Urinary levels of thymine dimer as a biomarker of exposure to ultraviolet radiation in humans during outdoor activities in the summer

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The incidence of skin cancer is rising rapidly in many countries, presumably due to increased leisure time exposure to solar ultraviolet radiation (UVR). UVR causes DNA lesions, such as the thymine dimer (T=T), which have been causatively linked to the development of skin cancer. T=T is clearly detectable in urine and may, thereby, be a potentially valuable biomarker of UVR exposure. The objective of this study was to evaluate the relationship between UVR exposure and urinary levels of T=T in a field study involving outdoor workers. Daily ambient and personal exposure of 52 beach lifeguards and agricultural workers to UVR were determined (employing 656 personal polysulphone dosimeters). In 22 of these subjects, daily urinary T=T levels (120 samples) were measured, the area of skin exposed calculated and associations assessed utilizing mixed statistical models. The average daily UVR dose was approximately 600 J/m2 (7.7 standard erythemal doses), i.e. about 20% of ambient UVR. T=T levels were correlated to UVR dose, increasing by about 6 fmol/μmol creatinine for each 100 J/m2 increase in dose (average of the three preceding days). This is the first demonstration of a relationship between occupational UVR exposure and urinary levels of a biomarker of DNA damage. On a population level, urinary levels of T=T can be used as a biomarker for UVR exposure in the field.

Introduction

Ultraviolet radiation (UVR) causes mutations in cellular DNA and exposure, mainly in the form of sunlight, is considered to be the predominant cause of the skin cancers, squamous cell carcinoma (SCC), basal cell carcinoma and cutaneous malignant melanoma (CMM) (1–3). A relationship between latitude and the incidence of especially SCC has been clearly demonstrated (1). Individuals suffering from xeroderma pigmentosum, who repair UVR-induced DNA damage poorly (4), run an exceptionally high risk of developing skin cancer (5). In the skin cancer tumour cells, apoptosis genes such as p53 contain mutations, specific for certain types of UVR-generated DNA lesions (6). Formation and repair of DNA damage are highly important in cancer induction by UVR.

In many countries, the incidences of SCC and CMM have risen rapidly during the last four decades (7). Part of this increase can be explained by prolonged life expectancy, but in Sweden for example, even the age-standardized incidences of SCC and CMM rose 3- and 4-fold, respectively, between 1970 and 2005 (8). Moreover, during the past decade, incidences increased by an average of 4% annually (9). This has been attributed to enhanced exposure to UVR in connection with leisure outdoor activities such as sunbathing.

Time spent outdoors, body orientation in relation to incoming sunlight and sun protection habits are key determinants of individual exposure to UVR. Moreover, the albedo effect (reflections from the surroundings) and local atmospheric conditions also influence personal exposure (10,11), which is generally measured by thermal or photon detectors (12). The commonly employed polysulphone film dosimeter (PSF) to estimate the individual UVR exposure from sunlight (13) is considered reliable and cost effective (14–16).

Biological monitoring of DNA damage due to UVR may provide a valuable complement to assessment with personal dosimeters. Ideally, such monitoring could take into account protection of certain areas of the skin by clothing or sunscreen, as well as individual sensitivity due to pigmentation and other factors. Cyclobutane pyrimidine dimers (CPDs), the most common lesions produced in human skin in situ by UVR (17,18), are removed to a substantial extent through enzymatic repair during the first 48 h following exposure (18). Thymine dimer (T=T), the most common CPD, is excreted in the urine (19,20) after repair. A 32P-postlabelling assay for analysis of T=T in human urine (19) has been validated using sunbed exposure (21). In that study and in a recent field study (22), it was shown that there is a correlation between UVR exposure and amount of urinary T=T, which is an important observation if using this product as a biomarker.

Materials and methods

Subjects and diaries

Forty-five lifeguards working at a beach (Tylosand) on the west coast of Sweden in the summer of 2006 were recruited through contact with the Swedish Life Saving Society. Some of them also participated in a pilot study in 2005 (U. Wester and A. Blomqvist, unpublished data) where it was discovered that the UVR doses received were substantial and this prompted the current more extensive investigation. In addition, we recruited seven farm workers engaged in the cultivation of sugar beets in open fields and greenhouses.

These 52 subjects (28 men and 24 women) had a mean age of 28 (range 18–54) years. The lifeguards wore a uniform the nature of which was adapted to the weather, as was the clothing of the farm workers. The lifeguards entered the study on different dates during June–August 2006, and 17 of them participated for at least 18 consecutive days. Those 17 individuals were selected for analysis of urinary T=T, but one was later excluded because of low creatinine levels. Six out of the seven farm workers participated during 12 days (June 27–July 9) and those were included in the study of urinary T=T. The different subgroups of the investigation are given in Table I.

