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Reduced nitric oxide synthesis in winter: A potential contributing factor to increased cardiovascular risk

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ABSTRACT

Background: Nitric oxide is a key signalling molecule that elicits a range of biological functions to maintain vascular homeostasis. A reduced availability of nitric oxide is implicated in the progression of cardiovascular diseases and increases the risk of pathogenic events.
Aims: To compare the concentration of nitric oxide metabolites in healthy adults between winter and summer months.
Design: An observational study of healthy adults (age 32 ± 9 years) living in central Scotland.
Methods: Thirty-four healthy adults (13 females) were monitored for 7 days in summer and winter to record sunlight exposure (ultraviolet-A (UV-A) radiation), diet, and physical activity. At the end of each phase, blood pressure was measured, and samples of blood and saliva collected. The samples were analysed to determine the concentrations of plasma and salivary nitrate and nitrite and serum 25-hydroxyvitamin D (25(OH)D).
Results: The participants maintained similar diets in each measurement phase but were exposed to more UV-A radiation (550%) and undertook more moderate-vigorous physical activity (23%) in the summer than in winter. Plasma nitrite (46%) and serum 25(OH)D (59%) were higher and blood pressure was lower in the summer compared to winter months. Plasma nitrite concentration was negatively associated with systolic, diastolic, and mean arterial blood pressure.
Conclusions: Plasma nitrite, an established marker of nitric oxide synthesis, is higher in healthy adults during the summer than in winter. This may be mediated by a greater exposure to UV-A which stimulates the release of nitric oxide metabolites from skin stores. While it is possible that seasonal variation in nitric oxide availability may contribute to an increased blood pressure in the winter months, the overall impact on cardiovascular health remains to be determined.

1. Introduction

Nitric oxide (NO) is a gaseous singling molecule that plays an important role in vascular homeostasis [1] and is essential for cardiovascular health [2]. NO can be synthesised from L-arginine by NO synthases (NOS) in the presence of oxygen [3] or via the stepwise reduction of nitrate in the nitrate – nitrite – NO pathway [4]. Nitrate is generated from the oxidation of NO and it is absorbed when nitrate-rich foods such as green leafy vegetables and roots are consumed [5]. A proportion of circulating nitrate is concentrated in the saliva [6,7] where bacteria from genera such as *Actinomyces, Coynebacterium, Haemophilus, Kingella, Neisseria, Rothia, and Veillonella* can reduce it to nitrite [8–11]. When swallowed, a portion of the nitrite is further reduced to NO in the acidic environment of the stomach [12,13]. Once the remaining nitrite is absorbed into the circulation it can be converted to NO when hypoxic and acidic conditions prevail [14,15].

In addition to significant concentrations in the circulation, skeletal muscle, and organs, there are large stores of NO metabolites in the skin. Some of these metabolites are released into the circulation following exposure to radiation in the ultraviolet-A (UV-A) wavelength range

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(315–400 nm) [16,17]. Previous research has reported that plasma [nitrite] increases by over 40% following 15–30 min exposure to 20 J cm^{-2} of UV-A [18,19]. This is clinically relevant as plasma nitrite has been shown to be the best measurable marker of vascular NO synthesis [20,21]. Therefore, it can be inferred that exposing the skin to the UV-A component of sunlight can increase NO synthesis. In a sample of healthy human participants, Liu and colleagues (2014) demonstrated that irradiation of the skin with two standard erythemal doses of UV-A caused NO release from skin stores in a dose dependent manner. The authors also reported that UV-A irradiation reduced blood pressure and increased forearm blood flow, which they speculated was a consequence of the translocation of NO from the skin to the circulation. This is important as a 5% reduction in diastolic blood pressure can decrease the risk of stroke by 35% and reduces the risk of mortality from cardiovascular issues [22,23]. This is supported by epidemiological data which show that, despite increasing the risk of developing skin cancers [24], increased sunlight exposure is associated with a reduced risk of all-cause and cardiovascular mortality [25,26].

The beneficial effects of sunlight exposure on cardiovascular health are generally presumed to be a consequence of the physiological actions of vitamin D, which plays a key role in calcium homeostasis and metabolism. Indeed, an inverse relationship exists between markers of vitamin D status and the risk of cardiovascular disease [27]. Vitamin D is synthesised when skin is exposed to light in the UV-B wavelength range (280–315 nm) [28,29]. This compound is then converted to the biologically active 25-hydroxy-vitamin D_3 (25-(OH)D) in the liver [30]. It is, therefore, unsurprising that the bioavailability of 25-(OH)D is typically higher in the summer months [31] when UV-B exposure from sunlight is more pronounced [32]. There have been numerous randomised-controlled trials to explore whether oral supplementation with vitamin D supplementation can protect against a variety of non-communicable diseases. While there is some evidence that vitamin D supplementation can improve isolated disease risk factors, such as blood pressure, dyslipidaemia, and inflammation [33] other large-scale studies report that vitamin D supplementation did not significantly reduce all-cause mortality and vascular disease mortality or cancer incidence and mortality in older adults [34]. Thus, the link between cardiovascular disease and vitamin D status may be an epiphenomenon and there may be health benefits to sunlight exposure that go beyond vitamin D [35].

