Exposure to solar ultraviolet radiation is associated with a decreased folate status in women of childbearing age

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List of abbreviations

Food Frequency Questionnaire (FFQ), 5-Methyltetrahydrofolate (5-MTHF), Ultraviolet (UV), Neural Tube Defects (NTDs), Reactive Oxygen Species (ROS)

Abstract

In vitro studies indicate that folate in collected human blood is vulnerable to degradation after exposure to ultraviolet (UV) radiation. This has raised concerns about folate depletion in individuals with high sun exposure. Here, we investigate the association between personal solar UV radiation exposure and serum folate concentration, using a three-week prospective study that was undertaken in females aged 18-47 years in Brisbane, Australia (153 E, 27 S). Following two weeks of supplementation with 500µg of folic acid daily, the change in serum folate status was assessed over a 7-day period of measured personal sun exposure. Compared to participants with personal UV exposures of <200 Joules per day, participants with personal UV exposures of 200-599 and >600 Joules per day had significantly higher depletion of serum folate ($p=0.015$). Multivariable analysis revealed personal UV exposure as the strongest predictor accounting for 20% of the overall change in serum folate (Standardised $B=-0.49$; $t=-3.75$; $p=<0.01$). These data show that increasing solar UV radiation exposures reduces the effectiveness of folic acid supplementation. The consequences of this association may be most pronounced for vulnerable individuals, such as women who are pregnant or of childbearing age with high sun exposures.

1. Introduction

Folate is a vitamin that plays an essential role in one-carbon transfer reactions and DNA synthesis [1]. One carbon methylation reactions are dependent on folate for a diverse range of important molecular functions including; the synthesis of purines and pyrimidines, homocysteine metabolism and methylation of DNA, proteins and lipids; functions which are essential in cell division and metabolic processes [2]. The function of folate in these cellular processes has led to a number of hypotheses regarding the role of folate in disease aetiology. Folate deficiency is classically associated with megaloblastic (large cell type) anaemia and a particularly active area of research, with mixed outcomes, is the role of folate in the development and progression of cancerous cells (due to its essential requirement in DNA synthesis and methylation reactions) [3]. However, it is the importance of folate in the prevention of Neural Tube Defects (NTDs), such as spina bifida, where the strongest evidence exists for folate in reducing the risk of human disease [4,5]. Consequently, the need for childbearing age women to obtain adequacy in folate status is of particular importance and has been the rationale for the introduction of mandatory folic acid fortification in several countries [6].

Folate is acquired through the intake of natural folates found in foods; with rich sources including green leafy vegetables, legumes and yeast extracts, and the synthetic form, folic acid which is found in supplemental form or added as a fortificant to food [7]. Similarly to the National Institutes of Health in the United States, the National Health and Medical Research Council (NHMRC) in Australia has set the Recommended Daily Intake (RDI) for folate at 400μg a day for adults [8,9]. Higher levels of 600μg/day are recommended for pregnant women to reduce the risk of NTDs [9].

In vitro data show that folate and its synthetic derivative, folic acid, are both vulnerable to degradation by UVB radiation (280-315nm), although only folic acid is vulnerable to direct degradation by the longer wavelength UVA(315-400nm)[10]. However, only longer wavelength UVA radiation (315-400nm) penetrates to the dermal circulation where direct impact on unmetabolised folic acid in the blood could occur [11,12]. Other processes that may impact folate status in humans include UVA exposure-derived Reactive Oxygen Species (ROS) that can oxidise the main circulatory form of folate, 5-Methyltetrahydrofolate (5-MTHF) and a possible direct role of UV radiation in the degradation of folate in the skin, which has the potential to enhance photo and oxidative damage to folate-depleted skin cells [13-15]. For a more detailed treatment of these various mechanisms please refer to Borradale and Kimlin [16].

