Exposure to Solar UVR Suppresses Cell-Mediated Immunization Responses in Humans: The Australian Ultraviolet Radiation and Immunity Study

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Ashwin Swaminathan[1,2], Simone L. Harrison[3], Natkunam Ketheesan[4], Christel H.A. van den Boogaard[3,5], Keith Dear[6], Martin Allen[7], Prue H. Hart[8], Matthew Cook[9,10] and Robyn M. Lucas[1,11]

Animal and human studies show that exposure to solar-simulated UVR is immunomodulatory. Human studies that used natural sun exposure and controlled for confounding are rare. We immunized 217 healthy adults (age range = 18–40 years) with a T-cell–dependent antigen, keyhole limpet hemocyanin, and measured personal clothing-adjusted UVR exposure (for 5 days before and after immunization), lifetime cumulative UVR exposure, serum 25-hydroxyvitamin D concentration at immunization, and potential confounding factors. We tested cellular and humoral immune responses in relation to UVR exposure. The delayed-type hypersensitivity response to keyhole limpet hemocyanin recall challenge was lower in individuals with higher personal clothing-adjusted UVR exposure on the day before immunization ($P = 0.015$) and during intervals spanning the day before to 2–3 days after immunization. There was an incremental increase in T helper type 17 cells (as a proportion of $CD4^+$ T cells) from preimmunization to postimmunization in the high, compared with the low, personal clothing-adjusted UVR exposure group (0.31% vs. −0.39%, $P = 0.004$). Keyhole limpet hemocyanin-specific antibody titers were not associated with acute or cumulative UVR exposure or serum 25-hydroxyvitamin D levels. Higher UVR exposure at antigen sensitization was associated with a reduced delayed-type hypersensitivity response and altered T helper type 17 kinetics. This has implications for the effectiveness of vaccinations and susceptibility to infections that rely on cell-mediated immune responses.

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INTRODUCTION

The immunomodulatory properties of exposure to UVR were first recognized in animal experiments showing that UVR-induced immune suppression was important in the development of skin cancer (Kripke and Fisher, 1976). Since then, it has become clear that UVR exposure has complex effects on the human immune system—suppressing antigen-specific cell-mediated immune processes but up-regulating aspects of innate immunity (Glaser et al., 2009; Hart and Norval, 2018; Schwarz, 2008). UVR exposure also induces the synthesis of vitamin D, and the active form of this prehormone also has regulatory effects on immune function (Hart et al., 2011).

The effects of UVR on responses to infections or vaccination have been studied in animals (reviewed in Sleijffers et al., 2004). Overall, UVR exposure appears to decrease animals' resistance to intracellular infections and response to vaccinations that require integrity of T helper (Th) type 1 cell-mediated immune processes. Host resistance is affected both locally (at the site of infection or UVR exposure) and systemically for noncutaneous disease (e.g., murine leukemia virus). Other animal studies have shown that UVR impairs humoral responses via suppressive effects on T-follicular-helper cell function and germinal center formation (Chacón-Salinas et al., 2011) and the promotion of regulatory T cells (Wang et al., 2008).

To date, controlled human studies of the immunomodulatory properties of UVR exposure have used laboratory irradiation with artificial UVR that does not closely simulate the intensity, spectrum, and skin exposure of solar UVR experienced under natural conditions (Sleijffers et al., 2001), or they have used proxies for exposure to natural solar UVR,

[1]National Centre for Epidemiology and Population Health, Research School of Population Health, The Australian National University, Canberra, Australia; [2]Departments of General Medicine and Infectious Diseases, The Canberra Hospital, Canberra, Australia; [3]College of Public Health, Medical and Veterinary Sciences, James Cook University, Townsville, Queensland, Australia; [4]Science and Technology, University of New England, School of Science and Technology, University of New England, Armidale, New South Wales, Australia; [5]University of Adelaide, Adelaide, South Australia, Australia; [6]Global and Tropical Health Division, Menzies School of Health Research, Darwin, Northern Territory, Australia; [7]Department of Electrical and Computer Engineering, University of Canterbury, Christchurch, New Zealand; [8]Telethon Kids Institute, University of Western Australia, Perth, Western Australia, Australia; [9]Department of Immunology, The Canberra Hospital, Canberra, Australia; [10]Centre for Personalized Immunology, John Curtin School of Medical Research, Australian National University, Canberra, Australia; and [11]Centre for Ophthalmology and Visual Science, University of Western Australia, Perth, Australia

Correspondence: Robyn Lucas, National Centre for Epidemiology and Population Health, Research School of Population Health, Australian National University 62 Mills Road, Acton, Canberra Australian Capital Territory 2601. E-mail: robyn.lucas@anu.edu.au

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; AusUVI, Australian Ultraviolet Radiation and Immunity Study; ca-UVR, clothing-adjusted dose of UVR; CI, confidence interval; DTH, delayed-type hypersensitivity; KLH, keyhole limpet hemocyanin; SED, standard erythemal dose; Th, T helper

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such as season or latitude, without individual exposure data (Colditz et al., 1994; Linder et al., 2011).

Here, we describe the results of the Australian Ultraviolet Radiation and Immunity (AusUVI) Study, which was designed to assess the influence of natural sun exposure on the sensitization phase of a primary immune response to a model protein antigen, keyhole limpet hemocyanin (KLH) (Swaminathan et al., 2014). We measured the UVR exposure experienced during daily living in two cohorts of healthy young adults residing in regions with very different ambient UVR conditions, and we measured potential confounding factors, including physical activity, psychological state, and ethnicity. We measured T-cell–dependent antibody responses, differentiation of T cells to effectors, and delayed-type hypersensitivity (DTH) responses as a composite measure of immunity.

RESULTS

Participant characteristics

We recruited 222 healthy adult participants but excluded five from analysis because screening tests suggested possible infection at the time of KLH immunization. The characteristics of the remaining 217 participants are summarized in Table 1. Participants from Canberra were more likely than participants from Townsville to have a university qualification. Townsville participants were more likely to report occupational settings described as "half indoors/half outdoors," were less likely to be employed predominantly "indoors," were less likely to report light recent physical activity, and had higher melanin density at the (sun-protected) upper inner arm than Canberra participants.

Measurement of acute UVR exposure

The 10-day total clothing-adjusted UVR (ca-UVR) exposure (in standard erythemal doses [SEDs]) was approximately one third the unadjusted dosimeter measurement, although this ratio varied by season and study site (Figure 1). The largest difference in ca-UVR between study sites occurred in the winter months (June through August) (Townsville = 2.3 SEDs vs. Canberra = 0.32 SEDs, $P < 0.001$). Participants with northern European ethnicity or an outdoor work setting had higher ca-UVR exposure. Each unit increase in ca-UVR exposure was linearly associated with increasing serum 25-hydroxyvitamin D (25[OH]D) of 3.9 nmol/L (95% confidence interval [CI] = 2.4–5.5, $P < 0.001$) sampled at day 8 (see Supplementary Table S1 online).

On the day before immunization (day 7), on average, the forearm (i.e., immunization site) was uncovered for 64.4% of the recorded day (7 AM–7 PM), with differences by study site (Townsville = 81.6% vs. Canberra = 46.9%, $P < 0.001$).

Measurement of lifetime cumulative UVR exposure

Silicone skin cast scores were normally distributed (mean = 3.7, standard deviation = 1.0). In a mutually adjusted multiple regression model, higher ordinal skin cast score was associated with Townsville residence (adjusted odds ratio = 2.3, 95% CI = 1.4–3.7; $P = 0.001$), older age (adjusted odds ratio = 1.1 per year, 95% CI = 1.08–1.18; $P < 0.001$), male sex (adjusted odds ratio = 2.8, 95% CI = 1.6–4.8; $P < 0.001$), and northern European parental ethnicity (adjusted odds ratio = 3.3, 95% CI = 1.9–5.8; $P < 0.001$).

Cell-mediated immune response

DTH testing. DTH responses were recorded in 211 (97%) participants; 6 participants did not attend the final study visit for the DTH response to be measured. One extreme DTH response (29.5 mm) (55% higher than the next measured response) was excluded, given its disproportionate influence on regression coefficients. The mean of the remaining DTH responses was 7.0 (standard deviation = 4.0) mm. Females had significantly larger DTH responses than males (7.4 mm vs. 6.5 mm, $P = 0.038$). Other putative immunomodulatory factors were not significantly associated with the DTH response (see Supplementary Table S2 online).