While seasonal changes in UVR exposure [36], blood pressure [37], and vitamin D status [31] are well established, no previous study has compared the concentration of circulating NO metabolites between summer and winter months. This is important since NO is a potent vasodilator and has antimicrobial and cytoprotective effects. Deficiencies in NO availability are characterised by oxidative stress, inflammation, and endothelial dysfunction [38] and levels of plasma nitrite are inversely correlated with cardiovascular risk load [39]. A reduced NO availability may, therefore, contribute to the increased number of deaths from cardiac events in the winter compared to the summer [40]. Therefore, the primary aim of this study was to determine whether there was a difference in NO markers between winter and summer. A secondary aim was to determine whether the concentration of plasma nitrite (as a marker of NO synthesis) and blood pressure were associated with UV-A exposure, diet, and physical activity. In comparison to winter, we hypothesised that healthy adults would be exposed to more UV-A radiation, have a higher concentration of plasma nitrite, and lower blood pressure in the summer. Furthermore, we hypothesised that plasma nitrite would be positively associated with UV-A exposure and negatively associated with blood pressure.

2. Methods

2.1. Participants

Thirty-four healthy and recreationally active participants (21 males and 13 females, age 32 ± 9 years, stature 174 ± 8 cm, and body mass 77.7 ± 14.1 kg) volunteered to participate in the study. Exclusion criteria were cardiovascular or metabolic disorders or anything limiting mobility and movement. Additionally, high BP at summer examination (systolic ≥140 mmHg or diastolic ≥90 mmHg) or a diagnosis of hypertension. All participants were living in central Scotland at a latitude of ~55.7° N. Participants were not asked to self-declare their ethnicity, but all were considered to be Caucasian. Written informed consent was obtained from all participants and the study was approved by the School of Science and Sport Ethics Committee at The University of the West of Scotland. All procedures were performed in accordance with the 1964 Declaration of Helsinki and its later amendments with the exception that the trial was no registered prior to participant recruitment.

2.2. Study design

Data were collected in the two-month period following each solstice (June 21st – August 21st, 2017 and December 21st – February 21st, 2018). Each participant attended the laboratory on four separate occasions, twice in the summer and twice in the winter. The laboratory temperature was set to 20 °C and maintained between 19 and 21 °C for all trials. During the first visit in each season, participants were provided with personal monitoring devices, a food diary, and instructions on their use. In the subsequent seven days, participants recorded everything they ate and drank in their food diary. Physical activity and sun exposure (light intensity) were continuously measured using a tri-axial accelerometer and daily UVR exposure was measured using a bespoke wrist worn dosimeter. The participants were instructed not to alter their lifestyle due to study participation. Participants then returned to the lab to return monitoring devices, provide samples of blood and saliva, and to have blood pressure measured. The follow-up visits in both seasons were completed at the same time of day for each participant and following an overnight fast if the trial was completed in the morning. For individuals who could attend the laboratory in the morning, this control measure ensured the acute effects of nitrate/nitrite ingestion in foods did not affect the outcomes. If the trial was completed in the afternoon, the participant consumed an identical breakfast prior to both visits. Participants were instructed to avoid caffeine, foods high in nitrite and nitrate (e.g. green leafy vegetables and cured meats), alcohol, mouthwash, and strenuous exercise 24 h prior to the experiment, and to attend the laboratory in a euhydrated state.

2.3. Procedures for follow-up visit

After stature and body mass were recorded, participants were seated in a semi-supine position. Blood pressure was collected in triplicate at 15 min, using a validated oscillometric automated device (Omron 705IT, Omron Global. Hoofddorp, Netherlands). The mean of the second and third measurement was used in the analyses. Unstimulated samples of saliva were collected at 10 and 20 min, and venous blood (4 ml) was drawn immediately after the second saliva sample as previously described [41].

2.4. Accelerometers

During the two monitoring periods, each participant wore a tri-axial accelerometer (GT3X+, ActiGraph™; LLC, Pensacola, FL, USA) on their non-dominant wrist and on their hip. The participants were instructed to wear the wrist monitor over clothing in order to maximise light exposure on the device when outdoors. Participants were instructed to take the accelerometers off when washing, when underwater, or when sleeping. The duration of time that the accelerometers were worn each day was determined by calculating "wear time". Non-wear was defined by an interval of at least 60 consecutive minutes of zero physical activity counts [42]. The duration and intensity of daily physical activity was assessed using the tri-axial vector magnitude (VM3) from the hip-worn

accelerometer [43]. Light intensity was measured with the built-in lux monitor from the wrist-worn accelerometer to provide an indication of time spent indoors and outdoors. The physical activity and light intensity measurements were recorded at a frequency of 30 Hz and monitored continuously throughout the monitoring period. Accelerometer physical activity data are presented as the average daily duration and proportion of accelerometer wear time spent undertaking light physical activity (LPA) and moderate-vigorous physical activity (MVPA).

