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Children sustain high levels of skin DNA photodamage, with a modest increase of serum 25(OH)D₃, after a summer holiday in Northern Europe

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Running Head: Impact of summer sun on children's health

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Conflict of interest:
The authors state no conflicts of interest
What’s already known about this topic?

- Tenerife holiday studies in adults have shown a UVB dependent increase in 25(OH)D₃ and potentially mutagenic cyclobutane pyrimidine dimers (CPD) that may initiate skin cancer.
- Childhood solar UVR exposure increases the risk of skin cancer in adulthood, but no study has assessed the risks and benefits of solar UVR in children on holiday.

What does this study add?

- Relatively low daily UVR doses in children at a Baltic Sea summer camp resulted in a modest but significant improvement of 25(OH)D₃ (24%) but a very much greater increase in CPD (1262%).
- The children had the same level of CPD as adults who had higher UVR doses over a shorter holiday in Tenerife.
- These results stress the importance of rigorous photoprotection in the young.

**Abbreviations:** BSA: Body surface area, CPD: Cyclobutane pyrimidine dimer, SED: Standard erythema dose, SPF: Sun protection factor, T<>T: thymine dimer
Abstract

Background: Childhood solar ultraviolet radiation (UVR) exposure increases the risk of skin cancer in adulthood, which is associated with mutations caused by UVR-induced cyclobutane pyrimidine dimers (CPD). Solar UVR is also the main source of vitamin D, essential for healthy bone development in children.

Objectives: The impact of a 12-day Baltic Sea (54°N) beach holiday on serum 25-dihydroxyvitamin D (25(OH)D₃) and CPD was assessed in 32 healthy Polish children (skin types I-IV).

Methods: Blood and urine were collected, before and after the holiday, and assessed for 25(OH)D₃ and excreted CPD respectively, and personal UVR exposure was measured. Diaries were used to record sunbathing, sunburn and sunscreen use. Pre- and post holiday skin redness and pigmentation were measured by reflectance spectroscopy.

Results: The average daily exposure UVR dose was 2.4±1.5 (SD) standard erythema doses (SED) which is borderline erythemal. The mean concentration of 25(OH)D₃ increased (x1.24±0.19) from 64.7±13.3 → 79.3±18.7 nmol/L (p=1.59x10⁻⁷). Mean CPD increased 12.62±10.0-fold from 26.9±17.9 to 248.9±113.4 fmol/µmol creatinine (p=2.66x10⁻¹¹). Increased 25(OH)D₃ was accompanied by a very much greater increase in DNA damage associated with carcinogenic potential. Overall, skin type had no significant effects on behavioural, clinical or analytical outcomes, but skin types I/II had more CPD (unadjusted p=0.0496) than skin types III/IV at the end of the holiday.

Conclusions: Careful consideration must be given to health outcomes of childhood solar exposure, and a much better understanding of the risk/benefit relationships of such exposure is required. Rigorous photoprotection is necessary for children, even in Northern Europe.

Key Words: Children, UVR exposure, vitamin D, DNA damage

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Introduction
The growing global incidence of skin cancer is attributed to increased exposure to solar ultraviolet radiation (UVR). One study predicted that regular use of a sun protection factor (SPF) 15 sunscreen for the first 18 years would reduce the lifetime incidence of keratinocyte cancers (KC) by 78% ¹, assuming accumulation of ~50% of lifetime UVR exposure in that time ². In contrast, measured solar UVR exposure in Danish children and teenagers suggested that only 25% of lifetime UVR exposure is obtained by 20 years ³. Thus, measurements of childhood UVR exposure are important because this is a risk factor for malignant melanoma (MM) and basal cell carcinoma (BCC) in adulthood ⁴-⁶. Furthermore, the incidence of childhood and adolescent MM is increasing in the US ⁷, and the incidence of BCC is increasing in those under 30 years in the UK ⁸.

