

The tongue microbiome and nitric oxide bioavailability across the human lifespan

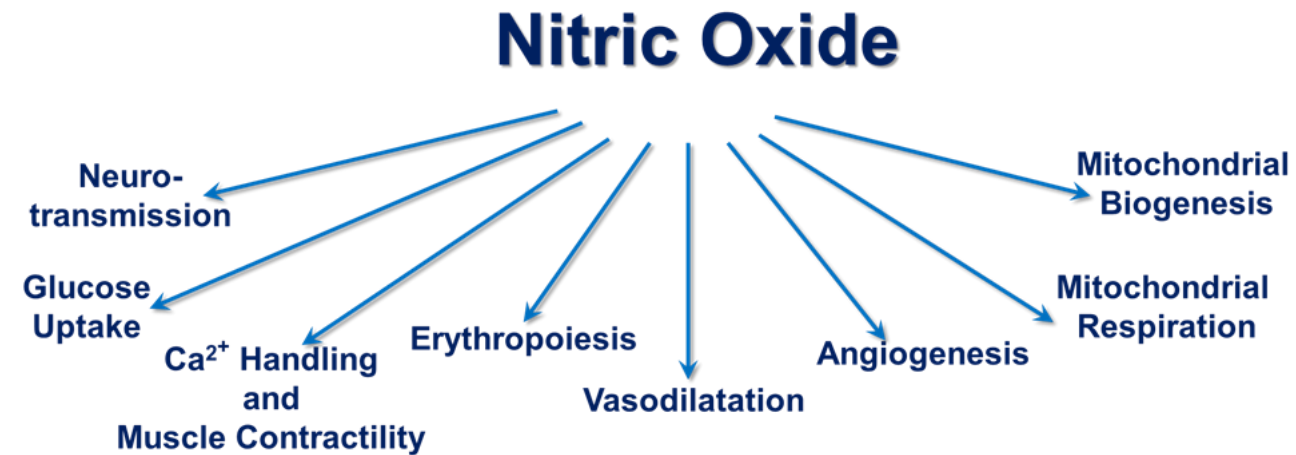
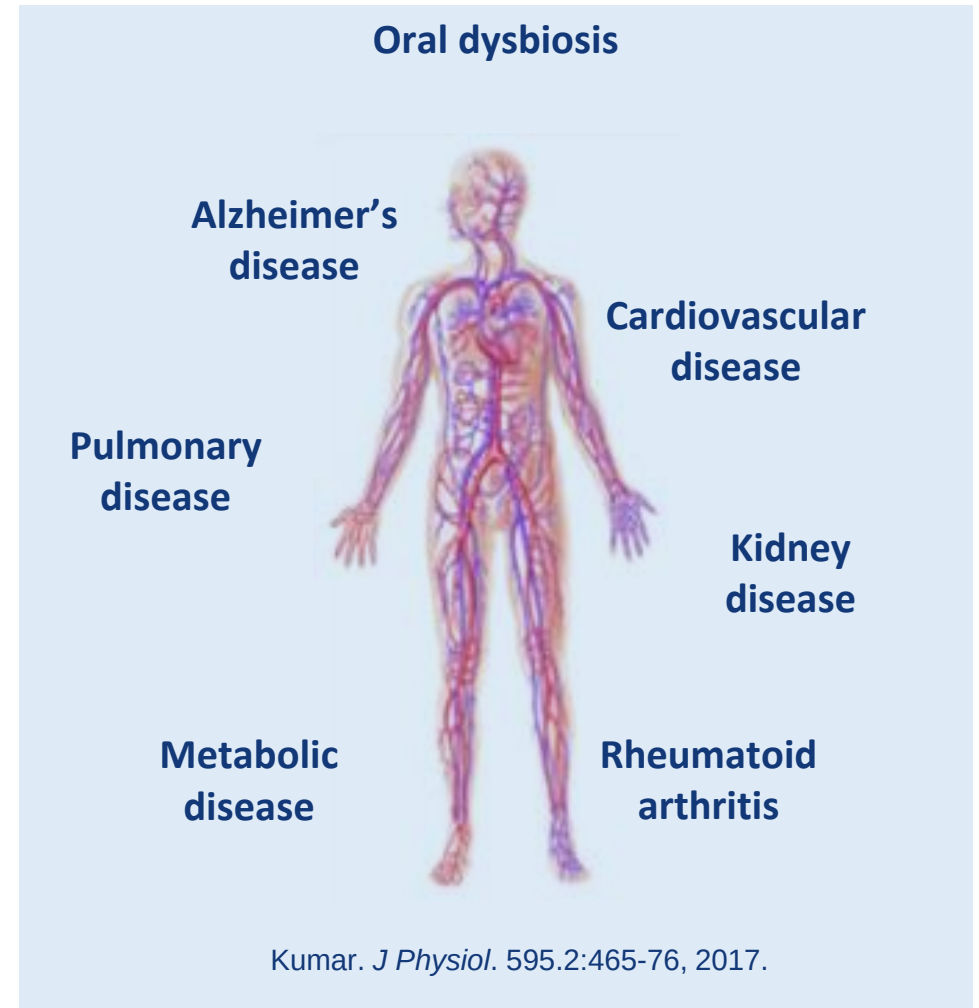
PI: Andy Jones