2.5. UV-A exposure

Bespoke personal UVR dosimeters (Scienterra, Ltd, Oamaru, New Zealand) were worn on the dominant wrist to provide a measure of daily UVR exposure. The wrist has previously been shown to provide representative measurements of whole body UVR exposure [44]. The personal UVR dosimeters directly measured erythema effective irradiance at a rate of 0.03 Hz throughout the monitoring period. The erythema effective irradiance is a product of the erythema action spectrum and solar spectral irradiance at each wavelength in the range of 280–400 nm [45]. The erythema action spectrum is an internationally recognised representation of the erythema-inducing effectiveness of wavelengths in the UV part of the spectrum [46]. The erythema effective and UV-A irradiances were measured by Public Health England (PHE) at a ground station local to the population study [47]. The dosimeters were calibrated throughout the course of a cloudless day at the Health Security Agency in Chilton, U.K. (51.575° N, 1.318° W), before and after the data collection periods using a Bentham DTMc300 double-grating spectroradiometer (Bentham Ltd, Reading, UK) as the reference instrument. Following analysis, the ground station at PHE in Glasgow (55°51′N, 4°20′W) was used to retrieve UV-A irradiances. All participants resided within a 9 km radius of this ground station. The UV-A and erythema weighted irradiances were measured in the horizontal plane in 1 s intervals. The dosimeter time stamp was used to determine the specific time and duration of the UVR exposure that was subsequently used to retrieve the UV-A irradiance ($J \bullet cm^{-2}$).

In winter, the personal UV dosimeters measured either minimal or no UVR exposure. To provide additional insight into the duration of daily time spent outdoors, lux data from the wrist-worn accelerometer were analysed. As previously shown [48], lux values can be used to indicate the type of light an individual is exposed to and whether they are indoors or outdoors. The periods of time spent outdoors (derived from the accelerometers) was then cross-referenced with the corresponding UV-A irradiance for the measured by solar monitoring ground station duration that participants were exposed to sunlight.

2.6. Assessment of diet

The participants used a food diary to log their dietary intake throughout the 7 day monitoring periods in the summer and winter. During a visit to the laboratory, participants also used the food diaries as a reference to complete a 7-day food frequency questionnaire that has been adapted to measure dietary vitamin D [49] and nitrate intake [50]. The participants noted the number of servings per day and per week which were subsequently calculated as the average daily intake of vitamin D (IU) and nitrate (mg).

2.7. Chemiluminescence nitrite and nitrate analysis

Plasma and salivary [nitrite] and [nitrate] were analysed as previously described [41]. Briefly, tri-iodide (nitrite) or vanadium (nitrate) reagents were placed into a customised glass purge vessel along with 100 μL of anti-foaming agent, infused with nitrogen and heated to 50 °C or 95 °C for nitrite and nitrate analysis, respectively. The purge vessel was connected to an NO analyser (Sievers NOA 280i, Analytix, UK). A standard curve was produced by injecting known quantities of nitrite and nitrate solutions and a control sample containing deionised water. The concentration of NO cleaved during the reaction was then measured by the NO analyser. The area under the curve was calculated using Origin software (version 7) and divided by the gradient of the slope. The coefficient of variation (CV) for the measurement of plasma nitrite, plasma nitrate, salivary nitrite and salivary nitrate was 1.3, 2.1, 1.2, and 0.9%, respectively.

2.8. 25-Hydroxy-vitamin D analysis

Total circulating serum 25-(OH)D was measured using an enzyme-linked immunosorbent assay kit (Demeditec Diagnostics GmbH, Kiel, Germany), as per the manufacturer's instructions. Each test was run in duplicate, with mean absorbance computed from the average for two wells normalized to a zero calibrator well. The absorbance was read at a dual wavelength of 450 and 630 nm reference filter. Levels of vitamin D were expressed in $ng \cdot ml^{-1}$. The intra-assay CV was 8.6% and the inter-assay CV was 4.7%.

2.9. Statistical analysis

All statistical analyses were carried out using jamovi (Version 0.9.1.5, The jamovi project, Sydney, Australia) and figures were created with GraphPad Prism (version 7, GraphPad Software Inc., San Diego, USA). Data are expressed as the mean ± standard deviation. The distribution of the data was tested using the Shapiro-Wilk test. Paired sample t-tests were performed to assess the differences between summer and winter for the plasma and salivary NO metabolites, blood pressure, UV-A, 25-(OH)D, physical activity, and dietary nitrate intake. Wilcoxon Signed Rank tests were used when data were not normally distributed. Pearson's or Spearman's rank correlation coefficients were used to determine whether the average daily UV-A exposure was associated with plasma [nitrite], [nitrate], 25-(OH)D, and blood pressure. The same method was also used to determine whether plasma [nitrite] was associated with daily physical activity levels, total dietary nitrate intake, 25-(OH)D, and blood pressure. Correlation coefficients were also calculated to determine whether there was any association between the delta change (from winter to summer) values of plasma [nitrite], blood pressure and 25-(OH)D.

Linear regression analyses were performed to assess the relationship between plasma [nitrite] and average daily UV-A exposure. The model was adjusted for age, body mass index, MVPA, dietary nitrate intake, and sex. As average daily UV-A exposure had a high clustering towards zero, the data were log transformed to assess the associations with basal plasma [nitrite] and 25-(OH)D. Statistical significance was declared when $P < 0.05$ and 95% confidence intervals (95% CI) are reported where relevant.

