The Link between Serum 25-Hydroxyvitamin D, Inflammation and Glucose/ Insulin Homeostasis Is Mediated by Adiposity Factors in American Adults

The Link between Serum 25-Hydroxyvitamin D, Inflammation and Glucose/ Insulin Homeostasis Is Mediated By Adiposity Factors in American Adults

Running Title: 25-Hydroxyvitamin D and Adiposity Factors

Mohsen Mazidi *[1], Mario Siervo[2]

1. *Department of Biology and Biological Engineering, Food and Nutrition Science, Chalmers University of Technology, SE-412 96 Gothenburg, Sweden.*
2. *School of Life Sciences, The University of Nottingham Medical School, Queen's Medical Centre, Nottingham, NG7 2UH, UK*

Conflicts of Interest: The authors state that there is no conflict of interest

Financial Disclosure: None

***Corresponding author:** Mohsen Mazidi, PhD, Department of Biology and Biological Engineering, Food and Nutrition Science, Chalmers University of Technology, *SE-412 96* Gothenburg, Sweden. mazidi@chalmers.se, tell: +46729414259.

Abstract

Prior studies have suggested a significant association between 25-hydroxyvitamin D (25(OH)D) concentrations with markers of inflammation and glucose and insulin homeostasis. However, it is unclear whether these associations are confounded or mediated by adiposity. We used an established mediation analysis to investigate the role of adiposity in the relation between serum 25(OH)D with markers of inflammation and glucose and insulin metabolism. We used data from National Health and Nutrition Examination Survey (2005-2010), to evaluate the associations between serum 25(OH)D and markers of insulin resistance or inflammation, and whether these associations are mediated by adiposity factors including body mass index (BMI, marker of body adiposity), waist circumference (WC, marker of central adiposity), anthropometrically predicted visceral adipose tissue (apVAT), and Visceral Adiposity Index (VAI). Analysis of co-variance and conceptual causal mediation analysis were conducted taking into consideration the survey design and sample weights. A total of 16,621 individuals were included; 8607 (48.3%) participants were men and the mean age of the population was 47.1 years. Mean 25(OH)D serum concentration for the overall population was 57.9±0.1 nmol/L with minimal differences between men and women (57.5±0.2 nmol/L and 58.2±0.2 nmol/L, respectively). After adjustment for age, sex, season and race/ethnicity, mean levels of C-reactive protein (CRP), apolipoprotein B (apo-B), fasting blood glucose (FBG), insulin, homeostatic model assessment of IR (HOMA-IR) and β cell function (HOMA-β), haemoglobin A1c (HbA1c), and 2-h glucose were lower for the top quartile of serum 25(OH)D (all $p<0.001$). Body mass index (BMI) was found to have significant mediation effects (to varied extent) on the associations between serum 25(OH)D and CRP, apo-B, fasting glucose, insulin, HOMA-IR, HOMA-B and HbA1c (all $p<0.05$). Both waist circumference and apVAT were also found to partly mediate the associations between serum 25(OH)D with CRP, FBG, HbA1c, triglycerides and HDL-cholesterol (all $P < 0.05$). VAI was found to have mediation effects on CRP only ($p<0.001$). Using a mediation model, our findings suggest that the relationship between serum 25(OH)D, insulin resistance and inflammation, may be in part mediated by adiposity. These findings support the importance of optimizing 25(OH)D status in conditions with abnormal adiposity (i.e., obesity) and treatments for the prevention of cardio-metabolic diseases affecting adipose tissue metabolism (i.e., weight loss).

Keywords: Serum 25(OH)D, Insulin Homeostasis, Glucose Homeostasis , Inflammation, Mediation Analysis

Word count: 356

Introduction:

Vitamin D (25(OH)D) is a hormone associated with maintenance of skeletal integrity (1). Furthermore, low serum 25-hydroxyvitamin D [25(OH)D] concentrations are inversely associated with several diseases with known or putative inflammatory aetiology such as rheumatoid arthritis, metabolic syndrome, type 2 diabetes (T2D), cardiovascular diseases (CVD), and some types of cancer (2-4).

Obesity has been inversely associated with 25(OH)D status in adults (5, 6) McGill et al. observed that serum 25(OH)D concentration was inversely related to body mass index (BMI) and waist circumference (WC) in overweight and obese adults(5). In a clinical-based sample, Hispanic adults with higher BMI, WC, and waist-to-hip ratio had a lower 25(OH)D status(7). As a steroid hormone, 25(OH)D is fat soluble, and thus deficiency of serum 25(OH)D levels associated with obesity is most likely due to the decreased bioavailability of 25(OH)D possibly related to the sequestration of 25(OH)D within the adipose tissue(8).

