DR ANTONY RICHARD YOUNG (Orcid ID : 0000-0002-4163-6772)
MR KARL P LAWRENCE (Orcid ID : 0000-0003-0124-961X)

Article type : Original Article

Optimal sunscreen use, during a sun-holiday with a very high UV index, allows vitamin D synthesis without sunburn

A.R. Young[1], J. Narbutt[2*], G.I. Harrison[1], K.P. Lawrence[1], M. Bell[3], C. O'Connor[3], P. Olson[4], K. Grys[1], K. Baczynska[5], M. Rogowski-Tylman[6], H.C. Wulf[4], A. Lesiak[2], P.A. Philipsen[4]

Corresponding author: antony.young@kcl.ac.uk

[1]King's College London, St John's Institute of Dermatology, London, SE1 9RT, UK; [2]Medical University of Łódź, Department of Dermatology, Pediatric Dermatology and Dermatological Oncology, Łódź, 90-647, Poland; [3]Walgreens Boots Alliance Inc., Nottingham NG90 5EF, UK; [4]Bispebjerg Hospital, Department of Dermatological Research, Copenhagen 2400, Denmark; [5]Public Health England, Centre for Radiation, Chemical and Environmental Hazards, Chilton, Didcot, Oxfordshire OX11 0RQ, UK;

[6]Dermoklinika Centrum Medyczne, Łódź, 90-436, Poland;

***Equal 1st author**

Running title

Optimal sunscreen use allows vitamin D synthesis

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/bjd.17888

Keywords

Vitamin D, sunscreen, UVA protection factor, holiday, UVR, SED, sunburn, erythema

What is known about this topic?

- Action spectra (wavelength dependence) for erythema and the cutaneous formation of vitamin D overlap considerably in the UVB region.
- Theoretically, sunscreens that inhibit erythema should also inhibit vitamin D synthesis.
- Studies to date on the inhibitory effects of sunscreens on vitamin D synthesis have given conflicting results; possibly in part because people typically apply sunscreen sub-optimally.
- Many studies have design flaws.

What does this study add?

- Sunscreens (SPF = 15) applied at sufficient thickness to inhibit sunburn during a week-long holiday with a very high UV index still allow a highly significant improvement of $25(OH)D_3$ concentration.
- An SPF=15 formulation with high UVA protection enables better vitamin D synthesis than a low UVA protection product. The former allows more UVB transmission.

What is the translational message?

- The UVB dose necessary for vitamin D synthesis is very low when a large fraction of body surface area is exposed.
- The benefits of sunscreen use can be advocated without concern about compromising vitamin D status, at least with SPF=15 used optimally.
- It is possible to tailor the optical properties of sunscreens to optimize the benefits and risks of solar UVR exposure.

Abstract

Background Sunlight contains UVA and UVB radiation. The latter is essential for vitamin D synthesis but is the main cause of sunburn and skin cancer. Sunscreen use is advocated to reduce the sun's adverse effects but may compromise vitamin D status.

Methods The impact of sunscreens on vitamin D status was studied during a one-week sun-holiday in Tenerife (28°N). Comparisons were made between two formulations, each with a sun protection factor of 15. The UVA protection factor (UVA-PF) was low in

one case and high in the other. Healthy Polish volunteers (n=20 per group) were given the sunscreens and advised on correct application. Comparisons were also made with discretionary sunscreen use (n=22) and non-holiday groups ($51^{o}5$N, n=17). Sunscreen use in the intervention groups was measured. Behaviour, UVR exposure, clothing cover and sunburn were monitored. Serum 25(OH)D, was assessed by HPLC MS/MS.

Results Use of intervention sunscreens was the same (p=0.599) and both equally inhibited sunburn, that was present in the discretionary use group. There was an increase ($p=9x10^{-8}$) of 28.0±16.5(SD) nmol/L 25(OH)D_3 in the discretionary use group. The high and low UVA-PF sunscreen groups showed statistically significant increases ($p\leq6.7x10^{-5}$) of 19.0±14.2 and 13.0±11.4 nmol/L 25(OH)D_3 respectively. The non-holiday group showed a fall (p=0.08) of 2.5±5.6 nmol/L 25(OH)D_3.

Conclusions Sunscreens may be used to prevent sunburn yet allow vitamin D synthesis. A high UVA-PF sunscreen enables significantly higher vitamin D synthesis than a low UVA-PF sunscreen because the former, by default, transmits more UVB than the latter.

Introduction

Terrestrial solar ultraviolet radiation (UVR ~295-400nm) contains UVB (280-315nm) and UVA (315-400nm). Maximal UVB content is ~5% but this region is 3-4 orders of magnitude more damaging than UVA per unit dose (J/m^2) for sunburn[1], potentially mutagenic epidermal DNA lesions, such as cyclobutane pyrimidine dimers (CPD) [1] and keratinocyte cancers (KC)[2].

However, UVB initiates cutaneous vitamin D_3 synthesis. Indeed, most (e.g. 80%) vitamin D is acquired from solar exposure[3], resulting in seasonal variations in temperate climates[3,4]. Vitamin D is essential for skeletal integrity and has been

associated with many other health benefits, though these remain controversial[5] or disputed[6]. It also enhances repair of epidermal DNA photolesions[7].

Solar UVR is responsible for an increasing incidence of melanoma, basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), especially in white-skinned populations[8-10], for which sunburn is a risk factor[11,12]. Much public health effort has been spent advising those at risk to minimize solar UVR exposure. The use of sunscreens is one approach and there is evidence, from randomized trials, that sunscreens inhibit SCC[13], actinic keratosis (AK; a surrogate risk marker for SCC)[14] and melanoma[15]. The role of sunscreens in melanoma prevention has also been supported by large population-based studies[16,17]. However, sunscreen use may impact vitamin D status. Reviews report that different studies reach different conclusions[18,19].

The sun protection factor (SPF) of a sunscreen is a quantitative measure of its ability to inhibit erythema. Regulatory authorities specify many requirements for SPF determination, one of which is an application thickness of 2mg/cm^2 [20]. However, people typically apply much less, e.g. 0.8mg/cm^2, with a commensurate reduction in SPF[21,22]. Furthermore, application is often patchy with, for example, missing facial coverage[23]. Additionally, people use sunscreens to prolong their intentional solar exposure time[24,25]. There is a consensus that typical sub-optimal sunscreen use is likely to have a limited effect on vitamin D production (Passeron et al under review, Neale et al under review).

