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News and Views

Vitamin D and the Evolution of Human Depigmentation

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In his recent commentary, Robins (2009) disputed the role played by ultraviolet radiation (UVR), namely, the vitamin-D-producing wavelengths of ultraviolet B (UVB), in the evolution of human skin. He questioned the theory that reduced levels of pigmentation in human skin were selected to facilitate absorption of UVB. He provided evidence to support his idea that people can produce enough vitamin D in their skin, regardless of pigmentation, if they are not pursuing a modern lifestyle. He asserted that, within his framework, rickets was the only selective force that could have influenced the evolution of light pigmentation because other detrimental effects of vitamin D deficiency are unproven. As rickets is increased by industrialization, Robins concluded that "... vitamin D status could not have constituted the fitness differential between lightly pigmented and darkly pigmented individuals at high latitudes that favored the evolutionary selection of the former" (Robins, 2009).

In this article, we examine the current evidence for what has been termed the "vitamin D theory," and highlight the importance of UVB penetration in the evolution of human skin. We begin with an overview of the solar processes involved in cutaneous vitamin D synthesis, followed by a discussion of causal arguments and causation in the context of the vitamin D theory, and conclude with a review of physiological mechanisms and their evolutionary significance.

SOLAR PROCESSES OUT OF AFRICA

The emergence of the human lineage and of *Homo sapiens* both occurred in equatorial Africa. The solar regimes at the equator and within the tropics are very different from those outside of the tropics. Within the tropics, the sun is directly overhead twice each year and creates two annual peaks of solar radiation. The solar flux is more intense and less variable, and it exhibits a different mixture of wavelengths. Levels of serum vitamin D [25-hydroxyvitamin D or 25(OH)D] in the human body are highly dependent on solar processes because UVB initiates vitamin D production in the skin. The amount of UVB that reaches a human body at any particular location is determined by global and local atmospheric parameters, by the amount of reflection from the substrate, by the posture and amount and type of clothing worn, and by personal behavior.

Humans living at the earth's surface manufacture pre-vitamin D_3 in their skin directly from UVB, optimally at 297 nm, but up to wavelengths of 310–315 nm. This radiation transforms 7-dehydrocholesterol (7-DHC or pro-vitamin D_3) in the skin into pre-vitamin D_3 (precholecalciferol). Pre-vitamin D_3 is unstable and is transformed in the skin into vitamin D_3 (cholecalciferol or calciol) within hours (Holick, 1987, 1995). Vitamin D_3 is itself biologically inactive, and exits the skin into the circulation bound to vitamin-D-binding protein. It is then converted into its biologically active form, 1,25(OH)2D, by two successive hydroxylation steps that take place in the liver and kidneys in the hours following UVB exposure (Lehmann, 2005; Norman, 2008). This is the steroid hormone form of vitamin D that acts through the vitamin-D receptor. It is significant that vitamin D status in humans is measured by the serum 25(OH)D (25-hydroxyvitamin D) clinical assay, and generally not by quantitative assessment of other metabolites. [The biologically active form of vitamin D is referred to in this paper as 1,25(OH)2D. It is known in the literature as 1,25-dihydroxyvitamin D, 1α,25-dihydroxyvitamin D_3, 1,25-$(OH_2)D_3$, and 1,25(OH)2D. Study of vitamin D physiology is dogged by inconsistent and incorrect use of chemical names for the compounds involved, and few workers follow the recommended standards of nomenclature (IUPAC–IUB Joint Commission on Biochemical Nomenclature, 1982).] Vitamin D_3 is a labile species and, once produced in the skin, is broken down by longer wavelengths in the ultraviolet A (UVA) range, specifically 315–345 nm (Webb and Holick, 1988; Webb et al., 1989). Because levels of vitamin D_3 and 25(OH)D are highly dependent on UVR, knowledge of the annual pattern of distribution of UVR at the earth's surface is important.

The amount of UVR reaching the earth's surface varies according to the solar zenith angle (SZA), the atmospheric ozone content overhead, and the transparency of the atmosphere (Johnson et al., 1976). Shorter the wavelength of UVR, more likely that the radiation will be absorbed or scattered by the atmosphere before reaching the earth's surface. Large SZAs and increased path lengths through the atmosphere at latitudes outside the tropics result in increased absorption and scattering of UVB, especially the shorter range of UVB wavelengths responsible for pre-vitamin D_3 production (Webb et al., 1988; Fligge and Solanki, 2000; Kane, 2002; Kimlin, 2004; Webb, 2006). UVB irradiance also varies with elevation above mean sea level. The highest UVB fluxes on earth occur at the highest elevations at low or relatively low

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latitudes, such as in the Andes, on the Tibetan Plateau, or in the Ethiopian highlands. The observation of sunlight is not a good guide to the insolation pattern of UVB (Devgun et al., 1983). UVB irradiance is highest when the sun is at its zenith during the day, that is, when the SZA is lowest and the path traversed by sunlight to the earth is shortest. Diurnal variation in the SZA causes visible sunlight to vary 30% between 1200 hr and 1500 hr, while UVB decreases by two orders of magnitude. At the equator, UVB irradiance exhibits peaks at the two equinoxes and reaches nadirs at the two solstices. This results in an annual range of variation of about 20% in vitamin-D-inducing wavelengths of UVB and 15% in vitamin-D-destructive wavelengths of UVA. At 50°N latitude, between the summer solstice and the equinoxes, there is a difference of 250% in vitamin-D-inducing wavelengths of UVB, and a 150% difference in vitamin-D-destructive wavelengths of UVA. Outside the tropics, people experience a single annual optimum of cutaneous pre-vitamin D_3 production at the summer solstice only, with lower levels during the rest of the year. At 50°N, there is effectively no UVB irradiance at the winter solstice, while vitamin-D-destructive wavelengths of UVA are still at 35% of their levels at the summer solstice. Local factors affecting UVB irradiance are mainly water vapor, and natural and industrial pollution. Light cloud cover decreases UVB by 50%, while heavy cloud cover almost completely occludes it. Pollution consisting of oxygen-rich molecules (including ground-level ozone) or sulfur oxides from combustion or volcanism significantly diminishes UVB irradiance. Very little UVB is reflected from most terrestrial substrates other than snow (Chadyšien and Girgždys, 2008), and so the amount of potential UVB gain from reflection is low. The final variables determining the amount of UVB reaching a human body relate to posture, clothing, and behavior. A person on the ground is an upright cylinder that is rarely exposed orthogonally to UVB rays except when prone or supine, when half of the cylinder would be exposed (Wheeler, 1984; Webb, 2006). Clothes and shade-seeking behaviors reduce effective solar exposure markedly (Matsuoka et al., 1992).