With regard to T2D, it has been proposed that activated vitamin D (25(OH)D) reduces the risk of T2D by promoting insulin secretion and reducing insulin resistance (IR) (9). Population-based investigations have revealed that 25(OH)D is inversely associated with the homeostatic model assessment of insulin resistance (HOMA-IR), plasma insulin, and fasting blood glucose (FBG) levels (9-12). Furthermore, the Australian Diabetes, Obesity, and Lifestyle Study showed that serum 25(OH)D3 concentrations were positively related with insulin sensitivity at the 5-years follow-up (13). However, in the CoLaus study, the risk of IR in healthy adults was not associated with serum concentrations of 25(OH)D3 and total 25(OH)D3 in Swiss adults (14).

Low serum levels of 25(OH)D have been suggested to cause mild acute phase response resulting in elevated concentrations of C-reactive protein (CRP), several hemostatic factors and different pro-inflammatory cytokines such as interleukin-6 (15-17). Previous observational and intervention studies have suggested that supplemental 25(OH)D may reduce circulating CRP levels as well as other plasma inflammatory cytokines; however, inconsistent results are reported across completed randomized trials (18, 19).

Mediation analysis is a statistical procedure that can be used to evaluate mechanisms underlying the relation between an exposure and outcome by quantifying the extent to which this relation is mediated by a third variable (20-22). The traditional approach to mediation analysis tends to produce a bias when there is an uncontrolled mediator outcome confounding or an interaction between the exposure and the mediator variables. In this study, by use of the "*counterfactual framework"* in "*causal mediation analysis"*, unbiased valid estimates of direct and indirect effects can be obtained (23, 24).

In general, it is unclear to what extent the association between serum 25(OH)D and cardio-metabolic risk, particular inflammation and glucose and insulin metabolism, is confounded or mediated by excess adiposity. We hypothesized that lower serum 25(OH)D levels would be associated with higher concentrations of inflammatory and insulin resistance biomarkers among adults and that these associations would be in part mediated by adiposity markers.

Hence, we evaluated the association between serum 25(OH)D, glucose/ insulin homeostasis and inflammation parameters, and assess the mediation effect of different adiposity indexes on the observed associations by applying on "*causal mediation analysis*". Specifically, mediation analysis could help clarifying the role of adiposity underlying the relation between serum 25(OH)D, markers of glucose/insulin homeostasis or inflammation (23). Furthermore, the degree of mediation may vary between different adiposity indexes (25).

Methods:

Population characteristics

NHANES is a series of ongoing repeated cross-sectional surveys conducted by the US National Center for Health Statistics (NCHS) (26). The NCHS Research Ethics Review Board approved the NHANES protocol and consent was obtained from all participants (26). The current study was based on analysis of data for two 2-year NHANES survey cycles: 2005-2010. Participants in this study were aged 18 years and above. All methods were performed in accordance with the Declaration of Helsinki regarding ethical standards for research involving human subjects (26). NHANES uses a complex, multistage and stratified sampling design to select a sample representative of the civilian and non-institutionalized resident population of the US. The sampling procedure consists of four stages: primary sampling units (mostly counties), segments, households and individuals, respectively. Data collection on demographic, information occurs through in-home administered questionnaires, while anthropometrical, inflammation and biochemistry data are collected by trained personnel using mobile exam centers (MEC). More detailed information is available elsewhere (26, 27). All methods were carried out in accordance with relevant guidelines and regulations approved by the National Centre for Health Statistics (27-30).

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(27).

A blood specimen was drawn from the participant's antecubital vein by a trained phlebotomist. 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)(31). The sensitivity of this assay has been shown to be 1·5 ng/ml and the coefficient of variance (CV) was 8% (31). From 2007–2008 onwards, 25(OH)D was measured using a standardized liquid chromatography–tandem mass spectrometry (LC-MS/MS) method (32) and in October 2015, updated 25(OH)D values for 2005–2006 were released, which had been converted from RIA to LC-MS/MS equivalents using ordinary least squares regression (31). As recommended by NHANES (31), these LC-MS/MS equivalents were used in the present study. The LC-MS/MS system consisted of an autosampler (PAL-CTC Analytics, Switzerland), a turbomolecular pump (1100 series, Agilent Technologies, USA) and a Triple Quadrupole mass spectrometer (PE-SCIEX API-3000, Applied Biosystems Division of MDS Health Group Ltd, Canada). Analyst software version 1.4.2 (AB SCIEX) was used for results acquisition and quantitation.

