Sun and Ski Holidays Improve Vitamin D Status, but Are Associated with High Levels of DNA Damage

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Skin cancer is caused by solar UVR, which is also essential for vitamin D production. DNA damage (thymine dimers: T-T dimers) and vitamin D (25(OH)D) synthesis are both initiated by solar UVB. We aimed to investigate the simultaneous adverse and beneficial effects of solar UVB exposure in holidaymakers. Sun-seekers and skiers ($n = 71$) were observed over 6 days through on-site monitoring, personal diary entries, and recording of personal UVB exposure doses with electronic dosimeters. Urine and blood samples were analyzed for T-T dimers and 25(OH)D, respectively. The volunteers had a statistically significant increase in vitamin D. There were strong associations between UVB exposure and post-holiday levels of T-T dimers and vitamin D, as well as between post-holiday T-T dimers and vitamin D. We conclude that UVB-induced vitamin D synthesis is associated with considerable DNA damage in the skin. These data, on two major health predictors, provide a basis for further field studies that may result in better understanding of the risks and benefits of "real life" solar exposure. However, vitamin D status can be improved more safely through the use of vitamin D dietary supplements.

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INTRODUCTION

Millions of people take sun holidays each year, exposing themselves to short-term high-dose solar UVR (Dadvand *et al.*, 2011). Epidemiological studies have shown associations between intermittent, non-occupational sun exposure and malignant melanoma at all latitudes, as well as an association between intermittent sun exposure and basal cell carcinoma. However, UVR is also beneficial by inducing vitamin D synthesis, which is crucial for human health. There are geographical variations in the incidence of skin cancer in Europe, with some of the highest incidences of malignant melanoma in Scandinavia (Forsea *et al.*, 2012). About 1.2 million Danes (Scandinavians) out of a population of 5.5 million travel to sunny destinations each year; 600,000 are estimated to do this for sunbathing purpose only. Each year, 100,000 Danes travel to Tenerife, and about 185,000 Danes travel to Austria to ski.

Solar UVR damages epidermal DNA, with the formation of cyclobutane pyrimidine dimers (CPD) as the most important lesions. The thymine dimer (T–T dimer) is the most frequent CPD (Mouret *et al.*, 2006). These DNA photoproducts are mainly induced by UVR B (UVB, 280–320 nm), but with some contribution from UVA(320–400 nm). The action spectrum for T–T dimers in the skin peaks at 300 nm (Young *et al.*, 1998). Such lesions are removed from DNA by nucleotide excision repair (Sancar *et al.*, 2004). It has been suggested that enzymatic processes degrade the excised oligomer containing the CPD, and at least in the case of T–T dimers a substantial fraction is excreted in the urine (Le and Hemminki, 2001). Cytosine containing CPD has been directly linked with p53 mutations that initiate non-melanoma skin cancer (Ling *et al.*, 2001). There is also some evidence that CPD may have a role in malignant melanoma (Pfeifer and Besaratinia, 2012). Apart from its mutagenic potential, there is evidence that CPD initiates photo-immunosuppression (Nishigori *et al.*, 1996), which has been implicated in skin cancer, and it is a trigger for the synthesis of matrix metalloproteinase-1 (Dong *et al.*, 2008), which degrades dermal collagen that probably results in skin photoaging. However, humans also benefit from UVR exposure of the skin because solar UVB is the major source of vitamin D (Holick, 2004). Sufficient levels of vitamin D are essential for bone development and maintenance (Wolpowitz and Gilchrest, 2006), and may reduce the risk for certain

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The research was conducted in Copenhagen (Denmark), Tenerife (Spain), and Wagrain (Austria).

Abbreviations: CPDs, cyclobutane pyrimidine dimers; PPF, pigment protection factor; SPF, sun protection factor; T-T dimers, thymine dimers

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cancers and influence other diseases (International Agency for research on Cancer (IARC), 2008; Ascherio *et al.*, 2010; Motiwala and Wang, 2011). Solar UVB (~295–320 nm) converts 7-dehydrocholesterol in the skin to previtamin D_3. The action spectrum for previtamin D_3 shows some efficacy in the non-solar UVC region (200–290 nm), but the maximal synthesis is between 295 and 300 nm, followed by a steep decline with no detectable production >320 nm (Boullion *et al.*, 2006). However, the validity of this action spectrum has recently been questioned (Norval *et al.*, 2010).