3. Results

3.1. Ultra-violet A exposure

The sample size for the measurement of UV-A exposure was reduced to n = 30 (17 male, 13 female) due to unknown technical faults with four of the UV dosimeters. The average daily UV-A exposure was lower during winter (0.4 ± 0.4 $J \cdot cm^{-2}$) when compared to summer (2.6 ± 3 $J \cdot cm^{-2}$, $P < 0.001$, 95% CI 0.5–3 $J \cdot cm^{-2}$).

3.2. Plasma and salivary markers

A freezer malfunction resulted in the loss of some biological samples meaning the sample size for all nitrite and nitrate measurements was n = 26 (16 male, 10 female). Plasma [nitrite] was lower in winter (137 ± 31 nM) when compared to summer (200 ± 56 nM, $P < 0.001$, 95% CI 43–84 nM, Fig. 1A). These differences were still observed when comparing the changes in plasma [nitrite] separately in males and

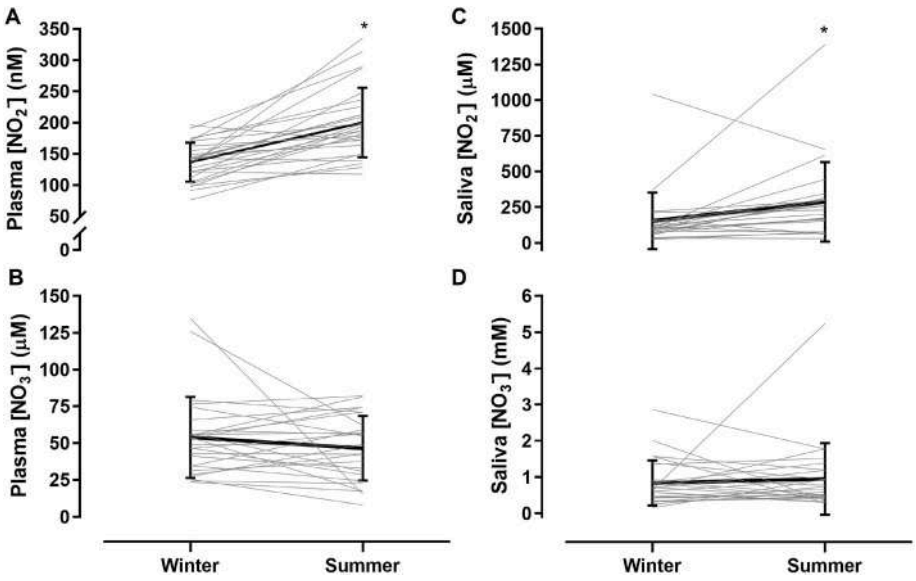

Fig. 1. Plasma nitrite (NO_2^-) (A) and nitrate (NO_3^-) (B) and salivary nitrite (C) and nitrate (D) comparison between winter and summer. The thing grey lines show individual participant data and the solid black line shows mean ± SD. * denotes significant difference compared to winter ($P < 0.01$).

females (both $P < 0.01$). Plasma [nitrate] was not different in winter (54 ± 28 μM) when compared to summer (47 ± 22 μM, $P = 0.57$, Fig. 1B). Salivary [nitrite] was lower in winter (154 ± 198 μM) compared to summer (288 ± 278 μM, $P < 0.01$, 95% CI 51–169 nM, Fig. 1C). Salivary [nitrate] was not different in winter (836 ± 624 μM) when compared to summer (945 ± 984 μM, P = 0.9, Fig. 1D). Due to the aforementioned freezer malfunction the sample size for 25-(OH)D was reduced to n = 30. Serum 25-(OH)D was lower in the winter (22 ± 8 $ng{\cdot}ml^{-1}$) compared to summer (35 ± 13 $ng{\cdot}ml^{-1}$, $P < 0.001$, 95% CI 9–18 $ng{\cdot}ml^{-1}$).

3.3. Blood pressure and heart rate

Systolic blood pressure was higher in winter (126 ± 13 mmHg) when compared to summer (119 ± 11 mmHg, $P < 0.001$, 95% CI 5–10 mmHg, Fig. 2A). Diastolic blood pressure was higher in winter (76 ± 9 mmHg) compared to summer (67 ± 8 mmHg, $P < 0.001$, CI 6–10 mmHg, Fig. 2B). Mean arterial blood pressure was also higher during winter (92 ± 10 mmHg) compared to summer (84 ± 8 mmHg, $P < 0.001$, 95% CI 5–10 mmHg, Fig. 2C). The resting heart rate was higher in winter (64 ± 13 bpm) when compared to summer (59 ± 9 bpm, $P < 0.01$, 95% CI 2–8 bpm, Fig. 2D).

3.4. Physical activity

There was no difference in daily accelerometer wear time between winter (754 ± 92 min) and summer (775 ± 96 min, $P = 0.17$). Daily average physical activity data are presented in Table 1. Total time ($P < 0.01$, 95% CI 4–22 min) and proportion of daily wear time ($P = 0.01$, 95% CI 0.2–2.5%) spent engaging in MVPA was lower in winter when compared to summer. The proportion of LPA was higher in the winter compared to summer ($P = 0.02$, 95% CI 0.3–2.9%).

