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

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**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)

## 1 **Abstract**

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

## 1 **1. Introduction**

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

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

24 *In vitro* data show that folate and its synthetic derivative, folic acid, are both vulnerable to  
25 degradation by UVB radiation (280-315nm), although only folic acid is vulnerable to direct  
26 degradation by the longer wavelength UVA(315-400nm)[10]. However, only longer  
27 wavelength UVA radiation (315-400nm) penetrates to the dermal circulation where direct  
28 impact on unmetabolised folic acid in the blood could occur [11,12]. Other processes that  
29 may impact folate status in humans include UVA exposure-derived Reactive Oxygen Species  
30 (ROS) that can oxidise the main circulatory form of folate, 5-Methyltetrahydrofolate (5-  
31 MTHF) and a possible direct role of UV radiation in the degradation of folate in the skin,  
32 which has the potential to enhance photo and oxidative damage to folate-depleted skin cells  
33 [13-15]. For a more detailed treatment of these various mechanisms please refer to Borradaile  
34 and Kimlin [16].

35 The impact of UV exposure on circulatory folate status in humans has not been widely  
36 investigated in population studies. Fukuwatari et al. [12] demonstrated that Japanese college  
37 students (N=7) supplemented with 250 µg of folic acid per meal for two days and  
38 subsequently asked to bathe in sunlight on the third day, had a significantly reduced plasma  
39 folate status (plasma folate pre-test =38.0±7.2 nmol/L vs. post-test =28.1±4.6 nmol/L,  
40  $p < 0.05$ ). Conversely, a control group of students (N=7) not supplemented with folic acid,  
41 but also asked to bathe in sunlight showed no significant depletion in plasma folate. Several  
42 other population studies have failed to observe a relationship between UV exposures and  
43 folate depletion. For example, in a controlled trial, Gambichler et al. [17] showed that  
44 participants exposed to both single and serial UVA radiation exposures via a sunbed did not  
45 have significantly lower serum folate levels to those not exposed (N=24, with eight  
46 participants exposed to UVA). Several other studies have also tested the effect of multiple  
47 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.

## 69 **2. Methods**

### 70 *2.1 Study population*

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

### 82 *2.2 Study design and measures*

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

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

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

103 Body Mass Index (BMI) was measured at the beginning of the study using a standard  
104 portable stadiometer (S+M) and rounded to the nearest cm. Weight was recorded to the  
105 nearest kg and was measured using a set of electronic scales placed on a hard surface.  
106 Measurement error was minimised with the use of the same set of scales and stadiometer for  
107 all measurements in the sample. Inter-rater bias was eliminated by the use of a single  
108 observer for all measurements who was trained in the anthropometric measurement of  
109 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:

$$124 \text{ Personal UV Exposure (J/m}^2\text{)} = \sum_{i=1}^{t=23} \text{UV (t)} \times \text{A (t)} \times \text{SAE (t)}$$

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

132 of daylight by multiplying personal UV exposure by each participant's BSA. BSA in m<sup>2</sup> was  
133 estimated for each participant using the Mosteller [30] equation:

$$134 \quad BSA (m^2) = ([Height(cm) \times Weight (kg)]/3600)^{1/2}$$

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

#### 138 *2.4 Serum folate analysis*

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

## 149 2.5 Data analysis

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

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

### 179 3. Results

#### 180 3.1 Participant demographics and univariate associations

181

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

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

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

### 204 *3.2 Multivariable association of personal UV exposure with folate depletion*

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

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

#### 223 4. Discussion

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

233 The findings from this study provide evidence that folate is vulnerable to degradation by  
234 solar UV radiation. Previous *in vitro* research has shown that folic acid is degraded by both  
235 UVA and UVB, although only UVA is able to penetrate through to the dermal circulation,  
236 while the main circulatory form of folate, 5-MTHF is only directly vulnerable to the non-  
237 penetrating UVB [12,22,23]. *In vivo* data reported by Fukuwatari et al. [12] has also shown  
238 significant depletion in serum folate in students supplemented with folic acid and  
239 subsequently exposed to natural solar radiation. This depletion of serum folate appears to be  
240 considerable in humans following sun exposure, with an average of 9.8nmol/L decrease after  
241 two days reported by Fukuwatari et al. [12] and an average 12.8nmol/L decrease after one  
242 week observed in our study. Personal UV exposure was the most significant predictor of  
243 change in serum folate status over the measurement period ( $p<0.01$ ) in the current study.  
244 Comparatively however, the majority of previous research conducted in participants who  
245 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.

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

301 In conclusion, our research found an association between increasing sun exposure and  
302 decreases in serum folate status. This adds to evidence suggesting that people whose folate  
303 levels have been raised by folic acid, are at increased risk of a higher degree of folate  
304 degradation following high levels of sun exposure, potentially reducing the efficacy of  
305 supplementation. The consequences of this association may be most pronounced for  
306 individuals with high sun exposure and poor sun protection habits. This study provides a  
307 strong case for continued investigation in this field with a larger sample or the establishment  
308 of a well-designed randomized controlled trial to provide data on the casual association and  
309 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**

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

<sup>a</sup>Dietary intake of folate measured as average daily Dietary Folate Equivalents (µg)

<sup>b</sup>Average daily Joules of UV radiation exposure over the measurement period

<sup>c</sup>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**

	<b>Standardised Beta</b>	<b>t</b>	<b>p-value</b>
<b>Personal Ultraviolet (UV) exposure (Joules)</b>	-0.49	-3.75	<0.01**
<b>Physical Activity</b>	-0.26	-2.00	0.05**
<b>Age</b>	-0.16	-1.20	0.24
<b>Body Mass Index (BMI)</b>	n.r		
<b>Dietary intake of folate</b>	n.r		
<b>Usual Alcohol intake</b>	n.r		
<b>Smoking</b>	n.r		
<b>Sunscreen Use</b>	n.r		
<b>Adjusted R<sup>2</sup></b>	.310		
<b>Significance of model (ANOVA)</b>	<0.01		

Note: n.r = not retained in final model

\*\* Statistically significant at p=0.05