Solar UVB (~295-320nm) is the main source of vitamin D ⁹,¹⁰. The most widely used indicator of vitamin D status is serum 25(OH)D. Vitamin D insufficiency (25(OH)D < 50 nmol/L), or deficiency (25(OH)D < 25nmol/L), prevalent in children ¹⁰-¹², impairs bone mineralization. A UK population based study of 1102 children (4-18 years) showed that insufficiency was 35% overall and 85% in those that were “non-white” ¹¹. Rickets is common in the developing world ¹³, and hospitalisation of children <15 years for this disease in England is at its highest rate for five decades, and not confined to those with pigmented skin ¹⁴. Sub-optimal serum 25(OH)D in childhood may also have adverse effects on tuberculosis and asthma ¹⁰,¹⁵. However, there is considerable controversy about the benefits of vitamin D to non-skeletal health ¹⁶.
The spectral region that initiates the synthesis of vitamin D, via the conversion of cutaneous 7-dehydrocholesterol to pre-vitamin D, is also the main cause of sunburn (erythema) and epidermal DNA damage \(^1\), especially the formation of cyclobutane pyrimidine dimers (CPD) that are associated with mutations that initiate KC \(^2\). CPD may also initiate photoimmunosuppression \(^3\), which has been implicated in KC and MM \(^4\). DNA damage in childhood may be a critical event for adult skin cancer risk. Most studies have made CPD assessments from skin biopsies but it is possible to measure CPD in urine because of their excretion after nucleotide excision repair (NER). This non-invasive approach has been validated \(^5\) and has ethical advantages. To date, only one study has assessed the urinary excretion of CPD in children after two consecutive days on a Swedish beach in late summer, and reported a linear increase with UVR dose over an estimated \(\sim70\%\) of body surface area (BSA) exposed \(^7\).

Laboratory studies in adults have shown that epidermal DNA damage accumulates \(^8\) and vitamin D synthesis \(^9\) occurs with repeated sub-erythemal UVR exposure. However, we lack data on molecular biomarkers of risk and benefit from “real life” UVR exposure in children. We report on changes in such biomarkers in children after a summer camp holiday. The primary aim was to determine the benefit of holiday solar UVR exposure. This was assessed by measuring serum 25(OH)D\(_3\) along with parathyroid hormone (PTH) and bone turnover markers \(^10\). The secondary aim was to assess the harmful effects, including erythema, pigmentation (a response to DNA damage) and urinary CPD. In addition, cumulative personal exposure to UVR was measured to determine any possible dose response relationships with these outcomes and, for CPD, also within sub-periods of UVR exposures because induction and repair kinetics of CPD are unknown in children. Additional aims were to (i) determine sun exposure behaviour by the use of daily diaries and any influence of skin type and gender.
on all outcomes (ii), determine 25(OH)D₃ in the Autumn, more than 3 months after the holiday and (iii) add to the literature on the proportion of ambient UVR exposure received by children.

Materials and methods

The Medical University of Łódź Bioethics Committee, Poland approved the study, which was done according to the “Declaration of Helsinki”. Thirty-two healthy children (girls 22; boys 10; mean age 8.9 years, range 8-10 years with Fitzpatrick 28 skin types I/II (n = 18) and III/IV (n = 14) were selected after a parents’ meeting. Exclusion criteria included regular oral or topical medication and sunny holidays within the previous two months. Two parents or a single parent, according to family circumstances, gave informed written consent. The children underwent a thorough paediatric assessment, which was normal in all cases.

The holiday (23rd June-6th July 2009) started the day after the end of the school year. The parents supplied sunscreens. The location was a Baltic Sea (Sztutowo 54°N) camp with an East–West unshaded sandy beach. Blood and urine samples were collected 24h before and 24h after the holiday, and stored at -20°C. Blood samples were also taken from 29/32 participants on 19-20 October 2009 for a follow-up assessment of 25(OH)D₃.

Diaries and measurement of UVR exposure

Each child completed a daily diary, based on previous studies 29,30, of which some parameters are listed in the first two columns of table 1, and wore a personal dosimeter “SunSaver” 31 on the wrist during the day. The dosimeter readout was the standard erythema dose (SED) integrated and reported every 0.5h. Two SunSavers, in watertight housing, on a 2m pole in an open unshaded area monitored ambient UVR.
Skin reflectance
Skin reflectance was measured, 24h before and 24h after the holiday, on 6 sites (back of hand, forehead, upper inner and outer arm, back and upper buttock) (UV-Opimize, Chromo-light, Espergærde, Denmark) at 555nm (erythema) and at 660nm (pigmentation).

Blood parameters
Measurement of 25(OH)D₃
The LC-MS/MS techniques have been described. Samples were run in triplicate, the mean value of which was used as the endpoint. Pre- and post-exposure samples from the same child were always run concurrently.

Serum PTH, crosslaps and osteocalcine
PTH was assessed by immunochemiluminescence (IMMULITE Turbo intact PTH, Diagnostic Products Corporation, Los Angeles USA) and ELISA was used for serum crosslaps and osteocalcine (bone gla protein (BGP) (ELISA Immunodiagnostic Systems GmbH (IDS GmbH), Frankfurt am Main, Germany). The mean of duplicates was used in the analyses.