In this study, we simultaneously determined the impact of personal holiday UVB exposure on DNA damage and vitamin D synthesis, and the association between these parameters. The study is a part of a European Community project and therefore different nationalities were included. Both, people on sun holidays (Danes and Spaniards) and those on ski holidays (Danes), were investigated, as both are extremely common with a high level of sun exposure but with different degrees of skin surface area exposed. There was no difference in age between the different groups of participants, but there were differences in gender, skin type, sunscreen use, and consumption of eggs and fish, which are rich in vitamin D (Table 1).

Table 1. Volunteer characteristics

Study	Sun holiday		Ski holiday
Nationality	Danes	Spaniards	Danes
N	25	20	26
Female	14 (56%)	14 (70%)	10 (38%)
Male	11 (44%)	6 (30%)	16 (62%)
Age[1]	39 (29–51)	38 (27–61)	39 (24–55)
Fitzpatrick skin type I[2]	0	0	2 (8%)
Fitzpatrick skin type II[2]	11 (44%)	6 (30%)	16 (61%)
Fitzpatrick skin type III[2]	11 (44%)	9 (45%)	8 (31%)
Fitzpatrick skin type IV[2]	3 (12%)	5 (25%)	0
Buttock-PPF[1,3] (objective skin type)	5.9 (4.1–9.2)	4.6 (2.6–6.2)	4.6 (2.2–6.4)
Days with sunscreen	85%	48%	90%
SPF (sun protection factor)[4]	16 ± 6	23 ± 10	21 ± 8
Number of sunscreen applications per day[4,5]	1.7 ± 0.9	1.5 ± 0.7	1.4 ± 0.9
Body area with sunscreen[4,5]	73.6% ± 21.5	61.3% ± 31.3	NA
Fish consumption (g)[6]	125 (0–600)	73 (0–250)	0
Egg consumption (g)[6]	180 (0–780)	120 (0–480)	465 (0–960)

[1]Mean (range) I.
[2]Supplementary Table S1 online (Fitzpatrick, 1988).
[3]See definitions.
[4]Mean ± SD.
[5]Only days with sunscreen use are included.
[6]Median (range).

RESULTS

When outdoors, the Danish sun-seekers exposed an average of 50% (SD 9.2) of their skin area, whereas the Spaniards exposed 44% (SD 9.2; $P=0.02$). Furthermore, the Danish skiers exposed an average of 4.0% (SD 2.6) of their skin area (calculated from diary and Supplementary Table S2 online). The UVB data, measured by personal UVR dosimeters (SunSavers), are presented in Table 2. We calculated the individual UVB exposure load ($kJ\,m^{-2} \times \%$) by multiplying the UVB dose ($kJ\,m^{-2}$) with the exposed skin area (%; see definitions). The Danish sun-seekers were exposed to UVB for 19% longer ($P=0.004$) and received 68% more UVB ($kJ\,m^{-2}$; $P=0.0003$) than did the Spanish sun-seekers. Furthermore, the UVB exposure ($kJ\,m^{-2} \times \%$) of the Danish sun-seekers was 71% higher than that of Spanish sun-seekers ($P=0.0002$).

DNA damage was measured as urinary T–T dimers. The Danish sun-seekers had significantly more T–T dimers after the holiday than did the Spanish sun-seekers ($P=0.007$), and all the sun-seekers as a group had a statistically significantly higher T–T dimer post-level compared with Danish skiers ($P<0.0001$; Table 3).

In about 90% of the Danish participants, the pre-holiday concentration of T–T dimers was below the detection limit of 0.5 fmol per 10 µl of urine, and in these cases the detection limit was used as the pre-level. The power equation was the strongest model for expression of the association between the T–T dimer pre-levels (where they could be detected) and T–T dimer post-levels, and between UVB exposure ($kJ\,m^{-2} \times \%$) and the T–T dimer post-levels. Data were therefore logarithmically transformed. The $\log_2$ (T–T dimer post-levels) were dependent on the $\log_2$ (T–T dimer pre-levels) expressed as (T–T dimer post-level $= 1.95 + 0.6 \times$ T–T dimer pre-level, $P=0.0001$, $R^2=0.19$). There was a strong association between $\log_2$ (UVB exposure) and $\log_2$ (T–T dimers) expressed as (T–T dimer post-level $= -10.85 + 0.92 \times$ UVB exposure, $P<0.0001$, $R^2=0.68$; Figure 1), and the model changed only minimally with the inclusion of the T–T dimer pre-level (T–T dimer post-level $= -9.23 + 0.85 \times$ UVB exposure ($P<0.0001$) $+ 0.25 \times$ T–T dimer pre-level ($P=0.012$), P (model) <0.0001, $R^2=0.71$). The effect of nationality, age, gender, objectively measured constitutive skin phototype, and self-assessed Fitzpatrick skin type on the model was tested in analysis of variance, but they had no statistically significant effect on the association.

