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The 25-hydroxyvitamin D₃ C-3 epimer: Distribution, correlates, and reclassification of 25-hydroxyvitamin D status in the population-based Atherosclerosis Risk in Communities Study (ARIC)

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Abstract

Background—Little is known about the vitamin D₃ epimer [3-epi-25(OH)D₃], particularly in adults. We describe characteristics of the D₃ epimer within the community-based ARIC cohort.

Methods—The vitamin D₃ epimer, 25(OH)D₃, and 25(OH)D₂ were measured using LC-MS/MS in stored serum collected in 1990–1992 from 9,887 white and 3,221 black ARIC study participants, aged 46–70 y. Cross-sectional characteristics were explored.

Results—Concentrations of the epimer were quantifiable (1.41 ng/ml) in 33.4% of whites and 15.0% of blacks, and made up on average 3.23% and 2.25% of total D₃ [epimer + 25(OH)D₃] concentrations, respectively. Epimer levels were positively correlated with 25(OH)D₃ in both whites ($r = 0.54$) and blacks ($r = 0.36$) and were unrelated to 25(OH)D₂ concentrations. Overall, epimer levels were associated with participant characteristics in a manner similar to that typically observed for 25(OH)D₃. Including the epimer in the calculation of total 25(OH)D resulted in approximately 2% of participants being reclassified from being clinically 25(OH)D deficient to having suboptimal levels.

Conclusions—Low concentrations of the D₃ epimer were present in adult serum and overall the epimer concentration is moderately correlated with the 25(OH)D₃ concentration. Reclassification of participant's clinical 25(OH)D status upon inclusion of the epimer was minimal.

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Keywords

25-hydroxyvitamin D₃ C-3 epimer; vitamin D; race; reclassification; Atherosclerosis Risk in Communities Study (ARIC)

1. Introduction

Vitamin D acts to regulate calcium homeostasis by increasing intestinal calcium absorption, stimulating calcium bone resorption, and suppressing parathyroid hormone (PTH) [1]. 25-hydroxyvitamin D [25(OH)D] is widely viewed as the most specific biomarker for assessing vitamin D sufficiency in clinical settings, although it is not the active form of vitamin D. The active form, 1,25(OH)₂D, does not reflect body stores of vitamin D as it is tightly regulated by serum calcium, phosphate, and PTH [2,3]. Severe vitamin D deficiency is known to cause rickets and osteomalacia [1]. Though controversial [4,5], suboptimal vitamin D has also been hypothesized to be associated with elevated risk of numerous non-skeletal conditions, such as cardiovascular disease, cancer, diabetes, impaired immunity, autoimmune disorders, and neuropsychological function. If a causal relationship is truly present between low 25(OH)D and these conditions, refining 25(OH)D measurement may enhance risk prediction and aid in targeting supplementation.

The presence of vitamin D₃ epimer [3-epi-25(OH)D₃] was first noted in neonates in 2001 [6], but only recently has been reported in adults [7–12]. Epimers have identical chemical structures except for a single site of carbon atom's asymmetry (in this case C-3α- vs. C-3β-hydroxy) [7]. As reviewed recently (2013), existing studies have estimated the percent of adults with detectable levels of the epimer to range from 0–100% [13]. This review calculated a weighted mean across studies of adults, and estimated that on average adults have a median (range) of the vitamin D₃ epimer of 1.72 (0–9.01) ng/ml* and that the epimer makes up 6.1% (0–47.0%) of the total 25(OH)D₃ [i.e. D₃ epimer + 25(OH)D₃] [13]. However, existing data are largely from clinical samples of Caucasians, and samples sizes have been relatively small (N<510).

Efforts to understand the physiological importance (if any) of the vitamin D₃ epimer are underway. Briefly, based on *in vitro* work and rodent models, it is believed that the D₃ epimer and the epimeric form of calcitriol (3-epi-1α,25(OH)₂D₃) bind to vitamin D binding protein at ~36–46% the affinity of 25(OH)D₃ and calcitriol (1α,25(OH)₂D₃), and to the vitamin D receptor (VDR) at 2–3% the affinity of 25(OH)D₃ and calcitriol [14]. However, the epimeric form of calcitriol has been shown to be capable of suppressing PTH secretion at similar concentrations as the non-epimeric form [15,16]. As this activity occurs despite lower VDR-binding, several authors have hypothesized that the epimeric form of calcitriol may be more metabolically stable than the non-epimeric form [14,17]. A detailed review of the physiological function of the vitamin D₃ epimer was recently published [13].