Urinary CPD
Urine aliquots of 10 µL were analysed for thymine dimers (T<>T), the most frequent CPD, by a postlabelling HPLC method. Creatinine concentration was used to adjust for urine dilution, using the Jaffe method.

Statistics
No holiday or laboratory UVR-intervention studies had been previously done in children. The study was overpowered based on a laboratory UVB intervention study of 50 adults.

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Under this study’s conditions, 16 children would have been sufficient to give a significance level of 5% and 90% power to detect Δ23.3 nmol/L 25(OH)D₃ with a SD of 26.5 using a paired design. Analyses were performed using SPSS Statistics version 22 (IBM New York, NY). All data were normally distributed (Kolmogorow-Smirmnov test p>0.134), except for the number of sunburns on the individual body sites listed in table 1. All analyses were performed with parametric tests, except for the influence of skin type on the number of sunburns for which the Mann-Witney U-test was used.

Comparisons of pre- and post-holiday values of 25(OH)D, T<>T, PTH, crosslaps, osteocalcin and skin reflectance measurements were made using paired t-tests assuming equal variance, because Levene’s (F test for equal variance) tests were non-significant in all cases. The influence of gender, skin type and low/high UVR exposure on numeric measurements, such as 25(OH)D, T<>T and their delta values (post-holiday minus pre-holiday), were made using independent t-tests.

The Chi-squared test was used to test for skin type and gender influence on the categorical diary data outcomes that are summarised in table 1. Pearson correlation was calculated to investigate relationships between individual measured parameters of UVR dose, 25(OH)D, T<>T, PTH, crosslaps, osteocalcin, skin reflectance and measurements at June, July and October time points and their Δ values. Analyses were also done with the temporally subdivided UVR-dose to determine if any relationships with T<>T were time-dependent. Only relevant correlations were performed and reported, an example of non-relevant correlation is between June PTH and July crosslaps.
General linear models (GLM), which correspond to multiple linear regression analysis, were used to determine the relationships between July and October values for 25(OH)D and their June (baseline) values, controlling for gender and/or skin type. A similar approach was used for T<<T except the baseline was not incorporated, because it would have been cleared during the holiday. All tests were two sided and p-values < 0.05 were considered significant. However, up to 50 biologically plausible associations were tested between 25(OH)D₃ and T<<T and other parameters (e.g. pigmentation, skin type). A p value of <0.001 (0.05/50) is necessary to maintain significance after adjustment for multiple comparisons (Bonferroni correction). Those that do not survive this test are identified in the text. Although these p-values cannot be considered significant, they are indicative of plausible trends that need to be confirmed with a larger sample size.

Results

Personal and ambient UVR exposure and diaries

Mean noon±2h ambient temperature was 19.4°C (range 17.0-25.0). The time outdoors was typically 6-7 hours/day giving a mean exposure of 2.4±1.5(SD) SED (median=2.1) with no differences between skin types I/II and III/IV (p=0.51). Figure 1a shows the accumulation of SED during the holiday. Skin type did not (p=0.43) influence cumulative exposure that was 28.4±4.2 SED in skin types I/II and 29.6±4.1 SED in skin types III/IV. This was also the case when UVR exposure was assessed on days 1-3 (p=0.40), 4-6 (p=0.64), 7-9 (p=0.97) or 9-12 (p=0.17). Days 1–6 were less sunny than days 7–12, so the ambient UVR data in Figure 1b have been split accordingly. Figure 1b also shows the mean SED/0.5h measured on the personal dosimeters. The cumulative exposures were 11.8±2.1 and 11.7±2.3 SED for skin types I/II and III/IV respectively during days 1-6 (~8% of ambient). The comparable data for
days 7-12 were 16.6±3.2 and 17.9±3.5 SED (~9% of ambient). There was no effect of gender on UVR exposure over the whole study period (p=0.72).

The diary results are summarised in table 1. Behaviour is likely to have been influenced by weather (Figure 1b). The children spent time of the beach for 6-8 days with no significant difference between the skin type groups (p = 0.261). Clothing was not recorded (apart from wearing T-shirts) but, when playing on the beach, the children wore hats; the boys wore shorts and the girls wore bikinis. The children were not able to apply sunscreen to their backs but used T-shirts to protect shoulders and back, with at least 56% using this strategy (table 1 – shoulders exposed) when playing on the beach. Sunscreens were used on 200/384 person/days (52%). The most common SPF was 30 (46.5% of the sunscreen/person days), with lower SPF (6-25) and higher SPF (35-50) on 28.0% and 25.5% of the person/days respectively with no significant difference, within the above SPF groupings, with skin type (p=0.410). Overall, there were no skin type dependent significant differences in sun exposure behaviour. However, skin types I/II had significantly more days with sunburn on any body site, than skin types III/IV (p=0.039), but this difference was not significant for specific body sites.

