Title: Nitrate supplement benefits contractile forces in fatigued but not unfatigued muscle

Authors: Neale A. Tillin, Sarah Moudy, Kirsty M. Nourse, Christopher J. Tyler

Affiliation: Department of Life Sciences, University or Roehampton, London, UK

Correspondence: Neale A. Tillin

University of Roehampton, Whitelands College, Holybourne Avenue, London, UK, SW5 4JD

Email: neale.tillin@roehampton.ac.uk

Tel: +44 (0)20 8392 3542

Short Title: Nitrate benefits muscle force when fatigued

Abstract

Purpose: Evidence suggests dietary nitrate supplementation enhances low-frequency (≤20 Hz) involuntary, but not voluntary, forces in unfatigued human muscle. We investigated the hypotheses that nitrate supplementation would also attenuate low-frequency fatigue and the loss of explosive-voluntary forces in fatigued conditions. **Methods:** In a counterbalanced double-blinded order, 17 male participants completed two experimental trials following 7 days of dietary supplementation with either nitrate-rich (NIT) or nitrate-depleted (PLA) beetroot juice. Each trial consisted of measuring isometric knee-extension forces during a series of explosive-maximal voluntary contractions (MVCs) and involuntary-tetanic contractions (at 10, 20 50, and 100 Hz) in unfatigued conditions, followed by a fatigue protocol of 60 MVCs and a repeat of the tetanic contractions immediately post the 60 MVCs. **Results:** In unfatigued conditions, there was no effect of NIT on any of the measured dependent variables; including maximal voluntary force, explosive-impulse, and tetanic peak forces or peak rate of force developments (RFDs) at any frequency. In contrast, the percentage decline in explosive-voluntary impulse from the first to the last 6 MVCs in the fatigue protocol was lower in NIT (51.1 ± 13.9%) than PLA (57.3 ± 12.4%; P=0.039; *d*=0.51). Furthermore, low-frequency fatigue determined via the percentage decline in the 20:50 Hz ratio was attenuated in NIT, for tetanic peak force (NIT, 12.3 ± 12.0% vs. PLA, 17.0 ± 10.1%; P=0.110; *d*=0.46), and tetanic peak RFD (NIT, 12.3 ± 10.4% vs. PLA, 20.3 ± 9.5%; P=0.011; *d*=0.83). **Conclusion:** Nitrate supplementation reduced the decline in explosive-voluntary forces during a fatiguing protocol, and attenuated low-frequency fatigue, likely due to reduced disruption of excitation-contraction coupling. However, contrary to previous findings, nitrate supplementation had no effect on contractile performance in unfatigued conditions.

Key words: low-frequency fatigue, rate of force development, force-frequency relationship, beetroot juice, excitation-contraction coupling, explosive strength

Introduction

Nitric Oxide (NO) is an important signalling molecule within the body, generated endogenously by the oxidation of L-arginine (1). NO can also be generated through the reduction of dietary inorganic nitrate to nitrite, by facultative bacteria in the oral cavity, with nitrite further reduced to NO within various tissues around the body (2). It is through this nitrate-nitrite pathway that supplementing the diet with inorganic nitrate appears to increase the bioavailability of NO and have measurable physiological effects, including reduced blood pressure (3, 4), increased exercise economy (4-6), and improved endurance performance (5, 7). These effects have been widely investigated over the last decade (8), but only recently has evidence suggested nitrate supplementation may also enhance the excitation-contraction coupling of skeletal muscle, resulting in greater force production for a given excitation (9-11).

Hernadez et al. (10) supplemented the diet of rats with nitrate for 7 days and reported improved tetanic peak force at low (≤50 Hz) stimulation frequencies in fast- but not slow-twitch muscle fibres, which was associated with increased release of Ca^{2+} from the sarcoplasmic reticulum (SR). Three *in vivo* human studies have since investigated involuntary contractile responses of the mixed-fibre quadriceps muscles following dietary supplementation with nitrate-rich beetroot juice, and whilst two reported 5-20% improvements in low-frequency (≤20 Hz) tetanic peak force (9, 11), the third reported no effects (12). These studies used different control conditions – nitrate-depleted beetroot juice (12), lime cordial (9), and no placebo (11) – to compare with the nitrate supplement condition, which may have contributed to the inconsistent results. Furthermore, the benefits of nitrate supplementation to contractile performance seem

47 specific to fast twitch fibres (10), and so will likely be diluted and thus variable in human whole
48 mixed-fibre muscles. Nevertheless, assuming it is possible to improve excitation-contraction
49 coupling and enhance low frequency force in human muscle, this would theoretically benefit
50 humans during voluntary contractions, where free cytosolic Ca^{2+} is low or rising, such as during
51 repeated submaximal voluntary contractions or during the rising slope of the force-time curve
52 of explosive voluntary contractions. The latter was investigated by Haider and Folland (9), and
53 whilst nitrate supplementation improved explosive force during involuntary twitch and 300-Hz
54 tetanic contractions, explosive force during voluntary contractions was unaffected. This poor
55 translation of effects from involuntary to voluntary contractions may have been due to the large
56 variability in neural drive during voluntary explosive contractions (13), which is a more
57 important determinant of explosive voluntary force than involuntary explosive force (13, 14).
58 It is conceivable however, that the influence of nitrate supplementation on explosive voluntary
59 force may become more apparent in fatigued conditions where excitation-contraction coupling
60 is disrupted.

61 A common feature of neuromuscular fatigue is the greater reduction of force at low (<50 Hz)
62 vs. high (≥50 Hz) stimulation frequencies (low-frequency fatigue; (15)), thought to be largely
63 caused by reduced SR Ca^{2+} release (16-18), and reflective of disruption to excitation-
64 contraction coupling. Low-frequency fatigue appears to have two components; one dependent
65 on metabolite accumulation, observed immediately after fatiguing exercise of sufficiently high
66 force-time integral (19, 20) but which recovers within minutes (16, 19); and one observed after
67 several minutes of recovery which can last for hours (15, 19) and is thus independent of
68 metabolite accumulation. Recent evidence of nitrate supplementation increasing SR Ca^{2+}
69 release in unfatigued rat muscle (10), and reducing metabolic perturbation during fatiguing
70 contractions in humans (21), raises the possibility that nitrate supplementation may attenuate
71 the first component of low-frequency fatigue, by countering the mechanisms causing it. Hoon

et al. (12) reported a reduction in the fatigue of low frequency (20 Hz) peak force during repeated contractions with hypovolemia, despite observing no effects in unfatigued conditions, suggesting the influence of nitrate supplementation becomes more evident during fatiguing conditions known to disrupt excitation-contraction coupling. However, Hoon et al. (12) did not quantify low-frequency fatigue (low vs. high frequency forces), so the extent of disruption to excitation-contraction coupling in their protocol, and benefit of nitrate supplementation to any disruption remain unclear. Moreover, it is unknown whether the effects of nitrate supplementation on explosive voluntary force also become more evident with fatiguing exercise that disrupts excitation-contraction coupling.

The purpose of this study was to investigate the effects of dietary nitrate supplementation on involuntary and voluntary contractile responses in human muscle, during both unfatigued and fatigued conditions. We hypothesised that in unfatigued conditions nitrate supplementation would enhance low-frequency force but not affect explosive voluntary force; whilst in fatigued conditions nitrate supplementation would attenuate both low-frequency fatigue and the loss of explosive voluntary force. The fatiguing protocol employed in this study was a 5-min all-out bout of 60 MVCs in which mean MVC force declines to a plateau representing a critical force threshold (22), due to the depletion of high energy phosphates and considerable metabolite accumulation (23). This protocol was chosen to enable between-condition differences in force-time characteristics of MVCs during the protocol, whilst simultaneously ensuring a plateau in fatigue and metabolic perturbation prior to testing the involuntary contractile responses immediately after the protocol. We also predicted the protocol would provide sufficient metabolic stress and force-time integral to observe the first component of low-frequency fatigue.

