances performed ranged from 50-100m). Participants were permitted to swim with no fins (two participants), bi-fins (five participants) or a monofin (two participants) according to their preference. Dynamic apnoeas were likewise preceded by a one and a half minute countdown before each breath-hold commenced.

Measurements

Heart rate and S_pO_2 were measured via fingertip pulse oximeter (Nellcor PM10N, Medtronic, MN, USA). The probe for registration of HR and S_pO_2 was attached to the forefinger for two minutes prior to each breath-hold. Measurements were recorded immediately prior to the

commencement of each static and dynamic breath-hold. As soon as the participant resurfaced from the breath-hold, the probe was placed back on the finger. HR was recorded as soon as data was presented on the screen, i.e. within a few seconds. The post-apnoea S_pO_2 nadir, which was defined as the lowest S_pO_2 was achieved within in the one minute post-apnoea period, was also recorded. An online gas analysis system (Cortex Metamax 3B, Leipzig, Germany), calibrated according to the manufacturer's instructions, was used to assess the effects of NO_3^- supplementation on pulmonary oxygen uptake ($\dot{V}O_2$) during breath-holds. Expired air was collected in the final minute pre-apnoea, and the first minute post apnoea, through a face mask (Hans Rudolph, inc, MO, USA.) attached to the gas analysis system. $\dot{V}O_2$ was calculated as the volume of inspired oxygen (VIO_2) minus the volume of expired oxygen ($\dot{V}EO_2$).

Statistics

Each subject served as their own control. Separate two-way repeated measures ANOVAs were used to compare the HR, S_pO_2 and $\dot{V}O_2$ data between BRJ and PLA for static and dynamic apnoeas. Statistical significance was accepted at $P \leq 0.05$. Data are presented as mean (SD). All statistical analysis was performed using IBM SPSS Statistics 24.

Results

There was no difference in HR measured immediately pre-apnoea between PLA (mean ± SD) (80 ± 3) and BRJ (82 ± 17); $P>0.05$). There were no differences between the PLA and BRJ conditions following any of the breath-holds. When averaged across all STA trials, the overall mean post apnoea HR was 59 ± 11 bpm for PLA and 61 ± 12 bpm for BRJ; following the DYN overall mean post apnoea HR was 76 ± 15 bpm for the PLA condition and 79 ± 16 bpm for the BRJ condition (Figure 1).

Figure 1 Near Here

There was no difference (P>0.05) in S_pO_2 between PLA and BRJ following any of the apnoeas. When averaged across all STA the mean SpO_2 for the PLA condition was 82 ± 6% and 81 ± 9% in the BRJ condition. Following the DYN, the mean responses were 83 ± 14% and 84 ± 9%for the PLA and BRJ conditions respectively. Figure 2 shows the changes in SpO_2 during static and dynamic apnoea. There were no differences in the absolute reduction of SpO_2 under both conditions; the reduction during the STA in the PLA condition was 26 ± 10% and 27 ± 11% in the BRJ condition. Following the DYN, the mean change responses were 18 ± 12% and 28 ± 18% for the PLA and BRJ conditions respectively.

****Figure 2 near here***

The results of $\dot{V}O_2$ following the STA and DYN apnoeas are presented in Figure 3. Two-way repeated measures analysis found no differences following any of the apnoeas between PLA and BRJ. Overall averages for post STA $\dot{V}O_2$ were 1.00 ± 0.22 $L.min^{-1}$ for PLA and 0.97 ± 0.27$L.min^{-1}$ for BRJ. The overall average values were 1.70 ± 0.38 $L.min^{-1}$ for PLA and 1.62 ± 0.40$L.min^{-1}$ for BRJ following the DYN apnoeas.

Figure 3 Near here

Discussion

Dietary NO_3^- supplementation has previously been reported to increase the bioavailability of NO and elicit an array of physiological responses that may be beneficial to

performance, including a lower oxygen cost at steady-state exercise (Larsen et al., 2007; Bailey et al., 2009; Pawlak-Chaouch et al., 2016), and elevated S_pO_2 in hypoxia (Masschelein et al., 2012; Muggeridge et al., 2014; Bourdillon et al., 2015; Shannon et al., 2016; 2017). The mechanisms underlying the oxygen sparing effect of NO_3^- supplementation remain to be fully elucidated, but may be related to an improvement in either the efficiency of mitochondrial respiration (Larsen et al., 2011), and/or a reduced oxygen cost of muscle force generation (Bailey et al., 2010). The reduction of NO_2^- into NO is enhanced in hypoxic conditions, yet oxygen-dependent generation of NO via the L-arginine nitric oxide synthase (NOS) pathway is suppressed in hypoxia. Thus, NO_3^- supplementation may be particularly effective at augmenting NO bioavailability, and hence influencing NO mediated physiological processes, in situations where oxygen availability is low (Lundberg et al., 2008). Considering this background, the present study explored the effects of acute NO_3^- supplementation on the physiological responses to submaximal static and dynamic apnoea. The principle finding of this study is that NO_3^- supplementation did not significantly alter any of the physiological responses to static or dynamic apnoeas. An emerging view in literature is that highly-trained individuals may experience a diminished response to NO_3^- supplementation (Jones, 2014). For example, in runners, the improvement in performance consequent to NO_3^- supplementation negatively correlated with $\dot{V}O_{2peak}$ (Porcelli et al., 2014). The training status of apneists is not easily defined, but may be indicated by maximal apnoea duration and tolerance to arterial desaturation (Schiffer et al., 2013). The participants in the current study were all competitive free-divers and as such may be considered well trained.

