



Effect of waist circumference on the association between serum 25-hydroxyvitamin D and serum lipids: results from the National Health and Nutrition Examination Survey 2001–2006

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Abstract

Objective: To examine the interaction between waist circumference (WC) and serum 25-hydroxyvitamin D (25(OH)D) level in their associations with serum lipids.

Design: Cross-sectional study. The associations of serum 25(OH)D with total cholesterol, HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), LDL-C:HDL-C and TAG were examined using multiple linear regression. Effect modification by WC was assessed through cross-product interaction terms between 25(OH)D and WC categories (abdominal overweight, 80–<88 cm in females/94–<102 cm in males; abdominal obesity, ≥88 cm in females/≥102 cm in males).

Setting: The US National Health and Nutrition Examination Survey waves 2001–2006.

Subjects: Non-pregnant fasting participants (*n* 4342) aged ≥20 years.

Results: Lower 25(OH)D levels were significantly associated with lower HDL-C levels as well as with higher LDL-C:HDL-C and TAG levels in abdominally obese participants, but not in abdominally overweight or normal-waist participants. In contrast, lower 25(OH)D levels were associated with lower levels of total cholesterol and LDL-C in abdominally overweight and normal-waist participants only, but this association was only partly significant. However, a significant difference in the association between 25(OH)D and the lipids according to WC category was found only for LDL-C:HDL-C (*P* for interaction = 0.02).

Conclusions: Our results from this large, cross-sectional sample suggest that the association between lower 25(OH)D levels and an unfavourable lipid profile is stronger in individuals with abdominal obesity than in those with abdominal overweight or a normal WC.

Keywords
Vitamin D
Serum lipids
Waist circumference
NHANES
Interaction

Insufficient levels of serum 25-hydroxyvitamin D (25(OH)D), the major circulating vitamin D metabolite, which is traditionally used to determine vitamin D status⁽¹⁾, are a global phenomenon⁽²⁾. Possible adverse effects of low 25(OH)D levels on health are thus of great interest. While the role of vitamin D in the regulation of calcium, phosphorus and bone metabolism and its consequential importance for skeletal health have been known for a long time⁽³⁾, research in recent decades has raised the question about non-skeletal health effects of vitamin D. Several cross-sectional studies have reported an association between low levels of 25(OH)D and an unfavourable lipid

profile⁽⁴⁾. In two large cohorts, 25(OH)D levels have also been found to be significantly associated with a decrease in TAG levels over 14 years⁽⁵⁾ and with lower TAG and VLDL cholesterol levels as well as with a reduced odds for hypercholesterolaemia after 5 years⁽⁶⁾. The results of a Mendelian randomization study, in which genetically instrumented 25(OH)D levels were positively associated with HDL cholesterol (HDL-C) and inversely with TAG⁽⁷⁾, support the notion of a causal relationship between vitamin D and the lipid profile. By contrast, serum lipids are generally not significantly influenced by vitamin D supplementation in randomized controlled trials⁽⁸⁾.

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However, the trials conducted so far provide limited evidence, as they were insufficiently powered and not specifically designed to evaluate the effect of vitamin D on serum lipids⁽⁸⁾.

If a causal relationship exists, there are several potential mechanisms on how low vitamin D levels might directly or indirectly impact serum lipids⁽⁹⁾. Some of these pathways, such as decreased insulin sensitivity⁽¹⁰⁾, decreased activity of lipoprotein lipase (LPL)⁽¹¹⁾ and lower levels of adiponectin^(12,13), are also related to obesity, a known risk factor for dyslipidaemia⁽¹³⁾. It is thus possible that the association between 25(OH)D and the lipid profile varies according to weight or waist circumference (WC) status. In a cohort of Chinese adults, the inverse association between 25(OH)D and the metabolic syndrome was significant only in overweight and obese individuals⁽¹⁴⁾, but little is known about a possible effect modification of obesity on the association between 25(OH)D and serum lipids alone. 25(OH)D levels are generally low in obese individuals⁽¹⁵⁾ and should an inverse association between 25(OH)D and serum lipids be stronger in the obese, or occur primarily in this group, 25(OH)D deficiency could be regarded as a currently unaccounted risk factor for dyslipidaemia in obese individuals. Thus, the objective of the present study was to examine the interaction between WC and serum 25(OH)D levels in their associations with serum lipids in adult participants of the cross-sectional US National Health and Nutrition Examination Surveys (NHANES) 2001–2006.

Methods

Study population

NHANES are continuous cross-sectional surveys of the non-institutionalized civilian resident US population, which are conducted annually by the Centers for Disease Control and Prevention's (CDC) National Center for Health Statistics (NCHS), Hyattsville, MD, USA. A nationally representative population sample is selected by means of a complex, four-stage probability sampling design. Certain subgroups (adolescents, adults aged 70 years or over, non-Hispanic blacks, Mexican Americans and persons with low income in the NHANES waves 1999–2006) are oversampled to increase reliability and precision. The surveys combine home-administered personal interviews with standardized physical examinations, interviews and laboratory tests conducted in specially equipped mobile examination centres. NHANES is conducted in accordance with the US Department of Health and Human Services' Policy for Protection of Human Research Subjects. The data are released to the public in 2-year cycles by the NCHS⁽¹⁶⁾. More details on sampling, interviews, examinations and laboratory measurements are given elsewhere^(16–18).

