Effect of serum folate status on total folate and 5-methyltetrahydrofolate in human skin\textsuperscript{1–3}

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ABSTRACT

Background: It has been suggested that human skin color adapts to balance the need for vitamin D synthesis in comparison with the protection of DNA and folate from photodegradation. However, the folate content of human skin is unknown and may affect the effectiveness of the antifolate methotrexate for the treatment of psoriasis. Objectives: We examined whether total folate and 5-methyl-(6\textsubscript{S})-tetrahydrofolate (5-MTHF) in human skin can be predicted by serum concentrations and whether there are differences in the proportion of 5-MTHF in dermis compared with epidermis.

Design: Total folate (by using a microbiological assay) and 5-MTHF (by using high-pressure liquid chromatography) were measured in fasting serum and fresh skin obtained at surgery by using a recovery validated extraction method.

Results: Total folate in human epidermis was shown to be low compared with in many other tissues, and dermal folate was an order-of-magnitude even lower. These concentrations were directly and linearly linked to serum folate status. Although the percentage of 5-MTHF of the total in the dermis was similar to that in other organs, it was especially high in the epidermis and increased to >65% as serum folate decreased.

Conclusions: The high proportion of 5-MTHF in the epidermis, which is further emphasized in subjects with a lower (10–20-nmol/L) serum folate status, points to a special role for this form of folate in skin, perhaps as a protectant from ultraviolet-induced photosensitization reactions. 5-MTHF may also maintain methylation reactions that influence the proliferative activity. These results may help to individualize the treatment of psoriasis patients with methotrexate and folate.


INTRODUCTION

Factors proposed to drive the adaptive variation in skin pigmentation include the need to prevent DNA and folate photodegradation compared with UV-dependent synthesis of vitamin D (1, 2). However, although darker skin protects against DNA damage and skin cancer (3), it is unknown whether skin folate is affected by UV radiation. Moreover, to our knowledge, the folate content of normal human skin has not been examined.

The concept that skin folate might be susceptible to UV was originally based on the photolability of synthetic folic acid (4). However, folate coenzymes are tetrahydrofolates that can be produced from folic acid by dihydrofolate reductase, although slowly in humans (5). Although the physiologically relevant 5-methyl-(6\textsubscript{S})-tetrahydrofolate (5-MTHF)\textsuperscript{4} is fairly stable to UVA or UVB, it is degraded when photosensitizers are present (6).

Naturally occurring photosensitizers have been shown in skin (7), the effects of which were subsequently examined on 5-MTHF in vitro (8). The photodegradation of 5-MTHF occurs while it scavenges \textsuperscript{1}O\textsubscript{2} and quenches excited photosensitizers. Even at low concentrations, these activities can protect supercoiled DNA from cleavage by UVA (6).

Although remethylation of homocysteine is the only enzymatic function of 5-MTHF, other one-carbon derivatives participate in de novo nucleotide synthesis (9). Risk of squamous cell carcinoma was reported to be 2-fold lower in subject with a high intake of green leafy vegetables and was proposed to be a result of the maintenance of DNA integrity by folate-enhanced repair (10). Because skin cancer has been linked to polymorphisms in 5,10-methylenetetrahydrofolate reductase (11, 12), this decrease in risk could also be related to 5-MTHF’s antiphotooxidative activity (6). Therefore, the contribution of this form to total skin folate is of relevance.

Several reports indicated increased cancer risk, and controversy surrounds UV tanning for cosmetic purposes (13–15), which is counterrecommended by the WHO (16). A few studies have examined whether exposure to UVA decreases plasma folate (17, 18), but the extent to which risk is related to changes in skin folate is, to our knowledge, not known. Moreover, it has been proposed that a diminished folate status by periconceptual intake of green leafy vegetables and was proposed to be a result of the maintenance of DNA integrity by folate-enhanced repair (10). Risk of squamous cell carcinoma was reported to be 2-fold lower in subject with a high intake of green leafy vegetables and was proposed to be a result of the maintenance of DNA integrity by folate-enhanced repair (10). Because skin cancer has been linked to polymorphisms in 5,10-methylenetetrahydrofolate reductase (11, 12), this decrease in risk could also be related to 5-MTHF’s antiphotooxidative activity (6). Therefore, the contribution of this form to total skin folate is of relevance.

