

# Vitamin D deficiency and non-lipid biomarkers of cardiovascular risk

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## Abstract

**Introduction:** Deficient 25-hydroxyvitamin D (25(OH)D) levels have been associated with dyslipidemia and cardiovascular diseases, though the underlying mechanism of these associations is uncertain. We analyzed associations between vitamin D and other non-lipid biomarkers of cardiovascular risk to better elucidate possible relationships between deficient 25(OH)D and cardiovascular disease.

**Material and methods:** We performed a cross-sectional analysis of 4,591 adults included in a clinical laboratory database from 2009 to 2011 with available measurements for 25(OH)D and the following biomarkers: homocysteine (Hcy), high-sensitivity C-reactive protein (hs-CRP), cystatin-C, creatinine,  $\gamma$ -glutamyltransferase (GGT), uric acid, and hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>). We calculated odds ratios (OR) of having high levels of each biomarker associated with 25(OH)D deficiency (< 20 ng/ml) compared to optimal levels ( $\geq$  30 ng/ml) using logistic regression adjusted for age, sex, and lipids.

**Results:** The mean  $\pm$  SD age was 60  $\pm$  14 years and 46% of patients were women. In multivariable-adjusted models, adults with deficient 25(OH)D compared to those with optimal levels had increased odds of elevated biomarkers as follows: Hcy (OR = 2.53, 95% CI: 1.92–3.34), hs-CRP (1.62, 1.36–1.93), cystatin-C (2.02, 1.52–2.68), creatinine (2.06, 1.35–3.14), GGT (1.39, 1.07–1.80), uric acid (1.60, 1.31–1.95), and HbA<sub>1c</sub> (2.47, 1.95–3.13). In analyses evaluating women and men separately, 25(OH)D deficient women but not men had increased odds of elevated levels of all biomarkers studied. There were significant interactions based on sex between 25(OH)D and Hcy ( $p = 0.003$ ), creatinine ( $p = 0.004$ ), uric acid ( $p = 0.040$ ), and HbA<sub>1c</sub> ( $p = 0.037$ ).

**Conclusions:** Deficient 25(OH)D is associated with elevated levels of many biomarkers of cardiovascular risk, particularly among women, in a United States population.

**Key words:** vitamin D, cardiovascular risk, biomarkers.

## Introduction

Vitamin D deficiency is a common condition that affects over 40% of the United States population [1]. Deficiency in 25-hydroxyvitamin D

(25(OH)D), the most widely used measure of vitamin D status, has been independently associated with risk of cardiovascular disease, severity of coronary atherosclerosis, and all-cause mortality [2–4]. However, clinical trials have not definitively shown that treatment of 25(OH)D deficiency with supplementation improves cardiovascular outcomes, and it remains uncertain why these associations exist [5]. The association between vitamin D deficiency and cardiovascular risk may be explained by underlying pathophysiologic mechanisms which have not been fully elucidated. We previously found that low 25(OH)D is associated with an atherogenic lipid profile [6, 7]. We now evaluate whether 25(OH)D deficiency is associated with other biomarkers of cardiovascular risk in a single study cohort, the Very Large Database of Lipids (VLDL-3B) Study.

### Material and methods

We used the Very Large Database of Lipids (VLDL), a dataset of 1,340,614 U.S. adults who were referred for Vertical Auto Profile (VAP) ultracentrifugation lipid analysis from 2009 to 2011 [8]. We studied 4,591 individuals who had available measurements for 25(OH)D, homocysteine (Hcy), high-sensitivity C-reactive protein (hs-CRP), cystatin-C, creatinine,  $\gamma$ -glutamyltransferase (GGT), uric acid, and hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>).