The SPF primarily quantifies protection from UVB, because this waveband is much more erythemogenic than UVA[1]. However, regulatory bodies require UVA protection, the

definition of which varies with regional domain[20]. This UVA protection factor (UVA-PF) is typically a qualitative index that describes the spectral profile of the sunscreen. One *de facto* consequence of increased UVA protection is a decrease in UVB protection for a given SPF. This would be expected to have a beneficial effect on vitamin D synthesis.

Holidays result in a highly significant enhancement of vitamin D_3 status in adults[26] and children[27]. However, this was accompanied with a high level of sunburn in the adults in Tenerife[28]. In both adults and children there were also very high levels of epidermal CPD, which is a determining event for skin cancer. Sunscreen use can inhibit CPD[29], even with very high UVR doses when applied at a typical user application thickness (e.g. 0.75mg/cm^2)[30]. It is therefore very important to determine conditions of sun exposure that maximize benefit and minimize risk.

The primary aim of this investigation was to assess the ability of two sunscreens to inhibit 25(OH)D_3 synthesis during a week's sun holiday in Tenerife. The study was designed to compare these sunscreens (intervention groups) under optimal use with typical sunscreen use (discretionary group). The secondary aim was to determine if the intervention sunscreens' different optical properties would affect 25(OH)D_3 synthesis. This was done by formulating two SPF=15 sunscreens with different levels of UVA protection. The hypothesis under test was that the sunscreen with high UVA-PF (by default more UVB transmission) would enable better 25(OH)D_3 synthesis than the product with low UVA-PF. We been previously reported that sunscreen intervention in the same participants inhibited erythema. In contrast, there was marked erythema with discretionary sunscreen use[31].

Materials and Methods

The study was approved by the Ethics Committee of the Medical University of Łódź, Poland and done according to the Declaration of Helsinki. All participants (n=79) gave written informed consent. Most were of Fitzpatrick skin type (FST) II and III[32]. Group demographics are summarized in table 1. Briefly, three groups of holidaymakers from Łódź, Poland, spent a week in March 2011 in Tenerife (28°N) with cloudless weather with a maximum UV index (UVI) of 9, which is classified as very high by the World Health Organization[33]. Sunscreen intervention groups A and B (each n=20) were given three ~50g tubes of SPF 15 sunscreens daily with high or low UVA-PF respectively[31]. Participants were instructed how to apply the sunscreens to achieve their labelled SPF, and to use 1 tube in the morning, 1 mid-day and 1 in the afternoon. Sun exposure behaviour was monitored half-hourly in diaries [34,35] that included clothing cover to estimate the percent body surface area (BSA) exposed. Time of sunscreen application was monitored, and application thickness was estimated (by weighing tubes before and after use) in cases when the 1st application per tube was on 85% BSA exposed (i.e. in swimwear). Discretionary use of sunscreen (group C) participants (n=22) were instructed to bring their own sunscreens to use as normal. No instructions were given, and use was not monitored. Control group D (n=17) remained in Łódź (51.5°N). The allocation of individuals to the 4 groups depended on several factors. Group C agreed to invasive procedures already reported[31] and Group D was unwilling or unable to travel. Groups A and B were randomized as room sharing pairs by being sequentially allocated to sunscreen A or B as they entered the study. Pairs were given the same sunscreen to avoid inadvertent mixing of product. Full details of the holiday, participants, sunscreens, personal UVR exposures and sunburn have been published[31]. Briefly, standard erythema doses (SED)[35] were measured using personal wrist-worn electronic

dosimeters and erythema was assessed at the end of each day by reflectance spectroscopy on 5 exposed body sites.

Assessment of 25(OH)D

Serum samples from groups A, B and C were prepared from blood taken 24 h before and 24-48 h after the holiday. Bloods from control group D were taken at the same times. All samples were stored at -80°C. 25(OH)D_3 was analysed by HPLC-MS/MS by two independent laboratories. One was the Department of Clinical Chemistry, Birmingham City Hospital (BCH), Sandwell and Birmingham Hospitals NHS Trust, Birmingham, UK. BCH is a UK Clinical Pathology Accreditation (CPA) laboratory and a member of the Vitamin D External Quality Assessment Scheme (DEQAS) [36]. The other was the Department of Dermatology, Bispebjerg Hospital (BBH)[37], Copenhagen University Hospital, Copenhagen, Denmark. Pre- and post-samples (n=158) from a given individual were analysed in the same batch. BCH and BBH ran 2 and 3 aliquots from each sample respectively and means from each laboratory were used. The double laboratory analysis gave data from 316 runs in theory, but in practice this was 307 (97%) because nine aliquots could not be analysed for technical reasons, but all participants provided data from at least one pre- and post-sample. BCH also assessed 25(OH)D_2.

Statistics

Sample sizes were based on a previous controlled laboratory non-solar UVB intervention vitamin D study of 50 adults, with changes in 25(OH)D_3 as the endpoint[37]. Sixteen people completing the study were deemed sufficient to detect Δ23.3±26.5(SD) nmol/L 25(OH)D_3 using a paired design (i.e. pre-holiday vs. post-holiday) with a significance level of 5% and 90% power. This was calculated by Power and Sample Size

Calculation version 3.1.2 available online on http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize. The larger sample sizes of those in Tenerife (20-22) allowed some leeway for the less controlled conditions of "real life" solar UVR behaviour and the possibility of dropouts. SPSS Statistics 22 (IBM, New York, United States) was used for the data analysis. Normality was determined by the Kolmogorov-Smirnov test and the Bland-Altman test was used to assess the difference between 25(OH)D_3 assessment in two laboratories. The relationship between the laboratories was determined by linear regression. Comparisons between treatment groups were made by ANOVA with *post hoc* tests where necessary. This adjusts for baseline 25(OH)D because this influences UVR response[37]. Pre- and post-holiday comparisons within the same individuals were made by paired t-tests. Group differences of categorical data were analysed by the Chi square test. The significance value was set at $p < 0.05$ and all tests were two-sided. Analyses were also made of total 25(OH)D (i.e. D_2 and D_3 combined).

Results

All non-vitamin D parameters have been previously reported[31] and were normally distributed. There was no overall difference in age between the groups p=0.202 (ANOVA), but sunscreen B group was just significantly older (mean diff 4.8±2.4 years) than the discretionary sunscreen group C (p=0.047 - post hoc test). BSA and mean sunscreen application thicknesses of intervention sunscreens are given in table 1. There was no difference (p>0.1) in BSA between any of the groups (M, F and M+F), though this was significantly greater in males. There was no difference in the amount of sunscreen applied in the intervention groups (p=0.599), based on 1st application from each tube over 85% BSA. 97.5% of participants were skin types II (59.5%) and III

(38.0%) with 2.5% type IV. There was a borderline significant difference in skin type between all groups (p=0.047), which was lost when the control group was excluded (p=0.146). Erythema, quantified by area under curve with time, showed virtually no change in both sunscreen intervention groups, and no difference between these groups (p≥0.36) but was marked in the discretionary sunscreen use group. The differences were highly significant (p<0.001), showing that the sunscreen interventions inhibited erythema.

