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Short Communication

Traditionally living populations in East Africa have a mean serum 25-hydroxyvitamin D concentration of 115 nmol/l

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Abstract

Cutaneous synthesis of vitamin D by exposure to UVB is the principal source of vitamin D in the human body. Our current clothing habits and reduced time spent outdoors put us at risk of many insufficiency-related diseases that are associated with calcaemic and non-calcaemic functions of vitamin D. Populations with traditional lifestyles having lifelong, year-round exposure to tropical sunlight might provide us with information on optimal vitamin D status from an evolutionary perspective. We measured the sum of serum 25-hydroxyvitamin D_2 and D_3 (25(OH)D) concentrations of thirty-five pastoral Maasai (34 (SD 10) years, 43 % male) and twenty-five Hadzabe hunter–gatherers (35 (SD 12) years, 84 % male) living in Tanzania. They have skin type VI, have a moderate degree of clothing, spend the major part of the day outdoors, but avoid direct exposure to sunlight when possible. Their 25(OH)D concentrations were measured by liquid chromatography–MS/MS. The mean serum 25(OH)D concentrations of Maasai and Hadzabe were 119 (range 58–167) and 109 (range 71–171) nmol/l, respectively. These concentrations were not related to age, sex or BMI. People with traditional lifestyles, living in the cradle of mankind, have a mean circulating 25(OH)D concentration of 115 nmol/l. Whether this concentration is optimal under the conditions of the current Western lifestyle is uncertain, and should as a possible target be investigated with concomitant appreciation of other important factors in Ca homeostasis that we have changed since the agricultural revolution.

Key words: **25-Hydroxyvitamin D: Evolution: Maasai: Hadzabe**

Evolutionary medicine tells us that our genes have been selected in an environment in which we successfully exploited hunting and gathering strategies for survival and procreation[1,2]. Since the agricultural (about 10 000 years ago) and industrial (100–200 years ago) revolutions, we have, however, drastically changed our conditions of existence and continue to do so with still increasing pace. These changes cause a conflict with our slowly adapting genome that basically still resides in the Paleolithic era[3]. Examples of such changes can be found in our current dietary composition, reduced physical activity, abnormal microbial flora, lack of sleep and environmental pollution[4]. These changes are intimately related and result in a state of homeostatic imbalance that is likely to be based on the pandemic of affluent diseases[3].

Other prominent changes include our current clothing habits and reduced time spent outdoors. The ensuing lack of exposure to direct sunlight negatively affects our vitamin D status, and thereby adds to the current state of homeostatic imbalance. Cutaneous synthesis of vitamin D by exposure to UVB is our principal source of vitamin D, which in reality is a prohormone with both rapid and slow effects and which also controls the expression of about 3 % of our genes[5]. The importance of vitamin D became apparent, for instance, from the loss of skin pigmentation in populations who migrated from Africa to settle at higher latitudes since about 100 000 years ago[6]. Skin depigmentation is likely to be an adaptation that enables vitamin D synthesis at low UVB exposure[7].

The current low vitamin D status of populations living in affluent countries is implicated in many diseases that are related to the calcaemic and non-calcaemic functions of vitamin D, including rickets, osteomalacia, osteoporosis, CHD (hypertension), cancer (colorectal cancer, breast cancer and prostate

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; nd, not detectable.

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cancer), infectious diseases (tuberculosis, influenza and HIV) and autoimmune diseases (type 1 diabetes, multiple sclerosis and rheumatoid arthritis)[8]. The optimal vitamin D status to prevent or treat all of the aforementioned diseases is intensively debated[9]. An important clue may come from the study of populations with traditional lifestyles who live under similar conditions of existence as those that shaped our genome to what it currently is. In the present study, we measured the vitamin D status of two traditionally living East African tribes. The members of these two tribes are exposed year-round to the natural abundance of tropical sunlight. The sum of serum 25-hydroxyvitamin D_2 and D_3 (25(OH)D) concentrations might provide us with information on a favourable vitamin D status for overall health from a Darwinian perspective[9].