Methods

Participants

Seventeen healthy, non-smoking, recreationally active males (mean ± SD; age, 23 ± 4 years; body mass, 74.04 ± 9.62 kg; height, 1.75 ± 0.06 m) volunteered to participate in this study which was approved by the University of Roehampton Ethical Advisory Committee. Participants provided written informed consent prior to their involvement.

Experimental Overview

Similar to the design of Haider and Folland (9), each participant visited the laboratory at a consistent time of day on four separate occasions to complete two familiarisation, and two experimental trials. Seven days separated each of the first three trials, whilst the two experimental trials were separated by 9 days and completed in a randomised, double-blinded order. In the seven days immediately prior to each experimental trial participants supplemented their diet with either nitrate rich (NIT) or nitrate depleted (PLA) beetroot juice. During the course of the study participants were requested to maintain habitual physical activity and diet, not use antibacterial mouthwash, and abstain from caffeine (for 6 hours), alcohol (for 24 hours) and vigorous exercise (for 36 hours) before experimental trials.

Each trial involved the same protocol of isometric voluntary and involuntary contractions of the knee extensors of the dominant leg, determined as the preferred leg to kick a ball with. In the protocol, participants first completed explosive-maximal voluntary contractions (MVCs) and electrically evoked 1-s tetanic contractions at 10, 20, 50, and 100 Hz, to determine neuromuscular function in unfatigued conditions. Participants then completed a fatiguing protocol of 60 MVCs, followed immediately by the same series of tetanic contractions as above to determine neuromuscular function in fatigued conditions. External knee-extensor force and

surface electromyography (EMG) were recorded throughout the measurement trials. Finger-prick blood samples were collected at the start of each experimental trial, to determine plasma nitrate and nitrate concentrations. Data analysis was completed before un-blinding the investigators to the condition order for each participant.

Supplementation

Participants supplemented their diet with 70-ml shots of concentrated nitrate-rich (NIT; 400-500 mg per 70ml) or nitrate-depleted (PLA; 0.35-1.26 mg per 70ml) beetroot juice (non-organic SPORT shot, Beet It, James White Drinks Ltd, Ipswich, UK). Two shots were taken per day for the 7-day supplementation period; one each morning and one each evening except for the day of the experimental trial when both shots were taken together 2.5 hours before the trial. Daily nitrate supplementation was ~12.9 mmol and ~0.01-0.04 mmol, in the NIT and PLA conditions, respectively.

Force and EMG recordings

Participants sat in a custom-built, low compliance, isometric knee-extensor strength testing chair (9), with hip and knee angles of 100° and 120°, respectively (180° = anatomical position). Shoulder and pelvis strapping secured participants tightly to the chair, minimising upper body movements. An ankle strap (35-mm width reinforced canvas webbing) was placed around the leg being tested, at a constant 4 cm above the lateral malleolus. The strapping was in series with a linear-response S-beam strain gauge load cell (1.5 kN maximum amplitude and amplitude resolution of 1/2000; Force Logic, Swallowfield, UK), positioned perpendicular and posterior to the shank. The force signal was amplified (x370), sampled at 2000 Hz via an AD convertor (Micro 1401; CED, Cambridge, UK) and recorded on a PC utilising Spike2 software (CED, Cambridge, UK). Offline, the force signal was low pass filtered at 500 Hz with a 4th

order zero lag Butterworth filter, and corrected for the weight of the shank by subtracting resting baseline force.

Following preparation of the skin (shaving, lightly abrading, and cleansing with 70% ethanol), single differential surface EMG electrodes (2-cm diameter Ag-Ag-Cl gel; 2-cm inter-electrode distance; Noraxon U.S.A., Inc., Scottsdale, Arizona) were placed over the belly of the muscle of the rectus femoris (RF), vastus lateralis (VL), and vastus medialis (VM). Electrodes were positioned parallel with the presumed orientation of the fibres; and at ~50% (RF), ~54% (VL), and ~88% (VM) of the distance between the greater trochanter and the lateral femoral condyle. EMG signals were filtered (10 Hz, high pass) and amplified (x200) at the source (TeleMyo DTS, Noraxon U.S.A., Inc., Scottsdale, Arizona), transmitted wirelessly to the DTS desktop receiver for further amplification (total system gain x500), and sampled at 2000 Hz via the same AD convertor and PC software as the force signal. Offline, EMG signals were corrected for the 156-ms delay inherent in the Noraxon wireless system, and band-pass filtered between 10-500 Hz with a 4^{th} order zero-lag Butterworth filter. All measurements of EMG amplitude (see Fatigue Protocol section) were averaged across the three quadriceps muscles to give a single mean value for the knee extensors.

Unfatigued Voluntary Contractions

Following a series of warm-up contractions (2, 3-s contractions each at 30, 50, 70, and 90% of perceived maximal effort), participants completed 10 MVCs (each separated by ~60 s), in which they were instructed to push as “fast and hard” as possible for ~3 s, from a relaxed (zero active tension) state and without prior countermovement. Participants were instructed to focus on pushing fast in the early phase (first second) of the MVC, followed by as hard as possible after that to maximize explosive and maximal forces, respectively (24). Biofeedback was provided on a computer monitor in front of participants, displaying; (i) the force signal with a

cursor on the greatest force achieved so far that session; (ii) the resting baseline force on a sensitive scale to provide feedback on whether a countermovement or pre-tension had occurred prior to the MVC; and (iii) the slope of the force-time curve (40-ms time constant) with a cursor on the highest peak slope achieved so far that session. Verbal encouragement was provided throughout.

All data were analysed using custom-developed computer programmes in Matlab (The MathWorks inc., Natick, MA, USA). Maximal voluntary force (MVF) was determined as the greatest peak force recorded in any MVC performed in that session. Explosive impulse (force-time integral) was measured over the first 50 (IMP_{0-50}), 100 (IMP_{0-100}), and 150 ms (IMP_{0-150}) from force onset, and averaged across the 3 valid MVCs with the highest IMP_{0-100}, in the unfatigued condition. Force onset was defined as the last data point before the slope of the force-time curve (2-ms time constant) crossed and remained above zero for the time it took force to reach 50% MVF. Valid MVCs for explosive impulse measures were considered those that had no pre-tension or countermovement prior to force onset, determined via the following criteria: (i) mean baseline force in the 200-ms immediately prior to force onset was between -1% and 1% MVF; and (ii) force at onset was within 1 N of this mean baseline force.

Unfatigued Tetanic Contractions

One-second, tetanic contractions were evoked with a train of square-wave electrical impulses (0.2-ms pulse width; DS7AH, Digitimer Ltd, UK), via two carbon rubber electrodes (14 x 10 cm; Electro Medical Supplies, Wantage, UK) placed ~8 cm apart at proximal (anode) and distal (cathode) ends of the anterior surface of the thigh. Starting at a near imperceptible electrical current (~20 mA), 100-Hz tetanic contractions were evoked at 20-s intervals, and the current intensity was gradually increased (20-30 mA steps) with each contraction until the peak force response reached 50% of the MVF measured in the familiarization session (typically at 100-

150 mA). At this stimulation intensity, 2 sets of 4 tetanic contractions were evoked with one contraction each at 10, 20, 50, and 100 Hz per set. The order of the four different stimulation frequencies within a set was randomized between participants, but remained constant for both sets and conditions (i.e., NIT and PLA) for each participant. Two seconds separated consecutive tetanic contractions within and between sets.

Tetanic peak force was determined as peak instantaneous force for 10 Hz contractions, or mean force calculated over a 300-ms period at peak instantaneous force (150-ms either side of peak, or 300 ms prior to peak if at the end of the plateau) for 20, 50, and 100 Hz contractions. Tetanic peak rate of force development (RFD) was determined as the peak instantaneous slope of the force-time curve over a 25-ms moving time window. For each frequency, peak forces and RFDs were averaged across the two sets, and calculated relative to peak force or RFD at 100 Hz, respectively, controlling for any differences in stimulation intensity between conditions. The 20:50 Hz ratio was also measured for both peak force and RFD to assess any differential effects of condition on low (20 Hz) vs. high (50 Hz) frequencies.

