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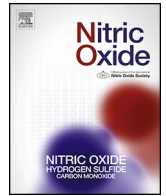
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Acute whole body UVA irradiation combined with nitrate ingestion enhances time trial performance in trained cyclists



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ABSTRACT

Dietary nitrate supplementation has been shown to increase nitric oxide (NO) metabolites, reduce blood pressure (BP) and enhance exercise performance. Acute exposure to ultraviolet (UV)-A light also increases NO bioavailability and reduces BP. We conducted a randomized, counterbalanced placebo-controlled trial to determine the effects of UV-A light alone and in combination with nitrate on the responses to sub-maximal steady-state exercise and time trial (TT) performance. Nine cyclists ($VO_{2\max}$ 53.1 ± 4.4 ml/kg/min) completed five performance trials comprising 10 min submaximal steady-state cycling followed by a 16.1 km TT. Following a familiarization the final four trials were preceded, in random order, by either (1) Nitrate gels (NIT) + UV-A, (2) Placebo (PLA) + UV-A, (3) NIT + Sham light (SHAM) and (4) PLA + SHAM (control). The NIT gels (2×60 ml gels, ~ 8.1 mmol nitrate) or a low-nitrate PLA were ingested 2.5 h prior to the trial. The light exposure consisted of 20 J/cm² whole body irradiation with either UV-A or SHAM light. Plasma nitrite was measured pre- and post-irradiation and VO_2 was measured continuously during steady-state exercise. Plasma nitrite was higher for NIT + SHAM (geometric mean (95% CI), 332 (292–377) nM; $P = 0.029$) and NIT + UV-A (456 (312–666) nM; $P = 0.014$) compared to PLA + SHAM (215 (167–277) nM). Differences between PLA + SHAM and PLA + UV-A (282 (248–356) nM) were small and non-significant. During steady-state exercise VO_2 was reduced following NIT + UVA ($P = 0.034$) and tended to be lower in NIT + SHAM ($P = 0.086$) but not PLA + UV-A ($P = 0.381$) compared to PLA + SHAM. Performance in the TT was significantly faster following NIT + UV-A (mean \pm SD 1447 ± 41 s $P = 0.005$; $d = 0.47$), but not PLA + UV-A (1450 ± 40 s; $d = 0.41$) or NIT + SHAM (1455 ± 47 s; $d = 0.28$) compared to PLA + SHAM (1469 ± 52 s). These findings demonstrate that exposure to UV-A light alone does not alter the physiological responses to exercise or improve performance in a laboratory setting. A combination of UV-A and NIT, however, does improve cycling TT performance in this environment, which may be due to a larger increase in NO availability.

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1. Introduction

Since Larsen and colleagues [1] first reported a reduced oxygen cost of exercise following ingestion of sodium nitrate, a growing number of studies have demonstrated the ergogenic effects of dietary nitrate supplementation on athletic performers [2–5]. Research has shown that ingestion of nitrate-rich food such as beetroot and spinach can increase circulating levels of nitric oxide (NO) metabolites via an NO synthase (NOS) independent pathway [6]. Following absorption of nitrate from the stomach into the plasma, nitrate is

transported into the saliva via the salivary glands. Bacteria then reduce the nitrate to nitrite [7] where, following ingestion, nitrite is potentially reduced to NO when exposed to hypoxic [8] or acidic environments [9].

This increase in NO related products, potentially leading to an increase in NO production, has been typically shown to reduce oxygen consumption (VO_2) during sub-maximal steady-state exercise [1,10] and improve both exercise capacity and performance [2,3,5,10]. Conversely, several other studies report exercise performance of varying modalities to be unaltered by dietary nitrate supplementation [11–13]. While there are various methodological differences between studies that may account for this disparity, it has become apparent that ergogenic effects appear minimal in highly-trained endurance cohorts [11,14]. This may be partly explained by the higher baseline nitrate/nitrite pool in endurance

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trained athletes compared to untrained matched controls [15]. Evidence from murine models also suggests that increases in muscle blood flow and contractile force production following nitrate supplementation only occur in Type II muscle fibers [16,17]. One may, therefore, reasonably assume that elite endurance athletes, who are known to have high proportions of type I muscle fibers, would be less likely to benefit from nitrate supplementation [18]. Alternatively, using only a single low dose of dietary nitrate (~4–5 mmol) may explain the resultant diminished ergogenic effect during exercise [12,13,19].

Intriguingly, exposing the skin to the ultra violet (UV)-A component of sunlight increases circulating plasma nitrite via decomposition of photo reactive nitrogen oxides stored in dermal cells [20,21]. Opländer and colleagues [21] observed that systemic blood pressure (BP) was reduced by 11% 30 min after UV-A exposure, a finding similar to that of dietary nitrate [22]. More recently, Liu and colleagues [23] provided some mechanistic basis for these findings by demonstrating UV-A induced NO production also increases forearm blood flow. Therefore, given that exposure to UV-A light increases NO bioavailability sufficiently to induce measureable physiological effects; it is plausible that it may also enhance exercise performance. Furthermore, it remains to be determined whether combining UV-A exposure with concomitant nitrate supplementation may potentiate a synergistic effect on NO bioavailability, given that both offer different routes for increasing plasma NO related products.

