Electronic Supplementary Information (ESI)

Major inter-personal variation in the increase and maximal level of 25-hydroxy vitamin D induced by UVB

Pameli Datta[1], Peter Alshede Philipsen[1], Peter Olsen[1], Bibi Petersen[1], Peter Johansen[2], Niels Morling[2], Hans Christian Wulf[1]

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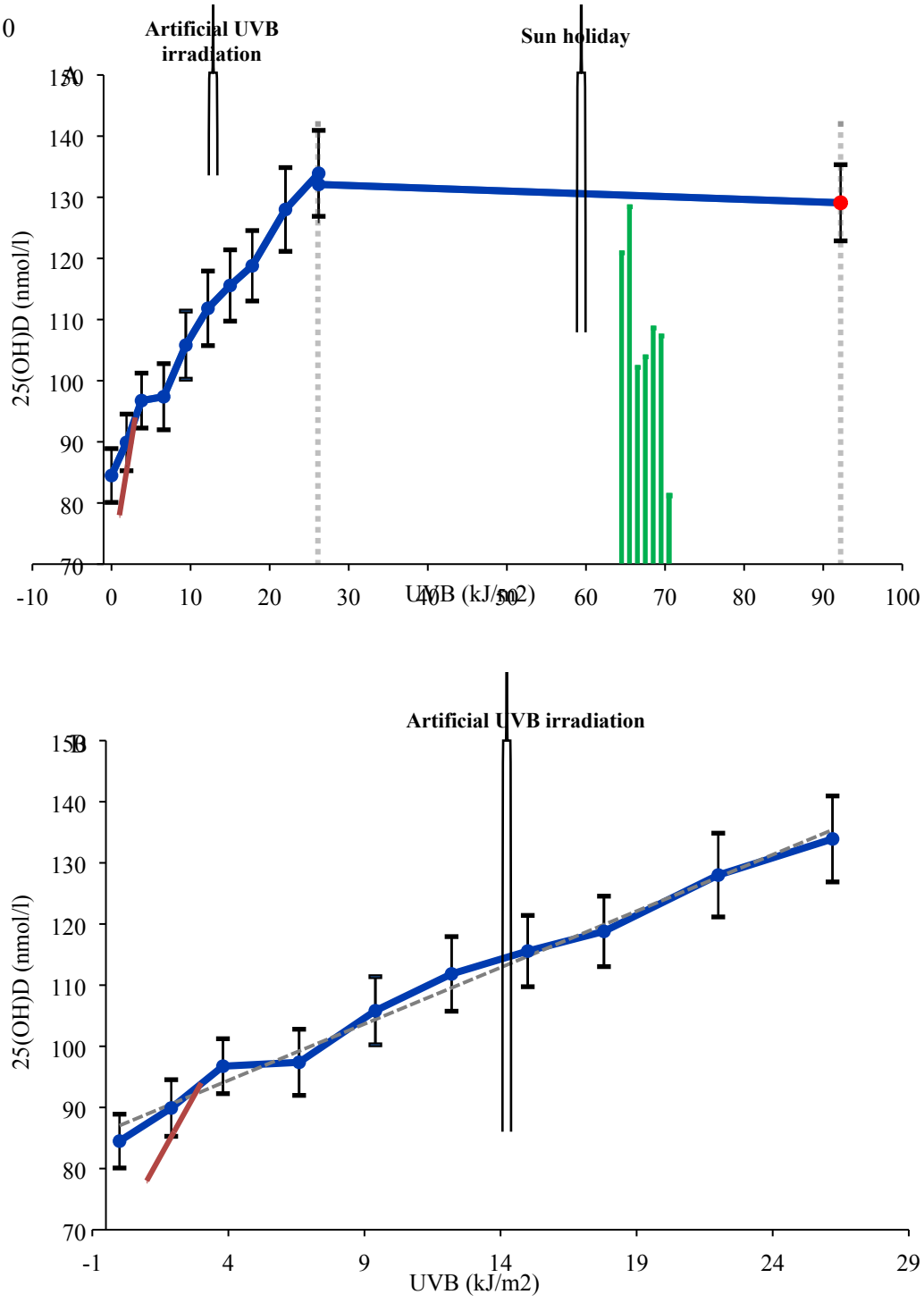