For the present study, data from the NHANES waves 2001–2006 were pooled. In these waves, a total of 31 509

individuals completed the interview (response rates varied from 79 to 84%) and 30 070 individuals completed the physical examinations (response rates varied from 76 to 80%)⁽¹⁸⁾. The analytic sample was restricted to the 6164 non-pregnant participants aged ≥ 20 years, who had been randomly assigned to an examination in the morning session after an overnight fast. Further, all participants with missing information on any of the variables used for the analyses were excluded, leaving a final analytic sample of 4342 participants as the study population. A detailed description of the study population and the excluded participants is shown in a flow diagram in the online supplementary material, Supplemental Fig. 1.

Laboratory measurements

During the physical examination at the mobile examination centres, blood samples were drawn via venepuncture by certified phlebotomists⁽¹⁸⁾. The samples were collected using Vacutainer tubes (Becton-Dickinson, Franklin Lakes, NJ, USA) and subsequently centrifuged, aliquoted and frozen to -20°C , before being transported to laboratories across the USA for analysis⁽¹⁸⁾.

Serum lipids were measured at the Johns Hopkins Hospital, Baltimore, MD, USA. HDL-C was measured using the heparin manganese precipitation method in NHANES 2001–2002 and a direct HDL-C immunoassay in NHANES 2003–2006. To control for these differences in methods, the HDL-C values were corrected by NHANES using quality controls⁽¹⁹⁾. Total cholesterol and TAG were measured enzymatically. LDL cholesterol (LDL-C) was calculated according to the Friedewald equation: $\text{LDL-C} = (\text{total cholesterol}) - (\text{HDL-C}) - (\text{TAG}/5)$ ⁽¹⁸⁾. The ratio LDL-C:HDL-C was generated for the present analysis by dividing LDL-C levels by HDL-C levels.

Total serum 25(OH)D was measured at the National Center for Environmental Health, CDC, Atlanta, GA, USA using a RIA kit (DiaSorin, Stillwater, MN, USA)⁽¹⁸⁾. The sensitivity of this assay has been shown to be 1.5 ng/ml and the CV for the years 2001–2006 varied between 4 and 13%^(20–22). From 2007–2008 onwards, 25(OH)D was measured using a standardized liquid chromatography-tandem mass spectrometry (LC-MS/MS) method and in October 2015, updated 25(OH)D values for 2001–2006 were released, which had been converted from RIA to LC-MS/MS equivalents using ordinary least squares regression⁽²³⁾. As recommended by NHANES⁽²³⁾, these LC-MS/MS equivalents were used in the present study.

Plasma glucose and serum insulin were measured at the University of Missouri-Columbia, Columbia, MO, USA from 2001 to 2004 and at the Fairview Medical Center Laboratory at the University of Minnesota, Minneapolis, MN, USA in 2005–2006. Glucose was measured using a hexokinase method. As different instruments were used in 2003–2004 (Roche/Hitachi 911) and 2005–2006 (Roche Cobas Mira), the glucose values from 2005–2006 were corrected to 2003–2004 values using linear regression, as suggested





by NHANES. Insulin was measured using a RIA kit (Pharmacia Diagnostics AB, Uppsala, Sweden) in 2001–2002, a two-site immunoenzymometric assay (Tosoh Corporation, Toyama, Japan) in 2003–2004 and an ELISA immunoassay (Merocodia, Uppsala, Sweden) in 2005–2006. Insulin values from 2001–2002 and 2005–2006 were adapted to 2003–2004 values using linear regression⁽¹⁸⁾. Insulin resistance was estimated from glucose and insulin using the homeostatic model assessment for insulin resistance (HOMA-IR), calculated with the following equation: [fasting serum insulin ($\mu\text{U/ml}$) \times fasting plasma glucose (mg/dl)]/405⁽²⁴⁾.

Anthropometric measurements

For the assessment of height and weight during the physical examination, participants were dressed in underwear, disposable paper gowns and foam slippers. A digital scale was used to measure weight to the nearest 100 g, a fixed stadiometer to measure height to the nearest millimetre. BMI was calculated as weight in kilograms divided by the square of height in metres. WC was measured at the iliac crest to the nearest millimetre, using a steel tape⁽¹⁸⁾.

Covariable assessment

The covariables were chosen based upon prior studies on the association between 25(OH)D and serum lipids^(5,14,25–33). Information on age, sex, self-identified ethnicity, level of education, physical activity, smoking behaviour, alcohol consumption and intake of prescribed cholesterol-lowering medication was obtained from the interview. Smoking behaviour was grouped into three categories. 'Never' applied to participants who reported never having smoked 100 cigarettes during their lifetime; 'former' applied to participants who reported having smoked at least 100 cigarettes during their lifetime but currently did not smoke, whereas 'current' applied to those who reported smoking either every day or some days or at least one cigarette per day. To categorize alcohol consumption, the reported number of alcoholic beverages consumed per week was calculated and grouped into: 'none' for women and men who reported no consumption of alcoholic beverages at all, 'moderate' for women and men who reported consuming at least one but not more than seven or fourteen alcoholic beverages per week, respectively, and 'heavy' for women and men who reported consuming more than seven or fourteen alcoholic beverages per week, respectively. For physical activity, all leisure-time activities in the past 30 d which were performed for at least 10 min were recorded. An activity which caused light sweating or slight to moderate increases in breathing or heart rate was considered to be moderate, an activity which caused heavy sweating or large increases in breathing or heart rate was considered to be vigorous. If participants performed both moderate and vigorous activities, the amount of vigorous activities defined the group allocation. The estimated glomerular

filtration rate was calculated from serum creatinine using the CKD-EPI equation⁽³⁴⁾ and chronic kidney disease was defined as a glomerular filtration rate <60 ml/min per 1.73 m^2 ⁽³⁵⁾.

