The effects of dietary nitrate supplementation on the adaptations to sprint interval training in previously untrained males

Abstract

Objectives: Dietary nitrate can improve repeated high-intensity and supramaximal exercise performance, although the effect on adaptations to training has received limited attention. The purpose of this study was to investigate the effects of dietary nitrate on the response to 3-weeks of sprint interval training (SIT). **Design:** Randomized control trial. **Methods:** Twenty-seven untrained males (Age: 28 ± 7 y, $\dot{V}O_{2Max}$: 42 ± 7 ml·kg^{-1}·min^{-1}) completed an incremental exercise test at the beginning and end of the study. Participants were matched for $\dot{V}O_{2Max}$ and randomly assigned to a control group (CON; n=8), SIT + placebo group (PLA; n=10), or SIT + nitrate group (NIT; n=9). The SIT comprised 4-6 repeated 15 s all out sprints on a cycle ergometer, interspersed with 4 min active recovery, 3-times per week. Approximately 2.5 h prior to exercise, participants consumed gels containing ~0.1 mmol (PLA) or ~8 mmol nitrate (NIT). **Results:** Following SIT, $\dot{V}O_{2Max}$ (PLA: 5%, p=0.057, *d*=0.34; NIT: 6.3%, p=0.041, *d*=0.34) and ventilatory threshold (VT) increased to a similar extent in both SIT groups. Maximum work rate tended to increase to a greater extent in NIT (8.7%, *d*=0.55) compared to PLA (4.7%, *d*=0.31, p=0.073). Fatigue index, calculated by the change in mean power from the first to the last sprint, tended to be reduced following SIT in NIT compared to PLA (PLA: -7.3 ± 7.4%, NIT: 0.5 ± 7.1%, p=0.058). **Conclusions:** While dietary nitrate supplementation does not augment improvements to $\dot{V}O_{2Max}$ and VT following SIT, it may improve WR_{max} and indices of repeated high-intensity exercise.

Keywords: Nitric Oxide; Nitrite; Exercise; $\dot{V}O_{2Max}$

Introduction

Research interest into the effects of dietary nitrate on the responses to exercise has increased exponentially since the seminal works of Larsen and colleagues [1]. Recent studies have demonstrated that dietary nitrate supplementation can improve tolerance to [2, 3], and performance of [4-6] short-duration, moderate-intensity aerobic exercise. In addition, there is also compelling evidence that dietary nitrate supplementation can improve repeated high-intensity[7, 8] and supramaximal[9, 10] exercise performance (for a detailed review see [11]). However, while these effects on acute bouts of exercise have been widely investigated, it is less clear how nitrate supplementation may affect chronic exercise training, with only one study to date investigating the supplement in this context [12].

Sprint interval training (SIT) has been consistently shown to improve aerobic capacity of healthy adults [13]. This mode of training requires participants to perform repeated supramaximal exercise for a short period of time (<30s), interspersed with active recovery [13]; imposing demands on both non-oxidative and oxidative metabolism. Furthermore, SIT elicits a wide range of positive cardiorespiratory, endocrine, metabolic, and peripheral adaptations. The interaction between dietary nitrate and the response to SIT, however, has not previously been investigated. Given that dietary nitrate supplementation is reported to increase in the total work done during repeated supramaximal sprints [10] it is plausible that dietary nitrate may favorably influence adaptations to SIT. Therefore, the primary purpose of this study was to investigate the influence of dietary nitrate supplementation on the physiological responses to 3-weeks of SIT in previously untrained males. We hypothesized that dietary nitrate supplementation would enhance the physiological responses to 3-weeks SIT.

Methods

Twenty-seven healthy males (age 28 ± 7 y, stature 177 ± 5 cm, body mass 82.3 ± 17.1 kg, and maximal oxygen consumption [$\dot{V}O_{2Max}$] 42.4 ± 7.2 mL·kg^{-1}·min^{-1}) volunteered and provided written informed consent to participate in the study. The participants were all untrained, defined by participation in less than two structured exercise sessions per week, but not sedentary. The study was approved by the University Ethics Committee at the University of the West of Scotland and all procedures were conducted in accordance with the Declaration of Helsinki.