Co-Is: Anni Vanhatalo, Paul Winyard, David Williams, Mark van der Giezen

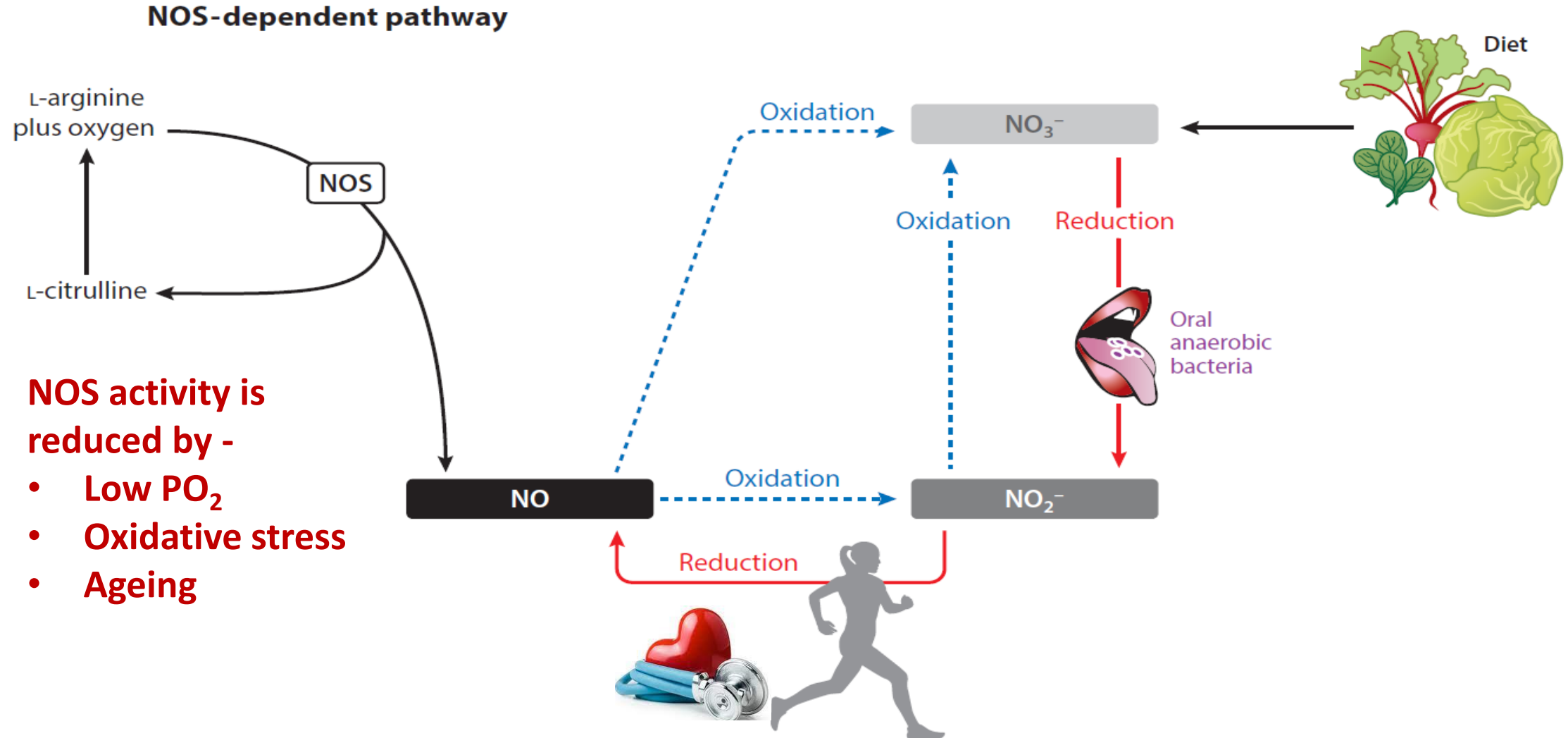
PDRAs: Matt Black, Joanna L'Heureux, Anne Smith, Chris Thompson