Fatigue Protocol

Following a 10-min recovery out of the strength testing chair, participants repeated the warm-up outlined above before completing a fatigue-protocol involving 60, 3-s MVCs (each separated by 2-s). This fatigue protocol has previously been shown to elicit a decline in mean force with each MVC to an asymptotic plateau (critical force threshold) by the last 6 MVCs, reflecting the highest force that can be maintained with a metabolic steady state (22, 23). The instruction with each MVC was as above for the unfatigued MVCs (i.e., “push fast and hard”), but participants were also instructed to relax as quickly as possible at the end of each MVC in preparation for the next MVC in the series. The timing of each MVC was maintained using a digital metronome (Tempo Application for IPad; FrozenApe.com). Participants were instructed

not to pace themselves, but to produce a maximal effort with each MVC, and were blinded from the time and MVC number until immediately before the last MVC. Two horizontal cursors were placed at 90 and 85% of MVF recorded in the unfatigued MVCs, and participants were required to exceed these forces in the first and second MVCs of the protocol, respectively. Failure to do so was considered indicative of sub-maximal efforts from the start, in which case the protocol was interrupted, 5-min recovery given, and the protocol re-attempted, for a maximum of three attempts. The same two sets of tetanic contractions as performed in unfatigued conditions were completed, commencing at a similar 3.0 ± 1.1 s (PLA) and 2.6 ± 0.8 s (NIT; paired comparison, $P = 0.278$) after the last MVC in the fatigue protocol.

For each MVC in the fatigue protocol, the force-time integral (impulse), mean force, and root mean squared (RMS) EMG amplitude were calculated between the data points where force increased above and decreased below 2% MVF. This threshold for detecting MVC on- and offset was selected for computational reasons to avoid misidentifying MVCs during recovery periods. Total impulse was determined from the sum of impulses of all 60 MVCs. Mean MVC force and EMG amplitudes were averaged across MVCs within 10 consecutive bins of 6 MVCs. End-test force reflecting the critical force threshold, was defined as the mean MVC force of the last 6 MVCs (bin 10). Fatigue indexes were calculated for mean MVC force and EMG amplitude, as the percentage decline from the first 6 (bin 1) to the last 6 MVCs (bin 10). Explosive impulse over the first 0-150 ms from force onset (IMP_{0-150}) was determined for each valid MVC using the same methods as above for the unfatigued MVCs, and averaged across the 3 valid MVCs in each bin with the greatest IMP_{0-150}. Explosive impulses over earlier phases (0-50 and 0-100 ms) were not calculated for the fatigue protocol due to the inherent variability in early phase explosive force (13), which appeared augmented by our fatigue protocol likely due to the limited recovery time between MVCs. Explosive RMS EMG amplitude over the first 0-150 ms from EMG onset (EMG_{0-150}), was also measured and averaged across the same three

MVCs used to determine $IMP_{0\text{-}150}$ in each bin. EMG onset was defined in the first muscle to be activated, as the last data point before the RMS EMG signal with a 2-ms moving time constant, increased and remained above the mean of the baseline RMS for 0.5 s. Fatigue indexes from bin 1 to 10 were calculated as above for $IMP_{0\text{-}150}$ and $EMG_{0\text{-}150}$.

Tetanic peak forces and RFDs at 10, 20, 50, and 100 Hz, and the 20:50 Hz ratio, were determined for the tetanic contractions post-fatigue protocol via the same methods explained above for the unfatigued conditions. The fatigue index (percentage decline) from pre- to post-fatigue protocol was determined for each frequency and the 20:50 Hz ratio, for both tetanic peak force and RFD. A positive fatigue index for the 20:50 ratio (i.e., a decline in this ratio following the fatigue protocol), was considered evidence of low-frequency fatigue.

Plasma Nitrate and Nitrite

Capillary, finger-prick blood samples were taken at upon arrival to the laboratory for each experimental trial. Whole-blood was collected into 3 x 300 μL EDTA-treated microvettes and immediately centrifuged in a micro-centrifuge for 15 min at 1000 x g. Following centrifugation the supernatant (300-400 μL) was removed and frozen at -80°C until analysis. Plasma nitrate and nitrite concentrations were determined by ozone-based chemiluminescence (model 88AM; Eco Physics) using previously reported methods (25). First, total NOx (all nitroso species) was measured by injecting an aliquot (50 μL) of each sample into a solution of vanadium (III) chloride (50mM) dissolved in 1M-HCl, within an airtight microreaction vessel connected to the chemiluminescence analyser. Plasma nitrite was then determined in a two-step process by: (i) injecting an aliquot (100 μL) of plasma into a solution of glacial acid acetic containing 45 mM-potassium iodide and 10 mM-iodide, at 60°C, and actively purged by inert He, which measured plasma nitrite + other nitroso species (but not nitrate); and (ii) treating the plasma with acidic sulphanilamide (1M-HCl) to scavenge nitrite, before injection (100 μL), allowing for

quantification of nitroso species not including nitrate or nitrite. Nitrite was then determined as the difference between the measures in step (i) and (ii), whilst plasma nitrate was determined as the difference between total NOx and the measure in step (i). Resources were only available to analyse plasma samples from the first 11 participants to complete the study.

Statistical Analysis

Two way repeated measures ANOVAs were used to determine the effects of supplementation on: unfatigued explosive impulse (2 supplements (PLA and NIT) vs. 3 time-epochs (IMP_{0-50}, IMP_{0-100}, and IMP_{0-150})); unfatigued normalised tetanic peak force and RFD (2 supplements (PLA and NIT) vs. 3 frequencies (10, 20, and 50 Hz)); and fatigue index of both tetanic peak force and RFD (2 supplements (PLA and NIT) vs. 4 frequencies (10, 20, 50, and 100 Hz)). In the instance of a main or interaction effect, paired t-tests were used for post-hoc paired comparisons. The effects of supplementation on all other dependent variables were determined via paired t-tests. Cohen's *d* effect sizes were determined for each paired comparison (26). To determine if mean MVC force had reached a plateau representing a critical torque threshold in the fatigue protocol, a linear function ($y = mx + c$) was fitted to the data plotting the relationship between mean MVC force (y) and MVC number (x) for the last 6 MVCs (bin 10). The slope (m) of this linear function was compared to zero using a paired t-test, for PLA and NIT separately, with no significant differences reflecting a plateau in mean MVC force. Statistical significance was considered where $P<0.05$, and statistical analysis was completed using IBM SPSS Statistics version 21. Data are reported as means ± standard deviation (SD).

Results

Nitrate and Nitrite

Plasma nitrate and nitrite were 10.3-fold greater ($P<0.001$; $d = 12.6$) and 1.8-fold greater ($P = 0.002$; $d = 4.66$), respectively, in NIT than PLA (Figure 1).

[INSERT FIGURE 1 HERE]

Unfatigued Conditions

Voluntary contractions. There was no effect of supplement on MVF ($P = 0.887$; $d = 0.01$; Table 1). There was also no main effect of supplementation ($P = 0.911$) or supplementation by time-epoch interaction effect ($P = 0.903$) on explosive impulse recorded during the MVCs performed in unfatigued conditions (Table 1).

