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Incidence rate of type 2 diabetes is >50% lower in GrassrootsHealth cohort with median serum 25–hydroxyvitamin D of 41 ng/ml than in NHANES cohort with median of 22 ng/ml

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ABSTRACT

Higher serum 25–hydroxyvitamin D [25(OH)D] concentrations have been associated with lower risk of type 2 diabetes. This study compared incidence rates of type 2 diabetes among participants aged ≥20 years in two U.S. cohorts with markedly different median 25(OH)D concentrations. The median 25(OH)D concentration in the GrassrootsHealth (GRH) cohort was 41 ng/ml (N = 4933) while in the 2005–6 National Health and Nutrition Examination Survey (NHANES) it was 22 ng/ml (N = 4078) ($P < 0.0001$). The adjusted annual incidence rate of type 2 diabetes was 3.7 per 1000 population (95% confidence interval = 1.9, 6.6) in the GRH cohort, compared to 9.3 per 1000 population (95% confidence interval = 6.7, 12.6) in NHANES. In the NHANES cohort, the lowest 25(OH)D tertiles (<17, 17–24 ng/ml) had higher odds of developing diabetes than the highest tertile (OR: 4.9, $P = 0.02$ and 4.8, $P = 0.01$ respectively), adjusting for covariates. Differences in demographics and methods may have limited comparability. Raising serum 25(OH)D may be a useful tool for reducing risk of diabetes in the population.

1. Introduction

According to the Centers for Disease Control (CDC), diabetes afflicts 29.1 million people in the United States, about 9.3% of the population [1]. Type 2 diabetes accounts for 90–95% of adult diabetes cases. The CDC expects the number of cases to double or triple in the next 40 years. Diabetes is the leading cause of kidney failure, non-traumatic lower limb amputations, and new cases of blindness among adults and is the seventh leading cause of death in the United States. The estimated total yearly cost of diabetes in the United States is $245 billion [1].

Those with type 2 diabetes have lower serum 25–hydroxyvitamin D [25(OH)D] concentrations than their healthy counterparts [2–4]. Prospective studies [5–16] and randomized controlled trials [17–20] have found a vitamin D association with type 2 diabetes and its metabolic indicators. A recent meta-analysis including both longitudinal cohort studies and randomized controlled trials of vitamin D supplementation found that higher baseline 25(OH)D levels in prospective studies predicted a lower diabetes risk [21]. There was a 43% reduction in type 2 diabetes incidence (95% confidence interval [CI] = 24–57%) comparing the highest (>25 ng/ml) to the lowest (<14 ng/ml) category of 25(OH)D.

We hypothesized that individuals in a cohort with higher serum 25(OH)D concentrations would have lower incidence of type 2 diabetes than those in a cohort with lower concentrations. This report presents the incidence of type 2 diabetes among United States residents aged 20 years and older in the GrassrootsHealth (GRH) study, a cohort with a median serum 25(OH)D level of 41 ng/ml, compared with estimates for the 2005–2006 National Health and Nutrition Examination Survey (NHANES) study, a cohort with a median serum level of 22 ng/ml. Additionally, the relationship between serum 25(OH)D and incidence of type 2 diabetes was examined. Serum 25(OH)D concentration was the variable of interest because it is a better indicator of vitamin D status than supplement intake or sun exposure because it accounts for multiple input sources and inter-individual variability in dose response.

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2. Materials and methods

2.1. GrassrootsHealth Cohort

This cross-sectional study utilized baseline data from GRH, a non-profit public health research organization running a large prospective population based study allowing participants to reach and sustain a serum 25(OH)D level of their choice, and tracking self-reported health status measures. Participants were individuals who responded to an invitation to attendees at a GRH seminar in 2008 and others recruited via internet invitations. There were no exclusion criteria for enrollment; participants included both genders, and wide ranges of ages and health statuses. Participation included submission of a home blood spot 25(OH)D test kit and completion of an online health questionnaire. Included in the test results were the normal reference ranges, information about potential toxicity levels, and suggested serum 25(OH)D concentrations (40–60 ng/ml) as recommended by a consortium of scientists and physicians [22]. All participants have given informed consent and this research study was approved by the Western Institutional Review Board (Olympia, WA) WIRB study 1126093.

