Osteoporos Int (2015) 26:1137–1146
DOI 10.1007/s00198-014-2905-0

ORIGINAL ARTICLE

Predictors of 25(OH)D half-life and plasma 25(OH)D concentration in The Gambia and the UK

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Received: 28 April 2014 / Accepted: 12 September 2014 / Published online: 3 October 2014

Abstract
Summary Predictors of 25(OH)D_3 half-life were factors associated with vitamin D metabolism, but were different between people in The Gambia and the UK. Country was the strongest predictor of plasma 25(OH)D concentration, probably as a marker of UVB exposure. 25(OH)D_3 half-life may be applied as a tool to investigate vitamin D expenditure.
Introduction The aim of this study was to investigate predictors of 25(OH)D_3 half-life and plasma 25(OH)D concentration.
Methods Plasma half-life of an oral tracer dose of deuterated-25(OH)D_3 was measured in healthy men aged 24–39 years, resident in The Gambia, West Africa (n=18) and in the UK during the winter (n=18), countries that differ in calcium intake and vitamin D status. Plasma and urinary markers of vitamin D, calcium, phosphate and bone metabolism, nutrient intakes and anthropometry were measured.
Results Normally distributed data are presented as mean (SD) and non-normal data as geometric mean (95 % CI). Gambian compared to UK men had higher plasma concentrations of 25(OH)D (69 (13) vs. 29 (11)nmol/L; P<0.0001); 1,25$(OH)_2$D (181 (165, 197) vs. 120 (109, 132)pmol/L; P<0.01); and parathyroid hormone (PTH) (50 (42, 60) vs. 33 (27, 39); P<0.0001). There was no difference in 25(OH)D_3 half-life (14.7 (3.5)days vs. 15.6 (2.5)days) between countries (P=0.2). In multivariate analyses, 25(OH)D, 1,25$(OH)_2$D, vitamin D binding protein and albumin-adjusted calcium (Ca_{alb}) explained 79 % of variance in 25(OH)D_3 half-life in Gambians, but no significant predictors were found in UK participants. For the countries combined, Ca_{alb}, PTH and plasma phosphate explained 39 % of half-life variability. 1,25$(OH)_2$D, weight, PTH and country explained 81 % of variability in 25(OH)D concentration; however, country alone explained 74 %.
Conclusion Factors known to affect 25(OH)D metabolism predict 25(OH)D_3 half-life, but these differed between countries. Country predicted 25(OH)D, probably as a proxy measure for UVB exposure and vitamin D supply. This study supports the use of 25(OH)D half-life to investigate vitamin D metabolism.

Keywords 24,25$(OH)_2$D · Gambia · Half-life · Vitamin D binding protein · Vitamin D metabolism

Introduction

Vitamin D is essential for human health, and a growing body of evidence links vitamin D status to health outcomes beyond those associated with the musculoskeletal system [1]. Functional biomarkers are required to determine the adequacy of vitamin D status and to determine physiological requirements during growth, pregnancy or ageing and how requirements may be modified by ethnicity, sex, and disease or body composition. Biomarkers are also required to address specific questions such as elucidating the vitamin D-response relationship and its functional outcomes. Plasma 25-hydroxyvitamin D (25(OH)D) concentration is the most commonly used marker of vitamin D status. It is determined by endogenous vitamin D synthesis and dietary intake, conversion into 25(OH)D and,

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finally, distribution and usage (metabolism and excretion). The concentration of 25(OH)D in plasma is largely unregulated, and it has a relatively long half-life of 2–3 weeks [2–5].

Plasma 25(OH)D half-life, as a marker of vitamin D usage, may be useful for elucidating the determinants of the vitamin D dose-response relationship and for examining relationships between vitamin D, calcium, phosphate metabolism and balance, their regulating hormones and other factors related to vitamin D metabolism. In clinical and animal studies that used radiolabelled isotopes, lower plasma $1,25(OH)_2D$ [6, 7], lower parathyroid hormone (PTH) [8] and calcium supplementation were associated with a longer half-life [6, 8]. Fibre intake [9] and vitamin D binding protein (DBP) concentration and genotype may also influence 25(OH)D half-life [10].

We previously described a method to measure 25(OH)D half-life [5, 10] that uses stable isotope tracers. Here, we report an investigation of $25(OH)D_3$ half-life as a biomarker of 25(OH)D expenditure by examining relationships between half-life and known drivers of vitamin D metabolism in two distinct populations. The study was performed in healthy men resident in a rural area of The Gambia, West Africa, and in Cambridge, UK, populations known to differ in vitamin D status, calcium intake and markers of vitamin D metabolism (e.g. $1,25(OH)_2D$ and PTH) [11]. We tested the hypothesis that 25(OH)D half-life in these populations differs due to these differences in dietary calcium intake and vitamin D supply. We also investigated predictors of plasma 25(OH)D concentration using the same variables.

Subjects and methods

Study setting

Detailed descriptions of the study design and subject characteristics were published previously [10]. In short, the study was performed at two centres: MRC Keneba, a rural field station in The Gambia (latitude 13° N), and at MRC Human Nutrition Research (HNR), Cambridge, UK (latitude 52° N). The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and procedures involving the participants were approved by the joint Gambian Government-MRC ethics committee and the UK National Research Ethics Service (NRES) Cambridge committee, respectively. Trained staff explained the study to the participants, and informed written consent was obtained. There is year-round ability to synthesise vitamin D in The Gambia where field work was performed at the end of the dry season, in May and June 2010. In the UK, study procedures were carried out between January and April 2011, when there is little or no cutaneous synthesis of vitamin D and an exclusion criterion was recent (previous 3 months) or planned (during study period) travel to tropical or subtropical countries or high-altitude areas.