Tetanic contractions. Tetanic peak force at 100 Hz in unfatigued conditions was 47 ± 4% of MVF and 46 ± 3% of MVF in PLA and NIT, respectively, with no difference in the absolute value between the two conditions (PLA, 348 ± 60 N; NIT, 343 ± 60 N; $P = 0.176$; $d = 0.08$). There was also no difference in tetanic peak RFD at 100 Hz between PLA (4468 ± 942 $N.s^{-1}$) and NIT (4542 ± 982 $N.s^{-1}$; $P = 0.608$; $d = 0.08$). These results suggest stimulation intensity was constant across conditions. There were no main effects of supplementation ($P \geq 0.718$) nor supplementation by frequency interaction effects ($P \geq 0.382$) on either tetanic peak force or RFD recorded at 10, 20, and 50 Hz, in unfatigued conditions (Table 1). Consequently, the 20:50 Hz ratio in unfatigued conditions was also similar between NIT and PLA for both tetanic peak force ($P = 0.317$; $d = 0.16$; Table 1) and RFD ($P = 0.657$; $d = 0.10$; Table 1).

[INSERT TABLE 1 HERE]

Fatigued Conditions

Validity of fatigue protocol. Three participants achieved <90% MVF in the first MVC and/or <85% MVF in the second MVC, of the fatigue protocol, in either PLA and/or NIT, and so were excluded from all fatigued-condition measurements on the assumption they were not performing maximal efforts from the start. In the remaining 14 participants, mean MVC force averaged across the 6 MVCs within each consecutive bin, declined in an exponential manner (Figure 2), so by the last 6 MVCs (bin 10) the slope of the linear relationship between mean MVC force (relative to MVF) and MVC number was statistically similar to zero in both PLA (-0.17 ± 0.74 %MVF/MVC; P = 0.394) and NIT (0.52 ± 1.23 %MVF/MVC; P = 0.140). This suggests the 14 remaining participants were consistently producing maximal efforts throughout the protocol, and mean MVC force declined to an asymptote likely representative of a critical force threshold (22). One participant of the 14 remaining was unable to record a valid MVC for explosive impulse analysis (i.e., there was countermovement or pre-tension before force onset) during the first 6-MVCs (bin 1) in PLA, and so was removed from analysis of explosive impulse and EMG during the fatigue protocol.

[INSERT FIGURE 2 HERE]

Voluntary contractions. There were no effects of supplementation on total impulse (P = 0.326; d = 0.05), end-test force (P = 0.388; d = 0.07), mean MVC force fatigue index (P = 0.198; d = 0.19), or mean MVC EMG amplitude fatigue index (P = 0.308; d = 0.21), recorded during the fatigue protocol (Table 2). However, the fatigue index for $IMP_{0\text{-}150}$ was greater in the PLA compared with NIT for 8 of the 13 participants (Figure 3) resulting in a moderate and statistically significant effect of supplementation on explosive impulse fatigue (P = 0.039; d = 0.51; Table 2; Figure 3). This was despite a similar fatigue index for $EMG_{0\text{-}150}$ in both conditions (P = 0.286; d = 0.39; Table 2).

[INSERT TABLE 2 AND FIGURE 3 HERE]

Tetanic Contractions. There was a main effect of stimulation frequency on tetanic peak force fatigue index ($P<0.001$), due to greater fatigue at 10 Hz than all other frequencies ($P<0.001$; $d = 0.62$-1.27), and greater fatigue at 20 Hz than 50 or 100 Hz ($P<0.001$; $d = 0.60$-0.76), whilst fatigue at 50 and 100 Hz was similar ($P = 1.000$; $d = 0.01$-0.11), in both PLA and NIT (Figure 4A). There was also a main effect of stimulation frequency on tetanic peak RFD ($P<0.001$), with fatigue tending to be greater at 10 than 20 Hz ($P = 0.011$-0.094; $d = 0.42$-0.56), greater at both 10 and 20 Hz than 50 or 100 Hz ($P<0.001$; $d = 0.41$-1.33), and greater at 50 than 100 Hz ($P<0.001$; $d = 0.43$-0.54), in both PLA and NIT (Figure 4B). The systematically greater fatigue at low (≤20 Hz) compared with high (≥50 Hz) frequencies resulted in reductions from pre- to post-fatigue protocol in the 20:50 Hz ratio for both peak force and peak RFD, in PLA and NIT (Figure 4C and D), showing the occurrence of low frequency fatigue in both conditions.

There was no main effect of supplementation ($P = 0.615$) nor supplementation by frequency interaction effect ($P = 0.253$) on the fatigue index for tetanic peak force. Whilst there was also no main effect of supplementation on the fatigue index for peak RFD ($P = 0.496$), there was a supplementation by interaction effect ($P = 0.042$); however, paired comparisons showed no differences in peak RFD fatigue index between PLA and NIT at any of the frequencies ($P \geq 0.647$; $d = 0.05$-0.27; Figure 4B). Interestingly, there was a smaller reduction in the 20:50 Hz peak force ratio from pre- to post-fatigue protocol in NIT than PLA for 12 of the 14 participants (Figure 4C), resulting in a low-moderate effect of supplementation ($d = 0.46$) that did not reach statistical significance ($P = 0.110$). There was also a smaller reduction in the 20:50 Hz peak RFD ratio from pre- to post-fatigue protocol in NIT than PLA (Figure 4D) that was a large effect ($d = 0.83$) and did reach statistical significance ($P = 0.011$).

[INSERT FIGURE 4 HERE]

Discussion

This was the first study to investigate the influence of dietary nitrate supplementation on voluntary and involuntary contractile performance, in both unfatigued and fatigued conditions. We found no evidence of improved contractile performance in unfatigued conditions following nitrate supplementation, with PLA and NIT recording similar MVF, explosive voluntary impulse over all measured time periods, tetanic peak forces and tetanic peak RFDs at all stimulation frequencies. In contrast, nitrate supplementation reduced fatigue of voluntary explosive impulse by ~11%, during a bout of 60 MVCs. Furthermore, low-frequency fatigue (reduction in 20:50 Hz ratio) was lower in NIT compared with PLA by ~28% and ~39% for tetanic peak force and RFD, respectively, despite fatigue indexes of peak force and RFD being statistically similar for NIT and PLA, at each separate frequency. This suggests nitrate supplementation attenuated the disruption of excitation-contraction coupling caused by the fatiguing protocol, which might explain the reduced fatigue of voluntary explosive impulse in the NIT condition. The benefits of nitrate supplementation to voluntary force production in fatigued conditions were specific to the rising force-time curve, as total force-time impulse, end-test force, and mean MVC force fatigue index during the bout of 60 MVCs were similar for NIT and PLA.

Plasma Nitrate and Nitrite

Seven days of nitrate supplementation successfully raised plasma nitrate and nitrite (10.3- and 1.8-fold, respectively) compared to the nitrate-depleted placebo. We believe these changes measured in only 11 participants likely reflect the responses in all 17 participants, given consistent observations of raised plasma nitrate and nitrite following both acute (≤24 hours)

and chronic (2-15 days) nitrate supplementation with smaller doses than the present study (<12.9 mmol per day; (5, 6, 26-28)). The measured increase in plasma nitrite is of particular importance as this appears to be required to realise typical physiological benefits (e.g., reduced blood pressure) of nitrate supplementation (29). Dietary nitrate intake was not restricted in the current study, and so baseline (PLA) plasma nitrite (352 ± 61 nm) was comparable to other studies without dietary nitrate restrictions (~216-454 nm; (6, 21)), but higher than that recorded in studies with dietary nitrate restrictions (~80-331 nm; (5, 27, 28)). In addition to not restricting dietary nitrate, our blood sampling method (capillary blood in EDTA microvettes) differed to that of other studies (venous blood in lithium heparin tubes (5, 6, 21, 27, 28)) and this may also have contributed to baseline plasma nitrite values in the upper end of the range reported in the literature. Dietary nitrate intake was not restricted in the current study, consistent with the first human study to measure improved contractile performance with nitrate supplementation (9).

