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The health benefits of UV radiation exposure through vitamin D production or non-vitamin D pathways

Blood pressure and cardiovascular disease.

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The detrimental effects of ultraviolet radiation are well known. Skin cancer, photo-aging, and induction or exacerbation of photosensitive dermatoses have been the focus of most photobiological research since 1928 when Findlay confirmed the carcinogenicity of ultraviolet radiation using a murine model of skin cancer[1]. The epidemiological, mechanistic and clinical trial data have enabled the classification by the International Agency for Research on Cancer of ultraviolet radiation as a Group 1 ('sufficient evidence') carcinogen for human skin[2]. Public health advice in most developed countries with a pale-skinned population following this has advocated limiting exposure to sunlight through use of clothing, sunblock and behavioural alterations[3-5]. Despite this plethora of data, one striking omission is evidence that ultraviolet radiation shortens life, and as I will lay out in this chapter, epidemiological and now mechanistic data suggest that UV may have significant benefits on health and in particular cardiovascular health.

Hypertension, latitude and season

The most recent World Health Organisation Global Burden of Disease survey assesses high blood pressure as the leading global risk factor for lost Disability Adjusted Life Years[6]. Hypertension is an important risk factor for cardiovascular and cerebrovascular disease, themselves two of the leading causes of death and disability. A considerable body of epidemiological data is consistent with the hypothesis that sunlight might reduce blood pressure and heart disease. Population blood pressure correlates inversely with latitude[7] as does atherosclerosis, for which high blood pressure is a risk factor. Temperature and exercise may account for some of this but UV appears to be an independent variable[8]. In a prospective Europe wide study to determine factors determining cardiovascular events, measurements of carotid artery intima-media thickness (C-IMT) were recorded. C-IMT is a marker of sub-clinical atherosclerosis and correlates closely with risk of cardiovascular death. Subjects were enrolled in 7 centres from Kuopio in Finland to Perugia in Italy. Multivariate regression analysis showed that latitude was the major independent determinant for C-IMT, accounting for almost half of variation, and significantly more important than other measured variables such as age, dietary factors, smoking, lipids, exercise, gender, age or blood pressure[9]. Correspondingly, risks of death from cardiovascular disease in Europe also correlate with latitude, being higher in northern countries[10-12]. Genetic or cultural differences such as dietary salt intake may account for these pan-European differences, but the linear relationship between latitude and cardiovascular endpoints is more consistent with a causative factor with a steady cline such as temperature or UV, than a discrete and random variable such as genetic variation or diet.

Further evidence differentiating between population determined effects (e.g. diet, genetics) and environmental effects (e.g. UV, temperature) can be obtained by examining seasonal effects. In those living in temperate climates, strong seasonal variations are seen in blood pressure which cannot be accounted for by inter-population differences at different geographical locations[13]. Within the UK, blood pressure is higher in untreated hypertensives in winter than summer, particularly in older patients, and this seasonal variation even occurs in patients on treatment with thiazide

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diuretics or propranolol[14]. Patients undergoing renal dialysis show the same seasonal variation with higher BP in winter than summer[15] and this effect also occurs following renal transplantation[16]. Temperature accounts for some of this variation[15], but an analysis of BP data from over 1/3 million dialysis patients that I am currently undertaking shows that UV is associated with reduced BP independently of temperature.

Vitamin D

Vitamin D has been extensively studied and is widely available as an over the counter health supplement, with many claimed benefits. Measurements of serum vitamin D levels (as 25-hydroxyvitamin D) show that populations with higher vitamin D levels are less likely to have hypertension, cardiovascular disease, cerebrovascular disease, or in fact death from any cause[17]. Those whose measured vitamin D levels are in the lowest quartile are around twice as likely to have one of these conditions as those whose levels are in the highest quartile[18]. Correlation is not evidence for causation however. Over 45 clinical trials have now been performed studying vitamin D supplementation as a treatment for hypertension. Earlier meta-analyses had raised the possibility of a vitamin D induced non-significant reduction of systolic but not diastolic blood pressure[18], or of diastolic but not systolic pressure[19]. The proliferation of trials of vitamin D supplementation on cardiovascular outcomes has reduced this uncertainty, and the most recent and thorough meta-analyses combining the data from all eligible studies confirm that intervention with oral vitamin D supplementation has no effect on blood pressure[20]. Meta-analyses looking at cardiovascular and cerebrovascular outcomes show a similar lack of effect on oral vitamin D supplementation[17, 18, 21]. They also confirm that the large number of patients enrolled in such studies exceeds the power needed to confirm any direct link between oral vitamin D supplements improved cardiovascular health, and that no further studies are needed[21]