Skin folates may also be relevant to psoriasis, which is a chronic inflammatory and proliferative condition mediated in part by inappropriate T cell stimulation (21). Treatment with the antifolate methotrexate is often used but can lead to side effects.

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\textsuperscript{4}Abbreviations used: EB, extraction buffer; GGH, \textgamma-glutamyl hydrolase; 5-MTHF, 5-methyl-(6\textsubscript{S})-tetrahydrofolate; 5-MTHFglu\textsubscript{5}, 6-(R,S)-5-methyl-tetrahydrofolate pentaglutamate.

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These side effects can be combated by the coadministration of folic acid (22), but there is uncertainty whether this treatment offsets antifolate efficacy (23). Polymorphisms in folate-dependent enzymes and carriers are associated with toxic effects of methotrexate treatment of psoriasis (24). Perhaps the heterogeneity of response may also be related to the patient’s skin folate status. In the current study, we examined epidermal and dermal folates from fresh ex vivo human skin and how these are affected by serum folate and skin color.

SUBJECTS AND METHODS

Subjects and skin tissue collection

Fresh abdominal human skin was obtained during plastic surgery from 22 obese but otherwise healthy women undergoing an abdominoplasty. Patients were of different skin colors and age (age range: 29–55 y), and skin samples were not tanned or sun exposed. Nutrient-intake data were not available. Patient consent was obtained before surgery, and all procedures were approved by the University of South Alabama and Mobile Infirmary Institutional Review Boards. Skin samples were kept cold on ice until they were cleaned from blood with cold phosphate-buffered saline. With the use of a Goulian skin-graft knife (George Tiemann & Co) equipped with a depth gauge, the epidermis and dermis were separated from the hypodermis while kept on ice and were stored at −80°C. The thickness of the 2 layers when placed between glass slides and measured by using a caliper was typically 0.30 and 1.8 mm, respectively. The interval between skin collection and freezing was ≤2 h. Fasting blood samples were also collected from the same subjects immediately before surgery and centrifuged within 2 h, and the serum was stored at −80°C until analysis.

Skin grinding and storage

Because skin is exceedingly difficult to disrupt, a tissue pulverizer (Cellcrusher) was used to grind the dermis and epidermis under liquid nitrogen within 1 mo of collection. These were further pulverized with a mortar and pestle under liquid nitrogen to a very fine powder and stored at −80°C.

Preparation of rat plasma γ-glutamyl hydrolase

Endogenous folates were removed from 1.5 mL Sprague-Dawley rat plasma by incubation with 150 mg charcoal for 1 h at 4°C and centrifuged at 32,000 × g for 10 min at 4°C, and the supernatant fluid was stored at −80°C in aliquots as a source of γ-glutamyl hydrolase (GGH) (25). The complete removal of 5-MTHF was confirmed by using HPLC. The activity of the rat plasma GGH was checked by measuring the rate of conversion of folic acid pentaglutamate (Schircks) to folic acid monoglutamate (see Supplemental Methods under “Supplemental data” in the online issue) and was shown to be 2.8 pmol · min⁻¹ · μL⁻¹ plasma.