Total 25(OH)D was measured using the LIASON 25(OH) Vitamin D Reagent Integral: Ref 310600 kit and Liaison chemistry analyzer (DiaSorin). Direct measurements of high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), non-HDL-C, and low-density lipoprotein cholesterol (LDL-C) were conducted using inverted rate zonal single vertical spin, density gradient ultracentrifugation by the VAP technique. Triglycerides (TG) were measured with the Abbott ARCHITECT C-8000 system (Abbott Park, IL). Hcy and cystatin C were measured using marker-specific reagents manufactured by Diazyme Laboratories (Poway, CA) and the Architect clinical chemistry analyzer (Abbott Laboratories, Abbott Park, IL). Creatinine, GGT, and uric acid were measured using marker-specific reagent and Architect clinical chemistry analyzer manufactured by Abbott Laboratories (Abbott Park, IL). HbA<sub>1c</sub> was measured with an assay based on the AxSym chemistry analyzer and an immunoassay reagent (Abbott Diagnostics). The methodology was changed on 07/20/2010 to one based on the Tosoh Automated Glycohemoglobin Analyzer and G8 Variant Elution Buffer No. 1 (S), No. 2 (S), and No. 3 (S) (Tosoh Corporation). As is typical for a laboratory dataset, other clinical characteristics such as body mass index, physical activity levels, and medication/supplement usage were not available.

Atherotech Diagnostics Lab recorded patient data as part of routine clinical measurements, removed duplicates, and de-identified data before transfer to investigators. The database is housed at the Johns Hopkins Hospital in Baltimore, Maryland. All investigators had unrestricted access to the data and authority over the manuscript. The Johns Hopkins University School of Medicine Institutional Review Board granted the study an exemption from informed consent.

We examined the distribution of demographics and biomarkers across the following clinical cutoff levels of 25(OH)D defined by the Endocrine Society guidelines [9]: deficient (< 20 ng/ml), intermediate ( $\geq$  20–30 ng/ml), and optimal ( $\geq$  30 ng/ml). To convert 25(OH)D levels from ng/ml to nmol/l, multiply by 2.496. Elevated non-lipid biomarkers were defined as follows: Hcy ( $\geq$  18.6  $\mu$ mol/l), hs-CRP ( $\geq$  2.0 mg/l), cystatin-C ( $\geq$  1.65 mg/l), creatinine ( $\geq$  1.5 mg/dl), GGT ( $\geq$  73 U/l), uric acid ( $\geq$  6 (women),  $\geq$  7 (men) mg/dl), and HbA<sub>1c</sub> ( $\geq$  6.5%). We conducted logistic regressions to determine the odds of elevated non-lipid biomarker levels for deficient (compared to optimal) 25(OH)D groups after adjustment for age, sex, HDL-C, directly measured LDL-C, and TG. We also tested for interactions by sex and age based on 25(OH)D status.

### Statistical analysis

Statistical analyses were generated using Stata version 12.0 (College Station, TX).

### Results

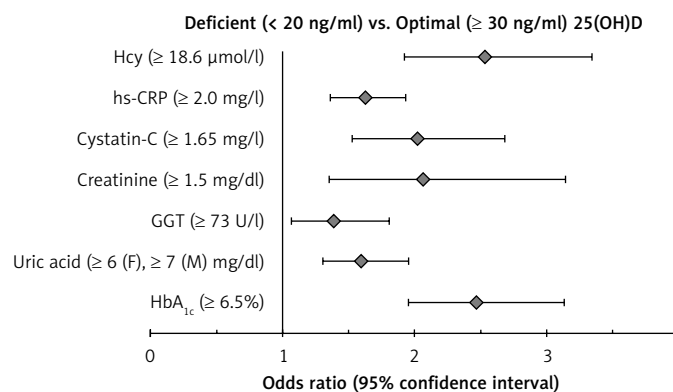
Our study population consisted of 4,591 individuals with a mean  $\pm$  SD age of 60  $\pm$  14 years, and 46% were women. The clinical characteristics of individuals with deficient, intermediate, and optimal 25(OH)D are shown as median and interquartile range in Table I. After using multivariable adjustment to account for age, sex, LDL-C, TG, and HDL-C, increased odds of elevated biomarkers for 25(OH)D deficient individuals persisted for all variables (Figure 1): Hcy (OR = 2.53, 95% CI: 1.92–3.34), hs-CRP (1.62, 1.36–1.93), cystatin-C (2.02, 1.52–2.68), creatinine (2.06, 1.35–3.14), GGT (1.39, 1.07–1.80), uric acid (1.60, 1.31–1.95), and HbA<sub>1c</sub> (2.47, 1.95–3.13). We performed separate analyses adjusting for age, sex, and non-HDL-C and obtained similar results ( $p < 0.001$  for all variables).