Glycated haemoglobin (HbA1c) was measured using a Tosoh A1C 2.2 Plus Glycohemoglobin Analyzer. Fasting plasma glucose was measured by a hexokinase method using a Roche/Hitachi 911 Analyzer and Roche Modular P Chemistry Analyzer (Boehringer Mannheim Diagnostics, Indianapolis, Indiana). Other laboratory-test details are available in the NHANES Laboratory/ Medical Technologists Procedures Manual (31). Insulin was measured using an ELISA immunoassay (Merocodia, Uppsala, Sweden). Details on Information on C-reactive protein (CRP) concentrations measurement are available elsewhere (27). HOMA-IR and β-cell function (HOMA-B) were calculated as follows: the HOMA-IR = [glucose (nmol/L) * insulin (mU/mL)/22.5] using fasting values, and HOMA-B=[20 × fasting insulin (μU/ml)]/ [fasting glucose (mmol/l) − 3·5] (33). The anthropometrically predicted visceral adipose tissue (apVAT) was estimated with sex-specific validated equations that included age, BMI, and circumferences of the waist and thigh (34). The equation for men was: 6 *waist circumference – 4.41 * proximal thigh circumference + 1.19 * age – 213.65; and the equation for women was: 2.15 * waist circumference – 3.63 * proximal thigh + 1.46 * age + 6:22 * BMI - 92.713(34). Visceral Adiposity Index (VAI) was calculated using sex-specific formulas: men [WC/39.68 + (1.88 ×BMI)] × (TGs/1.03) × (1.31/HDL); Women: [WC/36.58 + (1.89 × BMI)] × (TGs/0.81) × (1.52/HDL), where both TGs and HDL-cholesterol levels are expressed in mmol/L(35). Smoking status indicates whether the participant is a current smoker or not. Smoking status determined by participants "self-report". Metabolic equivalent of task (MET) is used to measure the intensity level of physical activity and indicated the rate of energy consumption for a specific activity. A MET is

defined as 1 kcal/kg/hour that is roughly equal to the energy cost of being at rest. Physical activity was categorized into three intensity levels upon MET score: light, moderate and vigorous (36).

Statistical analysis

Data were analysed using SPSS® complex sample module version 22.0 (IBM Corp, Armonk, NY). according to the CDC guidelines for analysis of complex NHANES datasets, accounting for the masked variance and using the proposed weighting methodology (37). We used means and standard deviations for continuous measures (analysis of variance) and percentages for categorical variables (chi-square). Kolmogorov-Smirnov test was used to evaluate the normality of data. We computed age, race/ethnicity, season, and sex-adjusted mean of markers of insulin resistance or inflammation across the quartiles of serum 25(OH)D, using analysis of covariance (ANCOVA). All tests were two sided, and $p<0.05$ was the level of statistical significance.

In this study we assessed the total, direct, and indirect effects of serum 25(OH)D on markers of insulin resistance or inflammation with BMI, WC, apVAT and VAI as a mediator by using the counterfactual framework (21, 22, 38-44). In this approach, the total effect can be decomposed into a direct (not mediated by BMI, WC, apVAT, VAI) and indirect effect (mediated by BMI, WC, apVAT, VAI). The SPSS Macro developed by Preacher and Hayes (44-46) was used to evaluate the direct and indirect effects of serum 25(OH)D on markers of insulin resistance or inflammation with BMI, WC, apVAT, VAI as mediators. A product-of-coefficients test was used as it has the potential to detect significant mediation effects in the absence of a significant intervention (38, 39, 44). Utilizing single mediator models, the SPSS macro was used to calculate all regression coefficients which were adjusted for baseline values. In brief, the macro generates output that includes the following steps. Firstly, the total effect (C coefficient) of the intervention on the outcome variable (e.g., markers of insulin resistance or inflammation) is estimated by regression. The action theory test is then used to examine the effect of the serum 25(OH)D on the hypothesized mediators (α coefficient, BMI, WC, apVAT, VAI). The conceptual theory test examines the association between changes in the hypothesized mediators and changes in dependent variables (i.e., markers of insulin resistance or inflammation; β coefficient). The program also estimates the direct (£' coefficient) and indirect (α#β product of coefficients) effects. The proportion of the mediation effect was calculated using the following equation [α#β /(α#β + £)]. Full or complete mediation is present when the total effect (the £-path) is significant, the direct effect (the £'-path) is non-significant and α#β is significant, whereas partly or incomplete mediation is present when the direct effect (the £'-path) is also significant. Inconsistent mediation is present when neither total nor direct effect is significant and α#β is

significant (22, 40-42, 44, 46). All estimates were adjusted for age, sex, race/ethnicity, level of education, smoking and level of physical activity.