Further proof that Vitamin D does not play an important part in maintenance of cardiovascular health comes from Mendelian randomisation studies, which behave like a natural randomised, controlled clinical trial. Random assortment of genetic variants at the time of gamete formation acts in the same manner as randomisation for a clinical trial and prevent confounding, as long as there is no linkage disequilibrium. Polymorphisms in the genes *DHCR7* and *CYP2R1* lead to lifelong reduced 25-hydroxy vitamin D formation. Nearly 100,000 Danes were studied in the Copenhagen City Heart Study, General Population Study, and Ischaemic Heart Study. All participants were genotyped for these two variants and an allele score given dependent on how many low vitamin D coding variants they carried. An allele score associated with reduced genetic vitamin D production correlated with an increased cancer and all-cause mortality, but no increase in cardiovascular mortality, (in fact a trend to reduced cardiovascular mortality)[22]. Similar Mendelian randomization studies using BP as an outcome measure have shown no effect of polymorphisms affecting Vitamin D synthesis, metabolism or signaling[23]

Vitamin D independent mechanisms of cardiovascular benefit.

Measured vitamin D levels inversely correlate with cardiovascular health, but Vitamin D is not causally related to cardiovascular health. Several explanations can be made for this.

Poor cardiovascular health and hypertension may directly reduce vitamin D levels. Vitamin D levels are a function of a balance between synthesis and inactivation pathways[24]. Levels inversely correlate with inflammatory markers in patients with heart failure[25], although there are no published data showing any direct effect of heart disease on this homeostatic balance.

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Vitamin D may be a marker for poor cardiovascular health, as impaired health may lead to poor diet and less time spent outside in the sunlight. This explanation seems unlikely to account for the hypertension data, as this is a symptomless condition and should not therefore affect dietary or exercise choices. Symptomatic cardiovascular disease will have an effect on exercise and thus time spent outside, although epidemiological studies generally sought to exclude individuals with this confounder.

Measured vitamin D levels may be a marker for exercise performed outside. Exercise has a potent beneficial effect on cardiovascular health, lowering blood pressure and incident heart disease. Healthy lifestyles will thus be reflected by higher vitamin D levels.

Finally and perhaps most controversially given the concerns of the dermatological community on the hazards of UV exposure, sunlight may have beneficial cardiovascular effects, independently of Vitamin D production. Vitamin D could in these circumstances act as a marker for sunlight exposure and its postulated beneficial effects[26].

A recent retrospective study from Denmark study looked at all Danes over the age of 40 from 1980 to 2006. Denmark has extremely thorough health records, and records 98% of cancer diagnoses and all myocardial infarctions. Using a case-control design, all incident cases of skin cancer were matched with 5 controls by age, birth year and gender. A diagnosis of non-melanoma skin cancer conferred an odds ratio of myocardial infarction of 0.90 (95% confidence intervals 0.88–0.92) and cutaneous melanoma an odds ratio of 0.74 (0.68–0.81)[27]. Ultraviolet radiation is the major risk factor for non-melanoma skin cancer in a fair skinned population such as the Danes, and as such, NMSC can act as a proxy for cumulative sun exposure[28]. Other environmental risk factors for NMSC include use of photosensitising anti-hypertensive medication, chronic inflammatory skin conditions (Marjolin's ulcer), immunosuppression-either iatrogenic, or due to conditions such as HIV, and smoking[29]. As these risk factors were not controlled for, the reduction in the odds ratio of developing a myocardial infarction or indeed mortality from any cause (0.97 95% confidence intervals 0.96–0.99) in this case-control study is striking[28]. Factors associated with development of NMSC such as sunlight, or outdoor lifestyle, must be beneficial enough to outweigh the adverse effects of the other exposures.

