HHS Public Access

Author manuscript

Nitric Oxide. Author manuscript; available in PMC 2016 May 01.

Published in final edited form as:

Nitric Oxide. 2015 May 1; 47: 85–90. doi:10.1016/j.niox.2015.04.003.

The Relationship between Plasma and Salivary NO_x

William H. Clodfelter[a,b], **Swati Basu**[b,c], **Crystal Bolden**[d], **Patricia C. Dos Santos**[a,b], **S. Bruce King**[a,b,*], and **Daniel B. Kim-Shapiro**[b,c,*]

[a]Department of Chemistry, Wake Forest University, Winston-Salem, NC 27109, USA

[b]Translational Science Center, Reynolda Campus, Wake Forest University, NC 27109, USA

[c]Department of Physics, Wake Forest University, Winston-Salem, NC 27109, USA

[d]Department of Molecular Medicine and Translational Science, Wake Forest University School of Medicine, Winston Salem, NC 27157, USA

Abstract

Several studies have shown that fasting plasma nitrite (NO_2^-) is an indicator of endothelial nitric oxide synthase (NOS) activity while plasma nitrate (NO_3^-) or the sum of NO_2^- and NO_3^- (NO_x) do not reflect NOS function. Plasma NO_2^- can also be elevated through dietary NO_3^- where the NO_3^- is partially reduced to NO_2^- by oral bacteria and enters the plasma through the digestive system. NO_3^- is taken up from plasma by salivary glands and the cycle repeats itself. Thus, one may propose that salivary NO_2^- is an indicator of plasma NO_2^- and consequently of NO production. Many brands of nitric oxide (NO) saliva test strips have been developed that suggest that their product is indicative of circulatory NO availability. However, data supporting a relationship between salivary and plasma NO_2^- or NO bioavailability is lacking. Here we have measured basal salivary and plasma NO_2^- and NO_3^- to determine if any correlation exists between these in 13 adult volunteers. We found no significant correlation between basal salivary and plasma NO_2^-. Also no correlation exists between salivary NO_3^- and plasma NO_2^-. However, we did see a correlation between salivary NO_3^- and plasma NO_3^-, and between salivary NO_2^- and plasma NO_3^-. In a separate study, we compared the efficiency of salivary NO_3^- reduction with the efficacy of increasing plasma NO_3^- and NO_2^- after drinking beet juice, a high NO_3^--containing beverage, in 10 adult volunteers. No significant correlation was observed between the *ex vivo* salivary reduction of NO_3^- to NO_2^- and plasma increases in NO_3^- or NO_2^-. These results suggest that measures of salivary NO_3^-, NO_2^- or NO_x are not good indicators of endothelial function. In addition, the efficiency of saliva to reduce NO_3^- to NO_2^- ex-vivo does not demonstrate one's ability to increase plasma NO_2^- following consumption of dietary NO_3^-.

[*]These authors share senior authorship, address correspondence to: S. Bruce King, Department of Chemistry, Wake Forest University, Winston-Salem, NC 27109, kingsb@wfu.edu, or Daniel Kim-Shapiro, Department of Physics, Wake Forest University, Winston-Salem, NC 27109, shapiro@wfu.edu.

Keywords

Nitrite; nitrate; nitric oxide bioavailability

1. Introduction

Determining a diagnostic marker of endogenous NO bioavailability has been a major topic of research, one which would have clinical implications for monitoring cardiovascular disease, metabolic syndrome, and other conditions [1; 2; 3; 4]. Endothelial dysfunction has been noted as a key event in early atherosclerosis. Due to defective synthesis, decreased levels of endothelium-derived NO characterize endothelial dysfunction [5]. In addition, increased scavenging of NO by oxygen radicals results in low NO bioavailability associated with endothelial dysfunction [6; 7]. Individuals with endothelial dysfunction show a decrease in flow-mediated dilation (FMD) and an increase of intima media thickness (IMT), both representative of atherosclerosis [8; 9; 10; 11]. Given the cost of procedures to measure FMD and IMT, it would be useful to establish a simple blood test to diagnose endothelial dysfunction or low NO bioavailability due to other conditions. The Kelm and Moncada labs have demonstrated that the majority of plasma NO_2^- is derived from constitutive NOS-activity [12; 13]. Observations have shown that upon regional nitric oxide synthase (NOS) inhibition in forearm circulation, vascular resistance increases linearly as plasma NO_2^- levels decrease, thereby establishing plasma NO_2^- as a potential measure of endothelial function [12].

In some of the same studies, plasma NO_3^- was ruled out as an indicator of NOS function. The Moncada lab showed that only 16% of isotopic L-arginine infused into circulation was represented in plasma NO_3^- levels versus 90% of plasma NO_2^- [13]. The Kelm laboratory demonstrated no significant change in plasma NO_3^- in mammals with inhibition of NOS activity [12]. These data are somewhat expected since plasma NO_3^- has many NOS-independent factors which can drastically change the basal levels such as dietary NO_3^- intake, denitrifying liver enzymes, and renal function [14; 15]. Interestingly, Hibbs and colleagues showed that inducible-nitric oxide synthase (iNOS) is one of the main contributors to circulating NO_3^- due to the increase of NO production after the addition of the cytokine-IL2 [16]. However, the general consensus is that, under most conditions, plasma NO_3^- does not reflect NOS function or NO bioavailability. In addition, there are even some investigators who suggest that plasma NO_2^- does not accurately reflect eNOS function and NO bioavailability [17].