A schematic of the experimental design is presented in Figure 1. Following standard anthropometric measurements, $\dot{V}O_{2Max}$, ventilatory threshold (VT), and maximal work rate (WR_{max}) were assessed using a continuous graded incremental exercise test (IET1) on an electronically braked cycle ergometer (Lode Excalibur, Groningen, The Netherlands). Participants performed an initial warm-up; cycling at 50 W for 5 min followed by 5 min of static stretching. The IET1 commenced at an initial work rate of 50 W and increased by 15 W·min^{-1} in a ramp protocol until volitional exhaustion. Heart rate (HR) was continuously measured via telemetry (Polar Electro, Oy, Finland) and respiratory variables were measured breath by breath via indirect calorimetry (Medgraphics Ultima, MGC Diagnostics, MN, USA) which was calibrated immediately prior to each test. Following data collection, oxygen consumption ($\dot{V}O_2$) data were filtered and smoothed data were analyzed to determine $\dot{V}O_{2max}$. A plateau in $\dot{V}O_2$ (determined by a rise in $\dot{V}O_2$ of <50% of the expected increase for the given WR) was used to confirm achievement of $\dot{V}O_{2max}$. Based on these criteria, valid determinations of $\dot{V}O_{2max}$ were obtained from all participants at each time point. The coefficient of variation for our lab utilizing this protocol and method of assessment is 1.9%. VT was determined by the 'V-slope' method as the break point in the association between carbon dioxide production and $\dot{V}O_2$ [14].

Following IET1, participants were matched for $\dot{V}O_{2Max}$ and randomly assigned to either a SIT + nitrate supplementation group (NIT: *n*=9, Age: 31 ± 9 y, Stature: 178 ± 5 cm, Body Mass: 80.8 ± 17.1 kg), a SIT + placebo supplementation group (PLA: *n*=10, Age: 26 ± 4 y, Stature: 178 ± 4 cm, Body Mass: 83.7 ± 19.2 kg), or a control group (CON: *n*=8, Age: 27 ± 6 y, Stature: 177 ± 5 cm, Body Mass: 74.0 ± 14.7 kg). There were no differences in descriptive characteristics between all groups (all p>0.05). The NIT group consumed two nitrate-rich, peach-flavored gels (~8 mmol nitrate [0.06 – 0.15 mmol·kg^{-1} body mass], Science in Sport Go+ Nitrates, Lancashire, UK), 2.5 h prior to each SIT session. The PLA group ingested two identical peach-flavored gels but with the nitrate source not added by the manufacturer, 2.5 h prior to each SIT session. The nitrate-rich and placebo gels were provided in identical packaging which ensured a double blind supplementation protocol. Participants provided verbal confirmation that they had ingested the supplements prior to each trial or training session. Prior to each experimental trial, participants were asked to abstain from the use of anti-bacterial mouthwash and were provided with a list of high nitrate foods to avoid for 48 h, not to exercise or consume alcohol for 24 h, not to consume caffeine for 6 h or to consume anything other than water or their supplement in the 3 h prior to testing. The control group was instructed to maintain current physical activity levels and diet and received no supplements.

Within seven days of IET1, participants in the NIT and PLA groups each commenced nine instructor led sessions of SIT over a period of 3-weeks. Upon arrival at the laboratory in sprint session 1 (SS1), participants lay supine for 10 min after which 4 ml of venous blood was collected from the cephalic or antecubital vein. Blood samples were collected in tubes containing EDTA and immediately centrifuged at 4000 rpm at 4ºC for 10 min. The plasma was then separated into two cryovials and immediately frozen and stored at -80ºC. Plasma nitrite was subsequently assessed via ozone-based chemiluminescence [15] using procedures we have described previously [16]. The coefficient of variation for plasma nitrite in the present study was

5.4 %. A further sample of venous blood was also collected for measurement of blood glucose and blood lactate concentration prior to exercise using a bench top automated analyser (Biosen C-line analyzer, EKF Diagnostics, Germany).