Participants

Participants (n=36) were healthy, nonsmoking males, aged between 25 and 39 years. Exclusion criteria were recent illness (within 2 weeks); a broken bone within the last 3 years; known bone, kidney or liver disease; taking any prescription medicines; or haemoglobin less than 10 g/dL. All Gambian participants were of Mandinka ethnicity (defined by self-reported ethnicity of the father), and UK subjects were all self-classified as white.

Study protocols

The same study protocols were followed in The Gambia and the UK. On day 1 and day 21 of the study, and following an overnight fast and voiding of the first morning urine, a 2-h fasting urine [5] was collected from approximately 7:00 a.m. A blood sample was collected after 1 h. Height (Leicester Stadiometer, Chasmoors Ltd, London, UK), weight (Tanita HD305 scale, Tanita, Amsterdam, The Netherlands) and body composition by bioelectrical impedance (Tanita BC418-MA Tanita, Amsterdam, The Netherlands) were measured. On day 1, and after completion of the urine collection, participants were given an oral tracer dose of 40 nmol (16 μg) of stable isotope-labelled $25(OH)D_3$ (d_3-$25(OH)D_3$) (deuterated (6, 19, 19) 25-hydroxy vitamin D_3) (product number 705888; 97 atom %; purity 98 %; Sigma-Aldrich, Poole, UK) dissolved in vegetable oil with a standard breakfast [10]. Fasted blood samples were collected into tubes containing lithium heparin anticoagulant on day 6 and (±2 days) days 9, 21, 24, 27, 30 and 33 for measurement of the plasma tracer concentration for the calculation $25(OH)D_3$ half-life [5, 10].

Dietary nutrient intakes were quantitatively assessed with methods appropriate to each country. In The Gambia, trained field workers weighed all meals, snacks and drinks consumed over 2 days and coding of the dietary records was performed using The Gambian Food Composition Tables [12]. In the UK, a 4-day food diary was completed during one weekend and two week days. All dietary data were entered at MRC HNR using DINO (Diet In Nutrients Out), an in-house analysis program to calculate nutrient intakes [13].

Sample processing and laboratory analysis

Blood was collected into tubes containing either ethylenediaminetetraacetic acid (EDTA) or lithium heparin anticoagulant (S-Monovettes, Sarstedt Ltd, Leicester, UK) and plasma separated by centrifugation at 1800g for 20 min at 4 °C. Samples were stored at −70 °C. Collected urine was mixed thoroughly and acidified (Hydrochloric acid, 10 mL/L Fisher

Scientific, Loughborough, UK), and aliquots were stored at −20 °C. Gambian samples were shipped for analysis to MRC HNR, Cambridge, on dry ice.

Plasma calcium, phosphate, creatinine and albumin and urinary calcium (*u*Ca), urinary phosphate (*u*P) and urinary creatinine (*u*Cr) were measured on the Kone Lab 20i clinical chemistry analyser platform (Kone, Espoo, Finland). Plasma osteocalcin (OC) and bone alkaline phosphatase (BAP) were measured on the DiaSorin Liason platform (DiaSorin Ltd., Dartford, UK). Carboxy-terminal cross-linking telopeptide of type I collagen (β-CTX) was measured by ELISA (Immunodiagnostics Systems Ltd., Tyne & Wear, UK). EDTA plasma was used for analysis of PTH by immunoassay (Immulite, Siemens Healthcare Diagnostics Ltd, Camberley, UK), and C-terminal fibroblast growth factor-23 (FGF23) was analysed using a second-generation C-terminal, two-site enzyme-linked immunosorbent assay (Immutopics Inc., San Clemente, CA). Plasma $1,25(OH)_2D$ was measured with a radioimmunoassay (IDS Ltd., Tyne and Wear, UK). DBP was measured by radial immunodiffusion assay with polyclonal antibody [14], and $24,25(OH)_2D$ was analysed by UPLC-MS/MS (method modified from [15], both at KU Leuven, Belgium).

Assay performance at MRC HNR was monitored using kit and in-house controls and under strict standardisation according to ISO 9001:2000. Quality assurance of $25(OH)D$,$1,25(OH)_2D$ and PTH assays was performed as part of the Vitamin D External Quality Assessment Scheme (www.deqas.org) and the National External Quality Assessment Scheme (www.ukneqas.org.uk) and was within accepted limits. In addition, an aliquot of a pooled plasma sample was assayed in each batch to monitor possible drift over time and to provide running quality assurance for analytes where no external reference material was available. Further details of assay performance are described in [16].

Mass spectrometry

$25(OH)D_2$, $25(OH)D_3$, and d_3-$25(OH)D_3$ were measured by UPLC-MS/MS as described previously [10]. Intra- and inter-assay coefficients of variation were <10 % for all analytes. Total 25(OH)D was calculated from the sum of $25(OH)D_2$ and $25(OH)D_3$.

Data analysis

$25(OH)D_3$ half-life was calculated from the slope of plasma d_3-$25(OH)D_3$ disappearance using the line of best fit of the natural log of d_3-$25(OH)D_3$ concentration against time [10]. One additional Gambian participant was removed from the analysis because $25(OH)D_3$ half-life values were more than 3 interquartile ranges beyond the 75th centile [10] and had a major impact on relationships between half-life and other variables when analysed together with the remaining participants (n=36). Body mass index was calculated as weight divided by the square of height (kg/m^2). Plasma calcium was adjusted for albumin (Ca_{alb}) by normalising to an albumin concentration of 40 g/L and a correction factor of ±0.02 mmol calcium/g albumin above or below 36 g/L [16]. Fasting 2-h *u*Ca and *u*P were divided by *u*Cr to derive *u*Ca/*u*Cr and *u*P/*u*Cr ratios. Statistics were performed in Stata 11 (Stata Corp, TX, USA). Normally distributed data are presented as means and standard deviation (SD). Skewed data were log-transformed and are presented as geometric means and 95 % confidence interval. There were no differences between day 1 and day 21 values of plasma and urinary analytes as tested with paired Student's t tests, so subsequent analysis was performed with the mean values. Unpaired Student's t tests were used to test for differences between populations (Table 1). Statistical analyses were performed on countries separately, due to clustering of data and country-specific associations, and subsequently with data for countries combined with the inclusion of country in the model. In the combined country models, a country×independent-variable interaction was also tested.