In recent years, dietary NO_3^- has become a known contributor to the pool of bioavailable NO [18]. It is known that dietary NO_3^- is reduced in the oral cavity by tongue flora, specifically by *Actinomyces* and *Veilonella* species [19; 20]. Once ingested, NO_2^- is non-enzymatically reduced to NO in the gastric acidic milieu [21]. NO_3^- and remaining NO_2^- are rapidly absorbed in the small intestine. Plasma NO_2^- can then be reduced to NO by various mechanisms [18; 22]. Though most of the circulating NO_3^- is excreted in urine, approximately 25% is extracted by the salivary glands and recycled through the enterosalivary circulation [23]. Complementary to endogenous NO production, this cycle of

dietary NO_3^- being converted to NO in physiology is referred to as the nitrate-nitrite-nitric oxide pathway [18].

Through this physiological pathway, it has been shown that dietary NO_3^- will increase plasma NO_3^- and NO_2^-. In addition, dietary NO_3^- has been shown to lower blood pressure with short and long term effects, be vasoprotective and reduce platelet aggregation, along with having acute effects on cerebral blood flow and an increase in exercise tolerance and performance [18; 24; 25; 26; 27; 28; 29; 30]. Daily dietary NO_3^- ingestion also improves endothelial function and vascular stiffness in hypercholesterolemia [31; 32; 33; 34].

As evidence suggests that basal plasma NO_2^- levels reflect NOS function and bioavailable NO, these measurements may have clinical utility. However, based on the nitrate-nitrite-NO cycle, one may also suggest that salivary NO_2^- could have the same utility, as recently pointed out [1]. Indeed, commercially available products exist that measure salivary NO_2^- and claim to report NO bioavailability. However, until now, no published studies have shown a positive correlation between basal plasma NO_2^- and salivary NO_2^- levels. Thus, in this work, we sought to investigate the basal levels of plasma and salivary NO_2^- and NO_3^-.

When studying increased plasma NO_2^- after a high NO_3^- load, Lundberg et al. observed attenuation after using an antibacterial mouthwash [35], suggesting saliva's conversion from NO_3^- to NO_2^- greatly affects plasma NO_2^-. Consumption of high NO_3^--containing food or drinks increases plasma NO_3^-, NO_2^-- and thus NO_x. However, many studies have observed a significant variation with the increase in plasma levels among individuals [13; 24; 25; 28; 35]. It appears that some individuals are poor or non-responders with respect to dietary NO_3^- interventions as measured by increases in plasma NO_2^-[24; 27; 36; 37]. It would be useful to easily determine individuals' efficacy at converting oral NO_3^- to plasma NO_2^-. In this study we hypothesized that saliva would reflect ability to convert NO_3^- to NO_2^- in the oral cavity and that this ability would correlate with an individual's dietary NO_3^- to plasma NO_2^- conversion efficacy. Thus we conducted a second study where we examined both ex-vivo conversion of NO_3^- to NO_2^- in saliva as well as *in vivo* conversion of dietary NO_3^- to plasma NO_2^-.

2. Methods

2.1 Study Design

All human subjects use was approved by an internal review board following federal and institutional guidelines. For the basal levels study, 13 volunteers (8 male and 5 female) participated between the age of 18 and 80 years old. Volunteers reported to the lab at 9:00 am on the day of their participation. Individuals did not eat or drink within two hours of their participation. If the volunteers had eaten any food the morning of the sampling, they were told to avoid any high NO_3^- foods (ex. spinach, beets, lettuce, and other green leafy vegetables). In addition, volunteers did not use mouthwash but were permitted to brush their teeth. Upon arrival, blood was drawn from each volunteer from an antecubital vein and collected in a 4 mL lithium heparin vial. Simultaneously, volunteers expectorated 5 mL of saliva which was collected in a sterilized 50 mL Corning tube.

The beet juice study was ancillary to a larger study aimed at investigating potential additional benefits of beet juice combined with supervised exercise compared to supervised exercise alone. The larger study provided a great opportunity for the ancillary one discussed here to compare in vivo conversion of oral nitrate to plasma nitrite and ex vivo salivary conversion efficiency. 10 participants (5 male and 5 female) above the age of 55 were recruited. All recruits consumed a bottle of concentrated beet juice (Beet it Sport shot, 500 mg NO_3^-) a day for 6 weeks. On the first day of weeks 1, 3 and 6 participation, each recruit came in for sampling. As described for the study above, volunteers did not use mouthwash or eat any high NO_3^- foods but were permitted to brush their teeth. Blood was drawn before and 1 hour after beet juice consumption. Immediately before the blood draw, participants expectorated a 5 mL saliva sample into a 50 mL sterilized Corning tube. One plasma sample was excluded due to hemolysis during sample preparation. In addition, two anaerobic saliva samples were excluded due to their having dried out during the deoxygenation procedure.

2.2 Measurements

Since salivary NO strips claim to be indicative of physiological NO, we sought to determine what these strips actually test in saliva. Two brands of NO test strips, Nitric Oxide Test Strips (Berkeley Test) and Nitric Oxide Indicator Strips (Neogenis; Austin, TX), were placed in solutions of $NaNO_3^-$ and $NaNO_2^-$. Since the strips have a colorimetric indicating tip, concentrations were varied in order to darken the strip with more reactant.

Similar measuring techniques were used for both basal and beet juice studies. Blood was taken from an antecubital vein and collected in a 4 mL Lithium heparin vial. The tubes were immediately centrifuged at 11,500 g for 2 min. The supernatant plasma was removed and immediately frozen on dry ice in aliquots of ~0.4 mL of plasma and stored in a −80 °C freezer. Plasma NO_3^- and NO_2^- were determined and labeled “basal levels.” For the beet juice studies, plasma NO_2^- levels were determined in the before- and 1 hr after-beet juice consumption timepoints. The difference was reported as the Δ plasma NO_2^-.