The SS1 was performed on the same Lode Excalibur bicycle ergometer used in the IET and comprised four intermittent supramaximal sprints (S1, S2, S3, S4). Following a 2 min warm-up at 50 W, a load corresponding to 0.07 $kg \cdot kg^{-1}$ of body mass was applied to the bike and participants were verbally encouraged to maintain the highest cadence possible for 15 s. Peak power and mean power during the sprint were calculated using device software and fatigue index (FI) during sprint sessions assessed as: [(mean power S1 – mean power S4)/mean power S1 * 100]. Upon completion, the load was reduced to 50 W and participants completed 4 min of active recovery before repeating the sprint and recovery period a further three times. Following completion of SS1, participants lay supine and a second plasma sample was collected and stored, and glucose and blood lactate were analyzed from whole blood. Each of SIT sessions 2 – 8 were performed on a Wattbike Pro cycle ergometer (Wattbike Ltd, Nottingham, UK) to allow several participants to train simultaneously. Each of the instructor-led sessions followed a similar format to that of SS1 with the exception that blood samples were not collected. An air brake resistance was applied from a setting of 5 – 10 based upon the WR_{max} that the participant obtained in IET1. Pilot data from our lab has shown that peak power can reliably be achieved on a Wattbike Pro ergometer which has since been confirmed in a recent study by Herbert, Sculthorpe (17. Sprint session progression is outlined in figure 1. During the final SIT session (SS9), participants repeated the procedure of SS1 precisely to allow comparison between pre- and post-training. At least 48 h following the final SIT session (max 72 h), or after three weeks in the control group, participants returned to the laboratory to repeat the IET (IET2) as previously described.

Taylor et al. [18] have suggested that to evaluate the fidelity of any exercise intervention, data on session attendance and compliance (exercise intensity) should be reported. On this basis, we can confirm that there was perfect adherence to the SIT intervention as each participant completed 100% of the prescribed exercise sessions. The relative intensity for each training session was determined by measuring the average power during each 15 s bout and expressing this as a percentage of each individual's WR_{max} from IET1 (included as a supplementary data file). A complete data set (n=19) was analyzed for SS1 and SS9 which were completed on the Lode Excalibur Ergometer. Unfortunately due to firmware update on the Wattbikes, power data from several training sessions in SS 2-8 were lost. Nevertheless, a complete data set was obtained from nine participants in SS 2-8. These data confirm that while there was considerable within-subject variability between sprints and training sessions, the mean relative intensity in each 15 s bout was between 216 – 300% of WR_{max}. The between-subject coefficient of variation for each individual sprint ranged from 12.5 – 24.5%. Taken together, these data confirm that the fidelity of the exercise regime was high for all participants for whom we have a complete data set.

The distributions of the data were assessed using Shapiro–Wilk tests and when normality was violated the skew was assessed, and appropriate transformation was applied. Data are reported as mean ± SD or the geometric mean and mean confidence interval (CI) for log transformed data. Differences in the indices of aerobic fitness were assessed using two-factor repeated measures ANOVA (condition x time). The indices of anaerobic performance and blood parameters measured during training were assessed using three-factor repeated measures ANOVA where the main effects were 'group', 'sprint' (1, 2, 3, and 4) and 'time' for anaerobic performance and 'group', 'time' and 'session' (SS1 and SS9) for blood parameters. *Post-hoc* analysis of significant within-subject effects was performed using a Bonferroni correction. Statistical significance was set at $p \leq 0.05$. The 95% CI are included together with p values,

where appropriate. Effect sizes (Cohen's *d*) were calculated and interpreted as: small effect > 0.2; medium effect > 0.5; large effect > 0.8. All statistical procedures were completed using SPSS for Windows version 22.

