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ORIGINAL CONTRIBUTION

Vitamin D status indicators in indigenous populations in East Africa

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Abstract
Purpose Sufficient vitamin D status may be defined as the evolutionary established circulating 25-hydroxyvitamin D [25(OH)D] matching our Paleolithic genome.
Methods We studied serum 25(OH)D [defined as $25(OH)D_2 + 25(OH)D_3$] and its determinants in 5 East African ethnical groups across the life cycle: Maasai (MA) and Hadzabe (HA) with traditional life styles and low fish intakes, and people from Same (SA; intermediate fish), Sengerema (SE; high fish), and Ukerewe (UK; high fish). Samples derived from non-pregnant adults (MA, HA, SE), pregnant women (MA, SA, SE), mother–infant couples at delivery (UK), infants at delivery and their lactating mothers at 3 days (MA, SA, SE), and lactating mothers at 3 months postpartum (SA, SE). Erythrocyte docosahexaenoic acid (RBC-DHA) was determined as a proxy for fish intake.
Results The mean ± SD 25(OH)D of non-pregnant adults and cord serum were 106.8 ± 28.4 and 79.9 ± 26.4 nmol/L, respectively. Pregnancy, delivery, ethnicity (which we used as a proxy for sunlight exposure), RBC-DHA, and age were the determinants of 25(OH)D. 25(OH)D increased slightly with age. RBC-DHA was positively related to 25(OH)D, notably $25(OH)D_2$. Pregnant MA (147.7 vs. 118.3) and SE (141.9 vs. 89.0) had higher 25(OH)D than non-pregnant counterparts (MA, SE). Infant 25(OH)D at delivery in Ukerewe was about 65 % of maternal 25(OH)D.
Conclusions Our ancient 25(OH)D amounted to about 115 nmol/L and sunlight exposure, rather than fish intake, was the principal determinant. The fetoplacental unit was exposed to high 25(OH)D, possibly by maternal vitamin D mobilization from adipose tissue, reduced insulin sensitivity, trapping by vitamin D-binding protein, diminished deactivation, or some combination.

Keywords 25-Hydroxyvitamin D · Evolution · East Africa · Sunlight exposure · Fish intake · Pregnancy

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Introduction

Homo sapiens is widely considered to originate from East Africa where our genes have probably become selected in a land–water ecosystem [1–3] that is both characterized by abundant tropical sunlight and a diet that is rich in brain-selective nutrients [4–6], including vitamin D [7]. To us, vitamin D is a prohormone that is synthesized in our skin by UV-B exposure and to a lesser extent derives from foods that notably originate from the water ecosystem, such as (fatty) saltwater and freshwater fish [8, 9].

Since the out-of-Africa diaspora, starting some 100,000 years ago [10], we have changed the latitude at which we live. The concomitant lower exposure to UV-B is likely to have favored skin depigmentation [11, 12] as an adaptation to the risk of vitamin D deficiency [13], while other mechanisms, such as mobilization of vitamin D from adipose tissue [14, 15], might have enabled us to preserve an adequate status during winters characterized by negligible UV-B exposure and limited food sources. With the advent of the agricultural revolution (some 10,000 years ago) and notably the industrial revolution (100–200 years ago), we started to jeopardize our evolutionary established optimal

vitamin D status that at first became clearly noted by the epidemic of rickets in the nineteenth century caused by UV-B absorbing air pollution and sunlight avoidance, but also traces back to our changing clothing habits, diminishing fish intake [16], a lifestyle behind glass, fear for skin cancer, sunscreen usage, increasing body weight without periods of prolonged fasting, a high vitamin A status [17], and probably many others.

The current situation is that many populations with typically Western lifestyles have marginal, and often deficient, vitamin D status [18, 19] and that we increasingly recognize that this dinosaur molecule also has many non-classical functions next to the better known functions in systemic calcium homeostasis, bone metabolism, and neuromuscular functioning [20]. Some diseases and conditions, such as rickets, osteomalacia, osteoporosis, and muscle weakness [15], but also influenza [21], have proven relations with vitamin D shortage, but most of them are as yet "associated," including coronary heart disease (hypertension, heart failure), type 2 diabetes, cancer (colorectal, breasts, and prostate), neuropsychiatric diseases (depression, Alzheimer and Parkinson's disease, schizophrenia), obstetric conditions (cesarian section, preeclampsia), infectious diseases (tuberculosis, HIV), and autoimmune diseases (type 1 diabetes, multiple sclerosis, rheumatoid arthritis) [15, 18]. What vitamin D status reduces each of these risks at best experiences an upward trend [15], but is heavily debated in view of the reigning paradigm of "evidence based medicine" [22, 23], which in nutrition is named "evidence based nutrition" and by many is confused with the outcome of randomized controlled trials (RCT) and preferably their meta-analyses [24]. RCTs with single nutrients and hard end points are inherently difficult to perform and have many poorly appreciated limitations [25].

Because of the lack of useful information from RCTs, current establishments of dietary reference intakes are dominated by the "precautionary principle" [26], implying that they are notably inspired by arguments pointing at vitamin D's toxicity such as experienced after the second World War in children [15] and nowadays largely attributed to the Williams syndrome and *CYP24A1* mutations [27]. Other concerns include hypercalcemia/hypercalcuria [18], kidney stone formation [28, 29], vascular calcification [30, 31], retarded growth [15], and the higher mortality rate associated with a vitamin D status ranging from about 75 to 120 nmol/L [15, 32], while a higher risk of skin cancer, melanoma included [33], argues against augmentation of the vitamin D status by sunlight exposure. Taken together, the rapidly expending information from non-RCT-based studies pointing at the benefits of a vitamin D status beyond the currently recognized >50 nmol/L 25-hydroxyvitamin D [25(OH)D] [15, 34] is largely ignored for the establishment of vitamin D's dietary reference intakes [35, 36].

Evolutionary medicine acknowledges that our genome is adapted to a certain environment, which implies that traditionally living people might provide us with valuable information on our evolutionary established vitamin D status. Based on the studies of the hunting–gathering Hadzabe and the pastoral Maasai, we have previously estimated this status at 115 nmol/L 25(OH)D [37]. To further explore the determinants of our ancient vitamin D status, notably during the perinatal period, we went "back to East Africa". We chose five populations in Tanzania, that is, the Hadzabe and Maasai people, and individuals from Same, Sengerema, and Ukerewe. The Hadzabe and Maasai have traditional lifestyles, while the latter two have high intakes of local freshwater fish. Each of these populations has skin type VI. We studied the influence of pregnancy and lactation, ethnicity, age, gender, body mass index (BMI), and fish intake on vitamin D status.