Body surface area was calculated from height and weight, using a nomogram (23). Daily information concerning hours spent at work, tasks, clothing, sunscreen use, time spent outdoors and hours wearing dosimeters (see below) was obtained from the diaries kept by the participants. On the basis of these data, the area of skin exposed to sunlight during each working day was estimated. Skin type was self-reported, and skin type 1 was defined in the questionnaire as ‘burn easily, seldom tan’, skin type 2 as ‘often burn sometimes tan’ and skin type 3 as ‘sometimes burnt, always tan eventually’.
Table I. Description of the subgroups of the study

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Employed for</th>
<th>Number of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole-day dose measurements</td>
<td>Range of doses</td>
<td>52 (45 lifeguards and 7 farm workers)</td>
</tr>
<tr>
<td>Subjects with correctly filled questionnaires, exposed skin surface area known and work-shift dosimeters used</td>
<td>Range of personal doses (skin surface area adjusted) and comparisons of doses received during work and leisure</td>
<td>31 (24 lifeguards and 7 farm workers)</td>
</tr>
<tr>
<td>Subjects with an exposure period of at least 12 consecutive days</td>
<td>Urine analysis</td>
<td>22 (16 lifeguards and 6 farm workers)</td>
</tr>
</tbody>
</table>

(Underline: Informed consent was obtained from all participants, and the study design was approved by the Regional Ethical Review Board in Stockholm (DNR: 246/03 and 247/03).

UVR dosimeters

Each subject wore two PSF (thickness 40 µm), pinned to the top of one shoulder, one during the working shift (mean 5.9 h, up to 10 h) and one during the entire day (mean 11.5 h, up to 19.3 h). The dosimeters were replaced daily, and before and after use kept separate in light-proof envelopes. The absorbance was measured before and after exposure at 330 nm in a standard UV spectrophotometer. The erythema-weighted UVR doses thus obtained are expressed in joule per square metre, as suggested by Commission Internationale de l’Eclairage (CIE) (24). The accuracy of the PSF employed was confirmed previously (25).

Altogether, 730 whole-day measurements with personal dosimeters were collected, but due to loss of dosimeters, dates or identification codes, 74 of them had to be excluded. See Table I for the number of dosimeters of different subgroups. Appropriate questionnaire information regarding clothing was available for 348 of them. Dosimeter data for the work shifts were obtained for 256 days. In 76 cases, the work-shift dosimeter exhibited a higher value than the other and it was assumed that the dosimeters had been interchanged by the subjects, so that the highest value was taken to represent whole-day exposure.

Modelling UVR exposure

Estimates of UVR exposure for all days during the study period were obtained from the Swedish Meteorological and Hydrological Institute. These estimates are derived from a mesoscale model for solar radiation, referred to as STRANG, which involves several measures of radiation, including the CIE-weighted UVR, within an 11 × 11-km grid. These CIE-weighted UVR doses are expressed here as joule per square metre (26).

Table II. Exposure to UVR and urinary levels of thymine dimer (T=T) for the 22 subjects analysed