With increasing distance from the equator, the proportion of vitamin-D-productive wavelengths in the sunshine diminishes daily. Hominids dispersing out of equatorial Africa in the late Pliocene and again in the Late Pleistocene faced reduced UVB levels that significantly affected their physiology. This problem is acute beyond 37°N (approximately the level of northern Tunisia), where the potential for annual production of cutaneous pre-vitamin D_3 is reduced and the length of time that vitamin D stores need to be maintained increases primarily because of the nature of UVB distribution (Kimlin, 2004) (see Fig. 1). These problems existed prior to urbanization and modern human behavior, but have only been exacerbated by air pollution, ozone, and the habits of human work, clothing, and sun protection.

TESTING THE VITAMIN D THEORY

Maintenance of the ability of human skin to produce pre-vitamin D_3 from sunlight has been viewed for a long time as a causal factor in the evolution of light skin pigmentation (Murray, 1934; Loomis, 1967). In our previous papers, we demonstrated very high correlations between skin reflectance and UVMED (Jablonski and Chaplin, 2000; Chaplin, 2004). [UVMED is a measure of erythemally weighted UVB (298 nm) exposure expressed in arbitrary units (Herman and Celarier, 1999).] For Robins, socioeconomic circumstances and opportunities for outdoor exposure are the primary determinants of the ability to produce pre-vitamin D_3 in the skin, with melanin concentration playing a secondary role (Robins, 2008). Robins contends that facilitation of UVB penetration of the skin sufficient to catalyze pre-vitamin D_3 production was an insufficient cause for the evolution of depigmentation. His contentions can be readily tested.

The very high correlations between skin reflectance and UVMED (Jablonski and Chaplin, 2000; Chaplin, 2004) suggested a causal relationship between skin pigmentation and UVR, namely, that pigmentation phenotypes had been modified by natural selection to maintain an optimum balance between photoprotection and photosynthesis over spatially varying conditions of ultraviolet irradiation. In epidemiology, the separation of causal from noncausal associations is of paramount importance and philosophical debates over what constitutes the evidence of disease causality abound (Maldonado and Greenland, 2002; Olsen, 2003; Rothman and Greenland, 2005; Vineis and Kriebel, 2006). Conditions for establishing causation (variations on "Hill's Criteria") are commonly employed (Rothman and Greenland, 2005). These criteria are as follows: 1) strength; 2) consistency; 3) specificity; 4) temporality; 5) biological gradient; 6) plausibility; 7) coherence; and 8) experimental evidence (Rothman and Greenland, 2005). It is useful to examine the evidence pertaining to the vitamin D theory within this ontological framework.

Strength

Strong associations are more likely to be causal than weak ones. The correlation of $r^2 = 0.927$ between skin reflectance (of green visible light wavelengths) and the autumnal UVMED is extremely high, especially for a biological system (Chaplin, 2004). The strength of the association suggests a close coupling between skin pigmentation, UVR absorption by the skin, and physiological processes therein.

Consistency

Consistency refers to the recurring observation of an association in different populations under different circumstances. The convergent evolution of lightly pigmented skin was predicted for all hominids dispersing outside of the tropics because of the importance of maintaining the potential for producing pre-vitamin D_3 in the skin under conditions of low annual UVB (Jablonski and Chaplin, 2000; Jablonski, 2004). These predictions have been borne out by recent genetic studies, which have demonstrated that depigmented skin evolved independently by different molecular mechanisms multiple times in the history of the human lineage. This occurred twice in modern humans—in the ancestors of western Europeans and eastern Asians (Lamason et al., 2005; Norton et al., 2007; Norton and Hammer, 2008)—and by yet a different mutation in Neanderthals (Lalueza-Fox et al., 2007).

Specificity

This criterion requires that a cause leads to a single effect rather than multiple effects. UVB radiation is involved in two major types of biological activities, only