Simple regression was used to evaluate anthropometric, biochemical and dietary predictors of $25(OH)D_3$ half-life (Table 2) or 25(OH)D plasma concentration (Table 3) for the countries separately and for the countries combined. Multiple linear regression was used to identify variables that independently predicted $25(OH)D_3$ half-life (Table 4) or $25(OH)D_3$ (Table 5). Throughout, the same full starting models were used with variables for inclusion selected a priori on the basis of a theoretical biological mechanism and/or a significant relationship as assessed with simple regression. These variables were 25(OH)D, $1,25(OH)_2D$, DBP, Ca_{alb}, weight, age, PTH, FGF23, plasma phosphate and dietary calcium intake. Dietary phosphate intake and the plasma concentration of $24,25(OH)_2D$ were not included in multiple regression because of their highly significant and strong correlation with calcium intake (phosphate intake r=0.87, P<0.0001 for countries combined) and plasma 25(OH)D ($24,25(OH)_2D$ r=0.94, P<0.0001 for countries combined), respectively. Variables that were not significant and did not reduce variance were removed, and the final models were chosen based on robustness after the examination of relationships between all variables. Regression coefficients (slope) represent the change in $25(OH)D_3$ half-life or 25(OH)D plasma concentration for a 1-unit change in the predictor variable. For log-transformed variables ($1,25(OH)_2D$, PTH, FGF23, *u*Ca/*u*Cr, *u*P/*u*Cr, and plasma BAP, OC and β-CTX), the regression coefficient represents the change in $25(OH)D_3$

Table 1 Participant age, anthropometric data, biochemistry and nutrient intakes (mean (SD))[b] by country

	The Gambia (n=18)	UK (n=18)	P value[a]
Age and anthropometry			
Age, years	29.2 (3.2)	29.3 (4.4)	1.0
Weight, kg	64.5 (8.3)	73.3 (10.9)	0.01
Height, m	1.74 (0.06)	1.80 (0.07)	0.009
BMI, kg/m^2	21.3 (2.0)	22.6 (2.3)	0.08
Fat mass, kg	7.5 (2.6)	10.3 (4.3)	0.02
Fat-free mass, kg	55.7 (6.7)	62.6 (8.0)	0.009
Biochemistry			
25(OH)D_2, nmol/L	0.6 (0.2)	2.1 (1.0)	<0.0001
25(OH)D_3, nmol/L	68.4 (13.1)	26.5 (10.8)	<0.0001
25(OH)D, nmol/L	69.0 (13.2)	28.6 (11.0)	<0.0001
1,25(OH)$_2$D, pmol/L[b]	181 (165, 197)	120 (109, 132)	<0.0001
24,25(OH)$_2$D, nmol/L	6.5 (1.3)	1.9 (1.4)	<0.0001
DBP, mg/L	259 (33)	268 (23)	0.4
Albumin, g/L	36.4 (2.2)	41.0 (2.4)	<0.0001
Ca_{alb}, mmol/L	2.25 (0.06)	2.27 (0.06)	0.2
Phosphate, mmol/L	1.04 (0.14)	1.05 (0.10)	0.9
PTH, ng/L[b]	50.1 (41.9, 60.0)	32.8 (27.4, 39.3)	<0.01
FGF23, RU/mL[b]	79.2 (58.6, 106.9)	57.0 (52.5, 62.0)	0.03
Plasma BAP, μg/L[b]	17.3 (12.8, 23.3)	11.8 (10.1, 13.9)	0.02
Plasma OC, μg/L[b]	15.0 (12.7, 17.6)	10.8 (9.3, 12.6)	0.004
Plasma β-CTX, μg/L[b]	1.0 (0.9, 1.2)	0.6 (0.5, 0.7)	<0.0001
uCa/uCr, mmol/mmol[b]	0.06 (0.04, 0.09)	0.17 (0.13, 0.26)	<0.0001
uP/uCr, mmol/mmol[b]	0.73 (0.59, 0.90)	0.99 (0.83, 1.17)	0.02
Nutrient intake[c]			
Energy, kJ/day	8267 (2383)	9804 (1931)	0.04
Fat, g/day	42 (17)	94 (27)	<0.001
Protein, g/day	68 (26)	90 (19)	0.005
Carbohydrate, g/day	358 (115)	281 (60)	0.02
Calcium, mg/day	358 (177)	1087 (248)	<0.0001
Phosphate, mg/day	803 (332)	1603 (300)	<0.0001
Magnesium, mg/day	506 (222)	361 (94)	0.02

DBP vitamin D binding protein, *Ca_{alb}* plasma calcium corrected for albumin, *PTH* parathyroid hormone, *FGF23* fibroblast growth factor 23, *BAP* bone alkaline phosphatase, *OC* osteocalcin, *β-CTX* β-carboxy-terminal collagen crosslinks, *uCa/uCr* creatinine corrected fasting 2-h urinary Ca, *uP/uCr* creatinine corrected fasting 2-h urinary phosphate

[a] Country differences as tested by unpaired, two-tailed Student's t test

[b] Data are presented as mean (SD) or geometric mean (95 % CI) of samples collected on day 1 and day 21

[c] Daily nutrient intakes were assessed by 2-day weighed dietary assessment in The Gambia and 4-day food diary in the UK

half-life or 25(OH)D plasma concentration for a 100 % change in the predictor variable. Relationships were considered significant where P<0.05, and a trend for a relationship was noted where P≥0.05 and P<0.1.