UVR exposure

Figure 1a shows the mean daily ambient UVR exposure and the exposure profiles of the holiday groups that obtained 13-17% of ambient. The mean cumulative SED exposures in the three groups (table 1) were not significantly different from each other (p=0.08). The mean cumulative exposure of the groups combined was 53.2±16.5 SED, that is equivalent to ~18 and ~11 minimal erythema doses (MED) in FST II and III respectively[38]. There was however a difference in the number of hours outside (p=0.02), also shown in table 1. *Post hoc* multiple comparisons, of hours outside, showed no differences between the two sunscreen intervention groups (~ 5h 30m daily, p=1.0), each of which had significantly fewer hours exposure than the discretionary use group (~ 6h 40m daily, p≤0.014). The mean cumulative exposure in the control group was 1.9±3.4 SED which is <1 MED, irrespective of FST.

BSA exposed, sunscreen transmittance properties and application, and erythema assessments

We have previously shown a relationship between holiday UVB dose and production of 25(OH)D_3 after adjustment for BSA exposed[26]. In effect, this is a product of exposed BSA

and SED. Thus, figure 1b shows the half-hourly product of BSA exposed (m^2) x SED ($100J/m^2$) to give the total energy received (J) at the skin surface. There was no significant difference between the three holiday groups (p=0.747 ANOVA) using individual area under curve as the outcome. The individual data, shown in figure S1 in the supplementary material, show a very wide range of individual behavioural patterns.

The sunscreens' UVR transmittance spectra are shown in figure 2. The inset shows that high UVA-PF sunscreen (A) transmitted about 20% more UVB than the low UVA-PF sunscreen (B).

$25(OH)D_2$, $25(OH)D_3$ and total 25(OH)D

Values of $25(OH)D_2$ were low with means ranging from 3.5 to 5.6 nmol/L. All $25(OH)D_2$ data were normally distributed (p>0.148) apart from pre-holiday sunscreen A (p=0.022). In 26 of the 158 pre and post-samples (16.5%), both aliquot runs were at the limit of detection (2.8 nmol/L). The pre- and post-holiday $25(OH)D_2$ results are given in supplementary figure S2, with additional statistical information in the figure caption. There was no significant difference between pre- and post-holiday samples for the control and sunscreens A and B groups (p>0.09). However, the post-holiday value was lower in the discretionary sunscreen group (p=0.003).

The Bland-Altman test (see figure S3) showed a significant ($p=4.38x10^{-18}$) systematic mean difference of 7.3 (95% CI=5.9-8.8) nmol/L $25(OH)D_3$ between the two laboratories. The inter-laboratory results were compared by linear regression (figure 3) to give an equation of y=1.01x+6.73 (95% CI of slope=0.96-1.06 and intercept=3.06-10.40) with $r^2=0.91$ and slope $p=2.94x10^{-77}$. A slope of 1.01 means there is no

laboratory bias for the Δ values (i.e. post-holiday – pre-holiday). Thus, given the excellent correlation, the mean values from two laboratories were used in the statistical analyses. Data from a single laboratory were used in the 9 missing cases.

Analyses were done for 25(OH)D_3 and total 25(OH)D. Pre-, post-holiday and Δ25(OH)D_3 were normally distributed ($p>0.31$ with exception pre-holiday for sunscreen A with $p=0.054$). All total 25(OH)D data (pre-, post- and delta) were also normally distributed ($p>0.268$) except for sunscreen A ($p=0.054$).

The 25(OH)D_3 results are shown in table 1. The overall mean baseline (pre-holiday) 25(OH)D_3 value was 58.9±26.7 nmol/L. There was no significant difference ($p=0.191$ - ANOVA) between the baseline 25(OH)D_3 values of any group (nor total 25(OH)D ($p=0.216$) -ANOVA). However, *post hoc* analysis showed there was a significant difference between the baseline 25(OH)D_3 of groups D and A $p=0.031$ ($p=0.041$ for total 25(OH)D); importantly, such analyses showed no differences between the 3 holiday groups ($p>=0.341$).

Table 1 also shows a non-significant decline of 25(OH)D_3 of the Łódź control group during the study, but highly significant post-holiday increases in all Tenerife groups. The ranking of this increase is discretionary (group C)>high UVA-PF sunscreen (group A) >low UVA-PF sunscreen (group B).

ANOVA showed a highly significant ($p=2.5\times10^{-13}$) difference between the post study 25(OH)D_3 values for the 4 groups ($p=2.74\times10^{-12}$ for total 25(OH)D), after adjustment for pre-level (baseline), and differences between the groups were tested by *post hoc*

analyses. The greatest differences (for $25(OH)D_3$ and total 25(OH)D) were between the Łódź control and the three Tenerife groups with $p \leq 6.9 \times 10^{-6}$. The post study value in the discretionary sunscreen group was greater than the low UVA-PF ($p=2.09 \times 10^{-5}$ for $25(OH)D_3$ and $p=7.16 \times 10^{-5}$ for total 25(OH)D) and high UVA-PF ($p=0.037$ for $25(OH)D_3$ and $p=0.068$ for total 25(OH)D) sunscreen groups, and the increase in the latter was significantly greater ($p=0.022$ for $25(OH)D_3$ and $p=0.025$ for total 25(OH)D) than the former. The baseline adjusted post-holiday group differences for $25(OH)D_3$ are; C>A by 7.2 (±3.4 SEM) nmol/L, C>B by 15.3 (±3.4) nmol/L and A>B by 8.1 (±3.5) nmol/L (note – p values same as above). The latter comparison supports the hypothesis of the secondary aim, i.e. that better vitamin D synthesis would occur with the sunscreen that transmitted more UVB.

Table 1 shows the percentage with $25(OH)D_3$ >50nmol/L (D_3 sufficiency) in each study group before and after the holiday. In all holiday groups, this percentage was reduced and $25(OH)D_3$ level increased in all individuals. The sunscreen B group had 3 volunteers with a post-holiday $25(OH)D_3$ level <50 nmol/L, 2 of whom had 25(OH)D <25 nmol/L pre-holiday. Only one person remained insufficient in the discretionary sunscreen group but increased from 39.7 to 48 nmol/L. The Łódź control group D had a much higher pre-holiday percentage of insufficiency.