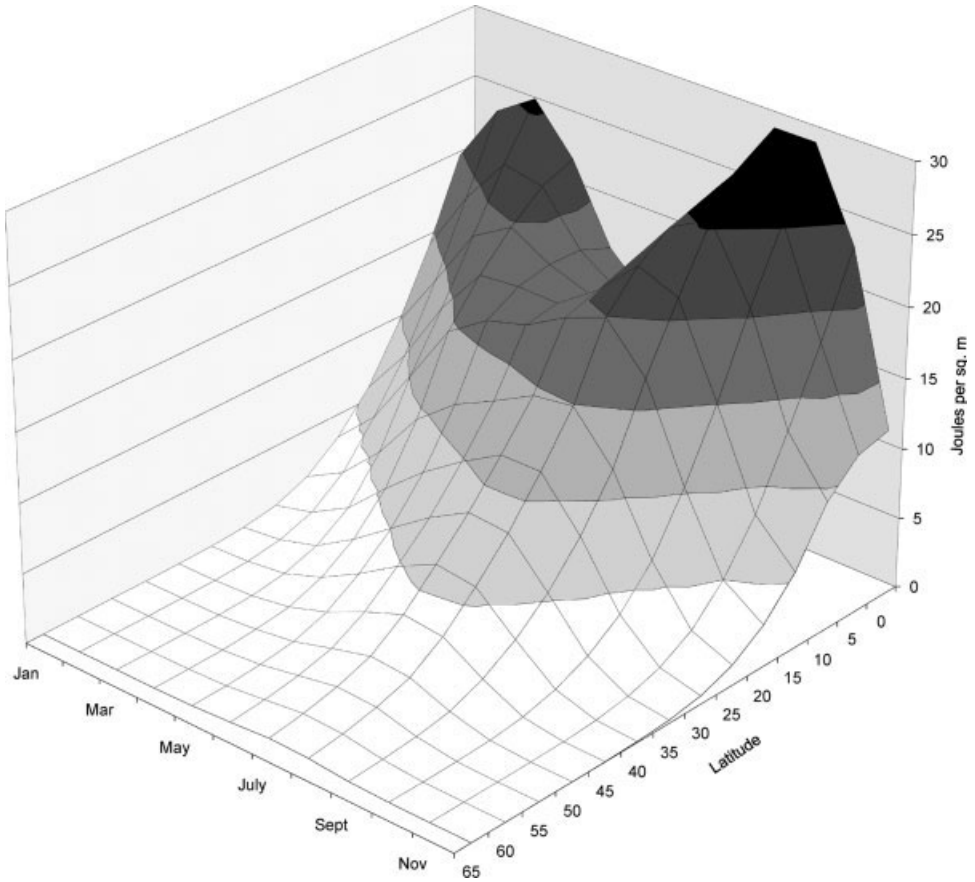

Fig. 1. Graph of calculated daily average influx of solar UVB 295 nm radiation for an idealized clear sky, for the year, calculated at the middle of each month, and at 5°-latitude intervals for the northern hemisphere. Data from Johnson et al. (1976).

one of which will lead to depigmentation. The first is that it harms biological systems through direct and indirect damage to DNA, breakdown of cell membranes, and photolysis of micronutrients (Caldwell et al., 1998; Cleaver and Crowley, 2002; Off et al., 2005). The second is that it acts as the essential catalyst during the first step in the conversion of the vitamin D precursor in the body (7-DHC or provitamin D_3) into pre-vitamin D_3 in the skin of terrestrial vertebrates (Holick, 2003; Norman, 2008).

In connection with the first action of UVB, it has been argued that lightened skin pigmentation resulted from relaxation of stabilizing selection for darkly pigmented skin when members of the human lineage dispersed from areas with high to lower UVB. Biological damage from sunburn or skin cancer actions mediated by UVB is more likely to be more highly correlated with peak UVB in the summer than with winter and autumnal UVB. This would not have been the case for dispersal into northern Eurasia. When a system involved in the production of a phenotype is no longer of active benefit to the organism, it can undergo structural reduction because of the probable mutation effect, as illustrated by the eye and pigment systems of cave fishes (Brace, 1963; Zilles et al., 1983). It was suggested that depigmentation of human skin followed such a course in northern latitudes because of clothes wearing in the Pleistocene of Europe (Brace, 1963). The genes for light skin show evidence of positive directional selection for light skin in multiple lineages (Lamason et al., 2005; Izagirre et al., 2006; Norton et al., 2007; Alonso et al., 2008; Norton and Hammer, 2008), and not structural reduction related to relaxation of the selective pressure of high UVB.

In connection with the second action of UVB, exposure of human skin to short-wavelength UVB induces the production of pre-vitamin D_3 from 7-DHC. Initiation of pre-vitamin D_3 production by UVB is its single most important biological effect. Without the biologically active form of vitamin D—1,25(OH)2D—normal life and reproduction are not possible. The potential for producing pre-vitamin D_3 in the skin from ambient UVB is strongly related to skin pigmentation (Brunvand and Haug, 1993; Goor and Rubinstein, 1995; Harris and Dawson-Hughes, 1998; Jablonski and Chaplin, 2000; Chaplin, 2004; Webb, 2006; Armas et al., 2007; Chen et al., 2007; Gilchrest, 2008; Kimlin, 2008). The predicted global disease burden caused by very low UVB levels and associated low 25(OH)D levels has been estimated at four billion cases or 3.3 billion disability-adjusted life years, based on morbidity estimates of bone diseases (rickets, osteomalacia, and osteoporosis) alone (Lucas et al., 2006). This predicted disease burden far exceeds that connected with high UVR exposure (Lucas et al., 2008a).

Temporality

This refers to the necessity for a cause to precede an effect in time. The cascade of chemical events initially catalyzed by UVB leads eventually to the production of the active metabolite of vitamin D, 1,25(OH)2D, which

has ramifying positive effects on the musculoskeletal, immune, and nervous systems. Deficiencies or insufficiencies of vitamin D can be caused by lack of UVB exposure, the breakdown of pre-vitamin D_3 by UVA (Webb et al., 1989), and by natural decomposition of the stored form of 25(OH)D (Mawer and Davies, 2001).

Biological gradient

This refers to the presence of a unidirectional dose-response curve. There is a linear relationship between skin reflectance and UVB for the autumn and winter quarters that is consistent with a one-to-one dose-dependent response (Chaplin, 2004). This dose response translates into salient biological effects: Lower values for skin reflectance (darker pigmentation) are associated with less damage to DNA caused by UVB and diminished capacity for pre-vitamin D_3 synthesis, while higher values of skin reflectance are associated with greater susceptibility to DNA damage in the skin and enhanced capacity for pre-vitamin D_3 production (Tadokoro et al., 2003, 2005; Miyamura et al., 2007).

Plausibility

This refers to the biological plausibility of the hypothesis. Are there other confounding cocorrelated plausible variables? The number of mechanisms that fit the observed (Chaplin, 2004) response requirement of a simple, linear relationship is very low. Robins contends that the 25(OH)D deficiencies experienced by darkly pigmented populations are due primarily to social factors, but these factors would not be expected to follow a clear, strongly linear response to UVB, nor with the nearly collinear variable of latitude. Other social mechanisms advanced to account for the evolution of light skin pigmentation, including sexual selection (Frost, 1988, 2007), also fail to satisfy this requirement.