Results

Country comparisons

Anthropometric, biochemical and dietary data are shown in Table 1. Gambian participants were significantly shorter and lighter than UK participants and had lower fat mass and fat-free mass. Plasma 25(OH)D, 1,25(OH)$_2$D, 24,25(OH)$_2$D, PTH and FGF23 concentrations were higher and plasma DBP, Ca_{alb} and plasma phosphate were not different in Gambian compared to UK participants. Markers of bone resorption and formation were also higher in Gambian participants. Gambian macro- and micronutrient intakes were generally lower than those in the UK with the exception of carbohydrate and magnesium which were significantly higher. Consistent with the lower dietary calcium and phosphate intakes, fasting 2-h urinary excretions in The Gambia were lower than those in the UK. Mean 25(OH)D_3 half-life was 14.7 (3.5)days and 15.6 (2.5)days in The Gambia and UK, respectively, and was not significantly different between countries (difference 0.9 days; P=0.2) [10].

Predictors of 25(OH)D_3 half-life

In Gambian participants, 25(OH)D_3 half-life was negatively associated with Ca_{alb} and positively associated with age and DBP (Table 2) in simple regression models. There were trends towards a negative association with PTH (P=0.09) and positive with uP/uCr (P=0.08). In contrast, in the UK participants in simple regression models, none of the measured variables were significantly associated with 25(OH)D_3 half-life. With the countries combined, where the country was also included in the model, Ca_{alb} was negatively associated and age, DBP and plasma phosphate were positively associated with 25(OH)D_3 half-life. Near-significant relationships were also observed with PTH (inversely) and uP/uCr (a positive association); in none of these models was country a significant predictor of 25(OH)D_3 half-life. However, the pattern of the relationship between half-life and both Ca_{alb} and age differed between countries. There were significant country×age (P=0.03) and country×Ca_{alb} interactions (P=0.006) such that the positive association with age and negative association with Ca_{alb} was of a greater magnitude in the Gambians and not significant in the UK participants.

Results of the multivariate model (Table 3) were partly consistent with simple regression analysis. Ca_{alb} and 1,25(OH)$_2$D negatively predicted and 25(OH)D and DBP positively predicted 25(OH)D_3 half-life in Gambians. Together, these variables explained 79 % (adjusted R^2) of the variation in 25(OH)D_3 half-life. There were no significant associations in UK participants (Table 3). In the model for the

Table 2 Regression analysis of $25(OH)D_3$ half-life and other variables by country and for the countries combined

	The Gambia		UK		Countries combined and corrected for country[a]	
	Slope (95 % CI)	*P* value	Slope (95 % CI)	*P* value	Slope (95 % CI)	*P* value
Age and anthropometry						
Age, years	*0.63* (*0.16*, *1.10*)	*0.01*	0.07 (−0.23, 0.37)	0.6	*0.27* (*0.002*, *0.54*)	*0.05*[b]
Weight, kg	0.09 (−0.13, 0.31)	0.4	−0.02 (−0.14, 0.10)	0.7	0.02 (−0.09, 0.13)	0.7
BMI, kg/m^2	0.34 (−0.61, 1.28)	0.5	−0.19 (−0.75, 0.37)	0.5	0.03 (−0.48, 0.54)	0.9
Fat mass, kg	0.14 (−0.56, 0.86)	0.7	−0.02 (−0.33, 0.28)	0.9	0.02 (−0.28, 0.33)	0.9
Fat-free mass, kg	0.12 (−0.15, 0.39)	0.4	−0.03 (−0.19, 0.14)	0.7	0.04 (−0.11, 0.18)	0.6
Markers of vitamin D, Ca and P metabolism						
25(OH)D, nmol/L	0.09 (−0.04, 0.22)	0.2	−0.02 (−0.14, 0.10)	0.7	0.04 (−0.04, 0.13)	0.3
$1,25(OH)_2D$, pmol/L[c]	4.70 (−5.49, 14.88)	0.3	−1.67 (−8.48, 5.12)	0.6	1.27 (−4.54, 7.08)	0.7
$24,25(OH)_2D$, nmol/L	0.64 (−0.72, 2.01)	0.3	−0.09 (−1.04, 0.86)	0.8	−0.26 (−0.53, 1.05)	0.5
DBP, mg/L	*0.06* (*0.14*, *0.11*)	*0.01*	0.004 (−0.05, 0.06)	0.9	*0.04* (*0.007*, *0.08*)	*0.02*
Albumin, g/L	−0.18 (−1.03, 0.67)	0.7	0.25 (−0.28, 0.78)	0.3	0.06 (−0.41, 0.53)	0.8
Ca_{alb}, mmol/L	*−44.6* (*−65.5*, *−23.6*)	*<0.0001*	−1.31 (−23.6, 21.0)	0.9	*−23.0* (*−39.3*, *−6.58*)	*0.007*[b]
Phosphate, mmol/L	8.37 (−4.07, 20.8)	0.2	9.20 (−2.85, 21.26)	0.1	*8.66* (*0.43*, *16.8*)	*0.04*
PTH, ng/L[c]	*−4.06* (*−8.87*, *0.71*)	*0.09*	−1.42 (−5.02, 2.18)	0.4	*−2.73* (*−5.59*, *0.13*)	*0.06*
FGF23, RU/mL[c]	0.19 (−2.92, 3.31)	0.9	−0.71 (−8.66, 7.24)	0.9	0.13 (−2.32, 2.59)	0.9
*u*Ca/*u*Cr, mmol/mmol[c]	−0.78 (−2.97, 1.40)	0.5	−1.21 (−3.38, 0.96)	0.3	−0.92 (−2.38, 0.53)	0.2
*u*P/*u*Cr, mmol/mmol[c]	*3.46* (*−0.51*, *7.42*)	*0.08*	1.33 (−2.50, 5.16)	0.5	*2.64* (*−0.006*, *5.28*)	*0.05*
Markers of bone metabolism						
Plasma BAP, μg/L[c]	−0.94 (−4.05, 2.17)	0.5	1.10 (−3.01, 5.22)	0.6	−0.49 (−2.75, 1.78)	0.7
Plasma OC, μg/L[c]	−0.26 (−6.07, 5.54)	0.9	−0.49 (−4.83, 3.85)	0.8	−0.37 (−3.82, 3.09)	0.8
Plasma β-CTX, μg/L[c]	−2.02 (−8.30, 4.27)	0.5	0.47 (−3.57, 4.50)	0.8	−0.64 (−4.12, 2.83)	0.7
Nutrient intake						
Calcium intake, mg/day	0.003 (−0.007, 0.14)	0.4	0.003 (−0.002, 0.008)	0.2	0.004 (−0.001, 0.008)	0.1
Phosphate intake, mg/day	0.003 (−0.002, 0.01)	0.2	0.00003 (−0.004, 0.004)	1.0	0.002 (−0.002, 0.005)	0.3

