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The oral nitrate-reducing capacity correlates with peak power output and peak oxygen uptake in healthy humans.

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Abstract

Interest in inorganic nitrate and nitrite has grown substantially over the past decade as research has revealed the role of these anions in enhancing nitric oxide (NO) availability through an oral pathway. Nitrite synthesis in the mouth has been shown to be a key source of the circulatory levels of this anion. This is interesting since greater plasma nitrite concentration has been associated with better exercise capacity in humans, but this question has not been investigated in relation to salivary nitrite concentration. Additionally, no previous study has investigated the nitrate-reducing activity of oral bacteria in regards to exercise performance in humans. Thus, the main goal of this study was to investigate whether salivary nitrite and nitrate concentration and the nitrate-reducing capacity of oral bacteria were associated with aerobic exercise capacity in healthy humans. Fifty individuals (22 females and 28 males; 38.8 ± 14.3 years/old; BMI= 22.8 ± 3.9) performed a graded exercise test on a cycle ergometer to assess their maximum aerobic capacity (VOpeak). Unstimulated salivary samples were taken before and 20 min after exercise to measure nitrate/nitrite, pH and lactate. The nitrate-reducing capacity of oral bacteria was also assessed in 25 subjects before and after exercise. Nitrate-reducing capacity of oral bacteria was positively associated with muscle power (rs= 0.64; P=0.001) and the VO2peak (rs= 0.54; P=0.005). Similar correlations were found when these variables were analysed after exercise. In addition, a significant decrease in salivary pH (pre:7.28 ± 0.361; post-exercise: 7.16 ± 0.33; P = 0.003) accompanied by an increase of salivary lactate (pre: 0.17 ± 0.14 mmol/L; post-exercise: 0.48 ± 0.38; P < 0.001) was found after exercise. However, these changes did not have any impact on salivary nitrate/nitrite concentration and the nitrate-reducing activity of oral bacteria after exercise. In conclusion, this is the first evidence showing a link between the nitrate-reducing activity of oral bacteria and aerobic fitness levels in healthy humans.

Keywords	nitrate, nitrite, exercise, oral bacteria
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Figure 1_Salivary_lactate_pH.JPG [Figure]

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13th November 2018

To the Editor— NITRIC OXIDE JOURNAL

Dear Prof. Hogg:

Please find uploaded our manuscript entitled *'Nitrate-reducing capacity of oral bacteria correlates with aerobic exercise performance in healthy humans'*. This is the largest study (*n* = 50) that has been performed to date looking at salivary nitrate and nitrite concentration, and the nitrate-reducing activity of oral bacteria in regards to exercise capacity in humans. Interestingly, we found an strong correlation between the nitrate-reducing activity of oral bacteria and the main two parameters of aerobic exercise performance (power and maximal oxygen uptake). This finding is very novel, and it may explain, at least partially, the lack of effect of nitrate supplements in well-trained individuals. On the other hand, we did not find an association between salivary nitrate and nitrite and exercise performance in this study. Differences between gender were not observed either. The manuscript is original, is not under concurrent consideration elsewhere, and was prepared in accordance with the requirements and style of the NITRIC OXIDE. All authors have participated in this study and they have read the manuscript being agreed with the submitted work.

Yours sincerely,

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Nitrate-reducing capacity of oral bacteria correlates with aerobic exercise performance in healthy humans

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Keywords: nitrate, nitrite, exercise, oral bacteria

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Abstract

Interest in inorganic nitrate and nitrite has grown substantially over the past decade as research has revealed the role of these anions in enhancing nitric oxide (NO) availability through an oral pathway. Nitrite synthesis in the mouth has been shown to be a key source of the circulatory levels of this anion. This is interesting since greater plasma nitrite concentration has been associated with better exercise capacity in humans, but this question has not been investigated in relation to salivary nitrite concentration. Additionally, no previous study has investigated the nitrate-reducing activity of oral bacteria in regards to exercise performance in humans. Thus, the main goal of this study was to investigate whether salivary nitrite and nitrate concentration and the nitrate-reducing capacity of oral bacteria were associated with aerobic exercise capacity in healthy humans.

Fifty individuals (22 females and 28 males; 38.8 ± 14.3 years/old; BMI= 22.8 ± 3.9) performed a graded exercise test on a cycle ergometer to assess their maximum aerobic capacity (VO_{peak}). Unstimulated salivary samples were taken before and 20 min after exercise to measure nitrate/nitrite, pH and lactate. The nitrate-reducing capacity of oral bacteria was also assessed in 25 subjects before and after exercise.

Nitrate-reducing capacity of oral bacteria was positively associated with muscle power (r_s= 0.64; P=0.001) and the VO_{2peak} (r_s= 0.54; P=0.005). Similar correlations were found when these variables were analysed after exercise. In addition, a significant decrease in salivary pH (pre:7.28 ± 0.361; post-exercise: 7.16 ± 0.33; P = 0.003) accompanied by an increase of salivary lactate (pre: 0.17 ± 0.14 mmol/L; post-exercise: 0.48 ± 0.38; P < 0.001) was found after exercise. However, these changes did not have any impact on salivary nitrate/nitrite concentration and the nitrate-reducing activity of oral bacteria after exercise.

In conclusion, this is the first evidence showing a link between the nitrate-reducing activity of oral bacteria and aerobic fitness levels in healthy humans.

Introduction

Nitrate and nitrite have emerged over the last decade as key regulators of nitric oxide (NO) metabolism in humans (1). Nitrate is the main circulatory anion in healthy humans, and it has been estimated that about 25% of circulatory nitrate is absorbed in the salivary glands (2). There, nitrate is excreted with saliva into the mouth where different species of oral bacteria can reduce it to nitrite. Once nitrite is swallowed, a small part is rapidly absorbed into the circulation across the upper gastrointestinal tract (2). Circulating nitrite can be reduced to NO through different pathways (3).