Despite this, the effects of UV-A exposure, either alone or in combination with nitrate supplementation, on plasma nitrite and parameters of exercise performance are currently unknown. This is of interest given the reported benefits to exercise performance associated with an increased availability of NO related products [2,3,5,10]. Therefore, the aim of this study was to determine the effects of acute UV-A light exposure with and without simultaneous nitrate supplementation on plasma nitrite, the physiological responses to steady-state exercise and cycling time trial (TT) performance. We hypothesized that: (1) UV-A exposure would increase plasma nitrite and improve exercise performance and (2) UV-A exposure combined with nitrate would coalesce to increase plasma nitrite and improve exercise performance to a greater extent than either intervention alone.

2. Methods

2.1. Participants

Nine male trained-cyclists and triathletes (age 36 ± 6 years, stature 182 ± 5 cm, body mass 78.9 ± 6.0 kg, and VO_{2max} 53.1 ± 4.4 mL·kg⁻¹·min⁻¹) volunteered and provided written informed consent to participate in the study that was approved by the School of Science Ethics Committee at The University of the West of Scotland. All participants were amateur competitive athletes who typically completed a minimum of two cycling training sessions per week and regularly competed in TT competitions. All procedures were conducted in accordance with the Declaration of Helsinki.

2.2. Experimental design

Each participant attended the human performance laboratory at the University of the West of Scotland on six separate occasions between July and August 2013. The laboratory is located in the Hamilton campus at latitude 55.78 degrees north. During the first visit participants completed a maximal incremental test to exhaustion in order to determine VO_{2max} and maximum work rate (WR_{max}). This was followed by a familiarization trial and then four performance trials conducted at the same time of day (± 1 h), at least five days apart. The familiarization trial followed a similar protocol to the

performance trials but was not preceded by any intervention. Each of the four performance trials were preceded by ingestion of either nitrate gels (NIT) or a low-nitrate placebo (PLA). The NIT comprised 2×60 ml concentrated peach-flavored nitrate-rich gels with extracts of Swiss chard and Rhubarb (~8.1 mmol nitrate, Science in Sport GO + Nitrates, Lancashire, UK). This nitrate dose, equivalent to ~0.1 mmol nitrate kg⁻¹ body mass, is similar to previous studies that have reported a significant increase in circulating plasma nitrate and nitrite following ingestion [1,2,5,19]. Furthermore, data from a recent dose response study suggests that 8.4 mmol is the optimal dose of nitrate (compared to 4.2 and 16.8 mmol) to induce beneficial physiological and performance responses [19]. The PLA was composed of peach-flavored gels that were similar in taste and texture to NIT but with the nitrate source not added by the manufacturer. The NIT and PLA were delivered in identical packaging to ensure that neither participants nor lead investigators were able to identify them. Despite a small discrepancy in taste none of the participants had consumed a gel prior to testing and were therefore unable to detect which supplement they had consumed. Supplements were ingested 2.5 h prior to arrival at the lab as plasma nitrite peaks 2.5–3 h after ingestion of an acute dose of nitrate-rich beetroot juice (BR) [6].

Prior to the performance trials, participants were also exposed on one side of the body (equating to the skin surface area exposed when wearing a short-sleeved shirt and shorts) to either UV-A light or sham light (SHAM) for a total of 22 min. A Waldmann GH-8 ST unit (Herbert Waldmann, GmbH, Villingen-Schwenningen, Germany) containing eight, F851 100W UV-A bulbs (320–410 nm; maximum 351 nm), was positioned 20 cm from the body. At this distance a dose of 20 J/cm² was produced which is the equivalent to two standard epithelial doses or 30 min exposure to sunlight at noon on a sunny day in Southern Europe [23]. This dose of UV-A light has been shown to significantly increase plasma nitrite immediately after irradiation with values remaining elevated at least 40 min after exposure [23]. During SHAM trials an aluminum foil space blanket covered the participants, blocking UV-A radiation from the skin while allowing surface and body temperature to rise [23]. Participants were aware of the significance of the aluminum space blanket prior to the start of the study. During the light exposures, participants wore only cycling shorts and a visor to protect the face and eyes.

The ingestion of NIT or PLA and exposure to UV-A or SHAM light was administered in a randomized counterbalanced design so that each of the four possible combinations of interventions was administered: (1) PLA + SHAM (control), (2) PLA + UV-A, (3) NIT + SHAM, (4) NIT + UV-A. One of the researchers, who was not involved in the data collection or analysis, randomized the orders of supplementation and light exposure using a Latin-square model. Prior to each trial participants were asked to abstain from the use of antibacterial mouthwash and consumption of high nitrate food 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 for 3 h prior to testing.