Vitamin D was measured as serum 25(OH)D. The Spanish sun-seekers had a statistically nonsignificant higher vitamin D pre-level compared with all the other groups (Table 3), which was probably due to adventitious early-year UVR exposure in Spain before the study. All groups had a statistically significant increase in vitamin D (Table 3). The Danish sun-seekers had a 31% greater vitamin D increase compared with the Spanish sun-seekers (21.5 $nmol\,l^{-1}$ versus 16.6 $nmol\,l^{-1}$, $P=0.24$, SD = 4.1, 95% confidence interval $= -3.4$–13.1). Overall, 52% of the sun-seekers had vitamin D insufficiency (25–50 $nmol\,l^{-1}$) before the holiday, which was 56% of the Danes and 45% of the Spaniards. After the holiday, only 13% of all sun-seekers had vitamin D insufficiency. In contrast to this, the number of skiers with vitamin D insufficiency was only reduced from 52 to 35% during the holiday. Three skiers and one Danish sun-seeker had vitamin D deficiency

Table 2. UVB data

Measures	Sun-seekers				Skiers
	Danes	Spaniards	Difference[2]	*P*-value for difference	Danes
Cumulative UVB dose ($kJ\,m^{-2}$)[1]	101 ± 37.5	60 ± 23.6	40.65 (19.9–61.4)	0.0003	109 ± 14.0
Cumulative UVB exposure time (h)[1]	38 ± 5.4	32 ± 8.8	6.62 (2.2–11.0)	0.004	44 ± 4.8
UVB exposure[3] ($kJ\,m^{-2} \times \%$)[1]	6394 ± 3042	3736 ± 1742	2658 (1005–4310)	0.002	473 ± 164

[1]Mean ± SD.
[2]Mean (95% confidence interval).
[3]UVB exposure is estimated by multiplying the UVB dose from the SunSaver for every 30 minutes with the area of skin exposed during that 30 minutes according to the diary, and then these numbers are summed up to a total for all the study days (see definitions).

($<25\,nmol\,l^{-1}$) before the holidays, but none was vitamin D deficient after the holiday.

The vitamin D post-level was linearly dependent on the vitamin D pre-level (Vitamin D post-level $= 31.47 + 0.69 \times$ vitamin D pre-level, $P<0.0001$, $R^2 = 0.67$) and was dependent on UVB exposure expressed as (vitamin D post-level $= 57.73 + 0.001 \times$ UVB exposure, $P<0.0001$, $R^2 = 0.17$) or as (vitamin D post-level $= 18.18 + 4.01 \times \log_2$ (UVB exposure), $P = 0.001$, $R^2 = 0.15$). When both vitamin D pre-level and UVB exposure were included as independent factors, the logarithmic model with $\log_2$ (UVB exposure) was the best way to express the association with vitamin D post-level (Vitamin D post-level $= -9.70 + 0.66 \times$ vitamin D pre-level ($P<0.0001$) $+ 3.57 \times \log_2$ (UVB exposure) ($P<0.0001$), R^2 (model) $= 0.75$, P (model) <0.0001) (Figure 2). The 0.66 (i.e., <1) regression coefficient above shows that vitamin D increases for individuals with higher vitamin D pre-levels are less than those for individuals with lower vitamin D pre-levels for a given UVB exposure. The effect of nationality, age, gender, consumption of fish/eggs, objectively measured constitutive skin phototype, and self-assessed Fitzpatrick skin type on the association was tested, but they had no statistically significant effect on the model.

We studied models in which UVB exposure was adjusted for body surface area, but they had no significant effect on the strength of the models, and percentage of body exposure was included because it adjusts for body size. Furthermore, as some of the study participants used sunscreen, we calculated a sun protection factor (SPF)-adjusted UVB exposure (see definitions) to analyze whether this adjustment increased the strength of the T–T dimer/UVB exposure response model or the vitamin D/UVB exposure response model, but these models became slightly weaker (data not shown), probably because of variations in the way people apply sunscreen compared with what they report in the diary.