Subjects and methods

Subjects and cultural circumstances

All studied subjects live 2°–4° south of the equator in Tanzania have skin types VI and neither of them use sunscreens. We included 5 different ethnic groups, that is, the Hadzabe, Maasai, and people from Sengerema, Same, and Ukerewe.

Hadzabe are the traditional hunter–gatherers who live in small bands of 10–30 people in the arid bush lands around lake Eyasi. They are nomadic and build their shelters from local wood, leaves, and grass. Hadzabe have no personal belongings. Their shelters are only used for protection during the rain or night. Their diet is composed of fruits, tubers, honey, meat, and an occasional fish from the alkaline lake. Their clothes cover mainly their upper body and upper legs, or just the upper legs (in males). They avoid direct exposure to the fierce sun whenever possible, and most of their activities are planned in the early morning and late afternoon, while spending the middle part of the day sleeping, eating, or talking in a cooler place under a tree or rock [38].

The Nilotic Maasai live in the Maasai Steppe where they used to be pastoralists, but their lifestyle is currently better characterized as settled or semi-nomadic. The selected subjects live near Ruvu and Loliondo. They live in "bomas", which consist of several small mud houses belonging to one family, encircled by thorn bushes to protect cattle and keep out wild animals. Due to their daily activities, Maasai spend most of their days in the sun, wearing clothes that cover mainly their upper body and upper legs. Also the Maasai avoid direct sun exposure whenever possible and prefer a shady place especially during midday. Their diet consists of curdled milk and

Table 1 Anthropometrics, serum 25(OH)D, and red blood cell docosahexaenoic acid of the studied populations

