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Could serum levels of calcidiol predict the onset of chronic inflammatory conditions?

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Previous research studies had suggested that low serum calcidiol could lead to the onset of chronic inflammatory conditions (CIC). This study used the dataset of National Health and Nutrition Examination Survey (NHANES) 2005-2006 to investigate whether serum calcidiol can predict CIC. The linear correlation between serum calcidiol and body mass index (BMI) was explored for therapeutic purposes. A point prevalence of serum calcidiol deficiency was 84.0% in each CIC group. People of African origin were eleven times more likely to suffer from vitamin-D deficiency (crude ORc=10.57[7.98-13.99]). Linear regression elicited strong negative correlation between calcidiol and C-reactive protein (CRP) after adjustment (R^2=0.33; r=-0.57; p<0.001). Logistic regression showed non-significant association between calcidiol and CIC after adjustment OR_a=1.16[0.93-1.44], 1.03[0.81-1.31] and 0.76[0.55-1.05] for asthma, arthritis and malignancy, respectively. Linear regression study showed a strong linear negative correlation between calcidiol and (BMI) after adjustment R^2=0.27; r=-0.52; p<0.001. Although serum calcidiol is not an ideal predictor of CIC; however, we cannot completely rule out an association due to the complexity related to the presence of confounding, intermediate, and regulatory factors. Additional findings may suggest the potential for tailoring vitamin-D supplementation to individual's weight.

Key words: Calcidiol, NHANES 2005-2006, chronic inflammatory conditions, C-reactive protein, body max index.

INTRODUCTION

Vitamin-D is a steroid hormone and belongs to the family of fat-soluble vitamins. Reports from many research studies had shown that both low sunlight exposure and poor dietary intake of vitamin-D, may substantially alter serum levels of calcidiol also known as 25-hydroxyvitamin-D, in humans (Vitamin D Fact Sheet for Health Professionals, 2011; Reinhold V, 1999; Institute of Medicine, 2010).

When defining calcidiol deficiency as being less than 20 ng/ml in serum, approximately 42% of the US population is affected (Forrest and Stuhldreher, 2011). Investigators around the world have observed the same

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proportion (Hye-Rim Song et al., 2014; Edward Giovannucci et al., 2006; Thacher et al., 2011). Furthermore, its low serum levels have been linked to health conditions such as obesity, diabetes mellitus, osteoarthritis, cardiovascular diseases, neuropsychiatric disorders, and certain malignancies (Davis et al., 2011; Lappe et al., 2007).

The skin produces calcidiol endogenously from cholesterol during sunlight or Ultraviolet-B exposure (Anderson et al., 2003). The interaction between sunlight and skin cholesterol referred to as 7-dehydrocholesterol, leads to the formation of the pro-vitamin-D, also known as cholecalciferol. This endogenous production constitutes the major natural source of vitamin-D in humans, hence the name "*sunshine vitamin*". In contrast with endogenous production, exogenous dietary intake of vitamin-D from fish, meat, egg yolk, fortified milk, butter, and plants, represents a minor natural source of vitamin-D exposure (Institute of Medicine, 2010). Plant-sourced vitamin-D_2 is also referred to as ergocalciferol. Once released from their respective sources of production, both ergocalciferol and cholecalciferol undergo serial steps of hydroxylation (Anderson et al., 2003). The first hydroxylation happens in the liver under the action of 25-hydroxylase enzyme, transforming ergocalciferol and cholecalciferol into 25-hydroxyvitamin-D or calcidiol. Vitamin-D of this form is an inert and stable molecule, with a half-life estimated between fifteen and thirty days (Vitamin D Fact Sheet for Health Professionals, 2011; Reinhold V, 1999). After its production in the hepatocyte, the fat-soluble calcidiol is carried by a binding protein through the blood stream to the kidneys, where the second hydroxylation occurs under 1-α-hydroxylase enzyme (Hewison et al., 2000); which is mainly regulated by serum levels of parathyroid hormone (PTH) and calcium. This ultimate chemical reaction converts 25-hydroxyvitamin-D into *1-25-dihydroxyvitamin-D*, also known as calcitriol. In contrast to calcidiol, calcitriol is physiologically active, and has a short half-life of approximately 15 h (Lensmeyer et al., 2012; Christine Gonzalez, 2010). The 24-hydroxylase enzyme (Anderson et al., 2003) alters both molecules calcidiol and calcitriol to their forms of elimination in the bile (24-25-dihydroxyvitamin-D) and urine (1-24-25-trihydroxyvitamin-D).

Thus, calcidiol appears to be an ideal biomarker of exposure since it reflects both endogenous (sunlight) and exogenous (dietary supplement) vitamin-D intake. It has a fairly long circulating half-life conferring its temporal stability. Furthermore, calcidiol is less subject to regulation by other metabolites like parathyroid hormone (PTH) and calcium; and it is detectable at both low and high serum levels using a reliable laboratory test (Górriz and Estela, 2013; NHANES 2005–2006 Data Documentation, 2008; Wendy et al., 2013; Huiping Chen et al., 2008).

In contrast to calcidiol, it is well known that circulating calcitriol exerts a wider range of biological activities including the regulation of cellular differentiation and proliferation, immunity, and reproduction (Anderson et al., 2003; Eyles et al., 2003; Abou-Raya et al., 2013; David Reid et al., 2011). Furthermore, Calcitriol plays a primary role in the maintenance of calcium homeostasis by tightly regulating PTH, calcium, and phosphate serum concentrations (Anderson et al., 2003; Hewison et al., 2000). calcitriol is generally not a good predictor of vitamin-D exposure status due to its short half-life. Moreover, levels of calcitriol do not typically decrease until its deficiency becomes severe enough (Anderson et al., 2003). Thus, while calcidiol may be an excellent biomarker of exposure, the extent to which its deficiency can predict the onset of chronic inflammatory conditions (CIC) remains unclear.