Associations between personal UVR exposure and DTH response. We analyzed the association between ca-UVR exposure around the time of KLH immunization and subsequent DTH responses measured at day 31. We used the ca-UVR for each day that the dosimeter was worn and for combinations of days (by summing the individual daily ca-UVR exposures) as per the matrix shown in Supplementary Table S3 online.

In univariate analyses (Table 2), we observed a significantly reduced DTH response in association with higher ca-UVR on day 7 (the day before immunization) ($p = 0.019$) (Figure 2). Lifetime UVR exposure (using a dichotomous skin cast score cut point of 3.5, $P = 0.15$) and serum 25(OH)D levels ($P = 0.66$) were not associated with DTH response. Results were similar in the multiple regression model that included other immunomodulatory factors, except that ca-UVR over days 7–10 ($P = 0.039$) and 7–11 ($P = 0.025$) were also independent determinants of DTH response. The regression coefficients for all aggregated ca-UVR exposure variables showed inverse associations with DTH response. Adjustment for clothing coverage of the forearm (immunization site) on day 7 as a confounding or interaction variable did not change the association between ca-UVR and DTH response.

Effector T-cell lymphocyte subset assays. We enumerated effector $CD4^+$ T cells from a subset (55/216, ~25%) of participants with extremes of personal ca-UVR exposure. Mean 10-day aggregated ca-UVR exposures in the high (n = 27) and low (n = 28) groups were 5.3 and 0.36 SEDs, respectively ($P < 0.001$). Age and sex distribution were not statistically different from the overall sample, although the low-UVR group included more participants from Canberra.

We characterized $CD4^+$ T cells according to effector function and stratified by UVR exposure group and KLH-immunization status (see Supplementary Table S4 online). There were no significant differences in the abundance of effector or regulatory T-cell subsets at baseline between individuals with high or low UVR exposure. There was variability between individuals with regard to effector T-cell subsets, and because the impact of KLH immunization on overall effector abundance was expected to be small, we analyzed the responses for longitudinal changes within individuals comparing pre- and post-KLH immunization. From preimmunization to postimmunization, we observed a significant difference in Th17 as a proportion of all $CD4^+$ T cells

Table 1. Characteristics of eligible volunteer participants in the AusUVI Study

Characteristic	Canberra	Townsville	Overall
Number, n (%)	108 (49.8)	109 (50.2)	217 (100)
Female, n (%)	69 (63.9)	68 (62.4)	137 (63.1)
Age in years, mean (SD)	29.3 (5.7)	26.4 (6.4)	27.8 (6.2)
Range	(18.3–40.9)	(18.2–40.6)	(18.2–40.9)
Parental ethnicity, n (%)			
Northern European (both parents)	71 (65.7)	80 (73.4)	151 (69.6)
Highest education qualification, n (%)			
High school (not matriculated)	1 (0.9)	2 (1.8)	3 (1.4)
Higher school certificate	10 (9.3)	54 (48.7)	63 (29.2)
Apprenticeship, certificate, or diploma	7 (6.4)	11 (9.9)	18 (8.2)
Bachelor's degree	54 (50.0)	27 (24.3)	80 (37.0)
Postgraduate degree	36 (33.3)	17 (15.3)	52 (24.1)
Current smoker, n (%)	4 (3.7)	8 (7.3)	12 (5.5)
Work environment, n (%)			
Indoors	102 (94.4)	90 (82.6)	192 (88.5)
Half indoors/half outdoors	5 (4.6)	16 (14.7)	21 (9.7)
Outdoors	1 (0.9)	3 (2.8)	4 (1.8)
Body mass index in kg/m^2, mean (SD)	23.9 (3.3)	23.8 (4.2)	23.8 (3.7)
Recent physical activity category, n (%)			
Light	11 (10.2)	2 (1.9)	13 (6.0)
Moderate	37 (34.3)	34 (31.5)	71 (32.9)
High	60 (55.6)	72 (66.7)	132 (61.1)
Psychological—global scores			
Profile of Mood States, mean (SD)	1.9 (13.9)	1.4 (13.1)	1.6 (13.5)
Mental Health Inventory, mean (SD)	168.1 (19.2)	168.4 (21.2)	168.2 (20.2)
Cutaneous melanin density at visit 2			
Left inner arm, mean % (SD)	3.1 (1.4)	3.5 (1.2)	3.3 (1.3)
Left upper cheek, mean % (SD)	4.0 (0.77)	4.0 (0.77)	4.0 (0.77)
Serum 25(OH)D in nmol/L, visit 2, mean (SD)			
Winter (June–August)	39.7 (20.1)	80.0 (20.9)	65.7 (28.3)
Spring (September–November)	64.1 (20.9)	95.4 (23.2)	74.8 (26.2)
Summer (December–February)	82.1 (19.8)	98.6 (26.0)	89.9 (24.1)
Autumn (March–May)	76.2 (33.2)	93.7 (26.9)	84.3 (31.4)

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; AusUVI, Australian Ultraviolet Radiation and Immunity Study; SD, standard deviation.

between the high ca-UVR exposure group (mean increase of 0.31%) and the low ca-UVR exposure group (mean decrease of 0.39%, $P = 0.004$) (Figure 3). We did not detect any differences between the UVR exposure groups in the preimmunization to postimmunization periods for other effector or regulatory T-cell populations. Additionally, there was no correlation between an individual's DTH response and the change in Th17 cell proportions from preimmunization to postimmunization.

KLH-specific antibody response

The anti-KLH IgG_1 and IgG_2 titers on days 15 and 29 were higher than the preimmunization baseline levels (day 8) ($P < 0.001$). There were no significant associations between ca-UVR exposure on any day or combination of days and anti-KLH IgG_1 or IgG_2 titers at day 29 in multiple regression models (see Supplementary Tables S5 and S6 online). Furthermore, neither serum 25(OH)D level nor cumulative UVR exposure was significantly associated with anti-KLH IgG_1 or IgG_2 responses in the best-fitting multiple regression models.

DISCUSSION

The AusUVI Study is the largest study to date to have assessed the immunomodulatory effect of naturally acquired solar UVR on an immunization response in humans. We found that higher natural solar UVR exposure on the day before KLH immunization and the aggregate exposure from the day before to 2 and 3 days after immunization were associated with a reduced antigen-specific in vivo cell-mediated response, as assessed by DTH analysis performed 3 weeks later. This suggests that UVR exposure during the sensitization phase of the immune response is critical in inducing antigen-specific immunotolerance. One plausible mechanism would be impaired antigen processing and promotion of a regulatory T-cell environment within the draining lymph node (Schwarz, 2008). We also observed a small but significant difference in the preimmunization to postimmunization change in Th17 cells as a proportion of all $CD4^+$ T cells in individuals with low compared with high recent UVR exposure. We found no association between response to immunization (either cell-mediated or humoral) and lifetime UVR exposure or 25(OH)D level.