The impact of UV exposure on circulatory folate status in humans has not been widely investigated in population studies. Fukuwatari et al. [12] demonstrated that Japanese college students (N=7) supplemented with 250 μg of folic acid per meal for two days and subsequently asked to bathe in sunlight on the third day, had a significantly reduced plasma folate status (plasma folate pre-test =38.0+7.2 nmol/L vs. post-test =28.1+4.6 nmol/L, p=<0.05). Conversely, a control group of students (N=7) not supplemented with folic acid, but also asked to bathe in sunlight showed no significant depletion in plasma folate. Several other population studies have failed to observe a relationship between UV exposures and folate depletion. For example, in a controlled trial, Gambichler et al. [17] showed that participants exposed to both single and serial UVA radiation exposures via a sunbed did not have significantly lower serum folate levels to those not exposed (N=24, with eight participants exposed to UVA). Several other studies have also tested the effect of multiple UVB exposures on folate status with only one of these studies, by Shaheen et al. [18]

48 reporting significant effects on folate status in participants exposed to UVB radiation via
49 phototherapy unit [18-21]. With UVB not able to penetrate to the dermal circulation this one
50 significant result is surprising, however the participants in the study by Shaheen et al. [18]
51 were vitiligo patients whose depigmentation condition may have had a role in reducing the
52 skin's protective barrier to UV radiation. An important distinction between these studies and
53 that by Fukuwatari et al. [12] is that none of the other studies involved the use of folic acid
54 supplements by participants. The population evidence provided by Fukuwatari et al. [12] and
55 lack of significant effects observed in population studies where folic acid has not been
56 supplemented, suggests that folic acid may be the major vulnerable species of folate to
57 degradation by UV radiation.

58 Thus, while there is strong *in vitro* evidence for degradation of folate by UV radiation [11-
59 15,22,23], there remains a lack of population research in this area, with most human studies
60 involving the use of artificial UV exposures and lacking the use of folic acid supplementation
61 which has been shown *in vitro* to be highly vulnerable to UV radiation [12,17-21]. The
62 hypothesis that higher solar UV radiation exposures may lead to increased degradation of
63 circulatory folate levels for people taking folic acid supplements therefore requires further
64 investigation. This is particularly important for women of childbearing age due to the
65 consequences of low folate status for pregnancies. Our objective in the current study was
66 therefore to investigate the depletion of serum folate in a sample of females of childbearing
67 age with varying sun exposures, following a two week period of folic acid supplementation,
68 whilst also controlling for factors such as dietary folate.

2. Methods

2.1 Study population

Healthy female participants aged 18 to 47 years were recruited for this study. Participants were recruited from the Brisbane area (longitude: 153 E; latitude: 27 S), Australia, through advertisements placed within a university. Volunteers were excluded if they were pregnant or attempting to conceive, less than 18 years of age, had a diagnosed malabsorptive disease such as coeliac disease, liver diseases, a history of cancer or any condition that increased sensitivity to the effects of solar UV exposure, such as an inherited photosensitivity or lupus erythematous. Participants were also instructed to cease taking folic acid supplements prior to the beginning of the study. Data collection for the project occurred during October and November 2011. All participants provided written informed consent based on the study protocol that was approved by the Queensland University of Technology (QUT) Human Research Ethics Committee (Approval no. 1100000933).

2.2 Study design and measures

In this three week longitudinal study, participants were supplemented with a 500µg folic acid supplement daily for two weeks. After the two weeks of supplementation, serum folate was measured as a baseline measure, followed by a second serum folate measurement after one week of personal sun exposure measurements. The outcome variable for this investigation was the change in serum folate, between these two time points. A 500µg folic acid supplement was chosen due to this being the level of periconceptional folic acid supplementation recommended by the NHMRC for childbearing age women planning a family [24]. The supplement was supplied by Blackmores Limited and is a listed product on

the Australian Register of Therapeutic Goods (ID: 118091). The length of folic acid supplementation was chosen based on prior research showing significant increases in serum folate status following two weeks supplementation [25].

Dietary intake of folate was determined through the use of a validated food frequency questionnaire [26]. General health information, usual physical activity, use of supplements, and medication usage was collected at the beginning of the study using a general health and information questionnaire. The questions used in the general health and information questionnaire have been used in previous research undertaken in the AusD study (27). Due to alcohol's influence on folate status, a question was also included assessing participants usual intake of alcohol [28]. Additionally, participants were asked whether alcohol intake, medication or supplement usage had changed from that indicated in the general health information questionnaire over the course of the study.