Italicized values show significant ($P<0.05$) associations with $25(OH)D_3$ half-life or a trend for an association ($P<0.1$, ≥ 0.5)

[a] Country was a not significant predictor for any variable

[b] There were significant country × age ($P=0.03$) and country × Ca_{alb} ($P=0.006$) interactions

[c] Log-transformed variables. Coefficients represent change in $25(OH)D_3$ half-life for 100 % change in the predictor variable

Table 3 Predictors of $25(OH)D_3$ half-life in Gambian and UK participants and for countries combined in multivariate regression model

	Gambia ($n=18$)		UK ($n=18$)		Countries combined ($n=36$)	
	Slope (95 % CI)	*P* value	Slope (95 % CI)	*P* value	Slope (95 % CI)	*P* value
25(OH)D	0.12 (0.04, 0.19)	0.004	−0.02 (−0.18, 0.14)	0.8		
$1,25(OH)_2D$[a]	−6.33 (−12.1, −0.54)	0.04	−1.3 (−9.6, 7.0)	0.7		
DBP	0.04 (0.01, 0.07)	0.009	−0.003 (−0.07, 0.07)	0.9		
Ca_{alb}	−44.5 (−60.7, −28.2)	<0.0001	−2.56 (−30.5, 25.4)	0.8	−24.2 (−38.3, −10.0)	0.001
PTH[a]					−3.20 (−5.53, −0.86)	0.009
Plasma phosphate					8.38 (1.62, 15.14)	0.02
Country						0.9

[a] Log-transformed variables. Coefficients represent change in $25(OH)D_3$ half-life for 100 % change in the predictor variable

Table 4 Regression analysis of plasma 25(OH)D and other variables by country and for the countries combined

	The Gambia		UK		Countries combined and corrected for country[a]	
	Slope (95 % CI)	*P* value	Slope (95 % CI)	*P* value	Slope (95 % CI)	*P* value
Age and anthropometry						
Age, years	−0.17 (−2.33, 1.99)	0.9	0.71 (−0.57, 1.99)	0.3	0.40 (−0.71, 1.51)	0.5
Weight, kg	0.53 (−0.27, 1.32)	0.2	0.25 (−0.27, 0.76)	0.3	0.35 (−0.76, 0.77)	0.1
BMI, kg/m^2	1.83 (−1.63, 5.29)	0.3	0.74 (−1.73, 3.22)	0.5	1.19 (−0.77, 3.16)	0.2
Fat mass, kg	0.31 (−2.35, 2.98)	0.8	0.72 (−0.58, 2.01)	0.3	0.61 (−0.58, 1.79)	0.3
Fat-free mass, kg	0.72 (−0.25, 1.69)	0.1	0.25 (−0.47, 0.96)	0.5	0.44 (−0.12, 1.00)	0.1
Markers of vitamin D, Ca and P metabolism						
25(OH)D, nmol/L	–	–	–	–	–	–
1,25(OH)$_2$D, pmol/L[b]	*29.8* (*−5.97*, *65.6*)	0.1	20.9 (−6.98, 48.8)	0.1	*25.0* (*3.72*, *46.3*)	*0.02*
24,25(OH)$_2$D, nmol/L	*6.26* (*2.20*, *10.3*)	*0.005*	*6.94* (*5.02*, *8.87*)	*<0.0001*	*6.62* (*4.52*, *8.72*)	*<0.0001*
DBP, mg/L	0.04 (−0.16, 0.25)	0.7	−0.11 (−0.35, 0.13)	0.4	−0.006 (−0.16, 0.14)	0.9
Albumin, g/L	*−3.30* (*−5.96*, *−0.63*)	*0.02*	0.51 (−1.88, 2.90)	0.7	−1.22 (−3.03, 0.59)	0.2[c]
Ca_{alb}, mmol/L	2.46 (−115, 120)	1.0	−45.1 (−139.8, 49.7)	0.3	−21.3 (−93.2, 50.7)	0.6
Plasma phosphate, mmol/L	12.5 (−36.3, 61.4)	0.6	−11.5 (−68.1, 45.0)	0.7	4.38 (−30.3, 39.1)	0.8
PTH, ng/L[b]	−8.62 (−27.6, 10.4)	0.4	−11.4 (−26.3, 3.44)	0.1	*−10.0* (*−21.4*, *1.36*)	*0.08*
FGF23, RU/mL[b]	−5.34 (16.6, 5.94)	0.3	−6.98 (−41.6, 27.6)	0.7	−5.46 (−15.0, 4.08)	0.3
*u*Ca/*u*Cr, mmol/mmol[b]	−1.61 (−9.86, 6.64)	0.7	−3.81 (−13.5, 5.87)	0.4	−2.32 (−8.18, 3.53)	0.4
*u*P/*u*Cr, mmol/mmol[b]	−0.55 (−16.9, 15.8)	0.9	*−13.6* (*−29.0*, *1.78*)	*0.08*	−5.61 (−16.5, 5.30)	0.3
Markers of bone metabolism						
Plasma BAP, μg/L[b]	3.90 (−7.66, 15.5)	0.5	7.38 (−10.4, 25.1)	0.4	4.68 (−4.16, 13.5)	0.3
Plasma OC, μg/L[b]	−10.3 (−31.3, 10.7)	0.3	3.13 (−15.8, 22.1)	0.7	−3.98 (−17.6, 9.61)	0.6
Plasma β-CTX, μg/L[b]	5.20 (18.4, 28.8)	0.6	−1.82 (−19.5, 15.8)	0.8	1.31 (−12.5, 15.1)	0.8
Nutrient intake						
Calcium intake, mg/day	−0.007 (−0.05, 0.03)	0.7	0.0007 (−0.02, 0.02)	0.9	−0.002 (−0.02, 0.02)	0.9
Phosphate intake, mg/day	0.01 (−0.009, 0.03)	0.2	0.002 (−0.02, 0.02)	0.8	0.007 (−0.006, 0.02)	0.3