Thermal-injury hypotheses for the evolution of light skin pigmentation have been based on the relationship between latitude and solar processes, and must be examined. The correlation between the maximum or minimum temperature variables and skin reflectance is $r^2 = -0.82$ for average winter temperature and $r^2 = -0.55$ for average summer temperature (Chaplin, 2004). These correlations are lower than that between skin reflectance and UVB, and the effect of temperature is lost on detrending the data using PCA (Chaplin, 2004). Thermal-injury hypotheses would predict a threshold, not a linear, effect. A native Libyan with relatively dark skin (44–54% reflectance) moving to Lebanon is unlikely to suffer such catastrophic heat or cold injury that natural selection would favor directional selection for depigmentation to the local skin reflectance of 58.7% (Chaplin, 2004). When populations are not differentiated by social customs or temperature but only by latitude and UVB regime, as in the case of people living in the far south and far north of the British Isles, there is a 6% difference in skin reflectance (Chaplin, 2004).

Coherence

This means that a cause-and-effect interpretation for an association does not conflict with what is known of the natural history of the problem or disease under consideration and presence of conflicting information may indeed refute a hypothesis. This criterion is closely related to plausibility. The single variable of autumnal UVB accounts for 87% of the variation in skin reflectance (Chaplin, 2004). There are no coherent explanations that conflict with the mechanism.

Experimental evidence

Experimental evidence is not a criterion but a test of the causal hypothesis. Many studies have found that both supplemented diet and/or UVB exposure can recover low vitamins D status regardless of skin pigmentation (Hollis, 2005; Wolpowitz and Gilchrest, 2006; Holick and Chen, 2008). A risk assessment review of well-conducted human intervention trials recommended higher dietary intakes of vitamin D_3 because of its health benefits, and because insufficient exposure to UVB coupled with prolonged exposure to UVA destroyed vitamin D_3 in the skin (Hathcock et al., 2007).

QUESTIONING CUTANEOUS VITAMIN D PRODUCTION AS A MECHANISM FOR THE EVOLUTION OF DEPIGMENTATION

Any mechanism hypothesized to account for the evolution of lighter pigmentation in areas of lower UVB must be biologically reasonable, fit the effects observed, and pass the criteria of causality described above. Among the effects to be accounted for is the genetic evidence of positive selection for lightly pigmented skin under conditions of reduced UVB. The widely accepted vitamin D theory is the only one that satisfies the criteria of causality and that provides a mechanism for the evolution of lightly pigmented skin in northern Eurasia. If all of the causal criteria are equally well satisfied, the crucial consideration is mechanism.

Robins's (2008) challenge raises nearly the same issues as those posed by Neer (1975) concerning establishment of the fact that skin depigmentation increases cutaneous pre-vitamin D_3 production, and the consideration of its evolutionary significance. We have used Neer's criteria to structure our discussion of biological mechanism below. We also address two other issues, namely, whether vitamin D deficiency is sufficient to cause rickets and whether any vitamin D-related health impacts other than rickets could have promoted natural selection of lightly pigmented skin.

"Skin pigment should be proved, using well established vitamin D assay procedures, to decrease vitamin D production by skin irradiated in vitro or in vivo" (Neer, 1975, p 414).

Melanin is an essential sunscreen. The packaging of melanin differs between people with lightly and darkly pigmented skin. When melanin (primarily eumelanin) is distributed throughout the entire thickness of the epidermis, within the melanosomes of keratinocytes and as "melanin dust" (disintegrated melanosomes), it absorbs, reflects, or scatters most incident UVB (Kaidbey et al., 1979; Pathak, 1995; Sarna and Swartz, 1998; Alaluf et al., 2002; Ortonne, 2002; Thong et al., 2003; Zmudzka et al., 2006; Brenner and Hearing, 2008). Penetration of UVR into the skin is related to the amount and the distribution of melanin, with large, more superficial, melanin-filled melanosomes being more effective in reducing transmission (Nielsen et al., 2006). The penetration of UVB into the skin is minimal compared to that of UVA (Nielsen et al., 2006), because UVB typically travels only a few microns. Melanin in the skin competes with 7-DHC for UVB photons, thus preventing its penetration into the

basal and spinosal layers deep in epidermis where pre-vitamin D_3 production takes place (Clemens et al., 1982; Matsuoka et al., 1991; Chen et al., 2007). Because melanin content determines UVB penetration, the higher the melanin content, the lower the pre-vitamin D_3 production, all other things being equal (Clemens et al., 1982; Holick, 1987; Webb et al., 1988; Matsuoka et al., 1991; Goor and Rubinstein, 1995; Mitra and Bell, 1997; Jablonski and Chaplin, 2000; Kreiter et al., 2000; Skull et al., 2003; Vieth, 2003; Webb, 2006; Chen et al., 2007; Cosman et al., 2007; Hathcock et al., 2007; Tran et al., 2008). When volunteers of different skin phototypes were irradiated with UVB over the course of 12 weeks, individuals with darkly pigmented (phototype V) skin experienced a 40% increase in serum 25(OH)D levels, compared to a 210% ± 53% in lightly pigmented (phototype II) individuals (Chen et al., 2007).