<table>
<thead>
<tr>
<th>Gender</th>
<th>Skin type</th>
<th>Mean UVR exposure (range) (/m²)</th>
<th>Mean UVR exposure (range) (/subject)</th>
<th>T=T level on day 1 (fmol/µmol creatinine)</th>
<th>Mean T=T level (range) (fmol/µmol creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>3</td>
<td>421 (23–1350)</td>
<td>201 (0.4–594)</td>
<td>110</td>
<td>201 (110–363)</td>
</tr>
<tr>
<td>M</td>
<td>3</td>
<td>451 (110–1265)</td>
<td>396 (99–1039)</td>
<td>115</td>
<td>223 (115–359)</td>
</tr>
<tr>
<td>F</td>
<td>3</td>
<td>559 (120–1210)</td>
<td>469 (154–1187)</td>
<td>194</td>
<td>201 (148–302)</td>
</tr>
<tr>
<td>M</td>
<td>2</td>
<td>281 (20–630)</td>
<td>175 (28–352)</td>
<td>71</td>
<td>90 (67–133)</td>
</tr>
<tr>
<td>F</td>
<td>3</td>
<td>685 (100–1490)</td>
<td>531 (81–1131)</td>
<td>115</td>
<td>148 (115–176)</td>
</tr>
<tr>
<td>M</td>
<td>3</td>
<td>774 (10–1440)</td>
<td>413 (5–980)</td>
<td>94</td>
<td>167 (86–262)</td>
</tr>
<tr>
<td>F</td>
<td>3</td>
<td>1277 (130–3090)</td>
<td>700 (266–1000)</td>
<td>127</td>
<td>172 (100–286)</td>
</tr>
<tr>
<td>M</td>
<td>3</td>
<td>390 (140–1080)</td>
<td>690 (116–1921)</td>
<td>146</td>
<td>226 (146–338)</td>
</tr>
<tr>
<td>F</td>
<td>3</td>
<td>652 (50–1400)</td>
<td>398 (28–990)</td>
<td>20</td>
<td>76 (20–117)</td>
</tr>
<tr>
<td>M</td>
<td>3</td>
<td>330 (70–570)</td>
<td>284 (214–300)</td>
<td>129</td>
<td>184 (129–268)</td>
</tr>
<tr>
<td>F</td>
<td>2</td>
<td>1016 (30–2430)</td>
<td>577 (0.5–1771)</td>
<td>74</td>
<td>203 (74–333)</td>
</tr>
<tr>
<td>M</td>
<td>3</td>
<td>455 (60–1330)</td>
<td>559 (70–1783)</td>
<td>217</td>
<td>275 (217–364)</td>
</tr>
<tr>
<td>F</td>
<td>3</td>
<td>602 (120–1040)</td>
<td>496 (54–1328)</td>
<td>104</td>
<td>127 (94–157)</td>
</tr>
<tr>
<td>M</td>
<td>3</td>
<td>281 (60–780)</td>
<td>393 (71–1207)</td>
<td>132</td>
<td>164 (110–210)</td>
</tr>
<tr>
<td>F</td>
<td>3</td>
<td>759 (10–3120)</td>
<td>537 (6–1867)</td>
<td>147</td>
<td>348 (147–519)</td>
</tr>
<tr>
<td>M</td>
<td>3</td>
<td>390 (40–1170)</td>
<td>319 (30–872)</td>
<td>–</td>
<td>319 (202–423)</td>
</tr>
<tr>
<td>F</td>
<td>2</td>
<td>1214 (130–2540)</td>
<td>1109 (354–1659)</td>
<td>102</td>
<td>184 (81–381)</td>
</tr>
<tr>
<td>F</td>
<td>3</td>
<td>530 (50–1760)</td>
<td>533 (1–1907)</td>
<td>213</td>
<td>143 (69–235)</td>
</tr>
<tr>
<td>F</td>
<td>2</td>
<td>1214 (250–3240)</td>
<td>1198 (4–3679)</td>
<td>162</td>
<td>164 (148–182)</td>
</tr>
<tr>
<td>F</td>
<td>2</td>
<td>591 (70–1502)</td>
<td>459 (2–1583)</td>
<td>65</td>
<td>58 (2–115)</td>
</tr>
<tr>
<td>F</td>
<td>3</td>
<td>689 (80–1650)</td>
<td>690 (1–2059)</td>
<td>80</td>
<td>207 (2–751)</td>
</tr>
<tr>
<td>F</td>
<td>2</td>
<td>692 (60–2240)</td>
<td>660 (40–1657)</td>
<td>179</td>
<td>277 (117–404)</td>
</tr>
<tr>
<td>Overall mean</td>
<td>598 (281–1277)</td>
<td>603 (175–1198)</td>
<td>124 (20–217)</td>
<td>189 (58–348)</td>
<td></td>
</tr>
</tbody>
</table>

*Based on whole-day dosimetry from the first day of work to 1 day prior to collection of the last urine sample analysed.

**Corrected for the area of skin exposed.

†Calculated from urine samples collected on days 1, 3, 5, 7, 10, 14 and 18 (lifeguards) or days 1, 3, 5, 8 and 12 (farm workers) after entering the study.

‡Sample missing.

§Farm workers; all other subjects were lifeguards.
One goal was to assess the relationship between exposure and response on a population level, i.e. between T=T levels on a given day (timel T=T timel creatinine) and dosimetry data concerning UVR exposure on previous days (J m², where m² is the area of skin exposed). Our hypothesis was that the T=T level would increase as UVR exposure increased. Different combinations of previous exposure were considered, e.g. the exposure 3 days ago, UVR(j−3), denoted uv_3. Since many data were missing, we could not simply use the sum of the exposure during the three previous days, but considered the mean exposure, [UVR(j−1)+UVR(j−2)+UVR(j−3)]/3, denoted uv_123. Other combinations employed were [UVR(j−2)+UVR(j−3)+UVR(j−4)]/3, denoted uv_234 and [UVR(j−2)+UVR(j−3)+UVR(j−4)+UVR(j−5)]/4, denoted uv_2345.