Group	Origin	Age (years)	Weight (kg)	Lenght (m)	BMI (kg/m²)	25(OH)D mean ± SD (n)	25(OH)D range	$25(OH)D_3$ (nmol/L) mean ± SD (n)	$25(OH)D_2$ (nmol/L) median (range)(n)	$25(OH)D_2$ (%) median (range)(n)	<50 nmol/L (%)[a]	<80 nmol/L (%)[a]	RBC-DHA (g%)	Collection date
Non-pregnant														
Male	Hadzabe	33 ± 12 (21)	60.0 ± 6.7 (21)	1.62 ± 0.08 (21)	22.2 ± 2.2 (21)	106.3 ± 29.2 (21)	70.9 - 171.1	102.4 ± 30.2 (21)	5.1 (nd - 16.6)(21)	4.7 (nd - 23.4)(21)	0	24	2.8 ± 0.5 (7)	April 2009 and Aug 2010
Female	Hadzabe	38 ± 9 (4)	55.0 ± 5.6 (4)	1.60 ± 0.04 (4)	21.8 ± 2.6 (4)	123.8 ± 18.0 (4)	104.1 - 143.0	116.5 ± 17.9 (4)	7.6 (6.1 - 8.0)(4)	5.5 (5.4 - 7.7)(4)	0	0	3.8 ± 0.5 (4)	April 2009
Male	Maasai	35 ± 6 (15)	64.0 ± 11.5 (15)	1.74 ± 0.04 (15)	20.3 ± 3.8 (15)	120.9 ± 24.0 (15)	67.3 - 158.0	116.3 ± 23.7 (15)	5.9 (nd - 9.9)(15)	5.0 (nd - 9.4)(15)	0	7	2.4 ± 0.5 (13)	Dec 2008 and June 2009
Female	Maasai	33 ± 12 (20)	54.8 ± 10.1 (20)	1.63 ± 0.06 (20)	20.7 ± 3.6 (20)	118.3 ± 27.4 (20)	57.5 - 167.4	116.5 ± 26.0 (20)	nd (nd - 17.3)(20)	nd (nd - 11.2)(20)	0	10	2.6 ± 0.6 (19)	Dec 2008
Female	Sengerema	30 ± 7 (28)	57.1 ± 9.3 (28)	1.60 ± 0.06 (28)	22.3 ± 3.4 (28)	89.0 ± 22.8 (28)	31.1 - 130.9	73.0 ± 20.6 (28)	16.4 (nd - 31.1)(28)	17.3 (nd - 55.2)(28)	4	39	6.0 ± 1.4 (28)	July 2010
All		33 ± 10 (88)	58.0 ± 9.6 (88)	1.63 ± 0.08 (88)	21.7 ± 3.3 (88)	106.8 ± 28.4 (88)	31.1 - 171.1	99.2 ± 30.8 (88)	6.1 (nd - 31.1)(88)	5.4 (nd - 55.2)(88)	1	22	4.0 ± 1.9 (71)	
All traditional[b]		34 ± 11 (60)	58.4 ± 9.7 (60)	1.65 ± 0.08 (60)	21.5 ± 3.2 (60)	115.1 ± 27.0 (60)	57.5 - 171.1	111.5 ± 26.9 (60)	nd (nd - 17.3)(60)	nd (nd - 23.4)(60)	0	13	2.7 ± 0.7 (43)	
Pregnant														
Pregnant	Maasai	26 ± 8 (29)	55.1 ± 1.9 (15)	1.59 ± 0.07 (27)	20.8 ± 1.9 (15)	147.7 ± 35.0 (30)	65.4 - 218.1	142.5 ± 33.5 (30)	2.6 (nd - 21.0)(30)	1.3 (nd - 12.7) (30)	0	3	3.7 ± 0.5 (30)	December 2008
Pregnant	Sengerema	26 ± 6 (79)	59.4 ± 8.3 (77)	1.59 ± 0.07 (78)	23.6 ± 2.7 (76)	141.9 ± 35.2 (78)	76.3 - 262.4	129.7 ± 32.7 (79)	10.5 (nd - 67.0)(79)	7.2 (nd - 33.1)(79)	0	1	7.2 ± 0.9 (79)	February 2009
Pregnant	Same	26 ± 5 (30)	58.1 ± 8.7 (27)	1.58 ± 0.07 (30)	23.2 ± 3.3 (27)	120.7 ± 28.5 (30)	45.6 - 168.1	120.5 ± 28.5 (30)	nd (nd - 6.1)(30)	nd (nd - 4.8)(30)	3	3	5.1 ± 1.0 (29)	Oct/Nov 2008
All		26 ± 6 (138)	58.6 ± 8.1 (119)	1.58 ± 0.07 (135)	23.2 ± 2.9	138.5 ± 35.0 (138)	45.6 - 262.4	130.5 ± 32.7 (139)	8.1 (nd - 67.0)(139)	nd (nd - 33.1)(139)	1	2	6.0 ± 1.7 (139)	
Delivery														
Mother	Ukerewe	26 ± 7 (25)	54.6 ± 6.0 (25)	1.57 ± 0.04 (25)	22.3 ± 1.9 (25)	135.9 ± 31.8 (25)	87.2 - 195.7	115.8 ± 24.9 (25)	18.0 (nd - 58.3)(25)	15.7 (nd - 30.9)(25)	0	0	8.9 ± 1.2 (25)	July 2010
Infant	Ukerewe	0 (25)	3.0 ± 1.0 (25)			90.6 ± 28.2 (25)	57.6 - 176.0	77.7 ± 23.0 (25)	13.1 (nd - 37.4)(25)	14.7 (nd - 27.1)(25)	4	32	8.0 ± 1.2 (25)	July 2010
Infant	Maasai	0 (6)	3.2 ± 0.6 (5)			64.5 ± 23.4 (6)	26.8 - 89.2	64.5 ± 23.4 (6)	nd (nd - nd)(6)	nd (nd - nd)(6)	17	67	4.2 ± 0.9 (6)	Oct/Nov 2008
Infant	Sengerema	0 (28)	3.0 ± 0.6 (28)			80.3 ± 26.0 (28)	44.9 - 144.2	63.1 ± 24.8 (28)	14.5 (nd - 46.1)(28)	20.2 (nd - 51.4)(28)	7	61	6.6 ± 0.9 (27)	February 2009
Infant	Same	0 (23)	3.2 ± 0.5 (20)			68.6 ± 20.2 (23)	29.3 - 114.0	68.6 ± 20.2 (23)	nd (nd - nd)(23)	nd (nd - nd)(23)	17	74	4.9 ± 1.1 (22)	Oct/Nov 2008
All		0 (82)	3.1 ± 0.5 (78)			79.0 ± 26.4 (82)	26.8 - 176.0	68.4 ± 23.7 (82)	7.5 (nd - 46.1)(82)	9.9 (nd - 51.4)(82)	9	56	6.4 ± 1.7 (80)	
3 days PP														
Mother	Maasai	23 ± 5 (5)	52.4 ± 6.3 (5)	1.59 ± 0.07 (5)	20.9 ± 2.6 (5)	80.1 ± 27.5 (6)	38.8 - 113.2	80.1 ± 27.5 (6)	nd (nd - nd)(6)	nd (nd - nd)(6)	17	50	3.6 ± 0.7 (6)	Oct/Nov 2008
Mother	Sengerema	25 ± 7 (28)	54.8 ± 10.7 (25)	1.56 ± 0.06 (25)	22.4 ± 3.4 (25)	91.0 ± 22.9 (28)	50.7 - 131.1	72.3 ± 23.4 (28)	17.2 (nd - 49.4)(28)	20.4 (nd - 47.2)(28)	0	39	7.1 ± 1.1 (28)	March 2009
Mother	Same	24 ± 7 (22)	57.7 ± 7.0 (17)	1.54 ± 0.04 (20)	24.2 ± 2.0 (17)	97.6 ± 29.3 (23)	35.8 - 169.6	97.6 ± 29.3 (23)	nd (nd - nd)(23)	nd (nd - nd)(23)	4	26	4.5 ± 1.0 (23)	Oct/Nov 2008
All		25 ± 7 (55)	55.6 ± 9.1 (47)	1.55 ± 0.06 (50)	22.9 ± 3.1 (47)	91.5 ± 26.8 (57)	33.0 - 169.6	82.3 ± 28.8 (57)	nd (nd - 49.4)(57)	nd (nd - 47.2)(57)	4	30	5.7 ± 1.8 (57)	
3 months PP														
Mother	Sengerema	24 ± 6 (30)	54.7 ± 9.8 (30)	1.56 ± 0.06 (30)	22.3 ± 3.0 (30)	99.3 ± 20.9 (30)	41.8 - 138.7	86.9 ± 17.5 (30)	12.7 (nd - 30.3)(30)	12.8 (nd - 22.3)(30)	3	20	6.5 ± 0.7 (30)	February 2009
Mother	Same	25 ± 4 (23)	53.0 ± 12.4 (23)	1.55 ± 0.06 (23)	22.1 ± 4.6 (23)	81.8 ± 23.5 (28)	48.1 - 160.3	80.5 ± 23.3 (28)	nd (nd - 10.8)(28)	nd (nd - 22.4)(28)	11	50	3.5 ± 0.8 (28)	Oct/Nov 2008
All		24 ± 5 (53)	54.0 ± 11.0 (53)	1.56 ± 0.06 (53)	22.2 ± 3.7 (53)	90.8 ± 23.7 (58)	48.1 - 160.3	83.8 ± 20.6 (58)	5.5 (nd - 30.3)(58)	5.7 (nd - 22.4)(58)	7	37	6.5 ± 0.7 (58)	

Data are in mean ± SD (*n*) or median (range)

BMI body mass index, *GA* gestational age, *RBC-DHA* red blood cell docosahexaenoic acid, *PP* postpartum

25(OH)D, 25-hydroxyvitamin D, defined as $25(OH)D_2 + 25(OH)D_3$

[a] Percentage of subjects within the investigated population with 25(OH)D <50 or <80 nmol/L

[b] Joint data of the Hadzabe and Maasai

meat, which has recently become replenished with ugali (corn porridge) [39].

The subjects from Sengerema, Same, and Ukerewe are Bantu people. Most of them are of low socioeconomical background. Sengerema is located on the shores of Lake Victoria, the Same people live close to the Pare Mountains, and Ukerewe is an island in Lake Victoria. Their incomes derive from fisheries (Sengerema and Ukerewe) or agriculture (Same). Similar to the other ethnic groups, they avoid direct sun light exposure as much as possible, although most of the work is done outdoors and likewise their social lives happen outside. Their clothes cover basically the whole body apart from their lower arms and faces. Their diets are mainly composed of vegetables, beans, and fruits with ample ugali, rice, and chapati (corn–wheat pancakes). The people in Sengerema and Ukerewe have high freshwater fish intakes, while the Same people eat some meat and fish.

