

Sunlight-induced DNA damage in human mononuclear cells

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ABSTRACT In this study of 301 blood samples from 21 subjects, we found markedly higher levels of DNA damage (nonpyrimidine dimer types) in the summer than in the winter detected by single-cell gel electrophoresis. The level of DNA damage was influenced by the average daily influx of sunlight < 50 days prior to blood sampling. The 3 and 6 day periods before sampling influenced DNA damage the most. The importance of sunlight was further emphasized by a positive association of the DNA damage level to the amount of time the subjects had spent in the sun over a 3 day period prior to the sampling. The effect of sunlight was comparable to the interindividual variation, indicating that sunlight exposure and the individual's background were the two most important determinants for the basal level of DNA damage. Influence of other lifestyle factors such as exercise, intake of foods, infections, and age could not be detected. Our results suggest that sunlight penetrates the outer layer of the human epidermis and damages the DNA in mononuclear cells circulating in the vessels of the skin.—Møller, P., Wallin, H., Holst, E., Knudsen, L. E. Sunlight-induced DNA damage in human mononuclear cells. *FASEB J.* 16, 45–53 (2002)

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IN AN EARLIER report we presented data indicating that the level of DNA damage in human mononuclear cells, detected by single-cell gel electrophoresis, was related to the intensity of sunlight to which the subjects were exposed (1). The alkaline version of the single-cell gel electrophoresis assay detects a mixture of DNA strand breaks and types of DNA damage that are converted to strand breaks in alkaline solutions. The types of photolesions detected by the alkaline single-cell gel electrophoresis assay is a mixture of 'nondimer' types of DNA damage, since cyclobutane pyrimidine dimers are not converted to strand breaks by high pH. In addition to DNA damage, we also detected a parallel variation of UVC-induced unscheduled DNA synthesis in the same samples (1). Large transitional studies by research groups in Italy (2, 3) and in the U.S. (Ray Tice, personal communication) have reported that the level of DNA damage measured by single-cell gel electrophoresis was higher in the sunnier months of the year. However, none of the groups related the effect to

exposure to sunlight. In our study we observed a higher level of DNA damage in a sunny period for each subject in the study group. We therefore reasoned that the seasonal variation might be contributed by a single factor, namely, exposure to sunlight. In Denmark, most of the population has a light complexion. During the summer, many Danes are devoted to sun tanning and in general spend much time outdoors wearing less clothing. Exposure to sunlight for people in Denmark therefore varies considerably during the year, partly due to the varying length of the day and partly due to the habits of the population.

We hypothesize that rays of sunlight pass through the outer layers of the skin and induce DNA damage in mononuclear cells that circulate in the blood vessels of the skin. In addition to visible light at wavelengths > 400 nm, the solar radiation that reaches the face of the earth consists of 95% UVA and 5% UVB light. Solar irradiation produces different types of DNA lesions such as cyclobutane pyrimidine dimers, pyrimidine-pyrimidone (6–4) photoproducts, and thymine glycols (4). Whereas UVB light mostly produces pyrimidine dimers by a direct action, the UVA rays generate oxidative DNA damage indirectly by generation of reactive oxygen species (5). It has been found that both UVA and UVB radiation penetrate the stratum corneum of the skin (6). Chadwick and co-workers demonstrated that irradiation of human buttock skin with UVB light generated a significant amount of DNA photodamage (thymine dimers and (6–4) photoproducts) in the basal layer of skin cells, with only a small declining gradient in the level of DNA photodamage between the epidermal cells and basal cells (7). It has been possible to detect cyclobutane pyrimidine dimers by T4 endonuclease V in deep layers of an artificial skin preparation exposed to UVB light (8). Experimental studies in mice show that UVB light penetrates the outer layer of the skin of mice as thymine dimers were detected in both epidermal and dermal cells after UVAB irradiation (9). Whole-body UVB exposure to humans results in a decreased proportion of circulating E rosette-forming lymphocytes and decreased incorporation of tritiated thymidine into the DNA of lympho-

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cytes after stimulation by phytohemagglutinin (10). In general, these data indicate the outer layer of epidermis is not an effective barrier against UVB light. Thus, it is likely that lymphocytes are exposed to sunlight while they circulate in the skin.