Studies of the transmission of UVB through human skin and pre-vitamin D_3 production have increased in number and sophistication over the years. An early study found that the skin of native Europeans transmitted three times as much UVB as that of native Africans because of the thicker epidermis and higher melanin content of the latter (Thompson, 1955). Many experimental studies have demonstrated the inhibitory effect of melanin on pre-vitamin D_3 synthesis. Lightly pigmented (skin phototype II) volunteers exposed to UVB on a tanning bed for 12 weeks for a total of 216 min increased their 25(OH)D levels by 210%, while darkly pigmented volunteers (skin phototype V) increased theirs by only 40% even after 432 min of exposure (Chen et al., 2007). Whole-body (360°) exposure of lightly and darkly pigmented volunteers to UVB (1.5 MED for lightly pigmented skin) resulted in greatly increased 25(OH)D concentrations in the former group, but no significant change in the latter (Clemens et al., 1982). In the same study, exposure of one darkly pigmented subject to a sixfold greater dose of UVB did yield a sharp increase in serum vitamin-D levels, but the peak concentration attained was still below that observed in the lightly pigmented subjects (Clemens et al., 1982). A similarly designed study of whole-body UVR exposure involving larger numbers of human volunteers from a wider range of skin phototypes yielded similar results (Matsuoka et al., 1991).

The response of cutaneous pre-vitamin D_3 production to dose rate of UVB is not linear. More lightly pigmented individuals and those with higher starting levels of 25(OH)D exhibit steeper rates of increase in pre-vitamin D_3 production and end up with higher levels (Stamp, 1975). Darker individuals or those with lower starting levels of 25(OH)D exhibit moderate rates of increase following UVB exposure and generally have lower ending levels (Stamp, 1975). This study led Robins to conclude that "there is an intrinsic capacity for vitamin D_3 regardless of skin color, provided that UVB exposure is adequate." (Robins, 2009). Pre-vitamin D_3 production can occur in skins of all colors, but for darkly pigmented skin low doses of UVB do not raise 25(OH)D levels to physiologically adequate levels at which storage can take place; higher doses over longer periods of time are required for this, and these conditions are not met outside of the equatorial latitudes. The nonlinearity of pre-vitamin D_3 production results in lighter-skinned individuals achieving greater effect from higher doses of UVB than darker-skinned individuals (Harris and Dawson-Hughes, 1998; Armas et al., 2007).

"Dark skinned people should have lower blood vitamin D levels than white-skinned people exposed to equal, weak physiologic ultraviolet radiation" (Neer, 1975, p 414–415).

The potential capacity to produce pre-vitamin D_3 is enormous, but the rate is limited by the production of other photoproducts and by photodegradation of cutaneous vitamin D_3 by competing wavelengths of UVA of 315–345 nm (MacLaughlin et al., 1982; Webb and Holick, 1988; Webb et al., 1989; Holick, 1995). Prolonged exposure to natural sunlight (as opposed to narrow beam artificial sources) does not continue to increase pre-vitamin D_3 production. During times of low SZA, at latitudes above 37°, the balance of wavelength mix changes from net pre-vitamin D_3 production to cutaneous vitamin D_3 destruction (Webb and Holick, 1988). The same thing can happen at cloudier times when UVA can penetrate but UVB is mostly blocked.

Most studies of potential pre-vitamin D_3 production have been performed in high flux light boxes providing 360° of exposure at doses approximating noon at the equator on a clear day. Studies at Boston (42.2°N) performed outside over the summer (Webb et al., 1988) and those in which ecological exposure has been monitored in tropical settings show moderate levels of 25(OH)D increase.

Fewer studies comparing different groups in naturalistic settings are available. People from the Horn of Africa have common and unrecognized 25(OH)D deficiency when living in Australia or Israel (Fogelman et al., 1995; Skull et al., 2003). Congolese living in Belgium have much lower 25(OH)D levels compared to those in Africa or to native Belgians (M'Buyamba-Kabangu et al., 1987). Later studies contradict Robins's (2008) suggestion that Caribbean people do not have 25(OH)D deficiency in the UK (Hannam et al., 2004); it occurs even in Jamaica (Miller and Chutkan, 1976). Children of Pacific Islander ancestry compared to those of European ancestry living in New Zealand had much lower 25(OH)D status, with 56% exhibiting deficiency (Rockell et al., 2005). In South Africa, albinos have higher 25(OH)D status as compared to their heavily pigmented compatriots (Cornish et al., 2000). Seasonal changes are well known in all groups as has recently been documented in a review of 22 studies (Kristal-Boneh et al., 1999). Another review found that darker skin phototypes exhibited less pre-vitamin D_3 production on either the first or following repeated sun exposure (Gilchrest, 2008).

Regardless of urban or rural status, pregnant women from north India had a greater than 80% incidence of 24(OH)D deficiency (Sachan et al., 2005). People in southern India (13.4°N) showed less 25(OH)D deficiency. Of agricultural workers working 8 hours a day wearing only shorts or loin clothes only 16% had adequate 25(OH)D levels and an astounding 44% were deficient; women and children in the same population were worse off with >70% deficiency (Harinarayan et al., 2008). This poor 25(OH)D status is due to dark pigmentation in south Asians (skin reflectance 46.5%), but these people are not as dark as Africans at the same latitude (e.g., Burkina Faso 28.6%, Malawi 27%, and Sudan 35.5%) (Chaplin, 2004). A study of normal controls in a West African study showed high levels of 25(OH)D_3 deficiency and insufficiency, even in cases of non-Muslim peoples living on the coast and working as fisher-folk; their lowered vitamin status was exacerbated by the wet season (Wejse et al., 2007).

Among the general population of Córdoba, Spain (latitude 37.53°), over 80% of people exhibited unfavorable vitamin D status, with 14% of sampled individuals exhibiting severe 25(OH)D_3 deficiency (<25 nmol/L), 40.8% with 25(OH)D_3 insufficiency (25–50 nmol/L), and 17.6% with suboptimal 25(OH)D_3 levels (50–75 nmol/L) (Mata-Granados et al., 2008). In the state of Arizona, with the highest UVB regime in the USA, 25% of European-Americans, 56% of African Americans, and 38% of Hispanics exhibited 25(OH)D deficiency (<20 ng/mL) (Jacobs et al., 2008). Rural populations with some of the highest UVB levels on Earth like those inhabiting the Tibetan and Ethiopian plateaus also have low 25(OH)D status (Harris et al., 2001; Bereket, 2003).