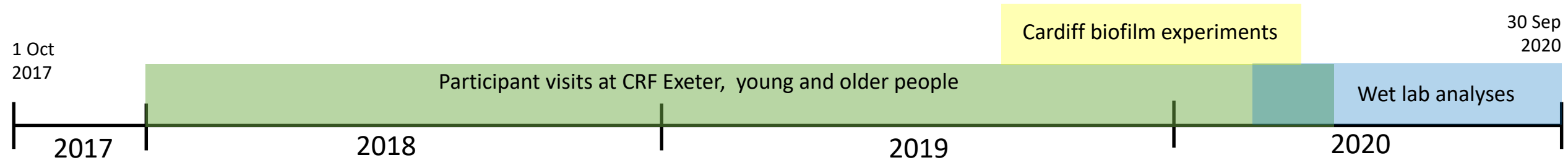
Project background - Nitric oxide production as a potential link between the oral microbiome and healthy ageing



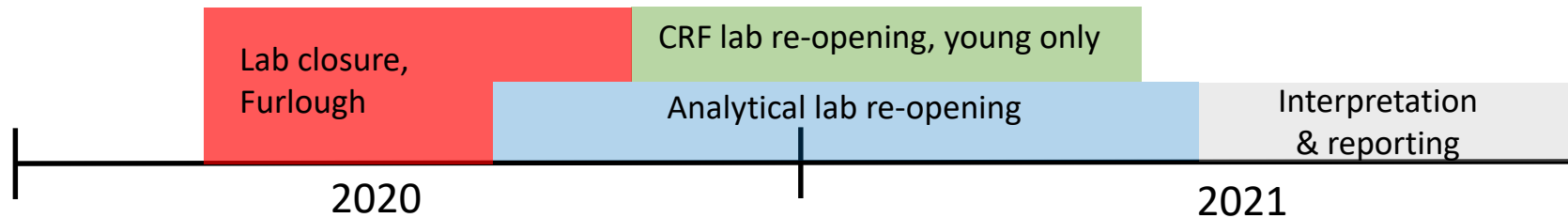
Project background - Pathways of nitric oxide production



Original timeline



Revised timeline beyond March 2020



- On 20 March 2020, the final 16 participants (10 young, 6 older) were on track to complete the 12 week intervention within ~5 weeks.
 - Furlough of PDRAs (5 months and 3.5 months)
 - Revision of risk assessments and ethics amendment
 - £50k COA funding to enable completion of the trial
- National Covid-19 restrictions precluded testing of human participants over the age of 40 during Nov 2020 - May 2021.
- First participant visit 10 Jan 2018, last participant visit 6 May 2021.