A previous study on the effect of UVR exposure on T=T levels involving a sunbed (21) concluded that these levels are maximal 3–4 days after a single 30-min exposure to UVR, with significant levels being detectable for several more days. For each subject in that investigation, the daily contribution to the cumulative 12-day increase in T=T was calculated resulting in mean contributions of 8, 17, 25, 19, 17, 6, 3, 2 and 2% (in total 99%) on days 1–9 after exposure, respectively. Based on these findings, we examined the following exposure variable, denoted uv_weather as well:

\[
\frac{8 \times \text{UVR}(j−1)+17 \times \text{UVR}(j−2)+25 \times \text{UVR}(j−3)+19 \times \text{UVR}(j−4)+17 \times \text{UVR}(j−5)+6 \times \text{UVR}(j−6)+3 \times \text{UVR}(j−7)+2 \times \text{UVR}(j−8)+2 \times \text{UVR}(j−9)}{99}.
\]

When measurements were missing, exposure was estimated from the data available, e.g. uv_123 was estimated as UVR(j−1)+UVR(j−3)/2 when UVR(j−2) was missing. We also considered exposure without adjustment for the area of skin exposed:

\[
\frac{\text{UVRraw}(j−1)+\text{UVRraw}(j−2)+\text{UVRraw}(j−3)}{3}, \text{denoted uvr}_{raw, 123}.
\]

The urinary level of T=T levels was determined at the beginning of the study and then at several time points during the period of each individual’s involvement in the study, while the UVR exposure was measured daily. With a single measurement per individual, an ordinary least squares regression model could have been used to estimate the relationship between UVR exposure and T=T level. However, since several measurements were taken for each subject, not all of the observations were independent and the analysis was, therefore, made using a mixed model in which T=T was assumed to be a linear function of the exposure and was applied as follows:

\[
T_{ij} = \alpha + a_i + b \times U_{ij} + s_i \times U_{ij} + e_{ij},
\]

where \(T_{ij}\) denotes the urinary level of T=T for subject \(i\) on day \(j\); \(U_{ij}\) denotes the exposure (one of the exposure variables uv_3, uv_123,...) for subject \(i\) on day \(j\); \(a_i\) and \(b\) are the stochastic intercept and slope for this same subject and \(e_{ij}\) is the stochastic error term for this same subject and day. The three stochastic terms \(a_i\), \(b\) and \(e_{ij}\) are assumed to be independent of one another and distributed normally with the variances \(\text{Var}(a) = \sigma_a^2\), \(\text{Var}(b) = \sigma_b^2\) and \(\text{Var}(e) = \sigma_e^2\), respectively.

The intercept \(a\) and slope \(b\) in the mixed model can be interpreted in a manner similar to an ordinary least squares regression model: \(b\) indicates the increase in T=T levels for each one unit increase in UVR exposure and \(a\) is the expected level of T=T during the summer for a zero UVR exposure during the previous couple of days (when \(U_{ij} = 0\), the expected level of T=T is \((\alpha + b \times 0)\)).

The mixed model employed here differs from an ordinary least squares regression model in that the components \(a_i\) and \(b\) are included, where \(a_i\) allows the intercepts and \(b\) allows the slope for the different subjects to vary. Ordinary least squares regression analysis involves only one component of variation, but here the variance could be divided into between-subject variance and within-subject variance.

Each UVR variable (uv_3, uv_123, etc.) was included individually into the mixed model and the fit assessed by comparing the variances (between-subject and within-subject) with those of an initial model without UVR exposure (\(T_{ij} = \alpha + a_i + e_{ij}\)). If the inclusion of the UVR exposure variable reduced either of the variances (in comparison with the initial model), this variable was considered a potential explanatory variable for the T=T level. This statistical analysis was conducted in SAS (version 9.2) using the MIXED procedure.

**Results**

**UVR exposure**

The different subgroups of the study are described in Table I. The mean whole-day exposure to UVR determined with the personal dosimeters was 607 J/m² (individual average 626; range 232–1228; calculated from 656 observations on 52 individuals). On the average, this value represented 19% (range 7–50%) of the ambient UVR exposure estimated from the 24-h STRANG and 23% (range 6–58%) of the STRANG estimation limited to the hours the dosimeters were worn, i.e. STRANG daytime (348 observations on 22 individuals). Three dosimeters indicated values higher than the global exposure, which remains unexplained.