Folate extraction from human skin

Duplicate samples of dermis and epidermis were extracted in 2-mL microfuge tubes bubbled with argon in each step to prevent oxidation of labile folates. Ground skin (50 mg) was incubated with 500 μL extraction buffer (EB) for 1 h at 37°C while being slowly stirred. The EB was composed of 100 mmol ascorbic acid/L, 71 mmol dipotassium hydrogen phosphate/L , 55 mmol β-mercaptoethanol/L, 15 mmol cysteine HCl/L, and 12 mmol tris base/L, with a final pH of 4.6. At this pH, folates are maximally stable (26, 27), and endogenous GGH is active. The ascorbic acid and β-mercaptoethanol were added to the buffer to prevent oxidation and interconversion of folates (28, 29). Argon was metered at a sufficiently slow rate such that no significant evaporation (<2%) occurred during incubation. The skin extract was centrifuged at 32,000 × g for 15 min at 4°C, the supernatant fluid was filtered by using 10,000-Da molecular-weight cutoff centrifugal filters (Millipore) at 14,000 × g for 15 min at 4°C and stored at −80°C. The pellet was weighed and washed twice with 10 volumes of fresh EB, and each wash was processed in the same manner as the first extract. Another 10 volumes of EB was added to the remaining pellet after the second wash, bubbled with argon for 3 min, and boiled for 15 min to release any folate still bound to protein. The boiled sample was cooled, centrifuged, and filtered as previously described. Reported concentrations were the sum of the 4 extraction steps corrected for recovery. To examine the endogenous GGH activity in human skin extract, the skin was spiked with 6-(R,S)-5-methyltetrahydrofolate pentaglutamate (5-MTHFglu5). Furthermore, in a separate experiment, 50 μL rat plasma GGH was added to 50 mg ground skin and 450 μL EB at the beginning of incubation to determine whether exogenous GGH has an effect on the recovery of folate. To determine the recovery of the extraction method, ground dermis or epidermis in EB was spiked with known concentrations of 5-MTHF (Merck & Cie) or 5-MTHFglu5 (Schircks) 3 min before incubation.

Analysis of folates

Reversed-phase HPLC with detection of intrinsic fluorescence was used to analyze 5-MTHF in the ultrafiltrates of the extracts. An A Luna phenyl hexyl 3.5 μm, 150 × 4.6-mm column equipped with a matching security guard was kept at 12°C and eluted with 30 mmol ammonium hydroxide/L plus phosphoric acid to pH 2.2 containing 5 mmol/L dithiothreitol (Gold Biotechnology): acetonitrile: methanol (18:1:1) (made fresh daily) at a rate of 0.8 mL/min. Samples (25 μL for epidermal extracts; 50 μL for dermal extracts) were injected with a model 420 autosampler (Varian Inc), with the sample tray at 4°C. A Waters 2475 multiwavelength fluorescence detector (Waters) monitored the eluate with excitation wavelengths of 315 and 320 nm and emission wavelength of 355 nm. Data were collected and analyzed with Waters Empower software (Waters). The limit of detection of this method was 1.0 nmol/L [signal to noise (peak to peak) = 3], and the limit of quantification was 3.5 nmol/L [signal to noise (peak to peak) = 10]. The method was linear up to 1,000 nmol/L (R² = 0.999) (see Supplemental Figure 5 under “Supplemental data” in the online issue). This method can be used also to analyze tetrahydrofolate, which is eluted 4 min earlier than 5-MTHF. Serum 5-MTHF was determined by using a somewhat different HPLC method (see Supplemental Methods under “Supplemental data” in the online issue for a description of the method).

Total folate in skin and freshly thawed serum samples was determined with a microbiological assay (30, 31) but by using the
American Type Culture Collection 7469 strain of *Lactobacillus rhamnosus*. The concentration of 5-MTHF standard was measured spectrophotometrically by using an extinction coefficient of 31,700 (mol/L)⁻¹ · cm⁻¹ at λ_max of 290 nm at pH 7 (32). The measured stock solution was diluted in EB to make HPLC standards of 10 and 30 nmol/L, which were stored at −20°C under argon.