Body Mass Index (BMI) was measured at the beginning of the study using a standard portable stadiometer (S+M) and rounded to the nearest cm. Weight was recorded to the nearest kg and was measured using a set of electronic scales placed on a hard surface. Measurement error was minimised with the use of the same set of scales and stadiometer for all measurements in the sample. Inter-rater bias was eliminated by the use of a single observer for all measurements who was trained in the anthropometric measurement of subjects.

110 *2.3 Personal sun exposure assessment*

111 A seven day sun exposure and physical activity diary was provided to participants for self-
112 completion each day. Participants reported the time spent outside in the sun in 15-minute
113 intervals for each hour of the day between 5am to 7pm. The use of sunscreen, clothing worn
114 and level of physical activity were also assessed during this time. The sun exposure and
115 physical activity diary has been used previously for sun exposure research in populations,
116 most recently in the AusD study [27]. Environmental UV radiation was measured with a
117 Solar Light Co. 501A biometer, located at the AusSun Research Laboratories at QUT Kevin
118 Grove campus. The detector collects solar UV data in five-minute intervals and reports the
119 exposure as J/m^2. Participants were physically located within a 25km radius of this detector
120 thus all were exposed to similar environmental UV radiation.

121 Participants' self-reported time in the sun (collected from the sun exposure diary) was
122 combined with environmental UV data to give an hourly personal UV exposure using the
123 following equation:

124 Personal UV Exposure (J/m^2) = $\Sigma^{t=23}_{i=1}$ UV (t) x A (t) x SAE (t)

125 Where i=time interval 1 hour, (t) = time interval in 15 minutes, UV = environmental UV
126 (from UV detector), A = activity for that 15 minute time interval (indoor/outdoor) taken from
127 the sun exposure diary. SAE (Skin Area Exposed) is the proportion of skin area exposed to
128 sunlight (obtained from the clothing section of the sun exposure diary), based on the clothes
129 cover index for Body Surface Area (BSA), described by Dixon et al. [29].

130 Body coverage area was provided by the common items of clothing illustrated in the sun and
131 physical activity diary. Personal UV exposure in total Joules could be estimated for each hour

of daylight by multiplying personal UV exposure by each participant's BSA. BSA in m^2 was estimated for each participant using the Mosteller [30] equation:

$$BSA\ (m^2) = ([Height(cm) \times Weight\ (kg)]/3600)^{1/2}$$

Hourly UV doses were summed to estimate UV dose for each participant each day over the one week outdoor measurement phase of the study. The average daily UV dose for each participant over the measurement week was then calculated.

2.4 Serum folate analysis

Analysis of serum folate for participants was undertaken at a commercial laboratory (Pathology Queensland). Serum folate was measured via an automated competitive binding assay with chemiluminescence detection (Roche Modular E170 and reagent - Folate III for Elecsys and Cobas e analyzers part no. 04476433 190). This test uses ruthenium labelled folate binding protein, with folate from the sample competing with biotin labelled folate for the binding sites of the protein. Measurements of chemiluminescence emissions were used for detection of the folate, of which the concentration is determined via a calibration curve. The inter-assay precision of the competitive binding assay used in this study has been reported with a coefficient of variation of 16%, compared to a standard QuantaPhase II radioassay [31].