**Skin reflectance**

There was more pigmentation in skin types III/IV than I/II on all exposed body sites. This was significant for the back (p=0.012), forehead (p=0.0078) and hand (p=0.00049) in June, and for the forehead in July (p=0.015). The holiday significantly (p<0.03) increased pigmentation on all these sites in all skin types, but this increase was not significantly different between skin types I/II and III/IV (p >0.14). However, there was a significant loss of pigmentation in all skin types (p=0.005) on the upper buttock with a significantly (p = 0.02) greater the loss in skin types III/IV compared with I/II. Skin redness was generally higher in skin types I/II than
III/IV, but this was only significant (p=0.038) for the hand in June. There was no significant increase in skin redness for any body site over the holiday (p>0.09).

**25(OH)D₃, PTH, crosslaps and osteocalcin**

Figure 2a shows the individual pre- and post-holiday 25(OH)D₃ values. Five/32 children (16%) were insufficient in June (<50 nmol/L 25(OH)D₃). The average increase (64.7±13.3 (median=64.9) → 79.3±18.7 (median=79.8) nmol/L) was highly significant (p=1.59x10⁻⁷), with Δ=14.7±12.4 nmol/L 25(OH)D₃. The mean fold increase was 1.24±0.19. There was no significant (p=0.626) correlation between baseline June 25(OH)D₃ and post-holiday response, i.e. Δ25(OH)D₃.

The boys had more 25(OH)D₃ than girls in June (73.98 vs. 60.41 nmol/L, p=0.0055 but significance lost after Bonferroni correction (SLABC)) and July (93.1 vs. 73.1 nmol/L, p=0.0032 (SLABC)), but there was no influence of gender or skin type (p≥0.17) on June-July Δ 25(OH)D₃. There was no relationship between total cumulative UVR exposure and the July 25(OH)D₃ levels (p=0.388) and for the Δ 25(OH)D₃ (p=0.741). However, there was a borderline association between total UVR exposure and Δ 25(OH)D₃ for skin types III/IV only (p=0.0502). The lack of an overall UVR dose effect is also supported by table 2, which shows a higher Δ 25(OH)D₃ value in the high vs. low SED exposure group but the difference did not reach significance. There were inverse relationships between July 25(OH)D₃ and pigmentation of the outer arm in June (r=−0.468, p=0.008 (SLABC)) and July (r=−0.396, p=0.025 (SLABC)), and Δ pigmentation of the forehead (r=−0.352, p=0.048 (SLABC)).

Figure 2b shows October post-summer (n=29/32) 25(OH)D₃ compared with post-holiday. In most cases (76% children) the values fell (mean of 79.3±18.7→68.2±13.7 nmol/L) and the

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average decline (7.6±10.1 nmol/L) is highly significant (p=3.55x10^{-4}) for the 29 children who could be compared, two of whom (7%) had 25(OH)D₃ measurements <50 nmol/L. The July (R²=0.56, p=7.6x10^{-7}) and October (R²=0.51, p=1.46x10^{-5}) 25(OH)D₃ levels were predicted by the June level, and the October level (R²=0.52, p=1.02x10^{-5}) was predicted by the July level, with no influence of gender, skin type or total UVR exposure in any case. There was no gender effect on October 25(OH)D₃ (boys=72.2 nmol/L and girls=66.7 nmol/L, p=0.34).

There were no significant differences between pre- and post-holiday (p>0.13) PTH (34.6±13.8 (SD) vs. 37.6±16.7 pg/mL (Δ=3.0±11.1)), crosslaps (1.9±0.5 vs. 2.0±0.5 ng/mL (Δ=0.1±0.4)) and osteocalcin (60.3±12.6 vs. 63.3±11.6 ng/mL (Δ=3.1±11.4)). There was a positive association between Δ crosslaps and Δ osteocalcin (p = 0.008, R² = 0.21) but no other associations between differences in 25(OH)D₃, PTH and the markers of bone turnover (p ≥ 0.060). However, the boys had a significant (p=0.016) fall of post-holiday osteocalcin compared with the girls (table 2).