**Statistics**

All data sets were examined for normality by using the Shapiro-Wilk test with α = 0.05. Unless otherwise specified, 1-factor ANOVA with Bonferroni’s post hoc analysis was performed to test the difference in concentrations and the recovery of 5-MTHF in the epidermis and dermis of white and black skin samples. P < 0.05 was considered statistically significant. A 2-sided unpaired t test was used to examine the difference between the percentage of 5-MTHF of total folate in the epidermis and dermis. Relations between serum 5-MTHF and epidermal and dermal 5-MTHF and total folate were modeled by using linear regression analysis, and differences in slope were examined by using an F test. The tests were done with Prism 6 software (GraphPad Software Inc). A multiple linear regression analysis was done with Microsoft Excel 2007 (Microsoft) was used to test the effect of age on the relation between serum and skin 5-MTHF.

**RESULTS**

**Folates and recovery of skin folate**

Folates in biological systems are present primarily as poly-γ-glutamate derivatives. To facilitate quantification of 5-MTHF by using HPLC and total folate by using a microbiological assay, the poly-γ-glutamates in skin extracts were first converted to the monoglutamate form. To examine whether endogenous poly-GGH in skin has sufficient activity, 5-MTHFglu₅ was added. At pH 9.2, when GGH is not active, no conversion was observed. However, when the pH of the skin homogenate was adjusted to 4.6, when the GGH is active, the conversion of most of the added 5-MTHFglu₅ to 5-MTHF monoglutamate occurred rapidly and was complete by 60 min (see Supplemental Figure 1 under “Supplemental data” in the online issue). The addition of rat plasma GGH to the reaction mixture did not increase the 5-MTHF extracted from human skin. Thus, human skin was shown to have sufficient endogenous GGH activity to hydrolyze endogenous poly-γ-glutamates. Therefore, only the endogenous GGH activity was used during the extraction procedure.

Folate was extracted from skin by using 4 consecutive steps (ie, the incubation of ground skin, a first wash, a second wash, and boiling of the resultant skin sediments) (see Supplemental Figure 2 under “Supplemental data” in the online issue). The efficiency of extraction was determined by spiking skin homogenates with known concentrations of 6S-5-MTHF chosen to be in the same range as the endogenous concentrations of folate in dermis and epidermis. The average recovery from the epidermis was 80.1 ± 4.5% (n = 4 whites and 4 blacks) and 77.1 ± 3.3 from the dermis (n = 3 whites and 2 blacks), with no significant difference between epidermis and dermis (P = 0.59) (Table 1). The average of recovery values for black and white skin for each layer was used to correct for recovery. The ~20% loss in recovery was shown to be mostly a result of the filtration of the supernatant fluid through a 10,000-Da molecular-weight cutoff filter (Millipore), which was an essential step for the subsequent HPLC analysis. This finding was confirmed by comparing the total folate concentrations (by microbiological assay) obtained from skin supernatant fluid before and after filtration (18% loss for both epidermal and dermal preparations), either with or without the addition of exogenous 5-MTHF. The epidermis was spiked with 5-MTHFglu₅ with a final concentration of 100 nmol/L, and the recovery of the resulting 5-MTHF monoglutamate was similar to that obtained by spiking with 5-MTHF monoglutamate. Incubation for longer times or additional Potter-Elvehjem homogenization after incubation did not produce a significant increase in the amount of 5-MTHF extracted.

**Folate concentrations in human skin**

5-MTHF in human skin extracts was quantified by using HPLC with fluorescence detection where it was well separated from other fluorescent compounds. To ensure the specificity of the method, fluorescence detection was done at 2 excitation wavelengths where the ratio of the response at 315 nm to that at 320 nm = 1.5 for 5-MTHF (see Supplemental Figure 3 under “Supplemental data” in the online issue). Dithiothreitol was shown to be a necessary component of the mobile phase to prevent on-column oxidation of tetrahydrofolates, especially at lower concentrations. Concentrations of 5-MTHF in the epidermis ranged from 308 to 802 pmol/g wet weight (n = 22), and concentrations of 5-MTHF in the dermis ranged from 17 to 119 pmol/g wet weight (n = 20) (Figure 1). With the use of differences between duplicate sample preparations, the CV for 5-MTHF was 8.0% for the epidermis and 16.4% for the dermis. 5-MTHF concentrations in the epidermis and dermis showed a normal distribution. There was no significant difference between white and black epidermis (P > 0.99) or white and black dermis (P > 0.99), with both comparisons having β = 0.31 given α = 0.05 and Hedges’ g = 1.0. However, 5-MTHF in the epidermis was an order of magnitude higher than that in the dermis (P < 0.0001) (Figure 1). Moreover, there was a linear correlation between dermal and epidermal 5-MTHF over the range observed (P = 0.0001) (Figure 2).