2.5 Data analysis

All analyses were conducted using the Statistical Package for the Social Sciences analysis software (SPSS Statistics, Version 19.0. Armonk, NY: IBM Corp. Inc.). Independent variables shown in previous research to impact folate status (BMI, smoking, dietary intake of folate, alcohol and age) and variables associated with sun exposure behaviours (country of birth, sunscreen use and physical activity) were included in the descriptive and univariate analysis (26,28,32-34) Averages were reported as means with standard deviations used to indicate the distribution of the data. Independent variables were grouped according to standardised cutoffs used for BMI, age and dietary folate intakes based on RDIs. Total UV exposure in joules was grouped according to adequate category sizes for statistical analysis at the univariate level. In cases where individual group sizes were small (<5), in our case for usual alcohol intake, the groups were combined into a two category variable. Univariate analysis using independent samples t-tests and ANOVA tests were used to investigate associations between independent variables and the dependent variable change in serum folate (nmol/L). Multiple linear regression was undertaken to investigate the association between personal UV exposure and change in folate status and the covariate variables of age, BMI, dietary folate intake, smoking, alcohol intake, country of birth, physical activity and sunscreen use. Covariates were included in the multivariable model if previous research evidence suggested that they had a likelihood of impacting folate status or if there were significant univariate associations from our own data analysis [26,28,32,33]. Multicollinearity between independent variables was assessed using matrix tables, with intercollinearity (>0.5) between variables resulting in a decision to omit or produce a composite variable for the highly interactive variables. Scatterplots of standardised residuals were used to assess the dependent variable for normality of residuals. The contribution to the

change in serum folate over the measurement period for each of the independent variables was reported using standardised beta coefficients. Adjusted R^2 value was used to describe the total variance that the independent variables explained for the change in serum folate over the measurement period and statistical significance of the overall model was reported. Two sided tests were used and a probability value (p-value) of <0.05 was considered statistically significant.

3. Results

3.1 Participant demographics and univariate associations

Of 48 participants recruited for the study, 45 completed all study components. Characteristics of the sample are presented in **Table 1**. No significant differences in change in folate by age group were found. Ten of the participants (22%) were classified as overweight or obese; this was not associated with any significant difference in serum folate status. The majority of participants (n=34) did not met the RDIs for folate of 400 μg/day, with nine of these participants reporting average daily dietary intakes of under 200 μg/day. Despite this, dietary intake of folate was not significantly associated with change in serum folate over the measurement time period.

Personal sun exposure during the week was strongly associated with a decrease in serum folate (p=0.015). Participants with lower sun exposures were observed to have only a slight decrease in mean serum folate over the one week measurement period. However, those participants with moderate and high personal UV exposures had greater decreases in serum folate, with average decrease in serum folate for those participants in the highest sun exposure group observed to be -22 nmol/L more than those in the lowest sun exposure group. Participants undertaking moderate to heavy physical activity had significantly higher folate depletion over this week than people taking part in light daily physical activity (p=0.019). Tobacco smokers (n=4) had significantly less decrease in serum folate than those who were non-smokers, although the low numbers of tobacco smokers in this sample increase the risk of error here. Intake of two or more alcoholic beverages per week over the study period was not associated with any significant change in serum folate. Likewise country of birth and

daily use of sunscreen was not associated with change in serum folate over the measurement time period.

3.2 Multivariable association of personal UV exposure with folate depletion

Multivariable regression analysis was utilised to assess the association between personal UV exposure and change in serum folate levels while controlling for possible confounding variables. The independent variables that showed associations with change in serum folate at the univariate level; physical activity and smoking, were included in this model. Age, BMI, dietary intake of folate and alcohol intake were also included in the model to observe any confounding effect that would adjust the relationship between personal UV and change in serum folate. Analysis of intercorrelations between independent variables also showed no evidence of multicollinearity.

Independent variables not showing trends ($p=<0.25$); smoking, BMI, dietary intake of folate and usual alcohol intake, were removed from the first model to produce the final model (**Table 2**). With the removal of such variables, the model produced an adjusted R^2 of .310 ($p<0.01$). Both personal UV exposure in Joules and physical activity were significant predictors of change in serum folate over the measurement week, while age did not show a significant association with change in serum folate. Thus, higher personal UV exposure and the highest category of physical activity were associated with significant decreases in serum folate over the measurement week. The final model explained 31% of the variance change in serum folate over the measurement week, with personal UV exposure being the largest contributor to this by explaining 20% of the variance.