Plasma NO_3^- and NO_2^- levels were measured using an HPLC-based Eicom NOx Analyzer, model ENO-20 according to instructions of the manufacturer. For all measurements, standard curves were obtained and used for quantitative measurements.

For the basal levels study, each tube of saliva was centrifuged at 11,500 g for 5 minutes to exclude a large pellet of bacteria. The supernatant salivary matrix was removed and immediately frozen on dry ice in aliquots of ~1.0 mL of saliva until time of analysis. Freezing saliva is a method tested and employed by our lab which maintains the integrity of the sample after exclusion of the bacterial pellet[38; 39]. Before instrumental analysis, saliva was thawed and mixed 1:1 by volume with methanol for deproteination (a method tested which maintains NO_3^- and NO_2^- but allows for a cleaner sample to eliminate possible syringe clogging of the HPLC system of the NO_x analyzer). Salivary NO_3^- and NO_2^- were determined and labeled “basal levels.”

For the beet juice study, each tube of saliva (5 mL) was immediately split into two samples, one designated for aerobic testing and the other for anaerobic testing. Both samples were placed in a round bottom flask and left to incubate in a 37 °C water bath. The anaerobic

sample was capped and flushed with argon for 1 hr to deoxygenate and induce anaerobic activity. After 1 hr of incubation, 10 mM $NaNO_3^-$ was added to each sample to mimic a high NO_3^- beverage. Starting with a 0 min time point, the NO_2^- produced was detected at 10 minute increments up to 90 minutes. The salivary rate (k) of NO_2^- production was calculated and reported in μM/min.

Salivary NO_3^- and NO_2^- levels were measured using chemiluminescence-based Nitric Oxide Analyzers (Sievers, Inc.) according to instructions of the manufacturer. For all measurements, standard curves were obtained and used for quantitative measurements.

2.3 Statistical Analysis

For the basal levels study, salivary NO_3^- and NO_2^- levels are reported as the mean value of three injections. Plasma NO_3^- and NO_2^- are reported as the sole value measured. For the beet juice study, the aerobic and anaerobic salivary rate of NO_2^- production was plotted against the change in plasma NO_2^-. A linear trendline was added to each plot to show relative correlation. Spearman correlation coefficients (r) were calculated to measure the linear correlation between the two variables plotted. An r value less than −0.5 or greater than 0.5 was considered significant. In addition, two-tailed p-values were calculated. A p-value less than 0.05 was considered significant.

3. Results

We first investigated sensitivity of oral commercial test strips to NO_2^- and NO_3^-. The salivary NO test strips reacted with $NaNO_2^-$ in solution, but not $NaNO_3^-$ (Fig 1). There was no colorimetric change when the strips were submerged in $NaNO_3^-$ solution of 1 and 10 mM concentrations. When the strips were submerged in $NaNO_2^-$, a very apparent colorimetric change was seen. No detectable reaction was observed using the low concentration of 10 μM $NaNO_2$. A light pink color was apparent at a concentration of 50 μM and the color darkened with increasing concentrations up to 10 mM $NaNO_2^-$.

As these test strips appear to measure salivary NO_2^-, then, assuming plasma NO_2^- reflects NO bioavailability, the strips would measure NO bioavailability as long as salivary NO_2^- correlates with plasma NO_2^-. Thus, we compared basal salivary NO_2^- to plasma NO_2^-. We found that salivary NO_2^- in volunteers ranged from 51 to 257 μM while salivary NO_3^- ranged from 9 to 681 μM. Plasma NO_2^- in volunteers ranged from 60 to 184 nM while plasma NO_3^- ranged from 19 to 61 μM. Importantly, plasma NO_2^- did not significantly correlate with salivary NO_2^- (Fig 2A, $p = 0.87$). Plasma NO_3^- significantly correlated with salivary NO_3^- (Fig 2B, $p = 0.05$). Plasma NO_3^- significantly correlated with salivary NO_2^- (Fig 2C, $p = 0.02$). Basal plasma NO_2^- did not correlate with basal salivary NO_3^- (Fig 2D, $p = 0.41$).

For the second study we investigated whether ex-vivo conversion of NO_3^- to NO_2^- in saliva correlates with *in vivo* conversion of dietary NO_3^- to plasma NO_2^-. Changes in plasma NO_2^- ranged from −270 to 740 nM. The average increase in plasma NO_2^- was 150 nM. The rates of salivary NO_2^- production ranged from 0 to 201 μM/min in aerobic saliva and 0 to 402 μM/min in anaerobic saliva. There was no correlation between the rate of aerobic

salivary NO_2^- production and the change in plasma NO_2^- (Fig 3A). The trendline R^2 value was 0.0240. The calculated *r* and p-values were 0.02 and 0.23, respectively. In addition, there was no correlation for the rate of anaerobic salivary NO_2^- production versus the change in plasma NO_2^- (Fig 3B). The trendline R^2 value was 0.0003. The calculated *r* and p-values were 0.08 and 0.76, respectively. Each plot includes all of the week 1, 3 and 6 time points for each individual. It is worth noting that there was no correlation when comparing only week 1, 3 and 6 time points individually, aerobic or anaerobic salivary reduction of NO_3^- (data not shown).

4. Discussion

We have investigated potential methods to assess individuals' basal NO bioavailability and a potential salivary-based method to assess the individuals' ability to convert dietary NO_3^- to plasma NO_2^-. Our major findings were (i) Nitric Oxide Test Strips indicate a relative level of NO_2^- in saliva, (ii) basal plasma and salivary NO_2^- do not correlate, and (iii) *ex vivo* conversion of salivary NO_3^- to NO_2^- is not an indicator of *in vivo* dietary NO_3^- conversion to plasma NO_2^-. We also found that plasma NO_3^- correlated with both salivary NO_3^- and NO_2^-. As expected, there was no correlation between plasma NO_2^- and salivary NO_3^-.

