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25-Hydroxyvitamin D, insulin resistance, and kidney function in the Third National Health and Nutrition Examination Survey

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Previous research has reported reduced serum 25-hydroxyvitamin D levels in patients with chronic kidney disease (CKD), although the relationship between vitamin D status and insulin resistance (IR) in patients with CKD has not been examined in the general population. We examined the association that kidney function, based on glomerular filtration rate (eGFR) estimated from serum creatinine, has with serum levels of 25-hydroxyvitamin D and components of the metabolic syndrome among 14 679 participants in the Third National Health and Nutrition Examination Survey (NHANES III). In this analysis, adjusted mean serum 25-hydroxyvitamin D was significantly lower only in the participants with a severe (15–29 ml/min/1.73 m^2) decrease in eGFR compared to those with normal kidney function (61.6 vs 73.3 nmol/l, $P = 0.0063$). Serum 25-hydroxyvitamnin D ($P = 0.0018$) and level of kidney function ($P = 0.0003$) were inversely associated, independent of each other, with homeostasis model assessment of insulin resistance (HOMA-IR), adjusting for confounders. Participants with high 25-hydroxyvitamin D levels (>81 nmol/l) had lower HOMA-IR. We conclude that 25-hydroxyvitamin D deficiency is not as prevalent in the US general population with decreased eGFR as previously reported in patients with CKD; and that vitamin D and kidney function have independent inverse associations with IR.

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Serum levels of 25-hydroxyvitamin D are the measure of body stores of vitamin D.[1] A reduction of serum 25-hydroxyvitamin D levels, the substrate for the kidney and other tissues for the generation of calcitriol ($1,25(OH)_2D_3$), produces secondary hyperparathyroidism,[2–4] reduced bone mineral density,[4] and increased rates of hip fracture[5] in individuals with normal kidney function. The parathyroid hormone–vitamin D axis plays a central role in calcium and phosphate homeostasis in patients with chronic kidney disease (CKD).[6] The recent Kidney Disease Outcomes Quality Initiative Clinical Practice Guidelines for Bone Mineral Metabolism and Disease in Chronic Kidney Disease have recommended the measurements of 25-hydroxyvitamin D levels in patients with CKD stages 3 and 4|the rationale being that low levels of 25-hydroxyvitamin D are likely to play a role in the development of secondary hyperparathyroidism by limiting the synthesis of calcitriol ($1,25(OH)_2D_3$).[7]

Vitamin D has also been recognized to have numerous non-calcemic functions.[8] Specifically, there is increasing evidence that vitamin D metabolism affects the risk of insulin resistance (IR), diabetes, and of the metabolic syndrome,[9–11] although the underlying molecular mechanism of this association remains to be elucidated.

Recently, human studies have shown diminished insulin-stimulated glucose uptake in uremic patients.[12] IR in patients with kidney disease is accompanied by hyperinsulinemia and glucose intolerance.[13,14] Chen *et al.*[15] identified a significant relationship between IR, insulin levels, and risk of CKD defined as an estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m^2 among 6453 persons without diabetes. Thus, kidney dysfunction appears to be associated with a syndrome of IR and several explanations have been proposed, including, vitamin D deficiency, anemia, or uremic toxins.[16,17] Given that a number of investigators[9–11] have shown that low vitamin D levels are associated with various measures of impaired glucose metabolism and diabetes risk in people with normal kidney function, it seems reasonable to propose that IR in patients with CKD may also be associated with reduced levels of 25-hydroxyvitamin D.

There appear to be no reports on the association between 25-hydroxivitamin D status and kidney function at the

population level. Previous reports come from small clinic-based samples[6,18,19] and may not represent the true association between vitamin D status and kidney function in the general population. However, the survey with the largest number of people with serum 25-hydroxyvitamin D measurements (nearly 20 000) appears to be the Third National Health and Nutrition Examination Survey (NHANES III), a national cross-sectional survey representative of the US population carried out in 1988–1994. The large number of people with concurrent blood measurements of 25-hydroxyvitamin D, fasting glucose, insulin, and kidney function provides an ideal opportunity to determine whether vitamin D status is related to IR in participants with CKD.