Results:

General characteristics

Characteristics of individuals (n=16,621) are summarised in Table 1. Overall 8607 (48.3%) participants were men and 9082 (51.7%) were women. The mean age of the total population was 47.1 years. Non-Hispanic white (69.4%) was the largest racial group and other Hispanic (4.5%) the smallest racial group. Furthermore, 56.1% of the participants were married, while 56.4% had an educational level greater than high school (Table 1).

Mean BMI, WC and apVAT were 28.7±0.05 kg/m^2, 98.2±0.1 cm and 179.2±1.2, respectively. Mean 25(OH)D concentrations for the overall population was 57.9±0.1 nmol/L, with minimal difference between men and women 57.5±0.2 nmol/L and 58.2±0.2 nmol/L, respectively. Totally, 20.2% were current smokers including 24.8% of men and 15.6% of women. The participants engaging in vigorous physical activity had the lowest percentage (5.3%) than those with little/none physical activity (24.1%). We used ANCOVA to calculate the age, sex, season and race-adjusted mean of markers of insulin resistance and inflammation across the quartiles of serum 25(OH)D (Table 2). Levels of serum CRP, serum apolipoprotein (B), fasting blood glucose, plasma Insulin, HOMA_IR, HOMA_B, HbA1c (%) and 2-hour blood glucose decreased with increasing concentrations of serum 25(OH)D (all p<0.001).

Association between serum 25(OH)D, BMI, WC, apVAT, VAI and markers of glucose/ insulin homeostasis and inflammation

There was a significant association between BMI, WC, apVAT and VAI with serum 25(OH)D (BMI= β: -3.10, p<0.001, WC= β: -5.59, p<0.001, apVAT= β: -22.14, p<0.001, VAI= β: -0.02, p=0.197, p<0.001, Table 3).

Furthermore, we examined the association between serum 25(OH) D and markers of glucose/insulin homeostasis or inflammation in multivariate models adjusted for demographics, education, smoking, and physical activity but without adjusting for the potential adiposity mediators (Table 3). The results showed that, except for serum apolipoprotein (β) (p=0.21), the other markers of glucose/ insulin homeostasis or inflammation were inversely and significantly associated with serum 25(OH)D. The 2-hour blood glucose (β: -13.76) concentrations showed the strongest association with serum 25(OH)D (all p<0.001, Table 3) .

In Table 4 we tested the "*conceptual theory*" (Figure 1) by evaluating the multivariate-adjusted associations between adiposity mediators (BMI, WC, apVAT and VAI) and markers of

glucose/insulin homeostasis or inflammation. We found that all potential mediators had significant, positive associations with markers of glucose/insulin homeostasis or inflammation (all p<0.001, Table 4).

Direct and indirect effects of the serum 25(OH)D on markers of insulin resistance and inflammation with BMI, WC and apVAT as mediators.

Table 5 shows the direct, indirect and proportion of mediation effects as well as the Sobel statistics for testing indirect effects.

BMI was found to significantly mediate (to various extent) the associations between serum 25(OH)D and CRP, serum apolipoprotein (B), fasting blood glucose, plasma insulin, HOMA_IR, HOMA_B, HbA1c and 2-hour blood glucose after full adjustment (all P < 0.001). Interestingly, WC, apVAT were also found to have mediating effects for the associations between serum 25(OH)D with the same markers of insulin resistance and inflammation including, CRP, serum apolipoprotein (B), fasting blood glucose, plasma insulin, HOMA_IR, HOMA_B, HbA1c and 2-hour blood glucose (all P < 0.001). VAI had a different pattern of association with a significant mediating impact on CRP (p<0.001).

Results of the direct effect estimates show that serum 25(OH)D are significantly associated with CRP, fasting blood glucose, serum apolipoprotein (B), plasma insulin, HOMA_IR, HbA1c and 2-hour blood glucose even after adjustment for BMI or WC. apVAT showed similar trends to BMI and WC but it did not have any direct effect on CRP. VAI was found to have a direct effect on all the glucose/insulin homeostasis and inflammatory factors but it showed no direct effect on serum apolipoprotein (B) (p=0.40).