2.3. Experimental procedures

2.3.1. Maximal exercise test

Following standard anthropometric measurements, VO_{2max} and WR_{max} were measured using a continuous graded exercise test on an electronically braked cycle ergometer (Lode Excalibur, Groningen, The Netherlands). Participants performed an initial warm-up that consisted of cycling at 50 W for 5 min followed by 5 min of static stretching. The exercise test commenced at an initial work rate of 50 W and increased by 30 W per min in a linear fashion until volitional exhaustion. Throughout the test 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). The indirect calorimeter was calibrated immediately prior to each test using a 3L syringe and both calibration and reference gases (calibration gas: 12% O₂, 5% CO₂; reference Gas: 21% O₂, 0% CO₂). Following data collection, VO₂ data were filtered to delete values that were less than or greater than the rolling seven breath mean \pm two standard deviations. All nine participants obtained a plateau in VO₂ (as determined by a rise in VO₂ of <50% of the expected increase for the given WR) fulfilling the criteria for VO_{2max}. Smoothed data were subsequently averaged over a rolling seven breath mean and the largest value obtained was determined as the participant's VO_{2max}. Pilot data from our laboratory have shown high reproducibility in measurement for these procedures. The coefficient of variation for VO_{2max} and WR_{max} were 1.9 and 0.5% respectively.

2.3.2. Performance trials

Upon arrival at the laboratory, participants were instructed to lie supine for 10 min after which BP was determined by standard auscultation using a stethoscope and sphygmomanometer (Accoson, London, UK) and 4 ml of venous blood was collected from the cephalic vein. Participants were then exposed to 22 min of either UV-A or SHAM light, before the measurement of BP was repeated and another venous blood sample collected. Blood samples were collected in tubes containing EDTA and immediately centrifuged at 4000 rpm at 4 °C for 10 min. The plasma was then separated into two cryovials and immediately frozen and stored at –80 °C.

After completion of the pre-exercise protocol, participants were seated on the cycle ergometer (Lode Excalibur, Groningen, The Netherlands) where resting respiratory measurements and HR were recorded. Subsequent to this participants cycled at 50 W for 5 min followed immediately by 10 min continuous steady-state cycling at 60% of WR_{max}. Respiratory variables and HR were monitored throughout the steady-state exercise period. VO₂ data were smoothed as previously described and the average value of the last 5 min of exercise was used for analysis.

Following the steady-state exercise protocol participants received a 5 min passive rest period where they were permitted to ingest water *ad libitum* and stretch. Following this, they each completed a 16.1 km TT on a cycle ergometer (Wattbike, Nottingham, UK) during which they were instructed to cycle at a freely chosen velocity and encouraged to complete the 16.1 km in the shortest time possible. Participants received verbal feedback on the distance they had completed at 1 km intervals and every 100 m for the last km.

2.3.3. Plasma analysis

Plasma samples were stored for a maximum of 4 months at –80 °C for later analysis of nitrite via ozone-based chemiluminescence [24]. We have described the procedures for the determination of nitrite previously [13]. Briefly, Triiodide reagent was heated in a water bath to 50 °C and nitrogen gas bubbled through. The purge vessel was linked to a trap containing a sodium hydroxide solution that was further connected to an NO analyser (Sievers NOA 280i, Analytix, UK). After obtaining a standard curve, blood plasma samples were thawed in a water bath at 37 °C for 3 min and injected into the purge vessel. Nitrite levels were determined by the area under the curve, calculated using Origin software (version 7), divided by the gradient of the slope obtained from the standard curve.

2.4. Pharmacokinetic response to SIS GO+ Nitrates

The majority of previous studies on dietary nitrate supplementation have utilized BR as the nitrate source. The pharmacokinetics of plasma nitrate and nitrite following ingestion of BR are already well-established [6], but it is unclear whether the response to the NIT used in this study is the same. Therefore, in a study run con-

currently to the principal investigation but with different participants, the pharmacokinetic response to NIT was determined. Seven healthy male volunteers participated (age 34 \pm 8 years, stature 179 \pm 8 cm, body mass 81.4 \pm 9.9 kg) who were not on any medication, maintained a healthy lifestyle but were not competitive athletes. Following a 12 h fast participants reported to the laboratory where a venous blood sample was collected and treated as previously described. Participants then ingested NIT (two 60 ml gels, 8.1 mmol nitrate, Science in Sport GO + Nitrates, Lancashire, UK) and blood samples were collected 30, 60, 90, 180, 360, 600 and 1440 min after ingestion. Plasma nitrite concentration was determined as described in section 2.3.3 and plasma nitrate was measured using the reductant vanadium chloride in hydrochloric acid at 80 °C. Participants refrained from eating until >90 min post NIT administration after which they were allowed a light breakfast. After 360 min participants received a sandwich lunch, and following 600 min were allowed a meal. They then fasted until 1440 min but were allowed nitrate-free water *ad-libitum*.

2.5. Data analysis

The estimated sample size for the study ($n = 9$) was based on the expected difference in the primary outcome measurement (16.1 km TT performance) using data previously collected in our laboratory (20 \pm 21 s) with an α set at 0.05 and power 0.8. The distributions of the data collected were assessed using Shapiro–Wilk tests and when normality was violated, the variable was logged transformed (\log_{10}) prior to statistical analysis. Data are reported as mean \pm SD or the geometric mean and mean confidence interval (CI) for log transformed data.