RESULTS

Variables associated with vitamin D

Mean serum 25-hydroxyvitamin D concentrations were higher in males than females and declined with increasing age. There were significant ethnic variations in 25-hydroxyvitamin D levels, reflected in means that were higher in non-Hispanic whites, intermediate in Mexican Americans, and lower in non-Hispanic blacks (Table 1). Mean serum vitamin D levels decreased with increasing body mass index (BMI) quintile, and increased with increasing frequency in the last month of physical activity and of drinking milk or having milk on cereal, and with increasing daily dose of vitamin D supplements (Table 1). The weighted percent of participants with mild, moderate, and severe kidney dysfunction taking vitamin D of >400 IU/day was 22.6% (95% confidence interval 20.8, 24.5), 24.6% (95% confidence interval 20.7, 28.5), and 11.2% (95% confidence interval 0.5, 22.0), respectively.

Vitamin D levels in participants with decreased kidney function

The adjusted mean serum 25-hydroxyvitamin D levels were mildly increased in participants with mildly decreased kidney function (eGFR, 60–89 ml/min/1.73 m^2) and decreased in those with severely decreased kidney function (eGFR, 15–29 ml/min/1.73 m^2), compared to participants with normal kidney function (eGFR >90 ml/min/1.73 m^2) (Table 1). The decreased mean 25-hydroxyvitamin D level in those with severe kidney dysfunction, being 61.6 (4.1) nmol/l remained after adjusting for age, gender, ethnicity, BMI, physical activity, intake of milk, and vitamin D supplements and month of year – indicating that none of these variables explained the low mean vitamin D level in this group.

Components of the metabolic syndrome, fasting insulin levels, and homeostasis model assessment of insulin resistance in participants with decreased kidney function

The components of the metabolic syndrome: (1) abdominal obesity, (2) blood pressure, (3) high-density lipoprotein cholesterol, (4) triglycerides, and (5) fasting glucose as well as insulin levels and homeostasis model assessment of insulin resistance (HOMA-IR) were evaluated by eGFR category (Table 2). Mean BMI and waist circumference, both measures of body fat, were raised in participants with mild, moderate, and severe kidney dysfunction compared to those with normal kidney function. The fasting insulin concentration was significantly higher in participants with mild, moderate, and severe kidney dysfunction, reflecting a state of hyperinsulinemia. Concomitantly, there was an inverse relationship between eGFR and insulin sensitivity after adjusting for age, sex, ethnicity, and BMI (Table 2). Thus, HOMA-IR was significantly higher in participants with mild, moderate, and severe decrease in kidney function, compared to those with normal kidney function, independent of obesity level. Furthermore, total cholesterol and triglycerides levels were higher in participants with kidney dysfunction, whereas high-density lipoprotein cholesterol levels were lower only in participants with moderate and severely impaired kidney function, compared to those with normal function. Both systolic and diastolic blood pressures were significantly higher in participants with moderate decrease in eGFR compared to those with normal eGFR. Participants with and without antihypertensive treatment had significantly higher HOMA-IR and insulin levels at all stages of kidney dysfunction when compared to patients with normal kidney function (eGFR >90 ml/min/1.73 m^2) (data not shown).

Decreased kidney function, vitamin D, and HOMA-IR

To further evaluate the relationship between eGFR, 25-hydroxyvitamin D levels, and IR, we stratified participants according to level of kidney dysfunction and quartile of serum 25-hydroxyvitamin D (Table 3). Stage of kidney dysfunction and quartile of 25-hydroxyvitamin D were each inversely associated with levels of HOMA-IR and fasting insulin, adjusting for demographic variables and BMI, indicating that eGFR function and 25-hydroxyvitamin D have independent associations with IR. No evidence of a statistical significant interaction between these two variables in level of IR was observed ($P>0.05$).

DISCUSSION

There are several important findings in this study. In this analysis, we have demonstrated in a large sample representative of the adult US population that significant vitamin D insufficiency (25-hydroxyvitamin D 40–75 nmol/l), as defined by the recent Kidney Disease Outcomes Quality Initiative Clinical Practice Guidelines for Bone Mineral Metabolism and Disease in Chronic Kidney Disease, is only present in participants with severely decreased kidney function (GFR, 15–29 ml/min/1.73 m^2) and that 25-hydroxyvitamin D levels in those with mildly (GFR, 60–89 ml/min/1.73 m^2) and moderately (GFR, 30–59 ml/min/1.73 m^2) decreased kidney function are not lower than levels in participants with normal kidney function (GFR >90 ml/min/1.73 m^2). Moreover, the decreased serum vitamin D levels in people with severely decreased kidney function is independent of age, gender, ethnicity, and lifestyle variables known to affect vitamin D status, such as BMI, physical activity, and intake of milk and