We tested the hypothesis that DNA damage in circulating mononuclear cells is increased by exposure to sunlight. To specifically address this question, we designed a study with repeated measurements of DNA damage in mononuclear cells from a series of healthy subjects. The influx of sunlight in the Copenhagen area was obtained from the Danish Institute of Meteorology. In questionnaires distributed at each sampling, the subjects answered questions concerning how much time they spent in the sun, food intake, exercise, and illnesses. In one or more reports, these factors have been proposed to influence the basal level of DNA damage (11). Our results indicate that DNA damage in circulating mononuclear cells may be increased by exposure to sunlight during the summer; the effect of other lifestyle factors and age were not detected in the study.

MATERIALS AND METHODS

Study design

The study was planned as a longitudinal study of repeated measurements in which each subject contributed measurements from his or her own time period as described by Diggle et al. (12). Our objective was to collect and analyze blood samples from each subject every third week throughout 1 year. However, neither interruptions in blood sampling nor unequal periods between the blood samples could be avoided in the study. The resulting set of data consisted of time series with different numbers of observations. The blood samples were collected from May 1997 to July 1998.

Subjects

Twenty-one healthy subjects were recruited among the personnel of the National Institute of Occupational Health: 7 were males with a mean age of 42 (range 35–59) and 14 were females with a mean age of 37 (range 26–51). One male and three females were smokers. Eighteen of the subjects were recruited at the beginning of the study. Three of these withdrew from the study during the study period (two because they left the Institute and one because of pregnancy). New volunteers of the same gender replaced them. All subjects worked and lived in the Copenhagen area, which is at 56° latitude N. The local Danish Medical Ethical Committee approved the study and subjects agreeing to participate submitted a written informed consent.

Blood sampling

The blood samples were taken on Tuesday mornings between 8 and 10 AM. Blood samples were taken by a finger prick. Thirty microliters of blood were drawn from the fingers of the subjects, mixed with 1 ml of RPMI 1640 media (Life Technologies, Gaithersburg, MD) with 10% fetal bovine serum (Life Technologies) in 1.5 ml Eppendorf tubes and kept on ice in a dark container until isolation of mononuclear cells.

Isolation of mononuclear cells

Mononuclear blood cells were isolated by applying 100 μ l Histopaque 1077 (Sigma, St. Louis, MO) under the RPMI 1640 medium, containing the blood, and centrifuging 3 min at 200 *g*. The mononuclear blood cells were retrieved, washed with 1 ml cold phosphate-buffered saline (pH 7.4), and centrifuged 3 min at 200 *g*. The cells were resuspended in a minimal volume of RPMI 1640 media, counted and diluted to a concentration of 10^6 cells/ml.

Control samples

The L1210 mouse lymphoma cell line was used for control samples to check for daily variation in the analyses and to estimate the uncertainty of the method. The control samples were dispensed in small aliquots and were cryopreserved. The DNA damage in these cells was within the range of that in the mononuclear cells. This assured that the person scoring the cells could not distinguish between a control sample and a test sample.

Determination of DNA damage

The single-cell gel electrophoresis was performed as described previously (1). A layer of agarose was prepared by transferring 95 μ l of high melting point agarose (Life Technologies) onto a fully frosted microscope slide (Richardson Supply Company, London, UK). It was allowed to solidify and a second agarose layer with 95 μ l of low melting point agarose (Life Technologies) with 10 μ l cell suspension (10^6 cell/ml) was added onto the first layer of agarose. The agarose-embedded cells were lysed in 1% Triton X-100, 2.5 mM NaCl, 0.1 mM Na₂EDTA, 10 mM Tris, pH 10. The slides were immersed into 0.3 M NaOH, 1 mM Na₂EDTA, pH > 13 at 4°C for 40 min and electrophoresed at 25 V and 300 mA for 20 min in the same solution. The slides were washed twice in 0.4 M Tris-HCl, pH 7.5 at 4°C and stained with 25 μ l of 0.6 μ M TOTOTM-1 iodide (Molecular Probes, Eugene, OR). From each blood sample, 100 randomly selected cells were analyzed under microscope by the Kinetics[®] image analyzing system (version 3.0). The tail of the image was defined as the area from the edge of the head to the end of the tail. The tail moment (μ m) of each cell was calculated by multiplying the tail length (μ m) by the fraction of DNA in the tail. The tail moment for the blood sample was the arithmetic mean of the tail moments for all the cells. The arithmetic mean was used because it represents the 'center of mass' of the distribution of tail moments. All blood samples and the mouse L1210 control samples were coded before analysis; the code was first broken at end of the study.