Differences in plasma nitrite and BP between conditions (PLA + SHAM; PLA + UV-A; NIT + SHAM; NIT + UV-A) were assessed using two-factor repeated measures ANOVA where the main effects were 'condition' and 'time' (pre- vs. post-light exposure) and the condition \times time interaction was also tested. Analysis of all other physiological variables and TT completion time was conducted using one-factor repeated measures ANOVA to establish differences between conditions. *Post-hoc* analysis of significant within-subject effects was performed with adjustments for multiple comparisons using the Bonferroni correction. The null hypothesis was rejected when $P < 0.05$. Effect size (Cohen's d) was calculated and interpreted as: small effect > 0.2 ; medium effect > 0.5 ; large effect > 0.8 . The 95% CI for mean differences are included together with P values, where appropriate. All statistical procedures were completed using SPSS for Windows version 20.

3. Results

3.1. Plasma nitrite responses during intervention study

Plasma nitrite data from the intervention study is presented in Fig. 1. There was a significant main effect of 'condition' ($P = 0.001$) on plasma nitrite concentration. There was no significant main effect for time ($P = 0.944$) or a condition \times time interaction ($P = 0.083$). Prior to the light-exposure, plasma nitrite in the NIT + SHAM (399 (345–461) nM) condition was higher than in the control (247 (179–343) nM, $P = 0.024$, 95% CI 17–257 nM). Plasma nitrite in the NIT + UV-A (391 (291–526) nM) condition tended to be higher than in the control ($P = 0.068$, 95% CI –20–320 nM). There was no difference in plasma nitrite pre-light exposure between the control and PLA + UV-A conditions (265 (203–347) nM, $P = 1.000$).

There was a significant decline in plasma nitrite from pre- to post-light exposure in the control condition ($P = 0.036$, 95% CI 4–82 nM) and a tendency for a decline in the NIT + SHAM condition ($P = 0.060$, 95% CI 4–143 nM). There was no change in plasma nitrite from pre- to post-light exposure in both UV-A conditions (PLA + UV-A,

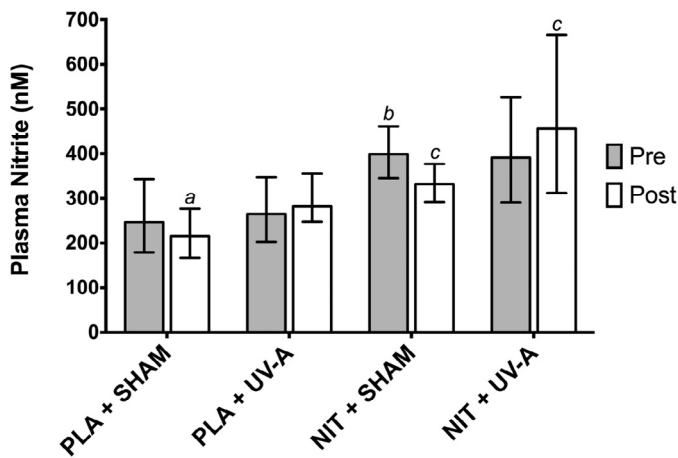


Fig. 1. Geometric mean and 95% CI (error bars) of plasma nitrite concentration in the control (PLA + SHAM) and experimental (PLA + UV-A; NIT + SHAM; NIT + UV-A) conditions for pre- (gray bars) and post-light (white bars) exposure. *a* denotes a significant decline in plasma nitrite from pre- to post-light exposure ($P=0.036$). *b* denotes a significantly higher plasma nitrite compared to PLA + SHAM pre-light exposure ($P=0.024$). *c* denotes a significantly higher plasma nitrite compared to PLA + SHAM post-light exposure (NIT + SHAM, $P=0.029$; NIT + UV-A $P=0.014$).

$P=0.645$; NIT + UV-A, $P=0.208$). In the post-light exposure measurements, plasma nitrite was higher in both NIT + SHAM (332 (292–377) nM; $P=0.029$, 95% CI 25–197 nM) and NIT + UVA (456 (312–666) nM; $P=0.014$, 95% CI -32–599 nM) conditions compared to the control (215 (167–277) nM). There were no differences in post-light exposure measurements of plasma nitrite between the control and PLA + UV-A conditions (282 (248–356) nM, $P=0.781$) or any other comparisons between interventions (all $P>0.20$). Magnitude based inferences suggest that all interventions resulted in a large increase in plasma nitrite concentration compared to the control (PLA + UV-A, $d=0.85$; NIT + SHAM, $d=1.64$; NIT + UV-A, $d=1.80$).

3.2. Blood pressure

The data for systolic, diastolic and mean arterial BP are presented in Table 1.

Table 1

Blood pressure variables measured pre-light and post-light exposure in the control (PLA + SHAM) and experimental conditions.