Anthropometric data were collected from measurements or questionnaires in Kiswahili by two of us (MFL and RSK). All subjects gave their informed consent. The study was approved by the National Institute for Medical Research in Dar-es-Salaam (NIMR/HQ/R.8a/Vol.IX/145, dated June 16, 2003 and NIMR/HQ/R.8a/Vol.IX/800, dated April 8, 2009, and the extension of ethical clearance NIMR/HQ/R.8c/Vol.II/05) and was in agreement with the Declaration of Helsinki 1975 as revised in 2000.

Serum 25(OH)D

About 4 mL of venous blood or cord blood was collected (BD Vacutainer, Plymouth, UK) by venipuncture in coagulation tubes. The samples were allowed to coagulate for 30 min at ambient temperature in the dark, stored at 4 °C in the dark, and processed within 2 h after collection, on the spot. The serum was isolated by centrifugation and stored at −20 °C. All samples were transported on ice to the University Medical Center Groningen (The Netherlands). Serum $25(OH)D_2$ and $25(OH)D_3$ [together referred to as 25(OH)D)] were determined by isotope dilution-online solid-phase extraction liquid chromatography–tandem mass spectrometry (ID-XLC-MS/MS). Briefly, serum was pretreated by the use of a protein disrupting buffer to dissociate the binding of 25(OH)D to the vitamin D-binding protein. Hexadeuterated $25(OH)D_3$ served as an internal standard. The calibration graph was prepared from dialyzed human plasma that was spiked with 0–280 nmol/L $25(OH)D_2$ and $25(OH)D_3$. Online extraction was performed as previously described [40]. The mass spectrometric conditions were essentially as described by Maunsell et al. [41]. The method specifications were level of quantification (LOQ) 4.0 nmol/L; intra-assay CV < 7.2 % and inter-assay CV < 14.1 % for three concentrations between 20 and 150 nmol/L; recovery 93–98 %; linearity $r^2 = 0.9972$. Accuracy was secured by the use of reference material from the National Institute of Standards & Technology (Gaithersburg, MD).

Erythrocyte docosahexaenoic acid

We used red blood cell (RBC) docosahexaenoic acid (DHA) as a proxy for fish intake [42]. For RBC-DHA analysis, we collected about 4 mL EDTA-anticoagulated venous blood of the adults and 4 mL EDTA-cord blood at delivery (BD Vacutainer, Plymouth, UK). The samples were stored at 4 °C in the dark and processed within 2 h after collection. RBC were isolated by centrifugation and washed three times with 0.9 % NaCl. After washing, 200 µL of the RBC suspension was transferred to a Teflon-sealable Sovirel tube containing 2 mL of methanol/6 mol/L HCl (5:1 v/v), 1 mg butylated hydroxytoluene (antioxidant), and 50 µg 17:0. All samples were transported at ambient temperature to the University Medical Center Groningen (The Netherlands) for fatty acid analysis. Analyses of fatty acid methyl esters were performed by capillary gas chromatography/flame ionization detection according to previously described procedure [43]. RBC-DHA contents were expressed in g/100 g fatty acids (g%).

Data evaluation and statistics

Vitamin D status was reported as 25(OH)D [defined as $25(OH)D_3 + 25(OH)D_2$], $25(OH)D_3$ (mean ± SD), and $25(OH)D_2$ [median, range, percentage of 25(OH)D]. Commonly used cutoff values of 25 [44], 50 [15, 45], and 80 nmol/L 25(OH)D [46, 47] were applied to calculate the percentage subjects with vitamin D deficiency (<25 nmol/L), vitamin D insufficiency (25–50 nmol/L), hypovitaminosis D (50–80 nmol/L), vitamin D sufficiency (80–250 nmol/L), and risk of toxicity (>250 nmol/L).

Statistical analyses were performed with SPSS version 18.0 (SPSS Inc, Chicago, IL, USA). Multivariate analyses were carried out by multiple linear regression, which allowed us to study the relationships between 25(OH)D and pregnancy, ethnicity, RBC-DHA, age, gender, and BMI. We used the forced enter model. Between-group differences were studied with a one-way ANOVA with a post hoc Student's t test at $p < 0.05$. All were corrected for type-1 errors. Scatter plots and equations were made by the aid of curve estimation. $25(OH)D_2$ and $25(OH)D_3$ values below 5 nmol/L were qualified as not detectable.

Results

Study group

We included 367 adults and 82 infants. The study group was composed of 88 males and non-pregnant females: 25

Table 2 Determinants of vitamin D status

Determinant	25(OH)D			$25(OH)D_2$			$25(OH)D_3$		
	B	95 % CI	*p*	*B*	95 % CI	*p*	*B*	95 % CI	*p*
Pregnant[a]	40.4	(28.80 to 52.02)	0.000	−2.8	(−6.08 to 0.51)	0.097	43.4	(32.22 to 54.56)	0.000
Maasai[c]	28.3	(11.26 to 45.37)	0.001	−5.9	(−10.75 to 1.07)	0.017	34.1	(17.73 to 50.54)	0.000
Delivery[a,b]	28.9	(11.09 to 46.72)	0.002	1.1	(−3.94 to 6.18)	0.663	27.4	(10.3 to 44.56)	0.002
RBC-DHA (g%)	5.2	(1.77 to 8.61)	0.003	1.7	(0.7 to 2.65)	0.001	3.6	(0.27 to 6.85)	0.034
Hadzabe[c]	34.6	(10.94 to 58.30)	0.004	−4.8	(−11.49 to 1.96)	0.164	39.4	(16.57 to 62.14)	0.001
Age (years)	0.6	(0.13 to 1 08)	0.014	0.1	(−0.04 to 0.23)	0.157	0.5	(0.02 to 0.94)	0.040
Male gender	−10.7	(−27.46 to 6.13)	0.212	−1.3	(−6.03 to 3.51)	0.604	−9.6	(−25.75 to 6.55)	0.243
BMI (kg/m^2)	−0.7	(−1.75 to 0.41)	0.222	−0.2	(−0.47 to 0.14)	0.288	−0.5	(−1.56 to 0.51)	0.317
3 months postpartum[a]	4.6	(−8.48 to 17.69)	0.489	−0.7	(−4.4 to 3.03)	0.718	5.1	(−7.48 to 17.69)	0.425
3 days postpartum[a]	2.3	(−11.71 to 16.28)	0.748	1.5	(−2.49 to 5.46)	0.463	0.6	(−12.84 to 14.09)	0.927
Same[c]	−1.1	(−13.12 to 10.89)	0.855	−9.9	(−13.31 to −6.5)	0.000	8.7	(−2.9 to 20.2)	0.141