Supplementary Figure S1 Mean 25(OH)D levels and accumulated UVB exposure doses. The dark blue curve shows mean 25(OH)D with 1 standard error of the mean bars (left y-axis) over accumulated UVB dose in kJ/m^2 (x-axis). Dark blue dots indicate data from the 22 participants exposed with artificial and identical UVB doses. (A) Red dots indicate data from the 19 participants who were

exposed to solar UVB doses (mean value, not corrected for sun screen use and UVB exposed body areas) during one week of sun holiday. For these 19 participants, the mean 25(OH)D level (132 nmol l^{-1}) before sun holiday did not change significantly (paired t-test, two-tailed, P=0.317) after sun holiday (129 nmol l^{-1}). (B) During UVB treatment the 25(OH)D increase and accumulated UVB dose relation was best described by a linear model.

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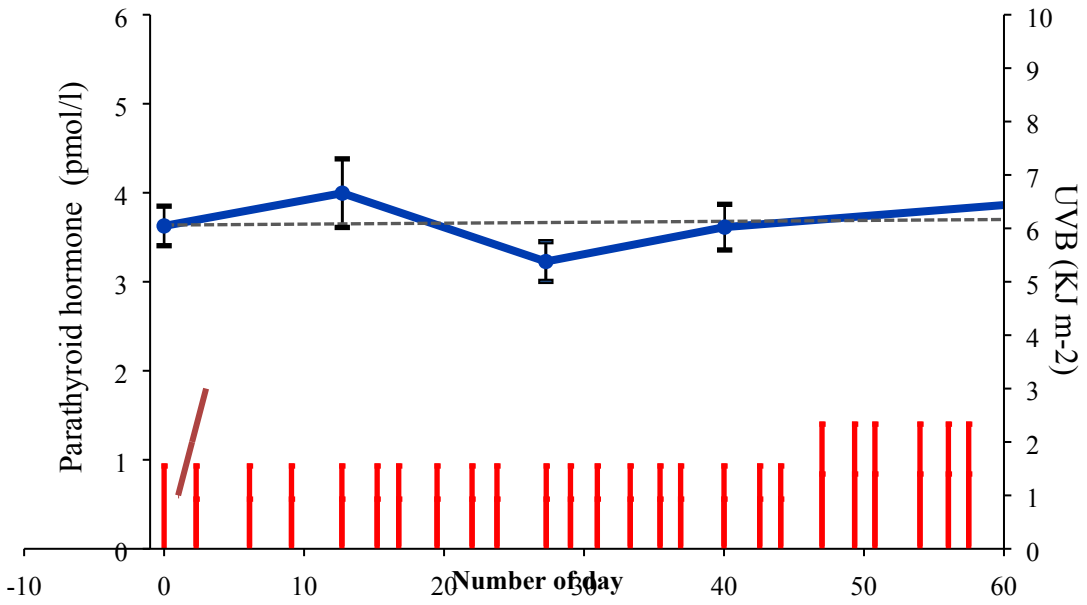