Questionnaire

The subjects filled out a questionnaire at the time each blood sample was taken and questions were answered concerning exposure to the sun, diet, physical activities, and ingested fried or smoked meat in the 3 day period prior to blood sampling. Intake of antioxidants was determined with questions about intake of fresh fruits, juice, and vitamin pills. The extent of physical exercise was determined by questions about the duration and intensity of exercise performed in the 3 days before the blood sample was taken. Subjects were specifically asked whether the exercise had been exhaustive. We defined exercise to be exhaustive when subjects reported that the level of exercise was too high to allow the exercise to continue. Illness was reported for the period of 7 days prior to the blood sampling. The subjects were asked to specify the

type of illness, the duration, and if they were ill at the time of blood sampling or how many days had passed since recovery. Exposure to sunlight was determined by asking the subjects how many hours they had been outside and how many hours they had spent in direct sunlight. The questionnaire also included questions about the use of sunscreens (frequency of application and factor number). As it turned out, sunscreens were used rarely and the factor number differed on the few occasions that subjects reported use of sunscreen. Thus, data on sunscreens were noninformative in the statistical analysis.

Measurement of sunlight

We obtained data on the intensity of sunlight by the Danish Meteorological Institute as described in our previous study (1). The sunlight was measured at two locations in the Copenhagen area. Since the two measurements were quite similar, we calculated the average of the two (indicated in this report as solar radiation). The solar radiation was determined as the daily power per square meter delivered by the sun (the unit is Wh m⁻² day⁻¹).

Statistics

In an attempt to quantify the influence of solar radiation and compare this to other data, we developed statistical models. The statistics were based on a linear regression model according to Diggle et al. (12). Denoting TM_{ij} as the tail moment (for the level of DNA damage) for individual no. i at time j, the general model is given as:

$$TM_{ij} = \mu + \tau_i + \beta_a + \gamma_a X_i + \sum_{k=1}^n \alpha_k Y_{kij} + \varepsilon_{ij} = \mu_{ij} + \varepsilon_{ij} \quad (1)$$

where:

μ is the common mean value for the whole population of individuals.

τ_i is the value specific for individual no. i.

β_a is the influence of gender (a=1 for male and a=2 for female).

γ_a is the age coefficient (a=1 for male and a=2 for female).

X_i is the age (in years) for individual no. i at the start of the study.

$\sum_{k=1}^n \alpha_k Y_{kij}$ is the sum of the time-dependent influence of n factors (e.g., sunlight)

where α_k is the coefficient for factor no. k and Y_{kij} is the variable for individual no. i at time j for factor no. k.

ε_{ij} is the residual for individual no. i at time j. The distribution of ε_{ij} is assumed to be normal with the variance σ^2 . The variance σ^2 is assumed to be the same for all individuals and is the variance within subjects. Therefore, σ should be a measure for the uncertainty of the method applied for measurement of the tail moment.

In the model given in equation 1, μ_{ij} is the expected value of the observation for individual no. i at time j. According to this model, the mean value for individual no. i without the influence from time is $\mu_i = \mu + \tau_i + \beta_a + \gamma_a X_i$, where β_a and $\gamma_a X_i$ are the correction terms due to gender and age, respectively, and τ_i is the correction term due to biological factors specific for individual no. i. The variance σ_i^2 of τ_i is often denoted as the variance between subjects.

Application of the linear regression model in the present experimental design

Application of the general linear regression model in this statistical analysis was set up to assess the influence from the

sunlight. It was unknown beforehand whether the influence of the sunlight was due to the received energy accumulated over a shorter or a longer period. We calculated an average solar radiation in 11 periods before the sampling. The 10 days immediately before the observation were divided into three periods of 3, 3, and 4 days. The remainder of the 50 days were divided into eight periods of 5 days each (denoted periods 1 through 11).

In the model given by equation 2, the individual no. i has at time j been exposed to sunlight from the 11 previous periods, where $\alpha_{1(r)}$ is the coefficient for the sun radiation in period no. r and $Y_{ij(r)}$ is the average sun radiation in period no. r, counted from time j.

$$TM_{ij} = \mu + \tau_i + \beta_a + \gamma_a X_i + \alpha_{1(1)} Y_{ij(1)} + \alpha_{1(2)} Y_{ij(2)} + \dots + \alpha_{1(11)} Y_{ij(11)} + \sum_{k=2}^n \alpha_k Y_{kij} + \varepsilon_{ij} \quad (2)$$

The model in equation 2 assumes that the influence of sunlight in one period is independent of the influence from the sunlight in other periods. Since there could be covariation between different periods, it was decided to consider two models for the influence of the sunlight. In the first model (the period model), the influence from each of the periods was estimated individually by using the following model:

$$TM_{ij} = \mu + \tau_i + \beta_a + \gamma_a X_i + \alpha_{1(r)} Y_{ij(r)} + \sum_{k=2}^n \alpha_k Y_{kij} + \varepsilon_{ij} \quad (3)$$

where $r = 1, 2, \dots, 11$.