Results

There was a significant main effect of 'time' on $\dot{V}O_{2Max}$ (p=0.013, Table 1). There was a small but statistically significant increase in $\dot{V}O_{2Max}$ (6.3%) following SIT in the NIT group (p=0.041, 95% CI 0.4 – 5.3 ml·kg·min^{-1}, *d*=0.34). There was also a small (5%) increase in $\dot{V}O_{2Max}$ in the PLA group that approached statistical significance (p=0.057, 95% CI -0.4 – 4.2 ml·kg·min^{-1}, *d*=0.34). The extent of the increase in $\dot{V}O_{2Max}$ from pre- to post-training was not different between PLA and NIT groups (*d*=0.21, p=0.646). There was no change in the CON group from IET1 to IET2 (p=0.725, *d*=0.05). Similarly, there was a significant main effect of 'time' and a 'time x group' interaction on VT (*P*<0.001, *P*=0.012, respectively). Work rate at VT increased significantly in both the PLA (p<0.001, 95% CI 10 – 28 W, *d*=0.61) and NIT (p<0.001, 95% CI 17 – 35 W, *d*=0.81) groups with no change in CON (p=0.188, *d*=0.16). The extent of the increase in VT from pre- to post-training was small although not statistically different between PLA and NIT groups (*d*=0.46, p=0.767). Lastly, there was a significant main effect of 'time' and a 'time x group' interaction on $WR_{max.}$ There was a significant increase in WR_{max} in both SIT groups (PLA: p=0.004, 95% CI 5 – 22 W, *d*=0.31; NIT: p<0.001, 95% CI 19 – 37 W, *d*=0.55) but it was not different in the CON group (p=0.812, *d*=0.01). The extent of the increase in WR_{max} from pre- to post-training between PLA and NIT groups was large and approached statistical significance (*d*=0.93, p=0.073).

Anaerobic power data from SS1 and SS9 are presented in Figure 2. There were significant main effects for the interaction of group*time*sprint for peak power, mean power and FI measures

during sprint sessions (all p<0.05). Post-hoc analysis revealed that in SS9 peak power in the PLA group was significantly higher in S1, S2 and S4 compared to SS1 (S1: p=0.014, 95% CI 33-257 W, *d*=0.40; S2: *P*=0.036, 95% CI 7 – 189 W, *d*=0.27; S4: p=0.003, 95% CI 75 – 304 W, *d*=0.69, Fig. 2A). In the NIT group, peak power was higher in S3 of SS9 compared to SS1 (p=0.047, 95% CI 1 – 164 W, *d*=0.22, Fig. 2B). There were no differences in peak power between groups for any sprint at either time point.

Mean power in the PLA group was significantly reduced in S4 compared to S1, S2, and S3 during both SS1 (all p<0.012, *d*>0.41) and SS9 (all p<0.04, *d*>0.19**)**. In the PLA group mean power was higher in all four sprints of SS9 compared to SS1 (S1: p=0.023, 95% CI 6 – 70 W, *d*=0.24; S2: p=0.045, 95% CI 1 – 61 W, *d*=0.19; S3: p=0.001, 95% CI 20 – 64 W, *d*=0.27; S4: p<0.001, 95% CI 43 – 103 W, *d*=0.59, Figure 2C). In the NIT group, there were no differences between sprints in either SS1 or SS9 (all p>0.300). Mean power was improved in S2, S3 and S4 of SS9 compared to SS1 (S2: p=0.007, 95% CI 14 – 77 W, *d*=0.29; S3: p=0.002, 95% CI 18 – 64 W, *d*=0.27; S4: p=0.001, 95% CI 27 – 90 W, *d*=0.41, Figure 2D).

In the NIT group, FI was lower in SS9 compared to SS1 (p=0.016 95% CI -11.6 – -1.4 %, *d*=0.96, Figure 2). In the PLA group FI was not different between sprint sessions (p=0.107, *d*=0.40, Figure 2E). The FI during SS9 tended to be greater in the PLA compared to the NIT group (PLA: -7.3%, NIT: 0.5%, p=0.058 95% CI -0.25 – 13.8 %, *d*=0.94 Figure 2E). There was no difference in FI during SS1 between the PLA and NIT groups.

There was a significant main effect for the interaction of group*time and time*sprint on plasma nitrite (p=0.034, p=0.002). During SS1 plasma nitrite concentration was significantly higher in the NIT group compared to the PLA group prior to exercise (p=0.037, *d*=1.28, Figure 2F). At the end of SS1, plasma nitrite concentration was significantly lower than pre-exercise in the NIT group (p=0.027, *d*=0.45) but not the PLA group (p=0.265, *d*=0.66, Figure 2**)**. In SS9,

plasma nitrite was higher in the NIT group compared to the PLA group prior to exercise, however did not reach statistical significance (p=0.066, *d*=0.94, Figure 2F**)**. Plasma nitrite concentration was lower in both groups following SS9 however did not reach statistical significance (PLA: p=0.549, *d*=0.47; NIT: p=0.329, *d*=0.35, Figure 2F). Blood lactate increased from pre- to post-exercise in both groups during SS1 and SS9, however there were no differences in blood lactate concentration between groups (data not reported). There were no main effects on blood glucose during training (data not reported).