Between January 2009 and July 2013, participants reported their gender, age, race, physical activity, and smoking status at study enrollment. Body mass index (BMI) was calculated using self-reported weight and height and categorized into 3 groups: <25, 25–30, >30. Regular exercise was defined as at least moderate physical activity for at least 20 min, 3 or more days/week. Race was categorized as "white" versus "non-white" and smoking status was categorized as "current smoker" versus "never/former smoker."

Serum 25(OH)D concentrations were determined by blood spot test kits analyzed at study enrollment using liquid chromatography-mass spectroscopy (LC-MS/MS) by ZRT Laboratory (Beaverton, OR). ZRT's assay has been validated against the LC-MS/MS consensus group reporting to the Vitamin D Quality Assessment Scheme (DEQAS), whose objective is to ensure the analytical reliability of 25(OH)D assays, with an R^2 value of 0.998. LC-MS/MS has been validated against the DiaSorin Radioimmunoassay (RIA) method with an R^2 value of 0.91 and with a slope not different from 1.0 [23].

In the GRH cohort, an incident case was defined as a self-reported diagnosis by a doctor of type 2 diabetes within the 12 months prior to enrollment based on the date of diagnosis. A date of diagnosis before this 12-month period was considered a prevalent case and excluded. This study included all GRH participants residing in the United States aged 20 years and older with no prior history of diabetes before the 12-month observation period (N = 4933). This age and residency group was chosen to match the NHANES cohort, which was a sample of the United States population and collected all covariate data for those aged ≥20 years. Age, gender, and race were available for all participants in this GRH cohort. BMI, smoking status, and physical activity were available for 90%, 89% and 89% of the cohort respectively.

2.2. NHANES Cohort

The 2005–2006 NHANES study population was a representative sample of the civilian, non-institutionalized United States population. NHANES used a complex, stratified, multistage, probability-cluster sampling design that oversampled low income persons, adolescents, the elderly, Blacks, and Mexican Americans to produce reliable estimates for these groups.

Detailed survey and examination methods can be found elsewhere [24]. Briefly, between January 2005 and December 2006, participants reported their gender, age, race, and physical activity via household interviews. BMI, regular exercise, race, and smoking status were defined, calculated, and categorized in the same manner as the GRH cohort mentioned above. Blood samples were collected by venipuncture in mobile examination centers and serum 25(OH)D concentrations were determined by using the DiaSorin RIA kit assay (Stillwater, MN) at the National Center for Environmental Health, CDC, Atlanta, GA.

In the NHANES cohort, an incident case was defined as a self-reported diagnosis of diabetes within the 12 months prior to the health interview. Specifically, the participant answered 'yes' to the question "Other than during pregnancy, have you ever been told by a doctor or health professional that you have diabetes or sugar diabetes?" and answered "12 months ago or less" to "When was your diabetes diagnosed?". Participants who answered "more than 12 months ago" were considered prevalent cases and excluded. To differentiate between type 2 and type 1 diabetes, participants who answered 'no' to both "Are you taking insulin now?" and "Are you now taking diabetic pills to lower your blood sugar?" were considered type 2 diabetes cases. Those who responded 'yes' to "Are you now taking diabetic pills to lower your blood sugar" (with or without insulin intake) were also considered type 2 diabetes cases.

This study included all 2005–2006 NHANES participants aged 20 years and older with no prior history of diabetes before the 12-month observation period and who completed the physical exam component where a valid 25(OH)D measurement was obtained (N = 4078). This age group was chosen because participants <20 years old were not assessed for smoking status, a key diabetes risk factor. All participants aged 1 year and older were eligible for the physical exam but among those ≥20 years, 484 did not participate in this component. Age, gender, race, smoking status, and physical activity were available for all participants in this NHANES cohort. BMI was available for all diabetes cases and 96% of the cohort.