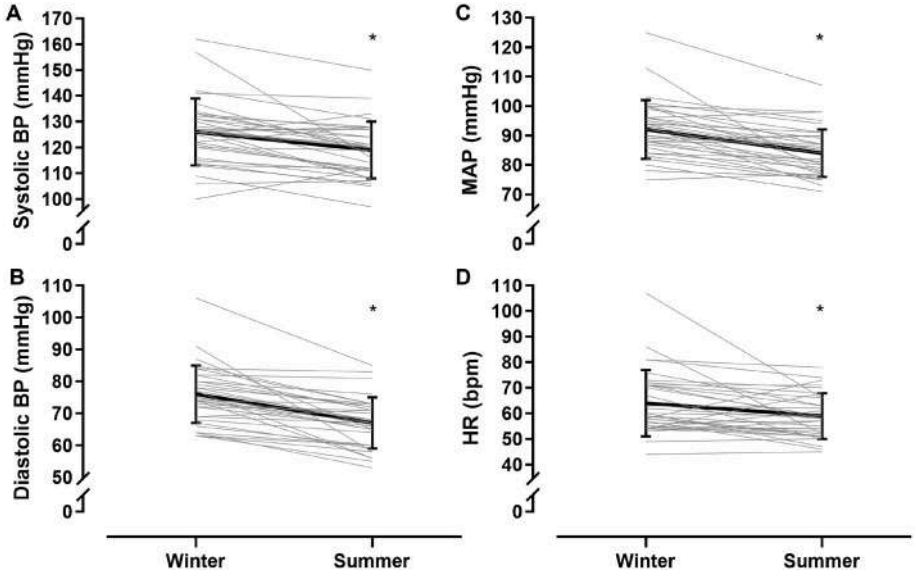

Fig. 2. Systolic (A) and diastolic (B) blood pressure (BP) with mean arterial pressure (C, MAP) and heart rate (D, HR) comparisons between winter and summer. The thing grey lines show individual participant data and the solid black line shows mean ± SD. * denotes significant difference compared to winter ($P < 0.01$).

Table 1
The average daily time and proportion of wear time spent undertaking physical activity in the summer and winter.

	Time (min)		Proportion of wear time (%)	
Activity intensity	Winter	Summer	Winter	Summer
LPA	698 ± 91	701 ± 90	92.4 ± 3.1	90.8 ± 4.3*
MPA	57 ± 23	70 ± 30*	7.3 ± 3.2	8.9 ± 3.5*

LPA, light physical activity; MVPA, moderate-vigorous physical activity.
* denotes significant difference compared to winter ($P \leq 0.01$).

3.5. Dietary vitamin D and nitrate intake

Vitamin D intake was similar in the winter (227 ± 261 IU) and summer (240 ± 532 IU, $P = 0.4$). Dietary nitrate intake was also not different in winter (73 ± 57 mg) when compared to summer (69 ± 49 mg, $P = 0.8$).

3.6. Associations between plasma nitrite and blood pressure

Average daily UV-A exposure was positively associated with plasma [nitrite] ($R = 0.4$, $P < 0.01$, Fig. 3A) and 25-(OH)D ($R = 0.411$, $P < 0.01$, Fig. 3B). Plasma [nitrite] and 25-(OH)D were also positively correlated ($R = 0.4$, $P < 0.01$). Average daily UV-A exposure was not associated with plasma [nitrate] or blood pressure (all $P > 0.06$). Plasma [nitrite] was negatively associated with systolic blood pressure ($R = -0.5$, $P < 0.01$, Fig. 4A), diastolic blood pressure ($R = -0.4$, $P < 0.01$, Fig. 4B), and mean arterial blood pressure ($R = -0.5$, $P < 0.01$, Fig. 2C). However, delta change (between seasons) values of plasma [nitrite] were not correlated with delta change values of blood pressure or 25-(OH)D (all $P > 0.05$). Plasma [nitrite] was not associated with physical activity levels at any intensity (all $P > 0.2$) or dietary nitrate intake ($P > 0.4$).

To assess the association between average daily UV-A exposure and plasma [nitrite], both unadjusted and adjusted linear regressions were performed. The models were performed with log transformed data. In unadjusted analyses, plasma [nitrite] and average daily UV-A exposure produced an R^2 value of 0.124 (β, 0.35; T = 2.25; $P = 0.03$). In the adjusted analyses, the R^2 value was 0.327 with average daily UV-A exposure as a significant predictor (β, 0.44; T = 2.39; P = 0.02). The only other significant predictor in the adjusted model was dietary nitrate intake (β, −0.35; T = −2.15; $P = 0.04$).