Two prospective cohort studies from Scandinavia have recently been published, each designed to identify the risks of sunlight exposure on melanoma induction and mortality. The strength of both these studies is that sun exposure data for each participant was assessed. The Melanoma in Southern Sweden study (MISS) recruited just under 30,000 Swedish women and gave them a sun exposure rating at the start of the study based on whether they sunbathed in summer, in winter, went on foreign holidays, or used artificial tanning lamps[30]. Extensive correction was made for known confounders both from questionnaire data and by cross-referencing their study records with health and tax databases. 25 years after study initiation, it was found that those with the highest sun exposure scores did indeed have an increased incidence of melanoma, but that there was a straight line inverse relationship between UV exposure and all-cause mortality. Extrapolating the findings of the study to the Swedish population, 3% of deaths in Sweden could be accounted for by inadequate sun exposure[30], and those with the lowest sun exposure scores carried a similar hazard for death compared to the maximally exposed group as smokers[31].

The Swedish Women's Lifestyle and Health Study also found that increased sunlight exposure correlated with reduced all cause and particularly cardiovascular mortality[32]. 38,472 women were recruited and completed a health questionnaire at initiation. 15 years later, incident malignant melanoma was higher in those with higher numbers of sunburns, mole numbers, red hair, and weeks

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spent on sunbathing holidays[33]. However, multivariately adjusted hazard ratios for all-cause mortality and for cardiovascular disease were significantly reduced in those with the highest sun exposure habits, as assessed by weeks spent on sunbathing holidays[32].

One other epidemiological study has studied UV and all-cause mortality, but used estimates of population UV exposure rather than individual estimates. The findings of this study are the only ones which suggest a link between UV and increased deaths, but weaknesses in the study make a causal relationship seem unlikely. Lin et al calculated incident environmental UV from NASA's Total Ozone Mapping Spectrometer and mapped this onto the residential addresses of 346,615 participants in the NIH-AARP Diet and Health study, recruited from 6 American states. The participants were not asked about UV related behaviour, and no occupational data (e.g. indoor *vs* outdoor) was collected so it is not known to what degree this outdoor environmental data indicates actual personal exposure. Those living in the highest sunlight areas had a higher standardised all- cause mortality. Although this might suggest a UV induced excess of deaths, the causes of death did not match known UV driven mechanisms. The major relative increased cause of death was for respiratory disease. There was a slight increase in deaths from cardiovascular disease and stroke in high versus low UV regions, but no dose-response relationship. Deaths from cancer were increased in men proportionately to the amount of UV, but women did not have higher death rates in higher UV areas. The cancers with the highest relative risk increase were, in descending order: liver, melanoma, and lung. Deaths from injury were increased in men living in sunnier areas. These data appear at odds with those from the Scandinavian studies, where UV exposure was assessed with reference to personal behaviour. Skin cancer is the only disease for which epidemiological, mechanistic and clinical trial data all confirm a UV-cancer induction cause-effect relationship. If the incident UV at the geographical location of participants homes correlates directly with personal UV exposure, one would have expected skin cancers (such as melanoma) to be a leading cause of UV related death. The high prevalence of respiratory causes of death, and cancers other than skin suggest that confounding may underlie this apparently higher hazard ratio for UV related deaths.

Nitric oxide and the skin

Avitamin D independent mechanism by which sunlight acting on skin can exert beneficial cardiovascular effects has recently been described by Christoph Suschek's group in Dusseldorf[35] and mine in Edinburgh[34]. It relies on the UVA mediated mobilisation of cutaneous nitric oxide stores to the systemic circulation.