There was no relationship between age and post-holiday $25(OH)D_3$ (with correction for baseline) for all groups combined ($p=0.533$) and for the individual groups ($p=0.526$-0.955). There was also no significant correlation between age and pre, post or delta $25(OH)D_3$ and total 25(OH)D ($p>0.230$ all 4 groups, $p>0.193$ holiday groups, $p>0.404$ individual groups), nor any significant effect of sex on vitamin D markers in holiday

groups (p=0.728 for 25(OH)D_3 and 0.785 for total 25(OH)D). There was also no relationship between duration of solar exposure and post-holiday vitamin D markers (with correction for baseline) for all holiday groups combined (p=0.233 for 25(OH)D_3 and p=0.298 for total 25(OH)D), and for the individual holiday groups (p=0.527-0.682 for 25(OH)D_3 and p=0.518-0.667 for total 25(OH)D).

Discussion

Holidays contribute substantially to UVR burden[39,40]; Over 5,500,000 Northern Europeans visited the Canary Islands in 2017[41], the latitude of which is comparable to the US holiday destination of Florida. Sunscreens are important for photoprotection, but concerns about their possible inhibitory effects on vitamin D_3 production have been largely based on laboratory studies with inappropriate UVB phototherapy sources that contain short wave non-solar UVB that is very effective at forming pre-vitamin D_3[42] or theoretical calculations[43]. In contrast, we studied sunscreen use under holiday conditions with excellent weather. Furthermore, the study was designed to test the possible inhibitory effect of sunscreens on vitamin D_3 synthesis under optimal conditions of use.

The study participants received an overall mean of 43.2±16.5 SED (range 12–93) that was 13-17% ambient erythemal UVR. It has been estimated that an indoor worker in Northern Europe receives about 150 SED annually on the face [40]. The SED measurements were made on the wrist, but studies have shown that this is about 50% of the facial dose[44], although this depends on behaviour. Without any body site adjustment, the wrist data confirm that a very high fraction of annual UVR exposure

(~30%) can be obtained in one-week sun holiday in spring. Many Northern Europeans take summer holidays, in which case the doses are likely to be considerably higher.

In a previous Tenerife study in March 2010, we reported that Danes (n=25) obtained a total of 57.0±24.7 SED (range 21-115) over 6 days that represented ~43% of their annual solar UVR burden[34]. This higher value than the current study may be because sun-seekers were specifically targeted during the recruitment of the Danes. Overall, these data from Tenerife support studies that estimate that a high fraction of annual UVR burden is received during sunny holidays [40]. Such exposure, especially if associated with sunburn, is likely to be an important risk factor for skin cancer[11,12].

There was no difference in cumulative SED between the three holiday groups, though the discretionary sunscreen use group spent significantly more time outdoors (about 1 hour/day) than the intervention groups. However, this additional time had no effect on any of the vitamin D outcomes. This is not surprising because photochemical reactions limit the production of pre-vitamin D_3 after ~3 hours, which in turn limits the production of 25(OH)D_3 [45]. Measurements on the same participants[31] showed that sunscreens A and B equally and significantly inhibited erythema, on five exposed body sites, in comparison with discretionary sunscreen group C that had marked erythema. Importantly, because BSA exposed affects serum 25(OH)D_3 [46], there was no significance difference between the product of BSA exposed and SED. Thus, we may conclude, that the overall patterns of UVR exposure of the three holiday groups were the same. The contemporaneous control group had very low UVR exposures in Poland where the average temperature was 5.8±4.1 (SD) ^{o}C with a maximum UVI of 2-3.

There was no significant difference between baseline 25(OH)D_3 in any of the groups, with an overall mean (n=79) of 58.9±26.7 nmol/L, even though the sunscreen A group had a 19.1±8.7 nmol/L higher 25(OH)D_3 than the Lodz control group D. At least 50nmol/L total 25(OH)D is regarded as sufficient by the Institute of Medicine (IOM), though different organizations use different levels for sufficiency[47]. The negative control group declined insignificantly by 2.5±5.6 nmol/L 25(OH)D_3, which is indicative of the gradual loss of UVB-induced vitamin D_3 reserves acquired in summer. It should be noted that food is not vitamin D fortified in Poland.

There was a highly significant increase of 25(OH)D_3 in all three holiday groups, which was greatest in the discretionary sunscreen use group (28.0±16.5 nmol/L) that showed sunburn[31]. We have previously reported a mean increase of 21.5 nmol/L 25(OH)D_3 after a mean cumulative exposure of 57.0 SED over 50% BSA (see table 1 in[48]) in a 6-day study of sun-worshiping Danes in Tenerife in March 2010[26]. All participants had sunburn[28] despite discretionary sunscreen use[34].

The increase in 25(OH)D_3 was significantly greater with high compared with low UVA-PF (19.0±14.2 vs. 13.0±11.4 nmol/L), which is almost certainly a consequence of greater UVB transmittance through the high UVA-PF sunscreen (see figure 2). The percentage with >50nmol/L (D_3 sufficiency) was reduced in all groups post-holiday (table 1). There was increased 25(OH)D_3 in all individuals, though 3 in sunscreen group B, and one in the discretionary sunscreen use group C, did not reach sufficiency post-holiday (i.e. 25(OH)D_3 <50 nmol/L). The higher pre-holiday percentage of insufficiency in the Łódź control group D may reflect its sun-behaviour habits as members chose not to travel.

Based on measurements from 21 tubes per person[31], the intervention sunscreens were applied at a mean thickness of 2.4 mg/cm^2, resulting in SPFs of at least 15. Thus, assuming a constant level of protection, the average cumulative UVR dose received through the sunscreen was ~40/15 = 2.7 SED (or ~0.4SED/day). This results in an increase of 7.0 and 4.8 nmol/L 25(OH)D_3 per SED through the high and low UVA-PF sunscreens respectively. A recent study, with an acute exposure of fluorescent SSR over 35% BSA, showed an estimated increase of ~3 and ~2.5 nmol/L per SED in FST II and III respectively (personal communication from L Rhodes) [49]. These results are compatible with ours given that the relationships between 25(OH)D synthesis and UVR dose and BSA exposed are complex [46]. It was not possible to estimate the doses received by the discretionary sunscreen use group C.