Unfatigued Conditions

In unfatigued conditions, nitrate supplementation had no effect on tetanic peak forces or RFD at any stimulation frequency nor on the 20:50 Hz ratios for peak force or RFD. These results are consistent with Hoon et al. (12), but in contrast to two recent studies reporting improvements in low-frequency (≤20 Hz) force (9, 11), increased 20:50 Hz peak force ratio (9), and increased twitch and 300 Hz tetanic explosive forces (9) following nitrate supplementation. The current investigation and the 3 cited studies all tested the quadriceps muscles of seemingly similar cohorts of healthy, young, low/recreationally active males, and involved chronic (4-7 days) supplementation of relatively high doses of nitrate (>9.7 mmol/day), so the reasons for the inconsistent findings are unclear. Nevertheless, here we offer 4 possible explanations to help direct future research. (I) We and the three cited studies used

beetroot juice for nitrate supplementation, but only the current study and Hoon et al. (12) compared the nitrate condition to a placebo of nitrate-depleted beetroot juice rather than blackcurrant cordial with lemon juice (9) or no placebo (11). Thus, other nutrients (e.g., polyphenols or antioxidants) in beetroot juice may have caused the effects observed by Haider and Folland (9) and Whitfield et al. (11). (II) Based on rodent models, it appears nitrate supplementation only improves excitation-contraction coupling in fast twitch fibres (10), so the contractile responses are likely to be diluted and variable in the mixed-fibre human quadriceps muscles, which are typically ~50% fast twitch (30) but can vary from 20-80% (31). (III) Increases in low-frequency force with nitrate supplementation appear to be negatively related to habitual dietary nitrate intake (9), so it is possible the habitual dietary nitrate intake of participants in the current study and that of Hoon et al. (12) was greater than that required to realise benefits from supplementation in unfatigued conditions. (IV) In a series of muscle contractions, such as those used in the methods of this and the other investigations, the first contraction/s in the series would potentiate force and RFD of subsequent low-frequency contractions, through phosphorylation of myosin regulatory light chains increasing Ca^{2+} sensitivity of the myosin-actin cross-bridge (32). It is conceivable potentiation may mask the benefits of nitrate supplementation in unfatigued conditions, and that the amount of potentiation differed between this and previous investigations; but as potentiation was not quantified in any of these studies, this remains speculative.

Maximum voluntary force was unaffected by supplementation, which is consistent with previous findings (9, 12, 21), and expected given nitrate supplementation does not affect force at the firing frequencies (>20 Hz; (9, 11)) typically expected at MVF (~25-40 Hz; (33, 34)). Explosive impulse recorded over all measured time periods from force onset in explosive voluntary contractions in unfatigued conditions was also unaffected by nitrate supplementation in the current study. Theoretically, improved excitation-contraction coupling characterised by

increased force at low frequencies – and thus low free cytosolic Ca^{2+} – may benefit voluntary explosive force where cytosolic Ca^{2+} is rising. However, given we found no evidence of improved excitation-contraction coupling in unfatigued conditions, it is no surprise that voluntary explosive impulse was also unaffected. Furthermore, nitrate supplementation does not appear to improve voluntary explosive force in unfatigued conditions, even when there is evidence of enhanced excitation-contraction coupling (9). The effects of nitrate supplementation on contractile performance might become more evident in fatigued conditions where excitation-contraction coupling is disrupted.

Fatigued Conditions

The fatigue protocol of 60 MVCs resulted in a decline in mean MVC force to a plateau reflecting a critical threshold, in both NIT and PLA. This threshold is thought to represent the highest force that can be maintained with metabolic steady state (22), which likely could not be overcome in the last 6 MVCs of the protocol because of high-energy phosphate depletion and considerable metabolite accumulation (23). Thus, we are confident a plateau in the fatigue and metabolic responses was reached in both conditions. As predicted, the 60 MVCs provided sufficient force-time impulse and metabolic perturbation to elicit the first component of low-frequency fatigue (19, 20), evidenced by greater fatigue at low (≤20 Hz) compared to high (≥50 Hz) frequencies, and a decline in 20:50 Hz ratios for tetanic peak force and RFD, in both NIT and PLA. This low-frequency fatigue reflects disruption of excitation-contraction coupling in both conditions, probably caused by a decline in SR Ca^{2+} release (16-18).

The disrupted excitation-contraction coupling likely contributed to the decline in voluntary explosive impulse from the first 6 to the last 6 MVCs in both NIT and PLA, during the fatiguing protocol. Interestingly, nitrate supplementation attenuated the decline in voluntary explosive impulse, as evidenced by the significantly smaller fatigue index in NIT compared with PLA;

446 although this effect was only observed in 8/13 participants, suggesting some variability in
447 individual responses. Nevertheless, this study provides novel evidence that nitrate
448 supplementation benefits explosive force production in fatigued but not unfatigued conditions.
449 The reduced explosive impulse fatigue in NIT does not appear to be due to differences in neural
450 drive between the conditions, as the explosive EMG fatigue index was similar for NIT and
451 PLA. Therefore, mechanisms at the muscle, which may be associated with attenuated
452 disruption of excitation-contraction coupling (discussed below), likely explain the effects of
453 nitrate supplementation on explosive impulse fatigue index.

454 Nitrate supplementation did not affect total impulse, end-test force, or mean MVC force fatigue
455 index recorded during the 60 MVCs. Thus, the benefits of nitrate supplementation on voluntary
456 force during fatiguing exercise appear to be specific to the rising slope of the force-time curve.
457 This provides some evidence for attenuated disruption of the excitation-contraction coupling
458 in NIT, which would theoretically have its greatest influence during contractile conditions of
459 low or rising free cytosolic Ca^{2+}, such as during the rising slope but not necessarily the plateau
460 of MVC force-time curves. One other study (21) has assessed voluntary forces during repeated
461 MVCs (50 MVCs) following nitrate supplementation and, similar to our results, found no
462 differences in the MVC forces recorded over the plateau of the force-time curve, but they did
463 not measure explosive forces during the rising slope.

464 Whilst the fatigue index for tetanic peak force at each frequency was similar for NIT and PLA,
465 there was evidence of reduced low-frequency fatigue in NIT compared to PLA. Specifically,
466 the decline in 20:50 Hz peak force ratio (a typical measure of low-frequency fatigue; (35)) was
467 ~28% lower in NIT compared to PLA. This difference was not statistically significant ($P = 0.110$), but there was a low-moderate effect ($d = 0.46$), and it occurred in 12/14 participants.
469 The reduced low-frequency fatigue in NIT compared with PLA becomes more evident when

470 considering the tetanic peak RFD results, where the decline in the 20:50 Hz ratio for peak RFD
471 was ~39% lower following nitrate supplementation, which was a large and statistically
472 significant effect ($P = 0.011$; $d = 0.83$). Furthermore, there was a supplementation by frequency
473 interaction effect on tetanic peak RFD fatigue index, which appears to be due to the low
474 frequencies (≤20 Hz) typically displaying less fatigue and the high frequencies (≥50 Hz) more
475 fatigue, in NIT compared with PLA (Figure 4B), although paired differences at each frequency
476 were not statistically significant. Collectively, these results provide novel evidence of reduced
477 low-frequency fatigue following nitrate supplementation, which is more evident during the
478 rising slope of the tetanic force-time curve compared to the peak, and suggests reduced
479 disruption of the excitation-contraction coupling during fatiguing exercise. The first
480 component of low-frequency fatigue appears dependent on metabolite accumulation causing
481 reductions in SR Ca^{2+} release (16, 17). In the current study, nitrate supplementation may have
482 attenuated the first component of low-frequency fatigue by: (i) lowering the PCr cost of force
483 production (21) and thus blunting the metabolite accumulation thought to cause reduced CA^{2+}
484 release; and/or (ii) increasing SR Ca^{2+} release for a given excitation in the fast (fatiguing) twitch
485 fibres (10). The same mechanisms likely also explain the reduced fatigue index of voluntary
486 explosive impulse during the 60 MVCs following nitrate supplementation. Furthermore, whilst
487 the current study assessed the first component of low-frequency fatigue, it is possible nitrate
488 supplementation – via mechanism (ii) – might similarly benefit the second component of low-
489 frequency fatigue, which is also caused by reduced SR Ca^{2+} release, but independently of
490 metabolite accumulation following several minutes of recovery (15, 16, 18).