It is possible that the dose of NO_3^- administered in this study (7.7 mmol) was insufficient to significantly alter NO bioavailability, and therefore influence NO mediated physiological signalling. Indeed, it is an acknowledged limitation of this study that we did not measure

101 plasma nor pulmonary markers of NO bioavailability. However, similar (Wylie et al., 2013)
102 and smaller (Muggeridge et al., 2014) doses of NO_3^- have previously been demonstrated to
103 increase plasma NO_3^-, and elicit physiological and ergogenic effects. Moreover, both Engan
104 et al. (2012), and Patrician and Schagatay (2016) reported significant physiological changes
105 following administration of a smaller NO_3^- dose (~ 5 mmol) and as such it would be expected
106 that the dosage used here would have been sufficient.
107
108 There are a number of methodological differences which may, in part, explain the disparate
109 findings observed between the present study and previous research. Apnoea duration may be
110 influenced by physiological changes brought about through the mammalian diving reflex,
111 demonstrated during breath-hold activity and characterised by bradycardia and peripheral
112 vasoconstriction (Schagatay & Holm, 1996). The current study did not find any differences in
113 HR between the conditions, which is in agreement with previous studies; suggesting that BRJ
114 had no effect on the bradycardia element of the diving reflex. However, peripheral
115 vasoconstriction is known to affect the values associated with pulse oximetry, with
116 vasoconstriction reportedly increasing S_pO_2 (Talke et al; 2006). It is well reported that NO_3^-
117 has a vasodilator response, although it is unclear how this may interact with the peripheral
118 vasoconstriction element of the diving reflex. Thus it is unclear how this may influence S_pO_2
119 measurements during apnoeic conditions which are also influenced by immersion, water
120 temperature, duration and the dynamic or static nature of the apnoea. Therefore, these
121 differences might be due to a lower and/or a delayed diving reflex effect, as a result of the
122 vasodilatory effect of the NO_3^-. Suppression of the diving reflex may lead to an attenuation of
123 the bradycardial effect and a lower peripheral vasoconstriction response, thus diminishing
124 and/or delaying the oxygen conserving mechanism of the diving reflex. This might lead to an
125 earlier consumption of the readily available oxygen stores and a reduction in the maximal

126 apnoeic durations that can be achieved by an individual. However, further research is required
127 to elucidate whether NO_3^- spares oxygen demand or increases it by blunting the diving reflex.
128
129 Splenic contractions have also been reported to occur, following a series of repeated apnoeas
130 (Schagatay, 2009). The spleen serves as a red blood cell reservoir; splenic contractions develop
131 progressively across apnoeas, and need several apnoeas to develop fully (3-5 repeated apnoeas)
132 (Prommer et al., 2007; Schagatay, 2009). During apnoeas Bakovic et al. (2003) identified a
133 positive correlation between the extent of splenic contractions and the level of S_pO_2, therefore
134 signifying that hypoxemia serves as a potent stimuli for evoking splenic contractions. During
135 contractions the spleen releases its stored red blood cells into circulation, increasing the oxygen
136 reserve and readily available oxygen supply, enhancing carbon dioxide buffering; thus
137 prolonging the apnoeic duration. However, neither the current study nor previous studies
138 (Engan et al, 2012; Patrician and Schagatay, 2016) reported values for splenic contractions
139 following the administration of NO_3^- supplementation. Thus, it is currently unclear as to
140 whether the vasodilatory effects of NO_3^- supplementation result in changes in splenic
141 contraction and whether this has any effect on breath-hold duration, HR or SpO_2. However, it
142 is tempting to speculate that NO_3^- supplementation may result in a delayed splenic effect.
143
144 **Conclusion**
145 The present study demonstrated that a consumption of 7.7mmol of beetroot juice
146 supplementation prior to a series of submaximal (~75%) static and dynamic apnoeas, did not
147 induce a change in S_pO_2, HR and $\dot{V}O_2$, when compared to placebo in nine (6 male; 3 female)
148 competitive freedivers. This suggests there is no apparent physiological response that may
149 benefit free-divers as a result of the supplementation.
150

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311 Figure Legends

312 Figure 1. Heart Rate (beats.min^{-1}) at cessation of apnoea for PLA and BRJ conditions.

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314 Figure 2. Change in SpO_2 from commencement to cessation of apnoea for PLA and BRJ

315 conditions.