2.3. Statistical Methods

Chi-square tests were used to test for differences between the GRH and NHANES cohorts. The unadjusted incidence rate of type 2 diabetes was calculated for the GRH and NHANES cohorts. An indirect rate adjustment was calculated according to the diabetes risk factors that were significantly different between the cohorts (age, gender, race, smoking status, and BMI). The NHANES population was used as the standard to simultaneously adjust for these risk factor differences in the GRH population. Specifically, gender, age, race, smoking status, and BMI-specific incidence rates from the NHANES population were applied to the gender, age, race, smoking status, and BMI distribution of the GRH cohort to estimate the expected number of cases. Participants with missing covariate data were distributed on the basis of existing data for each cohort. The ratio of observed to expected number of cases (standardized incidence ratio) and its 95% confidence interval (CI) were calculated. The Mantel-Haenszel (M-H) odds ratio estimate was calculated for the lowest 25(OH)D tertile (<17 ng/ml) versus the highest tertile (≥25 ng/ml) for the NHANES cohort, adjusting for age. Logistic regression was used to determine the association between 25(OH)D serum levels and the risk of developing type 2 diabetes within the NHANES cohort, adjusting for age, gender, race, BMI, physical activity, and smoking status. Modeling was confined to participants with valid values for all of the involved variables. The relationship between 25(OH)D serum levels and type 2 diabetes risk could not be assessed within the GRH cohort due to insufficient number of cases. Statistical analyses were performed using SPSS statistics version 22 (IBM, Armonk, NY).

3. Results

The median serum 25(OH)D concentration in the GRH cohort was 41 ng/ml (I-Q range: 31–55) and in the NHANES cohort was 22 ng/ml (I-Q range: 15–28) ($P < 0.0001$). In the GRH cohort, 77% of participants reported taking vitamin D supplements with a median daily intake of 2400 IU. In the NHANES cohort, 30% reported taking vitamin D supplements with a median daily intake of 400 IU. Among the 10 individuals who developed diabetes in the GRH cohort, 5 were below the median cohort serum level of 41 ng/ml and 5 were equal to or above it (Fig. 1). Among the 38 individuals who developed diabetes in the NHANES cohort, 31 were below the median cohort serum level of 22 ng/ml and 7 were equal to or above it. The GRH cohort had a statistically significant higher proportion of participants that were older, female, white, never/former smokers, and had a lower BMI (Table 1). Physical activity did not differ between the cohorts.

The unadjusted annual incidence rate within the GRH population was 2.0 per 1000 population (95% CI = 1.0, 3.6), compared to 9.3 per 1000 population (95% CI = 6.7, 12.6) within the NHANES population. After indirect adjustment for age, gender, race, smoking status, and BMI, the expected number of cases was 25 compared to the 10 observed GRH cases. The standardized incidence ratio was 0.40 (95% CI = 0.20, 0.71), or 60% lower risk in the GRH cohort (Fig. 2). The adjusted annual incidence rate within the GRH cohort was 3.7 per 1000 population (95% CI = 1.9, 6.6).

Using the Mantel-Haenszel (M-H) method to adjust for age, the lowest 25(OH)D tertile (<17 ng/ml) had higher odds of developing diabetes than the highest tertile (≥25 ng/ml) in the NHANES population (odds ratio: 8.0, $P = 0.001$). In the NHANES cohort, the lowest 25(OH)D tertiles (<17, 17–24 ng/ml) had higher odds of developing diabetes than the highest tertile (odds ratios: 4.9, $P = 0.02$ and 4.8, $P = 0.01$ respectively), adjusting for age, gender, race, BMI, physical activity, and smoking status (Table 2).