Subjects and methods

Subjects and cultural circumstances

We studied two traditional tribes, Maasai and Hadzabe, with hunter–gatherer-like lifestyles. Both tribes live 2–4° south of the equator in Tanzania. They have skin types VI and neither of the tribes uses sunscreens. Traditionally living tribes are difficult to access and the members are often reluctant to participate, thus proper sample conservation requires a swift operation. Several Maasai bomas and two Hadzabe bands (see below) were randomly selected. Subjects were recruited in their home 'villages'. Once the tribe members agreed to participate in the present study, all adults who met the inclusion criteria were included. The inclusion criteria were apparently healthy adults (>16 years old), non-pregnant and non-lactating. We aimed at a minimum of thirty participants per tribe and succeeded in the inclusion of thirty-five Maasai and twenty-five Hadzabe. We believe that the selected members were representative of their tribes.

The Nilotic Maasai live in the Maasai Steppe where they used to be pastoralists, but their lifestyle is currently better characterised as settled or semi-nomadic. The current Maasai population in Tanzania is estimated to comprise about 50 000 individuals[10]. The selected subjects live near Ruvu (latitude −4·1°, longitude 37·5°, mid-Tanzania) and Loliondo (latitude −2·1°, longitude 35·5°, North Tanzania). They live in 'bomas', which consist of several small mud houses belonging to one family, encircled by thorn bushes to protect cattle and to keep out wild animals. The daily life of Maasai is typically situated outside of the boma. Adolescent males take care of the cattle, while the young (semi) adult warriors typically hang around in the area looking after their families and protecting their cattle. Elder males take political decisions and congregate outside in small groups. Young and adult females milk the cattle, collect firewood and prepare food. 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. It is important to note that, whenever possible, they avoid direct exposure to the sun and prefer a shady place, especially during midday. Their diet consists of curdled milk and meat, which has recently become replenished with ugali (maize porridge)[11].

Hadzabe are traditional hunter–gatherers. The current tribe is composed of about 1000 individuals. The present study was conducted among the 300–400 individuals who still live as hunter–gatherers[12]. They live in small bands of ten to thirty people in arid bush lands around Lake Eyasi (latitude −3·7°, longitude 35·0°, North Tanzania). They are nomadic and build their shelters from local wood, leaves and grass. They move their camp from time to time to find better foraging areas. Hadzabe have no personal belongings. Their shelters are only used during the rain or night. Their diet is composed of fruits, tubers, honey, meat and an occasional fish from the alkaline lake. Fruits and tubers are gathered by females and children. Men gather honey and fruits, and hunt small animals in the wet season and bigger game in the dry season. Their clothes cover mainly their upper body and upper legs, or just the upper legs (in males). Hadzabe spend most of their days in the sun. Similar to the Maasai tribe, 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[13].

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

Samples and analyses

About 4 ml of venous blood were collected (BD Vacutainer) by venepuncture. The blood samples were allowed to coagulate for 30 min at ambient temperature in the dark, and subsequently stored at 4 °C in the dark. Within 2 h after collection, serum was isolated by centrifugation and stored at −20 °C on the spot. 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 referrede to as 25(OH)D) concentrations were determined by isotope dilution–online solid-phase extraction liquid chromatography–tandem MS. The outcome was summed to obtain 25(OH)D. Briefly, serum was pretreated using a protein disruption buffer to dissociate the binding of 25(OH)D to the vitamin D-binding protein. [$^2H_6$]25(OH)D_3 served as an internal standard. The calibration graph was prepared from dialysed human plasma that was spiked with 0–280 nmol/l 25(OH)D_2 and D_3. Online extraction was performed as described previously[14]. The mass spectrometric conditions were essentially as described by Maunsell *et al.*[15]. The method specifications were as follows: level of quantification 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.