As the Kelm lab has demonstrated, plasma NO_2^- may be indicative of endothelial function [8]. If salivary NO_2^- reflected plasma NO_2^-, it could be an easy way to measure endothelial function, as suggested recently [1]. However, we found no relation between basal and salivary NO_2^- (Figure 2A). These data suggest that salivary NO_2^- would not be a valid way to measure endothelial function and NO bioavailability. In addition, as we found that commercially available test strips measure salivary NO_2^- (Figure 1), those strips are not likely to accurately assess NO bioavailability. It should be noted that we used the test strips in a setting where liquid was applied to them. Future work may consider whether other employments, such as putting the strip directly on the tongue, give different results.

Our study for basal levels was designed primarily to determine if physiological levels of salivary NO_2^- and plasma NO_2^- correlated. Without recently using mouthwash, consuming a high NO_3^- food or beverage, or medications, salivary and plasma NO_2^- in volunteers should reflect normal physiological levels. We observed no correlation between basal salivary and plasma nitrite levels. Interestingly, plasma NO_3^- correlated with both salivary NO_3^- and NO_2^- (Fig 2B & 2C). As noted by the Kelm laboratory, plasma NO_3^- is influenced by many NOS-independent factors such as dietary NO_3^- intake, saliva formation, and bacterial synthesis in the bowel [12]. The positive correlations between plasma NO_3^- and salivary NO_2^- and NO_3^- could be understood in terms of the nitrate-nitrite-NO cycle [18]. Salivary NO_3^- is converted to salivary NO_2^- by oral bacteria and thus, these levels correlate with each other [18; 19; 20; 22; 23]. NO_3^- enters the plasma and is taken back up into saliva [18; 22; 23]. Thus salivary and plasma levels of NO_3^- are linked. However, under basal conditions, plasma NO_2^- levels are largely due to eNOS function [40] and thus do not correlate with salivary NO_3^- or NO_2^-. Indeed, we did not see a correlation between plasma and salivary NO_2^- or NO_3^-.

To further rationalize the lack of a correlation between plasma and salivary NO_2^- levels, it is important to consider nitrogen oxide chemistry occurring during consumption and in the gut. As suggested by work looking at nitrogen oxide production in the oral cavity, it is likely that salivary NO_2^- is converted to further reduced nitrogen oxides such as NO, nitrous oxide, ammonia, and/or nitrogen gas [41; 42] and also reactive nitrogen oxide species [43] by bacteria which make up the human oral microbiome [44]. Additionally, the acidic milieu of the gastric lumen will likely affect the levels of plasma NO_2^- as evident in research showing increased NO levels after swallowing NO_2^- containing saliva [21; 22]. Thus, variations in factors that influence gastric pH and nitrogen oxide absorption as well as those that influence chemistry in the oral cavity could lead to additional variations in plasma NO_2^- that are not reflected in salivary NO_2^-.

The beet juice study was designed for exploring a correlation between in-vivo conversion of dietary NO_3^- to plasma NO_2^- and the efficacy of ex-vivo salivary NO_3^- conversion. Several labs have shown that dietary NO_3^- increases plasma NO_2^- substantially [23; 24; 25; 26]. However, the efficacy of this conversion varies dramatically among individuals, perhaps due to their microflora [13; 24; 25; 28; 35]. It would be useful to be able to easily assess individuals' ability to convert dietary NO_3^- to plasma NO_2^-. We hypothesized that the rate of ex-vivo salivary NO_3^- to NO_2^- conversion would serve this purpose. However, we saw no correlation between the *in vivo* efficacy of converting dietary NO_3^- to plasma NO_2^- and the ex-vivo conversion rate. This lack of correlation may be due to the bacteria in the saliva samples we collected not being representative of the oral bacteria that are mainly responsible for NO_3^- to NO_2^- conversion *in vivo*. These bacteria are thought to form biofilms[19; 20] so that saliva may not necessarily reflect their number, distribution, or reflect optimum conditions for NO_3^- reduction.

Possible limitations to this study should be acknowledged. For the basal and beet juice studies, the sample sizes were 13 and 10 respectively. A larger sample size might show stronger correlations. For both studies we also used *ex vivo* salivary samples. As mentioned we used expectorated saliva to assess salivary NO_3^- to NO_2^- conversion. Tongue scraping or swabbing or NO_3^- to NO_2^- conversion in the oral cavity itself (gargling with NO_3^- and then expectorating) might show better correlations with *in vivo* dietary NO_3^- to plasma NO_2^- conversion. Plasma samples in the beet juice study were taken before and 1-hr after beet juice consumption. We have previously seen that plasma NO_2^- levels are close to maximum one hour after consumption [24]. However, others [25; 26] have found that plasma NO_2^- does not reach a semi-steady maximum value until three hours after consumption so that may have been a better choice for sampling. It's possible that several time points (such as at 1, 2 and 3 hours after consumption) are needed to obtain the maximum plasma NO_x levels after a high NO_3^- food or beverage for all individuals.

5. Conclusions

Our results argue against use of salivary NO_2^- as a marker for NO bioavailability. In addition, the efficacy of ex-vivo NO_3^- to NO_2^- conversion cannot be used to assess individuals' ability to convert dietary NO_3^- to plasma NO_2^-.

Acknowledgments

This work was supported by NIH grant HL058091 and by the Translational Science Center of Wake Forest University.