Nitric oxide (NO) is a small diatomic free radical with widespread physiological, immunological and cell signalling effects. Nitric oxide was originally identified from its first described actions as 'endothelial derived relaxant factor'[36]. This was subsequently delineated as nitric oxide, synthesised by a nitric oxide synthase in the endothelial cells[37]. It diffuses from its site of synthesis in the endothelial cells to the adjacent vascular smooth muscle cells to activate cyclic GMP and cause relaxation and vasodilatation. This pathway, and the novel idea that a small molecule might have tightly regulated and important biological roles earned its discoverers the 1998 Nobel Prize for medicine. NO synthesis was initially described following reduction of arginine to citrulline and NO by one of a family of three nitric oxide synthases. NOS I and III (neuronal and endothelial) are constitutive, calcium dependent enzymes broadly involved in homeostatic processes including neurotransmission, apoptosis and cell growth control and vasodilatation. NOS II is an inducible NOS which releases much higher amounts of NO and either alone or in combination with superoxide anion (which generates the potent oxidiser peroxynitrite) can drive inflammatory and cytotoxic effects in roles such as cytolysis, and pathogen destruction. Nitric oxide has a half-life of a few seconds, and is oxidised to nitrite (NO_2^-) in aqueous solution. Nitrite is more stable with a half-life of

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several hours and is often used experimentally as an indicator of NO concentration/production. Nitrite is finally oxidised to nitrate anion, NO_3^-. Nitrate has traditionally been thought of as a stable and biologically inactive molecule which was filtered to the urine by the kidneys to be cleared from the body. The major source of nitrate in man is from the diet, with green leafy vegetables such as spinach, and beetroot being particularly rich food sources. Nitric oxide oxidation can also be a source of nitrate however and as early as 1916 it was noted that more nitrate was excreted in human urine than was ingested[38], and that urinary nitrate excretion rose in sepsis, an effect now known to reflect increased iNOS activity.

The paradigm of nitrate as inert waste product of NO oxidation[39] has recently been overturned. Bacteria have been long known to contain nitrate reductases, and in fact, this was a traditional biochemical test for the presence of *Staphylococci*. A mammalian nitrate reductase was first described in 2008 and in both rodents and man this reduces inorganic nitrate to nitrite which in turn is reduced to NO with involvement of xanthine oxidoreducase[40]. Oral nitrate ingestion lowers blood pressure in man[41] and reduces oxygen demand during vigorous exercise[42] via effects on mitochondrial efficiency[43]. A further mechanism of reduction of nitrate to NO has also been identified dependent on photolysis. Thiols, particularly reduced thiols (R-SH), potently enhance UV induced reduction of nitrate (NO_3^-) to NO and S-nitrosothiols (R-SNO)[44]. My group observed that delivery of NOS antagonists to human skin via routes including topical application, iontophoresis, microdialysis and intradermal injection failed to prevent UV induced NO release. Problem solving these experiments led to the discovery by us[45] and colleagues[46] that the skin contains large stores of nitrogen oxides, in particular nitrate and S-nitrosothiols. Discussion of these data led us to hypothesise that the nitrogen oxides stored in human skin, and their mobilisation by UV irradiation to the systemic circulation might account for the observed epidemiological effects associating increased sun exposure with cardiovascular health described above[26] (Figure).