Condition and variable	Pre-light	Post-light
PLA + SHAM		
Systolic	124 ± 8	122 ± 7 ^b
Diastolic	72 ± 3	71 ± 3
MAP	89 ± 4	88 ± 4
PLA + UVA		
Systolic	122 ± 7	116 ± 5 ^b
Diastolic	72 ± 6	70 ± 7 ^b
MAP	89 ± 5	85 ± 6 ^b
NIT + SHAM		
Systolic	119 ± 7 ^a	116 ± 8 ^b
Diastolic	70 ± 6	70 ± 6
MAP	86 ± 6	85 ± 6
NIT + UVA		
Systolic	118 ± 8	115 ± 6 ^{b,c}
Diastolic	71 ± 4	69 ± 5
MAP	87 ± 4	84 ± 4 ^{b,c}

Data are presented as mean ± SD.

^a Denotes a difference from PLA + UVA pre-light measurement ($P<0.05$).

^b Denotes a difference from pre-light measurement in the same condition ($P<0.05$).

^c Denotes a difference from PLA + SHAM post-light measurement ($P<0.05$).

3.2.1. Systolic blood pressure

There was a significant main effect of both ‘condition’ ($P=0.002$) and ‘time’ ($P<0.001$) on systolic BP and a condition × time interaction ($P=0.008$). Prior to the light-exposure, systolic BP tended to be lower in the NIT + SHAM ($P=0.090$, 95% CI -1 to 9 mmHg) and NIT + UV-A ($P=0.052$, 95% CI 0–10 mmHg) conditions compared to the control. Compared to the PLA + UV-A trial, pre-light exposure systolic BP was lower in the NIT + SHAM condition ($P=0.031$, 95% CI 0–7 mmHg) and tended to be lower in the NIT + UV-A ($P=0.061$, 95% CI 0–9 mmHg) condition. There were no differences in systolic BP pre-light exposure between the control and PLA + UV-A conditions ($P=1.0$).

There was a significant decline in systolic BP from pre- to post-light exposure in the control condition and all three experimental conditions (all $P<0.05$). In the post-light exposure measurements, systolic BP tended to be lower in the PLA + UV-A condition ($P=0.057$, 95% CI 0–10 mmHg) and was significantly lower in the NIT + UV-A condition ($P=0.001$, 95% CI 3–10 mmHg) compared to the control. There were no differences in post-light exposure measurements of systolic BP between the control and NIT + SHAM conditions ($P=0.133$) or any other comparisons between interventions (all $P>0.20$).

3.2.2. Diastolic blood pressure

There were no main effects of ‘condition’ ($P=0.570$) or ‘time’ ($P=0.128$) on diastolic BP although there was a significant condition × time interaction ($P=0.042$). Diastolic BP declined from pre- to post-light exposure in the PLA + UVA trial ($P=0.045$, 95% CI 1–5 mmHg) with a trend for a reduction in the NIT + SHAM trial ($P=0.084$, 95% CI 0–5 mmHg). There were no differences in diastolic BP from pre- to post-light exposure in the other conditions (both $P>0.70$).

3.2.3. Mean arterial blood pressure

There was no main effect of ‘condition’ ($P=0.114$) on mean arterial BP although there was a significant main effect of ‘time’ ($P=0.012$) and a condition × time interaction ($P=0.010$). Prior to the light-exposure, there were no differences in mean arterial BP between conditions (all $P>0.10$). There was a significant reduction in mean arterial BP from pre- to post-light exposure in PLA + UVA ($P=0.002$, 95% CI 2–6 mmHg) and NIT + UVA conditions ($P=0.025$, 95% CI 0–5 mmHg) but no differences in the other trials (both $P>0.20$). In the post-light exposure measurements, mean arterial BP was significantly lower in the NIT + UV-A trial compared to the control ($P=0.030$, 95% CI 0–7 mmHg). There were no differences between other conditions (all $P>0.50$).

3.3. Cardio-respiratory variables

The data for VO_2 and HR at rest and during steady-state exercise are presented in Table 2.

Table 2

Oxygen consumption and heart rate at rest and during steady-state exercise in the control (PLA + SHAM) and experimental conditions.

	PLA + SHAM	PLA + UV-A	NIT + SHAM	NIT + UV-A
Rest				
VO_2 (ml/min)	305 ± 58	288 ± 58	321 ± 78	310 ± 61
HR (bpm)	61 ± 12	59 ± 13	61 ± 8	58 ± 10
Exercise				
VO_2 (ml/min)	2972 ± 171	2924 ± 181	2919 ± 179	2900 ± 209 ^a
HR (bpm)	146 ± 14	148 ± 14	148 ± 10	146 ± 11

Data are presented as mean ± SD.

^a Denotes a difference from PLA + SHAM measurement ($P<0.05$).

There were no differences in HR ($P=0.822$) or VO_2 ($P=0.385$) between conditions at rest. During steady-state exercise, VO_2 was lower in NIT + UV-A ($P=0.034$, 95% CI 7–136 ml/min, $d=0.38$) and tended to be lower in NIT + SHAM ($P=0.086$, 95% CI –10 to 115 ml/min, $d=0.30$) compared to the control condition. There was no difference in steady-state exercise VO_2 between the control and PLA + UV-A trials ($P=0.381$). There were no differences in HR during exercise between conditions (all $P>0.30$).