Overall, the conclusions from group comparisons of 25(OH)D_3 and total 25(OH)D were the same with one exception: The significance of the greater increase in the discretionary use (C) over high UVA-PF sunscreen (A) was lost when the total 25(OH)D was used. As previously reported by others in adults [50] the levels of 25(OH)D_2 were very low. Pre versus post-holiday 25(OH)D_2 did not change apart from a fall in group C that had the greatest increase in 25(OH)D_3. It is possible that 25(OH)D_2 and 25(OH)D_3 interact with each other, but this would require additional study.

Several laboratory studies have compared protection from UVR-induced epidermal and dermal damage after the application of high vs. low UVA protection, for a given SPF[51-53]. These investigations demonstrate benefits from better UVA protection. This is the first time that higher UVA protection, for a given SPF, has been shown to be beneficial for

vitamin D synthesis. Apart from biological benefits from increased UVA protection, the labelled SPF is a much more robust indicator of protection with a broad-spectrum product[54]. This is because solar UVB content, which varies considerably with the height of the sun, is a major determinant of SPF with low UVA-PF. Furthermore, high UVA protection is likely to be advantageous because there is increasing evidence that UVA, especially UVA1 (340-400nm), may be more harmful than previously thought[55]. For example, the basal layer of the epidermis, which contains melanocytes and proliferating keratinocytes, is especially susceptible to UVA1-induced DNA damage[56]. UVA may cause oxidative damage to DNA repair proteins[57]. There are also epidemiological data to suggest that solar UVA may be more important for melanoma in comparison to KC[58,59]. However, we lack definitive data for the action spectrum for melanoma in mammalian skin.

One strength of this investigation is that it was done under "real life" holiday conditions during a week of cloudless weather with very high UVI. Furthermore, there was no difference in cumulative SED exposure between the groups, including after adjustment for exposed BSA. Another strength is that the HPLC MS/MS 25(OH)D_3 measurements were independently assessed in two laboratories, including a DEQAS laboratory, with excellent inter-laboratory agreement. A major concern of many vitamin D studies is lack of standardization of measurements[36,60]. One weakness of the study was that the participants were not fully randomized; this was not possible for practical and logistical reasons. However, the baseline and demographic characteristics of the study groups were not significantly different from each other except that sunscreen group B (that has the smallest increase in 25$(OHD)_3$) was older than the other groups. This difference was of borderline significance and had no effect on any of the vitamin D outcomes.

However, it should be noted that vitamin D synthesis decreases with age[61] . Another weakness is the lack of data on sunscreen use in the discretionary group C but collecting such data might have altered sunscreen application behaviour; the so-called Hawthorn effect[62]. Our goal was to compare optimal with typical holiday sunscreen use; e.g. 0.79mg/cm^2 [22]. This is important because laboratory studies, with a UVB phototherapy source, have shown that sunscreen application thickness has an impact of serum 25(OH)D_3 [63].

In conclusion, there was an increase of 25(OH)D_3 during a week of cloudless weather with very high UVI, even when sunscreens were used to achieve their labelled SPFs and inhibit sunburn. We estimate that the measured increases of 25(OH)D_3 occur with ~0.4SED/day through the sunscreens, which is equivalent to ~0.1MED/day in fair-skinned people. Although labelled at SPF=15, the products used in the current study were in the region of SPF=18-19. A sunscreen with SPF=50+ (in fact 64±15.8(SD)) used at 0.75mg/cm^2 has an SPF of 20.9±3.3[30] . This means that typical use of high SPF sunscreens is likely to have a limited impact on vitamin D synthesis. However, the use of high SPF sunscreens in a way that achieves their labelled SPF may have an impact on vitamin D synthesis, but this needs to be tested under field conditions. There is interaction between BSA exposed and UVR dose[46] and this is likely to be complicated by the addition of sunscreens.

Significantly more 25(OH)D_3 synthesis occurred with a high UVA-PF (broad-spectrum) sunscreen when compared with a low UVA-PF sunscreen for a given SPF. This is what would be expected based on the action spectrum for pre-vitamin D_3 and the optical properties of the sunscreens. Thus, sunscreens can be designed to optimize the balance

between the adverse and beneficial effects of solar UVR exposure as suggested by theoretical calculations[64]. Our data support the use of sunscreens to prevent adverse effects of UVR, without compromising vitamin D synthesis.

Acknowledgements

The European Commission, under the Framework 7 Programme Environment Theme, funded the research. Contract No. 227020: The Impact of Climate and Environmental Factors on Personal Ultraviolet Radiation Exposure and Human Health (ICEPURE). Walgreens Boots Alliance Inc. supported the sunscreen intervention studies and formulated the sunscreens. The research was also funded by the Medical University of Łódź, Poland, research project number 503/5-064-01/503-01 and the National Institute for Health Research (NIHR) Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health. We that Professor Mary Norval for scientific advice during the project and also thank Dr Margarita Triguero Mas, Centre for Research in Environmental Epidemiology (CREAL), Barcelona, Spain for study logistics support.