There was a statistically significant association between T–T dimer post-levels and the vitamin D post-levels adjusting for vitamin D pre-levels (vitamin D post-level $= 24.4 + 0.71 \times$ vitamin D pre-level ($P<0.0001$) $+ 2.75 \times$ T–T dimer post-level ($P<0.0001$), P (model) <0.0001, R^2 (model) $= 0.76$; Figure 3). The inclusion of the T–T dimer pre-levels in the model did not affect this association (Vitamin D post-level $= 24.5 + 0.71 \times$ vitamin D pre-level ($P<0.0001$) $+ 2.77 \times$ T–T dimer post-level ($P<0.0001$) $- 0.6 \times$ T–T dimer pre-level ($P = 0.9$), P (model) <0.0001, R^2 (model) $= 0.76$).

DISCUSSION

This real-life study combined accurate personal UVB exposure, DNA damage, and vitamin D data. We found that a 1-week sun or ski holiday resulted in large UVB exposure doses that caused considerable DNA damage and induced vitamin D synthesis.

There has been a lack of field dose–response data on DNA damage and vitamin D status, and their interaction. Most UVB dose relationship human data, for short-term end points, are from laboratory studies under artificial or very controlled conditions, often with non-solar UVR sources that are rich in short-wave UVB that is very effective at vitamin D synthesis. To the best of our knowledge, observations from a real-life study with regular diary registrations of behavior, prospective measurements of personal UVB doses, and measurements of DNA damage and vitamin D during a short holiday are previously unreported. Simultaneous data, from environmental UVR exposure, on both hazardous DNA damage and beneficial vitamin D are unique, and provide further insight in this complex area, where risk and benefits cannot be fully separated.

Urinary T–T dimers are biomarkers of UVR-induced skin DNA damage and its repair. Laboratory studies show a correlation between UVB-induced T–T dimers in the skin and their excretion in urine (manuscript in preparation). Urinary T–T dimers have been shown to be absent in winter in Sweden (Liljendahl *et al.*, 2012), which is supported by our data because they were undetectable in almost 90% of all Danish volunteers when assessed before the holiday. The remaining Danes showed a level that was close to detection limits. A low level of T–T dimers was detected in the pre-holiday measurements in the Spaniards, which is not surprising given the climate. Thus, only post-holiday T–T dimers were analyzed because they could not be measured in the majority of Danish pre-holiday samples. A study in Sweden found a strong correlation between total urinary T–T dimers and UVR dose per m^2 skin after 1–2 days of sunbathing on the beach (Liljendahl *et al.*, 2012). In another field study, with repeated daily exposure to UVR, T–T dimers were correlated with the UVB exposure of the previous 3–9 days (Liljendahl

Table 3. Vitamin D and cyclobutane thymine dimers (T–T dimers)

Study	Nationality	Vitamin D pre-level (nmol l^{-1})[1]	Vitamin D post-level (nmol l^{-1})[1]	Vitamin D increase (nmol l^{-1})[2]	*P*-value vitamin D increase	T–T dimers pre-level (nmol)[1]	T–T dimers post-level (nmol)[1]	T–T dimers increase (nmol)[2]	*P*-value T–T dimers increase
Sun holiday	Danes	49.0 ± 23.3	70.5 ± 17.8	21.5 (16–27)	<0.0001	0.19 ± 0.2	3.8 ± 2.4	3.6 (2.6–4.5)	<0.0001
	Spaniards	55.8 ± 23.1	72.4 ± 18.1	16.6 (10–23)	<0.0001	0.38 ± 0.4	2.1 ± 1.3	1.7 (1.0–2.3)	<0.0001
Ski holiday	Danes	50.6 ± 23.1	59.2 ± 20.0	8.6 (4.8–12.5)	=0.0001	0.10 ± 0.1	0.5 ± 0.8	0.4 (0.1–0.7)	<0.0001

[1]Mean ± SD.
[2]Mean (95% confidence interval).