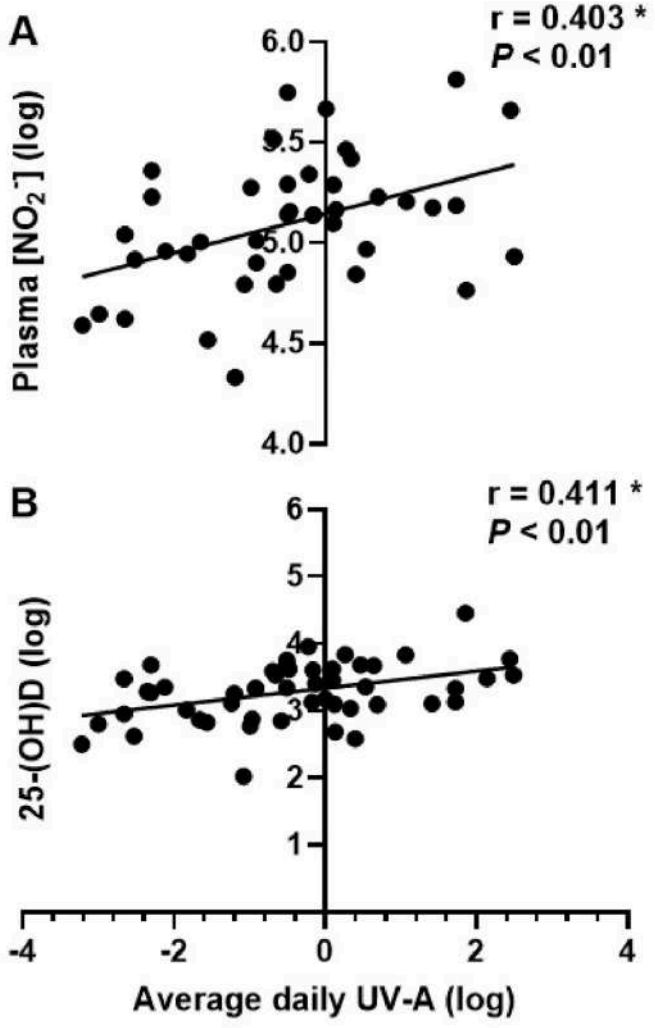

Fig. 3. Log transformed data to display the association between average daily UV-A exposure and plasma [nitrite] (NO_2^-) (Panel A) and serum 25-hydroxyvitamin D (25-(OH)D) (Panel B).

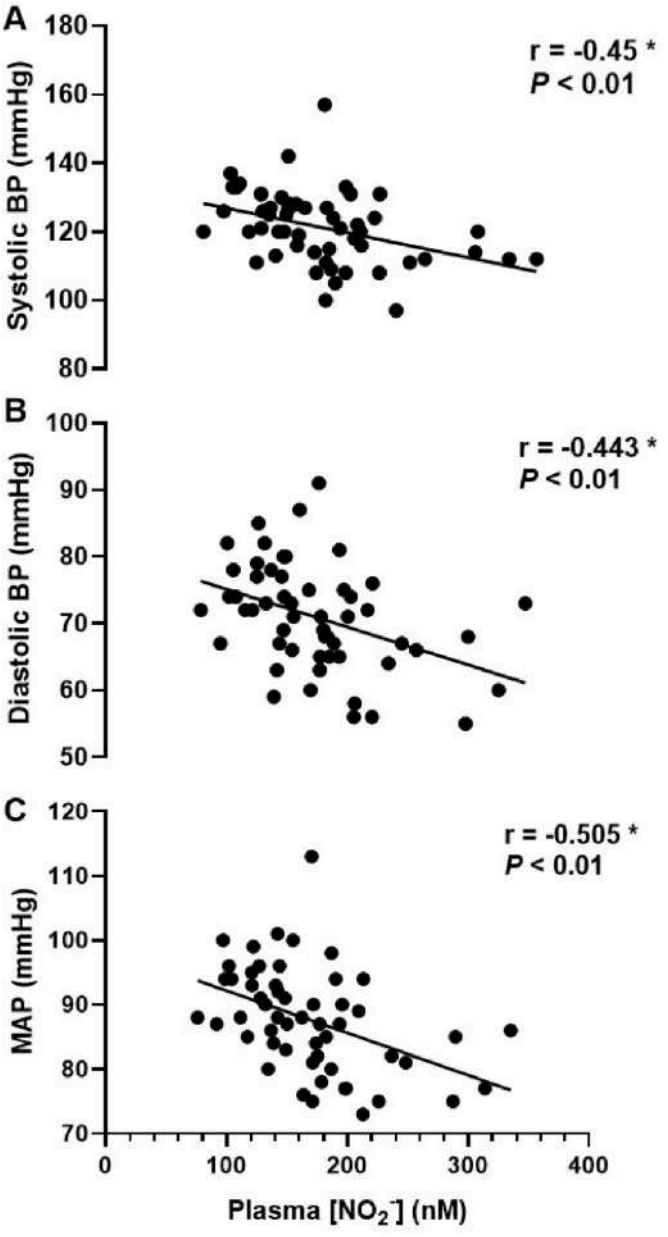

Fig. 4. Association between plasma [nitrite] (NO_2^-) and blood pressure (BP) measures.

4. Discussion

The principal novel finding of the present study is that, as hypothesised, the concentration of plasma and salivary nitrite were higher in the summer, suggesting a lower synthesis of NO in the winter. In contrast, plasma and salivary [nitrate] did not change between summer and winter. Further analyses revealed that plasma [nitrite] was positively associated with UV-A exposure and negatively associated with all measures of blood pressure. Given that plasma nitrite is an established marker of NO synthesis, the increased nitrite concentration in the summer suggests higher levels of NO. Intriguingly, these data suggest that, at least to some extent, basal NO synthesis may be influenced by habitual sunlight exposure which in turn may contribute to seasonal variations in cardiovascular risk factors.

