School of Sport and Health Sciences

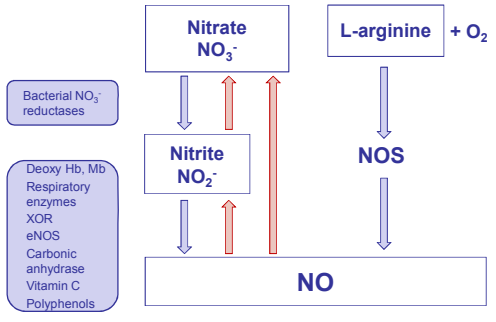
Why Popeye was Right: Dietary Nitrate and Skeletal Muscle Function

Andrew M. Jones PhD
Professor of Applied Physiology
University of Exeter

Why is NO important (for exercise)?

Control/regulation of:
Vascular tone and blood flow
Mitochondrial respiration
Muscle excitation-contraction coupling
Glucose and calcium homeostasis
Neurotransmission
Immune function
Etc.....

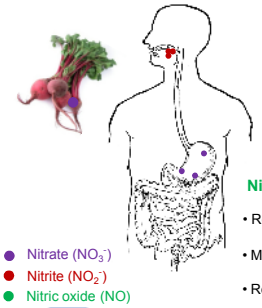
Pathways of NO synthesis



New concepts in nitrite biology

- The nitrate – nitrite – NO pathway is an essential 'back-up' system for NO generation
- It may compensate for dysfunctional NOS
- It may be particularly important in hypoxic conditions (such as during exercise?)

The Entero-Salivary Circulation of Nitrate



NO_3^- → NO_2^- (Bacterial anaerobes)

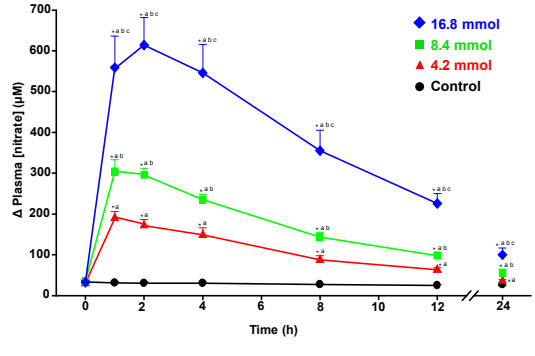
NO_2^- → NO (*Acidosis, Hypoxia*)

Nitric oxide:

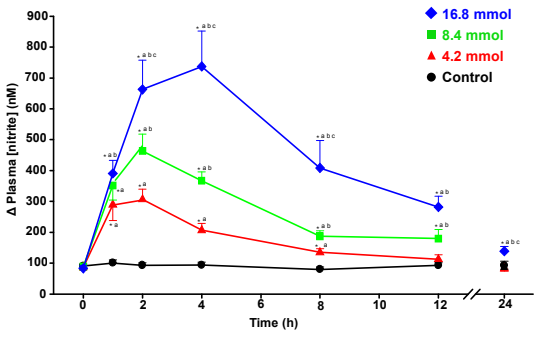
- Regulation of blood flow
- Modulation of excitation-contraction coupling
- Regulation of mitochondrial respiration

- Nitrate (NO_3^-)
- Nitrite (NO_2^-)
- Nitric oxide (NO)

Nitrate intake: pharmacokinetics



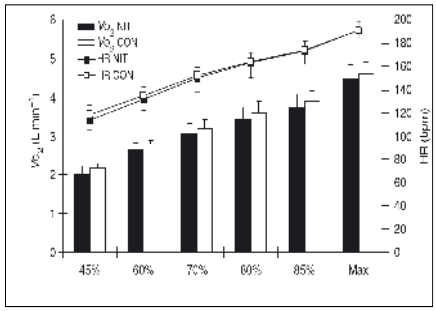
Nitrate intake: pharmacokinetics



Good sources of nitrate (mg/100g)