25(OH)D, 25-hydroxyvitamin D, defined as $25(OH)D_2 + 25(OH)D_3$

CI confidence interval, *BMI* body mass index

[a] Non-pregnant as reference

[b] Only applicable to Ukerewe

[c] Sengerema as reference

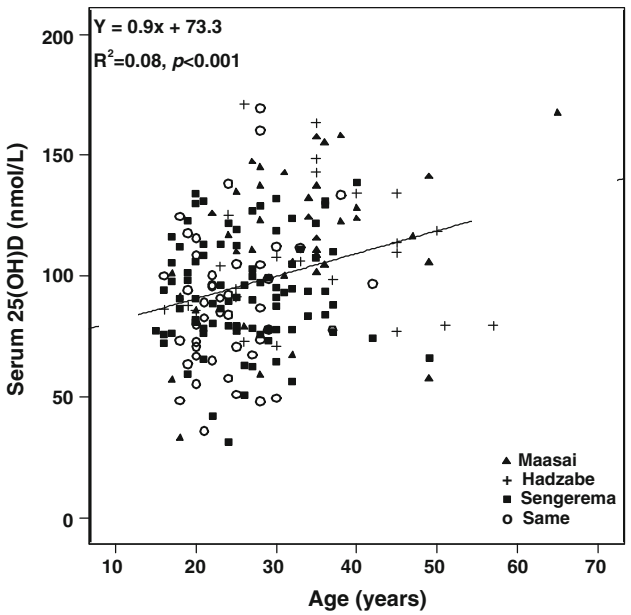

Fig. 1 Age dependency of 25-hydroxyvitamin D in non-pregnant adults in Tanzania. 25(OH)D, 25-hydroxyvitamin D; *filled triangle*, Maasai; *plus symbol*, Hadzabe; *filled square*, Sengerema; *open circle*, Same

Hadzabe (21 male and 4 female), 35 Maasai (15 male and 20 female), and 28 subjects from Sengerema (all female). Secondly, we included 139 pregnant women: 30 Maasai, 79 from Sengerema, and 30 from Same. A third group was composed of 25 mother–infant couples at delivery (all from Ukerewe). The fourth group was composed of 57 mother–infant couples of which we obtained cord blood at delivery and maternal blood at 3 days postpartum: 6 Maasai, 28 from Sengerema, and 23 from Same. Finally, we included 58 mothers at 3 months postpartum: 28 from Same and 30 from Sengerema. All infants were born at term. The anthropometric data, 25(OH)D (nmol/L) and RBC-DHA contents (in g/100 g fatty acids; g%) are presented in Table 1.

In all groups, 25(OH)D was remarkably high: non-pregnant adults 106.8 ± 28.4, pregnant women 138.5 ± 35.0, mothers at delivery 135.9 ± 31.8, cord blood 79.0 ± 26.4, mothers at 3 days postpartum 91.5 ± 26.8, and mothers at 3 months postpartum 90.8 ± 23.7 nmol/L. None of the subjects had 25(OH)D below 25 nmol/l. Of the entire non-pregnant non-lactating group, 1 and 13 % had 25(OH)D levels <50 and <80 nmol/L, respectively. Also, the percentages of pregnant women with 25(OH)D < 50 nmol/L (1 %) and <80 nmol/L (2 %) were low, although during lactation, higher percentages were found (7 % <50 nmol/L and 37 % <80 nmol/L). At birth, 9 % of the infants showed 25(OH)D < 50 nmol/L and 56 % <80 nmol/L. We found that $25(OH)D_2$ was significantly lower as compared to the $25(OH)D_3$ in all groups (all comparisons $p < 0.01$).

Indicators of serum 25(OH)D

Multivariate linear regression analysis, in which only adults were included, revealed that pregnancy ($p < 0.001$), delivery ($p = 0.002$), ethnicity ($p = 0.001$ and $p = 0.004$ for Maasai and Hadzabe, respectively), RBC-DHA ($p = 0.003$), and age ($p = 0.014$) related positively to the

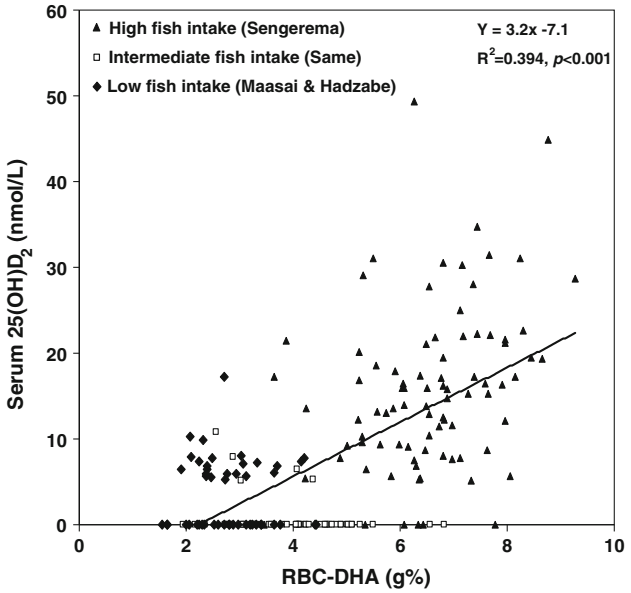

Fig. 2 Relation of serum 25-hydroxyvitamin D_2 with red blood cell docosahexaenoic acid in non-pregnant adults in Tanzania. 25(OH)D_2, 25-hydroxyvitamin D_2; RBC-DHA, red blood cell docosahexaenoic acid (a proxy for fish intake in g/100 g fatty acids; g%). Data were pooled for populations with no or low fish intakes (*filled diamond*; Hadzabe, Maasai), intermediate fish intake (*open square*; Same), and those with high fish intakes (*filled triangle*; Sengerema)

25(OH)D concentration (Table 2). No relationships between 25(OH)D and gender or BMI were found. Multivariate linear regression models with backward regression revealed similar results.