Group	A	B	C	D	All	Main conclusions from between group comparisons
Study Parameters						
Location	Tenerife	Tenerife	Tenerife	Łódź	NA	
Sunscreen use	High UVA-PF (label SPF 15)	Low UVA-PF (label SPF 15)	Discretionary	None	NA	
No. participants	20	20	22	17	79	
Age (yrs)	33±7	38±7	33±8	34±9	34±8	No age differences (p = 0.202), but B older than C (p = 0.047) [ANOVA and *post hoc*]
Mean BSA (m^2 ± SD)	1.81±0.23	1.90±0.25	1.74±0.12	1.82±0.20	1.82±0.20	No BSA differences for M, F and M+F (p > 0.1) [ANOVA]
Skin type II	13	12	15	7	47	Significant skin type difference in all groups (p=0.047) but lost without control D (p=0.146) [chi-square]
Skin type III	7	8	5	10	30	
Skin type IV	0	0	2	0	2	
Male	8	11	8	2	29	No sex difference in all groups (p=0.057) or holiday groups only (p=0.526) [chi-square]
Female	12	9	14	15	50	
Study Results						
Actual SPF	18.9±2.8	17.7±2.7	ND	NA	NA	No difference between SPF in A & B (p = 0.7) [unpaired t-test]
Sunscreen application thickness (mg/cm^2)	2.43±0.55	2.44±0.48	ND	NA	NA	No difference in sunscreen application thickness in groups A & B (p = 0.6) [unpaired t-test]
UVR exposure (SED)	41.0±13.6	38.6±15.4	49.4±18.4	1.9±3.4	43.2±16.5*	No SED difference in A, B & C (p = 0.08) [ANOVA]
Hours outside	39.5±6.5	38.0±6.4	46.7±10.0	3.7±3.9	41.6±8.7*	No differences in A & B (p = 1.0), but group C had more time outdoors than A or B (p = 0.014) [ANOVA and *post hoc*]
Erythema (reflectance spectroscopy)[31]	No	No	Yes	NA	NA	No differences in A & B on 5 exposed body sites (p ≥ 0.36), but C had more erythema than A & B (p < 0.001) [ANOVA and *post hoc*]
Pre-holiday $25(OH)D_3$ (nmol/L)	67.0±31.5	59.0±24.5	59.9±24.7	47.9±23.8	58.9±26.7	No baseline $25(OH)D_3$ differences in A, B, C & D (p = 0.19), but A but A>D (p=0.031) [ANOVA and *post hoc*]
Post-holiday $25(OH)D_3$ (nmol/L)	85.9±25.3	72.0±21.5	88.0±20.4	45.4±20.9	NA	With baseline adjustment, A, B & C higher $25(OH)D_3$ than D ($p \leq 2.8 \times 10^{-6}$), C higher than B ($p=2.09 \times 10^{-5}$) and A (p=0.037) and A higher than B (p=0.022) [ANOVA and *post hoc*]
$\Delta 25(OH)D_3$ (nmol/L)	19.0±14.2	13.0±11.4	28.0± 6.5	-2.5±5.6	NA	With baseline adjustment, A, B & C higher $25(OH)D_3$ than D ($p \leq 2.8 \times 10^{-6}$), C higher than B ($p=2.09 \times 10^{-5}$) and A (p=0.037) and A higher than B (p=0.022) [ANOVA and *post hoc*]
P values for pre- and post-holiday changes in $25(OH)D_3$	9.8×10^{-6}	6.7×10^{-5}	9.0×10^{-8}	0.087	NA	NA
Pre-holiday % <50 nmol/L $25(OH)D_3$	30	35	32	71	41	A high % with < 50nmol/L $25(OH)D_3$, especially in group D
Post-holiday % <50 nmol/L $25(OH)D_3$	0	15	5	65	NA	A reduction in % with < 50nmol/L $25(OH)D_3$ in holiday groups

Table 1 **Summary of demographic details, study conditions, sunscreen application, UVR exposure and 25(OH)D_3.**
Fuller details of all aspects apart from vitamin D status are described by Narbutt et al.[31] Ery = erythema, ND = no data, NA = not applicable. Note that the sunscreen application thickness data are based on the 1st application from each tube to 85% of BSA and that the group D exposure data are based 13/17 volunteers from whom there was a full 7-day data set. Values are mean ± SD. * excludes group D. Note: as previously reported[31] all non-vitamin D parameters are normally distributed, as are pre- and post-holiday 25(OH)D_3 and the differences between them and the addition of the 25(OH)D_2 data (see figure S2) will increase the combined means from about 4 - 6 nmol/L.

Figures

1. **Solar UVR exposure during the 7-day holiday in Tenerife.** (a) Mean half-hourly ambient and study groups' exposure (SED/0.5h). Groups A, B (sunscreen intervention) and C (discretionary sunscreen use) received 14% (95%CI, 11-17), 13% (10-16) and 17% (14-20) of ambient respectively (based on AUC). (b) Erythemally effective energy (J) received at skin surface. This is the product of SED (expressed as 100J/m^2) and BSA (m^2) exposed each 30 minutes in groups A, B and C. The individual data are shown in the supplementary material (figure S1).
2. **UVR transmittance of the two intervention sunscreens.** See Narbutt et al[31] for full details of sunscreen properties, including UVR absorption properties (as monochromatic protection factors (mPF)). The inset shows the transmittance in the UVB region. Based on AUC, sunscreen A (with high UVA-PF) transmits ~20% more UVB than sunscreen B (with low UVA-PF).
3. **Linear relationship between 25(OH)D_3 measurements from two independent laboratories.** The linear regression (n=149) equation is y = 1.01(±0.03)x + 6.73(±1.86) and the slope is highly significant ($p = 2.94 \times 10^{-77}$) with $r^2 = 0.91$. Errors are SE. The intercept is consistent with Bland-Altman test (see figure S3). Note: colour squares refer to study group and the shapes (O and Δ) refer to pre- and post-holiday respectively, and dotted lines represent 50nmol/L boundary between D_3 insufficiency and sufficiency.

Supplementary Figures (S1, S2 and S3)

1. **Erythemally effective energy (J) received at skin surface.** Individual data used to generate mean data in figure 1b. Figure S1a shows intervention sunscreen A (group A), figure S1b shows intervention sunscreen B (group B) and figure S1c shows discretionary sunscreen use (group C). These data show a wide range of individual sun behaviour patterns within each group. BSA exposed to UVR on a half-hourly basis. This is the product of SED (expressed as $100J/m^2$) and BSA (m^2) exposed each 30 minutes received in groups A, B and C.
2. **Mean serum $25(OH)D_2$ status pre- and post-holiday for all groups.** Note that the limit of detection is 2.8nmol/L and there is a lot of clumping at this value. Data are from a single laboratory (BCH). Pre-holiday values were different between the groups (ANOVA $p=6.07x10^{-5}$). *Post hoc* analyses showed the most significant differences were between group C and groups A or B ($p\leq0.0001$) but there was no difference between groups A and B ($p=0.72$).
3. **Bland Altman plot of serum $25(OH)D_3$ measured in two laboratories.** Birmingham City Hospital (BCH) that is a UK Clinical Pathology Accreditation (CPA) laboratory and a member of the Vitamin D External Quality Assessment Scheme (DEQAS) and the Department of Dermatology, Bispebjerg Hospital (BBH). The mean difference (n=149) of 7.33 nmol/L (central line, SD=9.0, SEM=0.74, 95%CI 5.9-8.8) was highly significant ($p=4.38x10^{-18}$) with BCH reading higher than BBH.

References

1 Young AR, Chadwick CA, Harrison GI *et al.* The similarity of action spectra for thymine dimers in human epidermis and erythema suggests that DNA is the chromophore for erythema. *The Journal of investigative dermatology* 1998; **111**: 982-8.

2 de Gruijl FR, Sterenborg HJ, Forbes PD *et al.* Wavelength dependence of skin cancer induction by ultraviolet irradiation of albino hairless mice. *Cancer research* 1993; **53**: 53-60.