The main sources of nitrate in the body are diet (mainly vegetables) and endogenous synthesis of nitric oxide through the enzymatic L-Arginine/Nitric Oxide Synthase (NOS) pathway (2). Substantial research over the last decade has focused on dietary nitrate from two different perspectives: 1) as a potential ergogenic aid in sport, and 2) as a potential blood pressure-lowering dietary compound in health and disease (4, 5). Taking the first approach, recent data suggest that supplementation of dietary inorganic nitrate may be useful to enhance exercise capacity especially in low or moderate trained individuals, but this is not evident in well-trained athletes (6). This lack of effect has been attributed to several factors such as physiological and metabolic adaptations of chronic exercise training (6). Years of training may lead to reconditioning responses on cardiovascular function, skeletal muscle vascularization, and mitochondrial efficiency, resulting in greater metabolic and mechanical efficiency (7). These exercise-induced adaptations can also enhance NO synthesis through the L-Arginine/NOS pathway during exercise increasing nitrate and nitrite bioavailability, and lowering any potential ergogenic benefit from supplementation (5). Two previous studies by Rassaf et al (8) and Totzeck et al (9) found a positive and significant correlation between circulatory nitrite and exercise capacity in healthy humans under fasting conditions. However, this question has not been investigated using salivary nitrite and nitrate. This is interesting since recent evidence (10), but not all (11), suggests that synthesis of oral nitrite is a key mechanism in sustaining the circulatory levels of this anion, and to support NO availability in the body (10). With salivary-nitrite test-strip manufacturers claiming that greater concentration of salivary nitrite enhances exercise performance, there is a need for research to investigate this question more in depth.

Another key question is the differences in NO metabolism between men and women. A recent study by Kapil et al (12) has shown that the nitrate-reducing activity of oral bacteria differs between males and females, but this has not been previously investigated in regards to gender and exercise capacity in humans. Thus, the main aims of this study were to investigate whether salivary nitrate and nitrite

concentrations were associated with maximum aerobic capacity in males and females. In a second part of this study, we investigated whether the nitrate-reducing ability of oral bacteria was related to parameters of aerobic exercise performance in a similar cohort of subjects. First, we hypothesised that higher exercise performance would be associated with greater salivary nitrate/nitrite levels in both males and females. Then, we hypothesised that greater salivary nitrite could be related to higher activity of oral bacteria and, consequently, better exercise capacity.

Materials and methods

Participants

Fifty-three participants were recruited for this study, but two participants could not complete the protocol due to physical problems, and another participant did not follow the full protocol. Thus, fifty participants were included in this study (Table 1). They were recruited from local cycling, triathlon and University sports clubs. A medical questionnaire was completed prior to the study, and individuals with any pathology that could affect the oral cavity, such as gingivitis or periodontitis, or which could affect vascular function such as diabetes, hypertension, smoking or psychological conditions were excluded. Furthermore, individuals under medication or treatment potentially affecting oral bacteria (e.g antibacterial mouthwash) were excluded. All participants gave their written informed consent to participate in the study. The procedures carried out were approved by the human ethics committee of the Faculty of Health and Human Sciences (17/18-415) (University of Plymouth, UK) in accordance with the Declaration of Helsinki.

Protocol

Participants were required to report to the laboratory on one occasion in a fully rested state having not participated in strenuous exercise in the preceding 24 hours. They were encouraged to refrain from eating at least 3 hours before the exercise test. Food rich in nitrate and caffeine were restricted 24 and 12 hours before the trial, respectively. On their arrival at the laboratory, body fat percentage and body mass were measured using a body composition analyser (Tanita, TBF-300MA, Tokyo, Japan). Height was measured using a stadiometer (Seca, Hamburg, Germany). Then, salivary nitrite was measured with a test strip (Nitric Oxide test strips, Berkeley, Chicago, US) before a whole non-stimulated saliva sample (~3 mL) was collected to analyse nitrate, nitrite, pH and lactate. Following this, the nitrate-reducing capacity of oral bacteria was measured in 25 participants (Table 1). They rinsed their mouth with a 10 mL solution of distilled water containing 80 µmol of sodium nitrate (Sodium Nitrate, Fisher Chemical, UK) for 5 minutes. The solution was collected in sterile tubes. All

saliva samples were centrifuged at 4,000 rpm for 10 min at 4°C. The supernatant was obtained, filtered and stored at -80˚C.

Following sampling, subjects performed a graded exercise test until exhaustion on a bicycle ergometer (Lode Corival, Groningen, Netherlands) to assess their maximal aerobic capacity (VO_{peak}) and peak power (W_{peak}). The test commenced with a five-minute warm-up with resistance set at 70W for females and 100W for males. Pedalling cadence was kept within the range of 80-100 rpm and on completion of the warm-up exercise intensity increased 20W every minute for females and 25W for males. The exercise test finished when the subject reached physical exhaustion or was unable to maintain a minimum pedalling cadence of 70 rpm. Oxygen uptake (VO_2) and carbon dioxide production (VCO_2) were measured at 10-second intervals by a computerized gas analyser (Cortex Metalyzer 3B, Leipzig, Germany), which was calibrated in accordance with the manufacturer's instructions prior to each test. Heart rate was measured via a wireless transmitter (Polar, Kempele, Finland). VO_2 and power output (W) averaged over the last 30-second period of the test, was defined as VO_{2peak} and W_{peak} respectively.

At the cessation of the exercise test the subject was seated and allowed to rest for 20 min. During this period water was permitted however food and other drinks were restricted. Then, salivary nitrite was again measured using a test strip (Nitric Oxide test strips, Berkeley, Chicago, US) before taking another salivary sample (~ 3 mL). Finally, the oral nitrate-reducing capacity was measured using the same procedure as before exercise.

Analyses

Salivary nitrate and nitrite

Samples were thawed and 100 µL of supernatant was collected and diluted 10 times with a solution containing 10% methanol, 0.15 M NaCl/NH_4Cl, and 0.5 g/L 4Na-EDTA. Then, 10 µL was directly injected in a HPLC system (ENO-30, Eicom, Japan) to measure nitrate and nitrite. The same procedure was followed to measure salivary nitrite from the rinsing solutions used to assess the nitrate-reducing activity of oral bacteria.