Discussion

In the present study we set out to determine whether ingesting dietary nitrate supplements prior to exercise would enhance the physiological adaptations to SIT in previously untrained participants. The principal findings of the present study were that SIT improved parameters of fitness in both groups, however, dietary nitrate supplementation administered prior to SIT did not improve $\dot{V}O_{2Max}$ or VT beyond a period of SIT alone. Despite this, the effect size suggests that dietary nitrate may have a positive impact on the increase in WR_{max} following SIT and reduce fatigue during repeated supramaximal sprints compared to ingestion of PLA.

Whilst SIT resulted in small increases in both $\dot{V}O_{2Max}$ and VT, the comparable improvement between PLA and NIT groups was contrary to our experimental hypothesis. Likewise, both PLA and NIT groups experienced similar increases in peak and mean power production from pre- to post-SIT during supramaximal sprints. As a consequence, the present study suggests that nitrate supplementation has no impact on these parameters of exercise following 3-weeks

SIT. Nevertheless, WR_{max} improved to a greater extent following SIT in the NIT group compared to PLA and FI reduced only in the NIT group from pre- to post-training which one may consider as a positive effect. Alternatively, given that nitrate supplementation has been shown to reduce the oxygen cost of exercise, it is also conceivable that the nitrate supplements masked any additional benefits on $\dot{V}O_{2Max}$ measured during IET2. For example, it has previously shown that dietary nitrate supplementation can result in a small, but significant (3%) reduction in $\dot{V}O_{2peak}$ [19], whilst maintaining WR_{max}. Whilst the participants in the present study did not supplement with dietary nitrate immediately prior to the IET, it is conceivable that NO availability within the skeletal muscle is greater following 3 weeks of supplementation, and therefore able to induce a reduction in $\dot{V}O_{2Max}$ at a given WR_{max}. Despite this, further work including the use of muscle biopsies for quantification of skeletal muscle NO status are required to explore these findings further. To our knowledge, only one other group has explored the impact of dietary nitrate supplementation on the response to training [12]. In this study, participants underwent 6 weeks of continuous exercise training in normobaric hypoxia, five times per week. The authors reported that nitrate supplementation did not augment improvements in $\dot{V}O_{2Max}$ and nor did it improve time-trial performance; findings that are similar to those presented in the present study. Nevertheless, issues with the regulation of training intensity and the dosing strategy utilized in this study may account for some of these findings.

Despite this, nitrate supplementation appeared to reduce the decline in mean power output during acute bouts of repeated sprints (Figure 2). In the PLA group, the mean power produced during S4 was lower than in S1-S3 during SS1 and SS9, and this decline was not observed in either trials of the NIT group. These acute ergogenic effects of nitrate supplementation on parameters of repeated supramaximal exercise are also reported elsewhere in the literature [9, 10]. For example, it was previously found that dietary nitrate improved total work done during

repeated short duration (6 s) sprint cycling [10]. Furthermore, a separate group reported that supplementation with nitrate-rich beetroot juice significantly increased the number of supramaximal sprints completed before volitional exhaustion [9]. The findings of these studies are perhaps unsurprising given that dietary nitrate supplementation attenuates the decline of muscle PCr and accumulation of adenosine diphosphate and phosphate ions, metabolites associated with fatigue[20]. In addition, recent studies in mice have also shown that it can increase muscle force production [21] and increase blood flow to type II muscle fibers [22]. The precise pathway underpinning this ergogenic effect is unclear but the reduction in exercise-induced PCr degradation following nitrate supplementation is a plausible mechanism[20].