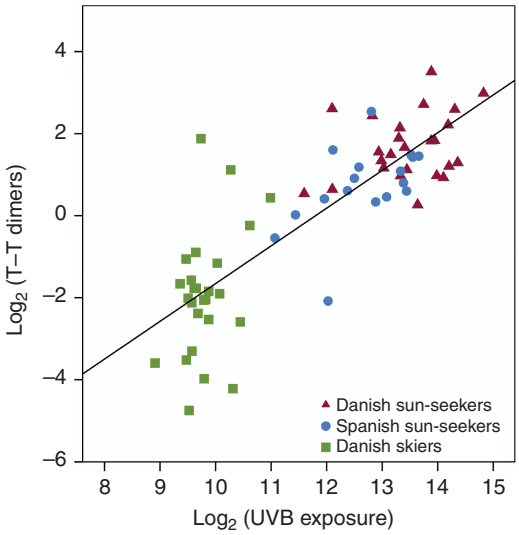

Figure 1. Association between cumulative UVB exposure and cyclobutane thymine dimers (T–T dimers). A power model of the association between the T–T dimer post-level (nmol) and UVB exposure (kJ m^{-2} × %) $\log_2$ (T–T dimer post-level) = −10.85 + 0.92 × $\log_2$ (UVB exposure), $P<0.0001$, $R^2=0.68$.

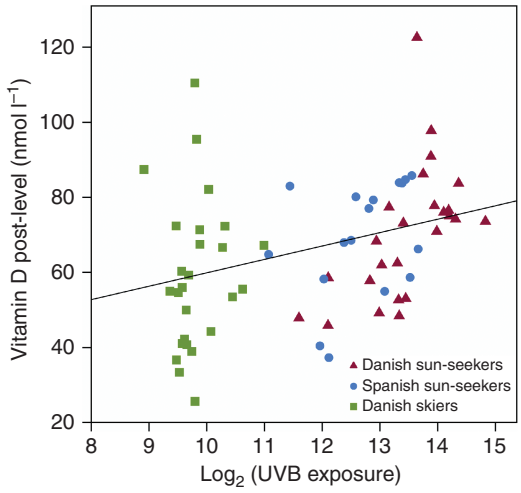

Figure 2. Association between cumulative UVB exposure and vitamin D. A logarithmic model of the association between UVB exposure (kJ m^{-2} × %) and vitamin D post-level adjusting for vitamin D pre-level. Vitamin D measured as 25(OH)D (nmol l^{-1}). (Vitamin D post-level = −9.70 + 0.66 × vitamin D pre-level + 3.57 × $\log_2$ (UVB exposure), $P<0.0001$, $R^2=0.75$.)

et al., 2013), but the diary registrations were much less detailed than ours and the UVR measurements were not time stamped. Furthermore, after a single dose of UVR, urinary T–T dimers increased post exposure, reaching a maximum at 3 days, and the time for 50% excretion was 55–76 hours. We found that the post-holiday T–T dimer levels were strongly associated with UVB exposure (Figure 1), and, in accordance with their higher UVB doses, the Danish sun-seekers had more DNA damage than those from Spain. Our dosimetric data show that 2/3 of all study participants received a higher cumulative UVB dose in the 2nd series of 3 days (mean 45.0 kJ m^{-2}) compared with the first 3 days (40.5 kJ m^{-2}), and this difference was significant ($P=0.02$—Wilcoxon signed-rank test). Thus, our post-holiday measurements of urinary T–T dimers are most likely to reflect events in the second part of the holiday. The presence of pre-holiday T–T dimers in Spain would have cleared by the time the post-holiday measurements were made and hence are unlikely to have influenced the outcome.

The Spanish sun-seekers had a statistically nonsignificant higher vitamin D pre-holiday level than the other groups, indicating that they were adventitiously exposed to early-year UVR before the study, as is possible in Spain. As in a previous laboratory study (Bogh *et al.*, 2010), we found a strong correlation between vitamin D post-level and vitamin D pre-level. The UVB exposure was also a strong predictor of the vitamin D post-level (Figure 2). Optimal vitamin D status is under debate, but insufficiency is widely considered <50 nmol l^{-1}, because this is necessary to prevent age-related bone decalcification (Wolpowitz and Gilchrest, 2006). An overall 52% of sun-seekers and 50% of skiers were vitamin D insufficient before the holiday, which changed to 13 and 35%, respectively, post holiday, but it should be noted that the skiers received lower UVB doses over smaller areas of skin. Vitamin D sufficiency can be obtained by exposing sufficient skin to much lower UVB doses than received in this study. A previous laboratory investigation showed that exposure to 1 standard erythema dose (SED—100 J m^{-2} erythemally weighted UVR) on 88% of the body area every second week was sufficient to maintain summer vitamin D levels (Bogh *et al.*, 2012), and that nutritional intake of vitamin D can reduce the dependence on UVR for maintaining vitamin D status. The higher UVB doses received by the Danish sun-seekers compared with the