A subset of epidermal and dermal samples was also analyzed for total folate by using a microbiological assay. The concentration of total folate in 9 subjects ranged from 684 to 1435 pmol/g wet weight of epidermis and 63–224 pmol/g wet weight of dermis. There was no significant difference between white and black epidermis (P = 0.76) and white and black dermis (P = 0.60), although because β was 0.75, this did not exclude a type 2
FIGURE 1. Mean (±SD) concentrations of 5-MTHF in human epidermis and dermis of subjects with different skin colors (age: 29–55 y). Values were 551 ± 102, 55.4 ± 27.4, 556 ± 225, and 53.9 ± 29.3 pmol 5-MTHF/g wet wt for the white epidermis and dermis and black epidermis and dermis, respectively. *Concentrations of 5-MTHF in the epidermis of white and black skin were significantly different from corresponding dermal 5-MTHF concentrations, P < 0.0001. However, there was no significant difference between white and black epidermal 5-MTHF (P > 0.999) or between white and black dermal 5-MTHF (< 0.999) (1-factor ANOVA with Bonferroni’s post hoc correction). wt, weight; 5-MTHF, 5-methyl-(6S)-tetrahydrofolate.

Correlation between serum and skin folate

5-MTHF in the serum of each subject (measured by using HPLC) was linearly correlated to their total serum folate measured by using a microbiology assay and composed ~ 80% of the total (see Supplemental Figure 4 under “Supplemental data” in the online issue). Linear relations between 5-MTHF in the serum and 5-MTHF or total folate in the epidermis and dermis were observed (Figure 4). A significant difference was shown between the slopes of the lines that represent epidermal 5-MTHF and total folate in relation to serum 5-MTHF (P = 0.013). There was also a significant difference between slopes of the lines that represented dermal 5-MTHF and total folate in relation to serum 5-MTHF (P = 0.033). Thus, the percentage of 5-MTHF of total folate was related to the serum folate concentration. The fitted lines show that, for subjects with the highest serum folate within the range examined, ~59% of epidermal total folate was 5-MTHF, whereas for subjects with the lowest serum concentrations, 65% of epidermal total folate was 5-MTHF. Concurrently, the percentages in the case of the dermis were only between 44% and 35% at the upper and lower end of serum folate, respectively. Multiple linear regression analysis showed that the age of the subject was not a significant factor that determined 5-MTHF in the skin (P = 0.83 and 0.87 for the epidermis and dermis, respectively).

DISCUSSION

People with dark skin color are less susceptible to UV-induced DNA damage (33) and subsequent skin cancer formation than individuals with light skin (3) due, in part, to the photoprotective effect of higher melanin (34, 35). In contrast, concentrations of different folate forms and their susceptibility to degradation in the skin by UV were not known (36). To our knowledge, our study is the first to determine 5-MTHF and total folate in epidermal and dermal layers of normal human skin.

Several precautions enabled valid measurements of skin folate. Skin samples were rapidly dissected and snap frozen in liquid nitrogen within 2 h after surgical removal. Extraction conditions optimized folate stability and the ability of endogenous GGH to hydrolyze polyglutamates. The multistep method promoted the release and extraction of cellular folate. Spiking with 5-MTHF and 5-MTHFglu5 showed that recovery was ~80% for both skin layers, and the addition of exogenous GGH did not increase the recovery. Because of the rapid dissection of skin, efficiency of extraction, and linearity and specificity of assays, the 5-MTHF and total folate measured likely reflected actual concentrations in living skin.