DNA damage (T<>T)

There was a highly significant (p=2.66x10^{-11}) increase in urinary T<>T (fmol/μmol creatinine) after the holiday (note that data from 4/32 July samples were not used because they were considered unreliable due to low creatinine levels). The mean value increased from 26.9±17.9 (median=22.4) fmol/μmol creatinine (range: 8.0-83.0) to 248.9±113.4 (median=220.9) fmol/μmol creatinine (range: 24.9-522.5). This represents a mean fold increase of 12.62±10.0. Pre- and post-holiday values are shown in figure 3a. June T<>T was not predictive for July (p=0.114). There was no relationship between total cumulative SED and T<>T in July (p=0.892), or between DNA damage and SED over any of the 4 sub-periods (p=0.093-0.934).
Figure 3b shows that skin type had no influence \((p=0.945)\) on the June T<>T measurements \((n=32)\) (mean=27.1±17.6 and 26.7±18.8 fmol/μmol creatinine for I/II and III/IV respectively with corresponding medians of 21.5 and 23.6). Figure 3c shows that there was a borderline significant skin type dependent difference \((p=0.0496 \text{ (SLABC)})\) in July T<>T \((n=28)\) (mean=287.7±100.0 and 204.1±114.9 fmol/μmol creatinine for I/II and III/IV respectively with corresponding medians of 305.0 and 184.4), which was independent of the June values. However, there was no skin type dependent difference \((p=0.057)\) for Δ T<>T (table 2).

There was a significant correlation for all skin types between pre-holiday pigmentation on the forehead and June T<>T \((p=0.041\text{ (SLABC)}, r=0.364)\), but no other pre-holiday correlations with the reflectance data \((p > 0.279)\). There was a significant correlation between Δ pigmentation on the back of all skin types and July T<>T \((p = 0.009 \text{ (SLABC)}, r = 0.484)\) and Δ T<>T \((p=0.020 \text{ (SLABC)}, r=0.438)\). This was also the case for buttock Δ pigmentation \((p = 0.010 \text{ (SLABC)}, r = 0.477 \text{ and } p=0.010 \text{ (SLABC)}, r=0.478 \text{ respectively})\). There were no other correlations between July T<>T and Δ T<>T with any of the reflectance data \((p >0.071)\).

### Relationships between T<>T and 25(OH)D₃

There was no association between July T<>T and 25(OH)D₃ with \(p=0.151\) for Δ (July–June) 25(OH)D₃ and \(p=0.247\) for July 25(OH)D₃. We did not use Δ (July–June) T<>T in these analyses because June T<>T represent DNA repair damage before the holiday, all of which would have been cleared during the holiday. Furthermore, there was no relationship between June and July T<>T (see figure 3a).
Discussion

Daily UVR exposure was 2.4 SED±1.5 (median=2.1) for all skin types which is borderline erythemal on non-acclimatized adult skin types I/II 35. In a study of 12 children (8-10 years) on holiday in Denmark (~56°N) over the same period in 2009, the mean daily dose was 3.4 SED (median=3.1, range 1.4–7.0 SED), with mean time outdoors of 5.9 hours (median=6.5, range 2.4–8.8 h) (data from a larger study by personal communication 36). These results are similar to our data, and to those for children in summer in Sweden 23.

The children received 8-9% of ambient erythemal UVR, which is little higher than the 6-7% reported for children of comparable age at 2-day summer schools in Valencia, Spain, while other studies show summer mean exposures ranging from 2.8% to 6.4% of ambient 37. The Polish children had a cumulative exposure of about 30 SED over 12 days, which represents ~20% of annual burden, based on a study of Danish children 3. Comparisons are approximations because exposure depends on body site 38.

The diary data (table 1) show no significant skin type differences in behaviour, sunscreen use and sunburn (except for any body site), although indicators of sunburn were generally higher in skin types I/II. The children self-applied sunscreen, and given that adults typically apply much less than necessary to achieve the labelled SPF 39, it is likely that this was also happened with the children. The reflectance data show no significant increase of skin redness, though values in skin types I/II were typically higher than types III/IV. However, there was a significant increase in pigmentation on exposed body sites, but a decrease in pigmentation in the upper buttock, especially in skin types III/IV. The reason for this is unknown, but this site
is the tan-line region and it is possible that it was more modestly covered in a group holiday than in previous family holidays.

Regular sub-erythemal exposure over small areas is advocated for maintaining optimal vitamin D status in adults, and at least 50 nmol/L (20 ng/mL) is recommended in children for bone health. One study on fully clothed (without hats) exclusively breastfed babies showed that 2h sunshine/week were needed to maintain 25(OH)D3 >27.5 nmol/L, compared to 0.5h/week for those wearing only a diaper/nappy. However, a recent global consensus on rickets prevention was unable to recommend a safe level UVR exposure to enhance vitamin D status.