4. Discussion

The adjusted incidence rate of type 2 diabetes in a cohort with markedly higher than usual median serum 25(OH)D values (GRH) was less than half that in a cohort with lower median 25(OH)D (NHANES). While the cross-sectional method of calculating incidence for the GRH prospective cohort was not traditional, this method was chosen to parallel the procedure used in the NHANES study. To assess how this may have influenced the results, the incidence rate for those diagnosed with type 2 diabetes after enrollment among the at-risk population was calculated and found to be 1.2 per 1000 person-years (95% CI = 0.37, 2.81). This rate was comparable to the cross-sectional GRH rate and significantly lower than the NHANES rate.

Within the NHANES cohort, those with serum 25(OH)D levels below 25 ng/ml had almost 5 times the odds of developing diabetes compared to those with serum 25(OH)D levels ≥25 ng/ml. Other studies have found a similar reduction in risk of type 2 diabetes. A nested case-cohort study found a 50% lower hazard of developing type 2 diabetes for those with serum 25(OH)D levels <20 ng/ml compared to those with levels ≥32 ng/ml (95% CI = 0.32, 0.76) [14]. Another study found a 42% reduction in the risk of progression to type 2 diabetes from prediabetes or normal glucose tolerance in the highest 25(OH)D quartile (>28 ng/ml) compared to the lowest quartile (<18 ng/ml) [11]. Among those with prediabetes, there was a 62% reduction in the risk of diabetes comparing the highest quartile to the lowest quartile. A study presented at the 2014 Endocrine Society Meeting found a 58% reduction in the progression from prediabetes to diabetes in a group treated with calcium and 60,000 IU/week of vitamin D for eight weeks and then monthly compared to a group given only calcium supplements [25].

The distribution of the GRH cases across the 25(OH)D serum level spectrum, shown in Fig. 1, allows a very interesting interpretation. A recently published article presented guidelines to standardize individual studies of nutrients and meta-analyses based on the biological response to nutrients [26]. The measureable benefit for a nutrient-specific response is found within a narrow response region, with a flat response above and below this region (Fig. 3). Taking into account the sigmoidal nature of the nutrient response, a recent study used successive regression models to localize the association of vitamin D status with insulin resistance to the range of 16–36 ng/ml; with a flat extension for levels >32–36 ng/ml [27]. In the GRH cohort, 71% of participants had serum levels above 32 ng/ml and outside the range of association found in that study. With this in mind, the results of both cohorts would be consistent with a sigmoidal response between 25(OH)D and diabetes risk where there is a flat response below ~10 ng/ml, a clinical response between about 10 ng/ml and 30–35 ng/ml, and a plateau of no additional effect above ~30–35 ng/ml.

A recent systematic review and meta-analysis of randomized controlled trials found no effect of vitamin D supplementation on diabetes prevention [28]. All four studies included in the meta-analysis regarding progression to diabetes reported null results [29–32]. One of these studies had a relatively small number of