4.1. Sunlight exposure

In the current study, personal average daily UV-A exposure was higher in the summer (2.6 J·cm^{-2}) compared to winter (0.4 J·cm^{-2}). The available daily UV-A dose at a similar latitude (60° N Oslo, Norway) can reach as high as ~170 J·cm^{-2} in the summer and as low as ~10 J·cm^{-2} in the winter [32]. Additionally, participants in Denmark (56° N) who wore personal electronic dosimeters had a lower average erythema effective UV dose in winter (0.03 J·cm^{-2}) compared to summer (1.33 J·cm^{-2}) [36]. Therefore, the reduced personal UV-A exposure (−86%) in winter compared to summer in the current study is not surprising. Although previous studies have found large variation in the UVR exposure between seasons using personal dosimeters, these have not isolated the UV-A or UV-B exposure [36,51]. Therefore, a strength of the current study is that we have been able to measure personal exposures of UV-A rather than utilise modelled proxies.

4.2. Nitric oxide metabolites

Here, we demonstrate that plasma and salivary [nitrite] are significantly higher during the summer compared to winter. Plasma [nitrite] is considered to provide the best approximation of vascular NO synthesis [20,52], and we show for the first time that plasma nitrite is higher in the summer. It is possible that this increased NO synthesis arises from the increased UV-A exposure on the skin in the summer. The skin (dermis and epidermis) has large stores of NO metabolites (nitrosothiols, nitrite, and nitrate) which are substantially higher in concentration than they are in circulating plasma [16,17]. *In vitro*, UV-A radiation of human skin leads to the release and photodecomposition of NO [16]. Whole body UV-A exposure of 20 J·cm^{-2} (~30 min of warm Mediterranean summer sunlight) has been shown to increase plasma [nitrite] [18,19]. Our data supports this with increased plasma [nitrite] and average daily UV-A exposure in the summer, which is moderately correlated.

The increased plasma [nitrite] during the summer may also be a consequence of the indirect effects of greater UV-A exposure, including a higher ambient temperature and increased vitamin D production. For example, hot water immersion has been shown to increase plasma [nitrite] [53], which is suggested to be a consequence of an acute inflammatory response that increases NO production via NOS. Likewise, vitamin D has been shown, *in vitro*, to increase NO production in human umbilical vein endothelial cells [54]. At present, we cannot separate the independent effects of UV-A, ambient temperature, and vitamin D on the seasonal changes in NO availability as our understanding of this complex physiological interplay is still in its infancy. Research using larger cohorts and in locations of varying latitudes would provide further insight. It is also important to highlight that changes in dietary nitrate intake [9] and regular exercise [55] also increase plasma and salivary [nitrite]. In the current study, we report no change in estimated dietary nitrate intake but do show an increase in MVPA during the summer. However, we found no association between MVPA and plasma [nitrite] suggesting it does not play a significant role in the regulation of basal plasma [nitrite].

In contrast to [nitrite], plasma and salivary [nitrate] did not change between seasons. This can be explained by the fact that dietary nitrate did not change between summer and winter. However, it should be acknowledged that dietary nitrate intake was based on self-report and that estimates of nitrate content in food and beverages does not account for seasonal or geographical fluctuations. For example, the nitrate content of some vegetables has been shown to vary considerably throughout the seasons [56] with higher nitrate levels in crops grown in winter compared with the summer [57]. On the other hand, salivary [nitrite] was higher during the summer (134 μM or 87%). Based on the established biological and analytical variation in this metabolite, we can conclude that this is a biologically meaningful difference [41]. It is important to highlight the crucial role that commensal bacteria play in the reduction of nitrate to nitrite in the oral cavity [8]. The abundance of nitrate-reducing bacteria will influence salivary [nitrite] production from nitrate in the diet [9]. In addition, fluctuations in nitrate-reducing bacterial abundances have been shown to occur in healthy adults [41], and these may affect salivary nitrite production. Therefore, it is possible that seasonal fluctuations in bacterial reduction of nitrate [58] may also contribute to increased nitrite in the summer compared to winter. Future research should determine the fluctuations in the function of the oral microbiome through the changing seasons.

4.3. Cardiovascular health

Our findings support previously published data showing seasonal variations in blood pressure for hypertensive patients and healthy controls [37,59]. The change in systolic blood pressure (−7 mmHg or −6%), diastolic blood pressure (−9 mmHg or −10%) and mean arterial blood pressure (−8 mmHg or −13%) from winter to summer are clinically meaningful [41]. As previously stated, small reductions in blood pressure can decrease the risk of stroke and reduces the risk of mortality from cardiovascular issues [22,23]. The importance of these findings is also highlighted by previous research showing that high blood pressure is the leading risk factor for disability adjusted life years lost globally [60] and for cardiovascular disease and mortality worldwide [61–63]. Blood pressure is regulated by a number of factors, including eNOS and NO [64]. The generation of endothelium-derived NO plays a crucial role in vascular homeostasis [1] and endogenous NO production is diminished in hypertensive patients [65].