Table 1 | Mean (s.e.) and mean difference in serum 25-hydroxyvitamin D (nmol/l) by level of demographic variables, lifestyle variables influencing vitamin D status, and eGFR category

Variable	*N*	Mean (s.e.)	Mean difference (s.e.)	*P*-value (*t*-test)
Gender				
Males	6950	78.0 (0.7)	6.3 (0.7)	<0.0001
Females	7729	71.6 (0.7)	0	—
P-value for Wald F	—	—	<0.0001	—
Age (years)				
20–29	3040	81.4 (1.2)	0	—
30–39	2932	78.5 (1.2)	−2.9 (1.2)	0.022
40–49	2315	73.3 (1.0)	−8.1 (1.3)	<0.0001
50–59	1650	71.8 (0.8)	−9.5 (1.5)	<0.0001
60–69	2085	68.9 (0.9)	−12.5 (1.7)	<0.0001
⩾70	2657	65.8 (0.9)	−15.5 (1.7)	<0.0001
P-value for Wald F	—	—	<0.0001	—
Ethnicity				
NH-white	6419	78.2 (0.7)	0	—
NH-black	4096	51.7 (0.7)	−26.5 (0.8)	<0.0001
Mex-Am	4164	68.1 (0.9)	−10.1 (1.0)	<0.0001
P-value for Wald F	—	—	<0.0001	—
BMI (kg/m^2)				
⩽22.4	2926	79.4 (0.7)	0	—
22.5–25.0	2912	77.8 (1.0)	−1.6 (1.0)	0.09
25.1–27.6	3002	74.4 (0.8)	−5.0 (0.9)	<0.0001
27.7–31.1	2883	72.2 (0.8)	−7.2 (0.8)	<0.0001
⩾31.2	2924	67.2 (0.9)	−12.2 (1.1)	<0.0001
P-value for Wald F	—	—	<0.0001	—
Leisure physical activity (times/month)				
Vigorous ⩾12	801	79.1 (1.5)	9.9 (1.6)	<0.0001
Vigorous 1–11	1463	74.5 (1.4)	5.3 (1.5)	0.0007
Moderate ⩾12	5100	77.4 (0.8)	8.2 (1.1)	<0.0001
Moderate 1–11	4097	72.7 (0.7)	3.5 (1.1)	0.0024
Inactive	3218	69.2 (0.9)	0	—
P-value for Wald F	—	—	<0.0001	—
Vitamin D supplements (IU/day)				
None	11265	73.2 (0.7)	0	—
>0 to <200	610	76.7 (2.0)	3.4 (1.8)	0.06
200 to <400	299	77.3 (2.2)	4.1 (2.4)	0.10
⩾400	2505	78.7 (0.9)	5.5 (1.1)	<0.0001
P-value for Wald F	—	—	0.0001	—
Milk (times/month)[a]				
None	2378	70.1 (1.2)	0	—
1–12	4232	71.7 (0.8)	1.6 (1.1)	0.15
13–30	6491	76.0 (0.7)	5.9 (1.3)	<0.0001
⩾31	1545	80.7 (0.9)	10.6 (1.3)	<0.0001
P-value for Wald F	—	—	<0.0001	—
eGFR category (ml/min/1.73 m^2)				
Normal (⩾90)	9687	73.3 (0.5)	0	—
Mild (60–89)	4094	77.3 (1.0)	4.0 (1.0)	0.0002
Moderate (30–59)	854	75.8 (1.6)	2.5 (1.5)	0.10
Severe (15–29)	44	61.6 (4.1)	−11.7 (4.1)	0.0063
P-value for Wald F	—	—	0.0004	—

BMI, body mass index; eGFR, estimated glomerular filtration rate; Mex-Am, Mexican-American; NH-black, non-Hispanic black; NH-white, non-Hispanic white.
[a]Times had milk to drink or on cereal.
Adjusted for all other variables in the table plus month of year.

vitamin D supplements. In conjunction with previous studies, this analysis highlights the direct relationship between kidney disease, IR, and other components of the metabolic syndrome. This analysis suggests that the association between kidney disease and IR is independent of vitamin D status.