Source	mg/100g
Carrot	92-195
Lettuce	12-267
Spinach	24-741
Chinese cabbage	43-161
Bok choy	102-310
Cabbage	26-125
Cole	77-137
Rocket (Arugula)	300-329
Rhubarb	281
Beet root	110

Oxygen consumption during exercise



Larsen et al. (2007): 3 days supplementation with 0.1 mmol/kg/day $NaNO_3^-$

'Beetroot Juice'

$NaNO_3^-$ restricted for human use in the UK

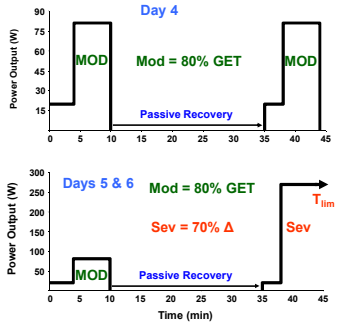
We wished to investigate whether:

- **1) Similar effects were manifest following dietary supplementation with nitrate-rich beetroot juice**
- **2) The potential improvement in exercise economy might enhance exercise tolerance**

Bailey et al., 2009, *J Appl Physiol*

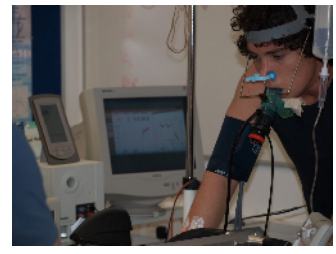
Experimental Design

Six day supplementation with Beetroot (NO_3^- = 5.6 mmol·day^{-1}) and Placebo (NO_3^-= negligible); 10 day washout period; randomized, double-blind, crossover design



Measurements

- **Pulmonary $\dot{V}O_2$ Dynamics**
  - **Breath-by-Breath**



Measurements

- Pulmonary $\dot{V}O_2$ Dynamics
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- Tissue Oxygenation
  - Near-infrared Spectroscopy

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- Pulmonary $\dot{V}O_2$ Dynamics
  - Breath-by-Breath
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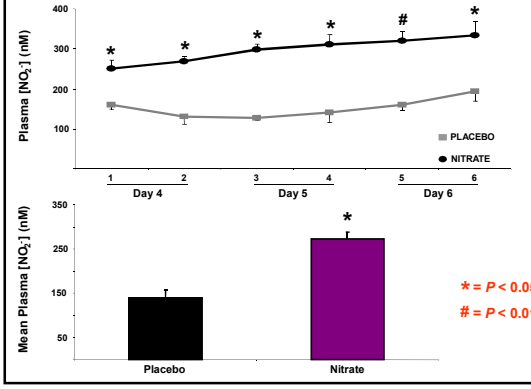
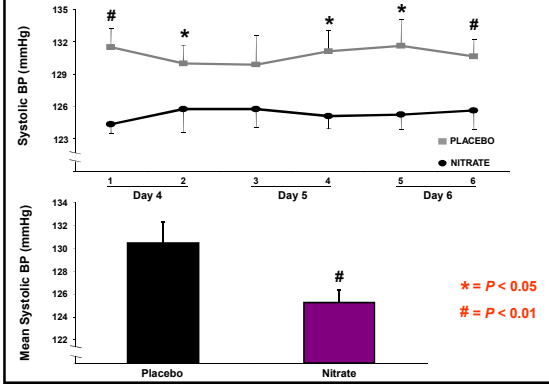
  - Automated Device
- Plasma $[NO_2^-]$ as biomarker of NO bioavailability
  - Chemiluminescence

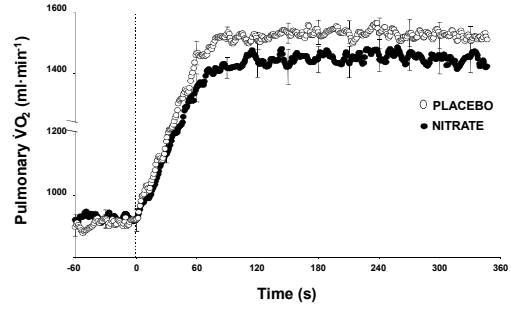
Influence of Nitrate on Plasma $[NO_2^-]$