**Figure 1A** illustrates the time trends of UVR exposure from June 26 to July 10 (after which very few subjects remained) for the 31 individuals who wore both whole-day and work-shift dosimeters and who filled in usable questionnaires (235 observations of each kind). The time courses for all three measures of exposure were similar. The whole-day exposure as indicated by personal dosimeter averaged 770 J/m² (based on individual averages), which corresponded to

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**Figure 1.** Mean ambient UVR exposure as calculated by STRANG or measured for the whole day or work shift by personal dosimeter. (A) As a function of date (June–July 2006) (based on 235 observations on 31 subjects). (B) As a function of days after entry into the study (based on the subjects whose urine samples were analysed for thymine dimer, days 1–12: 211 observations on 22 subjects; days 13–18: 67 observations on 16 subjects).
21% (range 7–38%) of the 24-h STRANG value, while the
shift dosimetry averaged 479 J/m², or 62% of the whole-
day dosimeter value (Figure 1A). The UVR exposures of the
subjects, whose urine samples were analysed, from the day
of entry into the study, independent of date, are shown in
Figure 1B. Twenty-two individuals are represented during
the first 12 days, but only the 16 lifeguards still participated
13 days after entry.

The correlation between the 24-h STRANG value and whole-
day dosimetry was reasonably strong, ($r_s = 0.54$, $n = 317$).
Whether adjustment was made using the STRANG value for
the time the dosimeter was worn (STRANG dtime), or by apply-
ing the dosimeter data and area of skin exposed, there were no
major effects on the correlations (0.51 and 0.62, respectively).

Multiple linear regression analysis using the mean whole-
day individual UVR exposure (adjusted for the area of skin
exposed) as the dependent variable and gender, skin type and
use of sunscreen as independent variables revealed that indi-
viduals with skin type 2 ($n = 13$) received a higher dose (615
J) than individuals with skin type 3 ($n = 34$; 428 J, $P = 0.013$).
Age, gender or use of sunscreen was not significantly associ-
ated with UVR exposure.

Urinary levels of T=T on the day that the subjects entered
the study ranged from 20 to 217 fmol/µmol creatinine
(mean 124) (Table II). They entered on different dates dur-
ing a 1-month period (June 22–July 24) and participated for
10–18 days. Figure 2 depicts the mean T=T levels on the day
of entry and thereafter. The average level for the lifeguards rose
significantly ($P = 0.002–0.012$) after entering the study and
basically remained at a higher level throughout the rest of their
participation. The smaller group of farm workers demonstrated
no clear elevation in levels of T=T until after 8 days.

There was no significant difference in T=T levels in relation-
ship to skin types 2 and 3 ($P = 0.84$) or gender ($P = 0.35$) and
no correlation to age ($r_s = 0.11, P = 0.62$). The individual T=T
levels are presented in Table II.

Association between UVR exposure and DNA damage
The association between urinary levels of T=T and UVR
exposure is exemplified using data from one subject who
entered the study on July 26 (Figure 3A). The aim was to find
a model to quantify this association, using a mixed model
in which the intercept and the slope can vary between the
subjects (‘individual parameters’), illustrated in Figure 3B
for the same subject. The T=T level was assumed to increase
linearly (slope $\beta$) with the UVR exposure. The results of the
mixed model analyses are presented in Table III. One subject
exerted a very large impact on the slope ($\beta$): the estimated $\beta$
was approximately 0.06 and 0.10 without and with this subject,
respectively.

As can be seen from the distribution of uv_123 displayed
in Figure 4, among the 22 subjects analyzed, the smallest
range was 280 J and the largest 3679 J (mean range 1121 J,
median range was 991). From the mixed model analysis, the
estimated effect of uv_123 (i.e. the mean daily UVR expo-
sure during the three previous days) on T=T levels was 0.06
(see Table III). This effect size can be illustrated by the fol-
lowing example: if, during the 3 days immediately preced-
ing measurement of urinary T=T, UVR exposure increases
from 200 to 1200 J/m² (which is realistic increase; see the
median ranges above), then the expected urinary level of T=T
would increase by 60 units $[0.06 \times (1200 - 200)]$. The varia-
tion in T=T between subjects and between days (within sub-
jects) can, in part, be attributed to different UVR exposure:
the variation (in T=T) is reduced around 10% by including
UVR exposure as an explanatory variable in the mixed model
(Table III, columns 5 and 7). However, there still remains a
large amount of unexplained variation, which is also illus-
trated in Figure 3B.

We also tested not to adjust for the area of skin exposed,
e.g. uvraw_123 (Table III). Since the slope did not vary
between subjects, a simpler model could be used: $T_{ij} = \alpha + \beta \times U_{ij} + a_i + e_{ij}$.