To our knowledge, there are few reports of 25-hydroxyvitamin D levels at varying degrees of decreased kidney

Table 2 | Mean (s.e.) levels of the components of the metabolic syndrome, fasting insulin levels, and HOMA-IR adjusted for age, sex, and ethnicity, by eGFR category

Variable	N	eGFR category (ml/min/1.73m^2)				P-value Wald F-statistic
		Normal (⩾90)	Mild (60–89)	Moderate (30–59)	Severe (15–29)	
BMI (kg/m^2)	14 647	26.4 (0.1)	26.8 (0.1)**	27.7 (0.3)***	27.2 (1.5)	0.0016
Waist circumference (cm)	14 049	91.9 (0.2)	92.6 (0.3)	94.9 (0.7)	95.3 (3.8)	0.0024
Blood pressure (mm Hg)						
Systolic	14 651	122.4 (0.3)	123.1 (0.3)	127.6 (1.1)***	125.1 (2.8)	<0.0001
Diastolic	14 649	73.8 (0.2)	74.8 (0.3)**	75.4 (0.6)**	71.5 (2.5)	0.0016
Cholesterol (mg/dl)						
Total	14 654	203 (0.77)	206.11 (1.16)*	217.32 (3.48)***	221.96 (15.47)	<0.0001
LDL	6340	126.45 (0.77)	129.54 (1.55)	133.8 (1.71)	121.4 (13.15)	0.13
HDL	14 564	51.43 (0.39)	50.66 (0.39)	46.79 (0.77)***	46.79 (3.09)	0.0001
Fasting Blood[a]						
Triglycerides (mg/dl)	6033	108.95 (92.12)[b]	116.92 (92.12)***	139.95 (97.43)***	168.29 (125.78)*	<0.0001
Glucose (mg/dl)	6058	97.1 (0.36)	96.74 (0.54)	98.18 (1.08)	96.02 (2.52)	0.57
Insulin (pmol/l)	6022	51.4 (1.02)[b]	54.6 (1.04)***	59.1 (1.10)**	73.7 (1.34)*	0.0002
HOMA-IR	6022	2.03 (1.04)[b]	2.14 (1.04)**	2.36 (1.10)**	2.92 (1.40)*	0.0006

BMI, body mass index; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HOMA-IR, Homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein.
*$P<0.05$, **$P<0.01$, ***$P<0.001$, compared with reference (GFR⩾90 ml/min/1.73 m^2).
[a]Also adjusted for BMI; restricted to those without past history of diabetes, attended the morning examination, and fasted ⩾8 h.
[b]Tolerance factor.

Table 3 | Mean level (tolerance factor) of fasting insulin and HOMA-IR, adjusted for age, sex, ethnicity, and BMI, by eGFR category and serum 25-hydroxyvitamin D quartile[a]

Variable	Fasting Insulin (pmol/l)		HOMA-IR	
	Mean (TF)	P-value	Mean (TF)	P-value
eGFR Category (ml/min/1.73 m^2)				
Normal (⩾90)	51.4 (1.02)	—	2.01 (1.04)	—
Mild (60–89)	54.6 (1.04)	0.0021	2.16 (1.04)	0.0057
Moderate (30–59)	59.1 (1.10)	0.0017	2.36 (1.10)	0.0020
Severe (15–29)	72.2 (1.34)	0.025	2.86 (1.40)	0.044
P-value for Wald F	0.0001	—	0.0003	—
25-OHD quartile (nmol/l)				
<44.2	56.3 (1.06)	—	2.23 (1.06)	—
44.3–60.7	53.0 (1.04)	0.053	2.10 (1.06)	0.069
60.8–80.9	53.5 (1.04)	0.11	2.10 (1.04)	0.09
>81.0	50.9 (1.02)	0.0012	1.99 (1.04)	0.0014
P-value for Wald F	0.0017	—	0.0018	—

BMI, body mass index; eGFR, estimated glomerular filtration rate; HOMA-IR, homeostasis model assessment of insulin resistance; 25-OHD, 25-hydroxyvitamin D; TF, tolerance factor.
[a]Restricted to those without past history of diabetes, attended the morning examination and fasted ⩾8 h.

function. Reichel *et al.*[18] noticed among 63 non-nephrotic CKD patients, median values of 25-hydroxyvitamin D levels in those with GFR of 60–90, 40–60, and 20–40 ml/min/1.73 m^2 were 30, 47, and 45 nmol/l. In a Japanese cohort[19] of 76 CKD patients with GFR of 20–50 ml/min/1.73 m^2, 47% had 25-hydroxyvitamin D levels below 40 nmol/l and 76% had 25-hydroxyvitamin D levels below 65 nmol/l. Most recently in a US study,[6] which only included 43 CKD patients not requiring dialysis with a calculated GFR of 111–11 ml/min/1.73 m^2, 86% had 25-hydroxivitamin D levels consistent with vitamin D deficiency or insufficiency. Interpretation of previous work is complicated by the lack of uniform definition of the categories of kidney dysfunction and by the diversity in the ethnicity of the patient population.