Italicized values show significant ($P<0.05$) associations with 25(OH)D_3 or a trend for an association ($P<0.1$, ≥ 0.5)

[a] Country was a significant predictor for all variables ($P<0.0001$) with the exception of 24,25(OH)$_2$D

[b] Log-transformed variables. Coefficients represent change in 25(OH)D plasma concentration for 100 % change in the predictor variable

[c] There was a significant country × albumin interaction ($P=0.03$)

countries combined, Ca_{alb} and PTH were negatively associated and plasma phosphate positively associated with half-life (Table 3). Country was nonsignificant.

In simple and multivariate regression, we obtained similar results if pH-adjusted ionised calcium or plasma calcium (independent of plasma albumin) were used instead of Ca_{alb}

Table 5 Predictors of plasma 25(OH)D in Gambian and UK participants and for countries combined in multivariate regression model

	Gambia ($n=18$)		UK ($n=18$)		Countries combined ($n=36$)	
	Slope (95 % CI)	*P* value	Slope (95 % CI)	*P* value	Slope (95 % CI)	*P* value
1,25(OH)$_2$D[a]	33.2 (0.13, 66.3)	0.05	21.3 (−3.50, 46.2)	0.09	29.3 (9.89, 48.8)	0.004
Ca_{alb}			−68.3 (−152, 15.8)	0.1		
PTH[a]	−15.7 (33.8, 2.4)	0.09	−14.6 (−28.4, −0.76)	0.04	−9.76 (−19.6, 0.12)	0.05
FGF23[a]	−10.5 (−21.4, 0.3)	0.06				
Weight					0.45 (0.08, 0.82)	0.02
Country						<0.0001

[a] Log-transformed variables. Coefficients represent change in 25(OH)D plasma concentration for 100 % change in predictor variable

for countries analysed separately and combined (data not shown).

Predictors of 25(OH)D

There were few significant associations with 25(OH)D in simple regression models: $24,25(OH)_2D$ was positively associated with 25(OH)D in both countries and for the countries combined. Plasma albumin inversely predicted 25(OH)D in Gambians, and there were trends for a positive association with $1,25(OH)_2D$ ($P=0.097$) in Gambians and a negative association with *u*P/*u*Cr ($P=0.08$) in UK participants (Table 4). When the countries were combined, there was a positive association with $1,25(OH)_2D$ and a trend towards a negative association with PTH ($P=0.08$) (Table 4); country was a highly significant predictor of 25(OH)D (The Gambia>UK, $P<0.0001$) in all models with the exception of $24,25(OH)_2D$. There was a significant country×albumin interaction ($P=0.03$), such that there was a strong negative association with albumin in the Gambians and a nonsignificant positive association in the UK participants.

In country-specific multivariate models, $1,25(OH)D_2$ positively predicted 25(OH)D, and there was a trend for inverse associations with FGF23 and PTH for Gambians (Table 5). In the UK, there was a significant inverse association with PTH, together with near-significant negative and positive associations with Ca_{alb} and $1,25(OH)_2D$, respectively (Table 5). When the countries were combined, country was the strongest predictor of 25(OH)D, accounting for 74 % (adjusted R^2) of the variation in 25(OH)D. However, PTH negatively predicted and $1,25(OH)_2D$ and weight positively predicted 25(OH)D and, together with country, accounted for 81 % of the variation (Table 5). There were no significant interactions with country.

Predictors of $24,25(OH)_2D$

The only consistently significant predictor of $24,25(OH)_2D$ was 25(OH)D, and this was true when data for the countries were combined (with country as a covariate) (coefficient, 0.08 nmol/L per 1 nmol/L change in 25(OH)D; $P<0.0001$) and when analysed separately (The Gambia (coefficient, 0.06 nmol/L; $P=0.005$); the UK (coefficient, 0.11 nmol/L; $P<0.0001$)). PTH also predicted $24,25(OH)_2D$ in the countries combined and The Gambia (a 10 % change in PTH predicts a 0.1 and 0. 2 nmol/L ($P=0.03$) decrease in $24,25(OH)_2D$, respectively). In multivariate models, there were no predictors of $24,25(OH)_2D$ other than 25(OH)D. There were no associations with FGF23. In combined country models, there were no country interactions.