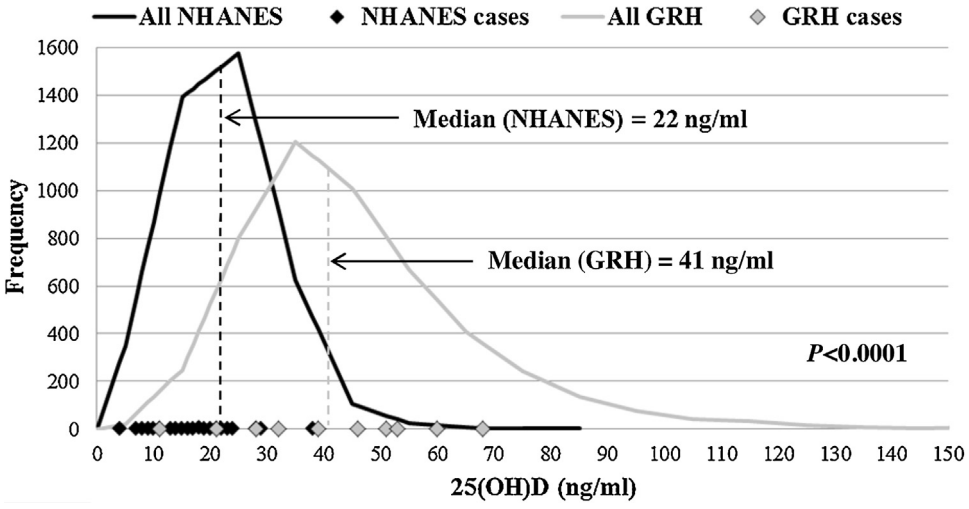
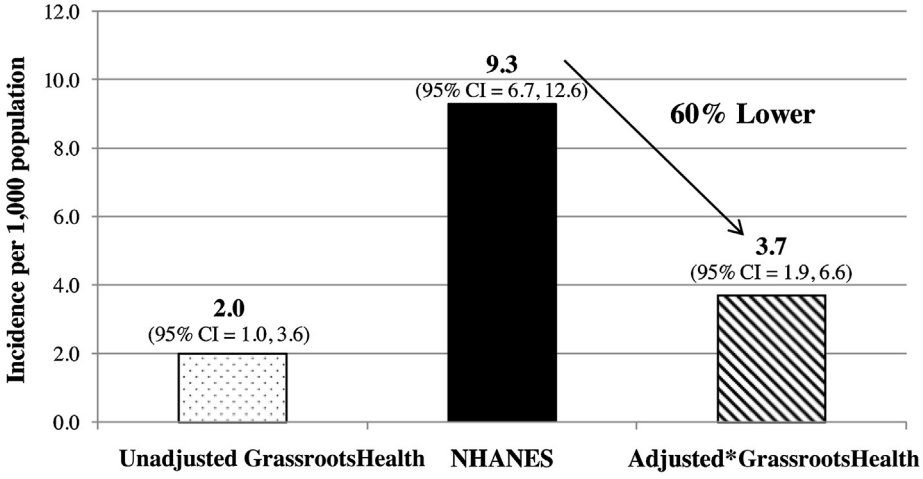


Fig. 1. Frequency distribution of serum 25–hydroxyvitamin D [25(OH)D] for GrassrootsHealth (GRH) ($N = 4933$) and NHANES ($N = 4078$) cohorts.

Table 1
Demographic characteristics of GrassrootsHealth (GRH) ($N = 4933$) and NHANES ($N = 4078$) cohorts.

Characteristic	GRH N (%)	NHANES N (%)	x^2	P
Age (years)			600.13	<0.0001
20–39	892 (18%)	1671 (41%)		
40–59	2382 (48%)	1260 (31%)		
≥60	1659 (34%)	1147 (28%)		
Gender			206.31	<0.0001
Male	1632 (33%)	1956 (48%)		
Female	3301 (67%)	2122 (52%)		
Race			1991.86	<0.0001
White	4584 (93%)	2107 (52%)		
Non–white	347 (7%)	1971 (48%)		
BMI (kg/m^2)			367.22	<0.0001
<25	2463 (55%)	1416 (36%)		
25–30	1332 (30%)	1395 (36%)		
>30	654 (15%)	1105 (28%)		
Physical activity			1.46	0.227
Regular exercise	3046 (69%)	2864 (70%)		
Non-regular exercise	1367 (31%)	1214 (30%)		
Smoking status				
Current smoker	161 (4%)	904 (22%)	656.83	<0.0001
Never/former smoker	4219 (96%)	3171 (78%)		



*Adjusted for age, gender, race, smoking status and BMI distribution differences between GrassrootsHealth and NHANES

Fig. 2. Type 2 diabetes incidence rates in GrassrootsHealth (GRH) ($N = 4933$) and NHANES ($N = 4078$) cohorts.

participants and three studies administered vitamin D doses ≤800 IU/day, an intake amount that is unlikely to raise basal levels to a sufficient status, and did not limit their study population to only vitamin D deficient individuals. The nutrient-specific response depends on an individual's basal status where an increase in intake will produce a benefit in a deficient individual but will have a null effect in a replete individual [26]. Also, treating the relationship as linear, spread across the entire 25(OH)D range instead of a sigmoidal response would dilute the effect and may explain why some studies did not find a vitamin D association with diabetes. Additional randomized controlled trials that base their design on nutrient physiology are needed to accurately assess the association between vitamin D and diabetes.