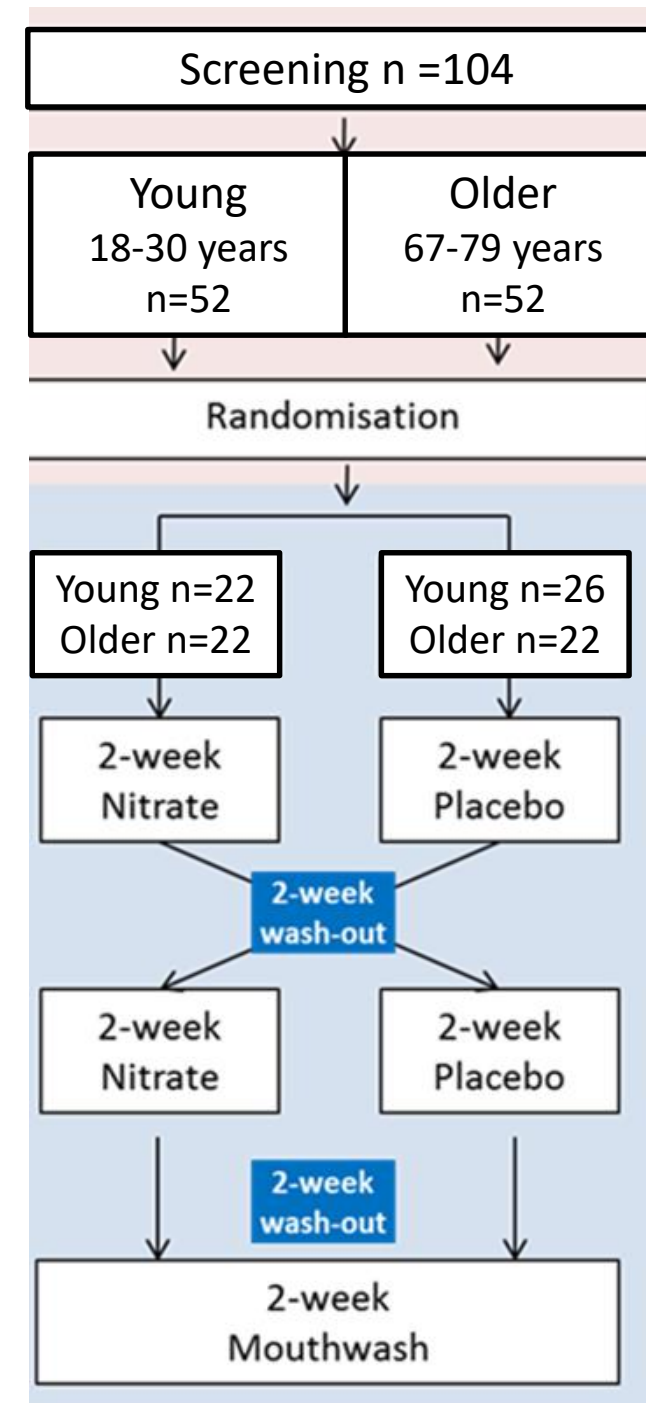
(I) The effects of dietary nitrate and antibacterial mouthwash on the oral microbiota, nitric oxide biomarkers and cardiovascular health in young and older adults

Placebo controlled, double blind cross-over design

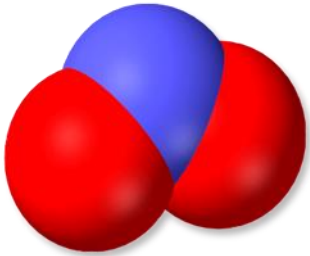
- Control: Placebo beetroot juice (**PL**) (BeetIt Sport, James White Drinks, Ipswich, UK)
- Positive control: Nitrate-rich beetroot juice (**BR**) (BeetIt Sport, James White Drinks, Ipswich, UK)
- Negative control: Mouthwash (**MW**) (Original Listerine® Antiseptic Mouthwash; Johnson & Johnson Consumer Inc., New Jersey, USA)

Trial was supported by Exeter Clinical Trials Unit and Exeter NIHR Clinical Research Facility.

	Young (n = 39)	Older (n = 36)	<i>P</i>
Age, y	24 ± 3	71 ± 3	<0.001
Height, m	1.73 ± 0.09	1.66 ± 0.09	0.001
Body mass, kg	71.5 ± 11.9	68.3 ± 11.2	0.224
BMI, kg/m ²	23.8 ± 2.9	24.5 ± 2.8	0.261
SBP (mmHg)	117 ± 9	126 ± 14	<0.001
DBP (mmHg)	72 ± 8	80 ± 7	<0.001
MAP (mmHg)	87 ± 7	95 ± 9	<0.001



Changes in plasma NO biomarkers and blood pressure



- 10-fold greater nitrate and nitrite concentration in high-nitrate compared to placebo and mouthwash.
- Greater increase in plasma nitrate and nitrite in high-nitrate condition in the older cohort compared to young.

