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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;  $38.8 \pm 14.3$  years/old; BMI=  $22.8 \pm 3.9$ ) performed a graded exercise test on a cycle ergometer to assess their maximum aerobic capacity ( $VO_{2peak}$ ). 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:  $7.28 \pm 0.361$ ; post-exercise:  $7.16 \pm 0.33$ ;  $P = 0.003$ ) accompanied by an increase of salivary lactate (pre:  $0.17 \pm 0.14$  mmol/L; post-exercise:  $0.48 \pm 0.38$ ;  $P < 0.001$ ) 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.

<b>Keywords</b>	nitrate, nitrite, exercise, oral bacteria
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13<sup>th</sup> 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,

A handwritten signature in black ink, appearing to read 'Raul Bescos Garcia', written in a cursive style.

Dr. Raul Bescos Garcia  
Faculty of Health & Human Sciences  
University of Plymouth  
Plymouth  
United Kingdom

# **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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1 **Abstract**

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

11 Fifty individuals (22 females and 28 males;  $38.8 \pm 14.3$  years/old; BMI=  $22.8 \pm 3.9$ ) performed a graded  
12 exercise test on a cycle ergometer to assess their maximum aerobic capacity ( $VO_{2peak}$ ). Unstimulated  
13 salivary samples were taken before and 20 min after exercise to measure nitrate/nitrite, pH and  
14 lactate. The nitrate-reducing capacity of oral bacteria was also assessed in 25 subjects before and after  
15 exercise.

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

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

25

26 **Introduction**

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

33

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

54

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

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

65

## 66 **Materials and methods**

### 67 **Participants**

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

79

### 80 **Protocol**

81 Participants were required to report to the laboratory on one occasion in a fully rested state having  
82 not participated in strenuous exercise in the preceding 24 hours. They were encouraged to refrain  
83 from eating at least 3 hours before the exercise test. Food rich in nitrate and caffeine were restricted  
84 24 and 12 hours before the trial, respectively. On their arrival at the laboratory, body fat percentage  
85 and body mass were measured using a body composition analyser (Tanita, TBF-300MA, Tokyo, Japan).  
86 Height was measured using a stadiometer (Seca, Hamburg, Germany). Then, salivary nitrite was  
87 measured with a test strip (Nitric Oxide test strips, Berkeley, Chicago, US) before a whole non-  
88 stimulated saliva sample (~3 mL) was collected to analyse nitrate, nitrite, pH and lactate. Following  
89 this, the nitrate-reducing capacity of oral bacteria was measured in 25 participants (Table 1). They  
90 rinsed their mouth with a 10 mL solution of distilled water containing 80  $\mu\text{mol}$  of sodium nitrate  
91 (Sodium Nitrate, Fisher Chemical, UK) for 5 minutes. The solution was collected in sterile tubes. All

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

94

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

107

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

113

## 114 **Analyses**

### 115 **Salivary nitrate and nitrite**

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

121 Change in colour of salivary strips (Nitric Oxide test strips, Berkeley, Chicago, US) was categorised on  
122 5 different levels according to the manufacturer's color chart.

123



124 **Salivary pH, lactate and glucose**

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

128

129 **Statistical analyses**

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

139

140 **Results**

141 **Baseline characteristics of study subjects**

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

147

-Table 1-

148

149 **Changes in salivary parameters between pre and post-exercise measurements**

150 A significant increase ( $P < 0.001$ ) in salivary lactate was observed in females (Pre:  $0.16 \pm 0.15$ ; Post:  
151  $0.45 \pm 0.37$ ;  $P < 0.001$ ) and males (Pre:  $0.17 \pm 0.15$ ; Post:  $0.53 \pm 0.40$ ;  $P < 0.001$ ) after exercise (Figure  
152 1). This response was associated with a significant reduction of salivary pH in males (Pre:  $7.26 \pm 0.33$ ;  
153 Post:  $7.11 \pm 0.25$ ;  $P < 0.001$ ). Although a similar trend was observed in females, the reduction in salivary  
154 pH was not statistically significant (Pre:  $7.29 \pm 0.39$ ; Post:  $7.18 \pm 0.37$ ;  $P = 0.64$ ). However, when all

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

157

158 In contrast, the concentration of salivary nitrate, nitrite and the nitrate-reducing capacity of oral  
159 bacteria did not differ ( $P > 0.05$ ) between pre and post-exercise measurements in either group (Figure  
160 2).