Abbreviations

NOS	nitric oxide synthase
FMD	flow-mediated dilation
iNOS	inducible nitric oxide synthase
IMT	intima media thickness

References

1. Bryan NS. The potential use of salivary nitrite as a marker of NO status in humans. Nitric Oxide. 2015; 45:4–6. [PubMed: 25542504]
2. Silver JH. Nitrite and Nitric Oxide as Potential Diagnostic Markers in Acute Vascular Diseases. Journal of Neurology and Neurophysiology. 2011; S1
3. Bredt DS. Endogenous nitric oxide synthesis: Biological functions and pathophysiology. Free radical research. 1999; 31:577–596. [PubMed: 10630682]
4. Clini E, Volterrani M, Pagani M, Bianchi L, Porta R, Gile' LS, Giordano A, Ambrosino N. Endogenous nitric oxide in patients with chronic heart failure (CHF): relation to functional impairment and nitrate-containing therapies. International Journal of Cardiology. 2000; 73:123–130. [PubMed: 10817849]
5. Heitzer T, Schlinzig T, Krohn K, Meinertz T, Münzel T. Endothelial Dysfunction, Oxidative Stress, and Risk of Cardiovascular Events in Patients With Coronary Artery Disease. Circulation. 2001; 104:2673–2678. [PubMed: 11723017]
6. Salisbury D, Bronas U. Reactive Oxygen and Nitrogen Species: Impact on Endothelial Dysfunction. Nursing Research. 2015; 64:53–66. [PubMed: 25502061]
7. Lu X, Guo X, Wassall CD, Kemple MD, Unthank JL, Kassab GS. Reactive oxygen species cause endothelial dysfunction in chronic flow overload. 2011
8. Kleinbongard P, Dejam A, Lauer T, Jax T, Kerber S, Gharini P, Balzer J, Zotz RB, Scharf RE, Willers R, Schechter AN, Feelisch M, Kelm M. Plasma nitrite concentrations reflect the degree of endothelial dysfunction in humans. Free Radical Biology and Medicine. 2006; 40:295–302. [PubMed: 16413411]
9. Mancini GBJ, Dahlöf B, Díez J. Surrogate Markers for Cardiovascular Disease: Structural Markers. Circulation. 2004; 109:IV-22–IV-30. [PubMed: 15226248]
10. Nambi V, Pedroza C, Kao LS. Carotid intima-media thickness and cardiovascular events. The Lancet. 2012; 379:2028–2030.
11. Davis PH, Dawson JD, Riley WA, Lauer RM. Carotid Intimal-Medial Thickness Is Related to Cardiovascular Risk Factors Measured From Childhood Through Middle Age: The Muscatine Study. Circulation. 2001; 104:2815–2819. [PubMed: 11733400]
12. Kleinbongard P, Dejam A, Lauer T, Rassaf T, Schindler A, Picker O, Scheeren T, Gödecke A, Schrader J, Schulz R, Heusch G, Schaub GA, Bryan NS, Feelisch M, Kelm M. Plasma nitrite reflects constitutive nitric oxide synthase activity in mammals. Free Radical Biology and Medicine. 2003; 35:790–796. [PubMed: 14583343]
13. Rhodes PM, Leone AM, Francis PL, Struthers AD, Moncada S. The L-Arginine: Nitric Oxide Pathway Is the Major Source of Plasma Nitrite in Fasted Humans. Biochemical and Biophysical Research Communications. 1995; 209:590–596. [PubMed: 7794389]
14. Tannenbaum SR, Witter JP, Gatley SJ, Balish E. Nitrate and Nitrite: Origin in Humans. Science. 1979; 205:1332–1337. [PubMed: 472751]