This study used data set from the National Health and Nutrition Examination Survey (NHANES) of the year 2005-2006 to assess two endpoints; firstly, whether are serum calcidiol levels associated with CIC; secondly, are serum levels of calcidiol linearly correlated with individual's body mass index (BMI).

MATERIALS AND METHODS

Study design

NHANES is serial cross-sectional study, conducted every two years on a random sample, representative of the US population (NHANES 2005-2006 Data Documentation). The survey sampling method is stratified multistage probability design of civilian population, aged one year or older who met eligibility criteria. After providing informed consent, participants agreed to complete household surveys conducted by trained NHANES staff members. Components of the survey questionnaire addressed demographics, health history and risk behaviors. The design also assessed the current health status of all participants by performing physical and laboratory examination at the mobile examination center.

Data collection

Investigators collected demographic data simultaneously with dietary supplement intake status twice a year (NHANES 2005-2006 Data Documentation, 2008). They defined two periods as summer (May-October) and winter (November-April) seasons. An interviewer administered questionnaires and performed physical examination record such as height, weight, and each participant's BMI. Investigators also collected and stored blood samples for the measurement of serum vitamin-D and C-reactive protein (CRP) among others. A proxy provided information for survey participants who were under 16-year-old and for individuals who could not answer the questions themselves. After data collection, the NHANES field office staff reviewed records for accuracy and completeness.

Vitamin-D and C-reactive protein ascertainment

The DiaSorin test is a radio-immunoassay (RIA) method that uses

Table 1. Summary statistics showing the distribution of each weighted continuous variable using Survey mean and non-missing completely at random (Nomcar) option.

Variable	N	Mean	SEM	95%CI
Age (year)	10348	36.22	0.26	35.71-36.74
BMI (kg/m^2)	8949	26.40	0.10	26.20-26.60
Vitamin D (ng/ml)	8306	23.88	0.14	23.62-24.14
CRP (mg/dl)	8172	0.38	0.01	0.35-0.40

N=Total number of observations; SEM: Standard error of the mean; 95%CI: 95% confidence interval; BMI: Body mass index (normal less than 30); CRP: C-reactive protein (normal less than or equal to 0.5 mg/dl). Vitamin-D normal higher or equal to 30 ng/ml. Weighted variable age with interview weight (WTINT2YR); weighted variables CRP, Vit-D, and BMI with examination weight (WTMEC2YR).
Source: NHANES 20005-2006 database.

an antibody specific to 25-hydroxyvitamin-D (NHANES 2005–2006 Data Documentation, 2008). In contrast to serum vitamin-D ascertainment, investigators used the Latex-enhanced Nephelometry method to measure serum levels of CRP. NHANES Website Laboratory Procedures Manuals provide detailed information about both methods (New vitamin-D blood tests are often highly inaccurate, 2013). Serum specimens for both Vitamin-D and CRP were processed, stored, and shipped for analysis to the Division of Laboratory Sciences, National Center for Environmental Health and Center for Disease Control and Prevention, Atlanta, Georgia; and University of Washington, Seattle, Washington. NHANES quality assurance and control protocols meet the 1988 Clinical Laboratory Improvement Act mandates (NHANES 2005–2006 Data Documentation, 2008).

Statistical analysis

In our dataset, the biomarker of the exposure was serum calcidiol in Nanogram per milliliter with the normal range being higher or equal to 30 ng/ml; while CRP in milligram per deciliter with the normal range less or equal to 0.5 mg/dl was the biomarker of effect, also known as CIC. To address our first endpoint, three outcome variables asthma, arthritis, and malignancy were identified as CIC; each of them was categorized in two levels “cases” and “non-cases” before being incorporated into the final analysis with logistic regression. Other categorized independent variables are family annual income “low” and “high”; seasonal examination “winter” and “summer”; vitamin-D dietary supplement intake “yes” and “no”; gender “female” and “male” and race/ethnicity “white”, “black”, and “other” defined as being non-black and non-white. In the final logistic regression model, we categorized continuous variables age with younger being less than 40-year; BMI with normal range less than 30 kg/m^2, calcidiol with deficiency defined as less than 30 ng/mL (Abou-Raya et al., 2013), and CRP with normal range less or equal to 0.5 mg/dL. For the second endpoint, we assessed whether BMI can predict serum levels of calcidiol through linear regression analysis by computing correlation and regression coefficient estimates. Confounding factors were ascertained when 10% change occurs in the crude parameter estimate after adjustment.

SurveyMeans, Surveyfrequency, Surveylogistic, and SurveyReg were used in the analysis to properly account for the weighted sampling design. Missing observations were treated as non-missing completely at random (NOMCAR) and performed $\log_{10}$ transformation of continuous variables with skewed distribution.

Because CRP is clinically used as a main biomarker of inflammation, a linear correlation between calcidiol and CRP can provide a comparative information to the odds ratio. Furthermore, a linear correlation between calcidiol and BMI may provide evidence that an adjustment for each patient’s weight is necessary for adequate therapeutic dosage of vitamin-D supplement. SAS version 9.2 was used for all statistical analyses. The level of significance was held at 5%.