We also investigated the determinants of 25(OH)D_2 and 25(OH)D_3 separately. RBC-DHA ($p < 0.001$) and ethnicity (Same $p < 0.001$ and Maasai $p = 0.017$) were determinants of 25(OH)D_2 (Table 2), whereas pregnancy ($p < 0.001$), delivery ($p < 0.002$), ethnicity ($p < 0.001$ and $p = 0.001$ for Maasai and Hadzabe, respectively), RBC-DHA ($p = 0.034$), and age ($p = 0.040$) were determinants of 25(OH)D_3 (Table 2). We investigated the most relevant determinants of 25(OH)D separately in non-pregnant adults.

Age and ethnicity

Multivariate linear regression analysis, using all adult samples, showed a positive relation with age. Figure 1 shows the relationship between age and 25(OH)D in the non-pregnant adults. The regression line was: 25(OH)D (in nmol/L) = 0.9*age (in years) + 73.3; $R^2 = 0.08$, $p < 0.001$. It should be noted that the 25(OH)D range was wide and the number of subjects in the higher age group was small. Removal of the single subject at age 65 conserved the positive relationship [25(OH)D (in nmol/L) = 0.8*age (in years) + 75.8; $R^2 = 0.062$, $p < 0.001$]. Age and 25(OH)D_3 proved positively related, but this was not the case for age versus 25(OH)D_2 (Table 2).

In the multivariate models, Maasai and Hadzabe origin related positively to 25(OH)D and 25(OH)D_3. Maasai and Hadzabe (males and females taken together) had higher 25(OH)D than Sengerema women (p values <0.001 and 0.016, respectively) (Table 1). 25(OH)D_3 of the Maasai and Hadzabe was also higher as compared to Sengerema (both $p < 0.001$), while 25(OH)D_2 was higher in Sengerema than in Maasai and Hadzabe (both $p < 0.001$).

Fish consumption

Serum levels of 25(OH)D, 25(OH)D_2, and 25(OH)D_3 related positively to the RBC-DHA content (Tables 1, 2). The relation of 25(OH)D_2 with RBC-DHA in non-pregnant adults is shown in Fig. 2 [25(OH)D_2 (in nmol/L) = 3.2*RBC-DHA (in g%) −7.1; $R^2 = 0.394$, $p < 0.001$]. This relation was notably on account of Sengerema (high fish intake from Lake Victoria), but was not found in Same (intermediate fish intake, different location) and the Maasai and Hadzabe. Also 25(OH)D_3 correlated positively with RBC-DHA in non-pregnant adults (Table 2, $p = 0.034$).

Pregnancy, delivery, and lactation

In the multivariate models, pregnancy proved positively related to both 25(OH)D and 25(OH)D_3. The 25(OH)D concentrations of Sengerema, Same, and Maasai women during pregnancy are graphically depicted in Fig. 3a–c, respectively. Data of the three trimesters were compared with those of non-pregnant women and counterparts at 3 days postpartum. Data from the women at 3 days postpartum were compared with those of women at 3 months postpartum. In the largest group of Sengerema women (Fig. 3a), 25(OH)D of women in the second and third trimesters were higher compared with counterparts who were non-pregnant or at 3 days postpartum (both $p < 0.001$). Higher 25(OH)D in the second and third trimester compared to 3 days postpartum was confirmed in the smaller data sets from Same (only third trimester) and the Maasai (Fig. 3b, $p = 0.009$; and Fig. 3c, $p = 0.004$ and $p < 0.001$, respectively). Pregnant (third trimester) Maasai exhibited higher 25(OH)D than non-pregnant Maasai ($p = 0.015$). Neither of the pregnant groups exhibited relationships of 25(OH)D with gestational age. We found that 25(OH)D and 25(OH)D_3 did not differ between 3 days and 3 months postpartum.

Mother–infant 25(OH)D relations

For the samples collected in Ukerewe at delivery, we found a linear relationship between maternal and infant 25(OH)D