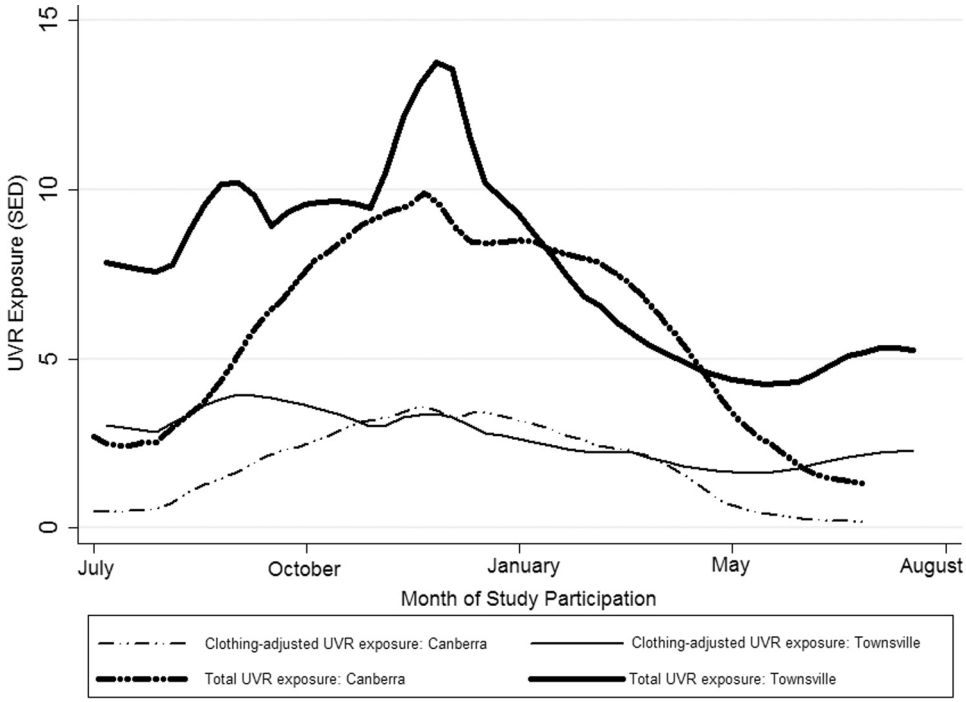

Figure 1. Aggregated 10-day clothing-adjusted UVR exposure for study participants from temperate (Canberra) and tropical (Townsville) study sites. The 10-day aggregated UVR doses for wrist dosimeter measurement (total UVR exposure) and clothing-adjusted UVR exposure for Canberra and Townsville study participants as they were recruited through the year. SED, standard erythemal dose.

This study is a major advance over previous photoimmunological work in this field, because we used KLH, a protein derived from the giant keyhole limpet, *Megathura crenulata,* which is a potent immunogen in humans. It is phylogenetically distant from mammalian proteins and produces a robust primary immune response in humans, with few false positive responses. Before and after immunization with KLH, we undertook comprehensive immune testing, innovative personal UVR exposure monitoring, and measurement of potential immunomodulatory confounding factors (Van Loveren et al., 1999).

Limitations of this study include the inability to adjust for any effect of sunscreen application on total personal UVR exposure. Sunscreen protection varies according to the thickness and distribution of application, sun protection factor, and decay in effectiveness over time. Optimal sunscreen use (at 2 mg/cm^2) has been shown to prevent solar UVR immunosuppression, as measured by contact hypersensitivity responses (Narbutt et al., 2018). There is currently no validated method for correcting UVR exposure for sunscreen use based on questionnaire-derived data. Additionally, our exposure monitoring was restricted to the antigen sensitization phase of the immune response, and therefore we cannot comment on the potential effect of sun exposure at the time of the elicitation phase of the DTH response. Nevertheless, extending the length of time for wearing the electronic dosimeter and/or completion of the sun diary would have been an additional participant burden that likely would have reduced compliance and the reliability of data recording.

Kelly et al. (2000) found a dose-dependent reduction in contact hypersensitivity response at 21 days after a single dose of solar-simulated radiation administered 24 hours before sensitization with a contact allergen (Kelly et al., 2000). The effect was stronger in lighter skin types and present at sub-erythemal doses. Damian et al. (1999) administered sub-erythemal solar-simulated radiation before a nickel challenge (in allergic participants) rather than at first exposure to the antigen. There was a significant reduction in the contact hypersensitivity response after 1 day of solar-simulated radiation, reaching maximal levels with 2 days of exposure and sustained suppression with up to 5 days of exposure (Damian et al., 1999). Other relevant in vivo models have used longer UVR exposure protocols (i.e., up to weeks), making it difficult to identify specific critical days for exposure before antigen sensitization (Damian and Halliday, 2002; Fourtanier et al., 2005). Our findings of a modest immunosuppressive effect of solar UVR, compared with the larger effects seen in experimental studies using artificial UVR, may relate to the comparatively low (i.e., typically sub-erythemal) UVR doses observed in this study.

We found no association between measures of acute personal UVR exposure and anti-KLH IgG_1 or IgG_2 titer at 21 days after immunization. This is consistent with a previous study, in which participants were randomized to a control group or to receive 5 days of whole-body UVB irradiation, before hepatitis B vaccination (Sleijffers et al., 2001). There were no significant differences in hepatitis B-specific IgG or T-cell lymphocyte responses between groups up to 60 days after initial vaccination, although natural killer cell activity and contact hypersensitivity responses were reduced in the irradiated group.

In the AusUVI Study, participants in the low ca-UVR exposure group had a higher percentage of Th17 cells (as a proportion of all $CD4^+$ T cells) before immunization than those in the high exposure group (0.75% vs. 0.56%. $P = 0.13$). Although this was not statistically significant, it is consistent with a more reactive T-cell milieu, and perhaps increased risk of autoimmunity, with low sun exposure (Lucas et al., 2015). When comparing Th17 cell percentages before

Table 2. Association between clothing-adjusted UVR exposure and DTH response

	Univariate Linear Regression			Multivariable Linear Regression[1]		
Day	**n**	**β (95% CI), ×10²**	***P***	**n**	**β (95% CI), ×10²**	***P***
Individual study day						
3	198	−3.7 (−108 to 32)	0.29	183	−59 (−150 to 32)	0.20
4	200	0.79 (−51 to 53)	0.98	182	6.1 (−57 to 69)	0.85
5	191	9.7(−23 to 43)	0.56	174	25 (−14 to 65)	0.21
6	185	3.3 (−33 to 40)	0.86	168	17 (−26 to 59)	0.45
7	192	**−60 (−110 to −9.8)**	**0.019**	175	**−78 (−140 to −15)**	**0.015**
8[2]	206	25 (−34 to 85)	0.40	188	50 (−23 to 123)	0.18
9	198	−40 (−97 to 17)	0.17	180	−47 (−115 to 22)	0.18
10	195	−26 (−68 to 16)	0.22	177	−45 (−95 to 4.3)	0.073
11	187	−31 (−78 to 17)	0.21	172	−34 (−93 to 25)	0.26
12	181	7.4 (−31 to 45)	0.70	164	21 (−24 to 65)	0.37
Study day combinations[3]						
A: days 3−12	195	−8.3 (−26 to 0.88)	0.34	177	−10 (−35 to 14)	0.41
B: days 4−11	195	−9.3 (−27 to 8.9)	0.31	177	−11 (−37 to 14)	0.38
C: days 5−10	195	−7.5 (−27 to 12)	0.45	177	−7.6 (−35 to 19)	0.58
D: days 6−9	195	−13 (−38 to 12)	0.30	177	−15 (−48 to 18)	0.38
E: days 7−8	195	−21 (−59 to 17)	0.27	177	−28 (−78 to 21)	0.26
F: days 7−9	195	−23 (−55 to 8.3)	0.15	177	−34 (−75 to 7.7)	0.11
G: days 7−10	**195**	**−22 (−47 to 4.1)**	**0.100**	177	**−36 (−70 to −1.7)**	**0.039**
H: days 7−11	**195**	**−22 (−45 to 1.0)**	**0.060**	177	**−35 (−66 to −4.4)**	**0.025**
I: days 6−7	195	−16 (−46 to 13)	0.27	177	−15 (−52 to 21)	0.41
J: days 5−7	195	−4.9 (−28 to 18)	0.67	177	1.2 (−28 to 30)	0.93
K: days 4−7	195	−6.2 (−28 to 16)	0.58	177	−1.1 (-29 to 27)	0.94
L: days 3−7	195	−9.6 (−31 to 12)	0.38	177	−7.7 (−36 to 21)	0.60
M: days 8−9	195	−11 (−51 to 30)	0.60	177	−11 (−63 to 40)	0.66
N: days 8−10	195	−17 (−46 to 122)	0.26	177	−26 (−63 to 11)	0.16
O: days 8−11	195	−18 (−43 to 6.6)	0.15	177	−27 (−59 to 5.4)	0.10
P: days 8−12	195	−9.9 (−32 to 12)	0.38	177	−12 (−40 to 17)	0.42

Boldface indicates statistical significance at $P < 0.05$.

Abbreviations: CI, confidence interval; DTH, delayed-type hypersensitivity.