15. Kelm M. Nitric oxide metabolism and breakdown. Biochimica et Biophysica Acta (BBA) - Bioenergetics. 1999; 1411:273–289.
16. Hibbs JB, Westenfelder C, Taintor R, Vavrin Z, Kablitz C, Baranowski RL, Ward JH, Menlove RL, McMurry MP, Kushner JP. Evidence for cytokine-inducible nitric oxide synthesis from L-arginine in patients receiving interleukin-2 therapy. Journal of Clinical Investigation. 1992; 89:867–877. [PubMed: 1541678]
17. Tsikas D. Circulating and excretory nitrite and nitrate: Their value as measures of nitric oxide synthesis, bioavailability and activity is inherently limited. Nitric Oxide. 2015; 45:1–3. [PubMed: 25576891]
18. Lundberg JO, Weitzberg E, Gladwin MT. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. Nature Reviews Drug Discovery. 2008; 7:156–67.
19. Hyde ER, Andrade F, Vaksman Z, Parthasarathy K, Jiang H, Parthasarathy DK, Torregrossa AC, Tribble G, Kaplan HB, Petrosino JF, Bryan NS. Metagenomic Analysis of Nitrate-Reducing Bacteria in the Oral Cavity: Implications for Nitric Oxide Homeostasis. PLoS One. 2014; 9
20. Doel JJ, Benjamin N, Hector MP, Rogers M, Allaker RP. Evaluation of bacterial nitrate reduction in the human oral cavity. European Journal of Oral Sciences. 2005; 113:14–19. [PubMed: 15693824]
21. Lundberg JO, Weitzberg E, Lundberg JM, Alving K. Intragastric nitric oxide production in humans: measurements in expelled air. Gut. 1994; 35:1543–1546. [PubMed: 7828969]
22. Lundberg JO, Gladwin MT, Ahluwalia A, Benjamin N, Bryan NS, Butler A, Cabrales P, Fago A, Feelisch M, Ford PC, Freeman BA, Frenneaux M, Friedman J, Kelm M, Kevil CG, Kim-shapiro DB, Kozlov AV, Lancaster JR, Lefer DJ, McColl K, McCurry K, Patel RP, Petersson J, Rassaf T, Reutov VP, Richter-addo GB, Schechter A, Shiva S, Tsuchiya K, Van Faassen EE, Webb AJ, Zuckerbraun BS, Zweier JL, Weitzberg E. Nitrate and nitrite in biology, nutrition and therapeutics. Nature Chemical Biology. 2009; 5:865–9.
23. Lundberg JO, Govoni M. Inorganic nitrate is a possible source for systemic generation of nitric oxide. Free Radical Biology and Medicine. 2004; 37:395–400. [PubMed: 15223073]
24. Presley TD, Morgan AR, Bechtold E, Clodfelter W, Dove RW, Jennings JM, Kraft RA, Bruce King S, Laurienti PJ, Jack Rejeski W, Burdette JH, Kim-Shapiro DB, Miller GD. Acute effect of a high nitrate diet on brain perfusion in older adults. Nitric Oxide. 2011; 24:34–42. [PubMed: 20951824]
25. Webb AJ, NP, Loukogeorgakis S, Okorie M, Aboud Z, Misra S, Rashid R, Miall P, Deanfield J, Benjamin N, MacAllister R, Hobbs A, Ahluwalia A. Acute Blood Pressure Lowering, Vasoprotective, and Antiplatelet Properties of Dietary Nitrate via Bioconversion to Nitrite. Hypertension. 2008; 51:784–790. [PubMed: 18250365]
26. Kapil V, Khambata R, Robertson A, Caulfield M, Ahluwalia A. Dietary Nitrate Provides Sustained Blood Pressure Lowering in Hypertensive Patients: A Randomized, Phase 2, Double-Blind, Placebo-Controlled Study. Hypertension. 2015; 65:320–327. [PubMed: 25421976]
27. Jones A. Dietary Nitrate Supplementation and Exercise Performance. Sports Medicine. 2014; 44:35–45.
28. Kelly J, Vanhatalo A, Bailey SJ, Wylie LJ, Tucker C, List S, Winyard PG, Jones AM. Dietary nitrate supplementation: effects on plasma nitrite and pulmonary O2 uptake dynamics during exercise in hypoxia and normoxia, American Journal of Physiology - Regulatory. Integrative and Comparative Physiology. 2014; 307:R920–R930.
29. Larsen FJM, Ekblom BMDP, Sahlin KP, Lundberg JOMDP, Weitzberg EMDP. Effects of Dietary Nitrate on Blood Pressure in Healthy Volunteers. The New England Journal of Medicine. 2006; 355:2792–3. [PubMed: 17192551]
30. Larsen FJ, Weitzberg E, Lundberg JO, Ekblom B. Effects of dietary nitrate on oxygen cost during exercise. Acta Physiologica. 2007; 191:59–66. [PubMed: 17635415]
31. Sindler AL, Fleenor BS, Calvert JW, Marshall KD, Zigler ML, Lefer DJ, Seals DR. Nitrite supplementation reverses vascular endothelial dysfunction and large elastic artery stiffness with aging. Aging cell. 2011; 10:429–437. [PubMed: 21276184]

32. Stokes KY, Dugas TR, Tang Y, Garg H, Guidry E, Bryan NS. Dietary nitrite prevents hypercholesterolemic microvascular inflammation and reverses endothelial dysfunction. Am J Physiol Heart Circ Physiol. 2009; 296:H1281–8. [PubMed: 19252084]
33. Heiss C, Meyer C, Totzeck M, Hendgen-Cotta UB, Heinen Y, Luedike P, Keymel S, Ayoub N, Lundberg JO, Weitzberg E, Kelm M, Rassaf T. Dietary inorganic nitrate mobilizes circulating angiogenic cells. Free Radical Biology and Medicine. 2012; 52:1767–1772. [PubMed: 22406434]
34. Rodriguez-Mateos A, Hezel M, Aydin H, Kelm M, Lundberg JO, Weitzberg E, Spencer JPE, Heiss C. Interactions between cocoa flavanols and inorganic nitrate: Additive effects on endothelial function at achievable dietary amounts. Free Radical Biology and Medicine. 2015; 80:121–128. [PubMed: 25530151]
35. Govoni M, Jansson EÅ, Weitzberg E, Lundberg JO. The increase in plasma nitrite after a dietary nitrate load is markedly attenuated by an antibacterial mouthwash. Nitric Oxide. 2008; 19:333–337. [PubMed: 18793740]
36. Wylie LJ, Kelly J, Bailey SJ, Blackwell JR, Skiba PF, Winyard PG, Jeukendrup AE, Vanhatalo A, Jones AM. Beetroot juice and exercise: pharmacodynamic and dose-response relationships. Journal of Applied Physiology. 2013; 115:325–336. [PubMed: 23640589]
37. Wilkerson D, Hayward G, Bailey S, Vanhatalo A, Blackwell J, Jones A. Influence of acute dietary nitrate supplementation on 50 mile time trial performance in well-trained cyclists. European Journal of Applied Physiology. 2012; 112:4127–4134. [PubMed: 22526247]
38. Salimetrics. Saliva Collection and Handling Advice. Salimetrics, LLC 3rd; 2013.
39. Veerman ECI, vandenKeybus PAM, Vissink A, Amerongen AVN. Human glandular salivas: Their separate collection and analysis. European Journal of Oral Sciences. 1996; 104:346–352. [PubMed: 8930581]
40. Lauer T, Preik M, Rassaf T, Strauer BE, Deussen A, Feelisch M, Kelm M. Plasma nitrite rather than nitrate reflects regional endothelial nitric oxide synthase activity but lacks intrinsic vasodilator action. Proc Natl Acad Sci U S A. 2001; 98:12814–9. [PubMed: 11606734]
41. Zetterquist W, Pedroletti C, Lundberg JO, Alving K. Salivary contribution to exhaled nitric oxide. European Respiratory Journal. 1999; 13:327–333. [PubMed: 10065676]
42. Schreiber F, Stief P, Gieseke A, Heisterkamp IM, Verstraete W, de Beer D, Stoodley P. Denitrification in human dental plaque. BMC BIOLOGY. 2010; 8:24–24. [PubMed: 20307293]
43. Takahama U, Hirota S, Oniki T. Production of nitric oxide-derived reactive nitrogen species in human oral cavity and their scavenging by salivary redox components. Free radical research. 2005; 39:737–745. [PubMed: 16036353]
44. Dewhirst FE, Chen T, Izard J, Paster BJ, Anne CRT, Yu WH, Lakshmanan A, Wade WG. The Human Oral Microbiome. Journal of Bacteriology. 2010; 192:5002–5017. [PubMed: 20656903]