Work is needed to delineate several correlates of the vitamin D₃ epimer. Using a community based sample of whites and blacks, we describe the distribution and correlates of the vitamin D₃ epimer, whether its inclusion in the calculation of 25(OH)D results in reclassification of clinical 25(OH)D status, and the repeatability of epimer measurements over a 3-y interval.

$$*1 \text{ ng/ml} = 2.496 \text{ nmol/l}$$

2. Materials and Methods

2.1 Study Design Overview

The ARIC Study began when, between 1987 and 1989, a cohort of 15,792 men and women aged 45–64 y were recruited from 4 U.S. field centers. Only blacks (27% of the total sample) were recruited by the Jackson, Mississippi field center, while participants in the other field centers reflected the underlying population (mostly white in the suburbs of Minneapolis, Minnesota and Washington County, Maryland, and white and black in Forsyth County, North Carolina). A total of 5 clinic visits have taken place. Stored serum from the second visit (1990–1992), which was attended by 14,348 participants, was used to measure the vitamin D3 epimer. Epimer measurements were also conducted in a subset of 1,900 participants (50% black) using serum from visit 3 (1993–1995).

Of the 14,348 participants who attended visit 2, we excluded 38 participants who were not white or black as well as blacks from the Minnesota and Maryland centers, and 1,202 with missing data for 25(OH)D₃, 25(OH)D₂ and/or the vitamin D3 epimer. A total of 13,108 participants were available for analysis. All participants gave written informed consent, and local Institutional Review Boards approved the study protocol.

2.2 25(OH)D₂, 25(OH)D₃ and D₃ Epimer Measurements

The 3-epi-25(OH)D₃ was measured concurrently with 25(OH)D₂ and 25(OH)D₃ in fasting (12 h) serum collected in 1990–1992, which was stored at –70°C until analyzed in 2012–2013 at the University of Minnesota Molecular Epidemiology and Biomarker Research Laboratory. The method involved liquid-liquid extraction steps that included a –80 °C freezing step in a 96-well format as described by Hoofnagle [18]. This methodology allowed for the simultaneous high throughput analysis of the 3 storage forms of vitamin D, 25(OH)D₂, 25(OH)D₃ and D₃ epimer. Briefly, a 200 µl serum sample was extracted with the addition of 100 µl of acetonitrile, followed with 0.75 ml of hexane and freezing at –80 degrees C. Deuterated internal standards were added to each sample for D₂ and D₃. The extract was transferred to a new plate. The organic layer was evaporated and reconstituted with 150 µl of 60/40 MeOH/H₂O solvent. The reconstituted sample was loaded on a Shimadzu Prominence HPLC System with a pentafluorophenyl column kept at 50°C for the separation of the D₃, D₂, and epimer peaks with an ammonium acetate/MEOH solvent system. Analyte detection was performed with an AB SCIEX Triple Quad™ 5500 LC/MS/MS System and quantitated using the Multiquant™ software. The instrument was calibrated with the use of certified standards from ClinCal®. In-house controls with high and low concentrations of the analytes are analyzed with each run and monitored on a daily basis. The lower limits of quantification were as follow: D₃ epimer = 1.41 ng/ml, 25(OH)D₃ = 1.23 ng/ml, 25(OH)D₂ = 0.21 ng/ml. The overall assay precision had CVs of <7% for all analytes and concentrations. The entire method was developed following CLIA guidelines and the assay was performed in a CLIA certified laboratory. The laboratory participated in the College of American Pathologists (CAP) proficiency program. Additional verification of

performance was indicated by participation in the CDC and DEQAS proficiency programs. Blind duplicate split specimens were collected at ARIC visit 2. These specimens were collected from single participant blood draws, and split into separate vials. Thus, they give an indication of variability due to post-draw processing (1990–1992) and long-term storage (1990–2013), as well as in the analytic measurement (2011–2013). The blind duplicate coefficient of variation (CV) and Pearson correlation coefficients were as follows: 25(OH)D3 CV = 6.9, $r = 0.97$; 25(OH)D2 CV = 20.8, $r = 0.98$; D3 epimer CV = 16.5, $r = 0.76$.