Change in colour of salivary strips (Nitric Oxide test strips, Berkeley, Chicago, US) was categorised on 5 different levels according to the manufacturer's color chart.

Salivary pH, lactate and glucose

One mL of saliva was used to measure the pH (25˚C) using an electronic pH meter (PH-208, Lutron, Taiwan) calibrated in accordance with the manufacturer's instructions. Salivary lactate and glucose were analysed by a biochemistry analyser (YSI 2300 Stat Plus, YSI Life Sciences, US).

Statistical analyses

Data are presented as means ± SD. All data were analyzed to determine the normal distribution using the Shapiro Wilk's test. Comparison between groups (males / females) were performed using the Mann-Whitney U-test as data were not normally distributed. Comparison between pre and post exercise measurements in saliva parameters were carried out using a Wilcoxon Signed Ranks Test for all the variables except for salivary pH as this was normally distributed, and a paired t-test was used in this case. The association between salivary markers, power and VO_{2peak} was analysed using two-tailed Spearman's rank correlation analyses (95% confidence). Additionally, Spearman's rank correlation analyses (95% confidence) were used to assess the level of agreement between the salivary strips and nitrite laboratory measurements.

Results

Baseline characteristics of study subjects

Table 1 lists the baseline characteristics of the subjects. Significant differences between males and females were found in age ($P = 0.048$), anthropometrical (body mass $P < 0.001$; BMI $P = 0.036$; body fat $P < 0.001$) and physiological parameters (VO_{2peak} $P = 0.002$; W_{peak} $P = 0.008$) as expected, but no differences were observed ($P > 0.05$) in any salivary parameter between males and females ($P < 0.001$) (Table 1).

-Table 1-

Changes in salivary parameters between pre and post-exercise measurements

A significant increase ($P < 0.001$) in salivary lactate was observed in females (Pre: 0.16 ± 0.15; Post: 0.45 ± 0.37; $P < 0.001$) and males (Pre: 0.17 ± 0.15; Post: 0.53 ± 0.40; $P < 0.001$) after exercise (Figure 1). This response was associated with a significant reduction of salivary pH in males (Pre: 7.26 ± 0.33; Post: 7.11 ± 0.25; $P < 0.001$). Although a similar trend was observed in females, the reduction in salivary pH was not statistically significant (Pre: 7.29 ± 0.39; Post: 7.18 ± 0.37; $P = 0.64$). However, when all

participants were analysed together salivary pH was significantly lower after exercise compared to the pre-exercise values (Pre: 7.28 ± 0.36; Post: 7.16 ± 0.33; P = 0.003) (Figure 1).

In contrast, the concentration of salivary nitrate, nitrite and the nitrate-reducing capacity of oral bacteria did not differ (P > 0.05) between pre and post-exercise measurements in either group (Figure 2).

-Figure 1-

-Figure 2-

Association between salivary strips and HPLC measurements on nitrite concentration

A moderate (r_s = 0.43) and significant (P = 0.002) association was found between salivary strips (Berkeley test®) and salivary nitrite laboratory measurements (Table 2). Although this association was still significant between measurements after exercise, it was weakened compared to the pre-exercise values (Table 2).

-Table 2-

Associations between salivary markers and exercise capacity

No associations were found between salivary nitrate and exercise capacity in males or females (Table 3). A moderate and significant association (r_s = 0.44, P= 0.04) between salivary nitrite and power was only found in females. However, this was not associated with a correlation between salivary nitrite and VO_{2peak} (r_s = 0.31, P= 0.16). Taking all participants together, power was significantly associated with salivary lactate (r_s = 0.33, P= 0.02).

-Table 3-

The nitrate-reducing capacity of oral bacteria was significantly associated with power (r_s = 0.64, P= 0.01) and VO_{2peak} (r_s = 0.54, P= 0.04) in males before exercise (Table 3). While the association between the nitrate-reducing capacity and power did not change after exercise (r_s = 0.64, P= 0.01), a lower and non-significant correlation was observed with the VO_{2peak} (r_s = 0.47, P= 0.08). In females, the oral nitrate-reducing capacity was not associated with power or VO_{2peak} before and after exercise (Table 3). On the other hand, a positive and significant association was found between the nitrate-reducing capacity of oral bacteria and power and VO_{2peak} before and after exercise when all the participants (males and females) were analysed together (Figure 3).

-Figure 3-

Discussion

The main finding of this study was the positive and significant association between the nitrate-reducing capacity of oral bacteria and aerobic exercise performance in terms of power (W) and respiratory capacity (VO_{2peak}) when all subjects were analysed together. To the best of our knowledge, this is the first evidence linking exercise capacity with the activity of oral bacteria in healthy humans. These results suggest that exercise training may modulate the activity and/or diversity of the oral microbiome. From this viewpoint, recent studies on the gut microbiome have shown a positive association between exercise levels and the diversity of bacteria (13-16), however no previous study has investigated a possible link between oral bacteria and exercise capacity. Thus, this new evidence may explain the lack of ergogenic effect of dietary nitrate supplements in well-trained individuals (6, 17). Higher nitrate-reducing capacity of oral bacteria in trained individuals may be an adaptive mechanism that helps sustain nitrite availability through endogenous sources under different physiological conditions.