Discussion

25(OH)D plasma concentration is a biomarker of vitamin D status and reflects the balance between vitamin D supply from cutaneous synthesis and diet, hepatic 25(OH)D production and 25(OH)D expenditure, which represents primarily hydroxylation into $1,25(OH)_2D$ and $24,25(OH)_2D$. Plasma 25(OH)D may also be influenced by the distribution volume, storage and release of vitamin D and 25(OH)D from fat or muscle [17]. Some of the mechanisms controlling 25(OH)D status (whether production or utilisation) and their relative importance in healthy people are not fully elucidated. The measurement of 25(OH)D half-life provides a measure of 25(OH)D expenditure that allows investigation of factors that influence plasma 25(OH)D concentration without the confounding contribution of newly synthesised 25(OH)D. We have investigated the predictors of $25(OH)D_3$ half-life and of plasma 25(OH)D in two distinct populations. In the Gambia and when data for the countries were combined, we found that the strongest and most consistent predictor of $25(OH)D_3$ half-life was Ca_{alb} inversely, but PTH inversely and total 25(OH)D, $1,25(OH)_2D$, DBP and *p*P plasma phosphate positively predicted half-life in multivariate models. However, predictors were different between countries and in models for the countries combined. For total 25(OH)D, PTH was a negative predictor, and $1,25(OH)_2D$ and weight were positive predictors, although country was the major influencing factor. As with half-life, the predictors of plasma 25(OH)D differed between countries. These data suggest that 25(OH)D half-life is primarily predicted by factors associated with 25(OH)D metabolism, whereas 25(OH)D is most strongly predicted by country, probably as a proxy measure of vitamin D supply from UVB exposure.

Plasma 25(OH)D half-life is likely to be determined by the metabolism of 25(OH)D to downstream metabolites and by its catabolism, driven primarily by the activities of the enzymes CYP27B1 (producing $1,25(OH)_2D$) and CYP24A1 (catabolising 25(OH)D and further downstream metabolites) [6]. The activities of these enzymes are stimulated by a large number of factors, the most important being increases in PTH and $1,25(OH)_2D$ and/or low plasma P. We observed associations between these factors and $25(OH)D_3$ half-life, although these were not consistent between countries, possibly due to genetic or environmental, especially dietary, differences. Low dietary calcium intake [7] and low calcium bioavailability, due to a high-fibre diet [9] or intestinal malabsorption [8, 18, 19], were reported to be associated with a shorter half-life. This is likely to be mediated through the PTH-CYP27B1-CYP24A1 axis, increasing the production of both $1,25(OH)_2D$ and $24,25(OH)_2D$ [7, 20]. In our study, dietary calcium intake was 3-fold lower in Gambian compared to UK participants, yet contrary to our expectations, there was no difference in mean $25(OH)D_3$ half-life between the populations [10] and no

association between half-life and calcium intake. This relationship may be difficult to prove in human studies over the habitual range of calcium intake, particularly in populations where there are other factors that may influence half-life and may require a large sample size. In addition, we may not have observed a difference in half-life between countries because of population differences due to long-term adaptation of vitamin D metabolism to either low vitamin D supply/high calcium intake (UK) or high vitamin D supply/low calcium intake (The Gambia). This may be due to genetic differences between populations, for example in DBP, vitamin D receptor or hydroxylase enzymes, or the activity of the hydroxylase enzymes regulating 25(OH)D synthesis and catabolism into $1,25(OH)_2D$ and $24,25(OH)_2D$ and further downstream metabolites. This could be investigated in future studies by the assessment of downstream metabolites of the tracer compound.

In this study, the strongest and most robust relationship with half-life was observed with plasma Ca_{alb} although this relationship was not observed in the UK participants when analysed separately. This association, which was not a reflection of differences in plasma albumin concentration, is surprising given that plasma calcium concentration is tightly regulated and thus half-life is more likely to be strongly associated with its regulators, PTH and $1,25(OH)_2D$. In addition, any relationship between plasma calcium and half-life would have been expected, on a theoretic basis, to be positive, with lower plasma calcium resulting in greater usage of 25(OH)D through the PTH-CYP27B1-CYP24A1 axis. The importance of $1,25(OH)_2D$ and PTH in the control of vitamin D metabolism was shown previously in clinical and animal studies. An increase in plasma $1,25(OH)_2D$ and hyperparathyroidism have been associated with an increase in 25(OH)D expenditure, whereas hypoparathyroidism was associated with a longer half-life [6]. Consistent with these results, we found a negative relationship between PTH and 25(OH)D half-life. A negative relationship between $1,25(OH)_2D$ and $25(OH)D_3$ half-life was only observed in Gambians, possibly because of the higher $1,25(OH)_2D$ concentration in this population, likely related to their very low calcium intake.

The mechanisms of the reciprocal regulation of vitamin D and phosphate metabolism are less well established than for calcium [21]. Phosphate deficiency may stimulate CYP27B1, leading to increased plasma $1,25(OH)_2D$ [22], and high phosphate intake or plasma phosphate concentration is associated with an elevated FGF23, which stimulates urinary phosphate excretion and CYP24A1 activity and downregulates CYP27B1. We found a positive relationship between plasma phosphate, *u*P/*u*Cr and $25(OH)D_3$ half-life, supporting a role for phosphate in vitamin D metabolism. However, we did not observe a relationship with c-terminal FGF23 or with dietary phosphate intake. This lack of association was also observed in a study in Gambian children where no relationship was found between 25(OH)D half-life and active (intact) FGF23 [23]. We found that FGF23 was elevated in Gambians compared to UK subjects. This may be due to low calcium intake and/or high dietary phosphate/calcium ratio driving elevated $1,25(OH)_2D$. FGF23 is thought to regulate vitamin D metabolism in conjunction with PTH; FGF23 has been shown to increase vitamin D receptor (VDR), calcium sensing receptor and CYP24A1 expression and inhibit CYP27B1 expression through which it suppresses plasma PTH and potentially influences 25(OH)D half-life [24].