In both epidermis and dermis, we showed that 5-MTHF and total folate were similar in white and black subjects and were not influenced by age. However, the concentration in the epidermis was an order of magnitude higher than in the dermis. Dermal and epidermal total folate were linearly correlated to each other, as were values for 5-MTHF. The observation that skin color has no effect on these concentrations suggested that people with white skin can maintain folate despite the lack of protection by higher melanin. However, skin samples examined were taken from the abdomen, which was not normally exposed to sunlight in our subjects. The lower folate in dermis than epidermis is likely influenced by the higher percentage of cell volume in the latter (37). A similar result has been reported for other nutrients in human skin; eg, ascorbic acid in the epidermis is ~5 times higher than in the dermis (38, 39). The higher folate in the epidermis might be a result of its function in normal continuous regeneration of skin from cells in the stratum basale.

Studies often attempt to relate the disease incidence or clinical response to the concentration of micronutrients in the blood. But the extent to which blood concentrations reflect those in relevant tissues is not always clear. Within the range studied, we showed that serum folate (either as 5-MTHF or total) was linearly and

FIGURE 2. Correlation between dermal and epidermal 5-MTHF (n = 18). R² = 0.74 and P = 0.0001 by using linear regression analysis. wt, weight; 5-MTHF, 5-methyl-(6S)-tetrahydrofolate.
respectively. The difference between epidermal and dermal total folate was also significant,
also significant, \( P = 0.001 \) (unpaired 2-tailed \( t \) test). The percentage of total folate, which is 5-MTHF in the epidermis compared with in the dermis was also significant, \( P = 0.001 \) (unpaired 2-tailed \( t \) test). 5-MTHF, 5-methyl-(6S)-tetrahydrofolate.

positively related to skin folate (either as 5-MTHF or the total) with no indication of reaching saturation. In rats fed defined diets with a varied folic acid content, each organ had a different linear relation of total folate to serum folate even down to 2 nmol serum folate/L. Rat tissues with large stores of folate, such as in the liver and kidney, decreased more sharply as a function of serum folate than in other tissues such as the heart and lung (40). Another study in rats showed that the brain is more protected than the liver from folate depletion during restricted intake (41). The relation between human epidermal folate and serum folate shown in the current study was similar to that between the rat gastrointestinal tract compared with plasma folate (40) and human colonic tissue compared with total serum folate (42). Thus, although having much less folate than liver, human skin appears to have a relatively high priority for maintaining its folate stores in the face of lower overall folate status. On extrapolation to very low serum folate, the current results suggested that the epidermis might still retain total folate at a minimum of \( \sim 350 \) pmol/g skin. Moreover, because folate intake correlates with serum folate status (43), the direct relation between the latter and skin concentrations is relevant to the decreased risk of squamous cell carcinoma in individuals with a high intake of green leafy vegetables (10).

The folate antagonist methotrexate is a first line of treatment of advanced psoriasis, but this is often accompanied by gastrointestinal, hematologic, and hepaticologic side effects. The American Academy of Dermatology recommends that folate be administered in conjunction with methotrexate to decrease such adverse consequences (22). However, although clinical efficacy was diminished by folic acid in some studies, in other studies, it was not (23). Patients with low serum or plasma folate status and, therefore, low epidermal folate, may respond differently to the methotrexate control of skin lesions and be at extra risk of systemic toxicity than individuals with a higher status. Phototherapy with UVB or UVA in conjunction with the photosensitizer psoralens (psoralen-UVA treatment) is also used for patients with extensive psoriasis. The relation between peripheral blood folate and skin status shown in the current work may aid in the optimization of treatment with antifolates by an appropriate dose and possibly timing of folate supplementation. It is suggested that clinicians determine the baseline blood folate status of their patients so that correlations with treatment efficacy or toxicity can be investigated. In addition, our previous work suggested that treatment with UV, and especially psoralen-UVA treatment, may deplete skin folate, particularly 5-MTHF (6), which may influence the therapeutic effects of phototherapy.