On the other hand, this study did not show an association between concentration of salivary nitrite and nitrate, and aerobic exercise capacity. A recent study has found that salivary nitrite production is well associated with higher abundance of oral nitrate-reducing capacity (18). According to this evidence and current results, it may suggest that highly trained individuals have similar levels of nitrate-reducing oral bacteria, but the activity of these species may differ between low and well-trained individuals. In order to answer this, there is a need to examine the oral biome of individuals with different fitness levels. On the other hand, our results in saliva were contrary to two previous studies showing a positive correlation between plasma nitrite concentration and aerobic exercise capacity in a similar cohort of individuals (8, 9). We based our hypothesis on these previous findings as well as on recent evidence showing that salivary nitrite correlated positively with the intensity and load of training (19). However, previous studies had some limitations requiring further examination. For instance, in the first study by Rassaf et al (8) the correlation coefficient was only 0.37 which suggests a large inter-individual variability in the cohort. In a second study by the same group, a stronger association was found (r= 0.65) between plasma nitrite and heart rate at lactate anaerobic threshold (9). From a physiological perspective this association is weak since heart rate at lactate anaerobic threshold cannot be considered a valid marker of aerobic exercise capacity in humans (20). Despite this, we found a positive and significant association between salivary nitrite and power in females after exercise, this association was moderate (r_s= 0.44), and we did not see the same effect

between these two variables before exercise. Additionally, we did not observe a similar correlation between salivary nitrite and the VO_{2peak}, which is another key marker of aerobic capacity analysed in this study. Thus, the observed correlation between salivary nitrite and power in females after exercise, should be treated with caution, as it may be a casual association.

As expected, this study found significant differences between males and females on anthropometrical and physiological variables, however no differences were found regarding salivary markers at baseline. These results differ from a recent study indicating that females had higher salivary nitrite concentrations than males which was linked to higher nitrate-reducing capacity, but not to a different composition of the oral microbiome (12). Regarding this discrepancy, a limitation of our study was that only 10 females took part in the study assessment of the nitrate-reducing capacity of oral bacteria while Kapil et al (12) investigated 25 females. In addition, the combination of this small sample size, and the lack of highly trained females (e.g VO_{2peak} > 60 mL/kg/min) may also explain the lack of association between the oral nitrate-reducing capacity and exercise performance in our group of females. This is supported by the fact that when collating all the subjects (males and females) together in order to increase the range of fitness levels we found that oral nitrate-reducing capacity was strongly correlated with W_{peak} as well as VO_{2peak} before and after exercise.

A significant increase of salivary lactate was observed in both groups after exercise. In males, this was accompanied by a significant decrease of salivary pH after exercise, but despite a similar pattern being observed in females, differences were not statistically different in this group. Perhaps, a reason that can explain, at least partially, these differences between males and females is the exercise-induced stress levels. Limitations in female leg muscle strength as opposed to cardiovascular capacity could limit their performance on the cycle ergometer test and explain the differences in the biological and physiological markers observed in this study. However, further research would be needed to elucidate this question. For instance, a previous study did not find an increase in salivary pH in a combined group of males and females (21). However, these authors did not take into account gender differences, and they measured salivary pH much earlier after exercise (5 min vs 20 min), which may reflect the different kinetics of this metabolite.

Another interesting finding of this study was the lack of changes in salivary nitrite levels as well as the oral nitrate-reducing capacity after exercise. It has been suggested that the oral nitrate/nitrite pathway is enhanced under acidic conditions (2), but in spite of the low salivary pH and higher lactate

concentrations observed after exercise, this did not induce any change on salivary nitrate and nitrite. It is possible that these variations on the oral pH balance were too small to stimulate the nitrate/nitrite reduction response compared to the changes that have been observed in the stomach under much more acidic conditions (22).

We also compared the level of agreement between salivary strips and a standard laboratory assay in order to assess salivary nitrite concentration. A stronger correlation was found between pre-exercise measurements compared to post-exercise measurements. However, the level of agreement of both correlations (pre and post-exercise) was much lower than values (r= 0.76 and r= 0.59) reported by other two previous studies (23, 24). Perhaps, this discrepancy may be explained by differences in the population and methodology of studies. For instance, Modi et al (23) recruited twenty overweight and obese individuals with an average BMI of 34, and showing low levels of salivary nitrite compared to our participants. Another study by McDonagh et al (24) found a significant correlation between laboratory measurements and the salivary strip indicators following an increase of salivary nitrite due to the ingestion of beetroot at different doses in 10 subjects. This could provide high readings that are much easier to compare with reference values from the salivary strips than low readings. However, from a statistical viewpoint the current study was much more powerful than previous studies by Modi et al (23) and McDonagh et al (24) as measurements were taken from 50 individuals compared to 20 and 10, respectively.

This study has some limitations that are important to highlight. For instance, a direct screening to assess oral health was not performed. While participants completed a medical questionnaire to assess their medical and oral health before the start of the study, this did not exclude participants carrying some degree of gum disease. It has been estimated that prevalence of gum disease in adults living in the UK is over 45% (25). This is relevant since periodontal disease can seriously disrupt the equilibrium of the oral ecosystem (26), and can also increase the levels of salivary nitrate and nitrite due to immune-mediated inflammatory response (27). Thus, future studies should examine the oral health of participants more in depth. Additionally, we did not analyse the salivary flow rate and salivary buffering capacity. It may be interesting in future studies to assess whether a higher salivary flow rate after exercise is a key mechanism for increasing the availability of circulatory nitrite. Furthermore, we did not assess the diversity of the oral microbiome due to funding constraints and because this question was not originally planned. Last but not least, it would be interesting to assess other non-

invasive cardiovascular parameters such as blood pressure and flow-mediated arterial dilation. The questions arising from the study are shaping our current research.

Conclusion

This is the first study to reveal that the nitrate-reducing activity of oral bacteria is related to the aerobic exercise capacity in healthy humans. Further research is needed to investigate more in depth the effect of exercise on the activity and diversity of the oral microbiome as well as inherited factors that can also play a key role on the oral ecosystem.

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Figures & tables

Figure 1: Salivary concentration of lactate and in males (*n* = 28), females (*n* = 22) and in all (*n* = 50) the participants together before and 20 min after exercise (mean ± SD).

Figure 2: Salivary concentration of nitrate in males (*n* = 28) and females (*n* = 22) as well as nitrate reducing capacity (*n* males = 15; *n* females = 10) before and after exercise (mean ± SD).

Figure 3: Spearman's rank correlation between the oral nitrate-reducing capacity of bacteria and maximum power (A) and aerobic capacity (B) in males and females (*n* = 25).