491 In conclusion, we provide novel evidence that nitrate supplementation attenuates the reduction
492 in explosive force production during fatiguing exercise, which appears likely due to attenuated
493 disruption of excitation-contraction coupling, evidenced by decreased low-frequency fatigue
494 in NIT compared to PLA. However, nitrate supplementation had no effect on voluntary or

involuntary contractile performance in unfatigued conditions, which is in contrast to two recent studies showing increased low-frequency tetanic force following nitrate supplementation.

Practical Implications

Explosive force production is functionally important where time to develop force is limited, such as during sprinting (36), joint stabilisation (37), and balance recovery (38). Thus, the considerable declines in explosive force observed during fatiguing exercise (39) and match play (40), will greatly impair exercise performance and increase injury risk. In the current study, nitrate supplementation reduced the decline in explosive force during fatiguing exercise and so may benefit exercise performance and reduce the risk of injury during fatiguing activity where explosive contractions are required.

Acknowledgements

This work was funded by the University of Roehampton. The authors would like to sincerely thank the following people for their help in producing this manuscript: Dr. Jonathan Folland (Loughborough University, for advice during the study design stage); Dr. Giulia Corona (University of Roehampton), for advice with plasma nitrate/nitrite analysis; Dr. Gunter Kuhnle (University of Reading) and Dr. Virag Sagi-Kiss (University of Reading) for completing the nitrate/nitrite analysis; Rupert Manwaring, Holly Medlock, Elisa Giacomelli Ranzi, and Filipe Campos De Souza, for their help in the data collection.

Conflict of Interest

The authors have no professional relationships with companies or manufacturers that may benefit from the results of the present study. The results of the present study do not constitute

endorsement by ACSM. The Authors declare the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

References

1. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. N Engl J Med. 1993;329(27):2002-12.

2. Lundberg JO, Weitzberg E, Gladwin MT. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. Nat Rev Drug Discov. 2008;7(2):156-67.

3. Lundberg JO, Carlstrom M, Larsen FJ, Weitzberg E. Roles of dietary inorganic nitrate in cardiovascular health and disease. Cardiovasc Res. 2011;89(3):525-32.

4. Larsen FJ, Ekblom B, Sahlin K, Lundberg JO, Weitzberg E. Effects of dietary nitrate on blood pressure in healthy volunteers. N Engl J Med. 2006;355(26):2792-3.

5. Bailey SJ, Winyard P, Vanhatalo A, Blackwell JR, Dimenna FJ, Wilkerson DP, Tarr J, Benjamin N, Jones AM. Dietary nitrate supplementation reduces the O2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. J Appl Physiol (1985). 2009;107(4):1144-55.

6. Vanhatalo A, Bailey SJ, Blackwell JR, DiMenna FJ, Pavey TG, Wilkerson DP, Benjamin N, Winyard PG, Jones AM. Acute and chronic effects of dietary nitrate supplementation on blood pressure and the physiological responses to moderate-intensity and incremental exercise. Am J Physiol Regul Integr Comp Physiol. 2010;299(4):R1121-31.

535 7. Cermak NM, Gibala MJ, van Loon LJ. Nitrate supplementation's improvement of 10-km
536 time-trial performance in trained cyclists. Int J Sport Nutr Exerc Metab. 2012;22(1):64-71.

537 8. Jones AM. Influence of dietary nitrate on the physiological determinants of exercise
538 performance: A critical review. Appl Physiol Nutr Metab. 2014;39(9):1019-28.

539 9. Haider G, Folland JP. Nitrate supplementation enhances the contractile properties of human
540 skeletal muscle. Med Sci Sports Exerc. 2014;46(12):2234-43.

541 10. Hernandez A, Schiffer TA, Ivarsson N, Cheng AJ, Bruton JD, Lundberg JO, Weitzberg E,
542 Westerblad H. Dietary nitrate increases tetanic [Ca2+]i and contractile force in mouse fast-
543 twitch muscle. J Physiol. 2012;590(15):3575-83.

544 11. Whitfield J, Gamu D, Heigenhauser GJF, VAN Loon LJC, Spriet LL, Tupling AR,
545 Holloway GP. Beetroot juice increases human muscle force without changing Ca2+-handling
546 proteins. Med Sci Sports Exerc. 2017;49(10):2016-24.

547 12. Hoon MW, Fornusek C, Chapman PG, Johnson NA. The effect of nitrate supplementation
548 on muscle contraction in healthy adults. Eur J Sport Sci. 2015;15(8):712-9.

549 13. Folland JP, Buckthorpe MW, Hannah R. Human capacity for explosive force production:
550 Neural and contractile determinants. Scand J Med Sci Sports. 2014;24(6):894-906.

551 14. Tillin NA, Jimenez-Reyes P, Pain MTG, Folland JP. Neuromuscular performance of
552 explosive power athletes versus untrained individuals. Med Sci Sports Exerc. 2010;42(4):781-
553 90.

554 15. Edwards RH, Hill DK, Jones DA, Merton PA. Fatigue of long duration in human skeletal
555 muscle after exercise. J Physiol. 1977;272(3):769-78.

556 16. Chin ER, Balnave CD, Allen DG. Role of intracellular calcium and metabolites in low-
557 frequency fatigue of mouse skeletal muscle. Am J Physiol. 1997;272(2 Pt 1):C550-9.

558 17. Hill CA, Thompson MW, Ruell PA, Thom JM, White MJ. Sarcoplasmic reticulum function
559 and muscle contractile character following fatiguing exercise in humans. J Physiol.
560 2001;531(Pt 3):871-8.

561 18. Westerblad H, Duty S, Allen DG. Intracellular calcium concentration during low-frequency
562 fatigue in isolated single fibers of mouse skeletal muscle. J Appl Physiol (1985).
563 1993;75(1):382-8.

564 19. Binder-Macleod SA, Russ DW. Effects of activation frequency and force on low-frequency
565 fatigue in human skeletal muscle. J Appl Physiol (1985). 1999;86(4):1337-46.

566 20. Griffin L, Anderson NC. Fatigue in high- versus low-force voluntary and evoked
567 contractions. Exp Brain Res. 2008;187(3):387-94.

568 21. Fulford J, Winyard PG, Vanhatalo A, Bailey SJ, Blackwell JR, Jones AM. Influence of
569 dietary nitrate supplementation on human skeletal muscle metabolism and force production
570 during maximum voluntary contractions. Pflugers Arch. 2013;465(4):517-28.

571 22. Burnley M. Estimation of critical torque using intermittent isometric maximal voluntary
572 contractions of the quadriceps in humans. J Appl Physiol (1985). 2009;106(3):975-83.

573 23. Burnley M, Vanhatalo A, Fulford J, Jones AM. Similar metabolic perturbations during all-
574 out and constant force exhaustive exercise in humans: A (31)P magnetic resonance
575 spectroscopy study. Exp Physiol. 2010;95(7):798-807.

576 24. Maffiuletti NA, Aagaard P, Blazevich AJ, Folland J, Tillin N, Duchateau J. Rate of force
577 development: Physiological and methodological considerations. Eur J Appl Physiol.
578 2016;116(6):1091-116.

579 25. Rendeiro C, Dong H, Saunders C, Harkness L, Blaze M, Hou Y, Belanger RL, Corona G,
580 Lovegrove JA, Spencer JPE. Flavanone-rich citrus beverages counteract the transient decline
581 in postprandial endothelial function in humans: A randomised, controlled, double-masked,
582 cross-over intervention study - CORRIGENDUM. Br J Nutr. 2017;118(8):638.

583 26. Cohen J. Statistical power analysis for the behavioural sciences. 2nd ed. Hillsdale (NJ):
584 Lawrence Erlbaum Associates; 1988; p. 21.