Exposure of human skin to physiologically relevant quantities of UVA leads to a fall in blood pressure and rise in heart rate, independently of temperature change[34, 35]. This is accompanied by a rise in circulating nitrite (the more stable oxidation product of gaseous NO), and fall in nitrate[34], with the rise in nitrogen oxides correlating in a linear fashion with the fall in BP[35]. Unexpectedly, the fall in nitrate levels was greater than the rise in nitrite, rather than following a simple stoichiometric ratio, a difference that as yet is unexplained[34]. A fall in BP with rise in heart rate indicates vasodilatation and a decrease in total peripheral resistance. Forearm venous plethysmography experiments confirm the direct vasodilatory effect of UVA on the human arterial vasculature. Infusion of the NOS antagonist L-NMMA into the brachial artery to block NOS derived NO production produced the expected vasoconstriction, and subsequent exposure of the NOS antagonised forearm to UVA led to vasodilatation, presumably by NOS independent NO release[34]. These experimental data are exciting, and the first description of a vitamin D independent UV driven-nitrate reduction and vasodilatation mechanism in man, but they may in fact be the rediscovery of an old story. Robert Furchgott won the Nobel prize for his description of 'Endothelial Derived Relaxant Factor'[36], now known to be nitric oxide generated by the action of endothelial nitric oxide synthase on arginine in endothelial cells[37]. Long before this, he observed that strips of rabbit aorta from which the endothelium (and thus endothelial NOS) had been removed were alternately relaxing and constricting in their organ bath. The day was bright but cloudy and he identified that this was not occurring in organ baths furthest from the window, and that the synchronous relaxation of those strips close to the window only occurred when the sun was uncovered by clouds[47]. He termed this process photorelaxation[48] and over the following two decades was able to define an action spectrum with a peak photorelaxation at 310nm, which was enhanced and shifted to 355nm when sodium nitrite was added[48, 49]. After the discovery of EDRF, and before its identification as nitric oxide Furchgott was able to compare the

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two muscle relaxing processes and found a number of similarities. Both photorelaxation and endothelial derived relaxation acted via cyclic GMP, and both could be inhibited by methylene blue and by haemoglobin[50]. Ultimately he proved that this *in vitro* photorelaxation was due to the release of NO from 'long wavelength' UV irradiated nitrite[51] and in his autobiography on the Nobel prize website, he speculates 'It is tempting to hypothesize that light (in the absence of added nitrite) produces relaxation of vascular smooth muscle by photoactivating the release of NO from some endogenous compound ..'[47].

Using rat aortic tissue, Rodriguez has shown a predominantly two component photorelaxation process[52]. A 330 to 340nm photorelaxation peak corresponded to the experimental NO release spectrum for S-nitrosothiols. Prolonged exposure to 335nm radiation exhausted S-nitrosothiol stores, revealing a flatter 310 to 360nm photorelaxation shoulder correlating with the nitrite derived NO release spectrum. The S-nitrosothiols required energies of UV around two orders of magnitude lower than those for nitrite, but correspondingly concentrations of nitrite in rat aorta were around two orders of magnitude higher, suggesting that these two nitrogen oxides play a similarly important role. Using human skin, Suschek has shown that human skin UVA induced NO release relies on stores of nitrite and nitrosothiols, which are present in higher concentrations than in plasma[46]. Reduced thiols strongly augment the photodecomposition of nitrite and thus enhance NO release. Short wavelength visible blue light can also mobilise NO in human skin[53] although higher fluences are required, and this may offer an ultraviolet free means of cardiovascular protection.

Nitrate stores exceed those of nitrite in the skin by an order of magnitude[45]. Nitrate is an important precursor to nitrite and thus NO in human cardiovascular protection[54] and can be photochemically reduced to inorganic nitrite[44], but the regulatory control of this range of oxidation, reduction and nitrosation reactions in human skin remains only partly understood. Redox status, sunlight and thiol presence affects these reactions, and interactions with sulfide groups add a layer of complexity and probable regulatory control[55]. Inter-individual variations in photorelaxation are seen in aortic strips taken from different rats which may reflect differences in stores of nitrogen oxides[52]. The source, size and precise location of nitrogen oxide stores in skin remains unclear[45] as does the extent of depletion of such stores on sunlight exposure. They may be dietary in origin[26], and it is known that a nitrate rich diet lowers BP[56]. Alternatively oxidation of NOS derived NO may be responsible(Figure).