3 Macdonald HM, Mavroeidi A, Fraser WD *et al.* Sunlight and dietary contributions to the seasonal vitamin D status of cohorts of healthy postmenopausal women living at northerly latitudes: a major cause for concern? *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA* 2011; **22**: 2461-72.

4 Kasahara AK, Singh RJ, Noymer A. Vitamin D (25OHD) Serum Seasonality in the United States. *PLoS One* 2013; **8**: e65785.

5 Autier P, Mullie P, Macacu A *et al.* Effect of vitamin D supplementation on non-skeletal disorders: a systematic review of meta-analyses and randomised trials. *The lancet. Diabetes & endocrinology* 2017; **5**: 986-1004.

6 Manson JE, Cook NR, Lee IM *et al.* Vitamin D Supplements and Prevention of Cancer and Cardiovascular Disease. *The New England journal of medicine* 2019; **380**: 33-44.

7 Dixon KM, Tongkao-On W, Sequeira VB *et al.* Vitamin D and death by sunshine. *International journal of molecular sciences* 2013; **14**: 1964-77.

8 Lomas A, Leonardi-Bee J, Bath-Hextall F. A systematic review of worldwide incidence of nonmelanoma skin cancer. *The British journal of dermatology* 2012; **166**: 1069-80.

9 Lens MB, Dawes M. Global perspectives of contemporary epidemiological trends of cutaneous malignant melanoma. *The British journal of dermatology* 2004; **150**: 179-85.

10 Whiteman DC, Green AC, Olsen CM. The Growing Burden of Invasive Melanoma: Projections of Incidence Rates and Numbers of New Cases in Six Susceptible Populations through 2031. *The Journal of investigative dermatology* 2016; **136**: 1161-71.

11 Wu S, Cho E, Li WQ *et al.* History of Severe Sunburn and Risk of Skin Cancer Among Women and Men in 2 Prospective Cohort Studies. *American journal of epidemiology* 2016; **183**: 824-33.

12 Wu S, Han J, Laden F *et al.* Long-term ultraviolet flux, other potential risk factors, and skin cancer risk: a cohort study. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2014; **23**: 1080-9.

13 van der Pols JC, Williams GM, Pandeya N *et al.* Prolonged prevention of squamous cell carcinoma of the skin by regular sunscreen use. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association*

for Cancer Research, cosponsored by the American Society of Preventive Oncology 2006; **15**: 2546-8.
14 Darlington S, Williams G, Neale R *et al.* A randomized controlled trial to assess sunscreen application and beta carotene supplementation in the prevention of solar keratoses. *Archives of dermatology* 2003; **139**: 451-5.
15 Green AC, Williams GM, Logan V *et al.* Reduced melanoma after regular sunscreen use: randomized trial follow-up. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2011; **29**: 257-63.
16 Ghiasvand R, Weiderpass E, Green AC *et al.* Sunscreen Use and Subsequent Melanoma Risk: A Population-Based Cohort Study. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2016; **34**: 3976-83.
17 Watts CG, Drummond M, Goumas C *et al.* Sunscreen Use and Melanoma Risk Among Young Australian Adults. *JAMA dermatology* 2018; **154**: 1001-9.
18 Springbett P, Buglass S, Young AR. Photoprotection and vitamin D status. *Journal of photochemistry and photobiology. B, Biology* 2010; **101**: 160-8.
19 Norval M, Wulf HC. Does chronic sunscreen use reduce vitamin D production to insufficient levels? *The British journal of dermatology* 2009; **161**: 732-6.
20 Young AR, Claveau J, Rossi AB. Ultraviolet radiation and the skin: Photobiology and sunscreen photoprotection. *Journal of the American Academy of Dermatology* 2017; **76**: S100-S9.
21 Petersen B, Wulf HC. Application of sunscreen--theory and reality. *Photodermatology, photoimmunology & photomedicine* 2014; **30**: 96-101.
22 Petersen B, Datta P, Philipsen PA *et al.* Sunscreen use and failures--on site observations on a sun-holiday. *Photochemical & photobiological sciences : Official journal of the European Photochemistry Association and the European Society for Photobiology* 2013; **12**: 190-6.
23 Pratt H, Hassanin K, Troughton LD *et al.* UV imaging reveals facial areas that are prone to skin cancer are disproportionately missed during sunscreen application. *PLoS One* 2017; **12**: e0185297.
24 Autier P, Boniol M, Dore JF. Sunscreen use and increased duration of intentional sun exposure: still a burning issue. *International journal of cancer. Journal international du cancer* 2007; **121**: 1-5.
25 Autier P. Sunscreen abuse for intentional sun exposure. *The British journal of dermatology* 2009; **161 Suppl 3**: 40-5.
26 Petersen B, Wulf HC, Triguero-Mas M *et al.* Sun and ski holidays improve vitamin D status, but are associated with high levels of DNA damage. *The Journal of investigative dermatology* 2014; **134**: 2806-13.
27 Narbutt J, Philipsen PA, Lesiak A *et al.* Children sustain high levels of skin DNA photodamage, with a modest increase of serum 25(OH)D3 , after a summer holiday in Northern Europe. *The British journal of dermatology* 2018.
28 Petersen B, Thieden E, Philipsen PA *et al.* A sun holiday is a sunburn holiday. *Photodermatology, photoimmunology & photomedicine* 2013; **29**: 221-4.
29 Olsen CM, Wilson LF, Green AC *et al.* Prevention of DNA damage in human skin by topical sunscreens. *Photodermatology, photoimmunology & photomedicine* 2017; **33**: 135-42.
30 Young AR, Greenaway J, Harrison GI *et al.* Sub-optimal Application of a High SPF Sunscreen Prevents Epidermal DNA Damage in Vivo. *Acta dermato-venereologica* 2018; **98**: 880-7.

31 Narbutt J, Philipsen PA, Harrison GI *et al.* Sunscreen applied at >/= 2 mg cm(-2) during a sunny holiday prevents erythema, a biomarker of ultraviolet radiation-induced DNA damage and suppression of acquired immunity. *The British journal of dermatology* 2018.

32 Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. *Archives of dermatology* 1988; **124**: 869-71.

33 WHO. http://www.who.int/uv/intersunprogramme/activities/uv_index/en/. In: World Health Organization.

34 Petersen B, Thieden E, Philipsen PA *et al.* Determinants of personal ultraviolet-radiation exposure doses on a sun holiday. *The British journal of dermatology* 2013; **168**: 1073-9.

35 Petersen B, Triguero-Mas M, Maier B *et al.* Sun behaviour and personal UVR exposure among Europeans on short term holidays. *Journal of photochemistry and photobiology. B, Biology* 2015; **151**: 264-9.