Statistical analysis

To account for the complex survey design, all analyses were adjusted for sampling probability, stratum and cluster effects using the SAS survey procedures SURVEYMEANS and SURVEYREG (SAS statistical software package version 9.3). A combined 6-year fasting weight for the three cycles was used⁽¹⁷⁾. For proper variance estimation, the analytic sample was examined using the DOMAIN statement⁽¹⁷⁾. The associations between 25(OH)D and the serum lipids were examined with multiple linear regression models, with the continuous lipids used as dependent variables. Normal distribution of the lipid variables was checked with visual inspection of the histograms. All lipids, except TAG, were considered normally distributed and after log transformation (\log_e) TAG attained a normal distribution as well. As the relationships of the lipids with both 25(OH)D and WC did not prove to be consistently linear, 25(OH)D and WC were categorized. Four 25(OH)D categories were used (<15 ng/ml, 15 – <20 ng/ml, 20 – <30 ng/ml, ≥ 30 ng/ml), based on cut-off points used by the US Institute of Medicine (16 and 20 ng/ml⁽³⁶⁾) and by the US Endocrine Society (20 and 30 ng/ml⁽³⁷⁾). For WC, three categories were used. Normal waist was defined as WC <80 cm in women or <94 cm in men, abdominal overweight as WC of 80 – <88 cm in women or 94 – <102 cm in men, and abdominal obesity as WC ≥ 88 cm in women or ≥ 102 cm in men.

At first, the associations between 25(OH)D and the serum lipids were assessed in models adjusted for WC (main effect models). Subsequently, effect modification by WC was examined in two ways. First, cross-product interaction terms between 25(OH)D and WC categories were added to the main effect models (interaction models). Additionally, the associations between 25(OH)D and the serum lipids were assessed in models stratified by WC category (stratified models). All models were adjusted for age, sex, ethnicity, season of examination, physical activity, alcohol consumption, smoking status, level of education, kidney disease and intake of prescribed cholesterol-lowering medication. Further, to account for the change in the HDL-C measurement method, the HDL-C and LDL-C:HDL-C models were additionally adjusted for survey cycle. Additionally, all models were further adjusted for HOMA-IR. Linear trends across HOMA-IR quartiles were tested and, accordingly, the HDL-C, LDL-C:HDL-C and TAG models were adjusted for HOMA-IR, whereas the total cholesterol and LDL-C models were adjusted for HOMA-IR as well as HOMA-IR squared. In a sensitivity analysis, BMI was used instead of WC to operationalize obesity. Like for WC, three BMI categories were used, with normal weight being defined

as BMI < 25.0 kg/m², overweight as BMI = 25.0–<30.0 kg/m² and obesity as BMI ≥ 30.0 kg/m². Sex- and ethnicity-specific differences in the interaction between 25(OH)D and WC with regard to the lipids were examined by adding three-way interaction terms (25(OH)D category × WC category × sex/ethnicity, together with the three respective cross-product terms) to the interaction models. A two-sided significance level of 0.05 was set, except for the interaction terms. Estimates of interaction effects have larger variances than estimates of additive effects, and thus the power of a statistical test to detect an interaction is lower⁽³⁸⁾. To compensate for this, a significance level of 0.1 was chosen for the interaction effects, which is considered more conventional for testing interactions⁽³⁹⁾.

Results

Characteristics of the study population

The characteristics of the study population in total and according to 25(OH)D category are shown in Table 1. Serum 25(OH)D levels ranged from 3.6 to 79.3 ng/ml, with the mean level being 25.0 ng/ml. Participants with 25(OH)D levels < 15 ng/ml were more likely to be female, non-white and inactive and more likely to have been examined between November and April. They were also more likely to be obese or abdominally obese. In participants with lower 25(OH)D levels, mean BMI, WC and HOMA-IR were considerably higher and LDL-C:HDL-C was marginally higher than in participants with higher 25(OH)D levels. No clear trend across the 25(OH)D categories was visible for the other serum lipids.

Associations between serum 25-hydroxyvitamin D and serum lipids

The results of the main effect models in the total sample are shown in the online supplementary material, Supplemental Table 1. Lower 25(OH)D levels were significantly associated with lower HDL-C and higher TAG levels, while no significant association was found between 25(OH)D and total cholesterol, LDL-C or LDL-C:HDL-C. The adjusted effect estimates for the 25(OH)D categories from the interaction models, which indicate the mean differences with regard to the reference category of ≥ 30 ng/ml, are shown in Fig. 1 (β coefficients for total cholesterol, LDL-C, HDL-C and LDL-C:HDL-C; geometric mean ratios for TAG). Compared with participants having 25(OH)D levels ≥ 30 ng/ml, abdominally obese participants with 25(OH)D levels < 15 ng/ml had a 0.15 mmol/l lower HDL-C level, a 0.28 units higher LDL-C:HDL-C and a 12% higher TAG level (all significant), whereas no significant association was found in abdominally overweight or normal-waist participants. By contrast, lower 25(OH)D levels were associated with lower levels of total cholesterol and LDL-C, but this association was significant only in abdominally overweight participants with 25(OH)D levels

between 15 and 20 ng/ml. The interaction between 25(OH)D and WC, however, was significant only for LDL-C:HDL-C (P for interaction = 0.02), while no significant difference between the WC categories was found for total cholesterol (P for interaction = 0.23), LDL-C (P for interaction = 0.19), HDL-C (P for interaction = 0.18) and TAG (P for interaction = 0.28). The results of the stratified models (Supplemental Table 1) are in accordance with the results from the interaction models.