[1]Regression model included the following explanatory variables: age, sex, study site, season, body mass index, physical activity (metabolic equivalent of task, minutes/week), psychological state (Mental Health Inventory−global index, Profile of Mood States [total mood disturbance], skin reflectance at the inner arm and left cheek, smoking status, 25-hydroxyvitamin D level (on day of vaccination), lifetime cumulative sun exposure (left hand skin cast grade), and parental ethnicity (both parents northern European vs. other). DTH data were square-root transformed.

[2]Day of immunization.

[3]Using imputed UVR data for days with missing data.

and after immunization, the marked fall in the low ca-UVR group and the more modest increase in the high ca-UVR group suggest an interactive effect of immunization with UVR exposure.

Recent work has shown that the exposure to UVR triggers a number of pathways mediated by the aryl hydrocarbon receptor (Youssef et al., 2019). These include wide-ranging effects on immune cells, including the proliferation and differentiation of Th17 cells (Baricza et al., 2016) and increased expression of cytokines IL-17 (A and F) and IL-22, particularly in the setting of antigen processing (Quintana et al., 2008; Veldhoen et al., 2008). Our observations suggest that further studies to determine the effect of UVR on antigen-specific T-cell responses are warranted.

In laboratory studies, the biologically active form of vitamin D, 1,25 dihydroxyvitamin D, suppresses adaptive immunity by modulating the differentiation and function of regulatory T, Th1, and Th17 cells (Wei and Christakos, 2015). Nevertheless, previous studies assessing the relationship between vitamin D status and antibody response to immunization have shown mixed results (Chadha et al., 2011; Zitt et al., 2012). Here, we did not observe any association between 25(OH)D concentration and responses to immunization in multivariable analysis, thus supporting the hypothesis of a direct UVR suppressive effect on the antigen-specific cell-mediated response.

Our results show that acute personal solar UVR exposure, occurring during daily activities, modulated the antigen-specific T-cell−mediated immune responses to sensitization with KLH, a highly immunogenic protein, but had no detectable effect on antibody responses. Observed changes in Th17 cell populations related to UVR exposure and immunization warrant further work to confirm and extend our findings. Reduced immune responses to vaccination during periods of higher natural solar UVR exposure could have major implications for vaccine efficacy, including that of the newer epicutaneous delivery systems, particularly for vaccines that rely on a cell-mediated immune response.

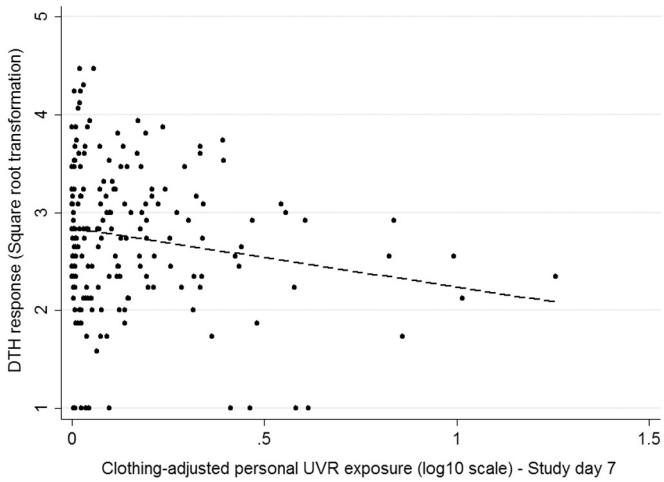

Figure 2. Association between clothing-adjusted UVR exposure on the day before immunization (study day 7) and subsequent DTH response. Each dot represents a study participant's clothing-adjusted UVR exposure for study day 7, the day before KLH immunization. The fitted regression line (dash) shows the significant inverse correlation between personal UVR exposure (log 10 scale) and DTH response (square-root transformed) (regression slope = −0.60 [95% confidence interval = −1.1 to −0.10]; $P = 0.02$). DTH, delayed-type hypersensitivity; KLH, keyhole limpet hemocyanin.

MATERIALS AND METHODS

Participants and settings

The AusUVI Study was conducted in two climatically distinct Australian cities: temperate Canberra, in the Australian Capital Territory (35° South latitude) and tropical Townsville, in Queensland (19° south latitude), with very different ambient UVR (Australian Government, Bureau of Meteorology, 2019). We recruited healthy volunteers aged 18–40 years. Exclusion criteria were shellfish allergy, preexisting immunosuppressive condition (e.g., diabetes, HIV infection, transplant recipient, chronic liver or kidney disease), use of immunosuppressive agents (systemic agents within 30 days, topical agents within 7 days), symptoms of infection or vaccination within 30 days, and pregnancy or breastfeeding.

Study protocol

Recruitment occurred uniformly over a 12-month period to account for seasonal variation, commencing in July (Southern Hemisphere winter). Each participant attended five study visits over a 31-day period (Figure 4).

Questionnaire data. On day 1, participants self-reported demographic information including age, sex, ethnicity of each parent, education level, occupational setting, smoking history, physical activity over the previous 7 days (using the International Physical Activity Questionnaire [Craig et al., 2003]), and medications, including vitamin D supplements. On day 8, participants completed the Profile of Mood States (short form) (Curran et al., 1995) and Mental Health Inventory (Snyder et al., 1993), which measure psychological well-being and distress.

Physical examination. On day 1, height and weight were measured to determine the body mass index. Skin pigmentation was assessed at the left inner upper arm (i.e., sun-protected site) and left upper cheek (i.e., sun-exposed site) on days 1, 8, and 15 with a reflectance spectrophotometer (CM2500d; Minolta, Tokyo, Japan).

Lifetime cumulative UVR exposure. Silicone skin casts were taken from the dorsum of both hands to assess lifetime cumulative solar UVR exposure (Cargill et al., 2013).

Sun exposure diary. Participants self-completed a sun exposure diary (Cargill et al., 2013) daily from days 3–12, inclusive. Time spent in the sun was recorded in 15-minute intervals. Types of clothing worn (i.e., for upper and lower body, headwear, footwear, and gloves) were recorded at hourly intervals, as previously described (see Supplementary Figure S1 online) (Brodie et al., 2013).

UVR dosimetry. Participants wore an electronic UVR wrist dosimeter (Seckmeyer et al., 2012) for the 10-day diary period. Wrist dosimeters have been previously shown to be reliable for recording personal UVR exposure (Thieden et al., 2000). Dosimeters recorded erythemally weighted UVR exposure at 8-second intervals between 7 AM and 9 PM. UVR exposure data were integrated to determine the received UVR dose in SEDs, where 1 SED = 100 Jm^{-2} of erythemally weighted UVR. Dosimeters were calibrated with a Yankee Environmental Systems erythemal radiometer at the National Institute for Water and Atmosphere (Lauder, Otago, New Zealand) before and after study deployment.

KLH immunization. After blood sampling on day 8, participants were injected subcutaneously at the anterolateral aspect of the midforearm with 125 μg high-molecular weight KLH (Biosyn Corporation, Carlsbad, CA).

Blood sampling. Blood was collected on days 8, 15, and 29 (Figure 4). Blood samples were assayed at accredited laboratories to quantify serum renal and liver function, random blood glucose, C-reactive protein, and full blood count. Sera were stored at −20 °C for analyses of KLH IgG_1 and IgG_2 titers (days 8, 15, and 29) and 25(OH)D concentration (days 8 and 29) at study completion. Serum 25(OH)D concentration was measured by liquid chromatography-tandem mass spectrometry (Royal Melbourne Institute of Technology Drug Discovery Techniques, Melbourne, Australia). Peripheral blood mononuclear cells were isolated (days 8 and 29) from whole blood within 16 hours of collection by centrifugation over a Ficoll step gradient using standard protocols (GE Healthcare Bio-Sciences AB, 2007). Separated peripheral blood mononuclear cells were frozen at −80 °C and stored in liquid nitrogen.