36 Carter GD, Berry J, Durazo-Arvizu R *et al.* Hydroxyvitamin D assays: An historical perspective from DEQAS. *The Journal of steroid biochemistry and molecular biology* 2018; **177**: 30-5.

37 Bogh MK, Schmedes AV, Philipsen PA *et al.* Vitamin D production after UVB exposure depends on baseline vitamin D and total cholesterol but not on skin pigmentation. *The Journal of investigative dermatology* 2010; **130**: 546-53.

38 Harrison GI, Young AR. Ultraviolet radiation-induced erythema in human skin. *Methods* 2002; **28**: 14-9.

39 Diffey BL. Time and Place as Modifiers of Personal UV Exposure. *International journal of environmental research and public health* 2018; **15**.

40 Diffey B. A behavioral model for estimating population exposure to solar ultraviolet radiation. *Photochemistry and photobiology* 2008; **84**: 371-5.

41 Gran Canarias Historic Tourism Record for 2017 Confirmed. In: The Canary News. 2018.

42 Matsuoka LY, Ide L, Wortsman J *et al.* Sunscreens suppress cutaneous vitamin D3 synthesis. *The Journal of clinical endocrinology and metabolism* 1987; **64**: 1165-8.

43 Sayre RM, Dowdy JC. Darkness at noon: sunscreens and vitamin D3. *Photochemistry and photobiology* 2007; **83**: 459-63.

44 Thieden E, Agren MS, Wulf HC. The wrist is a reliable body site for personal dosimetry of ultraviolet radiation. *Photodermatology, photoimmunology & photomedicine* 2000; **16**: 57-61.

45 Webb AR, Kline L, Holick MF. Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. *The Journal of clinical endocrinology and metabolism* 1988; **67**: 373-8.

46 Bogh MK, Schmedes AV, Philipsen PA *et al.* Interdependence between body surface area and ultraviolet B dose in vitamin D production: a randomized controlled trial. *The British journal of dermatology* 2011; **164**: 163-9.

47 Bouillon R. Comparative analysis of nutritional guidelines for vitamin D. *Nat Rev Endocrinol* 2017; **13**: 466-79.

48 Bais AF, Lucas RM, Bornman JF *et al.* Environmental effects of ozone depletion, UV radiation and interactions with climate change: UNEP Environmental Effects Assessment Panel, update 2017. *Photochemical & photobiological sciences : Official journal of the European Photochemistry Association and the European Society for Photobiology* 2018; **17**: 127-79.

49 Shih BB, Farrar MD, Cooke MS *et al.* Fractional Sunburn Threshold UVR Doses Generate Equivalent Vitamin D and DNA Damage in Skin Types I-VI but with Epidermal DNA Damage Gradient Correlated to Skin Darkness. *The Journal of investigative dermatology* 2018; **138**: 2244-52.

50 Tripkovic L, Wilson LR, Hart K *et al.* Daily supplementation with 15 mug vitamin D2 compared with vitamin D3 to increase wintertime 25-hydroxyvitamin D status in healthy South Asian and white European women: a 12-wk randomized, placebo-controlled food-fortification trial. *The American journal of clinical nutrition* 2017; **106**: 481-90.

51 Seite S, Christiaens F, Bredoux C *et al.* A broad-spectrum sunscreen prevents cumulative damage from repeated exposure to sub-erythemal solar ultraviolet radiation representative of temperate latitudes. *Journal of the European Academy of Dermatology and Venereology : JEADV* 2010; **24**: 219-22.

52 Fourtanier A, Moyal D, Seite S. UVA filters in sun-protection products: regulatory and biological aspects. *Photochemical & photobiological sciences : Official journal of the European Photochemistry Association and the European Society for Photobiology* 2012; **11**: 81-9.

53 Lejeune F, Christiaens F, Bernerd F. Evaluation of sunscreen products using a reconstructed skin model exposed to simulated daily ultraviolet radiation: relevance of filtration profile and SPF value for daily photoprotection. *Photodermatology, photoimmunology & photomedicine* 2008; **24**: 249-55.

54 Young AR, Boles J, Herzog B *et al.* A sunscreen's labeled sun protection factor may overestimate protection at temperate latitudes: a human in vivo study. *The Journal of investigative dermatology* 2010; **130**: 2457-62.

55 Lawrence KP, Douki T, Sarkany RPE *et al.* The UV/Visible Radiation Boundary Region (385-405 nm) Damages Skin Cells and Induces "dark" Cyclobutane Pyrimidine Dimers in Human Skin in vivo. *Scientific reports* 2018; **8**: 12722.

56 Tewari A, Sarkany RP, Young AR. UVA1 induces cyclobutane pyrimidine dimers but not 6-4 photoproducts in human skin in vivo. *The Journal of investigative dermatology* 2012; **132**: 394-400.

57 McAdam E, Brem R, Karran P. Oxidative stress-induced protein damage inhibits DNA repair and determines mutation risk and therapeutic efficacy. *Molecular cancer research : MCR* 2016.

58 Moan J, Baturaite Z, Porojnicu AC *et al.* UVA, UVB and incidence of cutaneous malignant melanoma in Norway and Sweden. *Photochemical & photobiological sciences : Official journal of the European Photochemistry Association and the European Society for Photobiology* 2012; **11**: 191-8.

59 Moan JE, Baturaite Z, Dahlback A *et al.* Ultraviolet radiation and cutaneous malignant melanoma. *Advances in experimental medicine and biology* 2014; **810**: 359-74.

60 Binkley N, Carter GD. Toward Clarity in Clinical Vitamin D Status Assessment: 25(OH)D Assay Standardization. *Endocrinol Metab Clin North Am* 2017; **46**: 885-99.

61 MacLaughlin J, Holick MF. Aging decreases the capacity of human skin to produce vitamin D3. *The Journal of clinical investigation* 1985; **76**: 1536-8.

62 McCambridge J, Witton J, Elbourne DR. Systematic review of the Hawthorne effect: new concepts are needed to study research participation effects. *J Clin Epidemiol* 2014; **67**: 267-77.

63 Faurschou A, Beyer DM, Schmedes A *et al.* The relation between sunscreen layer thickness and vitamin D production after ultraviolet B exposure: a randomized clinical trial. *The British journal of dermatology* 2012; **167**: 391-5.
64 Kockott D, Herzog B, Reichrath J *et al.* New Approach to Develop Optimized Sunscreens that Enable Cutaneous Vitamin D Formation with Minimal Erythema Risk. *PLoS One* 2016; **11**: e0145509.