Table 1. Anthropometric characteristics and serum 25-hydroxyvitamin D_2 and D_3 (25(OH)D) of Maasai and Hadzabe

(Mean values, standard deviations and ranges)

	Maasai (*n* 35)			Hadzabe (*n* 25)		
	Mean	SD	Range	Mean	SD	Range
Age (years)	34	10	17–65	35	12	16–57
Sex (% male)	43[b]			84[a]		
Weight (kg)	59	11·5	36–100	58	6·7	41–72
Height (m)	1·67[a]	0·08	1·49–1·85	1·62[b]	0·08	1·45–1·74
BMI (kg/m^2)	20·9	3·7	15·4–33·5	22·2	2·2	17·1–26·8
25(OH)D (nmol/l)	119·0	26·0	58–167	109·0	28	71–171
25(OH)D_2 (%)*	nd		nd–11·2	5·1		nd–23·4

nd, Not detectable.

[a,b] Mean values with unlike superscript letters were significantly different ($P < 0·05$).

* Median percentage of 25(OH)D_2.

Statistics

Statistical analyses were performed with SPSS version 18.0 (SPSS, Inc.). Between-group differences were analysed with the unpaired Student *t* test. $P < 0·05$ was considered to be statistically significant. Between-group differences in sex were determined using the χ^2 test. The relationships of 25(OH)D concentrations with regard to age, weight and BMI were investigated by Spearman's correlation analysis. We used cut-off values of 50 and 80 nmol/l 25(OH)D[16–20] and values of 100, 120 and 150 nmol/l 25(OH)D to construct frequency distribution graphs.

Results

Anthropometric characteristics and serum 25(OH)D concentrations of the included Maasai and Hadzabe tribes are shown in Table 1. The Maasai group was composed of fewer males than the Hadzabe group. The Maasai were taller compared with Hadzabe. The groups exhibited no differences in serum 25(OH)D. The mean serum 25(OH)D concentration of Maasai was 119 nmol/l (25(OH)D_3: 116·4 (SD 24·7) nmol/l; 25(OH)D_2: not detectable (nd) (range nd–17·3) nmol/l, percentage nd: 60 %) and that of Hadzabe was 109 nmol/l (25(OH)D_3: 104·6 (SD 28·8) nmol/l; 25(OH)D_2: nd (nd–16·6) nmol/l, percentage nd: 40 %). Their 25(OH)D concentrations were not related to age, sex, weight and BMI (data not shown). The overall mean 25(OH)D concentration was 115 nmol/l with a range of 58–171 nmol/l.

Fig. 1 shows the serum 25(OH)D frequency distributions for Maasai and Hadzabe. For the whole group, the percentage of subjects with 25(OH)D concentrations of 50–80, 81–100, 101–120, 121–150 and 151–175 nmol/l amounted to 13·3, 15·0, 28·3, 33·3 and 10·0, respectively. The percentages of subjects with serum 25(OH)D concentrations below 50, 80 and 100 nmol/l were 0, 13·3 and 28·3, respectively.

Discussion

We investigated the vitamin D status of two traditionally living populations in East Africa with lifelong exposure to abundant tropical sunlight. The mean 25(OH)D values of these populations, i.e. Maasai and Hadzabe, were 119 and 109 nmol/l, respectively. None of these populations had values below 50 nmol/l. The highest values were 167 and 171 nmol/l, respectively, which are well below the estimated toxicity concentrations of 250 and >600 nmol/l[21–23]. The presently encountered status is comparable with 25(OH)D concentrations above 100 nmol/l as measured in Caucasian lifeguards who had been working for at least 4 weeks at an open-air swimming pool in St Louis during May and June and Hawaiians receiving more than 3 h of sun exposure per d for more than 5 d/week during at least 3 months[24,25].