In the second model (the split model), the influence of sunlight was estimated for the first period, then the influence from the periods 1 and 2 was estimated, and so on. This process was continued until the influence was estimated for all 11 periods. The split model is expressed in the following way:

$$TM_{ij} = \mu + \tau_i + \beta_a + \gamma_a X_i + \sum_{k=1}^r \alpha_{1(k)} Y_{ij(k)} + \sum_{k=2}^n \alpha_k Y_{kij} + \varepsilon_{ij} \quad (4)$$

where $r = 1, 2, \dots, 11$.

The statistical analysis was performed by the mixed procedure in the SAS system.

To test the stability of the single-cell gel electrophoresis assay, a one-sample ANOVA model was applied to test for equality between the expected values for the control samples in the four seasons. The hypothesis of equal variances for the control measurements in the four seasons was tested by Levene's test.

RESULTS

Determination of DNA damage

DNA damage was determined in mononuclear cells of 21 healthy subjects over the course of 14 months. In **Fig. 1**, it is seen that the measurements of the DNA damage were larger in the summer and that the DNA damage seemed to correlate with the level of solar radiation during the season of the year the blood sample was taken. To test this, we plotted the level of DNA damage against the average solar radiation in the days before the blood sampling. In **Fig. 2**, it is seen that there was reasonable correlation between the DNA

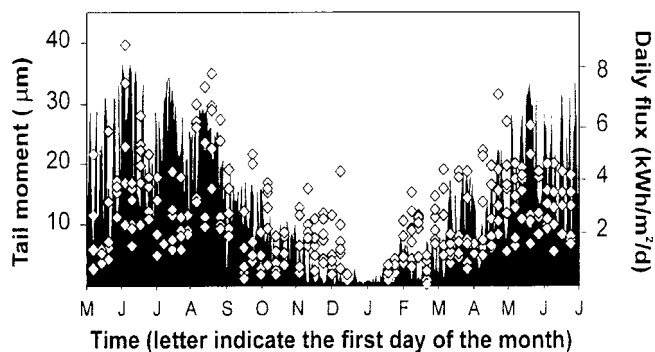


Figure 1. Distribution of DNA damage in mononuclear cell for person samples (diamonds) and the intensity of sunlight (daily flux) during the study period (shaded area).

damage and the solar radiation, with the correlation constants for the periods 1–3 and 1–10 days being slightly greater than those for the period 1–50 days.

Assay variation

To control for possible systematic variation in the assay procedure, frozen L1210 cell assay controls were in-