161 **-Figure 1-**

162 **-Figure 2-**

163

164

### 165 **Association between salivary strips and HPLC measurements on nitrite concentration**

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

170 **-Table 2-**

171

### 172 **Associations between salivary markers and exercise capacity**

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

178 **-Table 3-**

179

180 The nitrate-reducing capacity of oral bacteria was significantly associated with power ( $r_s = 0.64$ ,  $P =$   
181  $0.01$ ) and  $VO_{2peak}$  ( $r_s = 0.54$ ,  $P = 0.04$ ) in males before exercise (Table 3). While the association between  
182 the nitrate-reducing capacity and power did not change after exercise ( $r_s = 0.64$ ,  $P = 0.01$ ), a lower and  
183 non-significant correlation was observed with the  $VO_{2peak}$  ( $r_s = 0.47$ ,  $P = 0.08$ ). In females, the oral  
184 nitrate-reducing capacity was not associated with power or  $VO_{2peak}$  before and after exercise (Table  
185 3). On the other hand, a positive and significant association was found between the nitrate-reducing  
186 capacity of oral bacteria and power and  $VO_{2peak}$  before and after exercise when all the participants  
187 (males and females) were analysed together (Figure 3).

-Figure 3-

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**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

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

225

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

238

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

250

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

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

258

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

273

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

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

### 288 **Conclusion**

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

293

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365 **Figures & tables**

366

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

369

370

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

373

374

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

377

378 **Table 1:** Main characteristics of the participants (mean  $\pm$  SD).

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

379 VO<sub>2peak</sub>: maximum oxygen consumption; W<sub>peak</sub>: peak power in watts; NO<sup>3</sup>: nitrate; NO<sup>2</sup>: nitrite; ONRC: Oral-nitrate reducing  
 380 capacity; \* Statistical difference ( $P < 0.05$ )  
 381

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

	Salivary Nitrite HPLC Pre-exercise	Salivary Nitrite HPLC Post-exercise
<i>n</i> = 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<sup>3</sup>]; nitrite [NO<sup>2</sup>]; 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) <i>r<sub>s</sub></i> ( <i>p</i> )	VO <sub>2peak</sub> (mL/Kg/min) <i>r<sub>s</sub></i> ( <i>p</i> )	Power (W/kg) <i>r<sub>s</sub></i> ( <i>p</i> )	VO <sub>2peak</sub> (mL/Kg/min) <i>r<sub>s</sub></i> ( <i>p</i> )	Power (W/kg) <i>r<sub>s</sub></i> ( <i>p</i> )	VO <sub>2peak</sub> (mL/Kg/min) <i>r<sub>s</sub></i> ( <i>p</i> )	Power (W/kg) <i>r<sub>s</sub></i> ( <i>p</i> )	VO <sub>2peak</sub> (mL/Kg/min) <i>r<sub>s</sub></i> ( <i>p</i> )	Power (W/kg) <i>r<sub>s</sub></i> ( <i>p</i> )	VO <sub>2peak</sub> (mL/Kg/min) <i>r<sub>s</sub></i> ( <i>p</i> )	Power (W/kg) <i>r<sub>s</sub></i> ( <i>p</i> )	VO <sub>2peak</sub> (mL/Kg/min) <i>r<sub>s</sub></i> ( <i>p</i> )
Salivary NO <sup>3</sup>	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 <sup>2</sup>	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 ( $P < 0.05$ )