RESULTS

The data set has 10,348 observations. While calcidiol and BMI follow normal distribution; CRP however, is skewed in the right (median and mode not shown), therefore, logarithmic transformation of CRP is required for appropriate use of linear regression analysis. Independently of the outcome variables (asthma, arthritis and malignancy), the average serum level of Vitamin-D is below the normal range of 30 ng/ml, while average BMI and CRP are normal in Table 1.

In comparison with non-cases in Table 2, cases of asthma show statistically significant increased CRP (0.42 vs 0.37 mg/dl; p=0.045), increased BMI (27.5 vs. 26.2 kg/m^2; p=0.001), younger age (34.1 vs. 37.2 year-old; p=0.001). In contrast, calcidiol did not show significant statistical mean difference for asthma (p=0.095) when compared with arthritis and malignancy groups (p=0.001). Furthermore, the mean difference was evidenced for age and BMI in each CIC. CRP mean difference was only found with arthritis. Cases of arthritis show statistically significant lower calcidiol, higher CRP and BMI, and older age (all p=0.001). Cases of malignancy show statistically significant higher calcidiol (p=0.001), normal weight (p=0.005), older age (p=0.001), and non-significant CRP mean difference (p=0.163).

In Table 3, asthma group has 5% (539) of missing observations compared to 52% for arthritis and malignancy with 5379 and 5373, respectively. Although the study missed half of the data, the sample size remained larger enough to provide a robust power to this study. The point prevalence of Vitamin-D deficiency is approximately 84.0% in each CIC group.

Table 2. Comparative weighted Means and standard deviations of continuous variables in Case and Non-case of each chronic inflammatory condition (CIC), using T-test.

Variable	Asthma			Arthritis			Malignancy		
	Case (14%)	Non-case (86%)	p	Case (25%)	Non-case (75%)	p	Case (8%)	Non-case (92%)	p
Calcidiol	23.48±1162 N=1168 (14.1)	23.95±1624 N=7128 (85.9)	0.095	22.51±1804 N=1146 (25.5)	23.58±1968 N=3339 (74.5)	0.001	24.77±1690 N=372 (8.3)	23.17±1948 N=4119 (91.7)	0.001
CRP	0.42±133 N=1151 (14.1)	0.37±147 N=7011 (85.9)	0.045	0.56±211 N=1145 (25.5)	0.39±165 N=3337 (74.5)	0.001	0.48±136 N=371 (8.3)	0.43±181 N=4117 (91.7)	0.163
BMI	27.51±1449 N=1276 (14.3)	26.21±1279 N=7664 (85.7)	0.001	30.39±1620 N=1164 (24.9)	27.95±1315 N=3506 (75.1)	0.001	27.72±1232 N=381 (8.1)	28.62±1429 N=4295 (91.9)	0.005
Age	34.13±3570 N=1389 (14.2)	37.16±3786 N=8420 (85.8)	0.001	58.93±3144 N=1204 (25.3)	42.51±3295 N=3559 (74.7)	0.001	62.20±3210 N=395 (8.3)	45.17±3463 N=4374 (91.7)	0.001

N: Number of observations; p: p-value obtained either from the pooled or Satterthwaite method; CRP: C-reactive protein; BMI: Body mass index; case" is defined as having a chronic condition and "non-case" as not having it. ±: plus or minus weighted standard deviation. Weighted age with interview weight (WTINT2YR); weighted CRP, Vitamin-D and BMI with examination weight (WTMEC2YR).
Source: NHANES 2005-2006"

Table 3. Frequency distribution showing summary statistics for weighted categorical independent variables among case and non-case of response variables (Asthma, Arthritis, and Malignancy).

Variable	Asthma			Arthritis			Malignancy		
	Case	Non-case	P	Case	Non-case	p	Case	Non-case	p
Calcidiol (ng/ml)			**0.164**			**0.151**			**0.014**
<30 (low)	1183 (14.29)	7098 (85.71)		1056 (25.35)	3109 (74.65)		324 (7.77)	3846 (92.23)	
>=30 (Normal)	206 (13.48)	1322 (86.52)		191 (23.76)	613 (76.24)		90 (11.18)	715 (88.82)	
Age (year)			**0.003**			**0.001**			**0.001**
>=40	370 (12.13)	2680 (87.87)		1144 (37.53)	1904 (62.47)		386 (12.65)	2666 (87.35)	
<40	1019 (15.08)	5740 (84.92)		103 (5.36)	1818 (94.64)		28 (1.46)	1895 (98.54)	
Gender			**0.491**			**0.001**			**0.001**
Female	693 (13.78)	4335 (86.22)		716 (27.69)	1870 (72.31)		233 (9.00)	2357 (91.00)	
Male	696 (14.56)	4085 (85.44)		531 (22.28)	1852 (77.72)		181 (7.59)	2204 (92.41)	
Ethnicity			**0.001**			**0.001**			**0.001**
Black	449 (17.13) 381	2172 (82.87)		279 (24.87)	843 (75.13)		60 (5.35)	1062 (94.65)	

Table 3 ContD.