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**Fig. 2.** Mean urinary levels of thymine dimer (T=T) at different time points after entry into the study. Day 1 is the day of entry (regardless of date) and each bar
represents the mean value for 5–15 subjects (with the standard deviation indicated). For the lifeguards, the level of T=T on all subsequent days was significantly
higher than that of day 1 ($P = 0.012, 0.002, 0.005, 0.002$ and $0.004$, respectively; Wilcoxon matched pairs test).
UVR-induced DNA damage in a human field study

Fig. 3. The association between urinary levels of thymine dimer (T=T) and exposure to UVR is exemplified in the values for each subject, entering the study on July 26. UVR exposure is measured using personal dosimeters and the exposure (uv_123) is the mean of the whole-day dosimeters for the three previous days. (A) The co-variation between T=T and uv_123 over time. (B) T=T levels modelled as a linear function of UVR exposure (dotted line estimated using ordinary least squares regression).

Discussion

In the current evaluation, the 31 subjects for whom shift dosimetry was available received an average UVR dose of 487 J/m² during the work shift and 770 J/m² during the entire day, corresponding to 4.9 and 7.7 standard erythemal doses (SED) (29), respectively. In one Australian study, lifeguards received a mean dose of 9.2 SED between 10 AM and 4 PM (30). In a Spanish report, a small group of lifeguards received 11.4 SED between 10 AM and 4 PM (31). In a larger examination of 168 lifeguards at five different locations in the USA, median doses ranging from 0.9 to 4.6 SED were recorded between 10 AM and 4 PM (32). In these studies, the dosimeter was placed on the wrist.

Lower levels of exposure have been reported for other groups of outdoor workers. Gardeners in Denmark and in Ireland received median daily doses of 1.3 and 0.97 SED, respectively (dosimeter on the wrist) (33). For gardeners in Spain (dosimeter on the shoulder), a mean dose of 4.1 SED was reported for work shifts between 6 AM and 1 PM (31). Since the position of the dosimeter influences the proportion of ambient UVR measured (34), it is difficult to compare such investigations. However, the doses received in the present study must be considered to be relatively high.

If an individual is exposed to UVR during a single day or less, urinary levels of T=T will reach a maximum 3–4 days after exposure and thereafter fall (21). Both theoretical considerations and observations on experimental animals indicate that the level of binding of reactive chemicals to DNA (or other macromolecules), as well as the excretion of their degradation product(s) in the urine (in this case, the T=T dimer), reaches a steady state when the rates of formation and removal of the lesions are equal (assuming chronic administration and no inhibition or induction of the cellular processes involved) (35–37). Thus, an association between urinary levels of T=T and UVR exposure involves the assumption that the T=T levels attain a plateau at some time point. The kinetics in the sunbed study (21) suggest that if the daily UVR dose remains identical, such a steady state could be achieved after about 1 week. Figure 5 illustrates the expected kinetics of the levels of T=T in the urine following short-term and long-term exposure with an initial value of zero, as well as during long-term exposure with detectable background levels of urinary T=T due to previous UVR exposure (kinetics obtained from the sunbed study (22), also described in Statistical Analyses of the Materials and Methods).

The participants in the present investigation had been previously exposed to UVR (as confirmed by diaries), but the fact that urinary T=T levels for the lifeguards were lowest upon entering (Figure 2) indicates that the UVR doses received prior to were lower than those received during the study period. These levels increased and remained elevated for the rest of the period examined (Figure 2). In fact, these urinary levels of T=T are the highest noted in a field study to date. For the small group of farm workers, the increase was less obvious, maybe because they were all working during the same 2 weeks, making the impact of weather greater. Unfortunately the group of farm workers is small, but no additional exposed farm workers were available to us on this occasion.

Although the UVR dose received was influenced here by skin type, this variable exerted no significant effect on urinary T=T levels. One reason for this could be that there were too few subjects of skin type 2 (7) in the group whose T=T levels were analysed. Certain epidemiological studies have indicated that use of sunscreen could enhance the risk for CMM (38), perhaps due to a false sense of safety and thus longer exposure, application of inadequate amounts and/or lack of protection against certain wavelengths (39). In the present study, use of sunscreen on at least one occasion was not associated with higher UVR doses. Only six individuals in the entire study did not use sunscreen at all and this group was therefore too small to conclude if there are any effects of use of sunscreen on either dose or T=T level.