Table 1: Main characteristics of the participants (mean ± SD).

	Males			Females			All
	Without ONRC	With ONRC	Overall	Without ONRC	With ONRC	Overall	
Participants (*n*)	13	15	28	12	10	22	**50**
Age (y/o)	43.9 ± 14.2	40.6 ± 15.4	38.9 ± 14.8	38.8 ± 13.2	29.3 ± 10.7	38.1 ± 13.9*	**38.8 ± 14.3**
Body mass (kg)	76.0 ± 15.1	76.6 ± 9.3	72.1 ± 13.4	62.0 ± 9.5	59.6 ± 7.8	66.8 ± 13.6*	**69.7 ± 13.2**
BMI	23.3 ± 2.7	23.7 ± 3.5	23.0 ± 3.0	22.3 ± 2.7	21.6 ± 2.4	22.5 ± 2.6	**22.8 ± 2.9**
Body fat (%)	16.1 ± 4.8	16.4 ± 5.4	17.9 ± 5.8	25.7 ± 6.0	22.5 ± 5.5	21.5 ± 6.3*	**19.8 ± 6.7**
VO_{2peak} (mL/kg/min)	50.7 ± 10.7	52.7 ± 12.7	49.3 ± 11.5	42.2 ± 9.2	42.2 ± 8.1	42.7 ± 9.0*	**47.6 ± 11.4**
W_{peak} (W/kg)	4.5 ± 1.0	4.4 ± 1.1	4.3 ± 1.1	3.8 ± 0.9	3.6 ± 0.8	4.0 ± 0.9*	**4.2 ± 1.0**
Salivary NO^3 (µM/L)	189 ± 255	225 ± 234	225 ± 278	360 ± 355	154 ± 106	223 ± 281	**225 ± 264**
Salivary NO^2 (µM/L)	142 ± 110	74 ± 63	100 ± 88	154 ± 106	78 ± 69	128 ± 101	**113 ± 95**
ONRC (nitrite µM)	-	275 ± 138		-	231 ± 143		**257 ± 138**
Salivary glucose (µM/L)	0.04 ± 0.03	0.04 ± 0.03	0.03 ± 0.03	0.03 ± 0.02	0.02 ± 0.01	0.03 ± 0.02	**0.03 ± 0.03**
Salivary lactate (µM/L)	0.21 ± 0.20	0.18 ± 0.13	0.17 ± 0.15	0.13 ± 0.09	0.11 ± 0.09	0.16 ± 0.15	**0.17 ± 0.14**
Salivary pH	7.41 ± 0.37	7.25 ± 0.31	7.26 ± 0.33	7.34 ± 0.46	7.07 ± 0.23	7.29 ± 0.39	**7.28 ± 0.36**

VO_{2peak}: maximum oxygen consumption; W_{peak}: peak power in watts; NO^3: nitrate; NO^2: nitrite; ONRC: Oral-nitrate reducing capacity; * Statistical difference ($P < 0.05$)

Table 2: Correlations between salivary nitrite estimated with salivary strips and laboratory measurements using high-performance liquid chromatography (HPLC) system (mean ± SD).

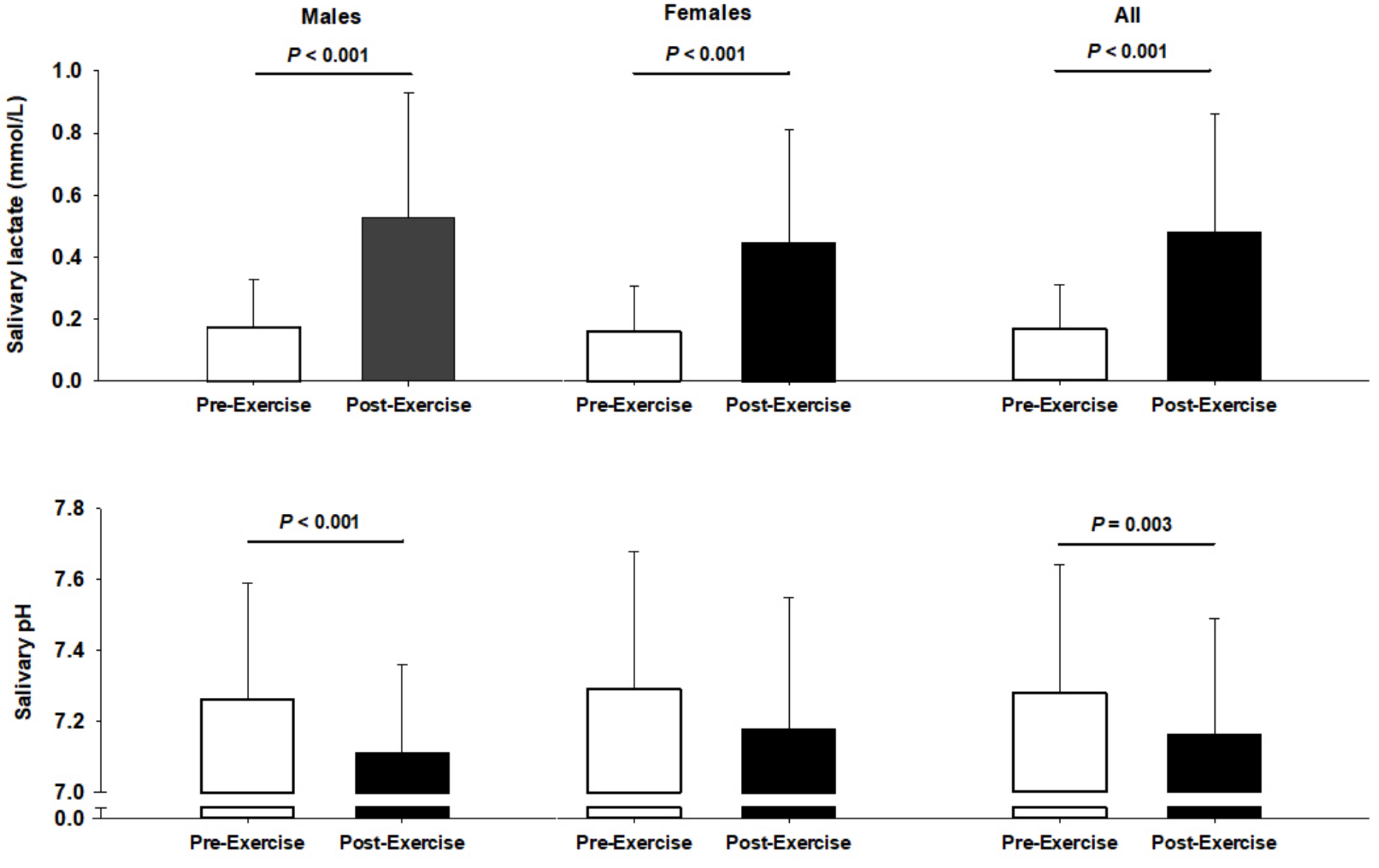
	Salivary Nitrite HPLC Pre-exercise	**Salivary Nitrite HPLC Post-exercise**
***n* = 50 (28 M; 22 F)**		
Salivary Nitrite (Berkeley Strips)	0.43 (0.002)*	0.32 (0.02) *

