Analysis of Nitrate/Nitrite Concentration in Blood Plasma Pre and Post Nitrate Supplementation

THOMPSON RIVERS UNIVERSITY

Juliana Hermiston, Gwen Freeze, Jesse Biddlecombe, MSc and Mark Rakobowchuk, PhD

Department of Biological Sciences

Introduction

- *Porphyromonas gingivalis* is the keystone pathogen found in patients with periodontitis and produces toxic proteases called gingipains.[1]
- This bacterium has also been found in the brains of patients with Alzheimer's disease.[1]
- In mouse models, inhibiting gingipain expression decreased the colonization of *P. gingivalis* in the brain.[1]
- The nitrate-nitrite-nitric oxide pathway relies on bacteria within the oral microbiome to convert nitrate to nitrite, and this pathway may be an important contributing factor to overall health.[2]
- Supplementing the diet with nitrate could alter the oral microbial community composition and reduce *P. gingivalis* abundance.

Methods

- Ten healthy participants underwent 10 days of twice daily 400mg KNO_3 supplementation.
- Venous blood samples were taken pre and post intervention.

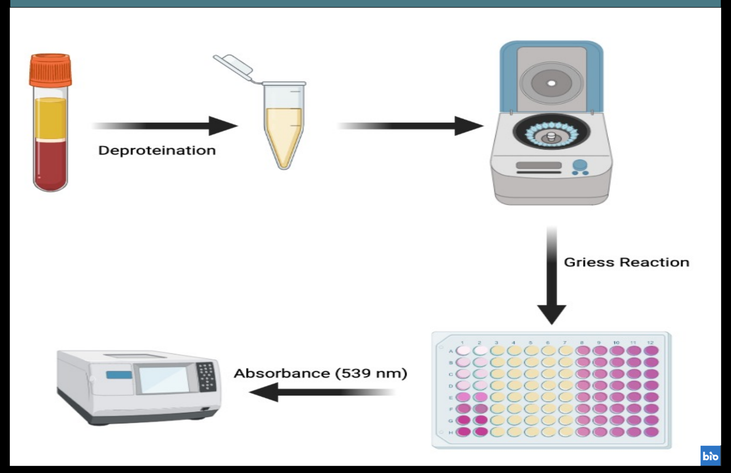

Figure 1. Procedure for the study presented here. Ten human blood plasma samples were deproteinated and subjected to a Griess reaction. Absorbances of the samples were subsequently read at 539 nm and nitrate/nitrite concentration determined.

Results

- All samples were spiked with a known amount of NO_3^- (32 μM) to provide a recovery value for this time-sensitive assay protocol.
- Concentrations of plasma NO_3^- /NO_2^- were calculated using absorbance values and the standard curve of the Griess assay (Figure 3).
- Percent recovery indicates proficiency with this time-sensitive assay although sensitivity within the range of concentrations measured in the blood samples is limited.

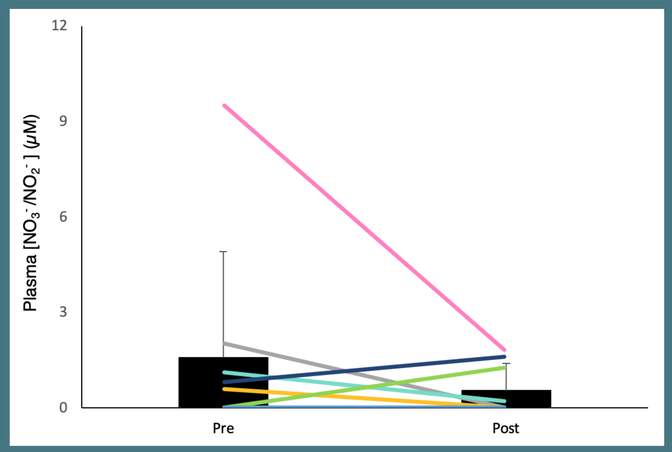

Figure 2. Plasma [NO_3^- /NO_2^-] for each of the participants pre- and post-nitrate supplementation as well as the average concentrations (n=10).