2.3 Other Variables of Interest

At each ARIC clinic visit trained study personnel gathered information on demographics, behaviors, and physiologic characteristics. Information was collected at visit 2, unless otherwise noted. Questionnaires were used to assess age, sex, race, educational attainment, smoking status, physical activity (visit 1 values carried forward) [19] and medication use. Participants were also asked to bring to the visit all medications, vitamins and supplements taken in the 2 weeks before the examination; all medication names were transcribed and coded. Dietary vitamin D intake was assessed at ARIC visit 3 (1993–1995) using a modified Harvard food frequency questionnaire. Due to the availability of more detailed information on supplements, information on multivitamin and vitamin D supplement use came from ARIC visit 3. Height (m) and weight (kg) were measured, and body mass index (BMI) calculated as $\text{weight(kg)}/\text{height(m)}^2$. Sitting blood pressure was measured in triplicate with a random-zero sphygmomanometer; the mean of the latter two measurements were used in this analysis. Diabetes was defined by fasting serum glucose >126 mg/dl, nonfasting glucose >200 mg/dl, a self-report of physician diagnosis, or current medication use for diabetes.

Lipids were measured in EDTA-plasma within a few weeks of ARIC visit 2 (1990–1992) at Baylor College of Medicine. Plasma total cholesterol [20] and triglycerides [21] were determined by enzymatic methods. HDL-C was measured after dextran-magnesium precipitation [22], and the Friedewald equation [23] was used to calculate LDL-C in those with triglyceride levels under 400 mg/dl. Several other relevant analytes were measured in serum in 2012–2013 at the University of Minnesota Advanced Research and Diagnostic Laboratory: serum phosphorus and calcium using colorimetric methods, high sensitivity C-reactive protein (hsCRP) using a latex-particle enhanced immunoturbidimetric assay kit, and cystatin C using the Gentian cystatin C reagent and calibrator; all on a Roche Modular P Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN). Intact PTH was measured in serum on a Roche Elecsys 2010 Analyzer (Roche Diagnostics Corp.) using a sandwich immunoassay method (Roche Diagnostics, Indianapolis, IN). Serum creatinine was measured within a few weeks of collection in 1990–1992 at the University of Minnesota Collaborative Studies Clinical Laboratory using a modified kinetic Jaffé reaction. Estimated glomerular filtration rate (eGFR) was calculated using the 2012 CKD EPI equation which incorporates both cystatin C and creatinine [24]. eGFR was categorized according to established clinical cut-points: 90, 60–89, and 15–59 ml/min/1.73 m².

2.4 Statistical Analysis

25(OH)D₃ concentrations are known to vary by season [25]. Therefore we adjusted 25(OH)D₃ for seasonal variation by computing the residuals from a linear regression model with 25(OH)D₃ as the dependent variable and month of blood draw (modeled categorically) as the independent variable. By definition, these residuals are uncorrelated with month of blood draw. The grand mean was then added to the 25(OH)D₃ residuals obtained from this model. We performed this adjustment separately for whites and for blacks, as seasonal variation in 25(OH)D₃ concentrations vary by race [26]. This new variable “25(OH)D₃ adjusted for month of blood draw” is an estimate of average annual 25(OH)D₃ levels, and was used in all analyses. After careful consideration, we did not use this approach to adjust D3 epimer concentrations for season, since the magnitude of seasonal variation for the D3 epimer was much smaller. In many participants the epimer was undetectable or below the limit of quantification, and using the aforementioned approach appeared to induce measurement error. Instead, where specified, we adjusted for seasonality using a dummy-coded variable, which incorporated the 1 month lag associated with the half-life of 25(OH)D₃ concentrations: Winter (Jan to Mar), Spring (Apr to June), Summer (July to Sept), Fall (Oct to Dec). A Fig. is also used to show the association between epi-25(OH)D₃ and month of blood draw. When the D3 epimer was modeled as a continuous variable (e.g. for correlation analyses), undetectable values were set to 0, and values below the limit of quantification were set to 0.70 ng/ml, which is the midpoint between 0 and the lowest level of quantification (i.e., 1.40 ng/ml).

Results are generally presented stratified by race, given inherent interest. Descriptive statistics are provided for the D3 epimer, 25(OH)D₃, and 25(OH)D₂ using means and proportions. Pearson’s correlations are provided to show the interrelations between the D3 epimer, 25(OH)D₃, and 25(OH)D₂. Restricted cubic splines also provide a visual depiction of the association between the D3 epimer and 25(OH)D₃.