We performed separate analyses stratified by sex and age. Among women, in multivariable-adjusted analyses, 25(OH)D deficiency compared to optimal levels was associated with increased odds of elevated levels of all biomarkers studied: Hcy (OR = 3.89, 95% CI: 2.52–5.99), hs-CRP (1.57, 1.22–2.03), cystatin-C (2.02, 1.33–3.08), creatinine (4.14, 1.88–9.13), GGT (1.72, 1.16–2.55),

**Table I.** Distribution of variables by clinical categories of 25(OH)D<sup>††</sup>

Parameter	Deficient 25(OH)D < 20 ng/ml (n = 850)	Intermediate 25(OH)D ≥ 20–30 ng/ml (n = 1430)	Optimal 25(OH)D ≥ 30 ng/ml (n = 2311)
Age [years]	58 (48–68)	59 (50–69)	62 (53–71)
Sex (% female)	50.8	44.0	46.4
Hcy [μmol/l]	11.6 (9–15.6)	11 (9–14.6)	10.7 (8.7–13.7)
hs-CRP [mg/l]	2.8 (1.3–6.3)	2.1 (0.9–4.6)	1.5 (0.7–3.7)
Cystatin-C [mg/l]	1.07 (0.93–1.32)	1.07 (0.93–1.28)	1.07 (0.93–1.28)
Creatinine [mg/dl]	0.9 (0.8–1.0)	0.9 (0.8–1.0)	0.9 (0.8–1.0)
GGT [U/l]	31 (21–53)	30 (20–44)	26 (18–39)
Uric acid [mg/dl]	5.6 (4.6–6.7)	5.6 (4.5–6.6)	5.1 (4.1–6.2)
HbA <sub>1c</sub> (%)	5.8 (5.5–6.3)	5.7 (5.5–6.1)	5.7 (5.4–6.0)
HDL-C [mg/dl]	46 (39–56)	49 (40–59)	52 (43–65)
TC [mg/dl]	197 (164–230)	185 (159–216)	175 (148–208)
Non-HDL-C [mg/dl]	148 (117–179)	134 (109–163)	118.5 (94–150)
LDL-C [mg/dl]	119 (91–147)	108.5 (85–135)	97 (76–125)
Triglycerides [mg/dl]	145 (102–213)	126 (89–183)	103 (74–148)

<sup>†</sup>Except for sex, values are median (interquartile range). HbA<sub>1c</sub> – hemoglobin A<sub>1c</sub>, BUN – blood urea nitrogen, HDL-C – high-density lipoprotein cholesterol, TC – total cholesterol, LDL-C – low-density lipoprotein cholesterol; <sup>††</sup>to convert 25(OH)D levels from ng/ml to nmol/l, multiply by 2.496.



**Figure 1.** Adjusted<sup>†</sup> odds ratios of elevated<sup>‡</sup> biomarkers in vitamin D deficient compared to optimal individuals

<sup>†</sup>Odds ratios were adjusted for age, sex, total cholesterol, directly measured LDL cholesterol, triglycerides, and HDL cholesterol.