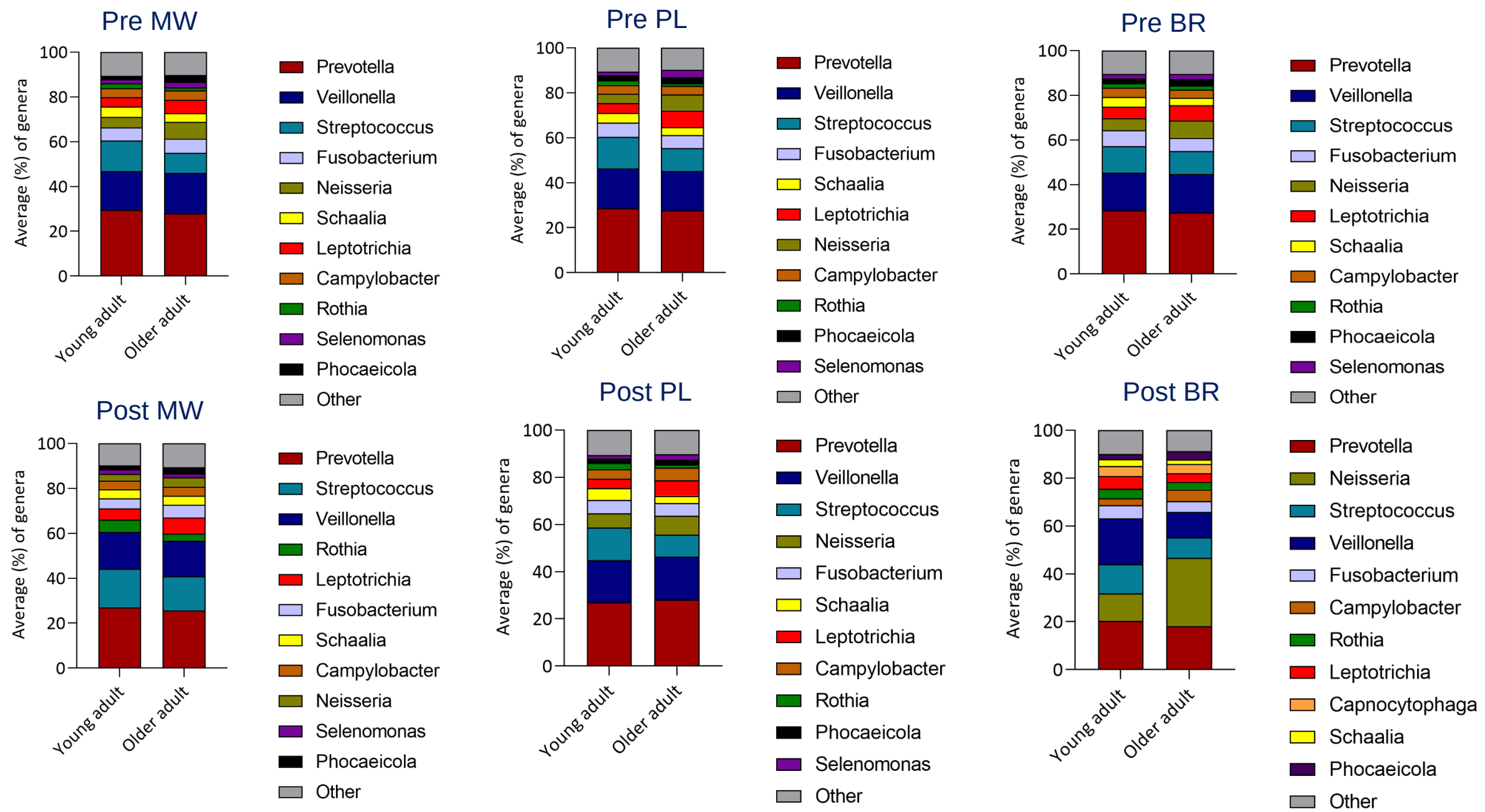
- No significant differences in blood pressure between conditions within the Young cohort.
- Greater decrease in blood pressure in BR compared to MW and PL in Older cohort.
- Greater decrease in systolic blood pressure in the Older cohort compared to Young in the BR condition.
 - Young ~ 1 mmHg reduction
 - Older ~ 7 mmHg reduction

- Those with elevated blood pressure at baseline benefited most from dietary nitrate supplementation.
- Changes in BP correlated with change in plasma $[\text{NO}_2^-]$.