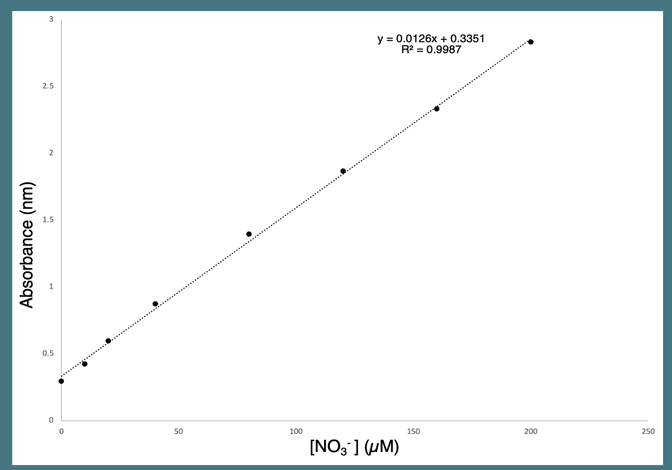

Figure 3. Standard curve of the Griess assay (n=8).

Table 1. Plasma nitrate/nitrite recoveries pre and post supplementation based on the standard curve of the Griess assay (Figure 3) as compared to plasma samples spiked with NO_3^-.

Participant	Percent Recovery of NO_3^- /NO_2^-
1	Pre: 91.37 %
	Post: 105.51 %
2	Pre: 96.58 %
	Post: 104.51 %
3	Pre: 97.35 %
	Post: 73.88 %
4	Pre: 88.79 %
	Post: 102.28 %
5	Pre: 93.11 %
	Post: 94.72 %
6	Pre: 86.19 %
	Post: 89.53 %
7	Pre: 82.84 %
	Post: 94.62 %
8	Pre: 55.31 %
	Post: 57.42 %
9	Pre: 82.44 %
	Post: 92.01 %
10	Pre: 96.70 %
	Post: 93.73 %

Discussion

- The results suggest that plasma [NO_3^-/NO_2^-] were not different before compared to after dietary supplementation.
- Kinetics of NO_3^- /NO_2^- in young healthy adults are rather fast as previously demonstrated by Wylie et al. (2013).
- They show, at similar dosage to the current study a return of plasma [NO_3-] over time with the administration of 4.2 mmol of nitrate by 12 hours.[3]

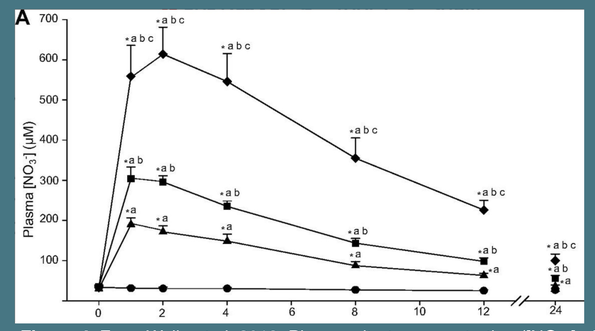

Figure 4. From Wylie et al. 2013, Plasma nitrate concentration ([NO_3^-]; *A*) and nitrite concentration ([NO_2^-]; *B*) following consumption of water (control; ●) and 4.2 (▲), 8.4 (■), and 16.8 (◆) mmol NO_3^- (group mean ± SE).

- This suggests [NO_3^- /NO_2^-] returned to baseline following acute administration.[3]

Future Work

- Time course with potassium nitrate supplementation should be performed to determine exposure of the oral microbial community with NO_3^- /NO_2^-.
- Future studies should consider taking blood samples within one hour post nitrate supplementation as to obtain peak plasma nitrate response.
- Salivary samples would provide more direct evidence of an altered oral environment capable of altering the microbial community.

References

1. Dominy SS, Lynch C, Ermini F, Benedyk M, Marczyk A, Konradi A, Nguyen M, Haditsch U, Raha D, Griffin C, Holsinger LJ, Arastu-Kapur S, Kaba S, Lee A, Ryder MI, Potempa B, Mydel P, Hellvard A, Adamowicz K, Hasturk H, Walker GD, Reynolds EC, Faull RLM, Curtis MA, Dragunow M, Potempa J. 2019. Porphyromonas gingivalis in Alzheimer's disease brains: Evidence for disease causation and treatment with small-molecule inhibitors. Sci Adv. [accessed 2022 Mar 23]; 5(1): 1-22. doi: 10.1126/sciadv.aau3333
2. Vanhatalo A, Blackwell JR, L'Heureux JE, Williams DW, Smith A, van der Giezen M, Winyard PG, Kelly J, Jones AM. 2018. Nitrate-responsive oral microbiome modulates nitric oxide homeostasis and blood pressure in humans. Free Radic Biol Med. [accessed 2022 Mar 24]; 124(3): 21-30. doi: 10.1016/j.freeradbiomed.2018.05.078
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