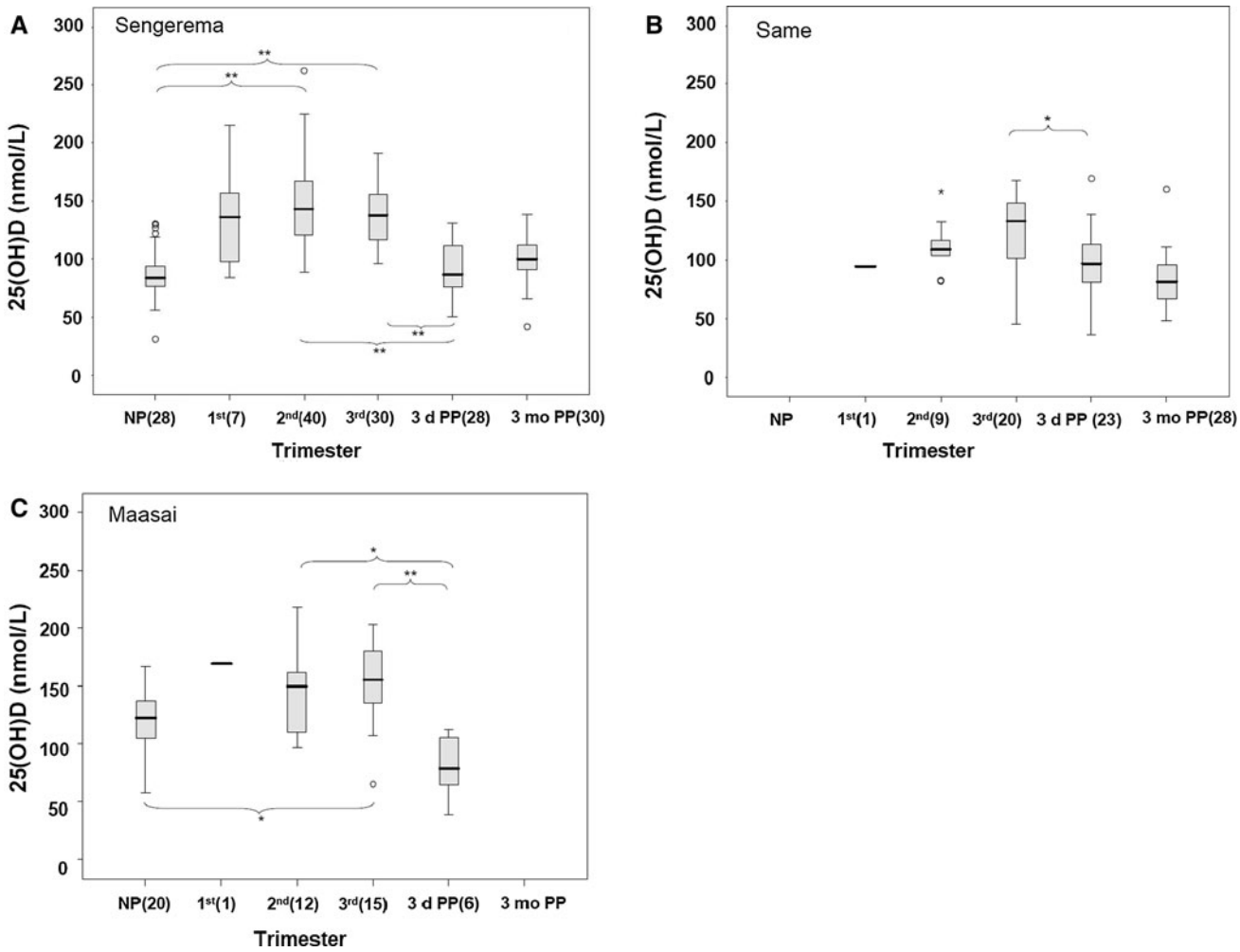

Fig. 3 Serum 25-hydroxyvitamin D of non-pregnant females, pregnant females in their first, second, and third trimester, and lactating mothers at 3 days and 3 months postpartum in Sengerema (**a**), Same (**b**), and the Maasai (**c**). *25(OH)D* 25-hydroxyvitamin D, *NP* non-pregnant, *3 d PP* 3 days postpartum, *3 mo PP* 3 months postpartum. **significance at $p < 0.001$, *significance at $p < 0.05$

concentrations (not depicted; graph forced through zero; maternal 25(OH)D = 0.65* infant 25(OH)D; $R^2 = 0.125$, $p = 0.022$). There was also a relationship between the maternal 25(OH)D at 3 days postpartum and infant 25(OH)D at delivery for the joint data of Maasai, Sengerema, and Same mother–infant pairs [maternal 25(OH)D = 0.78* infant 25(OH)D; $R^2 = 0.330$, $p < 0.001$].

Discussion

We investigated the determinants of vitamin D status across the life cycle in various East African populations. Among the most remarkable findings were (1) the high mean 25(OH)D levels of the entire study population (in nmol/L: non-pregnant adults 106.8, pregnant 138.5, mothers at delivery 135.9, cord blood 79.0, mothers at 3 days postpartum 91.5, and mothers at 3 months postpartum 90.8; Table 1); (2) the higher mean 25(OH)D concentrations in pregnant women in the second and third trimesters compared to non-pregnant counterparts (138.5 vs. 106.8 nmol/L; Table 1); and (3) the rapid postpartum fall of 25(OH)D at <3 days after delivery in all 3 groups (Table 1; Fig. 3a–c). The mean 25(OH)D concentration of all non-pregnant adults in this study was similar to the vitamin D status of Brazilian children living close to the equator [106 and 108 nmol/L in summer (September–March) and winter (April–August), respectively; [48]]. We encountered no cases with 25(OH)D <25 nmol/L (Table 1), which is the widely used cutoff for vitamin D deficiency [44]. In contrast, vitamin D deficiency is prevalent in many Western countries among elderly but also in younger age groups [49]. The highest value was 262 nmol/L in a pregnant woman in Sengerema. This value

is just above the conservative 250 nmol/L cutoff for toxicity [44].

Fish consumption

Fish consumption constitutes a major source of dietary vitamin D in many countries [18, 50, 51]. We have no information on the vitamin D contents of the fish eaten by the study populations, but estimated the amounts that should be consumed to reach the current vitamin D status. Hollis et al. [52] observed that a daily 4,000 IU supplement during the last two trimesters of pregnancy increased the 25(OH)D levels from 58.2 to 111.0 nmol/L, which implies an increment of 0.53 nmol/L per μg supplemental vitamin D. Using this figure and the currently encountered mean of 138.5 nmol/L 25(OH)D during pregnancy in Tanzania, one may estimate that these women would have to consume 3.4 kg/day of pelagic fish (mean of 7.72 μg vitamin D/100 g; [9]), if their vitamin D status derived solely from the principal dietary source that is available to humans. With the more often used increment of 0.7 nmol/L 25(OH)D per μg supplemental vitamin D for non-pregnant subjects [47], the presently studied non-pregnant subjects with a mean 25(OH)D of 106.6 nmol/L would have to consume 2.0 kg fish/day.

To further investigate the contribution of vitamin D in fish, we used the RBC-DHA content as a proxy for fish intake because aquatic food is the principal determinant of a high DHA status. In agreement with this notion, RBC-DHA was higher in the sequence Ukerewe, Sengerema (high fish intake) > Same (intermediate fish intake) > Maasai, Hadzabe (no or little fish intake) [53, 54] (Table 1). We found relations of $25(OH)D_2$ with RBC-DHA (Fig. 2) and of $25(OH)D_3$ with RBC-DHA (Table 2). A relationship between n-3PUFA in RBC and 25(OH)D was previously reported in South Korea [55]. The high vitamin D status in Tanzania and the limited contribution of 25(OH)D that might be expected from the consumption of freshwater fish [9] or milk render it likely that cutaneous vitamin D_3 synthesis overshadows its dietary intake. Since plankton and zooplankton may contain both ergosterol (provitamin D_2) and 7-dehydroxycholesterol (provitamin D_3), it is conceivable that in these populations, some $25(OH)D_2$ and $25(OH)D_3$ derive from fish. However, the Same people exhibited the lowest $25(OH)D_2$ levels, although they have intermediate fish intake with clearly higher median RBC-DHA than the Maasai and Hadzabe who have no or little fish intake (Table 1). It is therefore possible that other vitamin D_2 sources contributed to the high $25(OH)D_2$ levels in the people from Sengerema and Ukerewe, such as fungi, yeasts, or algae [56, 57], and that these sources notably enter the human food chain in the Lake Victoria environment.