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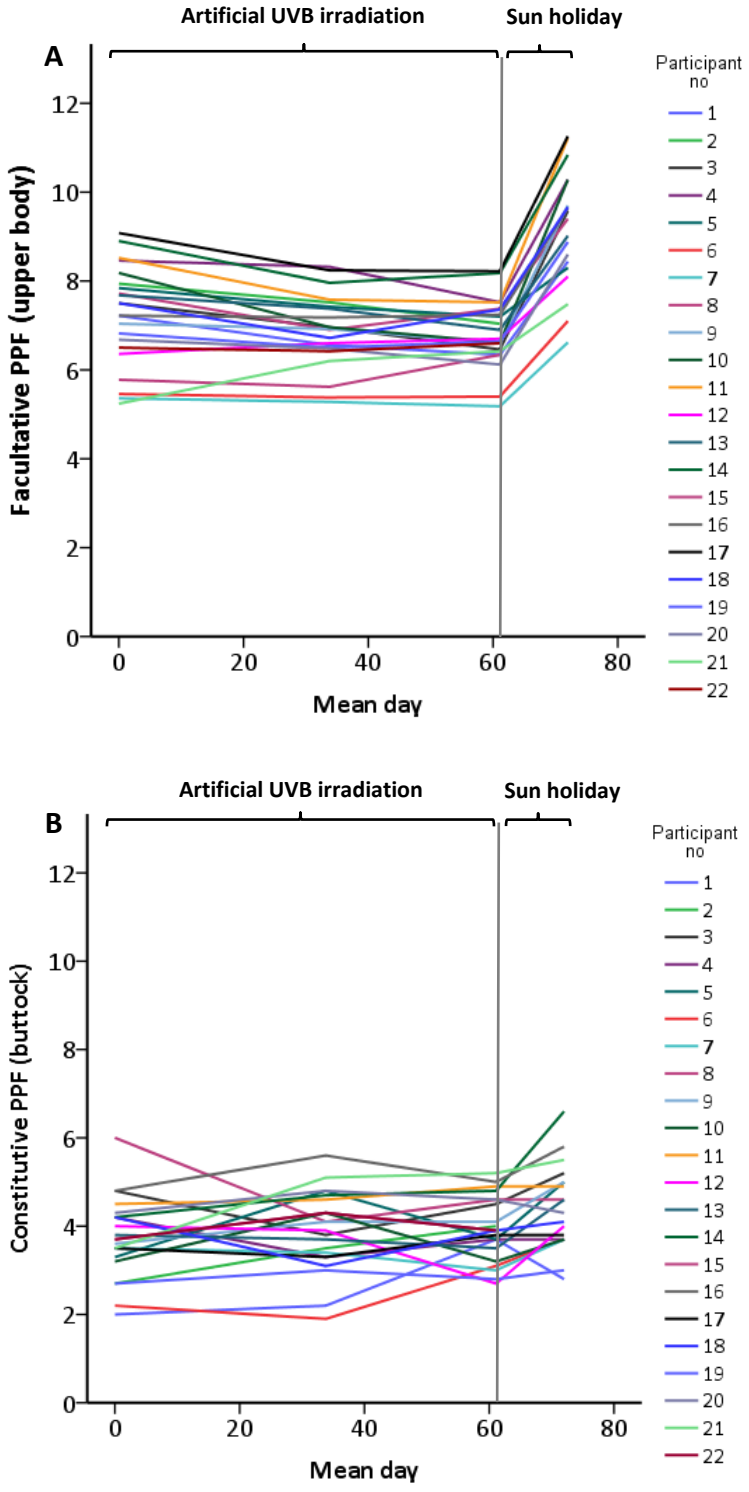
Supplementary Figure S2 Mean parathyroid hormone (PTH) levels during course of UVB treatment. Mean PTH levels (left y-axis) with 1 standard error of the mean over time in days (x-axis) are indicated by the blue curve. Artificial UVB doses (right y-axis) are indicated by red bars. Each bar represents a day with an UVB irradiation session. Mean 25(OH)D was 85 nmol l^{-1} baseline and 134 nmol l^{-1} at study-end. PTH did not change significantly during study period (linear regression analysis, P=0.323) as 25(OH)D levels were relatively high.



Supplementary Figure S3 PPF during study period. Constitutive (panel A) and facultative PPF (panel B) were measured in 22 participants at four time-points during the study period. PPF measurements on the first visit (Day 0) represent baseline values. PPF measurements at the two following visits show the change in PPF due to artificial UVB exposure. The last PPF measurement was taken after one week of sun holiday.

Table S1 PPF changes during study period. Pigmentation protective factor (PPF) is an objectively measurement of skin type, (range 1-25) with 1 being the least pigmented skin type. Constitutive pigmentation is defined at the PPF measurement of buttock. Facultative PPF is defined as a mean of PPF measurements on chest, midriff, back of shoulder, medial and lateral sides of arm. Three participants did not take part in sun holiday.

Mean PPF	Baseline (N=22)	After UVB treatment (N=22)	P value	Before sun holiday (N=19)	After sun holiday (N=19)	P value
Constitutive	3.7	3.9	0.270	3.9	4.4	4.2×10^{-3}
Facultative	7.2	6.8	5.0×10^{-3}	6.8	9.2	1.5×10^{-10}

DNA purification

The DNA was purified from extracted fluid from below the gel matrix of VACUETTE® Z Serum Sep Clot Activator tube using the QIAamp DNA Blood Mini Kit (Qiagen) as recommended by the manufacturer apart from dilution to 200 µl DNA was eluted in 50 µl AE buffer. A negative purification control was added using only the reagents from the DNA Blood Mini kit.