Others	(11.15) 559	3037 (88.85)		193 (14.23)	1163 (85.77)		35 (2.57)	1326 (97.43)	
White	(14.83)	3211 (85.17)		775 (31.11)	1716 (68.89)		319 (12.80)	2173 (87.20)	
BMI (kg/m^2)			**0.001**			**0.001**			**0.130**
>=30+	359 (17.50)	1693 (82.50)		533 (32.44)	1110 (67.56)		116 (7.05)	1529 (92.95)	
<30	1030 (13.28)	6727 (86.72)		714 (21.47)	2612 (78.53)		298 (8.95)	3032 (91.05)	
FA Income			0.361			0.001			0.064
Low	435 (14.45)	2576 (85.55)		442 (31.19)	975 (68.81)		125 (8.80)	1295 (91.20)	
High	903 (14.26)	5428 (85.74)		720 (21.78)	2586 (78.22)		260 (7.86)	3049 (92.14)	
CRP (mg/dl)			**0.001**			**0.001**			**0.010**
>0.5	260 (16.60)	1306 (83.40)		395 (32.24)	830 (67.76)		124 (10.11)	1102 (89.89)	
=<0.5	1129 (13.70)	7114 (86.30)		852 (22.76)	2892 (77.24)		290 (7.74)	3459 (92.26)	
Dietsup			**0.184**			**0.001**			**0.001**
No	887 (14.61)	5184 (85.39)		502 (20.32)	1968 (79.68)		141 (5.70)	2334 (94.30)	
Yes	501 (13.44)	3228 (86.56)		741 (29.74)	1751 (70.26)		273 (10.95)	2220 (89.05)	
Season			**0.984**			**0.079**			**0.061**
Winter	628 (13.88)	3895 (86.12)		507 (23.46)	1654 (76.54)		144 (6.65)	2021 (93.35)	
Summer	706 (14.40)	4198 (85.60)		697 (26.79)	1905 (73.21)		251 (9.22)	2353 (90.36)	
Total (N)	1389 (14.51)	8420 (85.49)		1247 (24.34)	3722 (75.66)		414 (8.22)	4561 (91.78)	
	9809			4969			4975		
Missing Data	539 (5%)			5379 (52%)			5373 (52%)		

"p": p-value from Wald Chi-square method; ng/ml: Nanogram per milliliter; kg/m^2: kilogram per square meter; mg/dl: milligram per deciliter; "<": less than; ">=": equal or higher than; "=<": equal or less than; FAI: Family annual income (high if more than 30.000 dollars per year); "Case" is defined as having a chronic condition and "non-case" as not having it. CRP higher than 0.5mg/dl is in favor of an inflammatory process; BMI higher than 30 kg/m^2 is in favor of overweight. Total (N): Sample size for each inflammatory condition (Asthma, Arthritis, and Malignancy) with respective missing observations; weighted age with interview weight (WTINT2YR); weighted CRP, Vitamin D and BMI with examination weight (WTMEC2YR).
Source: NHANES 2005-2006.

After continuous variables are categorized,the study of the relationship between cases of CIC group and calcidiol with covariates provides crude estimates of the association in Table 4. Asthma confirm evidence of lack of statistically significant association with calcidiol (p=0.164), gender,

Table 4. Distribution of crude and adjusted odds ratio of association between the main biomarker of exposure (Calcidiol), covariates, and their respective chronic inflammatory conditions.

Variable	Asthma				Arthritis				Malignancy			
	OR_c	95%CI	OR_a	95%CI	OR_c	95%CI	OR_a	95%CI	OR_c	95%CI	OR_a	95%CI
Calcidiol												
>=30 (ref)	1		1		1		1		1		1	
<30	1.16	0.95-1.42	1.16	0.93-1.44	1.14	0.93-1.41	1.03	0.81-1.31	0.71	0.53-0.94	0.76	0.55 -1.05
CRP												
=<0.5 (ref.)	1				1		1		1		1	
>0.5	1.36	1.11-1.66	N/A	N/A	1.85	1.54-2.22	1.44	1.16-1.78	1.45	1.09-1.92	1.43	1.05-1.96
Age												
<40 (ref.)	1		1		1		1		1		1	
>=40	0.77	0.65-0.92	0.66	0.55-0.79	7.71	5.97-9.94	6.83	5.26-8.87	7.44	4.60- 12.03	6.98	4.29-11.35
Gender												
F (ref.)	1				1		1		1		1	
M	0.98	0.83-1.15	N/A	N/A	0.64	0.54-0.75	0.69	0.57-0.83	0.56	0.44-0.75	0.60	0.46-0.78
Ethnicity												
W (ref.)	1		1		1		1		1		1	
B	1.13	0.96-1.33	0.99	0.83-1.19	0.72	0.60-0.86	0.72	0.58-0.90	0.37	0.27-0.53	0.43	0.30-0.61
O	0.76	0.62-0.92	0.69	0.56-0.85	0.43	0.34-0.55	0.53	0.41-0.69	0.33	0.20-0.54	0.42	0.25-0.70
BMI												
<30 (ref.)	1		1		1		1		1		1	
>=30	1.53	1.28-1.83	1.64	1.36-1.98	1.93	1.63-2.28	1.67	1.37-2.04	0.84	0.64-1.11	0.71	0.52-0.96
FA Income												
High (ref.)	1				1		1		1		1	
Low	1.11	0.94-1.32	N/A	N/A	1.60	1.35-1.90	1.70	1.40-2.06	1.29	0.99-1.69	1.34	1.00-1.78
Dietsup												
Yes (ref.)	1				1		1		1			
No	0.90	0.76-1.06	N/A	N/A	1.57	1.32-1.85	1.25	1.03-1.52	1.88	1.43-2.46	N/A	N/A

Table 4.Cont

Season												
Wint (ref.)	1				1				1			
Summer	0.98	0.83-1.15	N/A	N/A	1.16	0.98-1.37	N/A	N/A	1.27	0.97-1.66	N/A	N/A