Influence of adjustment for insulin resistance

Further adjustment of the models for HOMA-IR, as shown in the online supplementary material, Supplemental Table 2 (stratified models), as well as in Fig. 2 (interaction models: total cholesterol, LDL-C, HDL-C, LDL-C:HDL-C ratio and TAG), had little influence on the association of 25(OH)D with total cholesterol, LDL-C, HDL-C or LDL-C:HDL-C. For TAG, further adjustment for HOMA-IR resulted in changes in the adjusted geometric mean ratio, in particular in abdominally obese participants, where the association between lower 25(OH)D levels and lower TAG levels was attenuated and no longer significant. Consequently, after adjustment for HOMA-IR, the strength of the already non-significant interaction for TAG was further weakened (P for interaction = 0.32).

Sensitivity analyses

No significant differences in the interaction between 25(OH)D category and WC category were found according to sex (all P values for all three-way interaction terms > 0.1). However, a significant difference according to ethnicity was found for HDL-C, LDL-C:HDL-C and TAG. In order to examine these differences further, the respective interaction models were stratified by ethnicity. While the interactions between 25(OH)D category and WC category in non-Hispanic whites, the largest ethnic group in our study population, were similar to the interactions in the total sample, the analyses for the other ethnic groups were not sufficiently powered to draw a valid conclusion (data not shown).

Using BMI to operationalize obesity resulted in a different group allocation. Only 31% of the participants were classified as obese, but 34% as overweight and 35% as having a normal weight. As compared with abdominally obese and abdominally overweight participants, respectively, the β coefficients and geometric mean ratios for HDL-C, LDL-C:HDL-C and TAG were smaller in the obese and larger in the overweight participants (see online supplementary material, Supplemental Fig. 2). These differences were particularly strong for TAG, where the strongest association was found in overweight participants and where a significant association was no longer found in abdominally obese participants. For total cholesterol and LDL-C, the β coefficients were bigger in the obese and smaller in the overweight participants as compared with the main analysis (Supplemental Fig. 2).



Table 1 Characteristics of the study population in total and according to serum 25-hydroxyvitamin D (25(OH)D) category; non-pregnant, fasting adults aged ≥ 20 years, US National Health and Nutrition Examination Survey waves 2001–2006

Characteristic	N*	25(OH)D category									
		Total (N* 4342)		<15 ng/ml (N* 820)		15–<20 ng/ml (N* 808)		20–<30 ng/ml (N* 1799)		≥ 30 ng/ml (N* 915)	
		%	%	%	%	%	%	%	%	%	
Age group											
20–29 years	768	21	21	23	21	18	21	18	21	18	
30–39 years	711	20	20	19	20	23	18	22	20	23	
40–49 years	765	21	23	20	22	17	22	17	20	17	
50–59 years	572	16	14	16	17	11	17	11	17	11	
60–69 years	682	11	12	10	11	11	11	11	11	11	
≥ 70 years	844	11	9	11	11	11	11	11	11	10	
Sex											
Male	2260	49	40	46	53	50	53	47	50	50	
Female	2082	51	60	54	47	50	47	53	50	50	
Ethnicity											
Non-Hispanic black	842	11	39	16	6	2	6	6	2	2	
Non-Hispanic white	2314	73	37	58	79	90	79	79	90	90	
Mexican-American	894	8	11	11	8	3	8	8	3	3	
Other	292	9	13	15	7	5	7	7	5	5	
Physical activity (times/month)											
Vigorous (≥ 12)	738	19	12	14	20	25	20	20	25	25	
Vigorous (1–11)	561	16	11	13	16	19	16	16	19	19	
Moderate (≥ 12)	722	17	14	17	18	16	18	18	16	16	
Moderate (1–11)	563	14	12	16	15	14	15	15	14	14	
None	1758	34	51	41	31	26	31	31	26	26	
Alcohol consumption											
Heavy	308	8	6	5	8	11	8	8	11	11	
None	1549	30	38	35	29	23	29	29	23	23	
Moderate	2485	62	55	60	62	65	62	62	65	65	
Smoking status											
Current	962	25	31	24	22	27	22	22	27	27	
Former	1185	25	18	19	29	26	29	29	26	26	
Never	2195	50	52	57	49	46	49	49	46	46	
Season of examination											
Nov–Apr	2043	41	58	51	40	28	40	40	28	28	
May–Oct	2299	59	42	49	60	72	60	60	72	72	
Level of education											
Less than 9th grade	585	7	8	9	6	5	6	6	5	5	
9–11th grade	630	11	16	13	10	9	10	10	9	9	
High-school graduate	1073	27	29	24	26	28	26	26	28	28	
Some college/AA degree	1235	32	29	37	31	31	31	31	31	31	
College graduate/higher	819	24	17	18	27	26	27	27	26	26	
Kidney disease											
Yes	451	7	8	7	7	6	7	7	6	6	
No	3891	93	92	93	93	94	93	93	94	94	
Intake of medication†											
Yes	708	14	15	16	14	14	14	14	14	14	
No	3634	86	85	84	86	86	86	86	86	86	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
HOMA-IR (units)		2.60	0.06	3.69	0.22	3.05	0.14	2.48	0.08	1.95	0.11
WC (cm), mean		97.02	0.35	101.61	1.00	100.08	0.87	96.96	0.45	92.88	0.66
		%		%		%		%		%	
WC category‡											
Normal waist	1181	29	22	23	29	38	29	29	38	38	
Abdominally overweight	869	20	17	18	22	20	22	22	20	20	
Abdominally obese	2292	51	62	59	50	42	50	50	42	42	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
BMI (kg/m ²)		28.31	0.12	30.96	0.42	29.72	0.36	28.11	0.17	26.40	0.22