4. Discussion

In our study investigating the effects of natural solar UV radiation on serum folate status, we observed that significantly greater depletion of serum folate status occurred in participants with higher sun exposures compared to those with lower exposures ($p=0.015$). A dose-dependent effect was observed with regard to sun exposure and serum folate depletion, with participants who reported the highest sun exposures experiencing increased depletion compared to those with intermediate exposures and the lowest exposures. Multiple regression analysis continued to show a significant association between folate depletion and personal UV exposure, after adjusting for possible confounding variables of dietary and alcohol intake, BMI, age, sunscreen use and smoking status.

The findings from this study provide evidence that folate is vulnerable to degradation by solar UV radiation. Previous *in vitro* research has shown that folic acid is degraded by both UVA and UVB, although only UVA is able to penetrate through to the dermal circulation, while the main circulatory form of folate, 5-MTHF is only directly vulnerable to the non-penetrating UVB [12,22,23]. *In vivo* data reported by Fukuwatari et al. [12] has also shown significant depletion in serum folate in students supplemented with folic acid and subsequently exposed to natural solar radiation. This depletion of serum folate appears to be considerable in humans following sun exposure, with an average of 9.8nmol/L decrease after two days reported by Fukuwatari et al. [12] and an average 12.8nmol/L decrease after one week observed in our study. Personal UV exposure was the most significant predictor of change in serum folate status over the measurement period ($p=<0.01$) in the current study. Comparatively however, the majority of previous research conducted in participants who were not supplemented with folic acid has shown to not significantly decrease folate status

246 following UV exposure, [17,19,20,21]. This is despite *in vitro* research showing the
247 vulnerability of 5-MTHF to indirect degradation by UV derived reactive oxygen species
248 [11,13,14]

249 Thus, significant UV degradation of folate may only occur in people who are taking folic
250 acid supplements, i.e. the folic acid supplementation leads to the appearance of folic acid in
251 the blood, which is vulnerable to UVA photodegradation. If this is the case, people taking
252 folic acid supplements who have high sun exposures will be obtaining less benefit from the
253 supplements compared to people with lower solar UV exposures taking folic acid
254 supplements. This may be particularly important for vulnerable groups of the population such
255 as women of childbearing age with high sun exposures. Caution needs to be advised
256 however; in both the present study and that by Fukuwatari et al. [12] supplementation was
257 ceased and follow up observations showed accelerated degradation of folate with higher sun
258 exposures. However whether significant degradation of folate by solar UV exposure
259 continues when there is regular replenishment with a daily supplement still needs to be
260 elucidated. A randomised controlled trial is required to gain more insight into these questions.

261 Physical activity was the only other variable significantly associated with change in serum
262 folate at the multivariable level, with higher physical activity levels significantly associated
263 with larger decreases in serum folate status over the measurement week. Examinations of
264 multicollinearity showed no significant intercorrelation between physical activity and
265 personal UV exposure. Thus, it appears to be an independent association. The need for
266 increased folate for people with high physical activity, due to higher metabolic demands has
267 been raised previously, but research has not shown significantly lowered folate status in
268 athletes compared to inactive controls [35,36]. Data are therefore weak in this area and there

269 is a lack of research on whether folate requirements for physically active people are higher
270 than inactive people. Dietary intake of folate, alcohol intake, BMI, smoking and sunscreen
271 use were not associated with change in folate.

272 In showing a significant association between solar UV exposure and folate depletion, the
273 current study builds upon other research in this field and also adds several innovations;
274 including the measurement of the impact of natural sun exposure on folate degradation in a
275 population. Much of the previous *in vivo* research used sunbeds and UV lamps for the UV
276 exposure [17-21]. While use of artificial UV sources allows more control over dose received,
277 artificial UV sources are frequently limited to specific wavelengths, or due to ethical reasons
278 are only practical for participants who already require therapeutic use of artificial UV
279 sources, such as psoriasis patients. Additionally, the setting of the current study in a high UV
280 environment was important as the few previous studies investigating the effect of natural
281 solar UV on folate have been undertaken in areas of higher latitude, i.e. Fukuwatari and
282 colleagues [12] conducted their study in Japan, which has a substantially lower UV intensity
283 than Australia.