The percentage of 5-MTHF in the epidermis, with a median of 65% of the total folate, is higher than that reported in human colonic mucosal (also a rapidly proliferating tissue), human liver (44), or various rat tissues (41, 42, 45–48). Although the generally accepted function of 5-MTHF is to assist in the conversion of homocysteine to methionine, the skin protein content of methionine is not unusual compared with in other tissues. Still, \( S \)-adenosylmethionine–dependent epidermal methylation reactions (eg, DNA methyltransferase-1) have been reported to regulate proliferation (49). In addition, the prepponderance of FIGURE 3. Mean (±SD) concentrations of 5-MTHF and total folate in matched epidermis and dermis of human skin (6 whites and 3 blacks). SDs were ±146, ±284, ±54, and ±128 pmol 5-MTHF/g wet weight for the epidermis 5-MTHF and total folate, and dermis 5-MTHF and total folate, respectively. The difference between epidermal and dermal total folate was significant, \( P < 0.0001 \) (unpaired 2-tailed \( t \) test). The percentage of total folate, which is 5-MTHF in the epidermis compared with in the dermis was also significant, \( P = 0.001 \) (unpaired 2-tailed \( t \) test). 5-MTHF, 5-methyl-(6S)-tetrahydrofolate.

FIGURE 4. A: Correlation between serum 5-MTHF and epidermal 5-MTHF (\( n = 12 \)) and total folate (\( n = 7 \)). Red crosses denote epidermal total folate, and blue triangles denote epidermal 5-MTHF. Slopes of all lines were significantly different from 0: \( P < 0.0001 \) and \( P = 0.0002 \) for epidermal 5-MTHF and total folate, respectively (linear regression analysis). Slopes of epidermal 5-MTHF and total folate were different from each other, \( P = 0.004 \) (\( F \) test). B: Correlation between serum 5-MTHF and dermal 5-MTHF (\( n = 12 \)) and total folate (\( n = 7 \)). Red crosses denote dermal total folate, and blue triangles denote dermal 5-MTHF. Slopes of all lines were significantly different from 0, \( P < 0.0001 \) and \( P = 0.011 \) for dermal 5-MTHF and total folate, respectively (linear regression analysis). Slopes of dermal 5-MTHF and dermal total folate were different from each other, \( P = 0.033 \) (\( F \) test). 5-MTHF, 5-methyl-(6S)-tetrahydrofolate.
5-MTHF in human skin may also be a result of its ability to rapidly scavenge singlet oxygen and quench photosensitizers excited by UV (6). In a cellular context, the extent to which 5-MTHF hinders UV-induced damage may depend on the fraction that is free rather than protein bound. Although it has been suggested that free folate in cell culture and the liver is very low (50), free 5-MTHF in human skin is unknown. Moreover, even submicromolar 5-MTHF is effective in the prevention of DNA damage by UV (6).

Folate-restricted diets promote oxidative liver damage in rats (51). However, it is not yet known how low skin folate must be to affect skin biology and its susceptibility to oxidative stress. The potential consequence of such depletion might be detrimentally synergistic with decreased protection from UV damage. Extrapolation of the current results indicates that, at very low folate status, the epidermis retains a high percentage of 5-MTHF. Although 5-MTHF in the presence of photosensitizers is susceptible to degradation by UV (6), 2 studies of human subjects who were not taking folate supplements (one in Germany and the other in Japan) reported that exposure to UVA or sunlight light did not cause a significant decrease in plasma folate (17, 18). However, neither the possible transient depletion of blood nor skin folate was investigated. Because several photosensitizers are present in skin (7), this folate pool might be vulnerable to depletion by exposure to UV light. Its susceptibility might also be related to the ascorbate content of skin, which has been shown to help maintain 5-MTHF against photooxidation (6) and is also quite variable in skin (38). Although the current study showed a strong relation between serum folate and skin folate, it is not yet certain how rapidly this is reestablished after a perturbation by exposure to light. Moreover, our results showed that the total folate pool in the skin was only a small fraction (~0.97 μmol; see Supplemental Methods under “Supplemental data” in the online issue) of the total for the whole body.