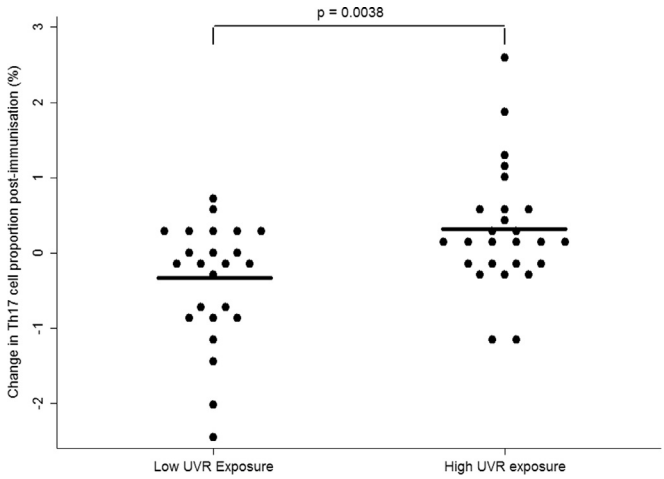

Figure 3. Change in Th17 lymphocytes (as a proportion of all $CD4^+$ T cells) from before to after immunization, according to clothing-adjusted UVR exposure category. Change in proportion of Th-17 cell subset (as a proportion of all T helper cells) was calculated by subtracting the preimmunization percentage from the day 21 postimmunization percentage in individuals with paired data only. Th, T helper.

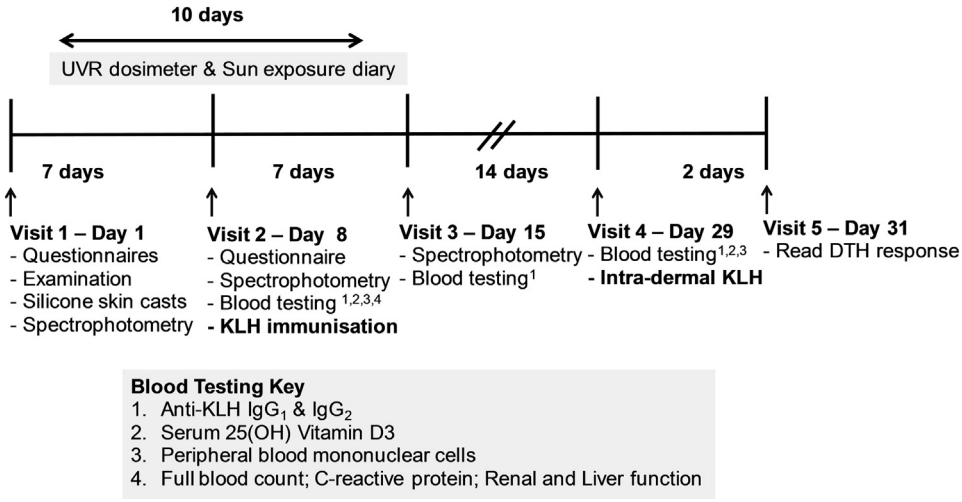

Figure 4. AusUVI Study Protocol. Each participant attended five visits over a 31-day period. Visit 1 included collection of demographic and lifestyle information (self-reported), cutaneous melanin density of sun-exposed and sun-protected skin, and silicone skin casts of the backs of the hands. At visit 2, immunization with KLH antigen occurred by subcutaneous injection. Personal UVR exposure was measured by digital dosimeter worn on the wrist and completion of a sun diary for 10 days (5 days before and 5 days after KLH immunization). Blood was taken for KLH antibodies at visits 2, 3 and 4, and for collection of peripheral blood mononuclear cells at visits 2 and 4. DTH testing was undertaken at visit 4 and read 48 hours later at visit 5. 25(OH)D, 25-hydroxyvitamin D; AusUVI, Australian Ultraviolet Radiation and Immunity Study; DTH, delayed-type hypersensitivity; KLH, keyhole limpet hemocyanin.

Participant data were excluded from analysis if screening blood test results suggested systemic inflammation (determined by C-reactive protein and white cell count greater than three standard deviations above the study population mean (C-reactive protein level > 20.7 mg/L, white cell count $> 11.3 \times 10^9$/L; n = 5), diabetes (random blood sugar level > 11.1 mmol/L, n = 0), moderate to severe kidney dysfunction (estimated glomerular filtration rate < 60 mL/min/1.73 m^2, n = 0), or moderate liver inflammation (alanine transaminase level > 120 U/L, n = 0).

Anti-KLH IgG assay. Anti-KLH IgG_1 and IgG_2 concentrations were determined by indirect ELISA, as described previously (Grant et al., 2008). Sera were tested in duplicate (and paired results averaged) for all three time points, along with a negative and positive control, on a single ELISA plate. Results were normalized across assays by dividing the raw absorbance by that plate's positive control result, and the background absorbance (defined as the difference in optical density reading between the negative control and the participant's preimmunization serum [i.e., day 8]) was subtracted from the day 15 and day 29 readings (Grant et al., 2008).

DTH testing. On day 29, participants received an intradermal injection of 10 μg KLH at the immunization site. The DTH response to intradermal KLH injection (an in vivo antigen-specific composite test of cell-mediated immunity) was measured 48 hours later by averaging the diameters of induration (in millimeters) along two perpendicular axes, as previously described (Jordan et al., 1987).

Effector T-cell assays. Effector T-lymphocyte populations (Th1, Th2, Th17) and regulatory T cells ($CD4^+CD25^+CD127^{lo}Foxp3^+$) were enumerated by flow cytometry. Briefly, 10^6 peripheral blood mononuclear cells were stimulated with Leukocyte Activation Cocktail with Golgi Plug (BD Biosciences, San Jose, CA) for 4 hours; then cells were surface stained, fixed, and permeabilized according to the manufacturer's protocol. For intracellular cytokine (IL-4, IL-17, IFN-γ) staining, cells were incubated on ice with antibodies for 20 minutes, washed, and acquired using the BD FACSCanto flow cytometer (BD Biosciences). For regulatory T cells, after surface staining, cells were incubated on ice for 30 minutes with Foxp3 antibodies. Data were then analyzed with FlowJo, version 8.7 (FlowJo, Ashland, OR). Antibodies to the following molecules were used for flow cytometry: CD3 FITC (BD Biosciences), CD4 PerCP (BD Biosciences), CD25 APC (BD Biosciences), CD127 FITC (eBioscience, San Diego, CA), Foxp3 PE-CF594 (BD Biosciences), IL-17 PE (BD Biosciences), IFN-γ APC (BD Biosciences), and IL-4 PE (Miltenyi Biotec, Bergisch Gladbach, Germany).

Statistical analysis

Cutaneous melanin density was estimated from an average of three reflectance spectrophotometer readings taken at wavelengths of 400 and 420 nm using the formula $MD_{400} = 100[0.035307 + 0.009974 (R_{420} - R_{400})]$ (Dwyer et al., 2002), where MD is the melanin density and R is reflectance. Skin casts were graded by two independent scorers (Cargill et al., 2013) (interrater agreement: weighted κ statistic = 0.55), with the average used in analyses.

For missing or clearly erroneous electronic dosimeter data, an interpolation of estimated personal UVR exposure was calculated using corresponding sun exposure diary and maximum cloud-adjusted UVR data (sourced from ground-based UVR monitors [Biometer Model 501; Solar Light Company, Glenside, PA] situated at the Australian National University, Canberra, and James Cook University, Townsville [Liley and Liley, 2010]).

To determine a participant's personal UVR exposure adjusted for the body surface area covered by clothing (ca-UVR) (see Supplementary Figure S1 online) for each hour that the electronic dosimeter was worn, we used the following formula:

$$ca - UVR = \sum_{i=1}^{12} (1 - BSAC_i) UVR_i$$

where

- i indexes the hours of the day, from 7 AM – 7 PM;

- $BSAC_i$ is the proportion of the body surface area covered by clothing for the hour *i*; determined by summation of clothing BSAC coefficients for different body regions for that hour (i.e., upper body + lower body + footwear + headwear + gloves) (theoretical range = 0–1) (Lee and Choi, 2009) (see Supplementary Table S7 online); and
- UVR_i is the UVR measurement from the electronic dosimeter (with interpolated values) for the hour *i*.

Agreement of DTH response between assessors was determined on a subset of participants (n = 22) and was found to be moderate to strong with a two-way mixed-method intraclass correlation coefficient of 0.63 (95% CI = 0.38–0.80).