N/A: Value not available due to the exclusion of the variable from the final regression model (the variable is not significant enough at level 0.05 to fit into the final model). OR_c: crude odds ratio from simple logistic regression. OR_a: adjusted odds ratio from multivariate logistic regression. 95%CI: 95 percent confidence interval. Ref: reference. Wint: winter. FA income: Family annual income. Calcidiol (ng/ml): nanogram per milliliter. BMI (kg/m^2): Body mass index in kilogram per square meter. CRP (mg/dl): C-reactive protein in milligram per deciliter. <: less than; Age: Age in year; F: Female. M: Male. P-value for crude odds ratio=0.154 (Asthma); 0.20 (Arthritis); 0.02 (Malignancy). P-value for adjusted odds ratio=0.20 (Asthma); 0.71 (Arthritis); 0.14 (Malignancy). P-value was generated from the method of Wald Chi-Square. Adjusted for vitamin D (Calcidiol) age, gender, income, body mass index, C-reactive protein, ethnicity, dietary supplement, and season.
Source: NHANES 2005-2006.

income diet supplement and seasons (p>0.05). However, CRP, BMI, Age and Ethnicity have statistically significant link (p<0.003). Arthritis cases show statistically significant relationship with diet supplement, CRP, income, BMI, ethnicity, gender and age (p=0.001); whereas there was no association with calcidiol (p=0.151) and season (p=0.079). Malignancy cases show statistically significant association with calcidiol (p=0.014), age, gender, ethnicity, diet supplement (p=0.001), and CRP (p=0.010), respectively; but no relationship with BMI, income and season, p=0.130, 0.064, 0.061, respectively.

Adjusted odds ratios (OR_a) provide the strength of association between each CIC and calcidiol after adjusted for covariates in Table 4.

Asthma cases shows non-significant statistical relationship with the main predictor calcidiol after adjustment OR_a with 95%CI =1.16[0.93-1.44], the same result was found with black ethnicity 0.99[0.83-1.19]; however overweight, age and other ethnicity displayed a positive or negative statistically significant association, OR_a=1.64[1.36-1.98], 0.66[0.55-0.79], and 0.69[0.56-0.85], respectively. Although OR_a could not be computed, the crude odds ratio for CPR showed significant statistical association OR_c=1.36[1.11-1.66]; while gender, family annual income (FAI), diet supplement, and season, displayed non-significant statistical association, 0.98[0.83-1.15], 1.11[0.94-1.32], 0.90[0.76-1.06], and 0.98[0.83-1.15], respectively.

Arthritis cases showed non-significant statistical relationship with calcidiol after adjustment OR_a= =1.03[0.81-1.31]; however, elevated CRP, higher BMI, adult-age, family low income, no diet supplement, male gender, black and other ethnicity have either positive or negative significant statistical association, OR_a=1.44[1.16-1.78], 1.67[1.37-2.04], 6.83[5.26-8.87], 1.70[1.40-2.06], 1.25[1.03-1.52], 0.69[0.57-0.83], 0.72[0.58-0.98], and 0.53[0.41-0.69], respectively. The OR_a for “season” could not be computed, but its OR_c was statistically non-significant, 1.16[0.98-1.37].

Malignancy cases showed non-significant statistical relationship with calcidiol and low FAI after adjustment OR_a=0.76[0.55-1.05] and 1.34[1.00-1.78], respectively; while elevated CRP serum level and adult-age are weakly and strongly related to it, OR_a=1.43[1.05-1.96] and 6.98[4.29-11.35], respectively. Conversely, overweight, male gender, black and other ethnicity, OR_a= 0.71[0.52-0.96], 0.60[0.46-0.78], and 0.43[0.30-0.61]/0.42[0.25-0.70], respectively displayed negative significant statistical relationship. The OR_a for “season” and not supplemented diet could not be computed, but their ORc=1.27[0.97-1.66] and 1.88[1.43-2.46] were statistically significant and non-significant, respectively.

An assessment of the relationship between calcidiol and covariates in Table 5 shows people of African origin to be eleven times, OR=10.57[7.98-13.99] compared to others (non-black/non-white) who are three times OR=2.98[2.43-3.66] more likely to suffer from serum calcidiol deficiency, than their counterparts of European origin. The category level summer season and diet supplementation have a protective effect against serum calcidiol deficiency OR=0.57[0.49-0.68] and 0.70[0.60-0.82], respectively.

Analysis of pairwise interaction terms (Results not shown) between calcidiol and covariates shows significant statistical positive interaction for variable age (β=1.14; p<0.001) and CRP (β=0.35; p=0.049) for malignancy and asthma groups; and age (β=1.35, p<0.001), BMI (β=0.29; p=0.005), and income (β=0.31; p=0.002) in arthritis group. On the other hand, statistical significant negative interaction is evidenced with variable gender (β=-0.64; p<0.001), ethnicity (β=-1.02 black; β=-1.22 other; p<0.001), and BMI (β=-0.57; p<0.001) for malignancy and asthma;whereas arthritis negative interaction term is only evidenced for gender

Table 5. Assessing association between weighted vitamin-D and covariates.

Variable	Calcidiol (25-hydroxyVitamin D)	
	Odds ratio	95% CI
Age_cat (adult vs. young)	1.44	1.21-1.71
Gender_cat (Male vs. Female)	1.07	0.92-1.25
Ethnicity_cat (Black vs. White)	10.57	7.98-13.99
Ethnicity_cat (Other vs. White)	2.98	2.43-3.66
Income_cat (Low vs. High)	1.17	0.97-1.40
BMI_cat (abnormal vs. Normal)	2.28	1.83-2.84
CRP_cat (abnormal vs. Normal)	0.97	0.76-1.20
Season_cat (Summer vs. Winter)	0.58	0.49-0.68
Dietsup_cat (yes vs. no)	0.70	0.60-0.82

Source: NHANES 2005-2006; _cat: category.

