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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 Borradale

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   erythematous. 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 500μg 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 500μg 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   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

132 of daylight by multiplying personal UV exposure by each participant's BSA. BSA in m^2 was

133 estimated for each participant using the Mosteller [30] equation:

134 *BSA (m^2) = ([Height(cm) x 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 met 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.

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.

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.

Acknowledgements

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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 ($\pm$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