Five (16%) children were insufficient in June but none in July. It should be noted that there is no vitamin D food fortification in Poland. There was a modest (x1.24±0.19) but highly significant increase (Δ=14.7±12.4 nmol/L) in 25(OH)D3 during the holiday (figure 2a) that was not correlated with UVR dose. Overall, this increase was equivalent to ~0.5 nmol/L 25(OH)D3 per SED (excluding any sunscreen effect). The lack of a baseline 25(OH)D3 effect in the children suggests that the increase in 25(OH)D3 is likely to have been limited by the relatively high June 25(OH)D3 values, because laboratory and holiday studies in adults have shown increased 25(OH)D3 in response to UVR is inversely related to its baseline. June 25(OH)D3 measurements predicted those for July and October, which might indicate individual behavioural trends and/or genetic/metabolic parameters.

The clinical significance of any increase in 25(OH)D3 depends its baseline level. The important factor is to achieve sufficiency. Thus, the relatively modest increase will have been more important in the children who were insufficient at the start of the holiday. However, it is
also important to build up summer reserves to maintain sufficiency in winter. We lack data on the half-life of solar UVR-induced 25(OH)D3 but a recent study in adults suggests that this is inversely related to the level attained when laboratory UVR intervention was stopped 45.

The study was insufficiently powered to detect skin type effects, but table 2 shows a trend for a greater increase of 25(OH)D3 in skin types I/II. A 4-week outdoor study on children in India showed a significantly greater increase in 25(OH)D3 in those with light (type IV) compared to dark brown (type V) skin 46. A review of studies on the role of skin type on vitamin D synthesis reported contradictory results 47.

Adult studies have shown that males have more serum 25(OH)D3 than females 48,49. The boys had higher 25(OH)D3 than girls in June and July (but significance was lost after correction for multiple comparisons), with no significant gender effect on the Δ 25(OH)D3 values, which suggests a comparable ability for 25(OH)D3 production because there was no gender difference in total holiday UVR exposure (p=0.72). These differences suggest that the boys spent more time outdoors than the girls before June and are consistent with the observation that serum 25(OH)D3 was best predicted (in adults) by solar UVR exposure 6 weeks prior to measurement 48. However, it should be noted that there was a trend (table 2) for a greater response in the boys. The gender difference in 25(OH)D3 was lost by October, by which time there was a significant decline of 25(OH)D3, with two (7%) children with < 50 nmol/L (insufficient), and two just above this level.

There was no overall effect of UVR exposure and other parameters on markers of bone turnover (osteocalcine, crosslaps) and PTH. The latter was within the expected range (11-54pg/mL) for children, and is in accord with a study that showed no association between PTH
and serum in children (n=271, mean age 10.4 years) when the concentration of 25(OH)D₃ was >25 nmol/L. The crosslaps values were similar to those of healthy French and Italian children of the same age (using the same assay) and the osteocalcin values were comparable to those of healthy Italian and Danish children of the same age (different assays). One study of 2798 10 year old German children showed that 25(OH)D₃ had an inverse seasonal relationship with osteocalcin and crosslaps. Our data show a positive correlation between Δ osteocalcin and crosslaps, and that there was a post-holiday significant reduction of osteocalcin in the boys compared to an increase in the girls. The reasons for this are unclear. Bone turnover markers in children are affected by many factors, especially growth, puberty and activity levels. It is quite possible that some of the girls had entered puberty with greater growth velocity, whereas boys typically have a later onset of puberty.

T<>T is the most frequent CPD, whereas cytosine containing CPD (C<>C, C<>T) are implicated in p53 and PTCH mutations in skin cancers. However, T<>T are indicative of cytosine containing CPD. There was a strong association between UVR dose and urinary T<>T in a laboratory study, and in children and adults sunbathing (after adjustment for BSA exposed) for 1-2 days in Sweden. In contrast, the association between UVR dose and urinary T<>T was weak in a 3-week study of Swedish lifeguards with a mean daily dose of 7.7 SED.

The highly significant increase in post-holiday urinary T<>T (see figure 3a) was not correlated with UVR exposure. A significant relationship between Δ back pigmentation and T<>T was lost after adjustment for multiple comparisons. Melanogenesis is a crude proxy for cumulative UVR exposure and there is considerable evidence that it is triggered by the CPD.
The back represents ~18% of total BSA in children and is the largest zone likely to have been exposed during the holiday.