General linear regression was used to describe the association between the D3 epimer and demographic, behavioral, and physiologic characteristics, after adjustment for season of blood draw and (where appropriate) age and sex. Least-squares means are presented for continuous variables, and percentages for categorical variables. P-trend values were calculated by including a linear term for category number. Associations between 25(OH)D₃ and demographic, behavioral, and physiologic characteristics are also provided, stratified by race.

In clinical settings total 25(OH)D levels are usually calculated as 25(OH)D₂ + 25(OH)D₃. In order to evaluate the influence of the epimer on clinical 25(OH)D classification we present the proportion of participants who would be categorized as 25(OH)D deficient (<20 ng/ml), suboptimal (20 to <30 ng/ml), and sufficient (≥ 30 ng/ml), both with and without the epimer included in the calculation of total 25(OH)D. Agreement was tested using weighted kappa statistics. Lastly, we provide correlations for the D3 epimer, 25(OH)D₃, and 25(OH)D₂ in samples collected ~3 y apart.

3. Results

3.1 Distribution of the Vitamin D3 Epimer

In this population-based sample of 9,887 whites and 3,221 blacks, aged 48–67 y, levels of the vitamin D3 epimer varied by race. Among whites, 33.4% had quantifiable levels (i.e.

1.41 ng/ml), 30.6% had levels that were detectable but below the level of quantification, and for 36.0% the vitamin D3 epimer was undetectable. Among whites with quantifiable levels of the epimer, the mean (25th and 75th percentile) was 2.12 (1.60, 2.33) ng/ml. Only 43 whites (0.43% of the total sample) had a D3 epimer value ≥ 5 ng/ml, and only 2 whites (0.02% of the total sample) had a D3 epimer level ≥ 10 ng/ml. Epimer levels constituted on average 3.23% (SD = 3.40%) of total D3 levels (epimer + 25(OH)D₃), with a range among ARIC whites from 0–59.1%.

In blacks, the epimer was quantifiable in 15.0% of samples, while for 24.4% it was below the limit of quantification, and in 60.6% it was undetectable. Among blacks with quantifiable levels, the mean (25th and 75th percentile) was 2.16 (1.55, 2.17) ng/ml. Only 12 blacks (0.37% of the total sample) had a D3 epimer value ≥ 5 ng/ml, and only 5 blacks (0.15% of the total sample) had a D3 epimer level ≥ 10 ng/ml. Epimer levels constituted on average 2.25% (SD = 3.98%) of total D3 levels (epimer + 25(OH)D₃), with a range from 0–57.5%.

Levels of 25(OH)D₃ also varied by race, with whites having higher mean (25th and 75th percentile) levels 24.3 (18.8, 29.3) ng/ml than blacks 17.6 (12.9, 21.3) ng/ml. Levels of 25(OH)D₂ did not vary by race; means (25th and 75th percentiles) in whites and blacks were 1.70 (0.47, 1.37) and 1.35 (0.37, 1.07) ng/ml, respectively. Race-stratified mean epimer levels by month of blood draw are presented in Fig. 1.

In whites, levels of the epimer were highly correlated with levels of 25(OH)D₃ ($r = 0.54$; p -value < 0.0001), but weakly correlated with levels of 25(OH)D₂ ($r = -0.05$; p -value < 0.0001). These correlations among blacks were $r = 0.36$; p -value < 0.0001 and $r = 0.001$; p -value = 0.97, respectively. The continuous association between the D3 epimer and 25(OH)D₃ is also visually depicted using restricted cubic spline models, adjusted for age, sex and season of blood draw (Fig. 2).

3.2 Participant Characteristics and the Vitamin D3 Epimer

Associations between vitamin D3 epimer category and demographic, behavioral, and physiologic characteristics are presented for whites in Table 1, adjusted (where appropriate) for age, sex, and season. Overall, participants with higher levels of the epimer were more likely to consume vitamin D supplements, had a greater daily intake of vitamin D, and tended to be male, more physically active, non-smokers, and overall had a better cardiovascular risk factor profile. These participant characteristic patterns were similar to those observed among whites when characteristics were instead stratified by quintile of 25(OH)D₃ (Table 2).