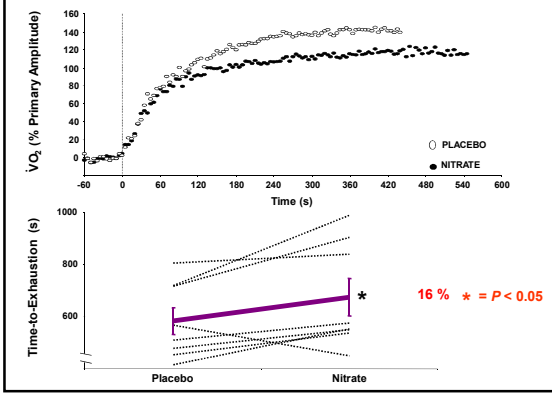
Influence of Nitrate on Systolic BP



Influence of Nitrate on O_2 Cost of Moderate Exercise



Influence of Nitrate on Exercise Tolerance



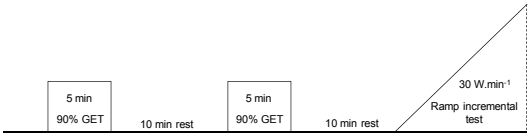
Summary

- Dietary nitrate supplementation increased markers of NO bioavailability.
- The O_2 cost of moderate-intensity exercise was reduced.
- During high-intensity exercise the $\dot{V}O_2$ slow component was reduced and exercise tolerance was enhanced.

Acute and Chronic Supplementation

Questions:

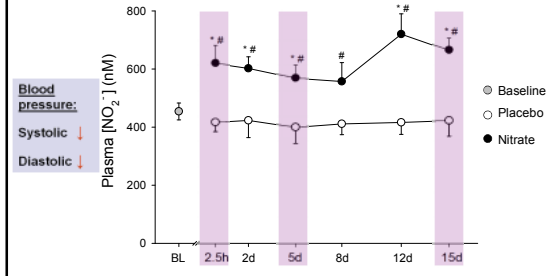
- What are the effects of acute and chronic supplementation?
- How might nitrate influence incremental test performance?



0.5 L beetroot juice per day for 15 days with exercise tests on day 1, 5 and 15

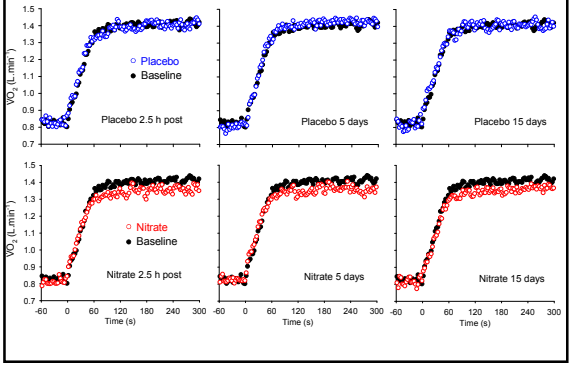
Vanhatalo et al., 2010, *Am J Physiol*

Plasma nitrite concentration



* Different from baseline; # Different from Placebo; $P<0.05$

Moderate intensity exercise: $\dot{V}O_2$



Beetroot Juice Constituents: Are There Other Potential 'Active Ingredients'?

- Beets are a rich source of:

- Antioxidants
  - Betaine
  - Vitamins

  → ↑ ROS Scavenging; ↑ NO Bioavailability; ↑ Fatigue Resistance
- Polyphenols
  - Quercetin
  - Resveratrol

  → ↑ Mitochondrial Biogenesis; ↑ Aerobic Capacity; ↑ Fatigue Resistance

• To what extent have these molecules contributed to our findings?