Laboratory studies show that \textit{ex vivo} skin type I/II is more susceptible to T<>T than skin type III/IV exposed to the same dose of UVB \textsuperscript{56} or solar simulated radiation (SSR) \textit{in vivo} \textsuperscript{61}. However, a review of the literature shows no consistent trends over the whole skin type range \textsuperscript{62}. Our study showed no effect of skin type on June T<>T. In July, skin types I/II had \textasciitilde40% more T<>T than III/IV, though of borderline significance (lost after correction for multiple comparisons) and not explained by differences in UVR exposure. Possible reasons include protection by melanin and/or better NER in skin types III/IV \textsuperscript{62,63}.

Adult data from a 7-day skiing holiday in Austria combined with a 7-day beach holiday in Tenerife showed a significant relationship between UVB dose, 25(OH)D\textsubscript{3} and urinary T<>T, after accounting for BSA exposed \textsuperscript{44}, as well as a relationship between post-holiday T<>T and 25(OH)D\textsubscript{3}. The lack of such relationships in the longer children’s study is a likely consequence of little interpersonal variation of UVR exposure doses, because of supervised activities, and the lack of adjustment for BSA exposed. Furthermore, both endpoints are likely to have been close to saturation at the end of the holiday. Mean 25(OH)D\textsubscript{3} at 79.3 nmol/L is likely to have been approaching a plateau and urinary T<>T at a steady state \textsuperscript{58}, where the formation and excretion of T<>T are equivalent. This state was attained after \textasciitilde5 days in the Swedish lifeguard study described above, with T<>T at \textasciitilde200-250 fmol/\mu mol creatinine, which is comparable to the children in July, even though their UVR doses were much lower.

Swedish children (n =12, skin type II/III) showed an increase of T<>T (means) from 106±35 to 258±61 fmol/\mu mol creatinine after two consecutive days of beach exposure with a mean
daily dose of 3.2±1.1 SED. The final T<>T value was very similar to ours, but the initial value was about 4 times greater, probably because the study was done in late summer. The Polish children’s post holiday median T<>T of 220.9 fmol/μmol creatinine was the same as Danish holidaymakers after a week in Tenerife (median=220.2 fmol/μmol creatinine), even though the Danes received much higher daily (mean=9.4±7.0 (SD) with range of 0.8 – 32.2 SED) and cumulative UVR doses (57±24.7 with range 21.0–115.0 SED) than the children. Overall, our data suggest that children are more susceptible to DNA photodamage than adults and/or have more efficient NER.

A laboratory study of skin type II adults exposed to 1.3 SED solar simulated radiation thrice weekly for 6 weeks, over ~35% BSA, showed dose-dependent increase of 25(OH)D3 that reached a plateau at 3 weeks (after 11.75 SED) in which the fold increase (1.55±0.24) was comparable to the children. There were no detectable urinary T<>T at 6 weeks. A complex UVR exposure (time) dependent combination of photochemical reactions limits the production of pre-vitamin D that reaches a maximal level in a relatively short time (~3h), which in turn limits the production of 25(OH)D3. Thus, daily exposure beyond that necessary for maximal pre-vitamin D will result in increased DNA damage. Thus daily exposure of 6-7 hours skews the outcome towards risk, as demonstrated in this study.

The study strengths are its relevance to “real life”; showing beneficial and harmful effects in the same children. The timing of the holiday, immediately after the school term, limited baseline confounding factors, especially T<>T. The weakness is the small sample size when the population is subdivided by skin type or gender. The significance of assessments based on these categories was lost after correction for multiple comparisons, although the skin type trends are biologically plausible. The study would have been improved if exposed BSA and
sunscreen application thickness had been measured. Group supervision limited the interpersonal variation of personal UVR exposure, which restricts the establishment of UVR dose relationships.

In conclusion, a temperate latitude beach holiday moderately increased 25(OH)D$_3$ (x1.24±0.19) in children but was associated with a much greater increase in DNA damage (x12.62±10.0), which was more evident in skin types I/II. Any comparison of risks and benefits should note that brief daily UVR exposures are the safest and most effective way to improve vitamin D status. Prolonged exposure has limited benefit 66, but enhances DNA damage. There is an urgent need for a better understanding of the consequences of childhood UVR exposure and to develop strategies for its optimization.

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Table 1  Summary of data from sun diaries during the summer camp. The back was not assessed because the children could not self-apply sunscreen. A day with shoulders exposed is indicative of not wearing a T-shirt. 