284 This study has also been the first investigation to study the effects of sun exposure on the
285 folate status in women of childbearing age. Possible degradation of folic acid by solar UV
286 radiation *in vivo*, is perhaps most concerning for this population group because of the
287 importance of folic acid supplementation and fortification strategies in reducing risk of NTDs
288 in planned and unplanned pregnancies. There were also several limitations to the study, one
289 of which is the observational nature of the study, thus we cannot state that sun exposure is the
290 *actual cause* for the increased folate degradation in those with higher sun exposure.

An important next step in folate and UV research would be the implementation of a study that could provide further exploration of the possible metabolic pathways responsible for this degradation. For this, a controlled trial where participants were randomised into a folic acid supplemented group and non-supplemented control group is required. This would also provide causal evidence for the association between sun exposure and folate degradation. Another potential area of future research is provided by the fact that the serum folate assays used in this study and in previous epidemiological research in this area have been unable to determine specific folate species. Future studies would therefore benefit from the measurement of the different folate species in circulation such as 5-MTHF and the free unmetabolised form of folic acid in the blood via techniques such as HPLC [19,37].

In conclusion, our research found an association between increasing sun exposure and decreases in serum folate status. This adds to evidence suggesting that people whose folate levels have been raised by folic acid, are at increased risk of a higher degree of folate degradation following high levels of sun exposure, potentially reducing the efficacy of supplementation. The consequences of this association may be most pronounced for individuals with high sun exposure and poor sun protection habits. This study provides a strong case for continued investigation in this field with a larger sample or the establishment of a well-designed randomized controlled trial to provide data on the casual association and strength of the relationship between UV exposure and folate degradation in the human body.

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Table 1: Variables and univariate relationship to change in serum folate status

Variable	N(%) (Total N =45)	Change in serum folate (nmol/L): Mean (+SD)	p-value*
Age (years)			
18-24	12(27)	-11.7(23.5)	0.96
25-34	19(42)	-13.9(22.3)	
35-49	14(31)	-12.3(24.7)	
Body Mass Index (BMI) (kg/m^2)			
<24.9	35(78)	-13.9(22.9)	0.54
>25	10(22)	-8.8(23.5)	
Dietary Folate intake (μg/day)[a]			
<200	9(21)	-11.2(24.4)	0.89
200-399	18(42)	-11.8(21.5)	
>400	16(37)	-15.0(24.6)	
Personal Ultraviolet (UV) exposure (average Joules[b] per day)			
<200	18(40)	-1.2(14.8)	0.015**
200-599	15(33)	-18.4(22.8)	
>600	12(27)	-23.2(26.6)	
Country of birth			
Australia	30(65)	-10.8(20.1)	0.47
Non-Australia	16(35)	-16.5(27.4)	
Smoking status			
Current smoker	4(8.7)	-3.6(2.8)	0.015**
Non-smoker	42(91)	-13.7(23.8)	
Usual Alcohol Intake			
Once a week or less	32(69)	-11.6(23.1)	0.60
Two or more times a week	14(31)	-15.5(22.8)	
Physical activity			
Light	25(54)	-5.0(13.6)	0.019**
Moderate to High	21(46)	-21.7(27.9)	
Sunscreen use[c]	28(64)		
Yes	16(36)	-9.2(23.8)	0.42
No		-15.2(22.8)	

[a]Dietary intake of folate measured as average daily Dietary Folate Equivalents (μg)
[b]Average daily Joules of UV radiation exposure over the measurement period
[c]Daily use of a sunscreen or moisteriser/makeup with sunscreen component
*Independent samples t-tests and ANOVA tests used to test associations
**Statistically significant (p=0.05)

Table 2: Multiple Regression model: Change in serum folate

	Standardised Beta	t	p-value
Personal Ultraviolet (UV) exposure (Joules)	-.49	-3.75	<0.01**
Physical Activity	-.26	-2.00	0.05**
Age	-.16	-1.20	0.24
Body Mass Index (BMI)	n.r		
Dietary intake of folate	n.r		
Usual Alcohol intake	n.r		
Smoking	n.r		
Sunscreen Use	n.r		
Adjusted R^2	.310		
Significance of model (ANOVA)	<0.01		

Note: n.r = not retained in final model
** Statistically significant at p=0.05