Placebo

We developed a process that selectively removes nitrate from beetroot juice

We wished to determine:

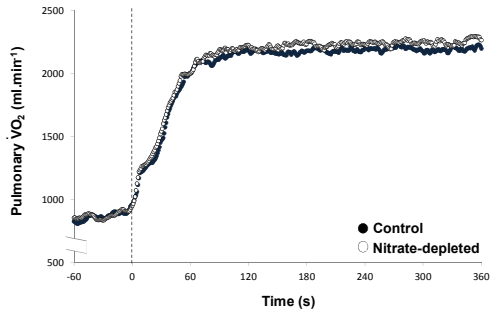
1) The effects of nitrate-rich vs. nitrate-depleted beetroot juice

2) The effects upon the oxygen cost of walking and running exercise

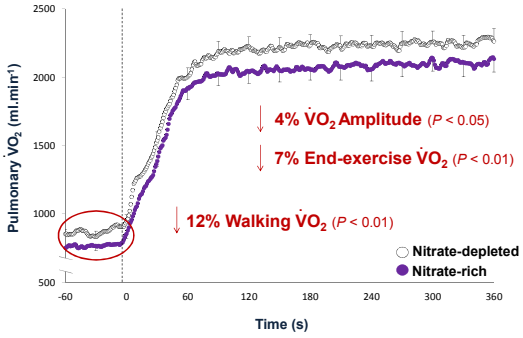
Lansley et al., 2011, *J Appl Physiol*

EXETER

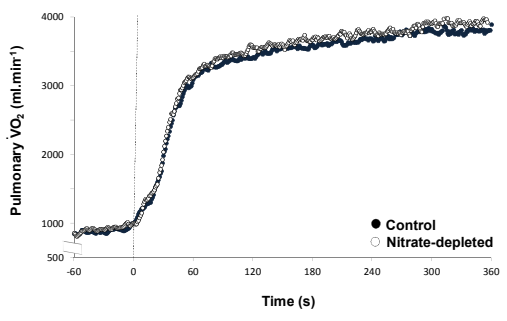
Nitrate-depleted Beetroot Juice and Moderate-Intensity Running



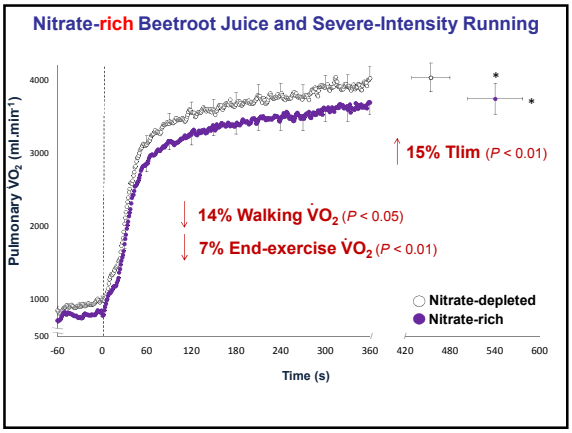
Nitrate-rich Beetroot Juice and Moderate-Intensity Running

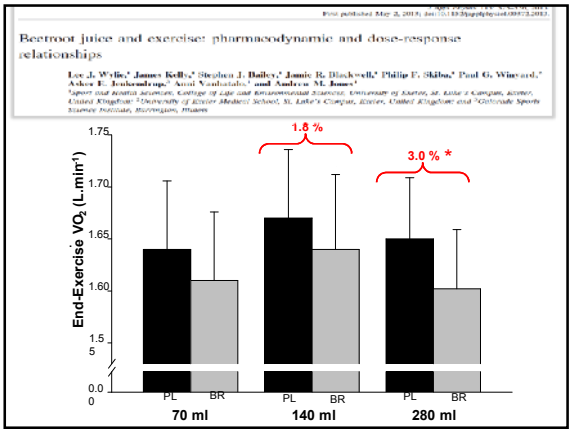


Nitrate-depleted Beetroot Juice and Severe-Intensity Running

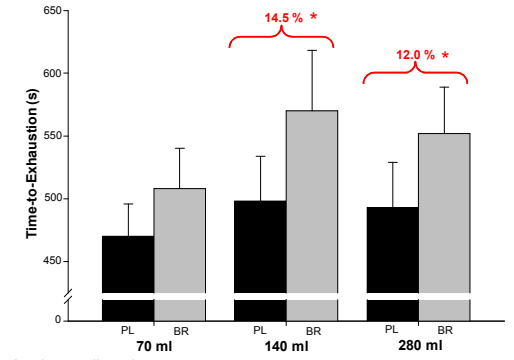


Nitrate-rich Beetroot Juice and Severe-Intensity Running





Exercise tolerance: the dose-response relationship



* Significantly different from PL ($P < 0.05$)