1 A maximum of 12 days was possible but behaviour was influenced by weather (See Fig 1b). 
2 Sunbathing is defined as intentional sun exposure with the upper body exposed. 
3 The participants reported that the sunburn was present for at least two consecutive days on the same body site, which is indicative of more severe sunburn that did not resolve within a day. 
4 Of 18 skin types I/II, 2 were skin type I. 
5 Of 14 skin types III/IV, 2 were skin type IV. M-W = Mann-Whitney, T = unpaired t-test
<table>
<thead>
<tr>
<th>Category</th>
<th>N</th>
<th>Mean total SED</th>
<th>Mean Δ T&lt;&gt;T (fmol/μmol creatinine)</th>
<th>Mean Δ 25(OH)D₃ (nmol/L)</th>
<th>Mean Δ PTH (pg/mL)</th>
<th>Mean Δ osteocalcin (ng/mL)</th>
<th>Mean Δ crosslaps (ng/mL)</th>
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<tr>
<td>All</td>
<td>32</td>
<td>28.9 ± 4.1</td>
<td>223.9 ± 109.7</td>
<td>14.7 ± 12.4</td>
<td>3.0 ± 11.1</td>
<td>3.1 ± 11.4</td>
<td>0.1 ± 0.4</td>
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<td>Skin type</td>
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<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>I/II</td>
<td>18</td>
<td>28.4 ± 4.2</td>
<td>260.5 ± 97.7</td>
<td>17.3 ± 15.6</td>
<td>2.5 ± 12.3</td>
<td>2.2 ± 13.6</td>
<td>0.2 ± 0.5</td>
</tr>
<tr>
<td>III/IV</td>
<td>14</td>
<td>29.6 ± 4.1</td>
<td>181.7 ± 111.1</td>
<td>11.3 ± 5.1</td>
<td>3.5 ± 9.7</td>
<td>4.1 ± 8.1</td>
<td>0.0 ± 0.2</td>
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<tr>
<td>p-value</td>
<td></td>
<td>0.428</td>
<td>0.057</td>
<td>0.182</td>
<td>0.806</td>
<td>0.640</td>
<td>0.443</td>
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<tr>
<td>F</td>
<td>22</td>
<td>29.1 ± 4.2</td>
<td>226.0 ± 115.1</td>
<td>12.7 ± 10.1</td>
<td>5.5 ± 11.3</td>
<td>6.2 ± 12.0</td>
<td>0.2 ± 0.5</td>
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<tr>
<td>M</td>
<td>10</td>
<td>28.5 ± 4.2</td>
<td>218.6 ± 102.1</td>
<td>19.1 ± 15.9</td>
<td>-2.5 ± 8.8</td>
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<td>Low SED</td>
<td>17</td>
<td>25.7 ± 1.8</td>
<td>222.4 ± 89.7</td>
<td>12.8 ± 12.7</td>
<td>4.2 ± 11.2</td>
<td>4.6 ± 9.4</td>
<td>0.2 ± 0.5</td>
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<td>High SED</td>
<td>15</td>
<td>32.6 ± 2.6</td>
<td>225.4 ± 130.2</td>
<td>16.8 ± 12.0</td>
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<tr>
<td>p-value</td>
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<td>6.102 x 10⁻¹¹</td>
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<td>0.509</td>
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</table>

**Table 2**  Total holiday exposure and impact on DNA damage (T<>T), 25(OH)D₃ and markers of bone turnover (±SD). Low SED are values below the mean (n = 32) and high SED above the mean. All p-values are from unpaired T-test.
Figure Legends

1. (a) Children’s mean cumulative SED (b) Mean daily ambient SED/0.5h and corresponding values from the personal dosimeters. The data are split into days 1 - 6 and 7 - 12 because of their different weather patterns.

2. (a) Pre- and post-holiday 25(OH)D$_3$ values (n = 32) with mean increase of 64.7 ± 13.3 → 79.3 ± 18.7 (SD) nmol/L (b) Post-holiday and October 25(OH)D$_3$ values with mean fall of 79.3 ± 18.7 → 68.2 ± 13.7 (SD) nmol/L. Note: October data missing for 3 children. Note: dotted lines on both figures show the 50nmol/L threshold for sufficiency.

3. (a) Comparison of DNA damage before and after holiday sun exposure (n = 28) with mean T<>T increase of 25.15 fmol/μmol (range: 7.97-68.64) to 248.9 fmol/μmol (range: 24.91-522.50). Note: 10-fold difference in scale. (b) There was no skin type dependent difference in T<>T on entry to the study (p = 0.945) but (c) Skin types I/II had more DNA damage than skin types III/IV at the end of the study (p = 0.0496 – significance lost after Bonferroni correction). Note: 4 samples taken in July not used because of low creatinine values.
Figure 3c

July T<->T (fmol/µmol creatinine)

Skin Type