For comparative purposes, participant characteristics by 25(OH)D₃ quintile among blacks are presented in Supplemental Table 1. Associations between participant characteristics and

25(OH)D₃ concentrations among blacks followed a similar pattern to those observed among whites, however associations tended to be weaker, and at times were not significant. We do not present an equivalent of Table 1 for blacks, as only 489 blacks had quantifiable levels of the epimer, and among those with quantifiable levels, concentrations were low [mean (25th and 75th percentile) = 2.16 (1.55, 2.17)].

3.3 The D3 Epimer and Clinical 25(OH)D Status

Table 3 presents, by race, the proportion of participants classified as having deficient, suboptimal, and optimal 25(OH)D status when 25(OH)D was calculated as the sum of 25(OH)D₂ + 25(OH)D₃ vs 25(OH)D₂ + 25(OH)D₃ + 3-epi-25(OH)D₃. Without the epimer, 22% of whites and 62% of blacks were classified as clinically deficient (<20 ng/ml). Inclusion of the epimer had little impact on these proportions, with less than 2% of whites and 3% of blacks reclassified from deficient to suboptimal. Concentrations of 25(OH)D calculated by 25(OH)D₂ + 25(OH)D₃ were optimal (≥ 30 ng/ml) in 29% of whites and 6% of blacks; with inclusion of the epimer in the calculation, optimal concentrations were observed in 33% of whites and 8% of blacks. Weighted kappa statistics indicated very good agreement between the two methods of classifying 25(OH)D status among both whites [κ (95% CI) = 0.91 (0.90–0.92)] and blacks [κ = 0.92 (0.91, 0.93)].

3.4 Repeatability of Epimer Concentrations Across Time

In a subset of 1,770 participants, vitamin D measurements were repeated in serum collected 3 years later (ARIC visit 3; 1993–1995). Among whites, correlations (r) between visit 2 and visit 3 were 0.43 for the D3 epimer, 0.71 for 25(OH)D₃, and 0.60 for 25(OH)D₂ (all p-values <0.0001). Among blacks, correlations (r) between visit 2 and visit 3 were 0.34 for the D3 epimer, 0.64 for 25(OH)D₃, and 0.33 for 25(OH)D₂ (all p-values <0.0001). Split-sample CV's of 25(OH)D₂, 25(OH)D₃, and the D3 epimer at visit 2 are reported in the Methods.

4. Discussion

In this population-based sample of 9,887 white and 3,221 black middle-aged adults we describe the distribution and correlates of the vitamin D3 epimer, whether its inclusion in the calculation of 25(OH)D results in reclassification of clinical 25(OH)D status, and repeatability of epimer measurements over a 3-year interval. Key findings are that D3 epimer levels were below our limit of quantification (1.41 ng/ml) in a large proportion of whites (66%) and blacks (85%), and that even when quantifiable, epimer concentrations are low, making up only 3.2% and 2.3% of total 25(OH)D₃ [epimer + 25(OH)D₃] levels in white and blacks, respectively. Accordingly, inclusion of the epimer in calculating total 25(OH)D levels had little impact on clinical 25(OH)D status. The epimer was correlated with 25(OH)D₃ in both whites (r = 0.54) and blacks (0.36), but was unrelated to 25(OH)D₂. Among whites, the D3 epimer was associated with participant characteristics in a manner similar to 25(OH)D₃. However, 3-year repeatability of the vitamin D epimer was modest in both whites (r = 0.43) and blacks (r = 0.34).

4.1 Distribution and Correlates of the D3 epimer

In the present sample, the epimer was quantifiable in 33% of whites and 15% of blacks, however even when quantifiable, absolute concentrations were low. On average the epimer made up only 2–3% of total D3 (epimer + 25(OH)D₃) levels. This is somewhat lower than the 6.1% calculated in a recent review [13]. The study samples used for that report were, however, obtained largely from clinical populations of Caucasians, with relatively small sample sizes. Furthermore, different methods were used to measure the D3 epimer, complicating comparisons across study populations. Importantly, about 0.4% of our analytic sample had D3 epimer levels ≥ 5 ng/ml. Thus for this very small proportion of individuals, the D3 epimer may make a meaningful contribution to their total D3 profile.