Limitations of this study include the use of self-report data where recall bias may have occurred, and differences in methods and demographics between the GRH and NHANES cohorts may have limited comparability. The GRH cohort of individuals was self-selected for health consciousness and NHANES is a population-based sample. Also, two different assays were used to determine serum 25(OH)D concentration and while these methods were both calibrated against LC-MS/MS with high correlation, systematic differences are possible. This analysis took into account cohort differences for many of the key risk factors for type 2 diabetes but there were some factors such as waist circumference, diet, and socio-economic indicators that were not available. Lack of adjustment for these covariates or other unavailable or unknown variables may have influenced the outcome. Since multivariate logistic regression was conducted only within the NHANES cohort, analysis of the association between serum 25(OH)D and risk of type 2 diabetes was not affected by this limitation. Also, this study assumed the 25(OH)D levels measured in participants at enrollment were similar to their levels in the 12 months prior, back to the beginning of the period used to measure diabetes incidence.

Table 2
Association between serum 25–hydroxyvitamin D [25(OH)D] levels and the risk of developing type 2 diabetes in the NHANES cohort, adjusting for covariates.

	NHANES (*N* = 4078)
Covariate	**OR (95% CI)**
Serum 25(OH)D (ng/ml)	
Lowest tertile (<17)	**4.90 (1.35, 17.86)**
Middle tertile (17–24)	**4.78 (1.37, 16.69)**
Highest tertile (≥25)	1.00
Age	
20–39 years	1.00
40–59 years	**3.75 (1.36, 10.34)**
≥60 years	**6.21 (2.22, 17.32)**
Gender	
Male	1.35 (0.70, 2.62)
Female	1.00
Race	
White	1.00
Non–white	1.84 (0.89, 3.80)
BMI	
<25	1.00
25–30	1.09 (0.37, 3.19)
>30	**3.83 (1.53, 9.59)**
Physical activity	
Regular exercise	1.00
Non-regular exercise	0.65 (0.31, 1.35)
Smoking status	
Current smoker	1.22 (0.56, 2.66)
Never/former smoker	1.00

Bold values signify significant adjusted odds ratios.

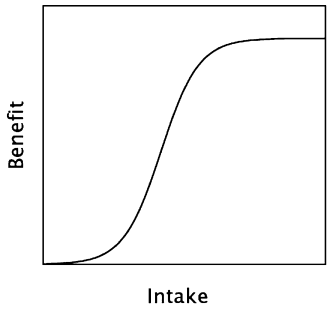


Fig. 3. A sigmoidal dose-response curve of the relation between nutrient intake and nutrient benefit.

While there are limitations to this study, these results add to the growing body of evidence supporting the association between higher serum 25(OH)D levels and a reduced risk of type 2 diabetes [5–21,25,27]. Higher vitamin D status has also been associated in other observational studies with a reduced risk of type 1 diabetes, cancer, myocardial infarction, and multiple sclerosis [22,33–38]. The Institute of Medicine (IOM) recommends levels of ≥20 ng/ml and considers 4000 IU/day as the safe upper intake level (SUIL) [39]. Other investigators and physicians have proposed a 25(OH)D concentration between 40 and 60 ng/ml as a safe range to achieve a reduction in risk of diseases associated with vitamin D deficiency [40–43]; this range can generally be achieved with the IOM SUIL of 4000 IU/day [44–45].

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Conflicts of interest

The authors have no conflicts of interest to disclose.

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