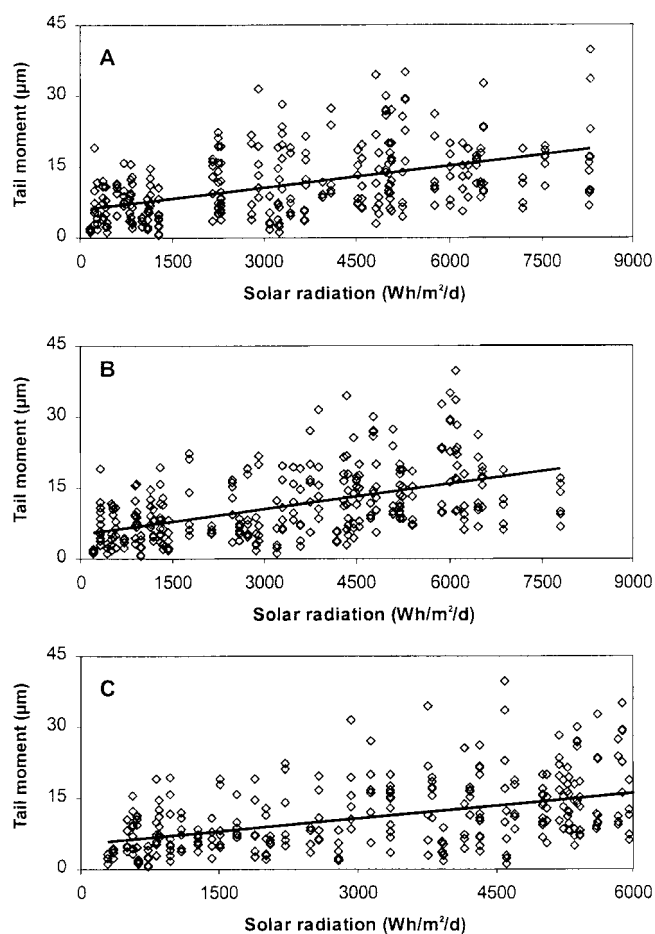


Figure 2. Correlation between the level of DNA damage and average solar radiation 3 (A, period 1), 10 (B, periods 1–3), and 50 days (C, periods 1–11) before sampling. Correlation coefficients were $r = 0.49$ ($P < 0.0001$), $r = 0.51$ ($P < 0.0001$), and $r = 0.47$ ($P < 0.0001$), respectively.

cluded in each analysis. Results from the statistical analysis of the control measurements indicated the measurements were stable during the period of the study. For statistical calculations, we stratified the data of the control samples into four seasons: winter (December–February), spring (March–May), summer (June–August), and autumn (September–November). **Table 1** outlines the data on control samples. The control samples did not display any difference among the four seasons ($P > 0.05$, one-sample ANOVA) and there was homogeneity of variation between seasons ($P = 0.18$, Levene’s test). The slightly higher level of DNA damage seen in the samples analyzed during the summer vs. winter was small compared with the effects seen in person samples. Furthermore, person samples still showed a marked seasonal variation if they were normalized against the control samples on the same day. The assay variation in our study was $6.9 \mu\text{m}$, and this is comparable to the variation described in other bio-monitoring studies using the single-cell gel electrophoresis assay (13, 14).

Test of the statistical model

For analyzing the influence of different factors on DNA damage, we developed the statistical models given in equations 3 and 4. The performance of the models can be evaluated from the calculated residuals. The results of the statistical analysis using the period model and split model are shown in **Table 2** and **Table 3**, respectively. The variation of the residuals did not differ significantly from the assay variation. This indicates that no factor with a significant influence on the level of DNA damage in the model defined in equation 1 has been omitted. There were only six outlying residuals. For both models, it was not reasonable to reject a hypothesis of normal distribution of the residuals (Anderson-Darling test).

Effect of sunlight

For statistical analysis of the influence of the solar radiation on the level of DNA damage, we used the period model (equation 3; Table 2) and the split model (equation 4; Table 3). In both models, the DNA damage was statistically significantly influenced by the

TABLE 1. Statistical analysis of the control measurements^a

Season	N	Mean (μm)	SD (μm)
Winter (Dec–Feb)	9	14.9	9.6
Spring (Mar–May)	17	17.9	6.5
Summer (Jun–Aug)	17	17.7	6.4
Autumn (Sep–Nov)	11	13.3	4.5
Whole study period	54	16.4	6.9

^a There was no difference on the means ($P > 0.05$, one-sample ANOVA) or in the variation ($P = 0.18$, Levene’s test) among different seasons.

TABLE 2. Regression analysis using the period regression model^a

Period (r) (days)	Intercept (μ)	Sun parameter ($\alpha_{1(r)} \cdot 10^{-3}$)	Gender (β_1) ^b	Sun hours (α_2)	Interindividual variation (σ_1^2)	Residual variation (σ^2)
1 (0–3)	5.87***	1.24***	-2.39*	0.19*	3.72	35.42
2 (3–6)	6.17***	1.18***	-2.36*	0.22*	3.20	36.74
3 (6–10)	5.62***	1.23***	-2.41*	0.23**	3.48	35.58
4 (10–15)	5.97***	1.11***	-2.41*	0.27**	2.67	37.37
5 (15–20)	5.70***	1.27***	-2.19*	0.23**	2.68	36.25
6 (20–25)	6.16***	0.93***	-2.34*	0.30**	2.83	38.17
7 (25–30)	6.53***	0.81***	-2.45*	0.32**	2.63	39.25
8 (30–35)	6.44***	0.99***	-2.70*	0.29**	2.94	38.42
9 (35–40)	6.49***	0.83***	-2.29*	0.32**	2.84	38.84
10 (40–45)	6.99***	0.55***	-2.55*	0.36**	2.84	40.11
11 (45–50)	7.07***	0.47***	-2.45*	0.38***	2.73	40.47

^a See equation 3 for the regression model. ^b Gender: coefficient for men when the coefficient for woman is zero. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

average daily solar radiation in different periods before the blood sampling. The influence of the solar radiation was strongest in the last two and fifth periods, but influence could be traced to all 11 periods (<50 days before the sampling). Significant solar radiation-related contributions to DNA damage were detected for the periods of 0–6, 15–20, 20–25, and 30–35 days in the split model. However, because the periods of 6–15 and 35–50 days did not contribute significantly, the statistically significant contributions in later periods may be a coincidence. In both models the greatest influence was from the period closest to the measurement. It was seen from the split model that incorporation of more periods in the model did not reduce the residual variance significantly. A comparison of the coefficients for the influence of the solar radiation in

the split model indicated interaction between the periods. The high value of the sun parameter in period five could be because the blood sampling usually was in 3 wk intervals. This is probably because the solar radiation in any two consecutive weeks is correlated. Our material is probably too small to identify all interactions in the model.