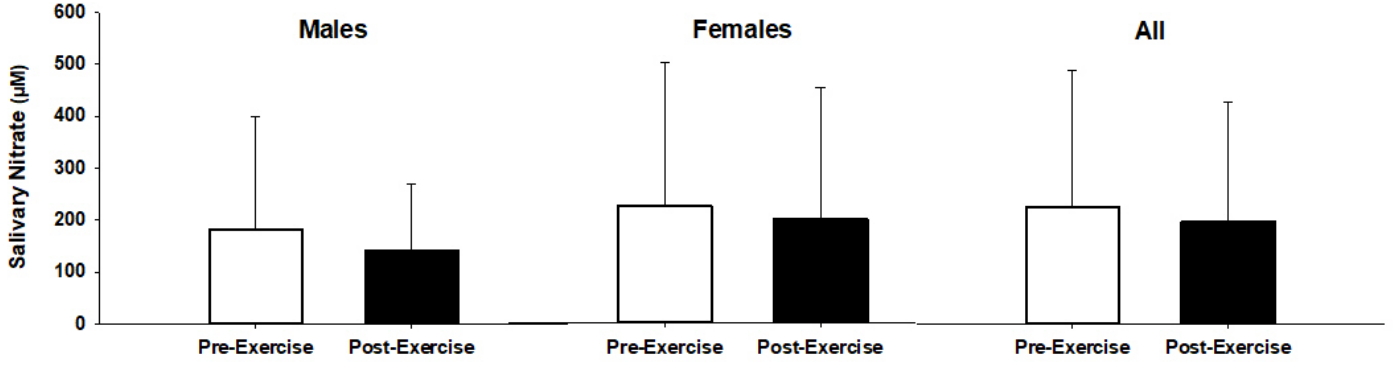
* Statistical difference ($P < 0.05$)

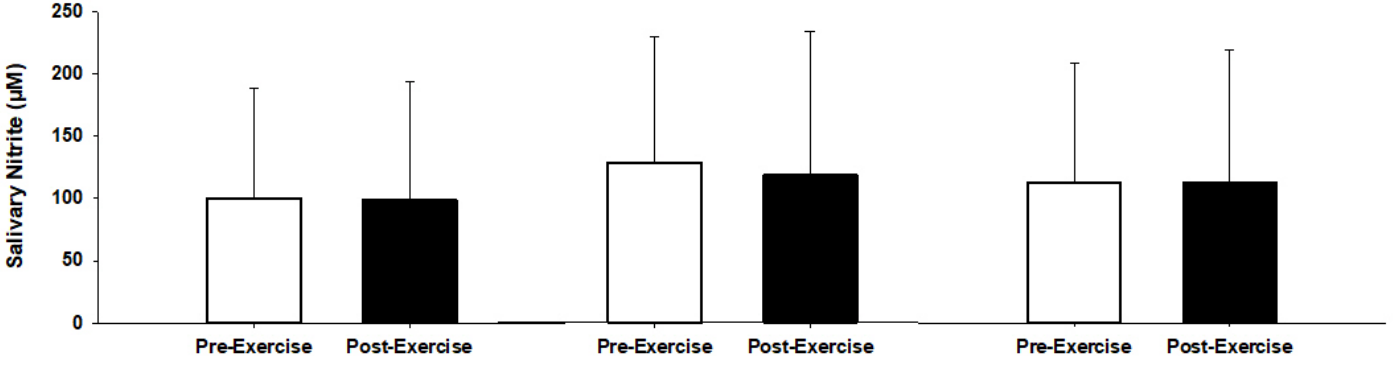
Table 3: Correlations between salivary markers (nitrate [NO^3]; nitrite [NO^2]; lactate; pH; and the oral nitrate-reducing capacity [ONRC]) and aerobic exercise performance parameters in males and females (mean ± SD).