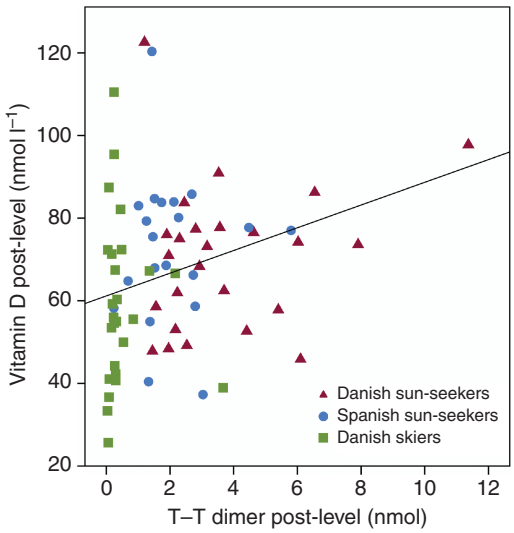

Figure 3. Association between cyclobutane thymine dimers (T–T dimers) and vitamin D. A linear model of the association between the T–T dimer post-level and vitamin D post-level adjusting for vitamin D pre-level. Vitamin D is measured as 25(OH)D (nmol l^{-1}). (Vitamin D post-level = 24.4 + 0.71 × vitamin D pre-level + 2.75 ×, $P<0.0001$, $R^2 = 0.76$.)

Spanish sun-seekers resulted in a higher, but statistically nonsignificant, increase in vitamin D (Table 2 and Table 3). This might be explained by saturation or by photodegradation of vitamin D. Both previtamin D and vitamin D can absorb UVR and this can lead to isomerization of these molecules to form inert photoproducts (Webb *et al.*, 1989).

T–T dimers and vitamin D post-levels were significantly correlated (Figure 3), strongly indicating that the harmful DNA effects of UVR are unavoidable when obtaining beneficial vitamin D in holiday situations. However, mouse studies have shown a protective effect of vitamin D against the harmful effects of UVB exposure in terms of suppression of cell proliferation, increased differentiation, and increased repair of DNA damage (Bikle, 2012), indicating that the vitamin D production may partly counteract the harmful effect.

Skin type had no effect on the models, but surprisingly the Danish sun-seekers had a higher buttock-pigment protection factor (PPF; see definitions) compared with the Spaniards, which we ascribe to more previous sunbathing and sunbed use among the Danes, possibly increasing their buttock pigmentation and affecting our measure of their constitutive skin type.

The strengths of our study include the measures of simultaneous beneficial and adverse effects of environmental UVR. These are supported by high-quality daily follow-up of the participants by on-site investigators and the time-stamped documentation of personal UVB exposure. Its limitations are the unavoidable uncertainties of diary registrations and the logistical restrictions that prevented the collection of urine and blood samples during the holiday period. Such data would have enabled the investigation of individual dose–response/kinetics data as opposed to the population approach that we have taken.

In summary, our study has assessed the risks and benefits of solar UVR exposure from holiday sun exposure. The Danes spent more time in the sun compared with the Spaniards and had significantly higher UVB exposure doses and consequent DNA damage. This may help explain the higher incidence of melanomas among Scandinavians compared with other European populations (Forsea *et al.*, 2012). Our real-life data confirm and extend laboratory studies in which exposure parameters are much more controlled; typically with non-solar UVR sources (Kotova *et al.*, 2005; Bogh *et al.*, 2011). We show a dose–response relationship between UVB exposure and DNA damage (Figure 1), and between UVB exposure and vitamin D formation (Figure 2). We also show a strong association between DNA damage and vitamin D synthesis (Figure 3). These data, on two major health predictors, provide a basis for further studies that may result in better risk/benefit analysis in the future. However, it is important to note that vitamin D status can be maintained by supplementation, which has the advantage of minimizing the risk of UVR-induced skin cancer. The mean daily dietary vitamin D intake in Denmark is 2.8 µg for women and 3.5 µg for men (Lyhne *et al.*, 2005). Intake of 1 µg vitamin D increases serum 25(OH)D by 0.6–2.0 nmol l^{-1}, depending on baseline status (Heaney, 2014). This, combined with an assessment of dietary intake and demographic factors, should be the basis of risk-free population recommendations for the maintenance of optimal vitamin D status.