These recent human data[34, 35] show the physiological relevance of photorelaxation. High blood pressure is the leading cause of disability adjusted life years lost worldwide and as a risk factor underlies 18% of all deaths[6]. Hypertension is a diagnostic category, but reductions in blood pressure, even within the healthy range confer health benefits such as reduced risk of cerebrovascular accidents and ischaemic heart disease[57]. So far, we have shown a vitamin D independent mechanism by which sunlight could lower blood pressure, but we do not yet know whether alterations in UV exposure- either artificially or by lifestyle changes allowing increased sunlight exposure- will lead to a sustained fall in blood pressure. We have recently started a clinical trial on the use of UVA home phototherapy as a treatment for mild hypertension (Clinicaltrials.gov Identifier NCT02621866) which should provide these answers for a patient population. The question as to whether improved sun exposure might to beneficial further lowering of the 'normal' blood

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pressure in a healthy population will require futher studies. Ultraviolet A radiation was used in our initial mechanistic studies as it does not induce vitamin D synthesis and could thus confirm a vitamin D independent effect of ultraviolet radiation on blood pressure. The action spectrum of nitrite release shows that ultraviolet B is also involved in nitrite reduction to NO[52], and thus sunlight may be more effective than a pure UVA source.

Pharmacological mechanisms such as vitamin D and nitric oxide for the suggested health benefits of sunshine may not be the sole explanation. Dopico and colleagues data mined gene expression sets from adipocytes and peripheral white blood cells collected from volunteers in studies in Australia, the Gambi, Europe and Iceland[58]. A remarkable seasonality in gene expression was found. 23% of genes are differentially expressed in subjects living in temperate climates. Broadly speaking, anti-inflammatory genes were upregulated in summer and pro-inflammatory genes in winter. Soluble IL-6 receptor and C reactive proteins, which are both markers of inflammation and risk factors for cardiovascular disease were also elevated in winter. It is not clear from the data whether the pro-inflammatory milieu in winter is an adaptive evolutionary response that prepares the body for combatting the higher incidence of infectious disease in winter, or is a consequence of exposure to such infections. Light exposure is the predominant zeitgeber entraining circadian rhythms. It thus seems likely that sunlight will also affect seasonal variations in gene expression. Inflammation is an important risk factor for cardiovascular disease[59] and sunlight entrainment of a seasonal variation in gene expression may thus also affect cardiovascular risk.

Medical and public health advice on sun exposure affects how our patients behave. We have robust evidence that sunlight is a risk factor for skin cancer, supported by epidemiological, mechanistic and trial data. However, the prevalence of cardiovascular and cerebrovascular deaths is around 100 times higher than those from skin cancer[6]. Interventions leading to small changes in the incidence of cardiovascular disease are thus of greater benefit to the health of the public even than large changes in skin cancer incidence. Epidemiological and mechanistic data now suggest that sunlight has cardiovascular benefits (Table). A priority of photobiological research should now be in developing advice that strikes a balance between the proven carcinogenic actions of ultraviolet radiation with the possible/probable benefits of the same UVR on cardiovascular health and all-cause mortality.

Figure and Table Legends

Table.

An overview of available evidence for skin carcinogenic effects of ultraviolet radiation, and cardiovascular benefits.

Figure.

Summary of nitric oxide production pathways involving the skin

Table

Evidence type.	**Epidemiological**	**Mechanistic**	**Intervention/trial**
Skin carcinogenic effects of UVR	Increased incidence of NMSC and MM in: • Sunnier climates. • Sun seeking behaviour. • Outdoor workers (SCC). • With tanning bed use. • In lower Fitzpatrick skin types.	• Mutagenic effects of UV radiation • UV signature mutations in skin cancers • Free radical generation by UVA. • Immunosuppressant effects of UVR[60]	• Ultraviolet radiation induces skin cancers in murine models[1]. • Sunblock reduces NMSC incidence in man. [61, 62]
Cardiovascular benefits of UVR	Observational data: • Low measured vitamin D correlates with increased hypertension and cardiovascular disease incidence[18] • Reduced blood pressure[14] and incident cardiovascular disease in summer[63] than winter • Anti-inflammatory:inflammatorygene expression ratio highest in summer[58] Case-control data: • Reduced odds ratio for myocardial infarctions in NMSC patients[27] Prospective cohort data: • Dose dependently sun seeking behaviour inversely correlates with all-cause mortality[30] • Sun seekers have reduced cardiovascular mortality[32]	Oral vitamin D supplementation does not reduce blood pressure or cardiovascular disease incidence[21] UV induced 'photorelaxation' of arteries[48] UV mobilises NO from nitrate in the presence of thiols[44] UVA exposure of skin vasodilates systemic arteries in man independently of NOS, vitamin D and temperature[34]	UVA exposure lowers BP in man independently of vitamin D and temperature[34, 35]