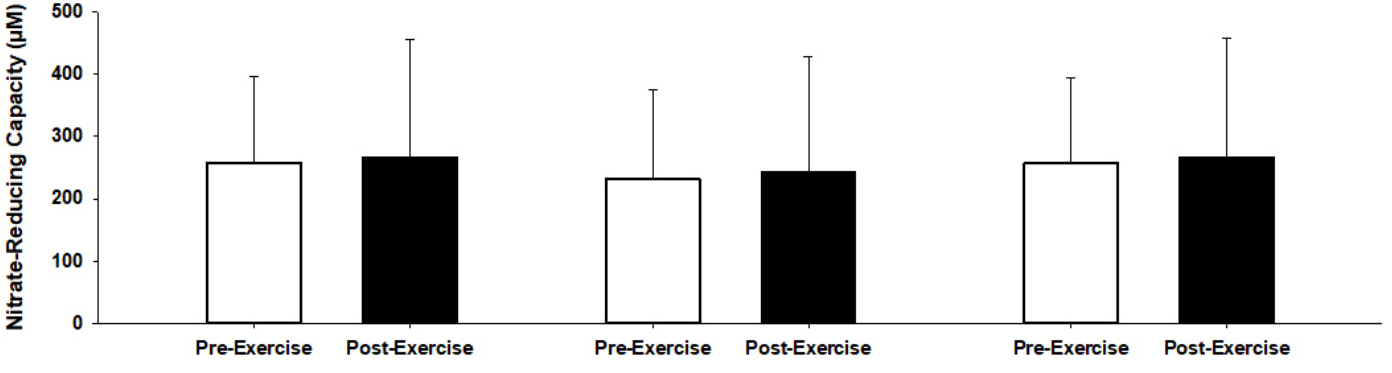
	Males (*n* = 28)				**Females** (*n* = 22)				**All** (*n* = 50)			
	Pre-Exercise		**Post-Exercise**		**Pre-Exercise**		**Post-Exercise**		**Pre-Exercise**		**Post-Exercise**	
	Power (W/kg) r_s (*p*)	**VO_{2peak} (mL/Kg/min)** r_s (*p*)	**Power (W/kg)** r_s (*p*)	**VO_{2peak} (mL/Kg/min)** r_s (*p*)	**Power (W/kg)** r_s (*p*)	**VO_{2peak} (mL/Kg/min)** r_s (*p*)	**Power (W/kg)** r_s (*p*)	**VO_{2peak} (mL/Kg/min)** r_s (*p*)	**Power (W/kg)** r_s (*p*)	**VO_{2peak} (mL/Kg/min)** r_s (*p*)	**Power (W/kg)** r_s (*p*)	**VO_{2peak} (mL/Kg/min)** r_s (*p*)
Salivary NO^3	0.16 (0.41)	0.15 (0.45)	0.07 (0.78)	0.05 (0.81)	0.38 (0.08)	0.19 (0.40)	0.37 (0.09)	0.19 (0.41)	0.18 (0.21)	0.11 (0.46)	0.09 (0.56)	0.01 (0.95)
Salivary NO^2	0.22 (0.27)	0.21 (0.28)	0.09 (0.65)	0.12 (0.55)	0.26 (0.24)	0.16 (0.49)	0.44 (0.04)*	0.31 (0.16)	0.20 (0.17)	0.16 (0.28)	0.19 (0.18)	0.18 (0.22)
Salivary lactate	0.19 (0.34)	0.14 (0.48)	0.02 (0.93)	-0.06 (0.75)	0.40 (0.07)	0.21 (0.35)	0.41 (0.06)	0.33 (0.14)	0.33 (0.02)*	0.26 (0.07)	0.19 (0.20)	0.14 (0.34)
Salivary pH	0.03 (0.86)	-0.90 (0.66)	0.21 (0.29)	0.15 (0.46)	0.12 (0.61)	0.19 (0.41)	0.11 (0.64)	0.14 (0.52)	0.04 (0.81)	-0.01 (0.99)	0.04 (0.80)	-0.01 (0.99)
	(*n* = 15)				(*n* = 10)				(*n* = 25)			
ONRC	0.64 (0.01)*	0.54 (0.04)*	0.64 (0.01)*	0.47 (0.08)	0.50 (0.14)	0.43 (0.22)	0.41 (0.24)	0.51 (0.13)	0.64 (0.001)*	0.54 (0.005)*	0.63 (0.001)*	0.54 (0.003)*

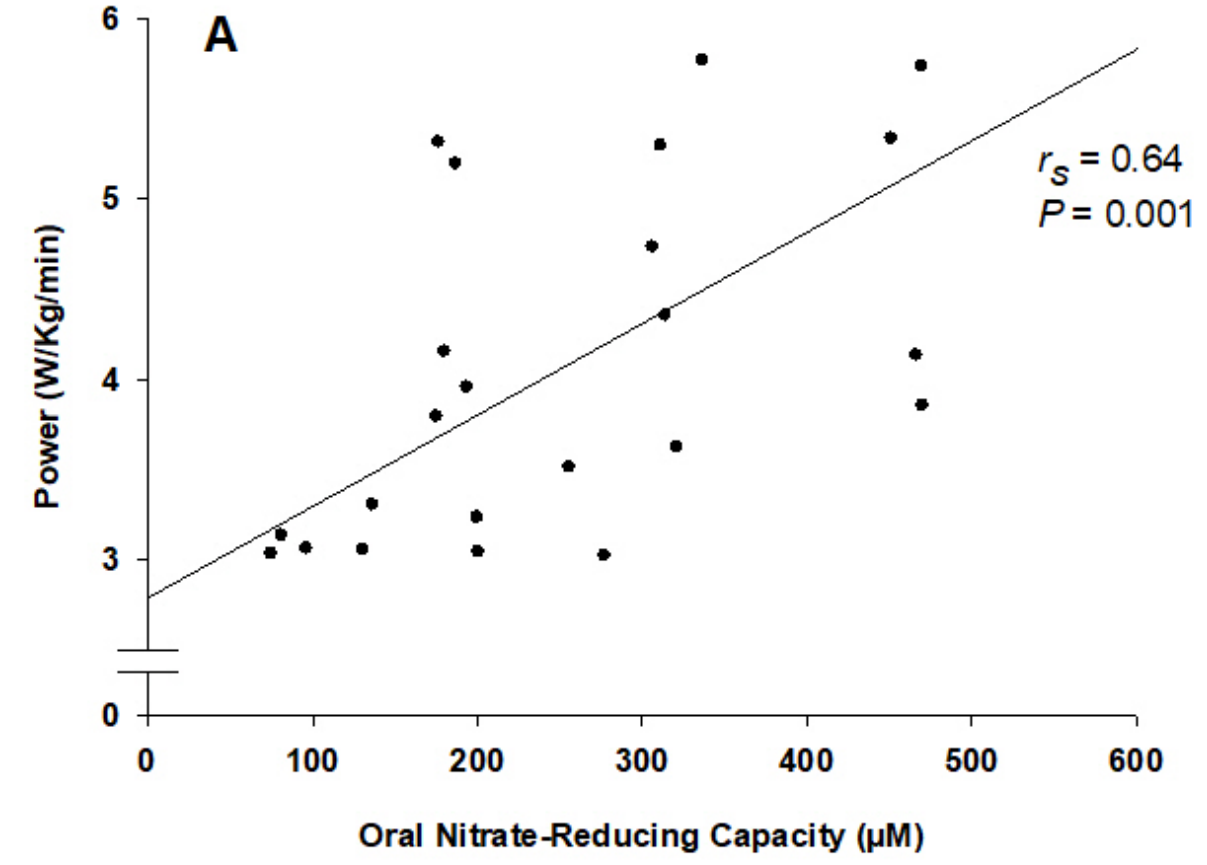
* Statistical difference ($P < 0.05$)