Regardless of the setting, and even including areas with high insolation, 25(OH)D status appears to be low to marginal for large proportions of indigenous populations. This indicates that even in long-settled populations, maintenance of healthy 25(OH)D status is difficult because of the effects and interactions of at least five variables: 1) skin pigmentation and the natural sunscreen properties of melanin; 2) the UVR wavelength mixture present at a particular place and time on the Earth's surface, and specifically the proportion of UVB (capable of inducing pre-vitamin D_3 production) to photodegrading UVA (capable of breaking down cutaneous vitamin D_3); 3) age, reproductive status (for females), and levels of stored 25(OH)D; 4) a lack of exposure to UVB (and failure to make pre-vitamin D_3 in the skin) due to use of clothing, shelter, or lifestyle choice; and 5) the quantity and seasonal pattern of consumption of vitamin D_3-rich foods in the diet. Alterations in any of these variables may upset the homeostatic mechanisms responsible for maintaining healthy 25(OH)D levels.

Skin pigmentation is the result of the action of two reciprocal clines working to promote the UVB-induced photosynthesis of pre-vitamin D_3 in the skin on the one hand and prevent the multifarious damage caused by UVB and UVA on the other. UVR damages DNA and degrades circulating micronutrients, particularly folate (Jablonski, 1992, 2004, 2006; Jablonski and Chaplin, 1999, 2000). The evolutionary significance of UV-induced folate photolysis has been demonstrated *in vitro* and *in vivo*, with UVA found to be particularly damaging (Off et al., 2005; Nielsen et al., 2006; Vorobey et al., 2006; Der-Petrossian et al., 2007). High concentrations of melanin significantly reduced folate destruction through absorption and scattering of UVA (Nielsen et al., 2006).

To maximize cutaneous pre-vitamin D_3 production by excessive depigmentation would endanger the protective aspects of darker skin. In a system of two competing clines, the UVR absorption by the organism is optimized. Individuals are just light enough—but no lighter—to make the required vitamin D_3 in their skin. Persons moving from UVB-rich zones to UVB-poor zones would be physiologically challenged by problems resulting from lower potential cutaneous pre-vitamin D_3 production. The stable clinal arrangement of human skin pigmentation among indigenous peoples indicates the action of stabilizing selection over a spatially varying optimum condition (Barton, 1999). This was achieved during the process of hominid dispersal and maintained in the face of migration (gene flow).

"The effects of skin pigment on blood vitamin D levels and intestinal calcium absorption should be eliminated if the skin is by-passed by oral vitamin D supplements" (Neer, 1975, p 416).

Many studies have found that, with both supplemented diet and/or UVB exposure, people can recover from low 25(OH)D status regardless of skin pigmentation (Wolpowitz and Gilchrest, 2006; Holick and Chen, 2008). A comprehensive risk assessment review recommended higher dietary intakes as the optimal solution for health (Hathcock et al., 2007).

Is vitamin D deficiency sufficient to cause rickets?

Rickets is a disease of calcium malabsorption arising from vitamin D deficiency, calcium or phosphate deficiency, and calcium mobilization (Shaw, 2003; Kovacs, 2008). The incidence of rickets skyrocketed as a result of industrialization due to release of low level ozone, sulphur oxides, and particulate matter (Robins, 1991, 2008), reaching 80% in children under 2 years of age (Shaw, 2003) and 33% in the general population in large public health studies before 1935 (Vieth, 2001). However, rickets is not just a disease of industrialization. Despite taphonomic biases, it has been recognized in early archeological and Neolithic materials at the rate of 1–2.7% (a reasonably high selective value) (Littleton, 1991; Robins, 1991, 2008; Mays et al., 2006). Written accounts of rickets exist in Greek, Roman, and Chinese literature dating from 900_{BCE}, 300_{BCE}, and 200_{CE}, and the disease was clinically described before industrialization by Glasson in 1651 (Arneil, 1975; Littleton, 1991; Shaw, 2003).

In developing countries today, there is still an epidemic of rickets. This is often associated with veil wearing and city living, but also occurs in rural populations in which breast feeding and/or a calcium-deficient diets are common. Rickets manifests itself in rural populations with some of the highest UVB levels on the Tibetan and Ethiopian Plateaus (Harris et al., 2001; Bereket, 2003). Breast-fed babies rely on their preterm supplies of 25(OH)D received from their mothers until they are exposed to the sun. A recent meta-analysis (Schroth et al., 2005) showed that large numbers of pregnant women are deficient in 25(OH)D. Recent studies conducted on populations of women and their newborn infants in the United States have shown that high percentages of African-American mothers and their newborns are vitamin D deficient or insufficient, despite mothers having taken multivitamin supplements during pregnancy (Bodnar et al., 2007; Lee et al., 2007). These levels were judged as representing an important risk factor for rickets. Parity is a risk factor in rickets and younger women having more offspring increase the risk of their own osteomalacia becoming more severe with each birth (Gannage-Yared et al., 2000; Vieth, 2001).

Rickets—even if it had an incidence no greater than 1–2.7% identified in the archeological record—would have had a selective effect. The incidence of vitamin D stress was probably higher in ancestral modern humans leaving Africa because of prolonged breast feeding, more seasonal environments, maternal 25(OH)D deficiency, low-calcium diets, younger age of first reproduction, and lack of adipose tissue in which to store the 25(OH)D. Coarse diets of legumes or containing husks of grass seeds, pulses, and grams contain calcium-chelating phytates that prevent dietary calcium from being absorbed (Harinarayan et al., 2008). The high fiber content of such diets also lowers the half life of 25(OH)D (Mawer and Davies, 2001). The content of phytic-acid-rich foods in the paleodiets of ancient

gatherers and hunters is not known, but human diets certainly did not contain calcium-rich dairy products, nor dehusked refined grains until after the start of animal domestication and agriculture.

Could any vitamin D-related health impacts other than rickets have promoted natural selection of lightly pigmented skin?

