

DIETARY NITRATE DOES NOT INDUCE HYPOXIA DEPENDENT AUGMENTED OXYGEN DELIVERY IN SKELETAL MUSCLE IN YOUNG HEALTHY SUBJECTS.

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Introduction: Dietary inorganic nitrate supplementation has recently been shown to improve skeletal muscle metabolic efficiency, through its conversion into nitrite and nitric oxide. In addition, it has been postulated that nitrate may induce a hypoxia dependent vasodilation, and in turn augment oxygen delivery to exercising muscle.

Hypothesis: We therefore hypothesise that dietary nitrate induces an augmented oxygen delivery to exercising skeletal muscle at high exercise intensity in young healthy subjects. This will manifest itself as a change in effective transverse relation rate (R_2^*), an indicator of tissue oxygenation

Methods: A group of 9 young healthy volunteers (age 33.3 ± 4.4 years, 5 male), with normal physical activity levels (International Physical Activity Questionnaire score 600-10,000 METs/week) were recruited. Each subject took part in three separate MRI visits interspersed by at least a week. The three scan visits consisted of identical experimental procedures, with 7 cl concentrated beetroot juice (containing 0.4g nitrate) was consumed 2.5 hours ahead of the third scan visit. Thus the reproducibility can be assessed through the comparison between visits 1 and 2, while the effect of nitrate revealed through the comparison between visits 2 and 3. Each visit consisted of baseline physiological measurements immediately followed by the MRI study. The study was approved by the local ethics committee and informed written consent was obtained prior to the study.

Physiological Measurements: Physiological monitoring was performed using a MAGLIFE physiological monitor during 10 min supine rest. Blood pressure (BP), respiratory and heart rate, oxygen saturation, inspired and end tidal CO_2 were recorded. The recordings of the final 5 min were averaged as the baseline physiology.

MRI Experiment: The MRI study was performed on a 3T Achieva TX scanner (Philips Medical System, Best, Netherlands), using a body coil for transmission and SENSE knee coil as receiver. Standard clinical anatomical images, angiograms and two quantitative fMRI runs were collected during each visit. Anatomical images were acquired using a standard clinical T_1 weighted sequence, so that muscle groups of soleus, medial and lateral gastrocnemius could be clearly identified. Angiograms were collected using phase contrast method, so that large vessels could be excluded from the analysis. Two fMRI runs were performed using a dual echo gradient echo fMRI sequence (FOV 200×200 mm², matrix size 80×80 , tem 5 mm slices, TR of 3 s TE_s of 21/60 ms) in concurrence with the plantar flexion exercise paradigm with a fixed amount of weight load (15% and 25% maximum voluntary contraction (MVC) in the two consecutive runs). The exercise paradigm consisted of 60s rest followed by 4 cycles, where each cycle consisted of 90s plantar flexion at 0.33 Hz followed by 120 s recovery.

Image analysis: fMRI images were motion corrected and spatially smoothed in SPM8 using default parameters. Regions of interest (ROIs) were generated in the soleus and gastrocnemius (containing medial and lateral components), using squares on each slice in the central 6 slices (Fig 1). The time course of each fMRI run was then extracted, and the R_2^* subsequently computed based on the Bloch equation. The time courses of the last 3 cycles were then averaged to generate a cycle averaged time course and normalised to the baseline value as percentage change curve (Fig 2). An exponential function was then fitted to the recovery portion of the curve, with the maximal percentage change (MPC) and peak to trough percentage change (PTC) quantified (Fig 2).

Statistical Analysis: One way ANOVA tests with repeated measures were performed on MPC and PTC. A correlation analysis using the Pearson product moment correlation coefficient was performed between the changes in MPC (visit 3-visit 2) and the changes in oxygen saturation, as well as between the changes in PTC and the changes in oxygen saturation.

Results: No significant changes in MRI measures were found between visits 1 and 2. A significant decrease was found in MPC in both soleus (5.12 ± 2.13 to 2.55 ± 1.42 , $p=0.004$, Fig 3) and gastrocnemius (6.04 ± 2.36 to 4.26 ± 1.50 , $p=0.017$, Fig 4) after dietary nitrate supplementation for 15% MVC. There was no significant decrease in MPC for 25% MVC. This decrease was not significantly correlated with oxygen saturation

Discussion: Our results indicate that the enhancement of muscle metabolic function from nitrate is mainly on type 1 fibres at lower exercise intensity, which does not support the postulation of hypoxia dependent augmented oxygen delivery. Transverse relation time (T_2) in skeletal muscle is known to increase with exercise intensity, as a result of vascular dilation effects. An hypoxia dependent vasodilation would increase T_2 and reduces R_2^* at higher exercise intensity. Therefore the consideration of T_2 effects would not alter the conclusion drawn.

Conclusion: Dietary nitrate does not induce a hypoxia dependent augmentation in oxygen delivery to exercising skeletal muscle in healthy young subjects, and its improvement in muscle efficiency is likely to originate from enhanced mitochondrial function.