Vitamin D receptor SNP typing

The samples were typed for two Vitamin D receptor (*VDR*) SNPs,rs1544410 and rs2228570 using the iPLEX® Gold kit (Sequenom). The PCR contained 2 µL DNA, 0.5µL 10x buffer, 0.8µL 25mM $MgCl_2$, 0.1µL 25mM dNTP mix, 1.30µL 0.5µM primer (Table S1) mix (DNA Technology), 0.2µL 5U/µl HotStarTaq, and 1.1µL H_2O. The PCR was performed in a GeneAmp® PCR system 9700 thermal cycler (Thermo Fisher) with the following conditions: denaturation at 94˚C for 2 min followed by 45 cycles of 94˚C for 20 sec., 62˚C for 30 sec., 72˚C for 1 min., followed by 72˚C for 3 min. The PCR products were treated with Shrimp Alkaline Phosphatase (SAP) (Sequenom) in a GeneAmp® PCR system 9700 thermal cycler (Thermo Fisher) at 37˚C for 40 min. and 85˚C for 5 min.. The SBE reaction contained 8µl SAP treated PCR products and 2µl iPLEX® mix (Sequenom). The iPLEX® mix contained 0.2µL 10x iPLEX® buffer, 0.2µL iPLEX®-Termination mix, 0.94µL primer mix (DNA Technology), 0.04µL iPLEX®-enzyme, and 0.62µL H_2O. The SBE reaction (Table S6) was performed in a GeneAmp® PCR system 9700 thermal cycler (AB) with the following conditions: denaturation at 94˚C for 30 sec. followed by 40 cycles of 94˚C for 5 sec., 52˚C for 5 sec., 80˚C 5sec., 52˚C for 5 sec., 80˚C for 5 sec., 52˚C for 5 sec., 80˚C for 5 sec., 52˚C for 5 sec., 80˚C for 5 sec., 52˚C for 5 sec., 80˚C for 5 sec., followed by 72˚C for 3 min..

A total of 40µL of molecular grade water and ion exchange resin (Sequenom) was added to each sample. The samples were rotated for approximately 4h and kept in the refrigerator for up to 4 days before spotting. The samples were spotted using the RS1000 Nanospotter (Sequenom) and analysed on the MassARRAY® Analyzer 4 System (Sequenom) using the autorun settings. The samples were analysed with Typer Analyzer 4 (Sequenom) and were auto-clustered using a signal to noise ratio=5. Cluster plots were visually inspected and outliers were further investigated. All samples were investigated in duplicate. The genotypes were compared between duplicate spotting and typing results using a custom made script (PlateCompare) developed with the statistic software R (R core team, version 2.11.0, URL http://www.R-project.org). Further details on the SNP typing method are described elsewhere[1].

Supplementary Table S2. Primers for vitamin D receptor SNPs.

rs1544410	Primer1	ACGTTGGATGTAGATAAGCAGGGTTCCTGG[1]
	Primer2	ACGTTGGATGAGCCCAGTTCACGCAAGAG[1]
	SBE primer	CCACAGACAGGCCTGC
rs2228570	Primer1	ACGTTGGATGTGGCCTGCTTGCTGTTCTTA[1]
	Primer2	ACGTTGGATGAAGTCTCCAGGGTCAGGCA[1]
	SBE primer	GCTGGCCGCCATTGCCTCC

Abbreviations: SNP, single nucleotide polymorphism; SBE, single base extension; A, adenosine; C, cytosine; G, guanine; T, thymine.
[1]Tag sequences are underlined.

References

1 C.Pietroni, J.D.Andersen, P.Johansen, M.M.Andersen, S.Harder, R.Paulsen, C.Borsting, and N.Morling, The effect of gender on eye colour variation in European populations and an evaluation of the IrisPlex prediction model, *Forensic Sci.Int.Genet.*, 2014, **11**, 1-6.