3.4. Time trial performance

There were no differences in time to complete the TT between control and familiarization trials (control: 1469 ± 52 s; familiarization: 1467 ± 61 s; $P=0.846$). Performance in the TT was significantly faster following NIT + UV-A, (1447 ± 41 s; $P=0.005$; 95% CI 9–35 s; $d=0.47$) but not PLA + UV-A (1450 ± 40 s; $P=0.122$; $d=0.41$) or NIT + SHAM (1455 ± 47 s; $P=0.106$; $d=0.28$) compared to the control. Although magnitude based inferences suggest that all experimental interventions result in a small benefit to TT performance, the *post-hoc* calculations of achieved power for the difference in TT performance compared to the control condition suggest a high likelihood of type II error for both PLA + UVA (0.474) and NIT + SHAM (0.509). The *post-hoc* calculation of achieved power for NIT + UVA was 0.968.

3.5. Pharmacokinetic response

Plasma nitrite and nitrate data from the pharmacokinetic experiment are presented in Fig. 2. Plasma nitrite peaked 90 min after ingestion of NIT and remained elevated above baseline until 6 h post-ingestion. Plasma nitrate peaked, on average, 1 h post-ingestion and remained elevated above baseline even at 24 h post-ingestion. The half-lives for plasma nitrate and nitrite were 6 h and 5 h, respectively.

4. Discussion

Exogenous supplementation with dietary nitrate increases the bioavailability of NO which has been shown in some conditions to reduce the oxygen cost of exercise and improve performance [2–5]. The present study explored the physiological and ergogenic effects of short-term exposure to UV-A light as a novel method to increase circulating NO metabolites both with and without ingestion of NIT. The principal finding was that exposure to UV-A light alone was not sufficient to significantly improve cycling TT performance although magnitude based inferences suggest a small positive effect. However, combining UV-A exposure with the ingestion of NIT reduced systolic and mean arterial BP at rest and VO_2 during steady-state exercise and significantly improved 16.1 km TT performance. As a result, these findings suggest that the extent of the rise in plasma nitrite following the intervention influences the extent of the physiological and ergogenic effects. Data from the present study suggest that combining short-duration UV-A exposure with acute supplementation of NIT may be an efficacious method to increase the availability of NO. It is important to highlight that the dose of UV-A in the present study was small, did not cause burning and was administered on only two occasions. However, regular exposure to acute bouts of UV-A or larger doses would not be recommended at present, given the possible increased risk of skin cancer, particularly for individuals with pale skin [25].

4.1. Effects of UV-A light on plasma nitrite and blood pressure

In contrast to our hypothesis and previous research [20,21,23], exposure to UV-A light resulted in only a small, non-significant increase in plasma nitrite concentration compared to baseline. However, plasma nitrite declined from pre- to post-light exposure

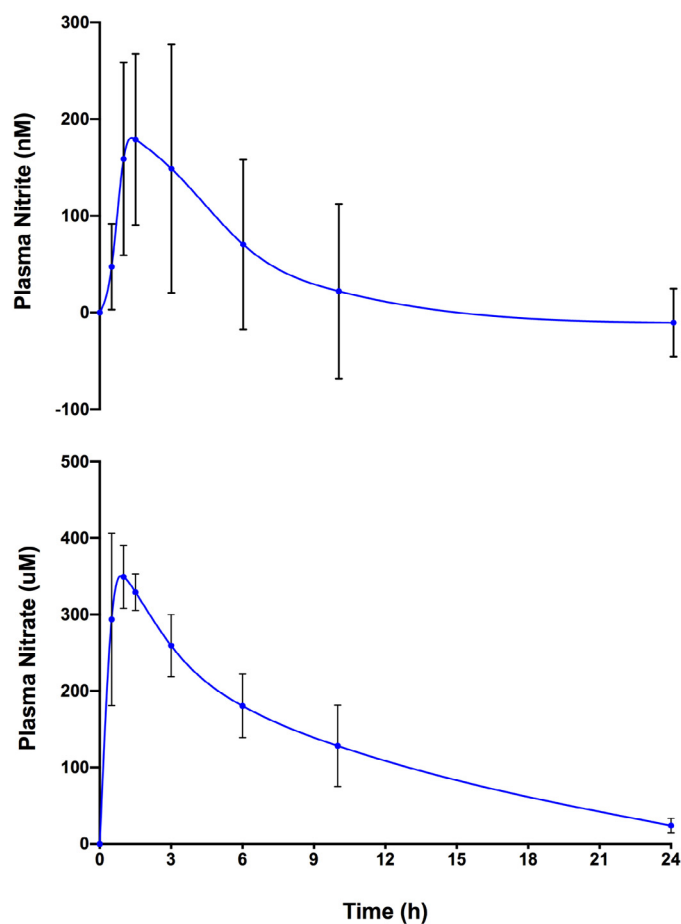


Fig. 2. Change from baseline in group mean \pm SD plasma nitrite (top panel) and nitrate (bottom panel) concentration after ingestion of 2×60 g SiS GO + Nitrates gels (~ 8.1 mmol nitrate).