The 'optimal' vitamin D status for overall health is controversial[9]. Currently, the recommended 25(OH)D concentrations by health authorities and other experts are diverse. Based on randomised controlled trials aiming at bone health, the Dutch Health Council recommends 25(OH)D concentrations above 30 nmol/l for women below 50 years old and men below 70 years old, and above 50 nmol/l for those above these ages[16]. The 14th Vitamin D Workshop

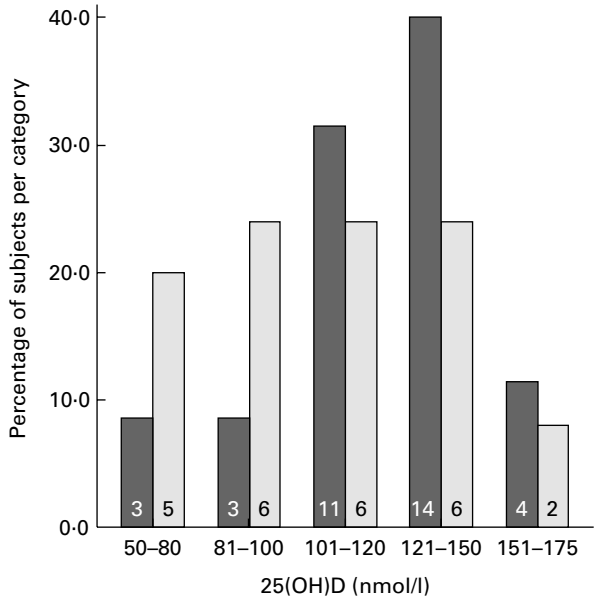

Fig. 1. Serum 25-hydroxyvitamin D (25(OH)D) frequency distributions for Maasai (■) and Hadzabe (□). The numbers in the bars refer to the absolute number of subjects.

consensus concluded that an absolute minimum 25(OH)D concentration of 50 nmol/l is necessary in all individuals to support all classical functions of vitamin D for bone and mineral health[17]. Likewise, the US Institute of Medicine recently advised a serum 25(OH)D concentration above 50 nmol/l as the main endpoint for bone health[18]. Taylor *et al.*[19] argued that the optimal concentration of vitamin D is the status in which sufficient vitamin D is secreted in the breast milk of a lactating mother to support the adequate vitamin D status of her infant. This maternal vitamin D sufficiency status was set at a minimum of 80 nmol/l[19]. Similarly, Heaney[20] reported a healthy 25(OH)D concentration of 80 nmol/l. The authors of a recent review have suggested that serum 25(OH)D concentrations of 75–110 nmol/l provide optimal benefits for the prevention of falls and fractures, cardiovascular health and colorectal cancer. They also showed that in twenty-five randomised controlled trials, mean serum Ca concentrations were not related to 25(OH)D at concentrations below 643 nmol/l[26]. The present study indicates a mean 25(OH)D concentration of 115 nmol/l, as based on traditionally living populations with sun exposure habits that might be comparable to our African ancestors before the out-of-Africa diaspora.

Whether the suggested 25(OH)D concentration of 115 nmol/l would constitute the present target for the prevention and treatment of diseases related to vitamin D insufficiency is uncertain. There is concern that a higher 25(OH)D concentration may moderately increase serum Ca leading to long-term renal stone formation and soft-tissue calcification, while Ca supplements with and without vitamin D have recently been implicated in CVD[27,28]. All of these potentially adverse effects might not argue against a higher vitamin D status, but would rather point at other imbalances that we have introduced since the agricultural revolution, such as our current low Mg status, low vitamin K status and the present high carbohydrate and low vegetable and fruit intakes[29,30]. Vitamin K, notably vitamin K_2, plays an important role in vascular Ca homeostasis since the vitamin K-activated matrix Gla protein has emerged as a potent inhibitor of arterial calcification[31]. A high intake of acid-forming carbohydrates together with a low intake of base-forming fruits and vegetables causes a state of diet-induced low-grade metabolic acidosis, with increased utilisation of base stores that leads to calciuria with net losses of body Ca[32]. A serum 25(OH)D concentration of 115 nmol/l in the current Western society might therefore only be appropriate in the context of a concerted correction of many other lifestyle factors that we have changed in our evolutionarily established Ca homeostasis.

We conclude that people with traditional lifestyles, living in the cradle of mankind, have a mean circulating 25(OH)D concentration of about 115 nmol/l. Whether this concentration is optimal under the conditions of the current Western lifestyle is uncertain, but it may serve as a target for further research. Such investigations should preferably be conducted with concomitant appreciation of many other important factors in Ca homeostasis that we have changed since the industrial revolution.

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