Table 1 Continued

Characteristic	N*	25(OH)D category									
		Total (N* 4342)		<15 ng/ml (N* 820)		15–<20 ng/ml (N* 808)		20–<30 ng/ml (N* 1799)		≥30 ng/ml (N* 915)	
		%	%	%	%	%	%	%	%	%	
BMI category§											
Normal weight	1396	35	26	26	34	45					
Overweight	1548	34	27	35	35	36					
Obese	1398	31	47	39	30	19					
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Total cholesterol (mmol/l)		4.99	0.02	4.96	0.04	4.92	0.03	5.01	0.03	5.03	0.04
LDL-C (mmol/l)		2.94	0.02	2.94	0.04	2.90	0.03	2.95	0.03	2.95	0.04
HDL-C (mmol/l)		1.40	0.01	1.35	0.02	1.34	0.02	1.38	0.01	1.48	0.02
LDL-C:HDL-C		2.28	0.02	2.35	0.04	2.35	0.04	2.30	0.03	2.17	0.04
TAG (mmol/l)		1.43	0.02	1.45	0.04	1.48	0.04	1.47	0.02	1.32	0.02

AA, associates degree; HOMA-IR, homeostatic model assessment insulin resistance; WC, waist circumference; LDL-C, LDL cholesterol HDL-C, HDL cholesterol.

% indicate column percentages.

*Unweighted N.

†Prescribed cholesterol-lowering medication.

‡Normal waist, WC <80 cm in women or <94 cm in men; abdominal overweight, WC of 80–<88 cm in women or 94–<102 cm in men; abdominal obesity, WC ≥88 cm in women or ≥102 cm in men.

§Normal weight, BMI <25.0 kg/m²; overweight, BMI = 25.0–<30.0 kg/m²; obesity, BMI ≥30.0 kg/m².

Discussion

In the present large, cross-sectional sample of adults aged ≥20 years, lower 25(OH)D levels were significantly associated with lower HDL-C levels, higher LDL-C:HDL-C and higher TAG levels in abdominally obese participants, but not in abdominally overweight or normal-waist participants. In contrast, lower 25(OH)D levels were associated with lower levels of total cholesterol and LDL-C in abdominally overweight and normal-waist participants. However, a significant difference in the associations between 25(OH)D and the lipids according to WC category was only found for LDL-C:HDL-C. Further adjustment for HOMA-IR attenuated the association between 25(OH)D and TAG in abdominally obese participants.

Our results are mainly in line with previous cross-sectional studies on the association between serum 25(OH)D and serum lipids. Few studies reported no significant association with any lipid at all^(31,32). By contrast, as in the total, unstratified sample in the present study, 25(OH)D levels in previous studies were found to be significantly positively associated with HDL-C^(5,14,25,28–30,33) and significantly inversely associated with TAG^(5,14,25,29,30) in most studies. For total cholesterol and LDL-C, the findings from previous studies are conflicting. A significant positive association with 25(OH)D has been reported before⁽⁵⁾, but more often, as in the total sample of the present study, a positive but non-significant association was found^(25,28). Other studies reported a significantly inverse association of 25(OH)D with total cholesterol and LDL-C^(27,29) or found no clear relationship^(26,30,33). The association between 25(OH)D and LDL-C:HDL-C has been examined

to a lesser extent, but results from previous studies report a significantly inverse association^(5,33), which is in line with our results. Little is known about differences in the associations between 25(OH)D and lipids according to weight or WC status. Only Jorde *et al.* examined this association stratified by BMI group⁽⁵⁾. Contrary to our results, a positive association of 25(OH)D with total cholesterol and LDL-C was found in all BMI groups, although it was significant only in overweight and obese individuals. They also found positive associations with HDL-C and negative associations with TAG in all three BMI groups, but strongest in overweight individuals, which is in line with the results of our sensitivity analysis using BMI to operationalize obesity.