Oxidation

$NO_3^- \rightleftarrows NO_2^- \rightleftarrows NO$

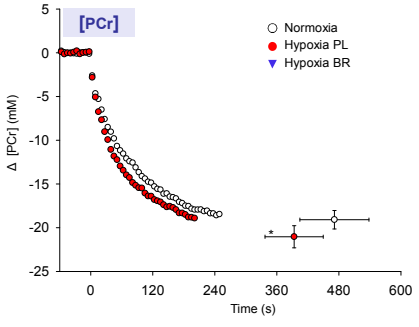
Reduction

Dietary nitrate reduces muscle metabolic perturbation and improves exercise tolerance in hypoxia

Anni Vanhatalo[1], Jonathan Fulford[2], Stephen J. Bailey[1], James R. Blackwell[1], Paul G. Winyard[3] and Andrew M. Jones[1]

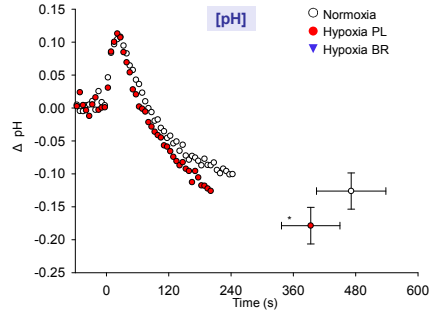
[1]College of Life and Environmental Sciences, [2]Peninsula NIHR Clinical Research Facility and [3]Peninsula College of Medicine and Dentistry, St Luke's Campus, University of Exeter, Heavitree Road, Exeter EX1 2LU, UK

Muscle metabolic perturbation



* T_{lim} different from CON and H-BR ($P<0.05$)

Muscle metabolic perturbation



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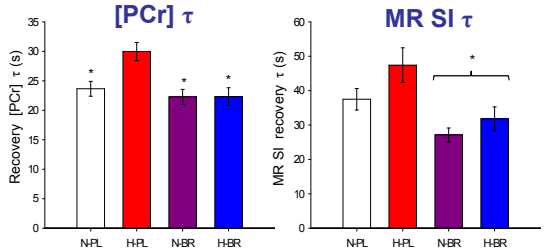
Nitrate speeded the PCr recovery in hypoxia

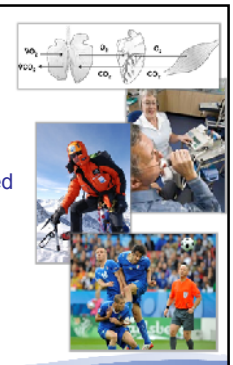
~~Hypoxia potentiated the rate of PCr recovery~~

hypoxia and normoxia



Implications

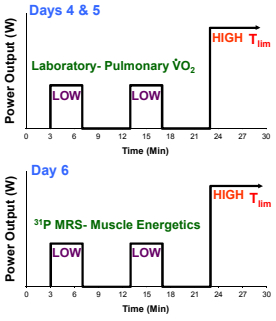
- Disease conditions in which muscle O_2 delivery is impaired
- High altitude exposure
- Intermittent high-intensity exercise



Mechanisms

- Six day intervention period- supplementation with: Beetroot (NO_3^- = 5.1 mmol·day^{-1}) and Placebo (NO_3^- = negligible)

Bailey et al., 2010, J Appl Physiol



Measurements

In Vivo Muscle Metabolism: Calibrated ^{31}P-MRS

[Pi] – comparing signal intensities from an external phosphoric acid source and the subjects' quadriceps.