MATERIALS AND METHODS

Participants

The Danish participants were recruited through the intranet at Bispebjerg Hospital, Copenhagen, Denmark. The Spanish participants were recruited from members and friends and family of the Centre for Research in Environmental Epidemiology (CREAL), Barcelona, Spain. The inclusion criterion for the sun holiday was as follows: sun-seekers of Scandinavian or Spanish ancestry defined as those who had been on at least five sun holidays in the previous 10 years with the primary goal being to enjoy the sun on the beach and/or pool. This inclusion criterion for sun-seekers was chosen because we believe this risky behavior is widespread among people traveling on sun holidays each year, and because we wanted to avoid people who did not have these habits but solely participated to get a free holiday. The inclusion criterion for the ski holiday was as follows: proficient skiers of Scandinavian ancestry, expected to be skiing most of the day. Participants were required to be able to read Danish or Spanish, respectively, and to participate in a pre- and post-examination. Seventy-one participants were recruited, with similar numbers of participants in each group (Table 1).

The exclusion criteria for both studies were as follows: presence of psoriasis or active eczema; present or previous skin cancer; diseases with increased UVR sensitivity; having undergone organ transplantation; disability; intake of medicines that increase photosensitivity; intake of vitamin D supplements >10 µg per day; solarium or intended sun exposure in the previous 6 months. Unintended sun exposure during everyday life was unavoidable in Spain, even in the first months of the year, and therefore this was not an exclusion criterion. All or part of the costs was covered by the project grant to enhance compliance. Skin type was self-determined by the volunteers according to Fitzpatrick criteria (Fitzpatrick, 1988; Supplementary Table S1 online: skin type definitions by Fitzpatrick, online).

The participants were instructed to behave as they would normally on sun-seeking or ski holidays. All 71 participants complied with all the requirements and underwent 6 full days of study.

Volunteer characteristics are presented in Table 1.

Locations

The sun holiday study was performed at a beach holiday resort on the ''Playa de Las Americas'', Tenerife (Canary Islands, Spain, 28° N, 16° W). The ski holiday study was performed at a ski resort in Wagrain, Austria (47° N, 13° E), with altitudes between 850 and 2680 m above sea level. Both studies were conducted in March 2010.

Personal electronic UVR dosimeter ''SunSaver''

Participants wore an updated version of the personal electronic UVR dosimeter (SunSaver) (Heydenreich and Wulf, 2005) that measures UVB (UVR in the B range (280–320 nm)) from sunrise to sunset, resulting in an accumulated UVB dose for each participant. The SunSaver is battery driven, records time-logged UVB data, and comprises a sensor and a data logger, which was set to measure every fifth second and to store an average of the last 24 measurements every second minute along with the time of day. A filtered silicon carbide photodiode (JEC1B-DE, Laser Components, Olching, Germany) was used as the sensor. The sensor has a built-in diffuser with a cosine response. The stability of the SunSaver was tested against a UV Biometer Model 501 Radiometer (Solar Light Company, Glenside, PA) under clear sky conditions in Denmark from 21 June to 21 July 2010 and showed a maximal daily variation of 3%. Furthermore, each dosimeter was individually calibrated against a spectroradiometer (Bentham DMc-150f double-grating monochromator plus a photomultiplier tube, Bentham Instruments, Reading, UK) using clear sky sun as a UVR source. The spectroradiometer was calibrated using a lamp traceable to the UK National Physical Laboratory (Baczynska *et al.*, 2011). The wrist has been shown to be a reliable body site for personal UVR dosimetry (Thieden *et al.*, 2000), and the participants were instructed to wear the SunSaver uncovered on the dorsal aspect of the wrist, in place of their usual wristwatch. The data were downloaded to a computer every evening. UVB data were completely missing for three Spanish participants because of bad data quality or failed calibration, and those were therefore excluded from analyses involving UVB. For eight participants, UVB data were missing for 1 or 2 days, and their total UVB doses were estimated by adding their average personal UVB dose of the observation days with intact data.

Sun exposure diary

The participants were asked to record their clothing, time of sunscreen application, the SPF, and dietary intake of fish and eggs (vitamin D) in a diary during the six study days.

Diary entries were made every 30 minutes during daylight hours from 07:00 to 18:30. The diaries were collected every evening and verified with help from the participants. Clothing was documented with a code (1–5) that was described in the diary for the upper and lower part of the body (Supplementary Table S2 online: percentage of skin area exposed for each clothing code eligible in the diary, online).