Robins (2008) dismissed current research into vitamin D by stating that, "Much of this work is still speculative and experimental, and the evidence linking vitamin D to the various disease states is inconsistent and dogged by confounding variables (Wolpowitz and Gilchrest, 2006)". Here he cited dermatologists who are antagonistic to calls for greater exposure to UVB because of the risks of skin damage (Gilchrest, 2008). Research on vitamin D has bloomed in the last few years, and, as of this writing, there are 3,714 studies in PubMed with vitamin D as a keyword that were added in the last 2 years alone. Of these, 393 were paired with the keywords of pathology, 638 with cancer, 87 with infection, 187 with immunity, 242 with cardiovascular, and 258 with mortality.

Vitamin D is essential to human health. Vitamin D receptors (VDRs) are found on 36 major organs. Vitamin D exerts paracrine functions in 10 organs, and performs essential regulation of B and T lymphocytes, the adaptive and innate immune system, pancreas, brain, and heart (Norman, 2008). It plays an important part in cancer prevention (Garland et al., 2006; Fleet, 2008). It has regulatory effects on inflammatory markers and autoimmune diseases such as diabetes and multiple sclerosis (Holick, 2008; Holick and Chen, 2008). Vitamin D deficiency is implicated in epidemics of infectious diseases like influenza (Cannell et al., 2008). Single nucleotide polymorphisms of the VDR gene are associated with susceptibility to pulmonary tuberculosis in West Africans (Olesen et al., 2007), and TB and pneumonia are frequently seen together with 25(OH)D deficiency. The lung has its own vitamin D paracrine function that indicates that the vitamin has special significance in protecting lung function (Muhe et al., 1997; Holick and Chen, 2008). Because vitamin D increases muscle performance, it positively affects cardiac output (Valdivielso and Ayus, 2008). Overall, there is an association with perinatal and childhood 25(OH)D status and mortality (Hollis and Wagner, 2004a,b; Lucas et al., 2008b). Because of the lack of comparative population-based studies, it is not yet known how assays of 25(OH)D represent health status, nor what is the essential determinant of pathophysiology: peak concentration, average throughout the year, the nadir, or the range (Millen and Bodnar, 2008).

Effects that work earlier in the developmental cycle have greater evolutionary effect. A likely new role for vitamin D in relation to sunlight is in the vitamin-D-based regulation of homeobox genes such as MSX 2 (Hox 8), which act during development to upregulate expression in embryonic cranial neural crest cells, which form bones of the skull and teeth (Hodgkinson et al., 1993; Davidson, 1995). In pregnant women with subclinical or moderate 25(OH)D deficiency, elevated parathyroid (PTH) concentrations work to maintain adequate plasma calcium concentrations through PTH-induced osteolysis (Okonofua et al., 1987). In women with severe pre-existing maternal 25(OH)D deficiency, however, pregnancy precipitates osteomalacia in women and nutritional rickets in neonates (Kreiter et al., 2000; Nozza and Rodda, 2001; Kovacs, 2008). Few prospective studies have examined the 25(OH)D status of women or neonates over the course of pregnancy and lactation (Hollis and Wagner, 2004a,b). Available evidence indicates that chronically depressed 25(OH)D levels in actively reproducing women compromise the female skeleton over successive pregnancies and lengthy periods of lactation, and adversely affect the integrity of the neonatal skeleton, and the future calcium status of children and juveniles so compromised as infants (Kimball et al., 2008; Kovacs, 2008). Many of the deleterious effects on the musculoskeletal and immune systems manifest themselves over the course of weeks or months, especially in children, following very low or no UVB exposure (Cannell et al., 2006; Wagner and Greer, 2008).

> "Dark-skinned people should have more clinical vitamin D deficiency than white-skinned people exposed to equal natural ultraviolet at high temperate latitudes" (Neer, 1975, p 415–416).

The numerous studies cited above have shown a decreased potential to form pre-vitamin D_3 in dark-skinned individuals compared to light-skinned individuals. Also, dark-skinned individuals have marginal 25(OH)D status in natural populations living in rural areas. There are higher levels of rickets in darker-skinned individuals compared to coresiding lighter-skinned individuals regardless of latitude, and greater pathology related to 25(OH)D deficiency in darker-skinned individuals. Evidence of tuberculosis of the bone associated with probable vitamin D deficiency in a *Homo erectus* fossil indicates the age of these selective forces (Kappelman et al., 2008). The fact that, as predicted by Jablonski and Chaplin (2000), multiple convergences of light skin evolved in different modern human populations and separately in Neanderthals indicates that the evolutionary force was strong enough to drive selection. Vitamin D deficiency is considered by some as an epidemic (Holick and Chen, 2008).

Levels of the active form of vitamin D, 1,25(OH)2D, are unaffected even by large differences in 25(OH)D status (M'Buyamba-Kabangu et al., 1987; Matsuoka et al., 1991; Harris and Dawson-Hughes, 1998). Tight control of an endocrine hormone system is to be expected. This is a compensatory mechanism, whereby, in the presence of vitamin D_3 suppression by melanin, the liver and kidney hydroxylating enzymes are activated in tandem to ensure that the concentration of the biologically active form is normalized and kept constant regardless of ethnic pigmentation (Robins, 2008). This compensatory mechanism has consequences for 25(OH)D storage. The circulating concentration of 1,25(OH)2D is about 1/1000th that of 25(OH)D from which it is produced by stimulation from the parathyroid glands and by low calcium or phosphate levels (Vieth, 2003). Besides being an endocrine system, it operates as a paracrine hormone within various organs having local ability to produce 1,25(OH)2D from circulating 25(OH)D (Vieth, 2003). The paracrine system operates in diverse organs from skin to uterus (Norman, 2008). 1,25(OH)2D has a half life of 4 h (Jones, 2008) compared to that of 10–21 days for serum vitamin D (Ellis et al., 1977; Vicchio et al., 1993) and up to 2 months for adipose stores (Vieth, 2001). 1,25(OH)2D levels show a 70% dose-dependent variation for high oral doses of vitamin D (Hathcock et al., 2007) but are usually very stable.