[PCr] – ratio of P_i:PCr

pH – chemical shift of the P_i spectral peak relative to the PCr peak

[ADP] – calculated using the procedures of Kemp et al. (2001).

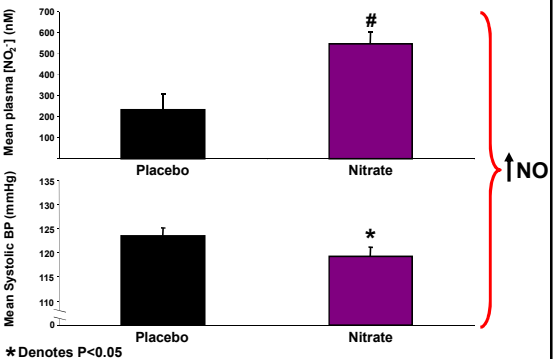
ATP_{Pcr} – rate of change of [PCr] from modeling [PCr] at each time point

ATP_{Gly} – determined by proton flux assuming 1 mol H^+ yields 1.5 mol ATP

ATP_{Ox} – hyperbolic relationship between ATP production rate and cytosolic [ADP]

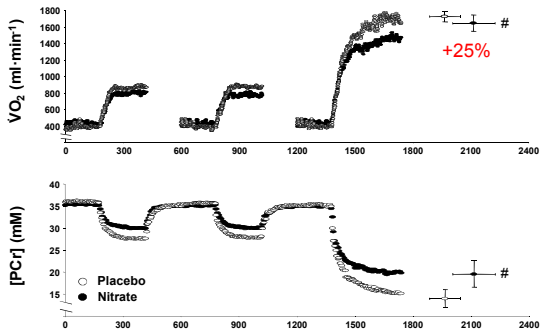
ATP_{TOTAL} = ATP_{Pcr} + ATP_{Gly} + ATP_{Ox} (Lanza et al., 2006; Layec et al., 2009).

Influence of Dietary Nitrate on Indices of NO Production



*Denotes P<0.05
# Denotes P<0.01

Influence of Dietary Nitrate on $\dot{V}O_2$ and Muscle [PCr] Dynamics



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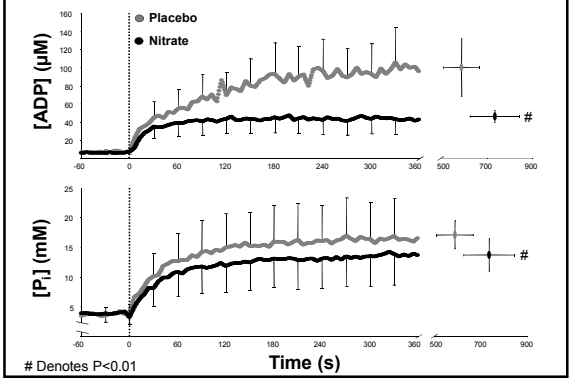
High-Intensity Exercise Intramuscular Metabolic Perturbation



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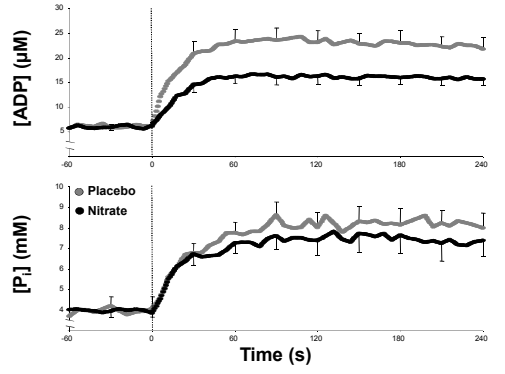
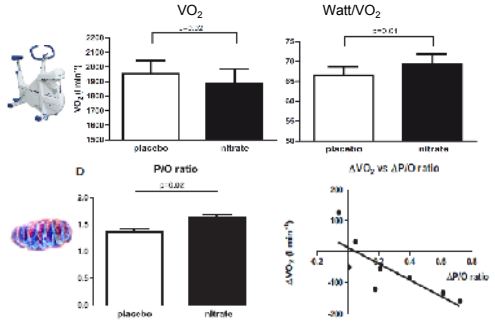
Low-Intensity Exercise Intramuscular Metabolic Perturbation