We used mean and standard deviation or median and interquartile range, depending on whether the data were normally distributed or skewed, to summarize participant demographics, examination findings, and blood test results. To compare groups, we used the contingency χ^2 statistic or Fisher exact test for categorical data, two-tailed *t* tests for normally distributed continuous data, and nonparametric tests (e.g., the Wilcoxon signed-rank test) for non-normally distributed data. Sinusoidal functions for time of year were used to adjust for seasonal variation in immune outcome variables at each study site. We used multiple linear regression or ordinal logistic regression models to test associations between outcome and exposure variables, adjusting for potential confounders. The best-fitting regression model was identified by the highest R^2 value; *P* less than 0.05 was considered statistically significant. Data were analyzed with the statistical software package Stata, version 12 (StataCorp, College Station, TX).

Study approval

This study was approved by the Human Research Ethics Committees of the Australian National University (2009/628), James Cook University (C6), and the Australian Capital Territory Health Department and was registered with the Australian and New Zealand Clinical Trials Registry (ACTRN12610000234011). Written informed consent was obtained from all participants at study enrolment.

ORCID

Robyn Lucas: http://orcid.org/0000-0003-2736-3541

CONFLICT OF INTEREST

The authors state no conflict of interest.

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We dedicate this article to the memory of Tony McMichael, a pioneering and internationally renowned medical epidemiologist, mentor, and friend, who was instrumental in the development and conduct of the AusUVI Study.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2018.12.025.

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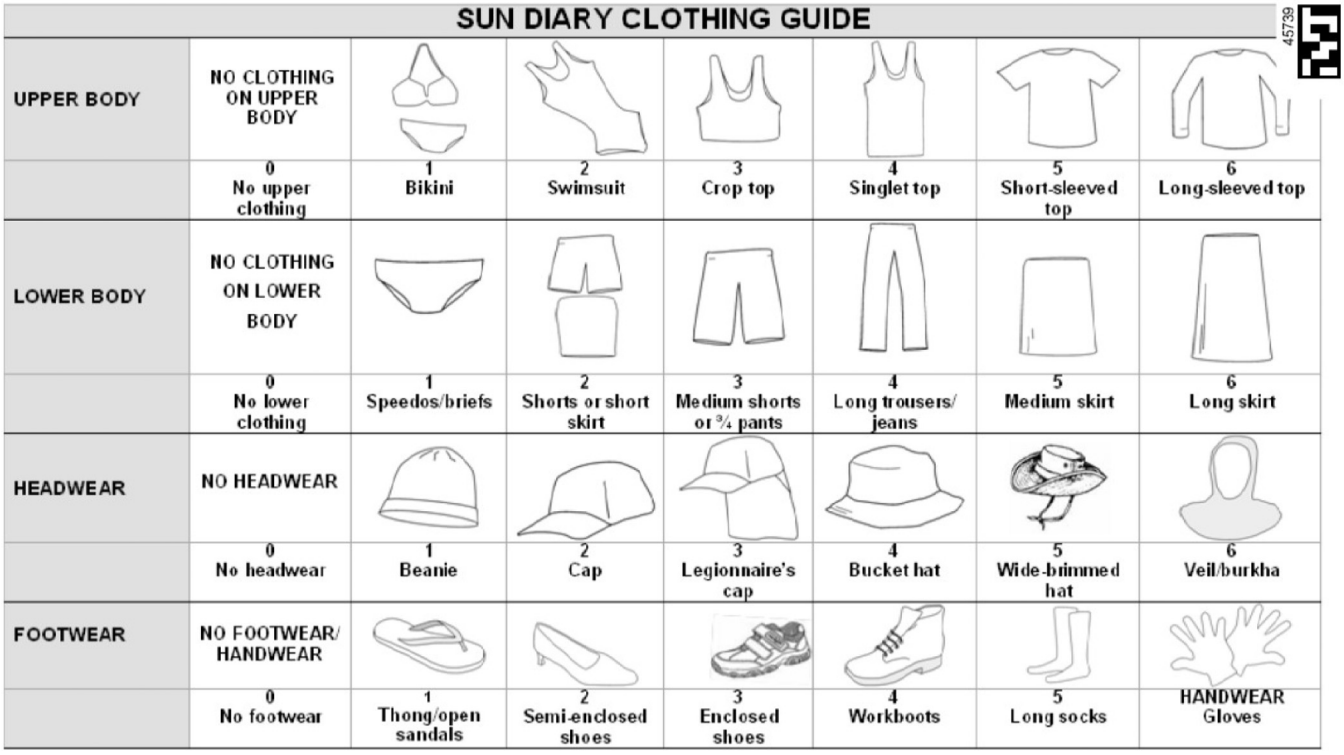

Supplementary Figure S1. Clothing options for sun and activity diary. Participants chose an item of clothing from the diagram that best represented clothing worn for the upper and lower body, head, hands, and feet. This information was recorded in the sun diary over the study period from days 3 to 12. Adapted from the clothing diary tool developed by the AusD Study Investigator Group for the AusD Study (Brodie et al., 2013).

Supplementary Table S1. Association between clothing-adjusted UVR exposure over 10 days and potential immunomodulating variables[1]

		Clothing-Adjusted UVR SED Range		
Potential Immune Modulators	**Subgroup (n)**	**Mean SED ± SD**	**SED Range**	***P*-Value[2]**
Overall	N = 211	2.1 ± 2.4	0.043–16.8	
Sex	Male (78)	2.2 ± 2.4	0.051–13.6	0.74
	Female (133)	2.1 ± 2.5	0.043–16.8	
Site	Canberra (106)	1.8 ± 2.2	0.043–13.6	**<0.001**
	Townsville (105)	2.5 ± 2.6	0.13–16.8	
Age in years	18–24 (77)	2.1 ± 2.1	0.051–11.1	Reference
	25–29 (67)	1.9 ± 1.9	0.043–9.5	0.47
	30–34 (30)	2.5 ± 3.5	0.074–16.8	0.89
	35–40 (37)	2.3 ± 3.0	0.094–13.6	0.89
Parental ethnicity	Northern European (147)	2.5 ± 2.6	0.043–16.8	**<0.001**
	Non–Northern European (64)	1.3 ± 1.6	0.051–10.1	
Indoor/outdoor worker status	Indoors (188)	2.1 ± 2.5	0.043–16.8	Reference
	Half indoors/half outdoors (19)	2.3 ± 1.3	0.11–5.10	0.15
	Outdoors (4)	5.3 ± 3.8	1.6–9.6	**0.042**
Smoking	Nonsmoker (199)	2.1 ± 2.4	0.043–16.8	0.22
	Current smoker (12)	3.1 ± 2.7	0.31–9.6	
Body mass index	Underweight (3)	1.1 ± 1.3	0.13–2.6	0.38
	Normal weight (138)	2.2 ± 2.4	0.051–16.8	Reference
	Overweight (56)	2.0 ± 2.3	0.043–9.5	0.37
	Obese (14)	2.3 ±3 .4	0.068–11.1	0.57
Psychological profile: POMS (TMD)	Below first quartile score (53)	2.8 ± 3.0	0.11–13.6	Reference
	Between 1st and 2nd quartiles (58)	1.5 ± 1.6	0.043–9.5	**0.005**
	Between 2nd and 3rd quartiles (43)	2.3 ± 2.3	0.084–9.6	0.57
	Above fourth quartile (54)	2.0 ± 2.6	0.051–16.8	0.056
Psychological profile: MHI (global score)	Below first quartile score (58)	1.9 ± 2.7	0.043–16.8	Reference
	Between 1st and 2nd quartiles (53)	2.0 ± 2.4	0.051–11.1	0.71
	Between 2nd and 3rd quartiles (49)	2.5 ± 2.0	0.084–9.5	0.061
	Above fourth quartile (48)	2.3 ± 2.7	0.11–13.6	0.27
Physical activity	Low activity (13)	2.0 ± 2.9	0.094–10.1	0.99
IPAQ category	Moderate activity (71)	1.5 ± 1.3	0.043–6.2	0.28
	High activity (126)	2.5 ± 2.8	0.051–16.8	Reference
Vitamin D status in nmol /L, baseline blood test, day 8	<25 (6)	0.23 ± 0.29	0.043–0.81	**<0.001**
	25–49.9 (32)	0.72 ± 0.63	0.068–2.5	**<0.001**
	50–74.9 (67)	1.9 ± 1.8	0.051–11.1	**0.018**
	>75 (106)	2.8 ± 2.9	0.11–16.8	Reference

Boldface indicates statistical significance at $P < 0.05$.