Finally, additional studies are needed to directly determine whether the skin folate pool, especially 5-MTHF, is more at risk in people with light skin than in those with dark skin during exposure to UV light as previously suggested (1, 2).

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REFERENCES

25. Aiso K, Tamura T. Trienzyme treatment for food folate analysis: Op-
32. Gupta VS, Huennekens FM. Preparation and properties of crystalline
5-methyl tetrahydrofolate and related compounds. Arch Biochem Biophy
33. Tadokoro T, Kobayashi N, Zmudzka BZ, Ito S, Wakamatsu K,
Yamaguchi Y, Korosy KS, Miller SA, Beer IZ, Hearing VJ. UV-induced
DNA damage and melanin content in human skin differing in racial/
34. Brenner M, Hearing VJ. The protective role of melanin against UV
35. Miyamura Y, Coelho SG, Wolber R, Miller SA, Wakamatsu K,
of human skin pigmentation and responses to ultraviolet radiation
37. Groenendaal W, von Basum G, Schmidt KA, Hilbers PA, van Riel NA.
Quantifying the composition of human skin for glucose sensor de-
38. Shindo Y, Witt E, Han D, Epstein W, Packer L. Enzymic and non-
enzymic antioxidants in epidermis and dermis of human skin. J Invest
39. Shindo Y, Witt E, Han D, Packer L. Dose-response effects of acute
ultraviolet irradiation on antioxidants and molecular markers of oxida-
tion in murine epidermis and dermis. J Invest Dermatol 1994;102:
470–5.
40. Clifford AJ, Heid MK, Muller HG, Bills ND. Tissue distribution and
41. Varela-Moreiras G, Sellhub J. Long-term folate deficiency alters folate
content and distribution differentially in rat tissues. J Nutr 1992;122:
986–91.
Colonic mucosal concentrations of folate are accurately predicted by
blood measurements of folate status among individuals ingesting
physiologic quantities of folate. Cancer Epidemiol Biomarkers Prev
43. Yetley EA, Pfeiffer CM, Pinney KW, Fazili Z, Lacher DA, Bailey RL,
status in NHANES: a roundtable summary. Am J Clin Nutr 2011;94:
303S–12S.
44. Liu J, Pickford R, Meagher AP, Ward RL. Quantitative analysis of
tissue folate using ultra high-performance liquid chromatography tan-
45. Gregory JF III, Sartain DB, Day BPF. Fluorometric determination of
folacin in biological materials using high performance liquid chro-
46. Duch DS, Bowers SW, Nichol CA. Analysis of folate cofactor levels in
tissues using high-performance liquid chromatography. Anal Biochem
1983;130:385–92.
47. Lumb M, Perry J, Deacon R, Chanarin I. Changes in tissue folates
accompanying nitrous oxide-induced inactivation of vitamin B12 in the
48. Wilson SD, Horne DW. Effect of nitrous oxide inactivation of vitamin
B 12 on the levels of folate coenzymes in rat bone marrow, kidney,
Progressive alopecia reveals decreasing stem cell activation probability
during aging of mice with epidermal deletion of DNA methyl-
transferase 1. J Invest Dermatol 2012;132:2681–90. (Published erratum
appears in J Invest Dermatol 2013;133:859.)
50. Suh JR, Herbig AK, Stover PJ. New perspectives on folate catabolism.
51. Huang RFS, Hsu YC, Lin HL, Yang FL. Folate depletion and elevated
plasma homocysteine promote oxidative stress in rat livers. J Nutr