Table 6. Assessment of weighted correlation between CRP and Vitamin-D, Vitamin-D and BMI.

Linear regression model	Unadjusted			Adjusted*	
	R^2	β	p	R^2	p
Log_CRP=Vit D	0.02	-0.01	<0.001	0.33	<0.001
VitD=BMI	0.07	-0.33	<0.001	0.27	<0.001

*R^2: Adjusted coefficient of determination of model Log_crp=VitD for covariates: bmi, age_cat, gender_cat, income_cat, season_cat, dietsup_cat, and ethni_cat. BMI has the strongest model effect (p<0.001), VitD has the weakest model effect (p=0.03), whereas ethnicity has no model effect at all (p=0.42). *R^2: adjusted coefficient of determination of model VitD=BMI for covariates: Log_crp age_cat, gender_cat, income_cat, season_cat, dietsup_cat, and ethni_cat; Ethnicity and BMI have the strongest model effect (p<0.001); CRP has the weakest model effect (p=0.03); whereas gender has no model effect at all (p=0.34).β: regression coefficient; p: p-value; Log: logarithm for C-reactive protein; VitD: Vitamin D, and BMI: body mass index. "Source: NHANES 2005-2006"

(β=-0.64;p=0.001) and ethnicity (β=-0.56; p=0.001). In contrast, there was no significant statistical interaction between calcidiol and variable season and dietary supplement (p>0.05) in any of the CIC. After adjustment, a 10% change was observed in the regression coefficient (β) for variable age, ethnicity, and BMI; making them confounding factors in asthma group, while season, diet, age, ethnicity, BMI, and gender were confounding with calcidiol in malignancy group; whereas all covariates were confounding in the arthritis group.

In linear regression analysis, Table 6 and Figure 1, the study for simple linear correlation between CRP as the outcome and calcidiol as the main exposure showed a weak linear negative correlation with a regression coefficient β=-0.01 (p<0.001), meaning that when serum calcidiol increases by one unit (1 ng/ml), serum CRP decreases by 0.01 mg/dl. Before the adjustment, the coefficient of determination was 2% (R^2=0.02), and the correlation coefficient r=-0.14 (p<0.001). The coefficient of determination increased substantially, with a negative strong correlation after adjustment for covariates in the final model (R^2=0.33; r=-0.57; p<0.001). Thus, calcidiol is responsible of 2% change in serum CRP levels before adjustment, and 33% change after adjustment. Further analysis showed that BMI has the strongest model effect (p<0.001), whereas calcidiol has the weakest model effect (p=0.03), and ethnicity has no model effect at all (p=0.42).

Also, the study of linear association between calcidiol as the outcome and BMI the main exposure in Table 6 and Figure 2, showed a weak linear negative correlation with β=-0.33 (p<0.001), meaning that, when the BMI increases by one unit (1 kg/m^2), serum calcidiol will decrease by 0.33 ng/ml. Before the adjustment, the coefficient of determination was 7% (R^2=0.07) with a correlation coefficient r=-0.26 (p<0.001). After adjustment for all covariates, we noted a strong negative correlation with an increase in the coefficient of determination R^2=0.27; r=-0.52 (p<0.001). Thus, BMI accounts for 7% change in serum calcidiol before adjustment, and 27% change after the adjustment. Further analysis showed that ethnicity and BMI have the strongest model effect (p<0.001); when CRP has the weakest model effect (p=0.03), and gender has no model effect at all (p=0.34).

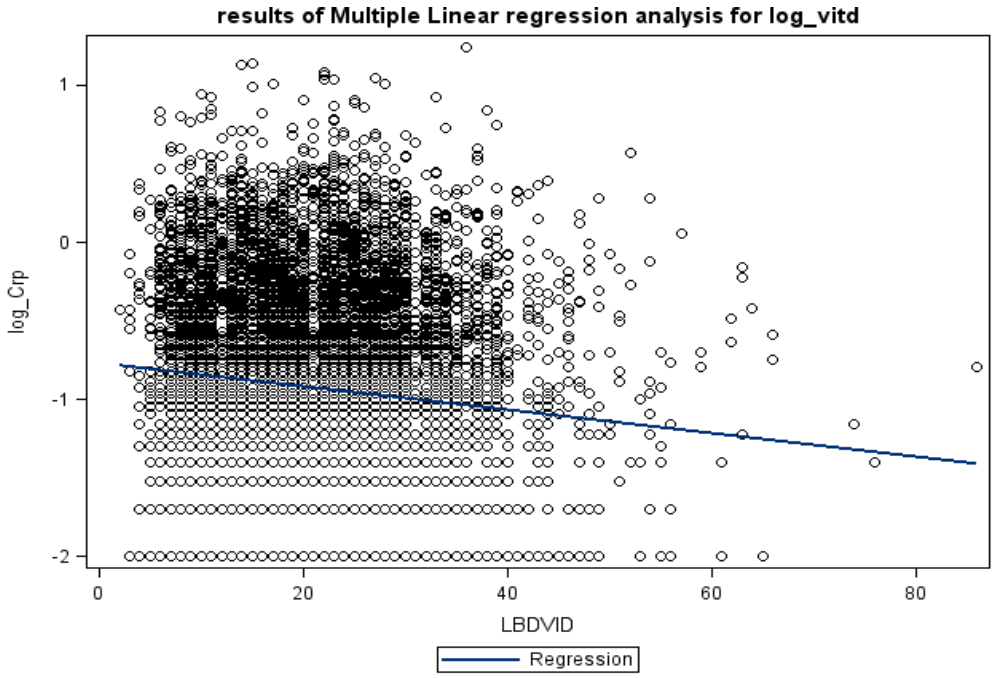

Figure 1. Scatterplot and regression line for C-reactive protein vs. vitamin D: β= -0.01; R^2= 0.02; r=-0.14; p<0.001; LBDVID=Vitamin-D.
Source: NHANES 2005-2006.