Consistent with prior reports, we found a positive correlation between concentrations of the D3 epimer and 25(OH)D₃ [10,12]. It is well established that neonates have higher absolute concentrations of the D3 epimer [13], but whether D3 epimer levels are associated with age in adults is unclear. Among ARIC whites, we observed no association between the 20-year age span and D3 epimer levels; this replicates findings from one prior study of adults [10] but is in contrast with another, which in its much larger age-range (1 to 94 y) identified an inverse association between D3 epimer levels and age [11]. Little is known about whether, and how, D3 epimer levels are associated with other individual-level characteristics. In our population-based sample, a fairly strong association was observed with sex, with men having higher D3 epimer levels than women. For most behavioral and physiologic characteristics, similar associations were observed with the D3 epimer as typically reported with 25(OH)D₃. Presently it is unknown whether this is a function of the epimer having an independent effect on these characteristics, or vice versa, or whether these associations are an artifact of the relatively high correlation between the D3 epimer and 25(OH)D₃. As noted in the introduction, basic science work has suggested that the D3 epimeric forms are physiologically active, albeit to a lesser degree than 25(OH)D₃ and calcitriol [13–17].

4.2 Epimer Inclusion and Clinical 25(OH)D Status

Distinguishing vitamin D3 epimeric forms from non-epimeric forms may be relevant clinically, given current evidence suggesting that epimeric forms are less active. There are presently several ways to measure 25(OH)D. Importantly, the commonly-used DiaSorin RIA does not appear to detect the D3 epimer [13]. Conversely, high-performance liquid chromatography tandem mass spectrometry, which has emerged as the preferred method for measuring 25(OH)D [10,27], generally separates the epimer from 25(OH)D₃ and provides a separate measurement for each. However, in some cases if the epimer is not explicitly measured levels of 25(OH)D₃ will be overestimated, as the D3 epimer values will be counted as 25(OH)D₃ given their similar structure and mass [7,10,13,28]. It has been demonstrated that in these cases the positive bias seen with LC-MS/MS is removed once the concentration of the epimer is subtracted, indicating that the lack of agreement between LS-MS/MS assays and the DiaSorin RIA method can most likely be attributed to the presence of the epimer [11]. Thus when interpreting findings from vitamin D screening, it may be of value for clinicians to be cognizant of the assay employed and how the assay handles the vitamin D3 epimer.

We found that inclusion of the D3 epimer in the calculation of total 25(OH)D had relatively little impact on clinical 25(OH)D status, as expected given the low epimer concentrations observed in our study sample. Adding the epimer to traditional 25(OH)D calculations (i.e. 25(OH)D₂ + 25(OH)D₃) resulted in only about 2% of participants being reclassified from being clinically deficient to having suboptimal 25(OH)D. The proportion of whites and blacks whose status changed from suboptimal to optimal 25(OH)D with the inclusion of the epimer were 4% and 2%, respectively. A similar analysis was conducted in a study of 501 outpatients aged 1–94 years. In that sample, inclusion of the epimer resulted in 3% of participants being reclassified as sufficient, whom with traditional 25(OH)D calculation were classified as deficient [11]. Overall, relatively few people appear to be reclassified based on whether or not the D3 epimer is used in the calculation of 25(OH)D.

4.3 Strengths & Limitations

Strengths of this study include the large community-based sample of blacks and whites which is well characterized in terms of demographics, behaviors, and physiologic characteristics, and repeat measurement of the D3 epimer in a subset of 1,770 participants. Perhaps the major limitation of this analysis is that the serum in which the epimer was measured was collected in 1990–1992. It is possible that contemporaneous (2014) epimer levels (or their proportions relative to 25(OH)D₃) in the general population differ from those obtained 20 years ago. Additionally, although 25(OH)D is incredibly stable with extended storage [29–32], it is unclear whether the D3 epimer is similarly stable. Also, it is unclear whether associations we observed between the D3 epimer and personal characteristics simply occurred as a consequence of levels of the D3 epimer and 25(OH)D₃ being correlated. Additional carefully controlled experimental studies are needed to determine the true physiologic relevance of the D3 epimer. Also relevant would be longitudinal studies that addressed whether the D3 epimer is associated with incident disease outcomes differently than total 25(OH)D, but given the moderately high correlation between the D3 epimer and total 25(OH)D, it would be very difficult to determine independence from total 25(OH)D.