Abbreviations: IPAQ, International Physical Activity Questionnaire; MHI, Mental Health Inventory; POMS (TMD), Profile of Mood States (total mood disturbance); SD, standard deviation; SED, standard erythemal dose.

[1]N = 211 because six participants did not have cumulative UVR dosimeter data available over this interval.

[2]Linear regression models using log-transformed UVR exposure distributions and adjusted for season.

Supplementary Table S2. Association between potential immunomodulatory factors and DTH response

Potential Immune Modulators	Subgroup (n)	DTH Response		*P*-Value[1]
		Mean ± SD (mm)	Range (mm)	
Overall	N = 210[2]	7.0 ± 4.0	0–19	
Sex	Male (79)	6.5 ± 4.2	0–19	**0.038**
	Female (131)	7.4 ± 3.8	0–19	
Site	Canberra (102)	7.2 ± 4.2	0–19	0.86
	Townsville (108)	6.9 ± 3.7	0–19	
Season	Winter (75)	7.3 ± 4.4	0–19	Reference
	Spring (47)	6.8 ± 3.7	0–16	0.61
	Summer (37)	7.6 ± 4.0	0–19	0.65
	Autumn (51)	6.4 ± 3.5	0–17	0.33
Age (years)	18–24 (79)	6.7 ± 3.9	0–19	Reference
	25– 9 (66)	7.3 ± 3.8	0–16	0.39
	30–34 (29)	7.1 ± 4.3	0–19	0.69
	35–40 (36)	7.3 ± 4.3	0–17	0.61
Parental ethnicity	Northern European (both parents) (146)	7.2 ± 3.9	0–17	0.62
	Other (64)	7.0 ± 4.0	0–19	
Melanin density, left inner arm, lightest to darkest skin	Quartile 1 (52)	7.5 ± 4.1	0–17.5	Reference
	Quartile 2 (51)	6.2 ± 3.6	0–19	0.16
	Quartile 3 (50)	7.1 ± 4.0	0–19	0.71
	Quartile 4 (55)	7.3 ± 4.2	0–17	0.74
Melanin density, left upper cheek, lightest to darkest skin	Quartile 1 (54)	6.5 ± 3.3	0–13.5	Reference
	Quartile 2 (56)	7.4 ± 3.9	2–17.5	0.29
	Quartile 3 (44)	6.4 ± 4.5	0–17	0.59
	Quartile 4 (53)	8.0 ± 4.2	0–19	0.11
Smoking	Nonsmoker (199)	7.0 ± 4.0	0–19	0.74
	Current smoker (11)	7.1 ± 2.9	2–13	
Body mass index categories	Underweight (4)	6.5 ± 1.9	4–8.5	0.87
	Normal weight (136)	7.2 ± 3.9	0–19	Reference
	Overweight (55)	6.4 ± 4.4	0–17.5	0.69
	Obese (15)	7.9 ± 3.0	3.5–14	0.58
Psychological profile: POMS (TMD), lowest to highest mood disturbance	Quartile 1 (54)	7.0 ± 4.1	0–19	Reference
	Quartile 2 (56)	7.5 ± 4.1	0–17.5	0.46
	Quartile 3 (44)	6.8 ± 4.5	0–19	0.67
	Quartile 4 (53)	6.6 ± 3.3	0–17	0.81
Psychological profile: MHI (global score), lowest to highest well-being	Quartile 1 (57)	6.5 ± 3.8	0–19	Reference
	Quartile 2 (53)	7.9 ± 4.3	0–17.5	0.083
	Quartile 3 (47)	6.9 ± 4.0	0–16	0.65
	Quartile 4 (50)	6.6 ± 3.9	0–19	0.86
Physical activity, IPAQ category	Low activity (12)	6.1 ± 4.8	0–14	0.26
	Moderate activity (70)	7.3 ± 4.2	0–19	0.79
	High activity (128)	7.0 ± 3.8	0–19	Reference
25(OH)D level in nmol/L at baseline, day 8	<25 nmol /L (6)	7.2 ± 7.8	0–14	0.99
	25–49.9 nmol /L (31)	7.4 ± 4.6	0–19	0.71
	50–74.9 nmol/L (65)	7.4 ± 4.4	0–19	0.56
	>75 nmol /L (108)	6.7 ± 3.5	0–17	Reference

Boldface indicates statistical significance at $P < 0.05$.

Abbreviations: 25(OHD, 25-hydroxyvitamin D; DTH, delayed-type hypersensitivity; IPAQ, International Physical Activity Questionnaire; MHI, Mental Health Inventory; POMS (TMD), profile of mood states (total mood disturbance).

[1]Parametric testing or regression performed with square-root transformed distribution of DTH responses.

[2]Seven participants were excluded from this analysis: six who were not evaluated for DTH response and one with an extreme DTH response (29.5 mm).

Supplementary Table S3. Combinations of UVR exposure daily totals used for regression models[1]

Day Combinations Analyzed[2]	Study Days Electronic Dosimeter Worn									
	3	**4**	**5**	**6**	**7**	**8[3]**	**9**	**10**	**11**	**12**
A	3	4	5	6	7	8	9	10	11	12
B		4	5	6	7	8	9	10	11	
C			5	6	7	8	9	10		
D				6	7	8	9			
E					7	8				
F					7	8	9			
G					7	8	9	10		
H					7	8	9	10	11	
I				6	7					
J			5	6	7					
K		4	5	6	7					
L	3	4	5	6	7					
M						8	9			
N						8	9	10		
O						8	9	10	11	
P						8	9	10	11	12

[1]Only days with complete UVR data (i.e., no missing data) were used to generate the aggregated combinations of days. To make this aggregated-day data set as complete as possible, missing daily totals that remained after the interpolation process were replaced by a simplified imputation method (based on the average of the remaining days that had complete data, differentiated by weekday and weekend). This most complete data set comprised 10 days of daily UVR exposure totals for 216 participants.

[2]Aggregated daily UVR exposure totals (measured + interpolated + imputed).

[3]Day of immunization.

Supplementary Table S4. T-helper cell subsets (%) before and after immunization by UVR exposure group

T-Helper Subset[1]	Cell Surface Markers/Intracellular Cytokine or Transcription Factor	Preimmunization Median % (IQR)			Postimmunization Median % (IQR)		
		Low UVR (n = 27)	**High UVR (n = 28)**	***P*[2]**	**Low UVR (n = 24)**	**High UVR (n = 26)**	***P*[2]**
Th1	$CD3^+CD4^+$/IFN-γ	5.4 (2.3–10.8)	5.8 (3.1–10.1)	0.95	5.3 (1.7–7.7)	7.9 (3.1–11.4)	0.12
Th2	$CD3^+CD4^+$/IL-4	0.28 (0.12–0.53)	0.51 (0.19–0.80)	0.13	0.31 (0.00–0.51)	0.27 (0.11–0.67)	0.35
Th17	$CD3^+CD4^+$/IL-17	0.75 (0.25–1.49)	0.56 (0.18–0.81)	0.13	0.54 (0.15–1.13)	0.62 (0.27–1.25)	0.38
T_{reg}	$CD3^+CD4^+CD25^+CD127^{lo}Foxp3^+$	0.06 (0.04–0.07)	0.05 (0.04–0.07)	0.11	0.05 (0.04–0.08)	0.052 (0.04–0.06)	0.63

Abbreviations: IQR, interquartile range; Th, T helper; T_{reg}, T regulatory cell.

[1]T-helper subsets are expressed as a percentage of total T-helper cells ($CD3^+CD4^+$).

[2]Wilcoxon rank-sum test of low versus high UVR group (unpaired) samples. The *P*-values pertain to a comparison of helper T-cell subset concentration in the low versus high clothing-adjusted UVR exposure groups, before and after immunization. They are unpaired (and nonparametric in distribution).