Plasma [nitrite] reflects eNOS activity in mammals [66] and a decreased synthesis of NO is associated with risk factors for cardiovascular disease [39]. We provide evidence of seasonal variation in plasma nitrite and, based on previous data that shows a reduction in systolic blood pressure following exposure to artificial UV-A radiation [67], we speculate that sunlight exposure is a significant underpinning factor. Here, we report a positive association between plasma [nitrite] and average daily UV-A exposure and a negative association between plasma [nitrite] and indices of blood pressure. However, the delta change values of plasma [nitrite] and blood pressure between winter and summer were not correlated. This suggests that seasonal changes in blood pressure were not entirely mediated by NO synthesis. Additionally, whilst it is possible that the correlations may be particularly important in showing indirect influences on blood pressure, it must be acknowledged that all statistically significant associations were only “moderate” in strength (R = 0.40–0.49), are likely underpowered, and do not necessarily imply “cause-effect”.

Instead, colder temperatures during the winter months may have a more significant impact on blood pressure. Frequent exposure to cold temperatures can increase sympathetic tone which, in turn, can increase blood pressure [78,68]. This is certainly plausible as, in the present study, the average ambient temperature was between 3 and 4 °C in the winter months and between 14 and 17 °C in the summer. It is also important to highlight that a lack of sunlight, leading to vitamin D deficiency, has been proposed as a contributing factor to this linear rise

in blood pressure during winter months [31,69]. In the current study, 25-(OH)D was higher in the summer (35 ± 13 ng ml^{-1}) than in the winter (22 ± 8 ng ml^{-1}). These values are higher than previous research which showed that 25-(OH)D concentrations in the Scottish Heart Health Extended Cohort for the 2 months following the winter and summer solstice were ~12 ng·ml^{-1} and ~23 ng·ml^{-1}, respectively [31]. The difference between these studies may be due to the analytical methods used to measure 25-(OH)D (ELISA in this study and a chemiluminescence immunoassay in the study of [31]), the weather, or lifestyle behaviours between study cohorts. Regardless, the low concentration of 25-(OH)D measured in this study and previous research is potentially of importance when one considers that cardiovascular disease is inversely associated with this marker [27]. It should be noted, however, that supplementing with vitamin D has been shown to have no or little effect on blood pressure, incidence of stroke, or ischemic heart disease [70,71]. On the other hand, increased nitrate in the diet has been shown to reduce the incidence of hypertension [72] and lowers the risk of cardiovascular disease ischemic events [73]. Furthermore, supplementing the diet with additional nitrate has been shown to consistently reduce blood pressure [74]. The present study provides further data to support the notion that NO may be relevant for cardiovascular health. While serum 25-(OH)D may reflect sunlight exposure it may not be the main metabolite which directly influences cardiovascular health. It should also be noted that UV-B was not directly measured in the current study, but the increased UV-A exposure can also be a proxy for increased sunlight exposure.

4.4. Implications

For the first time, the current study has reported that NO synthesis varies between seasons which may have important consequences for cardiovascular health. The variation in plasma [nitrite] is likely to be underpinned by the duration and magnitude of sunlight exposure and/or changes in temperature and circulating vitamin D, but seasonal fluctuation in the oral microbiome cannot be ruled out. Based on these data, it is plausible to suggest that this seasonal fluctuation in plasma [nitrite] could be an additional factor contributing to seasonal hypertension [75]. Therefore, future research should determine the effect of seasonal fluctuations in both plasma [nitrite] and blood pressure in populations who are at risk of developing cardiovascular disease and explore interventions to increase NO synthesis during winter, such as the Dietary Approach to Stop Hypertension (DASH) diet [76].

4.5. Limitations

The study, however, is not without limitation. Firstly, this is a pilot study with a small sample size and therefore, the associations should be interpreted with caution. Additionally, the participants in this study were all healthy and recreationally active. Consequently, the effects of UV-A exposure on markers of NO synthesis and blood pressure in other populations is not yet clear. Another potential limiting fact is the lack of monitoring of the menstrual cycle for female participants given influence of cyclical hormonal changes on NO production [77]. However, separate statistical analysis for male and female participants showed that plasma [nitrite] remained higher in summer compared to winter (both $P < 0.01$).

5. Conclusion

The main finding of the study was that plasma and salivary [nitrite] are lower in the winter compared to the summer, suggesting reduced synthesis of NO in the winter months. Our finding that absolute plasma [nitrite] was inversely correlated with blood pressure also extend previous research which has shown blood pressure to be higher in the winter compared to the summer months. Plasma [nitrite] was also positively correlated with UV-A exposure measured using personal UV dosimeters, which suggests that NO synthesis may be influenced by habitual sunlight exposure. In turn, sunlight exposure may cause seasonal variations in cardiovascular risk factors. Future research should focus on understanding the link between UV-A, plasma [nitrite], cardiovascular risk factors, and nitrate-reducing bacteria in the oral cavity.

Author contribution

CM, CE, DM and LL conceptualised and designed the study. LL and KB analysed the UVR data for this paper. LL, CM, and MB contributed to the acquisition of data. All authors contributed to the writing of the manuscript and agree to be accountable for all aspects of the work ensuring accuracy and integrity.

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Declaration of competing interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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