585 27. Wylie LJ, Kelly J, Bailey SJ, Blackwell JR, Skiba PF, Winyard PG, Jeukendrup AE,
586 Vanhatalo A, Jones AM. Beetroot juice and exercise: Pharmacodynamic and dose-response
587 relationships. J Appl Physiol (1985). 2013;115(3):325-36.

588 28. Christensen PM, Petersen NK, Friis SN, Weitzberg E, Nybo L. Effects of nitrate
589 supplementation in trained and untrained muscle are modest with initial high plasma nitrite
590 levels. Scand J Med Sci Sports. 2017;27(12):1616-26.

591 29. Webb AJ, Patel N, Loukogeorgakis S, Okorie M, Aboud Z, Misra S, Rashid R, Miall P,
592 Deanfield J, Benjamin N, MacAllister R, Hobbs AJ, Ahluwalia A. Acute blood pressure

593 lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to
594 nitrite. Hypertension. 2008;51(3):784-90.

595 30. Johnson MA, Polgar J, Weightman D, Appleton D. Data on the distribution of fibre types
596 in thirty-six human muscles. an autopsy study. J Neurol Sci. 1973;18(1):111-29.

597 31. Andersen JL. Muscle fibre type characteristics of the runner In: Bangsbo J, Larsen HB,
598 editors. Running & science in an inter-disciplinary perspective. Munksgaard Publishing,
599 Copenhagen; 2001; p. 49-65.

600 32. Tillin NA, Bishop D. Factors modulating post-activation potentiation and its effect on
601 performance of subsequent explosive activities. Sports Medicine. 2009;39(2):147-66.

602 33. Pucci AR, Griffin L, Cafarelli E. Maximal motor unit firing rates during isometric
603 resistance training in men. Exp Physiol. 2006;91(1):171-8.

604 34. Knight CA, Kamen G. Relationships between voluntary activation and motor unit firing
605 rate during maximal voluntary contractions in young and older adults. Eur J Appl Physiol.
606 2008;103(6):625-30.

607 35. Keeton RB, Binder-Macleod SA. Low-frequency fatigue. Phys Ther. 2006;86(8):1146-50.

608 36. Tillin NA, Pain MT, Folland J. Explosive force production during isometric squats
609 correlates with athletic performance in rugby union players. J Sports Sci. 2013;31(1):66-76.

610 37. Krosshaug T, Nakamae A, Boden BP, Engebretsen L, Smith G, Slauterbeck JR, Hewett
611 TE, Bahr R. Mechanisms of anterior cruciate ligament injury in basketball: Video analysis of
612 39 cases. Am J Sports Med. 2007;35(3):359-67.

613 38. Izquierdo M, Aguado X, Gonzalez R, Lopez JL, Hakkinen K. Maximal and explosive force
614 production capacity and balance performance in men of different ages. Eur J Appl Physiol
615 Occup Physiol. 1999;79(3):260-7.

616 39. Buckthorpe M, Pain MT, Folland JP. Central fatigue contributes to the greater reductions
617 in explosive than maximal strength with high-intensity fatigue. Exp Physiol. 2014;99(7):964-
618 73.

619 40. Thorlund JB, Aagaard P, Madsen K. Rapid muscle force capacity changes after soccer
620 match play. Int J Sports Med. 2009;30(4):273-8.

621

622 **Tables**

623 Table 1. Force parameters recorded in the unfatigued knee extensors during MVCs and
624 electrically stimulated tetanic contractions, in placebo and nitrate supplemented conditions.
625 Explosive impulse (force-time integral) was recorded over the first 0-50 (IMP_{0-50}), 0-100 (IMP_{0-100}), and 0-150 ms (IMP_{0-150}) from force onset. Tetanic peak force and peak rate of force
627 development (RFD) at stimulation frequencies of 10, 20, and 50 Hz are reported relative to
628 respective values at 100 Hz. Data are means ± SD (N = 17).

	Placebo	Nitrate
Voluntary Forces		
MVF (N)	739 ± 135	741 ± 136
IMP_{0-50} (Ns)	1.58 ± 0.52	1.52 ± 0.59
IMP_{0-100} (Ns)	15.2 ± 3.6	15.1 ± 4.2
IMP_{0-150} (Ns)	39.4 ± 7.6	39.4 ± 8.9
Tetanic Peak Forces		
10 Hz (%100 Hz)	40.0 ± 8.5	39.2 ± 8.1
20 Hz (%100 Hz)	66.8 ± 5.6	66.4 ± 5.4
50 Hz (%100 Hz)	90.4 ± 4.3	90.9 ± 3.8
20:50 Hz	0.74 ± 0.06	0.73 ± 0.05
Tetanic Peak RFDs		
10 Hz (%100 Hz)	50.1 ± 9.9	50.9 ± 10.4
20 Hz (%100 Hz)	51.4 ± 6.2	50.6 ± 7.5
50 Hz (%100 Hz)	80.7 ± 3.5	80.3 ± 3.6
20:50 Hz	0.64 ± 0.07	0.63 ± 0.08

629

630 Table 2. Force and EMG parameters recorded in the knee extensors during a fatigue protocol
631 involving 60 MVCs, in placebo and nitrate supplemented conditions. The fatigue indexes (FI)
632 document the percentage decline from the first 6 to last 6 MVCs for mean MVC force, average
633 MVC EMG amplitude, explosive impulse over the first 0-150 (IMP_{0-150}), and explosive EMG
634 over the first 0-150 (EMG_{0-150}). Data are means ± SD (N = 13 for IMP_{0-150} and EMG_{0-150}; N =
635 14 for all other variables). Paired difference is denoted by *($P<0.05$).

	Placebo	Nitrate
Total impulse (kNs)	70.5 ± 14.6	71.2 ± 14.4
End-test force (N)	261 ± 68	266 ± 61
MVC force FI (%)	54.2 ± 10.0	52.3 ± 8.5
MVC EMG FI (%)	31.2 ± 16.9	27.7 ± 17.2
IMP_{0-150} FI (%)	57.3 ± 12.4	51.1 ± 13.9*
EMG_{0-150} FI (%)	10.8 ± 18.8	3.5 ± 29.5

636

637 **Figure Legends**

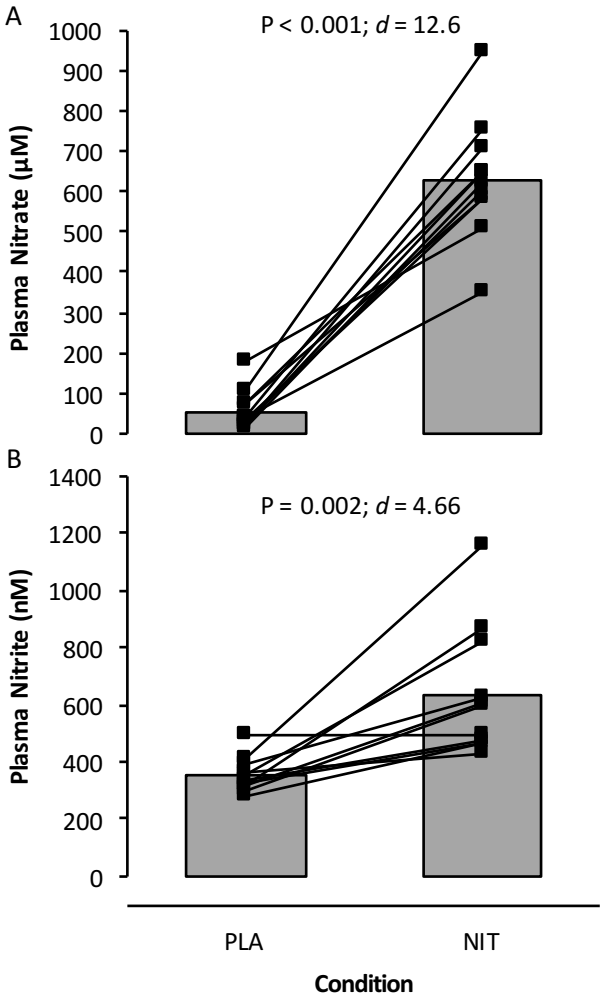
638 Figure 1. Plasma nitrate (A) and nitrite (B) concentration, in placebo (PLA) and nitrate (NIT)
639 supplemented conditions. Data are condition means (grey bars) and individual responses (black
640 lines) for N = 11. Between-condition paired comparisons (P) and effect sizes (*d*) are presented.