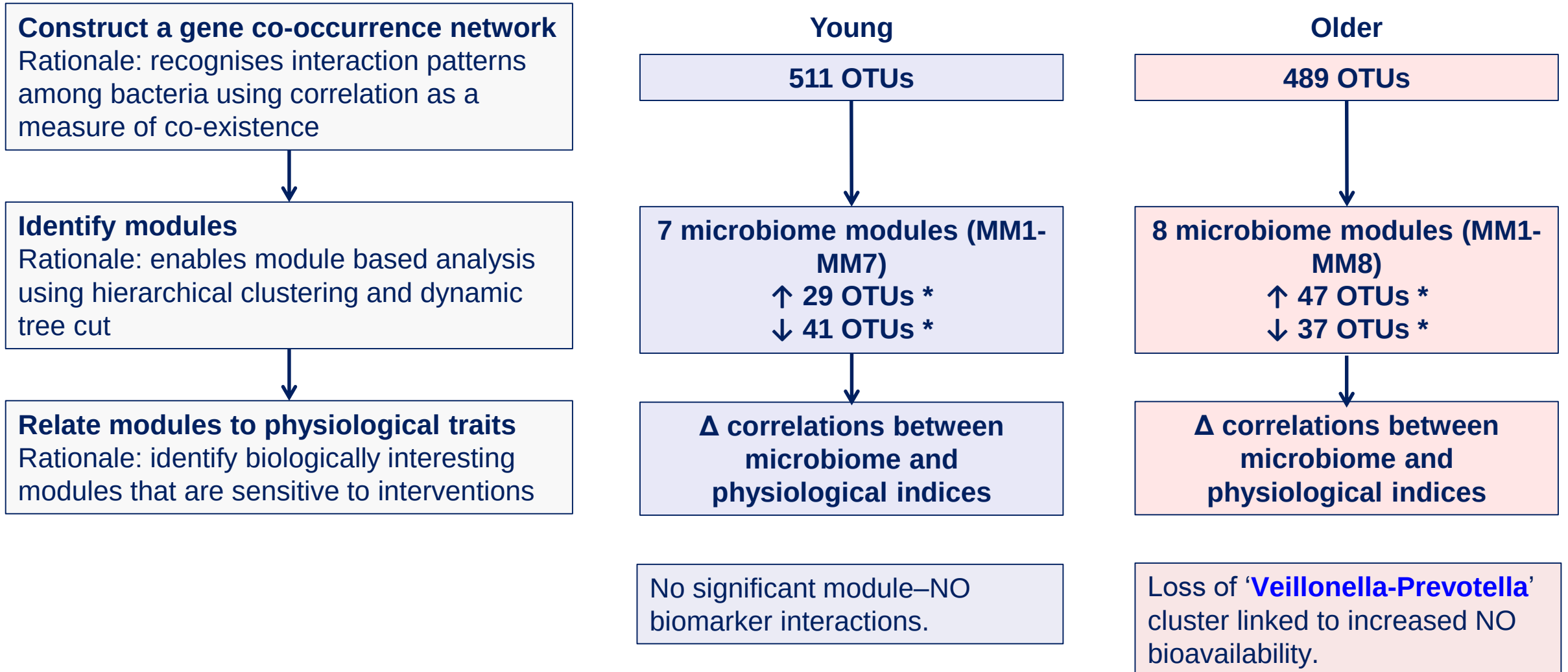
Influence of MW, PL and BR on oral microbiomes in young and older groups

- **There were no differences in oral microbiomes at baseline (pre-MW, pre-PL, or pre-BR) between young and older cohorts.**
- **PL and MW interventions did not alter the overall microbiome compositions in young or older groups, but there was a significant change in BR condition within both young and older cohorts.**
- **Post-BR microbiomes differed between young and older cohorts indicating different responses to supplemental nitrate across age groups.**