We estimated the UVB exposure by multiplying the UVB dose from the SunSaver for every 30 minutes with the percentage of body area exposed during that 30 minutes according to the diary, and then these numbers were summed for all study days.

Skin examinations

Before going on holiday, all the participants had their PPF measured on the buttock skin using a skin reflectance meter (UV Optimize Scientific 558; Chromo-Light, Espergaerde, Denmark) (Wulf, 1989). PPF is an objective, physical measure of UVR sensitivity based on pigmentation with a measuring range of 1.0–25.0 (1 is the PPF of the palest possible persons and 25.0 corresponds to the darkest black persons; See definitions). Buttock-PPF is an objective measure of constitutive photo-skin-type (Wulf and Lock-Andersen, 1997).

Analysis of urinary T–T dimers

The urine samples were collected before the holiday and on the third day after return. Urine was collected in 15 ml polyethylene tubes, immediately frozen, and kept at −20° until analysis. T–T dimers were purified, analyzed with the ^{32}P-post-labeling assay, and quantified as described before (Kotova *et al.*, 2005). To correct for variations in urine concentration, T–T dimer levels were adjusted for creatinine level, as determined by Jaffe's method (Seaton and Ali, 1984). Creatinine levels vary with gender, age, and body size, and to correct the measured values for such variations the table of Kampmann was used (Kampmann *et al.*, 1974). The resulting data are therefore expressed as nmol T-T (per 24 h).

Biochemical measurements of vitamin D (25(OH)D)

Blood was drawn before the holiday and on the third day after return. Samples were centrifuged (5,000*g* for 30 minutes) within 5 hours, and afterward the serum was poured into a dry tube and stored at −20°. The serum samples were analyzed for 25(OH)D by means of liquid chromatography—mass spectrometry, the recommended method for analysis of serum 25(OH) D. Every serum sample was analyzed in triplicate. Furthermore, we ensured that pre and post-samples from one volunteer were analyzed in the same run to eliminate batch variation.

Statistical analyses

SPSS Statistics 19 (IBM, New York, NY) was used for data analysis. The distributions were assessed graphically and by Kolmogorow–Smirnov test for normality. For the normally distributed data, the *t*-test was used. The Wilcoxon signed rank and the Mann–Whitney tests were used for paired and unpaired data, respectively, that were not normally distributed. Several equations were tested (linear, logarithmic, inverse, power, s-curve, exponential) to determine the best equation for the regression analyses. The significance level was set to $P<0.05$.

Ethics

The study was performed acccording to the Declaration of Helsinki. Informed consent was obtained from all participants. The national ethical committees from each participating country approved the study.

The Danish Ethical Committee: H-D-2009-034-23449.

The Spanish Ethical Committee: 2009/3692/I & 2088/3017/I.

The Regional Ethical Review Board in Stockholm: 2010/993-31/4.

Definitions

Pigment protection factor (PPF). Pigment protection factor is measured by skin reflectance and represents the number of

standard erythema doses (SEDs) needed to provoke a minimal erythema dose (MED) and is therefore a measure of the skin's sensitivity to the sun (Wulf and Lock-Andersen, 1997). A PPF of 3 means that the MED is provoked by 3 SED, which is typical of a fair-skinned person. PPF is linearly dependent on pigmentation (melanin) and is calculated from this using a spectroscopic approach (Wulf, 1989; Kongshoj *et al.*, 2006).

Effective SPF. The effective SPF is calculated for each volunteer using the average SPF of that day, assuming an average application thickness as found in a previous study (Petersen *et al.*, 2013), and an exponential relationship between application thickness and UVR transmission (Faurschou and Wulf, 2007). Effective SPF = labelled $SPF^{\left(\frac{0.79}{2}\right)}$.

UVB dose. The measured UVB dose is summed up to a total for all study days.

Time with UVB exposure. The measured time with UVB exposure is summed up to a total for all study days.

UVB exposure. The UVB dose for every 30 minutes is multiplied with the exposed body area percentage for that 30 minutes according to the diary, and then these numbers are summed up to a total for all study days. The variable is calculated for each day and then summed up to one total for all study days.

$$\text{SPF adjusted UVB exposure} = \text{UVB exposure before sunscreen application} + \left(\frac{\textit{UVB exposure after sunscreen application}}{\textit{Effective SPF}}\right)$$

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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