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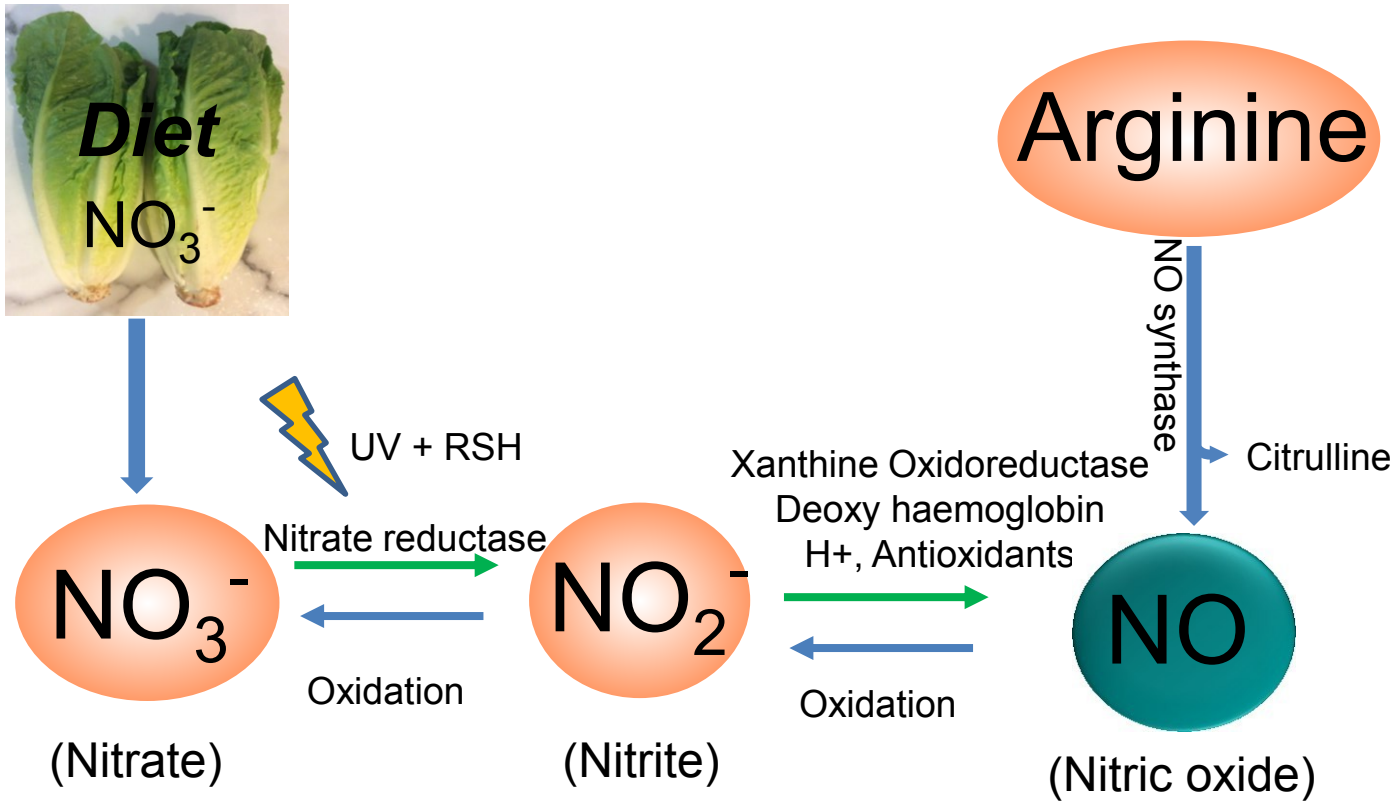