in the two SHAM trials (by 43 nM in PLA + SHAM and 69 nM in NIT + SHAM), a finding also reported by Liu and colleagues [23]. Thus while exposure to UV-A light did not increase plasma nitrite *per se*, it did attenuate the consequential decline of lying supine for ~ 30 min. Indeed, systolic BP following exposure to UV-A also tended to be lower than in the control trial, suggesting that this small absolute increase in plasma nitrite was substantial enough to induce physiological effects. While we can only speculate as to mechanisms underpinning the reduction in nitrite during SHAM trials, it is well established that circumferential wall tension and arterial strain are reduced in the supine position compared to standing [26]. A reduction in arterial shear stress is also associated with reduced NO production by the endothelial cells [27]. Circulating nitrite is a combination of oxidized NOS-derived NO, of which the eNOS component falls with reduced shear stress at rest, and exogenous nitrate derived from an individual's diet. We propose that this fall in eNOS derived NO offsets the rise from chemical reduction of the oral NIT. Furthermore, movement from a standing to a supine posture causes plasma volume to expand as fluid moves from the cells to the blood [28]. These fluid shifts may cause a dilution in some plasma metabolite concentrations.

Exposure to UV-A light has been consistently reported to increase plasma nitrite [20,21,23] which in the present study appears to have offset these postural effects. The skin is known to contain large stores of various NO metabolites (principally nitrate) [20] which appear to be liberated in response to UV-A irradiation [23]. In the present study, the individual response to the UV-A dose was

variable. Some individuals responded to the UV-A exposure with large increases in plasma nitrite whereas others experienced a reduction mirroring the SHAM trials (data not shown). With only a small sample size to draw upon it is difficult to offer any firm explanation for the current data although skin type, previous exposure to UV-A, skin surface area and training status are all factors that could have conceivably influenced the response. Nevertheless, inter-individual variability is a well-established facet of the dietary nitrate supplementation literature that is deserving of further attention. Moreover, the dose–response pharmacokinetics of UV-A exposure and NO metabolism are currently unknown, and therefore it is unclear whether a longer exposure to the UV-A light source would have increased plasma nitrite concentration beyond what we have reported.

Given previous findings it seems likely that the reduction in systolic BP following exposure to UV-A was mediated via a NO mechanism that is independent of temperature [23]. However, the photolytic process involved in the formation of NO may also contribute to these effects. While UV-A can produce NO directly from cutaneous nitrite stores [29–31] NO production from nitrate photolysis is reported to be low; however, thiols are known to enhance this process [32]. Therefore, some have suggested that the thiol side chain in the amino acid cysteine (which is present in the epidermis) may augment NO production from cutaneous nitrate stores. Therefore our interpretation of NO bioavailability in the present study is limited as circulating nitrite was the sole marker of changes to the NO pool. Additional measurements of systemic NO bioavailability (including nitrate and S-nitrosothiols (RSNO)) and cutaneous stores are worthy of further investigation.

4.2. Effects of nitrate ingestion on plasma NO related products and blood pressure

In the present investigations, the ingestion of NIT increased plasma nitrite (by 137 nM in NIT + SHAM, 150 nM in NIT + UV-A and 179 nM in the pharmacokinetics experiment), increased plasma nitrate (pharmacokinetic experiment) and tended to reduce systolic BP in the intervention study, which is consistent with the majority of other studies (e.g. Webb et al. [6], Kapil et al. [22]). However, it was surprising to note from the pharmacokinetic study that the peak occurrence of plasma nitrite following ingestion of NIT differs from BR. Plasma nitrite has been reported to peak 2.5–3 h after ingestion of a single dose of BR whereas the response appears to be substantially quicker following NIT. Whether these differences are due to the source of nitrate in NIT (rhubarb and Swiss chard), the viscosity and volume of the supplements or simply differences in sample populations is not clear at present and would only be revealed by comparing nitrate supplements directly in the same cohort. Accordingly, although plasma nitrite was still unquestionably higher 2.5 h after ingestion, one may argue that this unanticipated alteration in the pharmacokinetic response to NIT meant that the timing of the dose in the intervention study was not optimal. Additionally, as with the response to UV-A, the individual changes in circulating nitrite following NIT were variable.

4.3. Evidence for a cumulative response to UV-A light and nitrate ingestion

Our data show for the first time that the known effects of dietary nitrate on plasma nitrite and BP can be enhanced when taken in combination with an acute exposure to UV-A light. The increase in plasma nitrite was largest following NIT + UV-A (Fig. 1) and this intervention was the only combination in the present study to significantly reduce mean arterial BP. Given that consumption of dietary nitrate and irradiation with UV-A light are both known to separately increase NO availability through different mechanisms, these effects are perhaps not unsurprising. Where ingestion of dietary nitrate is known to increase plasma NO via a NOS independent pathway involving the reduction of

dietary nitrate to nitrite in the gut and oral cavity, UV-A light releases NO from pre-formed stores in the skin [23]. Indeed, Liu and colleagues [23] suggest that the response to UV-A irradiation is not dependent on systemic nitrate availability or NOS activity. Irrespective, the apparent symbiotic effects of diet (nitrate) and environment (UV-A from sunlight) on BP offers an intriguing new avenue for future research.