5. Conclusions

These findings confirm that vitamin D₃ epimer is present among adults, though concentrations tend to be low. It is correlated with 25(OH)D₃ but not 25(OH)D₂ in both whites and blacks, and cross-sectionally correlates with participant characteristics in a manner similar to 25(OH)D₃. However given the low concentrations of the epimer, its inclusion in the calculation of total 25(OH)D had little impact on reclassification of clinical 25(OH)D status. Additional efforts are needed to understand the whether the epimer is bioactive.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

ARIC Atherosclerosis Risk in Communities Study

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Highlights

- D3 epimer levels were below not quantifiable in most whites (66%) and blacks (85%).
- Even when quantifiable, the epimer made up, on average, only ~3% of total 25(OH)D₃.
- Inclusion of the epimer had little impact on clinical 25(OH)D status.
- The epimer was correlated with 25(OH)D₃ in whites ($r=0.54$) and blacks (0.36).
- The D3 epimer and 25(OH)D₃ associate similarly with participant characteristics.

Fig. 1. Monthly variation in concentrations of the vitamin D3 epimer in whites and blacks*: The ARIC Study 1990–1992. Dots and squares represent mean levels; error bars show 95% confidence intervals.

Fig. 2A: Whites **Fig. 2B: Blacks** **Fig. 2.**

Associations between of the vitamin D3 epimer and 25(OH)D₃ in whites and blacks: The ARIC Study 1990–1992.

Biomarkers modeled as restricted cubic splines. Only those with quantifiable levels of the D3 epimer were included. Knots are at the 5th, 27.5th, 50th, 72.5th and 95th percentiles (whites: 1.44, 1.61, 1.85, 2.25, 3.63; blacks: 1.43, 1.56, 1.77, 2.08, 3.82), and are adjusted for age and sex. Values shown are generated for a 55-year old male whose serum was collected between January and March.

Table 1

Characteristics of Caucasian participants by vitamin D3 epimer category: The ARIC Study 1990–1992

	Undetectable		Below Limit of Quantification	Quantifiable			p-trend
	Tertile 1	Tertile 2		Tertile 3			
Median (ng/ml)	0	0.7	1.53	1.85	2.62		
Range (ng/ml)			1.41 – 1.67	1.68 – 2.13	2.13 – 17.5		
N total	3559	3020	1102	1103	1103		
Vitamin D Intake & Biomarkers ^a							
Multivitamin, %	25.7	27.6	29.9	28.3	27.5	0.08 ↔	
Vit D Supplement, %	2.38	2.83	3.81	2.87	3.92	0.01 ↑	
Vit D Intake ^b , IU	238	251	256	253	255	0.0008 ↑	
Vitamin D2, ng/ml	1.92	1.58	1.55	1.49	1.67	0.002 ↓	
Vitamin D3 ^c , ng/ml	18.8	24.6	27.2	29.4	33.8	<0001 ↑	
Demographics							
Age, mean years	57.1	57.1	57.0	57.4	57.1	NS ↔	
Female, %	63.4	54.9	48.7	42.6	38.2	<0001 ↓	
Behavioral Characteristics ^a							
Sport Index, mean	2.39	2.55	2.61	2.67	2.80	<0001 ↑	
Current smoker, %	23.9	19.8	19.1	18.4	18.3	<0001 ↓	
Physiologic Characteristics ^d							
BMI, kg/m ²	27.8	27.3	27.2	26.9	26.5	<0001 ↓	
Prevalent diabetes, %	13.7	10.5	11.1	9.95	9.12	<0001 ↓	
Systolic BP, mmHg	120	119	120	120	119	NS ↔	
Hypertension med use, %	22.0	20.6	21.8	21.8	20.6	NS ↔	
Lipid Lowering med use, %	6.45	6.54	7.39	8.96	10.3	<0001 ↑	
HDL cholesterol, mg/dL	47.1	48.5	49.3	50.3	52.1	<0001 ↑	
LDL cholesterol, mg/dL	134	133	132	133	132	NS ↔	
Triglycerides, mg/dL	143	142	142	137	141	NS ↔	
hsCRP, mg/dL	4.37	3.71	3.90	3.29	3.64	0.0001 ↓	
PTH, pg/ml	42.5	39.5	39.6	39.1	37.1	<0001 ↓	