Our results suggest that an association between higher 25(OH)D levels and a favourable lipid profile in particular occurs in abdominally obese individuals. There are several mechanisms which could underlie such a relationship. Adiponectin, for instance, an adipokine whose levels are low in obese subjects, was found to have beneficial effects on lipid metabolism, such as on the HDL assembly in the liver, and low levels of adiponectin are associated with dyslipidaemia⁽¹³⁾. 25(OH)D levels were found to be positively associated with adiponectin levels, in particular in subjects with a high BMI, which was also found to be a significant effect modifier in the association between 25(OH)D and adiponectin⁽¹²⁾. The underlying pathway may be an inhibitory effect of 1,25-dihydroxyvitamin D, the active vitamin D metabolite, on the adipose tissue renin-angiotensin system, which is over-activated during obesity⁽¹²⁾. Another possible mechanism behind the association between 25(OH)D and lipids is LPL, an enzyme that

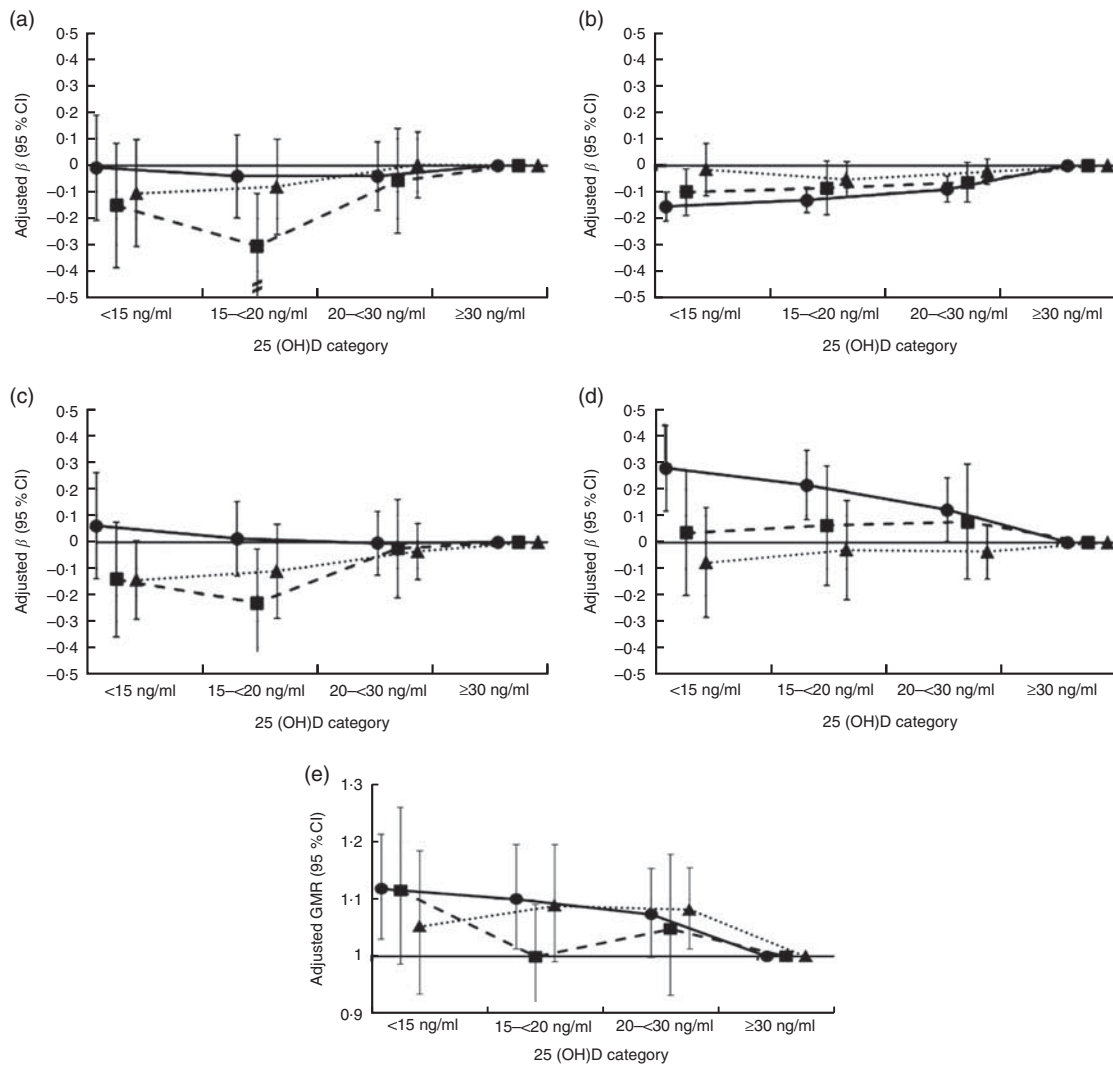


Fig. 1 Interaction between waist circumference (—●—, abdominal obesity; --■--, abdominal overweight; ···▲···, normal waist) and serum 25-hydroxyvitamin D (25(OH)D) levels in their association with serum lipids among non-pregnant, fasting adults (n 4342) aged ≥ 20 years, US National Health and Nutrition Examination Survey waves 2001–2006. Adjusted β coefficients, with their 95% confidence intervals represented by vertical bars, for the 25(OH)D categories from the interaction models on (a) total cholesterol (mmol/l), (b) HDL cholesterol (HDL-C; mmol/l), (c) LDL cholesterol (LDL-C; mmol/l) and (d) LDL-C:HDL-C. (e) Adjusted geometric mean ratios (GMR), with their 95% confidence intervals represented by vertical bars, for the 25(OH)D categories from the interaction model on TAG (mmol/l). Reference category: 25(OH)D ≥ 30 ng/ml; all models adjusted for age, sex, ethnicity, season of examination, physical activity, alcohol consumption, smoking status, level of education, kidney disease and intake of prescribed cholesterol-lowering medication. HDL-C and LDL-C:HDL-C models additionally adjusted for survey cycle