Evidence[7,20] exists that calcitriol ($1,25(OH)_2D_3$) levels may be more dependent on the availability of 25-hydroxyvitamin D in patients with impaired kidney function than in patients with normal kidney function. Our data would suggest that 25-hydroxyvitamin D stores in patients with kidney disease appear to be sufficient for calcitriol synthesis until severe decreased kidney function (GFR, 15–29 ml/min/1.73 m^2) is established.

Recent studies[15,16,21] have shown that IR correlates linearly with decline in kidney function. In our analysis, we have also

demonstrated that kidney function is associated with IR and other components of the metabolic syndrome, such as blood pressure and high-density lipoprotein cholesterol, even when the eGFR is above the severe range. Although no differences were noted in fasting glucose levels between the eGFR groups, triglycerides showed the same pattern as insulin, with levels increasing as eGFR declined, after adjustment for BMI. The presence of IR in patients with kidney dysfunction is of interest because it may contribute to preglomerular vasodilatation and glomerular hypertension.[22,23] Moreover, the association of IR and hypertriglyceredemia in patients with kidney disease might also explain the excessive cardiovascular risk of patients with CKD.[16,17,24]

Epidemiological studies of the general population have indicated a close association between insulin sensitivity, diabetes, and the metabolic syndrome with serum levels of 25-hydrovitamin D.[9–11] We have shown an inverse association between 25-hydroxyvitamn D levels and HOMA-IR in the general population. The lack of interaction between 25-hydroxyvitamin D and degree of kidney dysfunction on level of IR suggests that both variables affect IR by independent mechanisms. HOMA-IR was only decreased in participants with serum 25-hydrovitamin D >81 nmol/l. This cutpoint is well above the 25-hydrovitamin D level recommended by the recent Kidney Disease Outcomes Quality Initiative Clinical Practice Guidelines for Bone Mineral Metabolism and Disease in Chronic Kidney Disease. Almost all tissues and cells in the body possess vitamin D receptors, including brain, heart, skeletal muscle, smooth muscle cells, pancreas, activated T and B lymphocytes, and monocytes.[8,25,26] Pancreas and skeletal muscle,[27] key components of the IR syndrome might be a potential explanation of the association of IR and 25-hydroxyvitamin D deficiency.

The current study has several strengths. First, NHANES III used uniform methods to collect data on 25-hydroxyvitamin D, insulin levels, and HOMA-IR. Second, the NHANES III design allows results to be extrapolated to the entire US civilian non-institutionalized population of the early 1990s. Despite the comprehensive nature of the data set, there are limitations inherent in cross-sectional design. We cannot be certain that vitamin D status affected IR rather than *vice versa*. Further, there are no direct measures of GFR and insulin sensitivity (hyperinsulinemic euglycemic glucose clamp) in this study. However, the measurement error from estimating these two variables is likely to have been non-differential, resulting in attenuation of associations with other variables observed in this report.[28]

In summary, our study has shown that significant 25-hydrodyvitamin D insufficiency, as defined by the recent Kidney Disease Outcomes Quality Initiative mineral metabolism guidelines, is encountered in people with CKD stage 4, and that 25-hydroxyvitamin D stores in early stages of kidney disease appear to be adequate. In addition, IR is present in early stages of kidney dysfunction and has an inverse association with 25-hydroxyvitamin D levels. As IR is a known cardiovascular risk factor, the correlation found in our study would suggest the hypotheisis that low serum levels of 25-hydroxyvitamin D is a potential cardiovascular risk factor and that serum calcidiol levels higher than those presently reccomended for CKD patients might decrease the risk for cardiovascular disease.[29] Further studies are needed to confirm our findings and to determine possible mechamisms of any preventive effect from 25-hydroxyvitamin D against IR in patients with kidney dysfunction.