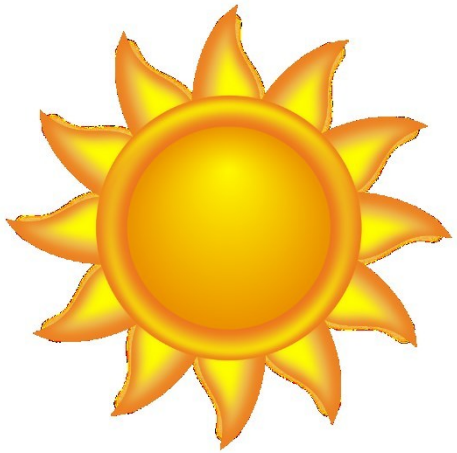

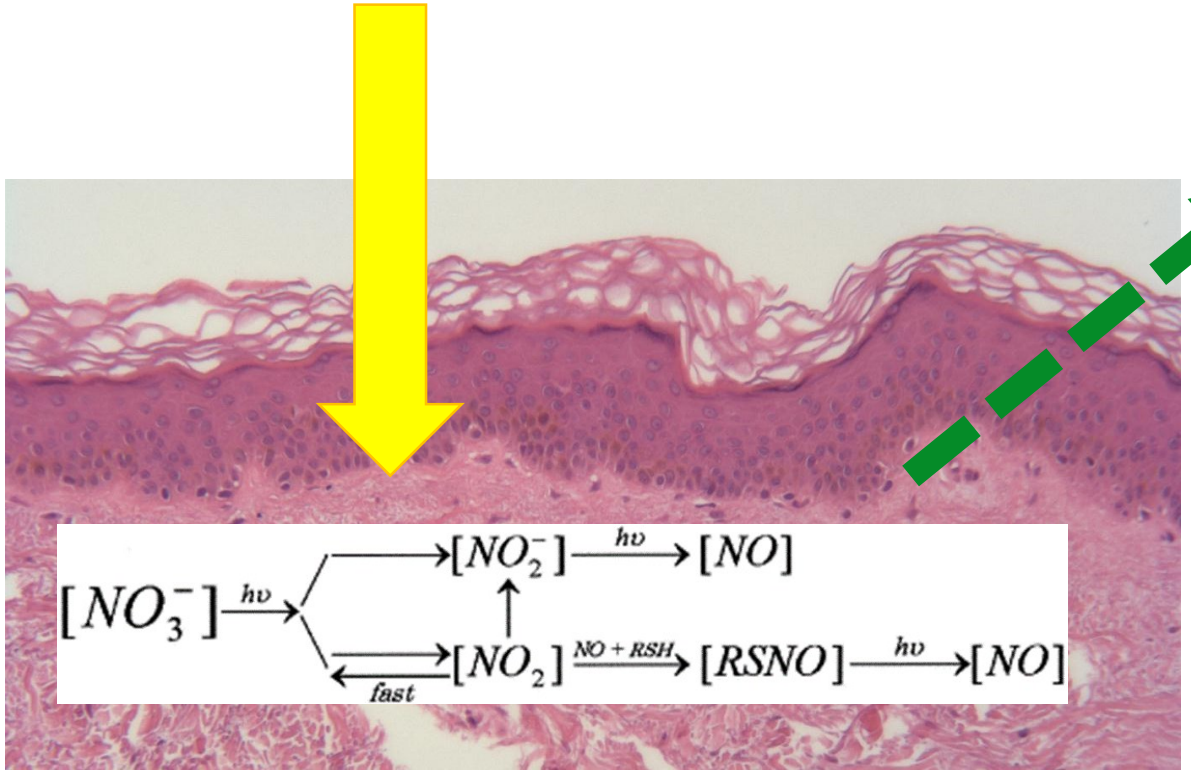

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