Supplementary Table S5. Association between clothing-adjusted UVR exposure on individual study days and combinations of days and anti-KLH IgG_1 response (measured at day 21 after immunization)

	Univariate Linear Regression			Multivariable Linear Regression[1]		
Day	**n**	**β (95%CI), ×10^{-3}**	***P***	**n**	**β (95%CI), ×10^{-3}**	***P***
Individual study day						
3	204	113 (−164 to 391)	0.42	188	−53 (−384 to 278)	0.75
4	205	165 (−41 to 372)	0.12	187	197 (−33 to 426)	0.093
5	196	1.4 (−119 to 147)	0.83	178	42 (−104 to 188)	0.57
6	190	−0.72 (−152 to 150)	0.99	173	−46 (−211 to 119)	0.58
7	197	3.9 (−169 to 248)	0.71	180	−11 (−249 to 226)	0.93
8[2]	211	−6.8 (−308 to 171)	0.57	193	−145 (−413 to 122)	0.29
9	203	7.8 (−157 to 314)	0.51	185	94 (−158 to 347)	0.46
10	200	112 (−5.5 to 280)	0.19	182	36 (−146 to 219)	0.70
11	192	1.0 (−179 to 200)	0.92	177	6.9 (−205 to 219)	0.95
12	185	3.2 (−111 to 175)	0.66	168	38 (−110 to 186)	0.61
Study day combinations[3]						
A: days 3−12	200	3.5 (−3.5 to 105)	0.33	182	16 (−75 to 107)	0.72
B: days 4−11	200	3.3 (−42 to 108)	0.38	182	−19 (−77 to 115)	0.70
C: days 5−10	200	3.0 (−51 to 112)	0.46	182	8.7 (−93 to 111)	0.87
D: days 6−9	200	6.5 (−9.6 to 109)	0.90	182	−22 (−147 to 102)	0.72
E: days 7−8	200	−12 (−170 to 146)	0.88	182	−61 (−249 to 127)	0.52
F: days 7−9	200	7.4 (−122 to 137)	0.91	182	−12 (−170 to 146)	0.88
G: days 7−10	200	3.4 (−7.3 to 140)	0.53	182	0.35 (−130 to 130)	1.0
H: days 7−11	200	2.4 (−7.2 to 119)	0.63	182	−1.4 (−119 to 116)	0.98
I: days 6−7	200	2.1 (−101 to 143)	0.73	182	0.088 (−139 to 139)	1.0
J: days 5−7	200	3.1 (−6.2 to 125)	0.51	182	27 (−82 to 136)	0.63
K: days 4−7	200	4.5 (−4.4 to 135)	0.32	182	48 (−59 to 154)	0.38
L: days 3−7	200	4.7 (−4.1 to 134)	0.30	182	38 (−70 to 145)	0.49
M: days 8−9	200	−1.8 (−184 to 147)	0.83	182	−39 (−232 to 155)	0.69
N: days 8−10	200	2.6 (−9.4 to 145)	0.68	182	−19 (−160 to 122)	0.79
O: days 8−11	200	1.4 (−8.9 to 117)	0.79	182	−17 (−140 to 107)	0.79
P: days 8−12	200	1.9 (−7.0 to 108)	0.68	182	−6.2 (−111 to 98)	0.91

Abbreviations: CI, confidence interval; IgG, immunoglobulin G.

[1]Regression model included the following explanatory variables: age, sex, study site, season, BMI, physical activity (MET-minutes/week), psychological state (MHI-global index, POMS-TMD), skin reflectance at the inner arm and left cheek, smoking status, 25(OH)D level (on day of vaccination), life-course UVR exposure (left hand skin cast grade), parental ethnicity (both parents northern European vs. other). Natural logarithm-transformed data were used for individual days and combinations of days to normalize the highly negatively skewed UVR exposure data.

[2]Day of immunization.

[3]Using imputed UVR data for missing days.

Supplementary Table S6. Association between clothing-adjusted UVR exposure on individual study days and combinations of days and anti-KLH IgG_2 response (measured at day 21 after immunization)

	Univariate Linear Regression			Multiple Variable Linear Regression[1]		
Day	**n**	**β (95%CI), ×10^{-3}**	***P***	**n**	**β (95%CI), ×10^{-3}**	***P***
Individual study day						
3	200	−117 (−349 to 115)	0.32	185	− 46 (− 331 to 239)	0.75
4	202	23 (−143 to 19)	0.78	184	146 (− 41 to 333)	0.13
5	193	−41 (−145 to 61)	0.42	176	60 (− 57 to 176)	0.31
6	187	−30 (−147 to 86)	0.61	170	− 13 (− 117 to 142)	0.85
7	194	152 (−323 to 17)	0.79	177	−74 (− 270 to 123)	0.46
8[2]	208	−62 (−271 to 146)	0.55	190	− 41 (− 278 to 196)	0.73
9	200	−35 (−253 to 182)	0.75	182	88 (− 151 to 327)	0.47
10	197	73 (−68 to 214)	0.31	179	123 (− 28 to 274)	0.11
11	189	−16 (−170 to 138)	0.84	174	101 (− 73 to 276)	0.25
12	183	44 (−66 to 156)	0.43	166	90 (− 25 to 204)	0.12
Study day combinations[3]						
A: days 3−12	197	−8.4 (−65 to 48)	0.77	179	−43 (−29 to 117)	0.24
B: days 4−11	197	−19 (−79 to 42)	0.54	179	33 (−44 to 111)	0.40
C: days 5−10	197	−21 (−87 to 44)	0.52	179	33 (−50 to 116)	0.43
D: days 6−9	197	−44 (−13 to 41)	0.31	179	−14 (−117 to 90)	0.80
E: days 7−8	197	−100 (−232 to 31)	0.14	179	−74 (−234 to 85)	0.36
F: days 7−9	197	−74 (−185 to 37)	0.19	179	−33 (−169 to 103)	0.63
G: days 7−10	197	−27 (−116 to 63)	0.56	179	19 (−90 to 129)	0.73
H: days 7−11	197	−28 (−107 to 51)	0.49	179	23 (−74 to 120)	0.64
I: days 6−7	197	−58 (−156 to 40)	0.24	179	−24 (−136 to 89)	0.68
J: days 5−7	197	−35 (−110 to 40)	0.36	179	26 (−62 to 114)	0.57
K: days 4−7	197	−27 (−98 to 44)	0.45	179	35 (−50 to 122)	0.41
L: days 3−7	197	−31 (−101 to 39)	0.39	179	31 (−55 to 119)	0.48
M: days 8−9	197	−45 (−193 to 102)	0.54	179	−7.9 (−169 to 185)	0.93
N: days 8−10	197	47 (−97 to 107)	0.93	179	56 (−65 to 177)	0.36
O: days 8−11	197	−8.5 (−94 to 77)	0.84	179	47 (−56 to 150)	0.36
P: days 8−12	197	14 (−58 to 88)	0.69	179	61 (−24 to 146)	0.16

[1]Regression model included the following explanatory variables: age, sex, study site, season, BMI, physical activity (MET-minutes/week), psychological state (Mental Health Inventory-global index, profile of mood states (total mood disturbance), skin reflectance at the inner arm and left cheek, smoking status, 25(OH)D level (on day of vaccination), life-course UVR exposure (left hand skin cast grade), parental ethnicity (both parents northern European vs. other).
[2]Day of immunization.
[3]Using imputed UVR data for missing days.

Supplementary Table S7. Assigned body surface area covered coefficients for individual clothing types[1]

Clothing Type	**0**	**1**	**2**	**3**	**4**	**5**	**6**
Upper body	No clothing	Bikini top	Swimsuit top	Crop top	Singlet top	Short-sleeved top	Long-sleeved top
	0	0.041	0.354	0.128	0.207	0.254	0.358
Lower body	No clothing	Speedos/briefs	Shorts or short skirt	Medium shorts or ¾-length pants	Long trousers/ jeans	Medium skirt	Long skirt
	0	0.070	0.200	0.294	0.423	0.304	0.431
Headwear	No headwear	Beanie	Cap	Legionnaire's cap	Bucket hat	Wide-brimmed hat	Veil/burka
	0	0.049	0.049	0.061	0.054	0.071	0.065
Footwear	No footwear	Thongs/open sandals	Semi-enclosed shoes	Enclosed shoes	Work boots	Long socks	
	0	0.032	0.056	0.077	0.111	0.154	
Gloves	No gloves	Gloves					
	0	0.049					

[1]Adapted from Lee and Choi, 2009.