4.4. Effects of NIT and UV-A on steady-state exercise and time trial performance

The cumulative effects of dietary nitrate and UV-A are also apparent in the physiological responses to steady-state exercise and TT performance. Although VO_2 tended to be lower following NIT + SHAM compared to the control trial, this parameter was significantly reduced following NIT + UV-A. Furthermore, NIT + UV-A was the only intervention that resulted in a statistically significant improvement in TT performance compared to the control. The precise mechanisms responsible for the reduced VO_2 during exercise are unquestionably related to an increase in NO related products but remain a matter of some controversy. In short, there are data suggesting that an increased bioavailability of NO improves the efficiency of mitochondrial respiration [33] and reduces the energy cost of muscle force production [34]. The various purported mechanisms underlying these effects are the subject of a recent review article by Jones [35] which neatly summarizes the extent of our knowledge on this topic.

While the present study does not advance our mechanistic understanding of dietary nitrate supplementation during exercise, it does support the notion that the response is dependent on the extent of the rise in plasma nitrite following the intervention. Where NIT + UV-A induced the largest increase in plasma nitrite, it also resulted in the largest reduction in VO_2 during steady-state exercise and improvement in TT performance. Wylie et al. [19] reported similar findings in their investigation into the dose–response to BR. In this study 8.4 mmol of BR increased plasma nitrite to a greater extent than 4.2 mmol which also coincided with a greater reduction in the oxygen cost of steady-state exercise and improvement in performance. The smaller increase in plasma nitrite following a smaller dose of dietary nitrate or the apparent blunted response in trained athletes compared to recreationally active individuals has been suggested by some authors to be the reason why dietary nitrate supplementation does not improve performance on all occasions [11–13]. Nevertheless, we did not observe a correlation between the changes in plasma nitrite and improvement in TT performance in the present study (data not presented). Additionally there is well-constructed mechanistic data suggesting a preferential effect of dietary nitrate on type II muscle fibers. Hernandez et al. [17] demonstrated that supplementation with BR increased force production of the fast twitch muscle fibers of mice which was also associated with an alteration in muscle protein expression. Again using a murine model, Ferguson et al. [16] also reported that the increase in muscle blood flow following supplementation with BR only occurred in type II muscle fibers. It is conceivable, therefore, that the blunted response to nitrate supplementation may be attributable to the training status or muscle fiber type distribution of the participants, or indeed the type of muscle recruited to meet the demands of the exercise task. The aforementioned expectation that elite endurance athletes will have a higher proportion of type I fibers [18] certainly offers some weight to this argument. In contrast, team sports such as rugby require short, intermittent bouts of anaerobic work and trained athletes in these sports are reported to have a higher percentage of type II fibers [36]. Recent work by Wylie et al. [4] supports this notion where performance gains were reported in trained team sports players when performing intense intermittent exercise.

Our work also highlights a pertinent methodological issue for researchers investigating the effects of dietary nitrate supplementation on markers of health and performance in human subjects. The UV-A

dose in this study was equivalent to only 30 min exposure to sunlight yet resulted in small alterations in the plasma nitrite response to lying supine, reduced BP and may cause small improvements in TT performance. Consequently, we suggest that the time of year when data are collected should be standardized where possible and the experimental location (latitude) should be a factor worthy of consideration in the interpretation and comparison of future NO related work. Indeed, this point also reveals a limitation of our own work. Although all 54 trials were conducted during a relatively narrow window (July–August 2013), this transpired to be during a period of sustained unusually warm weather in Scotland. Exposure to UV-A light outside of the laboratory was not controlled and nor was it measured. The effects of UV-A exposure on plasma nitrite and the consequent physiological responses are known to be sustained for at least 40 min after exposure [23]. The majority of participants, however, completed their trials in the late afternoon or evening after which they had been typically working in an indoor environment for the majority of the day. It is unclear at present whether additional basal exposure to sunlight would be substantial enough to cause a lasting physiological effect that would have impacted our findings.

5. Conclusion

The principal findings of the present study were that exposure to UV-A light subsequent to ingestion of a NIT improved the physiological responses to steady-state exercise and 16.1 km cycling TT performance. Furthermore, we provide some evidence of a cumulative effect of dietary nitrate and UV-A derived NO, whereby the increase in plasma nitrite was larger than with either intervention alone. This study offers the intriguing possibility that a combination of naturally occurring environmental and dietary factors may coalesce to enhance cycling performance. However, the potential long term risks of chronic exposure to UV-A light are unclear at present and further research is required to explore this. Researchers should also carefully consider the potential effects of natural sunlight exposure during study design and interpretation of their findings, given the important role of NO in energy metabolism and exercise performance.

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