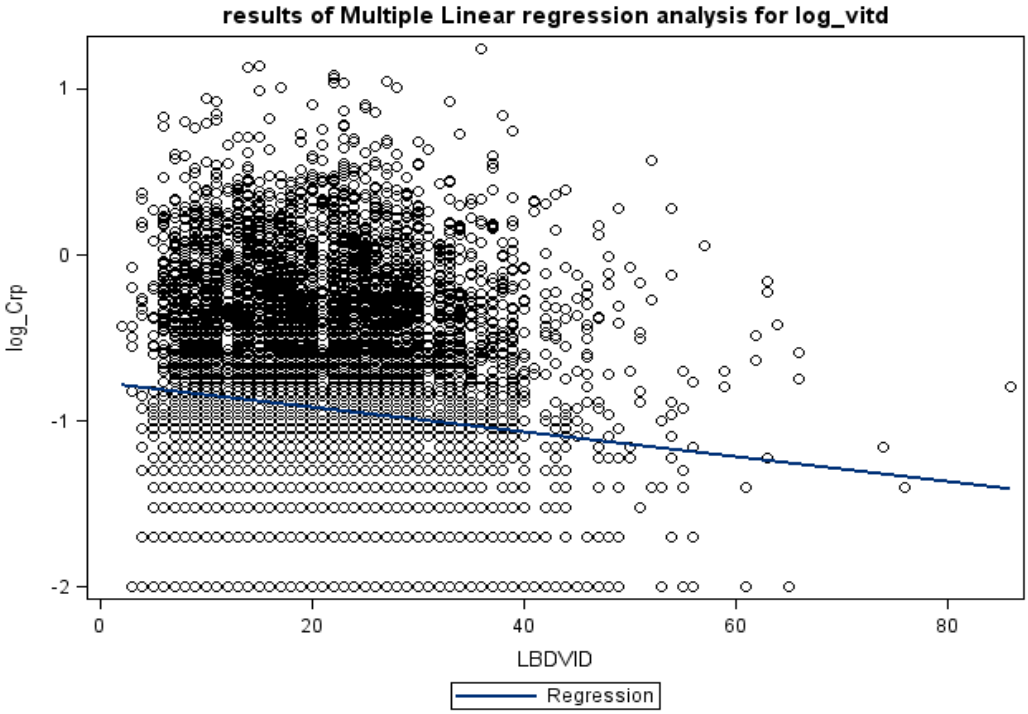

Figure 2. Scatterplot and regression line for vitamin D vs. body mass index: β= -0.33; R^2= 0.07; r= -0.26; p<0.001. There was one BMXBMI (Body Mass Index) outlier at 135 kg/m^2 that was removed in this scatterplot.
Source: NHANES 2005-2006

DISCUSSION

Statistical analysis of our dataset showed a point prevalence of serum calcidiol deficiency at 84.0% in each CIC group, with people of African origins being eleven times more likely to suffer from the deficiency than their European counterparts; a finding that is consistent with the work of Luque-Fernandez MA et al., (2014) in their study on "seasonal variation of 25-hydroxyvitamin D among non-Hispanic Black and White pregnant women from three US pregnancy cohorts.

When calcidiol is used as a categorized variable and adjusted for covariates, there is a lack of association with CIC in logistic regression analysis; this result is consistent with what David Reid had found in the study of the relationship between serum calcitriol, CRP and inflammatory conditions (David et al., 2011). In contrast however, when used as a continuous variable in linear regression analysis, calcidiol elicits a negative linear although weak correlation with CRP, a proven biomarker of effect for many CIC; this finding is consistent with what Abou-Raya reported from a randomized placebo-controlled trial, with significant improvement in disease activity in calcidiol supplemented group compared to the placebo (Abou-Raya et al., 2013).

Furthermore, malignancy and arthritis outcome showed significant mean difference for calcidiol between cases and non-cases; finding that is consistent with the work of Lappe et al. (2007) who found a significant reduction of cancer incidence in menopaused women supplemented with Vitamin-D. Previous other studies have shown association between calcidiol and CIC, especially with certain cancers, diabetes mellitus, and cardiovascular diseases (Davis et al., 2011; Michael 2004; Edward et al., 2006; Gröber et al., 2013). Still, our study of interaction terms showed that serum calcidiol significantly interacts with age, ethnicity, and BMI in malignancy and arthritis groups. The negative linear correlation observed between calcidiol and CPR is suggestive of a dose-response effect. However, the discrepancy in the results between linear and logistic regression analyses likely comes from the loss of information that naturally occurs when continuous variables are categorized.