catylases the lipolysis of TAG and whose reduced expression and activity is a pathway for dyslipidaemia in obesity⁽⁴⁰⁾. Low levels of LPL result in hypertriglycerolaemia, which in turn leads to decreased levels of HDL-C^(13,40). 25(OH)D was found to be significantly positively associated with LPL in a large Chinese cohort⁽¹¹⁾. In the same study, both 25(OH)D and LPL were found to be inversely associated with insulin resistance⁽¹¹⁾. Insulin is known to stimulate LPL activity and it is also an important regulator for the mobilization of NEFA from the adipose tissue⁽⁴⁰⁾; an uncontrolled release of NEFA is one of the main mechanisms of dyslipidaemia in obesity⁽¹³⁾. In our study, further adjustment for HOMA-IR attenuated the association

between 25(OH)D and TAG, which supports the notion of insulin resistance as an underlying mechanism. However, this relationship was found only in abdominally obese individuals. Thus, it is possible that the inverse association of 25(OH)D with TAG and, in turn, the positive association with HDL-C and the inverse association with LDL-C:HDL-C, is detectable only during obesity, when insulin sensitivity as well as the LPL action are reduced. In fact, being abdominally obese was previously found to significantly modify the association between 25(OH)D and insulin resistance in data from NHANES 2001–2006⁽⁴¹⁾. Further, in a meta-analysis of randomized controlled trials, vitamin D supplementation led to a non-significant decrease of TAG and a non-significant