Ethnicity

The Maasai and Hadzabe in the non-pregnant group had higher 25(OH)D compared with Sengerema (Table 1). The underlying association of vitamin D status with ethnicity (Table 2) is most probably caused by different cultural and behavioral habits, notably exposure time to the abundant sunlight and clothing habits, as also suggested by their distinct 25(OH)D versus RBC-DHA relations (Table 1). The observed between-group differences are unlikely to be explained by season, since there is little variation in seasonal sunshine around the equator (see also limitations). The Maasai and Hadzabe are pastoralist and hunter–gatherers, respectively, who, because of their daily activities, spend the major part of the day outdoors while they also wear less clothes compared to the Bantu populations in Sengerema, Same, and Ukerewe [37].

Pregnancy

Pregnant women in Sengerema and pregnant Maasai had higher 25(OH)D concentrations compared to non-pregnant counterparts (Fig. 3a, c, respectively). This observation contrasts with data from other studies reporting unaltered or even lower 25(OH)D levels during pregnancy [58–61]. One longitudinal study with pregnant women taking a 10 μg vitamin D/day prenatal supplement for ≥9 months showed higher 25(OH)D in the third trimester (about 118 nmol/L) as compared to non-pregnant controls (80 nmol/L) [62]. On the other hand, a study with Saudi women showed a 25(OH)D decrease during pregnancy from 54 ± 10 nmol/L in the first trimester to 33 ± 8 nmol/L by the third trimester and 35 ± 11 nmol/L at term [63]. It is well known that during pregnancy, the circulating vitamin D-binding protein (DBP) level increases as a response to the increasing estrogen concentrations [62, 64]. In the light of the circulating volume expansion in pregnancy and the higher serum vitamin D-binding capacity, the presently encountered higher 25(OH)D concentration represents a genuinely increased circulating 25(OH)D pool size, which occurs in the context of continuing 25(OH)D losses via transplacental passage and with no indications of altered dietary habits, sunlight exposure, or confounding influence of season. Reduced conversion to $1,25(OH)_2D$ is unlikely since $1,25(OH)_2D$ levels double early in pregnancy to remain at these levels until delivery, which has been attributed to an increased production rate [15, 59].

In search of an explanation for the higher circulating 25(OH)D, we consider it likely that the 25(OH)D surplus originates from enhanced vitamin D mobilization from adipose tissue stores. Moderate weight loss without changing vitamin D intake or sun exposure is indeed associated with higher 25(OH)D levels [14, 15]. In late

pregnancy, enhanced adipose tissue mobilization is caused by the reduced insulin sensitivity [65]. Any mobilized vitamin D from adipose tissue might become trapped in the circulation by DBP, of which the concentration increases during pregnancy. In this context, the above-mentioned 25(OH)D increase to 118 nmol/L in the third trimester by Cross et al. [62] might find its origin in improved vitamin D stores due to the supplement, while the decreasing 25(OH)D levels from 54 to 35 nmol/L in the pregnant Saudi women [63] are likely to have occurred in the context of seriously depleting maternal vitamin D stores.

Finally, it is also possible that we are dealing with increasing 25(OH)D and $1,25(OH)_2D$ half lives. Both of these become deactivated by 24-hydroxylation [27], which is catalyzed by 24-hydroxylase (CYP24A1). It has been shown that the placental CYP24A1 gene is downregulated by promoter methylation, suggesting epigenetic regulation mechanisms to maximize the availabilities of 25(OH)D and $1,25(OH)_2D$ at the feto-maternal interface [66]. Whatever the underlying mechanism, it seems that pregnancies in Tanzania occur at higher exposure to 25(OH)D when compared to most Western countries with as yet poorly known effects on the mother, development of the fetal brain [67, 68] and immune system [69], childhood respiratory tract infections [70], and bone health at elderly age [71].

Postnatal maternal 25(OH)D decrease

We found that the maternal 25(OH)D levels decreased sharply after delivery. This observation is in line with previous studies showing a rapid 25(OH)D decline after delivery [59, 72]. Explanatory factors might be the rapid return to the pre-pregnancy circulating volume and discontinued 25(OH)D losses by transplacental passage, but these would rather cause a 25(OH)D increase. The 25(OH)D decline might relate to the rapidly dropping estrogen levels and a concomitant drop of the DBP concentrations [72] resulting in return to the pre-pregnancy serum–adipose tissue vitamin D equilibrium. The half-life of DBP is indeed very short (2.5–3 days) [64]. However, at any time, only 1–2 % of the DBP sterol-binding sites are occupied [64], which implies that the vitamin D and 25(OH)D-binding capacities are huge, and that the observed 19–46 % 25(OH)D decline in the various populations seems unlikely to be explained by a decreasing 25(OH)D-binding capacity. The very rapid postnatal maternal 25(OH)D decline does not comply with a 25(OH)D half-life of 2–3 weeks [73] either, suggesting that other mechanisms might also be operational. It is also possible that the decline might in part relate to the well-known rapid postnatal restoration of maternal insulin sensitivity, which abolishes the continuous adipose tissue lipolysis of the third trimester [65], with a concomitant decline of vitamin D mobilization and subsequent 25(OH)D synthesis. Other likely options are losses of both vitamin D and 25(OH)D via breast-feeding [74], while a rapid postnatal abolition of the epigenetic silencing of CYP24A1 with concomitant augmentation of 25(OH)D deactivation should also be considered.

Limitations

This study has many limitations. First, we had no objective measurements that could, beyond doubt, exclude the influence of many other personal and environmental factors determining circulating 25(OH)D [75]. Although we documented the inclusion dates, we did not perform statistical analysis with season as a variable. The short and long rainy periods might be more important to vitamin D synthesis capacity: these are variable, last for minutes or hours rather than days, and are interspersed with sunny days and periods. We also had no information on the actual amounts of vitamin D in the diet, fish included. Second, the 25(OH)D data during pregnancy did not derive from the monitoring of pregnant women, although we interpreted them in terms of a longitudinal study. Because of logistic reasons, the local execution of a longitudinal study is difficult, but will probably provide more detailed insight because of its higher parametric power.

Conclusions

Our data suggest that the ancient vitamin D status was much higher than presently encountered in Western countries and that abundant sunlight was the principal source. Our ancient vitamin D status might have been higher during pregnancy and have normalized rapidly after delivery, suggesting that the feto-maternal unit was exposed to an even higher vitamin D status than encountered in non-pregnant conditions. The current about 115 nmol/L 25(OH)D is likely to be a target for Western populations, but needs to be investigated in more detail for its long-term safety because of the many other lifestyle changes that have occurred since the agricultural and notably industrial revolutions [37, 76].

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Conflict of interest There are no conflicts of interest.

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