641 Figure 2. Mean MVC force averaged across the MVCs within each consecutive bin of 6 MVCs,
642 during a 60-MVC fatigue protocol, performed in placebo (black diamonds) and nitrate (white
643 squares) supplemented conditions. Force is reported as a proportion of unfatigued maximal
644 voluntary force (MVF) recorded in the same session. Data are means ± SD (N = 14).

645 Figure 3. Fatigue index of explosive impulse recorded over 0-150 ms ($IMP_{0\text{-}150}$) from force
646 onset, in placebo (PLA) and nitrate (NIT) supplemented conditions. Fatigue Index is the %
647 decline from the first 6 to the last 6, of 60 MVCs. Data are condition means (grey bars) and
648 individual responses (black lines) for N = 13. Between-condition paired comparison (P) and
649 effect size (*d*) are presented.

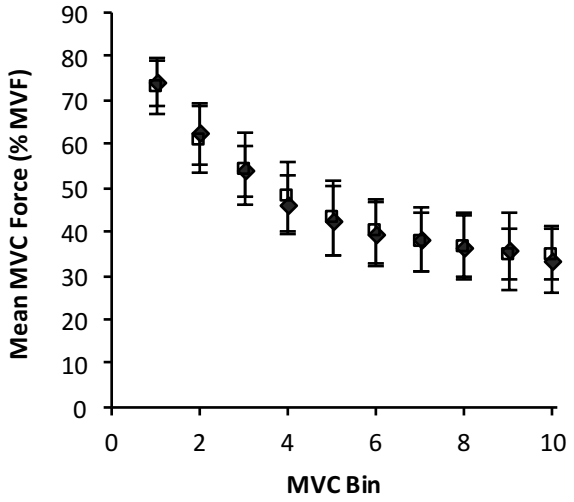
650 Figure 4. Fatigue indexes of tetanic peak forces (A and C) and RFD (B and D) recorded at 10,
651 20, 50, and 100 Hz (A and B) and the 20:50 Hz ratio (C and D), in placebo (PLA) and nitrate
652 (NIT) supplemented conditions. Fatigue Index is the % decline from pre- to immediately post-
653 60 MVCs. Data are condition means ± SD (A and B), or condition means (grey bars) and
654 individual responses (black lines; C and D) for N = 14. Between-condition paired comparisons
655 (P) and effect sizes (*d*) are presented for the 20:50 Hz fatigue indexes (C and D).

656



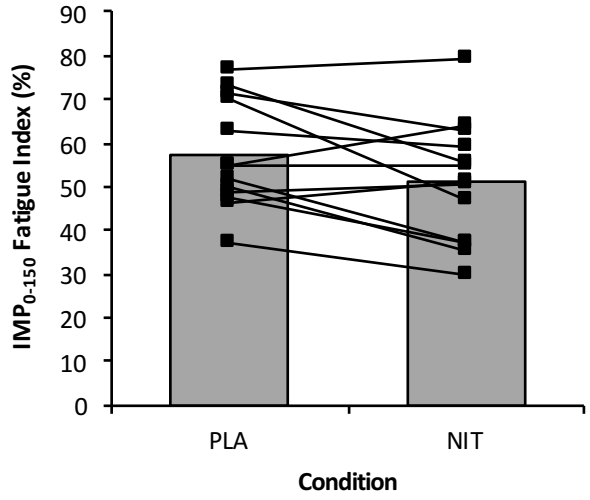
657

658 Figure 1.



659

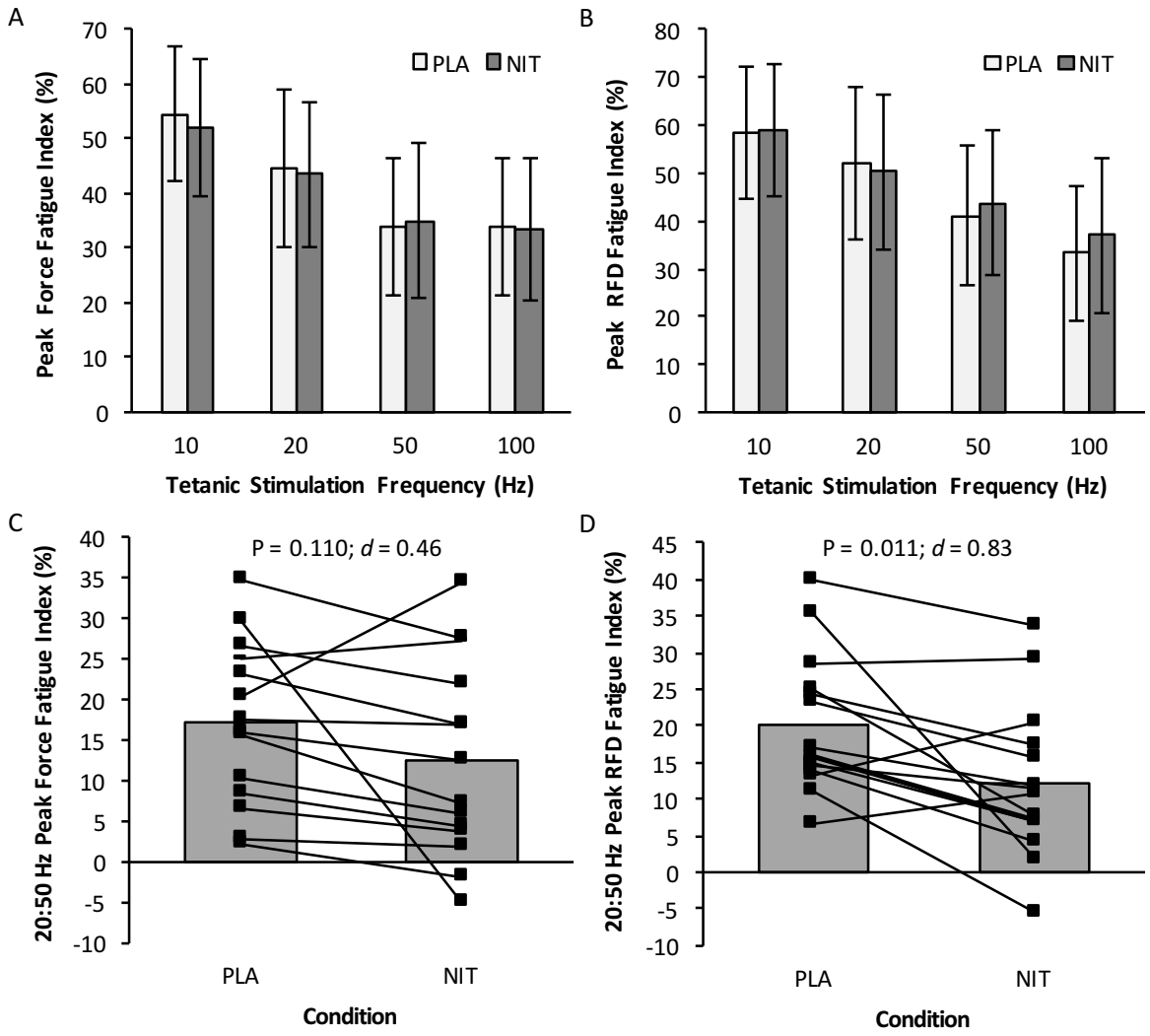
660 Figure 2.



661

662 Figure 3,

663



664

665 Figure 4.