	Undetectable	Below Limit of Quantification	Quantifiable			p-trend
			Tertile 1	Tertile 2	Tertile 3	
Calcium ^d , mg/dl	9.16	9.17	9.17	9.15	9.11	0.005 ↓
Phosphorous, mg/dl	3.48	3.52	3.53	3.51	3.51	0.047 ↑
Magnesium, mEq/l	1.64	1.63	1.63	1.63	1.61	<.0001 ↓
eGFR CysC, ml/min/1.73m ²	92.8	93.6	94.4	94.5	94.4	<.0001 ↑

^a Adjusted for age, sex and season of blood draw

^b Estimated intake from both diet and supplements

^c Adjusted for seasonality using the residuals approach

^d Corrected for serum albumin

Table 2

Characteristics of Caucasian participants by 25(OH)D₃ quintile: The ARIC Study 1990–1992

25(OH)D ₃	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	p-trend
Median (ng/ml)	12.8	17.9	22.1	26.5	33.6	
Range (ng/ml)	0.79 – 15.4	15.4 – 19.9	20.0 – 24.2	24.2 – 29.4	29.4 – 75.9	
N total	1270	1756	2111	2294	2456	
Vitamin D Intake & Biomarkers^a						
Take Multivitamin, %	25.5	25.8	26.7	28.3	28.6	0.01 ↑
Take VitD Supplement, %	0.89	3.26	2.88	2.80	3.81	0.0001 ↑
Vit D Intake ^b , IU	209	235	245	265	261	<0.0001 ↑
Vitamin D2, ng/ml	2.77	1.91	1.68	1.42	1.27	<0.0001 ↓
Vitamin D3 Epimer ^c , ng/ml	0.03	0.70	1.52	1.84	2.90	<0.0001 ↑
Demographics						
Age, mean years	57.1	57.1	57.0	57.1	57.3	NS ↔
Female, %	71.9	63.2	52.0	46.6	47.1	<0.0001 ↓
Behavioral Characteristics^d						
Sport Index, mean	2.29	2.40	2.51	2.57	2.75	<0.0001 ↑
Current smoker, %	31.8	21.1	19.3	18.4	18.7	<0.0001 ↓
Physiologic Characteristics^d						
BMI, kg/m ²	28.7	28.1	27.6	27.1	26.1	<0.0001 ↓
Prevalent diabetes, %	17.0	14.4	11.8	9.76	8.05	<0.0001 ↓
Systolic BP, mmHg	122	120	120	119	118	<0.0001 ↓
Hypertension med use, %	23.6	23.7	21.7	20.6	19.1	<0.0001 ↓
Lipid Lowering med use, %	9.08	7.11	6.94	6.83	7.24	NS ↔
HDL cholesterol, mg/dL	46.0	47.0	48.2	48.3	52.1	<0.0001 ↑
LDL cholesterol, mg/dL	133	134	133	135	131	0.08 ↔
Triglycerides, mg/dL	149	147	144	140	133	<0.0001 ↓
CRP-S, mg/dL	5.01	4.25	3.63	3.69	3.56	<0.0001 ↓
PTH, pg/ml	45.9	42.2	40.8	39.4	36.2	<0.0001 ↓
Calcium ^d , mg/dl	9.16	9.14	9.15	9.16	9.16	NS ↔

25(OH)D ₃	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	p-trend
Phosphorous, mg/dl	3.51	3.52	3.50	3.50	3.51	NS ↔
Magnesium, mEq/l	1.62	1.63	1.64	1.63	1.63	NS ↔
eGFR CysC, ml/min/1.73m ²	92.6	94.3	94.4	93.4	93.1	0.36 ↔

^a Adjusted for age, sex, season of blood draw, and 25(OH)D3

^b Estimated intake from both diet and supplements

^c Adjusted for seasonality using the residuals approach

^d Corrected for serum albumin

Table 3

Proportion of participants classified as vitamin D deficient, suboptimal, and optimal based on whether the vitamin D3 epimer was included in the calculation of total 25(OH)D

	Deficient	Suboptimal	Optimal
	<20 ng/ml	20–30 ng/ml	30 ng/ml
Whites, N (%)			
D2 + D3	2,277 (22.3)	4,783 (48.4)	2,827 (28.6)
D2 + D3 + Epimer	2,090 (21.1)	4,506 (45.6)	3,291 (33.3)
Blacks, N (%)			
D2 + D3	1,984 (61.6)	1,043 (32.4)	194 (6.0)
D2 + D3 + Epimer	1,898 (58.9)	1,060 (32.9)	263 (8.2)