DBP concentration was positively associated with $25(OH)D_3$ half-life. DBP concentration and genotype may affect vitamin D metabolism and function [25]. Vitamin D metabolites are maintained in the circulation by their binding to DBP. Higher circulating levels of DBP may reduce the fraction of free-25(OH)D, and a longer half-life may be expected. Further, these relationships may be affected by DBP genotype that may [25] or may not [26] alter affinity. However, we have shown earlier that the relationships between free-25(OH)D and half-life are not different to those with 25(OH)D [10].

We also observed a positive association between 25(OH)D and $25(OH)D_3$ half-life in Gambian participants. Early observations suggested an influence of 25(OH)D plasma status on 25(OH)D half-life, with more rapid turnover in vitamin D-deplete subjects [27], but other studies have failed to reproduce these findings in normal and diseased subjects [8, 18]. Our results suggest, in Gambian participants at least, that a higher plasma 25(OH)D concentration is associated with a longer half-life, or that a longer half-life results in a higher 25(OH)D concentration. We found no relationships between any of the anthropometric measures of size or fatness with $25(OH)D_3$ half-life (Tables 2 and 3).

The UK study was performed during winter and early spring when vitamin D status is at its lowest. In contrast, there is little seasonal change in the availability of vitamin D-producing UVB radiation in The Gambia. Consequently, we observed a highly significant difference in 25(OH)D plasma concentration between countries such that country may act as a surrogate for 25(OH)D in our analysis. Models that examined other predictors of 25(OH)D and that included country had significantly different intercepts, reflecting the effect of country (probably acting as a proxy for UVB exposure) on 25(OH)D. We did not formally assess skin pigmentation in this study and therefore cannot comment on the effect of differences in skin pigmentation on 25(OH)D production. $1,25(OH)_2D$ was also a positive predictor of 25(OH)D and may suggest that the greater supply of 25(OH)D permits greater renal production of $1,25(OH)_2D$ when calcium intake is low. This was partly unexpected because 25(OH)D and $1,25(OH)_2D$ do not generally correlate well, except when 25(OH)D is low [28], although others have reported a correlation across a range of 25(OH)D plasma concentrations [28, 29]. The other major product of 25(OH)D is $24,25(OH)_2D$,

and we found a very strong association between $24,25(OH)_2D$ and its precursor, consistent with previous studies [11, 31]. Multivariate models also indicated weight as a significant positive independent predictor of 25(OH)D. This is in contrast to other studies that have generally shown poorer vitamin D status with increasing adiposity. In our study, participants were relatively lean, and the BMI range was narrow (BMI, 18–27 kg/m^2). This may have precluded seeing the inverse relationship reported between 25(OH)D plasma concentration and adiposity [32]. If weight was replaced with fat mass, then no significant association was observed, but with fat-free mass, we observed a similar response to the weight model.

Our study has a number of limitations. It was designed to compare $25(OH)D_3$ half-life between different populations and its predictors. We restricted participants to men within relatively narrow age and BMI ranges, so we cannot report on how the relationships may be different in women, in different age groups or in overweight and obese individuals. Previous reports of changes in half-life, or strong correlations between half-life and other variables, have primarily been obtained from studies with an acute change, such as a $1,25(OH)_2D$ challenge or a very high-fibre diet, or in a disease state [6–9, 33]. It may be more difficult to detect associations in a small cross-sectional study such as this, especially in free-living individuals. The relatively small sample size limited the power of the statistical methods and means that the findings in this study should be interpreted with a degree of caution. Studies in larger cohorts are required to confirm the predictors of 25(OH)D half-life found here.

In conclusion, this study investigated predictors of the plasma half-life of stable isotope-labelled $25(OH)D_3$ in free-living individuals in two different countries. $25(OH)D_3$ half-life was predicted by factors associated with vitamin D metabolism (25(OH)D, $1,25(OH)_2D$, DBP, plasma phosphate and PTH), although an inverse association with Ca_{alb} was found. There were differences between countries in factors predicting $25(OH)D_3$ half-life, and it is perhaps surprising, given the large differences in calcium intake and $1,25(OH)_2D$ plasma concentration, that there was not a significant difference in $25(OH)D_3$ half-life between countries. This may suggest a degree of adaptation, or regulation of $25(OH)D_3$ half-life, either through expression of vitamin D hydroxylases or because of genetic differences. In contrast, 25(OH)D was primarily predicted by country, probably reflecting differences in UVB exposure between the UK in winter and The Gambia. 25(OH)D half-life has value as a marker of 25(OH)D expenditure that can disentangle the effects of vitamin D supply and utilisation. An understanding of factors that affect 25(OH)D expenditure are required to determine how vitamin D requirements may vary during the life course and between populations. Opportunities to explore either acute or longer-term interventions that may transiently change 25(OH)D expenditure should be explored to further enhance the understanding of vitamin D metabolism.

Acknowledgments We thank the participants from Keneba and Cambridge. We also thank MRC staff in Keneba, in particular Buba Sise, Ebrima Sise and Ebrima Danso for field and laboratory work, and Duangporn Harnpanich for coordinating study procedures in Cambridge. Biochemistry analysis was performed by Ann Laidlaw, Janet Bennett and Shailja Nigdikar at MRC HNR. We thank Erik van Herck and Ivo Jans (KU Leuven, Belgium) for plasma DBP and $24,25(OH)_2D$ analysis.

Conflicts of interest None.

Funding This research is jointly funded by the Medical Research Council (MRC) (programme codes U105960371, U123261351, MC-A760-5QX00) and the Department for International Development (DFID) under the MRC/DFID Concordat agreement.

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