The relationship between dosimetry and T=T

The estimates of the slope β in the various models ranged from 0.05 to 0.07 (Table III). For example, the slope of 0.07 for uv_3 (Table III) corresponds to a seven-unit increase in urinary T=T for each 100-J increase in UVR exposure (adjusted for the area of skin exposed) 3 days earlier. In our previous sunbed study involving a single dose of UVR [mean 315 J/m² or 516
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The distribution of urinary T=T (mean 2 fmol/µmol creatinine) and the mean increase 3 days after exposure was 16 fmol/µmol creatinine per 100 J (unpublished data), or about twice that observed here. This is close enough, considering the fact that when placed on the shoulder, the dosimeter does not measure the average dose received by all exposed skin (34). Usually, certain parts of the body are only slightly exposed to UVR, due to shading from other body parts and/or a non-optimal angle in relation to the sun. Our lifeguards were standing up, walking on the beach or sitting in a watch tower (normally without any protective roof) and the farm workers were also in an upright position most of the time. Thus, there is an inevitable uncertainty concerning the actual dose of UVR received by the exposed skin.

Some of the differences between the present and the sunbed studies might reflect the fact that the subjects in the latter were not as tanned as the present participants since they entered the study in the spring or the autumn. UVB tanning (occurring to a small extent in natural sunlight but not in a sunbed) offers protection corresponding to a skin protection factor of 2 (40), which would then result in a smaller increase of T=T in the present study compared with the sunbed study. However, the use of sunscreen during exposure is probably a more important difference between the studies, since 88% of our outdoor workers used sunscreen, which in adequate amounts protects strongly against UVR-induced DNA damage in the skin (41).

In a sunbed, both sides of the body are subjected to equally intense UVR. Our present estimate of the exposure–response relationship (i.e. an increase in urinary T=T by five to seven units per 100-J UVR) reflects more realistic scenarios, such as at a beach or in a garden. Possibly, the degree of skin pigmentation should also be taken into account.

The present investigation provides an estimate of the expected absolute increase in urinary T=T resulting from a certain dose. Obviously, the relative increase depends on the initial level and will be much higher for individuals starting from a low level of UVR-induced DNA damage (Figure 5). This is also true with

![Fig. 4. The distribution of uv_123 (mean exposure to UVR during the three previous days) within and between the individuals analysed (n = 22).](https://academic.oup.com/mutage/article-abstract/28/3/249/1293260)

![Fig. 5. The kinetics of urinary levels of T=T estimated using a spreadsheet based on the daily contributions to the cumulative 12-day increase in T=T levels in a previous sunbed study with known UVR doses (21). The circles illustrate estimated T=T levels following a single exposure to 500-J UVR with no background. The squares and triangles show the expected levels in connection with continuous daily exposure to 500-J UVR, without and with an initial background level, respectively.](https://academic.oup.com/mutage/article-abstract/28/3/249/1293260)

![Table III. The intercepts and slopes resulting from the different mixed models, in which the levels of thymine dimer (T=T) were modelled as a function of exposure to UVR.](https://academic.oup.com/mutage/article-abstract/28/3/249/1293260)

<table>
<thead>
<tr>
<th>Exposure</th>
<th>α (95% CI)</th>
<th>Between-subject variance (σ²)</th>
<th>Reduction in between-subject variance, %a</th>
<th>Within-subject variance (σ²)</th>
<th>Reduction in within-subject variance, %a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial modelb</td>
<td>199 n.a.</td>
<td>5596 n.a.</td>
<td>5757 n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>UVR exposure, J/m² (adjusted for the area of skin exposed)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uv_3</td>
<td>181</td>
<td>0.07 (0.01-0.12)</td>
<td>5111, 0.003</td>
<td>4321, 0</td>
<td>0</td>
</tr>
<tr>
<td>uv_123</td>
<td>172</td>
<td>0.06 (0.01-0.10)</td>
<td>5127, 0.001</td>
<td>5200</td>
<td>10</td>
</tr>
<tr>
<td>uv_234</td>
<td>173</td>
<td>0.06 (0.006-0.11)</td>
<td>5103, 0.002</td>
<td>5034</td>
<td>13</td>
</tr>
<tr>
<td>uv_2345</td>
<td>175</td>
<td>0.05 (-0.001 to 0.11)</td>
<td>5102, 0.003</td>
<td>5032</td>
<td>13</td>
</tr>
<tr>
<td>uv_wei9</td>
<td>172</td>
<td>0.06 (0.003-0.12)</td>
<td>4972, 0.002</td>
<td>5190</td>
<td>10</td>
</tr>
<tr>
<td>UVR exposure (unadjusted)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uvraw_3</td>
<td>180</td>
<td>0.05 (0.02-0.08)</td>
<td>5875, 0</td>
<td>4843, 0</td>
<td>0</td>
</tr>
<tr>
<td>uvraw_123</td>
<td>167</td>
<td>0.05 (0.02-0.09)</td>
<td>5448, 0</td>
<td>5250</td>
<td>9</td>
</tr>
<tr>
<td>uvraw_234</td>
<td>160</td>
<td>0.06 (0.03-0.10)</td>
<td>5588, 0</td>
<td>4956</td>
<td>14</td>
</tr>
<tr>
<td>uvraw_2345</td>
<td>160</td>
<td>0.06 (0.03-0.10)</td>
<td>5506, 0</td>
<td>5076</td>
<td>12</td>
</tr>
<tr>
<td>uvraw_wei9</td>
<td>161</td>
<td>0.06 (0.02-0.10)</td>
<td>5371, 0</td>
<td>5237</td>
<td>9</td>
</tr>
</tbody>
</table>