The most abundant genera of oral bacteria pre and post MW, PL and BR conditions



Interactions between oral bacteria and physiological variables

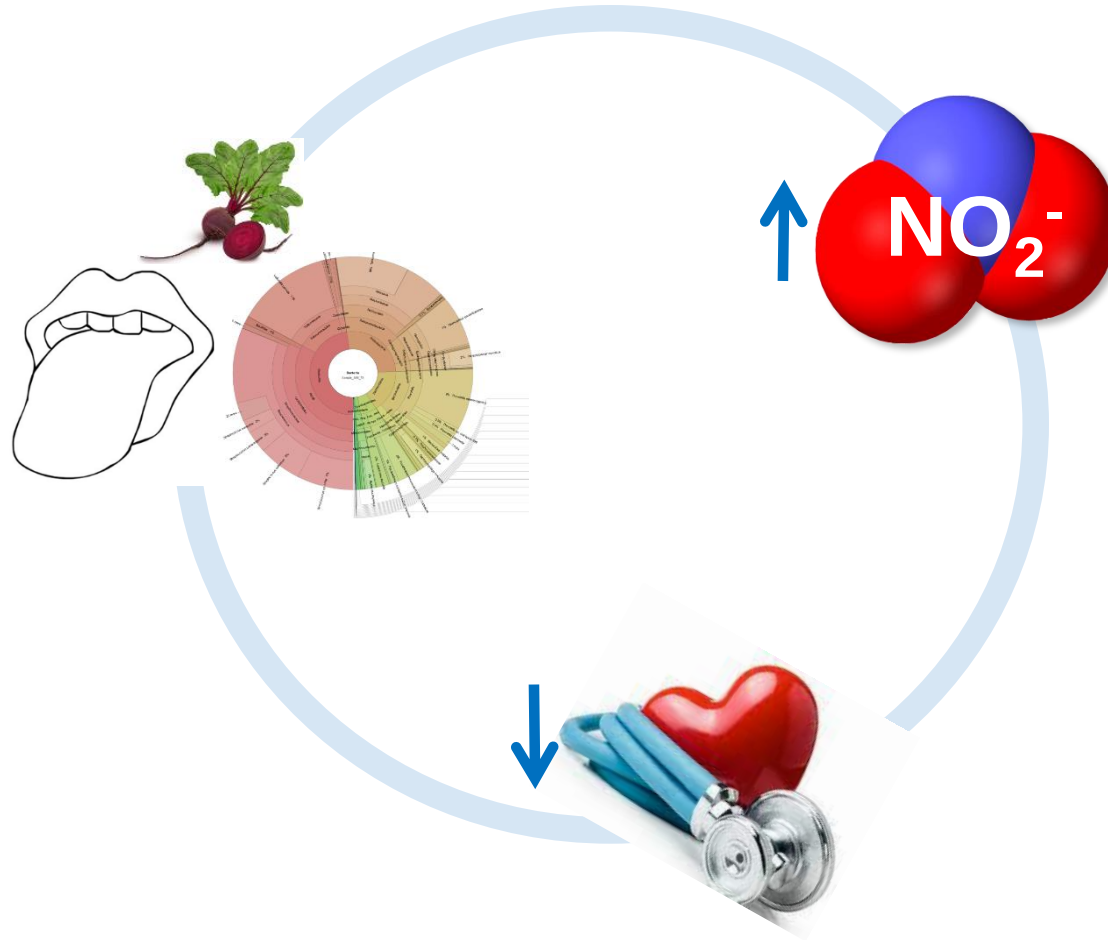


Interrelationships between changes in NO bioavailability, blood pressure and oral microbiome during nitrate supplementation

Dietary nitrate resulted in distinct microbiome alterations in Young and Older cohorts

Greater increase in *Neisseria* in the Older cohort compared to Young.

Increase in *Rothia* observed in the Older, but absent in the Young cohort.



Greater increase in NO biomarkers in the Older cohort compared to Young.

Increase in NO bioavailability correlated with changes in oral microbiome and with decrease in blood pressure in the Older cohort.

Greater decrease in blood pressure in the Older cohort compared to Young.

The effects of dietary nitrate and antibacterial mouthwash on the oral microbiome, markers of nitric oxide bioavailability and cardiovascular health in young and older adults

Key findings :

- The oral microbiomes of young and older adults responded differently to a 2-week high-nitrate diet, while the microbiomes of both cohorts were unaffected by 2-week placebo or antibacterial mouthwash interventions.
- Older people stand to gain greater vascular health benefits from dietary nitrate than healthy young adults.

Application, implications and future directions

Dietary nitrate represents a simple and cost effective dietary intervention to enhance cardiovascular health in older age via modulation of the oral microbiome.

- The finding that dietary nitrate diminishes the inflammatory *Prevotella-Veillonella* cluster of oral bacteria has important clinical implications for conditions underpinned by chronic, low level inflammation.
 - Cognitive decline
 - Recovery from radio therapy
 - Rheumatoid arthritis
 - COVID-19 severity
 - Low immunity, frailty, pneumonia

Acknowledgements

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