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317 Figure 3. Recovery $\dot{V}O_2$ following cessation of apnoea for PLA and BRJ conditions.

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328 Figure1.

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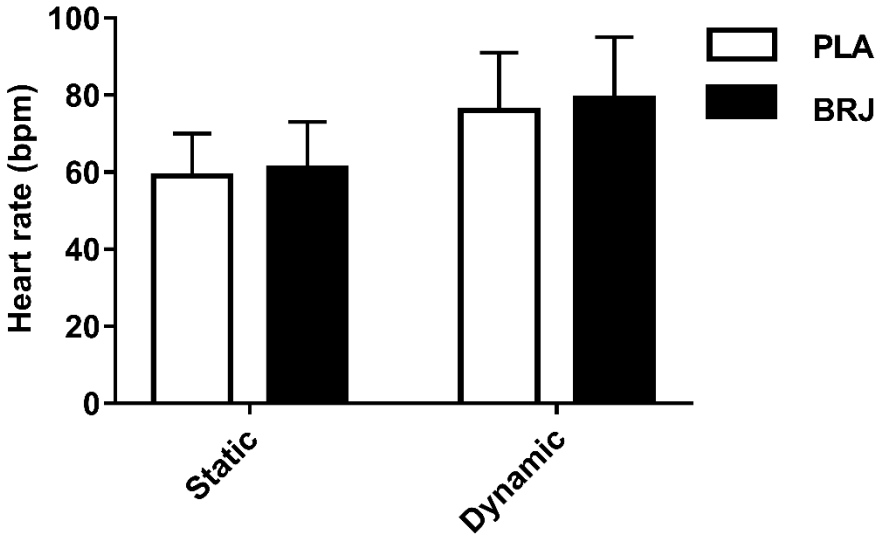

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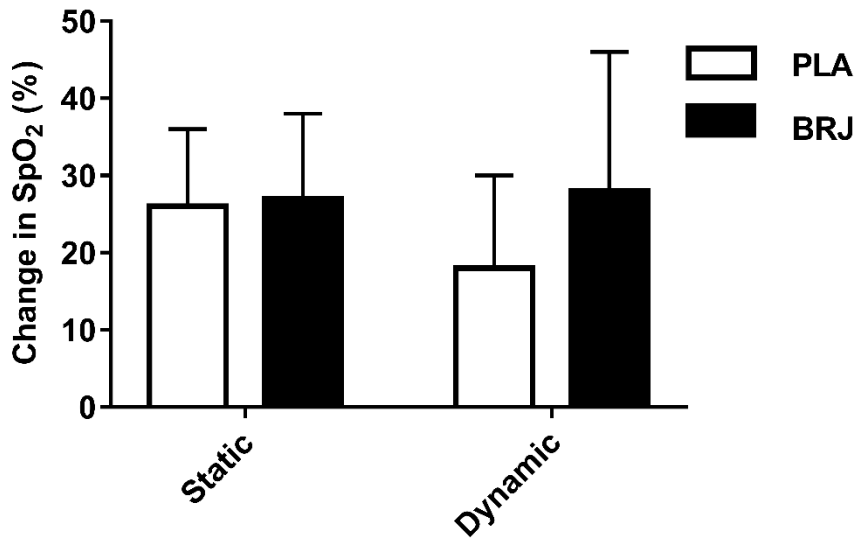
346 Figure 2.



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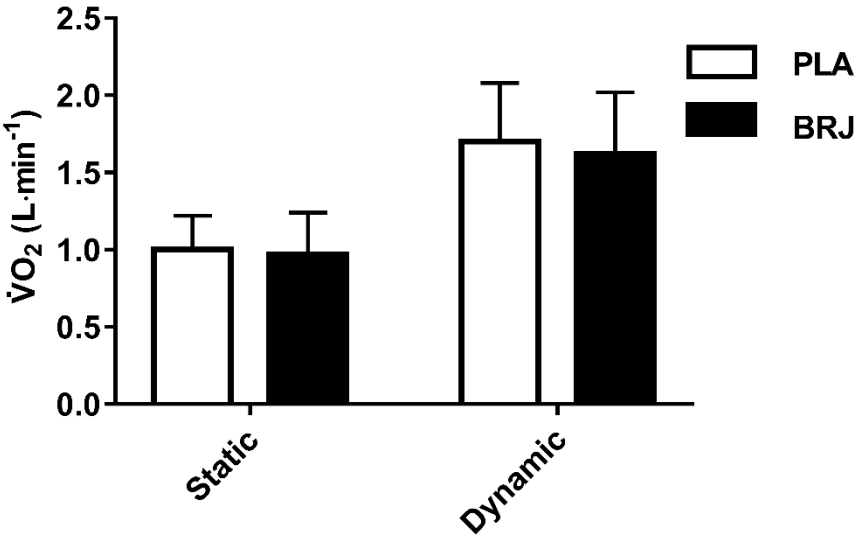
364 Figure 3.



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