Highlights

- The correlation of basal plasma and salivary NO_x are investigated
- Ex-vivo salivary NO_3^- reduction is compared to *in-vivo* dietary NO_3^- to plasma NO_2^-
- We report that basal salivary and plasma NO_2^- levels show no correlation.
- Salivary NO_3^- to NO_2^- does not correlate with *in vivo* dietary NO_3^- to plasma NO_2^-

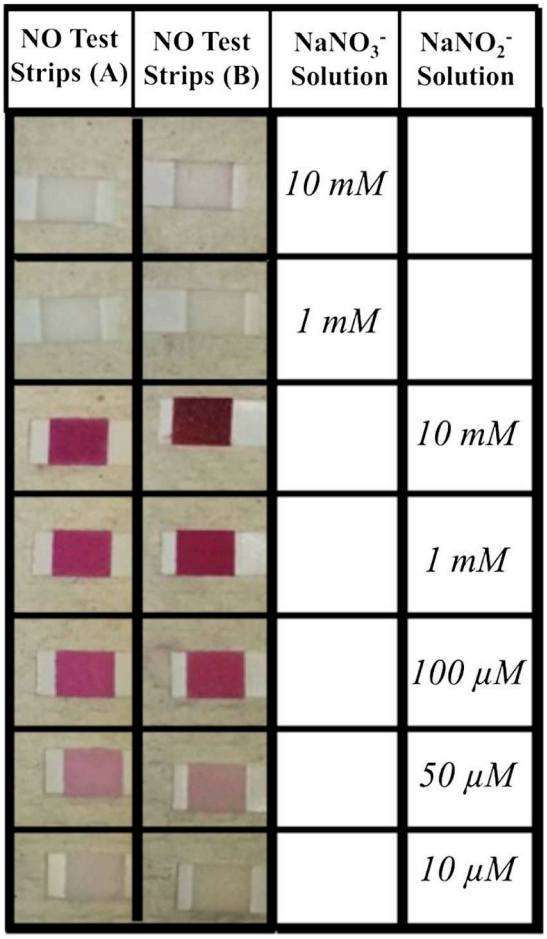

Fig. 1.

Sensitivity of test strips to NO_2^- and NO_3^-. Nitric Oxide Saliva Test Strips, Neogenis (A) and Berkeley (B), were dipped in a $NaNO_3^-$ or $NaNO_2^-$ solution. New unused test strips have a white tip where saliva is intended to be placed. Reaction with the strip caused a light pink to deep red colorimetric change.

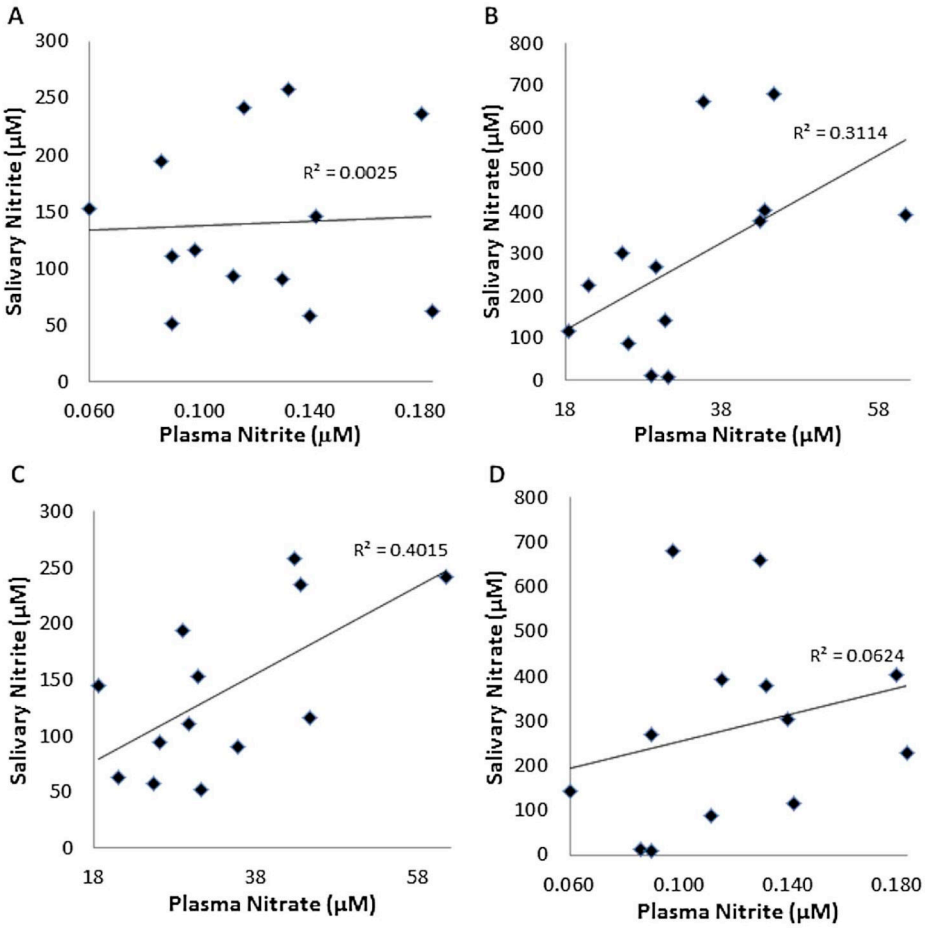

Fig. 2.