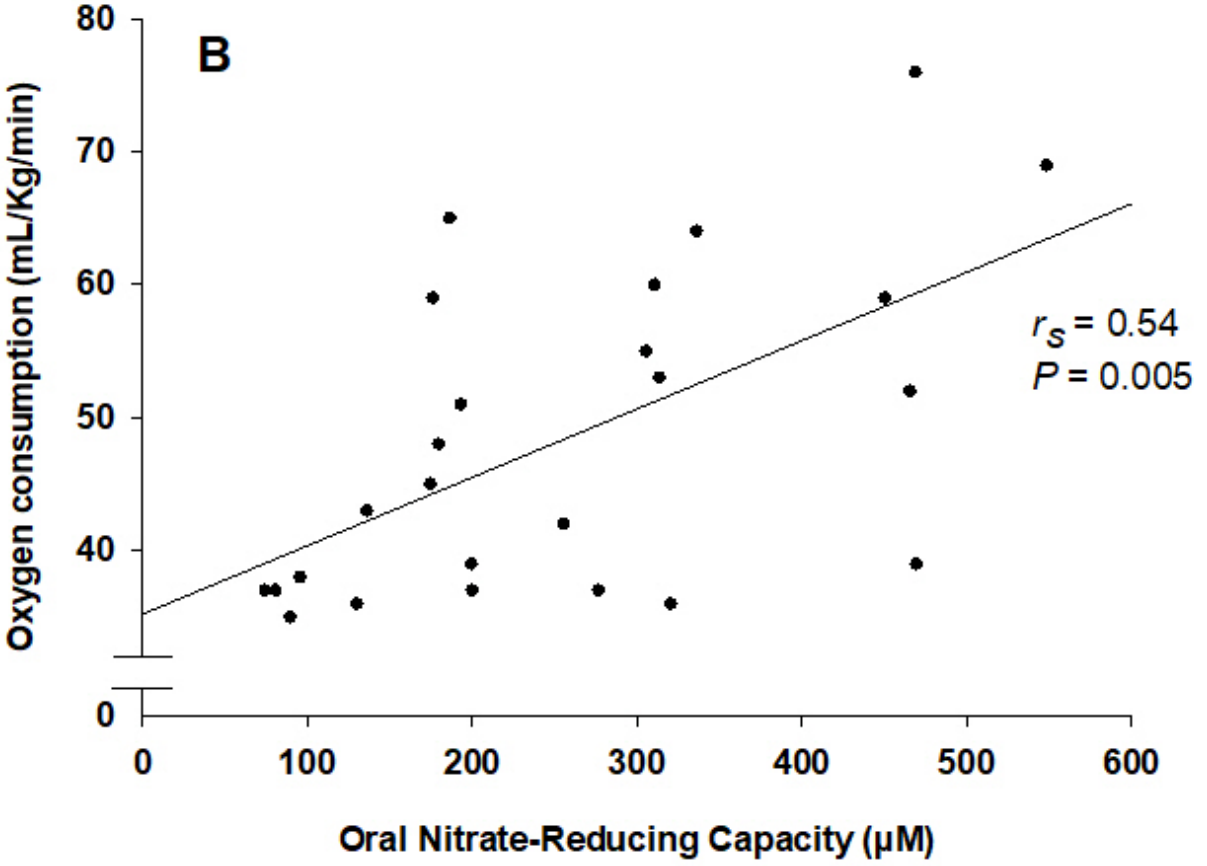

Males
Females
All
P < 0.001
P < 0.001
P < 0.001
Salivary lactate (mmol/L)
1.0
0.8
0.6
0.4
0.2
0.0
Pre-Exercise
Post-Exercise
Pre-Exercise
Post-Exercise
Pre-Exercise
Post-Exercise
P < 0.001
P = 0.003
Salivary pH
7.8
7.6
7.4
7.2
7.0
0.0
Pre-Exercise
Post-Exercise
Pre-Exercise
Post-Exercise
Pre-Exercise
Post-Exercise


Males
Females
All
Salivary Nitrate (µM)
600
500
400
300
200
100
0
Pre-Exercise
Post-Exercise
Pre-Exercise
Post-Exercise
Pre-Exercise
Post-Exercise


Salivary Nitrite (µM)
250
200
150
100
50
0
Pre-Exercise
Post-Exercise
Pre-Exercise
Post-Exercise
Pre-Exercise
Post-Exercise


Nitrate-Reducing Capacity (µM)
500
400
300
200
100
0
Pre-Exercise
Post-Exercise
Pre-Exercise
Post-Exercise
Pre-Exercise
Post-Exercise


A
Power (W/Kg/min)
6
5
4
3
0
0
100
200
300
400
500
600
Oral Nitrate-Reducing Capacity (μM)
r_S = 0.64
P = 0.001


B
Oxygen consumption (mL/Kg/min)
80
70
60
50
40
0
0
100
200
300
400
500
600
Oral Nitrate-Reducing Capacity (μM)
r_S = 0.54
P = 0.005