Sun hours

At each blood sampling, the subjects reported how much time they had spent in the sun during the last 3 days (sun hours). In both models, sun hours influenced level of DNA damage in mononuclear cells. In the period model, there was a statistically significant effect of the sun hours

TABLE 3. Regression analysis using a split regression model^a

Estimates of significant variables using the split regression model					
Period (days)	Intercept (μ)	Gender ^b (β_1)	Sun hours (α_2)	Interindividual variation (σ_1^2)	Residual (σ^2)
1	5.87***	-2.39*	0.19*	3.72	35.41
1–2	5.62***	-2.35*	0.16*	3.69	35.06
1–3	5.47***	-2.37*	0.17*	3.72	34.98
1–4	5.35***	-2.37*	0.16*	3.57	35.05
1–5	5.17***	-2.21*	0.14*	3.49	34.55

Estimates of the sun parameter using the split regression model

Period	Estimate (10^{-3})				
	$\alpha_{1(1)}$	$\alpha_{1(2)}$	$\alpha_{1(3)}$	$\alpha_{1(4)}$	$\alpha_{1(5)}$
1	1.24***				
1–2	0.91***	0.54**			
1–3	0.71***	0.23**	0.50		
1–4	0.74***	0.11**	0.35	0.31	
1–5	0.69***	0.16**	0.24	-0.32	0.85**

^a See equation 4 for the regression model. The results are only indicated for the first five periods: period 1: 0–3 days, period 2: 3–6 days, period 3: 6–10 days, period 4: 10–15 days, period 5: 15–20 days. ^b Gender: coefficient for men when the coefficient for women is zero. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

(Table 2). A contribution of the sun hours was seen in the first 5 periods of the split model, whereas inclusion of 11 periods produced statistically nonsignificant effects of sun hours in periods 6–11 (data not shown).

Interindividual variation and effect of gender

As can be seen in **Fig. 3A**, the means of DNA damage varied among the subjects. In the statistical models, interindividual variation was pronounced in both the period (equation 3) and the split model (equation 4). The two models provided similar estimates of the interindividual variation. In the period model, the variation of the residuals increased with the length of the period (Table 2), whereas the split model provided a stable interindividual variation and residual value. This suggests that the split model provided a more accurate estimate of the interindividual variation. As an example, within the first 3 days the mean tail moments