Vitamin D can be wasted in people with high-fiber and calcium-deficient diets because its half life is reduced (Mawer and Davies, 2001). Concentrations of 1,25(OH)2D will be normal or even elevated in the face of vitamin D deficiency as a result of secondary hyperparathyroidism (Wagner and Greer, 2008).

In contrast to Robins's claim (Robins, 2008), we did not misinterpret Mawer et al.'s (1972) research on vitamin D storage in our early paper (Jablonski and Chaplin, 2000). Mawer clarified in a later work (Mawer and Davies, 2001, p 153) that those with very low 25(OH)D status convert any available 25(OH)D into its short-lived (4-h half life) active form, 1,25(OH)2D, whence it is cleared from the body:

"The process can be modeled as a decreasing spiral in which low 25(OH)D and the ensuing low $1,25(OH)_2D$ result in poor calcium absorption. The falling calcium stimulates PTH secretion, thus increasing 1,25(OH)2D synthesis; this temporarily restores intestinal calcium absorption. This increased $1,25(OH)_2D$ synthesis will deplete still further an already compromised supply of 25(OH)D. The downward process will continue (provided vitamin D intake is not restored) until $1,25(OH)_2D$ can no longer be synthesized at the level required for adequate absorption of calcium, despite increasingly severe secondary hyperparathyroidism. In addition, the development of vitamin D deficiency may be accelerated at the stage when serum $1,25(OH)_2D$ is elevated, by a mechanism involving a shortening of the serum half-life of 25OHD" (Mawer and Davies, 2001, p 154–156).

Parathyroid hormone converts 25(OH)D to short-lived 1,25(OH)2D thereby reducing vitamin D stores, and together with 1,25(OH)2D causes the liver to clear vitamin D twice as fast (Clements et al., 1987b). The cumulative storage capability of 25(OH)D argued by Robins to allow people to survive the winter months at high latitudes is limited or nonexistent, because the 25(OH)D is converted, catabolized, or excreted (Mawer et al., 1972; Mawer and Davies, 2001). An intermittent supply of vitamin D_3 in the winter or early spring will not raise 25(OH)D and 1,25(OH)2D status; this problem will be exacerbated by higher pigmentation levels. In people with hyperparathyroidism, a "strong inverse relationship was demonstrated between the serum half-life of 25(OH)D and the prevailing concentration of 1,25 (OH)2D" (Mawer and Davies, 2001, p 156). These people will be more susceptible to rickets and osteomalacia (Clements et al., 1987a,b, 1992; Mawer and Davies, 2001; Pettifor, 2007; Prentice et al., 2008). Where 1,25(OH)2D is dominant over vitamin D, the nonsteroidal hormone actions of vitamin D are jeopardized, e.g., the paracrine systems of the colon, endothelium, dendritic cells, pancreatic islets, placenta, prostate, and skin (Norman, 2008). The classical view of vitamin D action has been that it exerts its effects on bone only indirectly, as a hormone, through regulation of absorption of calcium and phosphorus from the gut. This view is now being supplemented by the recognition that vitamin D directly modifies the activity of osteoblasts and chondrocytes, and many other nonclassical target tissues (St-Arnaud, 2008; Wolff et al., 2008)

DISCUSSION

On leaving Africa, hominids encountered UVB-impoverished environments with limited potential for cutaneous pre-vitamin D_3 production. The theoretical potential of large amounts of pre-vitamin D_3 manufacture in the skin is never realized in a natural setting. The conversion of 7-DHC is rarely complete, the rate-limited nature of the reaction leads to breakdown of the pre-vitamin D_3, and vitamin D_3 made in the skin can be readily broken down by UVA. When there is a shortage of vitamin D_3, the compensatory mechanism of hyperparathyroidism results in elevated clearance of existing stores of 25(OH)D. The ability to store large amounts of 25(OH)D in adipose tissues relies on the production of excess vitamin D_3 in the first place. The low 25(OH)D status of most people in the studies reviewed above shows that the potential for cutaneous pre-vitamin D_3 production and for storage of 25(OH)D often is not realized. Why would this be? Within the tropics with dual peaks of high UVB during the year, storage of 25(OH)D is never longer than a month on either side of the solstices. At the northern limits of large population settlements at 55°N, duration of storage would need to be at least 6 months for heavily pigmented skin. This is three times longer than the half life of 25(OH)D stored in adipose tissue, as described by Mawer's downward spiral (Mawer and Davies, 2001). This assumes a high level to start with. Because of the selective pressures of dual clines, humans have marginal vitamin D status regardless of location or lifestyle.

In all people, pigmentation increases slowly from birth through childhood and adolescence and peaks during the reproductive years (Robins, 1991). Lighter pigmentation prior to the commencement of reproduction helps to insure adequate vitamin D and calcium status, and normal bone growth and maturation. During the peak reproductive years, protection of folate status is preeminent because of the importance of high levels of cell division. After the reproductive career, pigmentation gradually fades. Thus, individual histories of pigmentation reflect the importance of the selective forces constituting the two clines influencing the evolution of human skin pigmentation.

Robins's (2008) opening quote from Huxley is an interesting 1870 antecedent of Popperian scientific philosophy. From a Kuhnian perspective (Kuhn, 1962), it does not apply very well to the paradigmatic role of vitamin D in the evolution of human skin pigmentation, because anomalies alone are insufficient to overthrow a paradigm without an alternative competing paradigm. As to the observation of a singular, linear correlation of skin pigmentation to UVB in the winter, the vitamin D hypothesis still holds explanatory scope and predictive success. Whatever be its specific actions, vitamin D is the only agent that can account for the observation that light skin is actively selected in areas where UVB is seasonal, absent, or more variable. In areas where UVB is strong and unwavering, dark skin is positively selected.

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