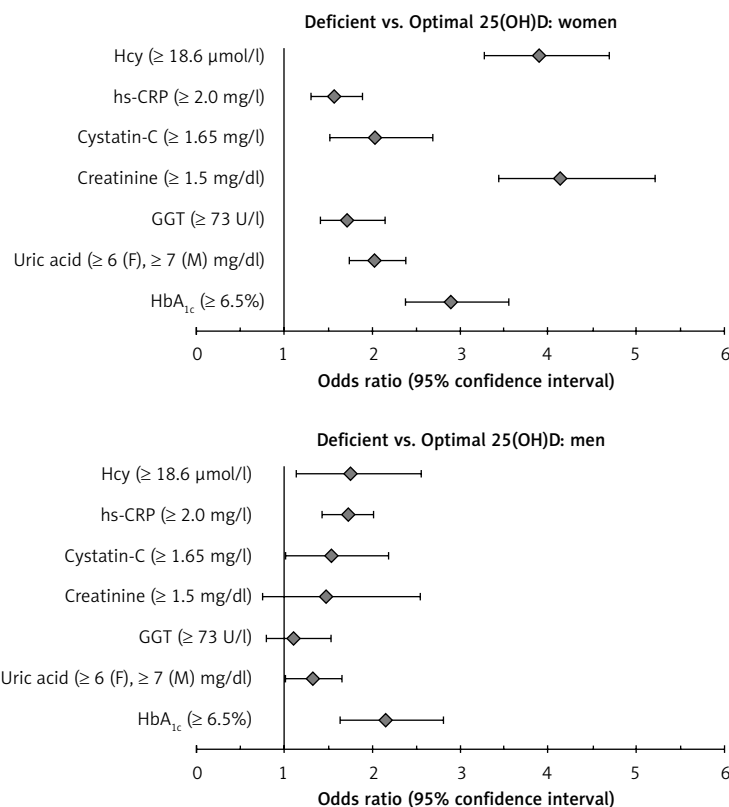
<sup>‡</sup>Elevated serum risk biomarkers were defined as follows (n = # of individuals with elevated levels out of total population of 4591): homocysteine ≥ 90<sup>th</sup> percentile = 18.6 μmol/l (n = 469), hs-CRP ≥ 2 mg/l (n = 2281), cystatin-C ≥ 90<sup>th</sup> percentile = 1.65 mg/l (n = 462), creatinine ≥ 1.5 mg/dl (n = 194), GGT ≥ 90<sup>th</sup> percentile = 73 U/l (n = 465), uric acid ≥ 7 mg/dl (men) or ≥ 6 mg/dl (women) (n = 992), HbA<sub>1c</sub> ≥ 6.5% (n = 640).

uric acid (2.02, 1.50–2.72), and HbA<sub>1c</sub> (2.90, 2.02–4.15). However, in men, there was a non-significant trend for associations with four out of seven biomarkers: cystatin-C (1.51, 0.96–2.37), creatinine (1.45, 0.86–2.46), GGT (1.10, 0.66–1.58), and uric acid (1.30, 0.99–1.72), but three biomarkers remained statistically significant: Hcy (1.73, 1.19–2.52), hs-CRP (1.70, 1.34–2.16), and HbA<sub>1c</sub> (2.14, 1.56–2.95) (Figure 2). We tested for interactions between 25(OH)D and each biomarker based on sex and found significant interactions for Hcy (p = 0.003), creatinine (p = 0.004), uric acid

(p = 0.040), and HbA<sub>1c</sub> (p = 0.037). In interaction testing based on age, we found a significant interaction for hs-CRP (p = 0.029) but not for any other biomarkers.

## Discussion

In this study we found that 25(OH)D deficiency is associated with increased levels of multiple non-lipid biomarkers of cardiovascular risk. This relationship was observed for all biomarkers included in our study and persisted after adjustment for age, sex, and lipids. We also found a trend towards



**Figure 2.** Adjusted<sup>†</sup> odds ratios of elevated<sup>‡</sup> biomarkers in vitamin D deficient compared to optimal individuals by sex