Examination of basal salivary vs plasma levels of NO_2^- and NO_3^- (A) Basal plasma NO_2^- and salivary NO_2^- levels, p=0.87. (B) Plasma NO_3^- and salivary NO_3^-, p=0.048. (C) Plasma NO_3^- and salivary NO_2^-, p=0.020. (D) Plasma NO_2^- and salivary NO_3^- levels, p=0.41.

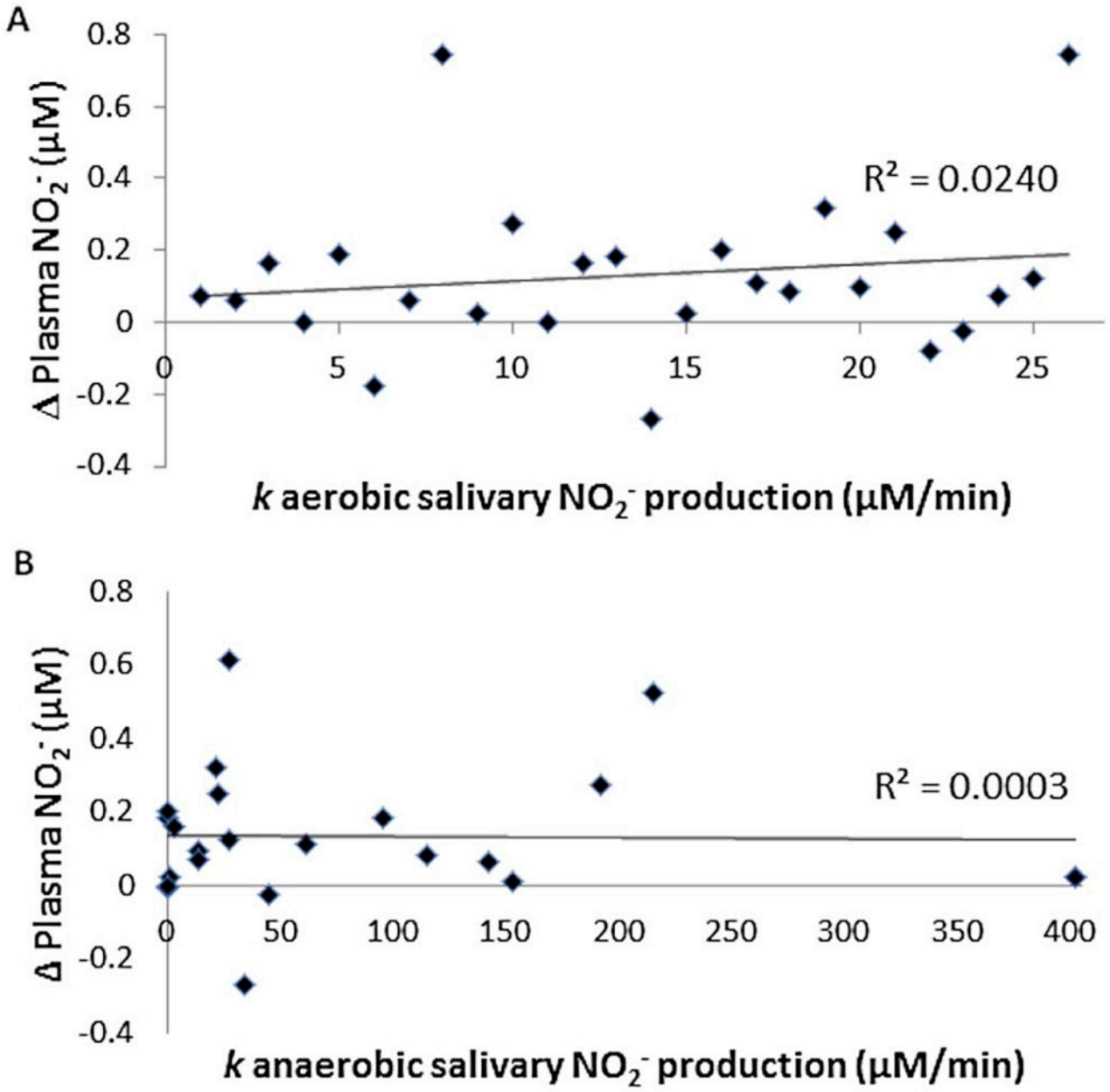

Fig. 3.

Comparison of *ex vivo* salivary conversion of NO_3^- to NO_2^- to *in vivo* conversion of oral NO_3^- to plasma NO_2^-. Patients (n = 10) were given 2.35 oz of concentrated beet juice (Beet-It) each day for six weeks. Blood was drawn from each patient before and 1 hr after (Δ Plasma NO_2^-) beet juice consumption at weeks 1, 3, and 6 (all 3 timepoints plotted for each individual). Saliva samples were expectorated early each morning (before breakfast or tooth brushing) at weeks 1, 3, and 6. Δ Plasma NO_2^- was plotted versus the rate of salivary NO_2^- production after 10 mM inorganic NO_3^- was added to (A) aerobic (p-value=0.23, corr=0.02) and (B) anaerobic saliva (p-value=0.76, corr=0.08). Neither condition shows a correlation.