In our study, the prevalence of calcidiol deficiency was two times higher than what Forrest et al. (2011) had reported using the same NHANES dataset. The discrepancy comes from the use of different thresholds in defining serum levels of vitamin-D as deficient, less than 30 ng/ml in our study (Abou-Raya et al., 2013) versus less than 20 ng/ml in Forrest and Stuhldreher (2011) study. Nevertheless, the distribution of serum levels of vitamin-D shows that a large proportion of research subject falls between 20 and 30 ng/ml without exhibiting clinical symptoms. Thus, higher threshold of serum calcidiol may have led to misclassification of many research subjects as vitamin-D deficient for value between 20 and 30 ng/ml, whereas they were in fact vitamin-D insufficient and clinically asymptomatic. However, comparison between studies remains daunting because of the difference in study designs and laboratory techniques used to ascertain serum levels of calcidiol (Wendy et al., 2013; Huiping Chen et al., 2008; Wallace et al., 2010; Lensmeyer et al., 2012; Craig, 2013).

Finally, linear regression analysis appears more sensitive for detecting the relationship between calcidiol and CRP, albeit weak. Our linear correlation analysis between calcidiol and BMI suggests that dietary supplementation of calcidiol would be clinically effective when the amount of Vitamin-D supplement is well correlated with the patient's weight.

Strengths

NHANES used DiaSorin-RIA, which has the closest sensitivity (95%) to the gold standard test, Liquid Chromatography Mass Spectrometry (98%) (Huiping Chen et al., 2008;Holmes et al., 2012).The high sensitivity and specificity of DiaSorin-RIA provide high precision and accuracy in the ascertainment of serum levels of calcidiol and help reduce misclassification of exposure (Huiping Chen et al., 2008; Górriz, and Estela, 2013; Wallace et al., 2010; Holmes et al. 2012). Although there is a high proportion of missing data; however, statistical analyses showed narrow confidence intervals, meaning that the sample size was large enough to compute statistical estimates with high precision.

Inconsistency and incoherence between studies may come from a lack of representativeness of the study population. NHANES used a nationally random sample; thus, our study has a vigorous external and internal validity. Trained interviewers ensured accuracy and precision in the ascertainment of outcome; therefore, occurrence of observational bias in this study is unlikely. Discrepancy between studies may also result from incomplete adjustment for confounding factors.

Limitations

Misclassification bias due to higher threshold of serum calcidiol may have led to a diluted association. However, the association failed to achieve significance even when we lowered the threshold of serum calcidiol to 15 ng/ml (results not shown).

Like many studies that involve metabolic processes, association between calcidiol and inflammatory condition is subject to residual confounding, mediators, and intermediate factors. Although, we adjusted effect size for many covariates, our study failed to adjust for kidney and liver diseases, genetic variants of 1-α-hydroxylase enzyme (Hewison et al., 2000), serum levels of calcium,

and PTH (David Reid et al., 2011), which can considerably affect the serum levels of calcidiol (Anderson et al., 2003). Furthermore, like other steroid hormones, calcitriol delivers its cellular messages through a receptor referred to as Vitamin-D receptor (VDR); its phenotypic expressivity is genetically determined with a fair proportion of dysfunctional genotypic variants (Nagpal et al., 2005; Pinette et al., 2003; Ramagopalan et al., 2010). The same reasoning applies to 1-α-hydroxylase enzyme, which converts calcidiol into its active form in the kidney; its deficiency may reduce the effect of vitamin-D on the tissue (Eyles et al., 2003). The combination of intermediate mediators, regulators factors and misclassification bias due to higher threshold of serum calcidiol could probably have led the association between calcidiol and CIC toward the null.

Correlation between calcidiol and body mass index: Impact in the treatment of Vitamin-D deficiency

There is an ongoing controversy about the adequate amount of vitamin-D needed to treat or prevent serum calcidiol deficiency. In its 2010 Dietary Reference Intakes, the Institute of Medicine recommended a dosage range between 400 and 800 IU of daily calcidiol supplement (Institute of Medicine, 2010). The Institute of Medicine also reported that based on current scientific knowledge, it does not support the policy of systematic screening for vitamin-D deficiency in US population: “The IOM finds that the evidence supports a role for vitamin-D and calcium in bone health but not in other health conditions.” However, high caliber scientists had expressed disagreement and called for the consideration of higher dosages (Michael and Holick, 2004; Zittermann et al., 2014).

The findings in the study of the correlation between serum levels of calcidiol and BMI are consistent with other studies (Zittermann et al., 2014) and suggest the necessity for prescribing a dosage of vitamin-D that is tailored on patient’s BMI or weight in the same way clinicians prescribe many drugs. Individuals suffering from Vitamin-D deficient should consider higher dosage to prevent the effects of many regulatory and intermediary factors on serum levels of calcidiol, which would not always correlate with serum levels of calcitriol (Giovannucci et al., 2006; Cannell and Hollis, 2008; Abou-Raya et al., 2013).

Conclusion

Although our findings suggest that serum calcidiol deficiency is not an ideal predictor of the onset of CIC; nevertheless, we cannot refute the observed weak association.Obviously, there is a need for further studies with new designs that take into consideration the complexity due to confounding, intermediate, and regulatory factors of this association. A two-step study design may be the best approach to deal with this complexity; the first step should investigate correlation between serum levels of calcidiol as a biomarker of exposure and calcitriol as a biomarker of effect; while the second step would assess the relationship between serum levels of calcitriol, at this time, as a biomarker of exposure and CIC or disease outcome as a biomarker of effect.

Finally, there is a necessity for implementing new public health policies that promote the prevention of calcidiol insufficiency and deficiency; while supplementation is tailored on individual’s body weight.

CONFLICT OF INTERESTS

The authors declared having no conflict of interests, and there was no funding granted to this work.

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