Despite these apparent acute benefits to supramaximal exercise resulting from dietary nitrate supplementation it is important to acknowledge that the timing of ingestion may have limited these effects. Following completion of data collection in the present study, we have since shown that NO metabolites appear to reach peak concentrations in the plasma faster when ingesting the nitrate gels compared to beetroot juice (1-1.5 h and 2.5-3 h, respectively) [23]. It remains to be determined whether these pharmacokinetic dissimilarities are due to individual differences or the inherent characteristics of the supplements themselves. Nevertheless, plasma nitrite concentration was higher in the NIT group prior to the SIT sessions compared to the PLA group suggesting the supplementation regimen was still sufficient to increase NO availability. It must also be recognised that there is a well-established heterogeneity in response to exercise training [24] and SIT [25, 26]. This variability in individual response makes it challenging to detect an additional effect of a supplement beyond that of the exercise training. Further research that increases both sample size and the duration of training would therefore be appropriate.

Conclusion

The principal findings of the present study were that dietary nitrate supplementation, administered throughout a 3-week SIT program, did not improve $\dot{V}O_{2Max}$ and VT beyond that of a period of SIT alone in previously untrained males. Nevertheless, we provide further evidence that dietary nitrate supplementation is effective for maintaining power output for the study population during acute bouts of repeated high-intensity exercise. In addition, this study suggests dietary nitrate supplementation may augment the increase in WR_{max} following SIT within this cohort.

Practical Implications

- Sports gels that are rich in nitrate improve maintenance of average cycling power when ingested prior to repeated bouts of very high intensity exercise in untrained individuals.
- Supplementing with nitrate rich gels throughout 3-weeks of sprint interval training does not improve physiological markers of aerobic fitness in untrained adults more than the training alone.
- Ingesting nitrate gels prior to training sessions of untrained male adults leads to a greater reduction in fatigue during repeated bouts of high intensity exercise and a greater increase in maximal power output during an incremental exercise test than 3-weeks of sprint interval training alone.

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Figure Legends

Figure 1. Schematic of the experimental design; IET = Incremental exercise test; CON = control group; PLA = placebo group; NIT = nitrate group; SIT = sprint interval training; PA = Physical activity

Figure 2. Peak power (A,D), Mean power (B,E) during repeated supramaximal sprints pre- (SS1) and post-training (SS9) in the placebo (D,E) and nitrate (A,B) groups. Fatigue Index (C) and plasma nitrite (F) for both groups during SS1 and SS9. * denotes a significant difference from SS1. # denotes a significant difference from the NIT group. † denotes a significant difference from S1 . ** denotes significant difference from PLA at SS1. ## denotes trend versus PLA at SS9. †† denotes significant difference from pre-exercise

Supplement Figure 1. Group (n=9) mean (column bars) and standard deviation (error bars) of the mean power output expressed as a percentage of WR_{max} for each sprint of the nine training sessions on either the Lode excaliber ergometer (A) or Wattbike ergometer (B).

Table 1. Indices of aerobic fitness pre- and post-training or control period.

	CON (*n*=8)		PLA (*n*=10)		NIT (*n*=9)	
	Pre: IET1	**Post: IET2**	**Pre: IET1**	**Post: IET2**	**Pre: IET1**	**Post: IET2**
Maximal Exercise Tests						
VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$)	44.0 (39.1 – 49.6)	44.7 (39.9 – 50.1)	40.2 (36.0 – 44.8)	42.2 (38.9 – 45.7)	41.8 (37.6 – 46.4)	44.4 (39.2 – 50.3)[a]
Ventilatory Threshold (W)	164 (139 – 193)	170 (145 – 199)	165 (148 – 185)	184 (164 – 207)[a]	170 (153 – 190)	196 (176 – 219)[a]
Maximal work rate (W)	288 ± 62	289 ± 61	274 ± 42	287 ± 42[a]	286 ± 47	314 ± 54[a,b]
Maximal Heart Rate (BPM)	184 ± 8	184 ± 7	189 ± 9	191 ± 8	185 ± 8	187 ± 9[a]

Data are presented as mean ± SD or geometric mean with 95% CI; [a] denotes differences between pre- and post-training within groups ($P<0.05$); [b] denotes a trend between PLA and NIT groups ($P<0.07$);