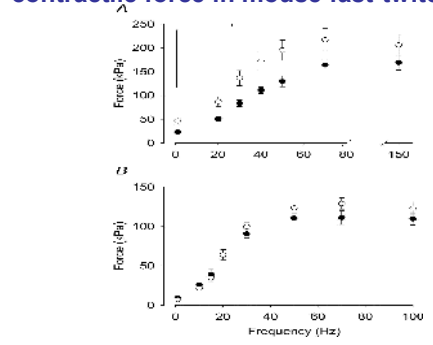


Mechanisms: nitrate and mitochondria



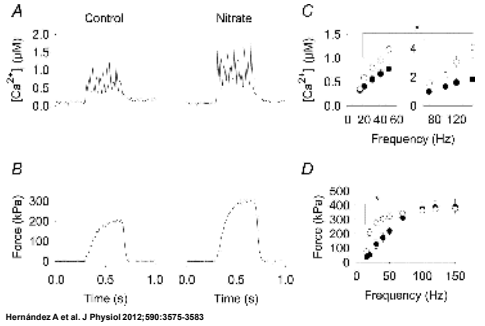
Larsen et al. (2011). Cell Metabolism, 13, 149-159

Dietary nitrate increases tetanic [Ca2+]i and contractile force in mouse fast-twitch muscle



Hernández A, et al., J Physiol.

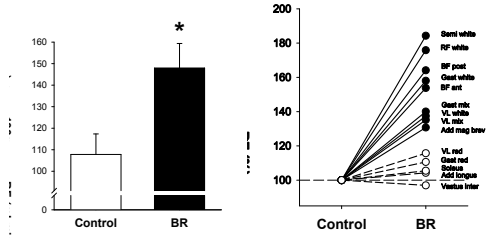
Tetanic [Ca2+]i and force are increased in FDB fibres of nitrate fed mice [Ca2+]i (A) and force traces (B) from representative Control and Nitrate fibres during 30 Hz stimulation



Hernández A et al. J Physiol 2012;590:3575-3583

The Journal of Physiology

Effects on Blood Flow and Flow Distribution



Ferguson et al., 2012

Performance

A 'time-to-exhaustion' or incremental test is a measure of 'exercise capacity' rather than athletic performance

Purpose:

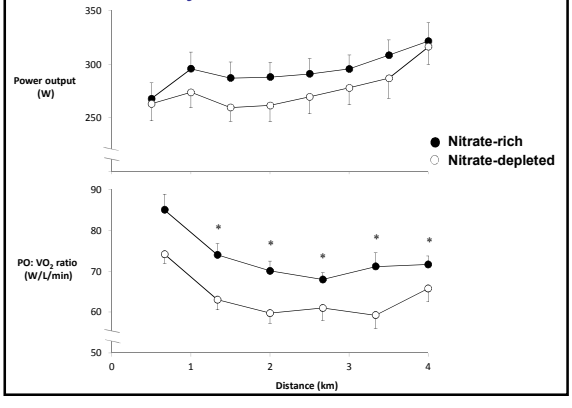
• Does acute (2.5 h) dietary nitrate supplementation affect cycling time trial performance in trained athletes?