MATERIALS AND METHODS

The National Center for Health Statistics of the Centers for Disease Control and Prevention carried out a cross-sectional survey representative of the US civilian non-institutionalized population during 1988–1994 (called NHANES III). A stratified, multistage sampling design was used, with over-sampling of non-Hispanic blacks and Mexican-Americans. Participants were recruited from household clusters, and initially interviewed at home, followed by an extensive physical examination carried out at mobile examination centers. Full details of the survey methods, including sampling, interview, examination, and laboratory measurement of blood samples, have been published.[30]

In the home interview, information was collected on a wide range of variables including: age, sex, ethnicity (self-assigned as either non-Hispanic white, non-Hispanic black, Mexican-American, Other), past history of ever being told by a doctor or other health professional of having hypertension or diabetes, and whether currently taking antihypertensive medication.[30] Information was also collected at the home interview on the following covariates: the frequency of intake of milk and supplements (including vitamin D) in the previous month; and the number of times a range of common physical activities were undertaken during leisure time in the previous month. Metabolic equivalents were assigned for each physical activity, and participants classified as doing moderate or vigorous activity based on their age.[31]

At the mobile examination centers, participants were dressed in underpants, disposable light clothing, and slippers while being weighed on electronic scales in kilograms, to two decimal places. Height was measured with a fixed stadiometer to the nearest millimeter. BMI was calculated as weight in kilograms divided by the square of height in meters. Blood pressure was measured at the mobile examination centers by physicians with mercury sphygmomanometers using a standard protocol.[30]

Blood samples collected during the examination were centrifuged, aliquoted, and frozen to -70°C on site, and shipped on dry ice to central laboratories where they were stored at -70°C until analysis.[30] Serum creatinine measurements were performed at a central laboratory (White Sands Research Center, Almogordo, NM, USA) by means of the modified kinetic Jaffe reaction using a Hitachi 737 analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN, USA) and reported using conventional units (1 mg/dl = 88.4 μmol/l). The levels of serum high-density lipoprotein cholesterol, total cholesterol, and triglycerides were measured enzymatically with a Hitachi 704 Analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN, USA). The serum low-density lipoprotein cholesterol concentration was calculated using the Friedewald equation (i.e., low-density lipoprotein = total cholesterol−high density cholesterol−triglyceride/5).[30]

eGFR was estimated using the following equation from the Modification of Diet in Kidney Dysfunction Study, after subtracting 0.23 mmol/l from serum creatinine measured in NHANES III:[32]

GFR (ml/min/1.73 m^2) = 186.3*(serum creatinine)$^{-1.154}$*age$^{-0.203}$*(0.742 for women)*(1.21 if African-American).

Plasma glucose was measured by a modified hexokinase enzymatic method, and separate radioimmunoassay methods used to measure serum insulin.[30] HOMA-IR was calculated using fasting glucose and insulin measurements ((fasting insulin (μU/ml)*fasting glucose (mmol/l))/22.5).[33] Serum 25-hydroxyvitamin D was measured by a radioimmunoassay after extraction with acetonitrile.[30] Serum 25-hydroxyvitamin D concentrations ranged from 8.7 to 243.6 nmol/l not including one person with a 25-hydroxyvitamin D value of 400.1 nmol/l.

A total of 16 573 adults $\geqslant$20 years attended mobile examination centers. Data in this report are restricted to non-Hispanic white, non-Hispanic black, and Mexican-American adults $\geqslant$20 years who attended the mobile examination centers ($n = 14\,679$). The following participants were excluded: 'Other' ethnicity ($n = 662$), missing serum creatinine ($n = 1019$), GFR category 5 ($n = 24$), calculated GFR abnormally high (> 200 ml/min/1.73 m^2; $n = 117$), and missing 25-hydroxyvitamin D including one individual with a very high outlying 25-hydroxyvitamin D value of 400.1 nmol/l ($n = 72$).

Statistical analyses were carried out with SUDAAN (version 9.0), using the sampling weights for the mobile examination centers to adjust for over-sampling of non-Hispanic black and Mexican-Americans, so that the data were representative of the US civilian non-institutionalized population, and to correct standard errors for any design effect arising from clustered sampling. For analyses of the blood variables (glucose, triglycerides, insulin, HOMA-IR) collected from those who attended the morning examination after fasting more than 8 h, analyses were restricted to those without diagnosed diabetes and with a sampling weight for the morning examination ($n = 6072$). The REGRESS procedure was used to estimate adjusted means of continuous outcome variables. For variables with skewed distributions (triglycerides, insulin, and HOMA-IR), the natural logarithm was used in statistical analyses and tolerance factors (anti-$\log_e$ ($1.96 \times$ s.e.)) calculated in place of standard errors. The Wald F-test was used to assess dose–response.

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