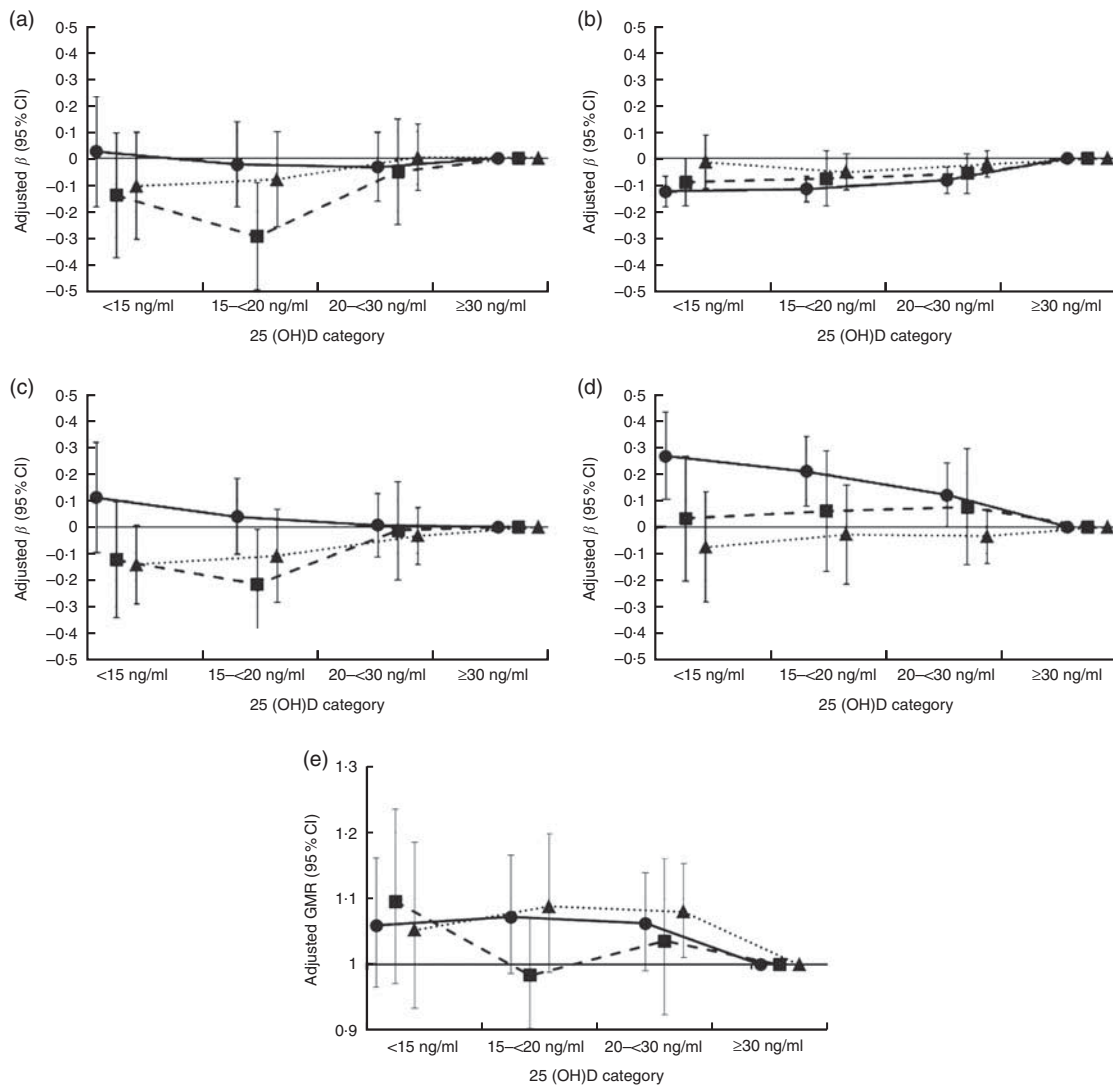


Fig. 2 Interaction between waist circumference (—●—, abdominal obesity; - - ■ - -, abdominal overweight; ··· ▲ ···, normal waist) and serum 25-hydroxyvitamin D (25(OH)D) levels in their association with serum lipids, additionally adjusted for homeostatic model assessment insulin resistance (HOMA-IR), among non-pregnant, fasting adults (n 4342) aged ≥ 20 years, US National Health and Nutrition Examination Survey waves 2001–2006. Adjusted β coefficients, with their 95% confidence intervals represented by vertical bars, for the 25(OH)D categories from the interaction models on (a) total cholesterol (mmol/l), (b) HDL cholesterol (HDL-C; mmol/l), (c) LDL cholesterol (LDL-C; mmol/l) and (d) LDL-C:HDL-C. (e) Adjusted geometric mean ratios (GMR), with their 95% confidence intervals represented by vertical bars, for the 25(OH)D categories from the interaction model on TAG (mmol/l). Reference category: 25(OH)D ≥ 30 ng/ml; all models adjusted for age, sex, ethnicity, season of examination, physical activity, alcohol consumption, smoking status, level of education, kidney disease and intake of prescribed cholesterol-lowering medication. HDL-C and LDL-C:HDL-C models additionally adjusted for HOMA-IR and survey cycle; total cholesterol and LDL-C models additionally adjusted for HOMA-IR and HOMA-IR²; TAG model additionally adjusted for HOMA-IR

increase of HDL-C in obese subjects, while the effect was opposite in normal-weight subjects⁽⁸⁾. It is thus possible that a significant effect of vitamin supplementation on TAG and HDL-C in obese individuals will be found in one of the large vitamin D trials that are currently being conducted⁽⁴²⁾.

The association of lower 25(OH)D levels with lower levels of total cholesterol and LDL-C, which was significant only in abdominally overweight participants with a 25(OH)D level between 15 ng/ml and <20 ng/ml, requires further investigation. To our knowledge, a similar relationship of 25(OH)D with total cholesterol and

LDL-C has not been found in previous studies. In the meta-analysis mentioned above, vitamin D supplementation led to an increase in LDL-C, but this was, contrary to our results, significant only in obese subjects⁽⁸⁾. As our result may be a chance finding, more research is necessary before further conclusions can be drawn.

Our study has several limitations. Due to the cross-sectional design of NHANES, our results cannot give evidence on the causality or direction of the observed associations. Further, our results were dominated by non-



Hispanic whites, due to the large proportion of this ethnic group in the study population. Although we tried to minimize confounding by adjusting our models for a variety of covariables, the possibility of residual confounding remains. Specifically, overall physical activity and smoking, which were used as covariables in the current analysis, might not be sufficient to capture a healthy lifestyle involving increased sun exposure due to more frequent outdoor physical activity as well as a diet rich in vitamin D. Such a lifestyle could be the common cause of both higher levels of 25(OH)D and a more favourable lipid profile. We also did not adjust our models for serum parathyroid hormone and calcium, which are both possible confounders in the relationship between vitamin D and CVD. However, in another study using data from NHANES 2001–2006, adjustment for both factors increased, rather than decreased, the strength of the association of 25(OH)D with TAG and HDL-C and only moderately decreased the strength of the already non-significant association with LDL-C⁽²⁸⁾. Finally, we did not correct our analyses for multiple testing, although we examined five different outcomes. As the evaluation of all lipids was planned and, as discussed above, we had a basis for expecting our results to be biologically plausible, we decided to not correct for multiple testing as suggested by Rothman⁽⁴³⁾. However, had we corrected our analyses for multiple testing, the association of 25(OH)D with HDL-C and LDL-C:HDL-C in abdominally obese participants as well as the interaction between 25(OH)D and WC on LDL-C:HDL-C would have still been significant.

Conclusion

Our results suggest that lower 25(OH)D levels are more strongly associated with an unfavourable lipid profile in individuals with abdominal obesity than in individuals with abdominal overweight or in normal-waist participants. Given that obese individuals generally have low 25(OH)D levels, vitamin D deficiency may be regarded as a currently unaccounted risk factor for dyslipidaemia in this population group. However, more research is needed to assess whether the interaction found in the present analysis is causal and if vitamin D supplementation is effective for the treatment of dyslipidaemia in obese individuals.

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interest: None. *Authorship:* S.V. and R.S. conceived the present study. S.V. performed the statistical analysis and drafted the manuscript. R.S. substantially contributed to the conception and interpretation of the statistical analysis and critically revised the manuscript. J.B., A.P. and B.T. gave advice on designing the present study, substantially contributed to the conception and interpretation of the statistical analysis and critically revised the manuscript. All authors approved of the final version of the manuscript. *Ethics of human subject participation:* The US NHANES 2001–2006 were reviewed and approved by the NCHS Research Ethics Review Board. Written informed consent was obtained from all participants. The data used for the present study are from anonymized public-use data files available for download on the website of the NCHS.

Supplementary material

To view supplementary material for this article, please visit <http://dx.doi.org/10.1017/S1368980016001762>

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