• Subjects asked to complete a 4 km (and 16.1 km) distance in the fastest possible time

Lansley et al., 2011, *Med Sci Sports Exerc*

EXETER

Influence of Dietary Nitrate on 4 km Time Trial Performance



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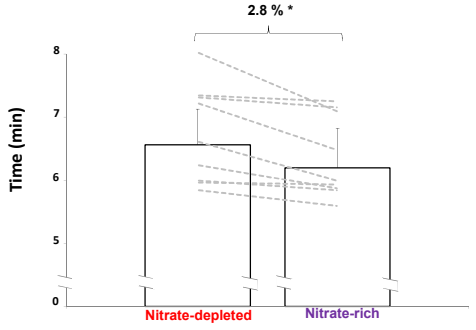
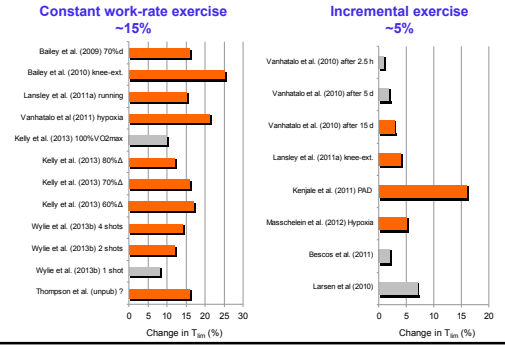
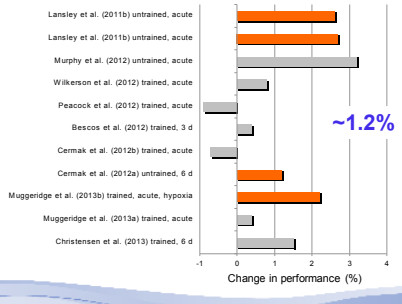


Effects of nitrate on exercise tolerance



How about time-trial performance?



EXETER

The Effect of Nitrate Supplementation on Exercise Performance in Healthy Individuals: A Systematic Review and Meta-Analysis

*"Across studies measuring time trial performance in trained cohorts, there was a ~**0.9% improvement** following nitrate supplementation. To put this in context, the measured difference between first and fourth place for elite swimming performance has been calculated to be **0.6%** (Trewin et al., 2004), and improvements as little as **0.3%** have been noted to be valuable to elite track and field athletes (Hopkins, 2005)".*

Hoon *et al., Int J Sport Nutr Exer Metab 2013*

Elite sports performance

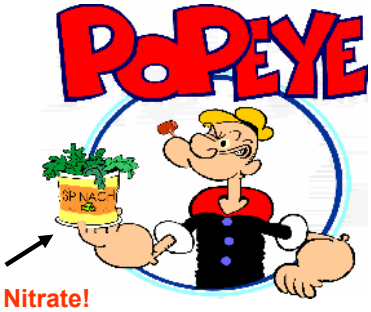
Recent Studies on Performance

What about élite athletes?

- Possible that nitrate supplementation may be less effective in very highly-trained endurance athletes
- Perhaps related to higher NOS activity, muscle oxygenation, mitochondrial function or baseline plasma [nitrite]
- Possible differences in effects of nitrite/NO on type I and type II fibres
- Nearly all nitrate studies with highly-trained endurance athletes have used acute supplementation of 'standard' nitrate dose

A (Mediterranean) diet that increases NO bioavailability may enhance:

- Cardiovascular health ('5 a day')
- O_2 delivery and distribution
- Skeletal muscle function
- Exercise economy
- Exercise tolerance / performance?



Rich in Nitrate!

With thanks...

a.m.jones@exeter.ac.uk
Twitter:@AndyBeetroot

• Jamie Blackwell • Stephen Bailey • Anni Vanhatalo • Jimmy Kelly • Fred DiMenna • Daryl Wilkerson • Paul Winyard • Jon Fulford • Ben Benjamin • Lee Wylie

Toxicology?

- **Nitrate consumption is regulated but evidence for it being harmful is weak; WHO says there is "no evidence that nitrate is carcinogenic"**
- **Diets with more nitrate associated with less c.v. disease and longer life**
- **The human body produces nitrite and nitrate endogenously**
- **Any possible harmful effect of nitrosation (formation of n-nitrosamines) is blunted by antioxidants which accompany nitrate in vegetables**
- **Nitrate is not toxic (due to limited and slow conversion to nitrite) but possibility of toxicity (methemoglobinemia) with nitrite salts**
- **Consensus is that nitrate supplementation with vegetable products is very unlikely to be harmful**