<sup>†</sup>Odds ratios were adjusted for age, total cholesterol, directly measured LDL cholesterol, triglycerides, and HDL cholesterol. <sup>‡</sup>Elevated serum risk biomarkers were defined as follows: homocysteine  $\geq$  90<sup>th</sup> percentile = 18.6  $\mu$ mol/l, hs-CRP  $\geq$  2 mg/l, cystatin-C  $\geq$  90<sup>th</sup> percentile = 1.65 mg/l, creatinine  $\geq$  1.5 mg/dl, GGT  $\geq$  90<sup>th</sup> percentile = 73 U/l, uric acid  $\geq$  7 mg/dl (men) or  $\geq$  6 mg/dl (women), HbA<sub>1c</sub>  $\geq$  6.5%.

increased odds of higher biomarkers for women compared to men, with significant interactions for Hcy, creatinine, uric acid, and HbA<sub>1c</sub> based on sex. Several studies have shown that 25(OH)D deficiency was associated with increased levels of several of the variables we analyzed, such as homocysteine, renal function, uric acid, and HbA<sub>1c</sub> [10–14]. However, to the best of our knowledge, this analysis is the first to evaluate the association between 25(OH)D and such an extensive panel of risk biomarkers in a single cohort of this size, and to show potential differences by sex.

Deficiency in 25(OH)D has been consistently associated with risk of myocardial infarction and cardiovascular death in multiple studies [4, 15], though the reason for this observation is unclear. The relationship between vitamin D and cardiovascular disease may be explained by mechanisms involving inflammation, the renin-angiotensin-aldosterone system, insulin sensitivity, or vascular calcification [16–22]. Alternatively, it is also possible that this association may be confounded by other variables related to overall health status including physical activity, sunlight exposure, and obesity [23–25]. Our work suggests that the association between 25(OH)D deficiency and cardio-

vascular risk may be related to a particular at-risk phenotype rather than one particular risk factor. Given that women appear to have increased odds of having elevated levels of several risk biomarkers compared to men, there may be additional sex-related factors such as hormones that influence the relationship between 25(OH)D deficiency and other cardiovascular risk factors.

Our study results should be considered in the context of several limitations. Our study is cross-sectional, therefore our results are limited to the associations between variables and do not prove a causal relationship between 25(OH)D deficiency and the biomarkers we evaluated. The demographic and clinical information available in our data set also did not include variables such as race, body mass index, and comorbid conditions which may have impacted the relationships we observed.

In conclusion, deficient 25(OH)D is associated with elevated levels of multiple non-lipid cardiovascular risk biomarkers in a cross-sectional U.S. population. These associations were stronger in women than in men. Further studies are needed to explore the relationship between 25(OH)D and other biomarkers to determine whether these as-

sociations may influence long-term cardiovascular risk and whether treating vitamin D deficiency can lead to improvement in these biomarkers.

### Conflict of interest

K.F. Faridi, J.R. Lupton, M. Banach, R. Quispe have no relevant disclosures to report. S.S. Martin has served as a consultant to Pressed Juicery, Abbott Nutrition, Quest Diagnostics, Sanofi/Regeneron, and the Pew Research Center, outside the scope of this article. Dr. Martin has been the recipient of prior or pending grants associated with Aetna Foundation, the American Heart Association, and Google, outside the scope of this article. Dr. Martin is listed as a co-inventor on a pending patent for a method of LDL-C estimation, not used in this article. K. Kulkarni received a salary as an employee of Atherotech and a modest royalty from the University of Alabama at Birmingham. S.R. Jones is listed as a co-inventor on a pending patent for a method of LDL-C estimation, not used in this article. Dr. Jones has received a charitable gift for support for the VLDL project from the David and June Trone Family Foundation. E.D. Michos has no disclosures to report relevant to this study. Unrelated to this study, Dr. Michos has served as a consultant for Siemens Diagnostic, Inc. as a blinded adjudicator of coronary events for a clinical trial.

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### Role of data sponsor

Atherotech provided the investigators with de-identified data generated from commercial lipid analyses and did not provide payments for the research or manuscript writing and did not participate in data analysis or influence the conclusions. This study was initiated by the investigators and did not receive any specific funding. The authors take responsibility for the accuracy of the statistical analyses and had the sole authority on manuscript preparation and submission for publication.

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