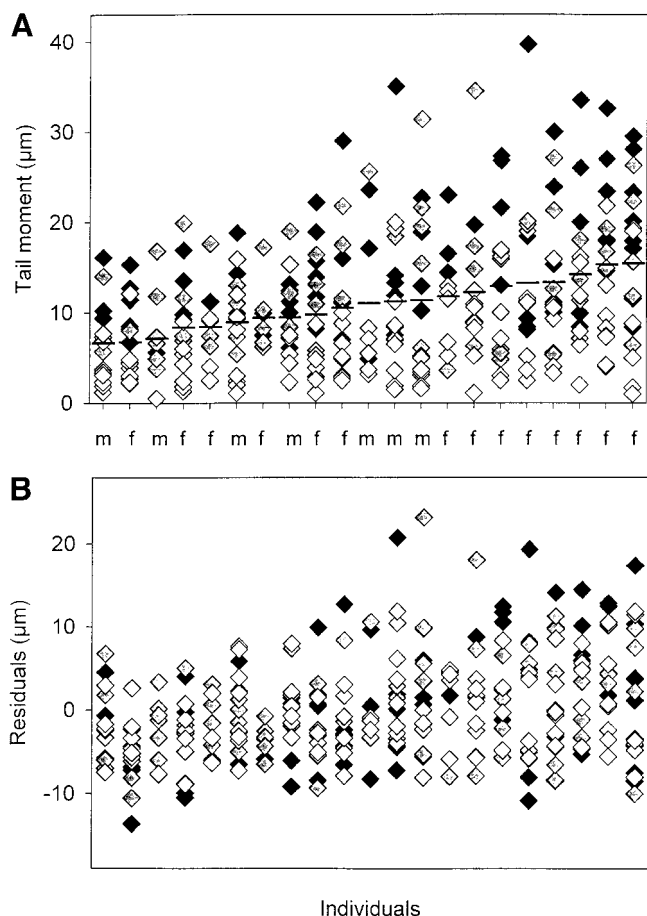


Figure 3. Distribution of DNA damage and residual values for males (m) and females (f), ranked by the means (—) of the tail moments of each individual. Blood samples taken in winter are indicated by open diamonds, spring by dark gray, summer by black, and autumn by light gray symbols (see ‘Assay variation’ in the Results section for definition of the seasons). Upper panel: Distribution of original measurements for each subject. Lower panel: Distribution of residual values for each subject based on calculations using the period model (1–3 days) described in Materials and Methods.

for all the subjects were $8.4 \mu\text{m}$, with a range of $10.2-5.9 = 4.3 (\mu\text{m})$. Thus, there was approximately a twofold difference between the subject having the lowest and highest level of DNA damage. The SD between the subjects was $1.9 \mu\text{m}$. Compared with the interindividual variation, the effect of gender was less pronounced, with women having a mean tail moment and SD of $5.9 \pm 1.2 (\mu\text{m})$ and men having $3.5 \pm 0.8 (\mu\text{m})$. Thus, the range was $5.9-3.5 = 2.4 (\mu\text{m})$, with women having $\sim 60\%$ more DNA damage than the men.

Intraindividual variation

An inspection of Fig. 3 clearly indicates a strong intraindividual variation, which in Fig. 3A is obscured by the seasonal variation. In Fig. 3B, each measurement has been corrected using the period model; the intraindividual variation was not decreased by this correction. The SD of the intraindividual variation was $6.0 \mu\text{m}$. This is similar to the assay variation, suggesting that the major contribution to the intraindividual variation is in fact assay variation. It also singles out the variation within subjects to be the type of variation that contributes the most to the overall variation. Previous estimations have indicated that the intraindividual variation varied more than the interindividual variation (13, 15).

Influence from other sources

We did not detect a significant influence from intake of smoked meat, fried meat, vitamin pills, fresh fruits, or coffee on the basal level of DNA damage. Being ill at the time of blood sampling or during the 7 days before blood sampling (in most instances, with the common cold or influenza) was not significantly related to DNA damage. However, only a few subjects had been ill during the study. No effect of exercise during a 3 day period prior to the blood sampling was detected whether the exercise was exhaustive or nonexhaustive. An effect of age could not be detected.

It should be emphasized that the statistical model we used does not include tests for interactions between factors. The existence of interaction between some of the factors cannot be ruled out. Yet since the assay variation is similar to the intraindividual variation, this indicates that statistically significant effects of interactions does not contribute to the level of DNA damage in this study.

DISCUSSION

We have previously suggested that the level of DNA damage in mononuclear cells in humans is affected by exposure to sunlight (1). In this paper, we present evidence that DNA damage in human mononuclear cells varies over the year and that higher levels of damage can be detected in the sunnier months of the year. The effect was detected in mononuclear cells

sampled over a period of > 1 year and was seen in both summer periods. A statistical analysis that included several other exposures and personal data fitted well to a dose-effect relationship between solar radiation and DNA damage. It corroborated that the average daily solar radiation in the periods directly before taking a blood sample had a strong influence. The strongest influence was in the previous 3 or 6 days; probably because the solar radiation in two short periods is strongly correlated, we cannot define which period is more important for causing DNA damage. Further evidence for an effect of solar radiation on DNA damage is the influence of how many hours the subjects had spent directly in the sun (sun hours in Tables 2 and 3). This also indicates that the exposure in the days preceding blood sampling is the most important. Besides influence of solar radiation, variation between subjects had a strong influence in the models, and this variation was comparable to the variation between a dark winter day and a sunny summer day. An influence of gender was detected, with women having more DNA damage than men.

These data indicate that UV radiation in sunlight is capable of inducing DNA changes in circulating mononuclear cells as they pass through the outer layer of the skin. In Denmark, there is ~7 h daylight on the shortest day in the winter whereas the daylight is 17.5 h from sunrise to sunset on the longest day of the summer. The variation in the length of the day is accompanied by overt temperature differences from below 0°C in the winter and occasionally to above 30°C in the summer. An effect of the seasonal variation in sunlight should therefore be more obvious at this latitude than at lower ones.