The slope (β) estimates the expected increase in T=T level as the UVR exposure increases by one unit and the intercept (α) is the expected T=T level when UVR = 0. Different combinations of previous days’ UVR exposure were tested (uv_3, uv_123, …), as were adjusted and unadjusted dosimeter values. The results are based on 91 observations (whole-day personal dosimetry) and 21 subjects (one of the 22 subjects being excluded as an outlier). n.a.: not applicable.

aIn comparison with the initial model.
bMixed model without any exposure variable, Tij = α + a + eij.
cBased on 61 observations, cannot be compared with the other.

The intercepts and slopes resulting from the different mixed models, in which the levels of thymine dimer (T=T) were modelled as a function of exposure to UVR.

J (21), the subjects demonstrated a very low initial level of urinary T=T (mean 2 fmol/µmol creatinine) and the mean increase 3 days after exposure was 16 fmol/µmol creatinine per 100 J (unpublished data), or about twice that observed here. This is close enough, considering the fact that when placed on the shoulder, the dosimeter does not measure the average dose received by all exposed skin (34). Usually, certain parts of the body are only slightly exposed to UVR, due to shading from other body parts and/or a non-optimal angle in relation to the sun. Our lifeguards were standing up, walking on the beach or sitting in a watch tower (normally without any protective roof) and the farm workers were also in an upright position most of the time. Thus, there is an inevitable uncertainty concerning the actual dose of UVR received by the exposed skin.

Some of the differences between the present and the sunbed studies might reflect the fact that the subjects in the latter were not as tanned as the present participants since they entered the study in the spring or the autumn. UVB tanning (occurring to a small extent in natural sunlight but not in a sunbed) offers
respect to the proportion of the variance in T=T levels that can be explained by the UVR dose [about 9% (based on the model for uv_123, Table III) in the present case].

Here, we have assumed a linear relationship between UVR dose and urinary T=T, which seems reasonable on the basis of previous knowledge concerning the relationship between UV radiation and DNA damage (17,21). The mixed effects model applied here provides stochastic intercepts and slopes. This seems biologically plausible, since the average urinary levels of T=T in individuals receiving the same UVR dose should differ due to variations in repair and excretion of the lesion, skin type and other skin-related factors. Moreover, elevations in T=T per unit UVR should be influenced by pigmentation and skin sensitivity.

Since UVR received on a certain day affects urinary T=T for more than a week (21), a model should theoretically include UVR exposure many days prior to urine sampling. Such a model would, however, be quite complicated and, as shown in Table III, a simpler model, involving the mean (unweighted) UVR exposure for the three preceding days (uv_123) is almost as good as a (weighted) model based on the preceding 9 days (uv_weight9).

As shown in Table III, models that do not involve adjustment for the area of skin exposed perform well with respect to within-subject variance, while they are much less effective in elucidating between-subject variance. This makes sense, since subjects may differ with respect to the average amount of clothing they wear, whereas the clothing of a given individual may be similar from day to day.

In conclusion, the average daily UVR exposure observed here (7.7 SED) was among the highest recorded in connection with outdoor activities in northern Europe. Urinary levels of T=T, a specific indicator of UVR-induced DNA damage, were high and exhibited a significant correlation to the estimated UVR dose received during the 3 days prior to urine sampling. Thus, on a population level, urinary levels of T=T should be similar from day to day.

In summary, the average daily UVR exposure observed here (7.7 SED) was among the highest recorded in connection with outdoor activities in northern Europe. Urinary levels of T=T, a specific indicator of UVR-induced DNA damage, were high and exhibited a significant correlation to the estimated UVR dose received during the 3 days prior to urine sampling. Thus, on a population level, urinary levels of T=T should be similar from day to day.

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Conflict of interest statement: None declared.

References

UVR-induced DNA damage in a human field study