Other research groups have reported that indicators of DNA damage and mutations are higher in the summer than the winter. It has been reported that the variation in UV-induced DNA repair synthesis was greater and UV tolerance was lower in the summer in lymphocytes repeatedly sampled over 2 years (16). From a retrospective study of 308 subjects, Bentham et al. reported that blood samples obtained during the summer had a higher frequency of mutations in the *hprt* gene of T cells and more translocations at the *bcl-2* gene in B cells (17). A solar UVB light index could be correlated to the mutation or translocation frequency.

Other data suggest a direct effect in mononuclear cells of sun tanning. A greater amount of DNA strand breaks in lymphocytes was detected in Danes after 1 wk of sunbathing vacation in the Canary Islands (18). There is also evidence that UV exposure after tanning under tanning lamps suppresses DNA repair activity in lymphocytes (19).

The greatest limitation of this study is that the type of DNA damage is not UV specific. The single-cell gel electrophoresis assay as used in this experiment detects DNA strand breaks. These may arise as frank breaks caused by sunlight, primarily resulting from indirect oxygen-mediated (i.e., UVA) effects. They may also arise from alkali-labile sites (e.g., abasic sites and Dewar

isomers) or as transient breaks caused by DNA repair enzymes. However, cyclobutane pyrimidine dimers, which generally are accepted as being UV specific are not alkaline labile and thus are not detected by the single-cell gel electrophoresis assay. The measured end point is thus a mixture of 'nondimer' types of DNA damage. This leaves open speculation of numerous other exposures besides sunlight that may contribute to the fluctuation in level of DNA damage seen during the year. For instance, ambient temperature is closely associated with sunlight.

It could therefore be speculated that the variation in DNA damage is due to another exposure that varies over the year, e.g., diet. However the subjects did not report a lower intake of fresh fruits in any season, although the types of fruits differed (for instance, strawberries were more frequently consumed in the summer and clementines more frequently in the winter). The food habits in Denmark include regular (typically daily) consumption of fresh fruit and juice. Dietary vitamin supplementation does not affect DNA damage, as detected by the single-cell gel electrophoresis assay, in a well-nourished population (20–22).

It may be that exposure to air pollution varies over the year. Several studies have reported that samples obtained during the summer had higher levels of polyaromatic hydrocarbon-DNA adducts in lymphocytes (23–26) and induced aryl hydrocarbon hydroxylase activity (27–29). However, probably only the effects of heavily polluted air can be detected. At least, Peluso et al. reported that laboratory controls had low levels of polyaromatic hydrocarbon adducts year-round (26). The subjects in our study worked indoors at our institute, and neither this area nor the residence areas of any of the subjects could be regarded as highly polluted. Therefore, it is unlikely that air pollution is the main contributor to the seasonal variation in level of DNA damage.

Even if exposure to sunlight is related to higher levels of DNA damage, repair, and mutations, it remains to be determined whether the elevated level of DNA damage is related to the development of lymphatic cancer. Several authors have suggested an association between exposure to sunlight and development of non-Hodgkin's lymphoma (30–34). The etiology of non-Hodgkin's lymphoma is not well understood, although environmental exposure seems to be important. It has been hypothesized that UVB light causes non-Hodgkin's lymphoma by a suppressive effect of the immune system (31). Indeed, such an immunosuppressive effect has been reported (especially for UVB), which is thought to be due to UVB-induced DNA damage in antigen-presenting cells in the irradiated skin (35). At least in animals, an increased incidence of lymphatic leukemia has been observed in mice irradiated with UVB light three times a week for 9 months (36). The evidence for humans is more circumstantial, with supporting lines of work coming from three directions. 1) In the past few decades, there has been a parallel increase in recreational sunbathing and the

incidence of skin cancer and non-Hodgkin's lymphoma (31). 2) A positive association between incidence of non-Hodgkin's lymphoma and estimated level of solar radiation has been reported (32). However, several reports have questioned this association because of a lack convincing data that support a latitudinal association (30, 37, 38). 3) Epidemiological studies have shown that patients with basal cell carcinoma or squamous cell carcinoma have a slightly increased risk of developing non-Hodgkin's lymphoma (34, 39–41). The major drawback of these epidemiological studies is that the secondary lymphoma may be caused by the treatment of the primary skin cancer or could be due to genetic predisposition. In general, it must be regarded as a highly speculative and controversial whether sunlight causes non-Hodgkin's lymphoma. In fact, there is more compelling evidence that certain chemicals and viruses are causal factors (42). **FJ**

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