Discussion:

Using a large, representative sample of the U.S. Population, we have applied a causal mediation analysis to investigate the associations between serum 25(OH)D with inflammatory and glucose/insulin homeostasis markers and explored the role of different adiposity factors (central and peripheral) as potential mediators of these associations. We found that individuals with higher levels of serum 25(OH)D have a more favourable profile of inflammatory and glucose and insulin metabolic markers; moreover adiposity factors could mediate (to a varied extent) the association between serum 25(OH)D and inflammatory and glucose/insulin metabolic parameters.

Our finding was in line with prior studies(47-49). The co-existence of low levels of 25(OH)D and abnormal glucose metabolism has also been reported in patients with type 2 diabetes mellitus (T2DM) compared to healthy controls (47). Also, a positive association between 25(OH)D and insulin

secretion has been reported in both glucose-intolerant Asian women (48) and healthy Caucasian elderly men (49). However, these studies were performed using an oral glucose tolerance test and findings have been supported by other studies using hyperglycaemic clamp technique (50, 51). A negative association between serum concentrations of 25(OH)D and future risk for hyperglycaemia and insulin resistance was found in a prospective study conducted over a 10-year follow up period(52). However, Orwoll et al. reported in a cross-sectional study a lack of association between serum 25(OH)D concentrations with fasting or post-challenge glucose and insulin secretion(53). A number of randomized controlled trials also reported non-significant effects of 25(OH)D supplementation on the risk of developing diabetes(54) and a recent meta-analysis demonstrated that 25(OH)D supplementation was not associated with improved glucose control, beta cell secretion or insulin sensitivity in patients with type 2 diabetes (55). Our group has recently conducted a meta-analysis of randomized controlled trials showing that 25(OH)D supplementation significantly increased serum IL-6 concentrations but had no significant effect on serum CRP, IL-10, and TNF-α (56). Possible explanations for this contrasting results include differences in study design, subject characteristics, baseline levels of serum 25(OH)D, seasonality, geographical place, study population, confounders and techniques used to assess glucose homeostasis and β-cell function.

Several biological mechanisms have been suggested by which 25(OH)D may contribute to the development of T2DM. 25(OH)D acts on multiple pathways that regulate insulin and glucose homeostasis including 1) insulin synthesis, 2) insulin signaling, 3) systemic and adipose inflammation, and 4) adipose tissue homeostasis. This evidence includes the presence of Vitamin D receptors (VDRs) and the expression of 1α-hydroxylase enzymes in the pancreatic β cell along with the existence of a 25(OH)D response element in the human insulin gene promoter (57, 58). In this regard, a significant reduction in the insulin secretion in VDR mutant mice (VDR null mice) has been observed (59) and the human insulin gene has been shown to be transcriptionally activated by 1,25(OH) D3 (60). Pancreatic β-cells express CYP27B1 [the gene encoding the enzyme 25(OH)D3-1a-hydroxylase], giving these cells the ability to synthesize active 1,25-dihydroxyvitamin D [1,25(OH)2D] from circulating 25(OH)D, which can then act locally in a paracrine fashion within the islets to regulate target genes (61). 1,25(OH)2D has also been shown to regulate insulin receptors in target cells(62). Thus, activated vitamin D (25(OH)D) exhibits the ability to stimulate both insulin synthesis and insulin signaling. Another possible mechanism explaining the involvement of 25(OH)D in the pathogenesis of T2DM is the role of hypovitaminosis D in enhancing insulin resistance in target tissues(63, 64). The presence of the VDR in extra skeletal target sites, such as skeletal muscle, together with the upregulation of insulin receptors (INS-R) after 1,25-hydroxyvitamin D3 treatment appears to support this hypothesis (65).

We have found that there is an inverse association between serum 25(OH)D and CRP levels in analyses adjusted for demographic, education, and lifestyle factors; however previous observational studies that investigated the relationship between 25(OH)D and inflammatory markers, such as CRP, have shown mixed results. In line with our results, Amer et al. found a significant inverse association between 25(OH)D and CRP in a cross-sectional setting in a population of 15,167 adults with a mean age of 46 years from the United States (66). Ngo et al. studied 253 adults (aged 51 to 77 years) with mean CRP level of 3.6 ± 4.0 mg/mL and found that serum 25(OH)D was inversely associated with CRP level (67). This inverse association was also seen in 147 morbidly obese participants whose CRP levels ranged from 1.88 to 4.01 mg/L (68). In contrast, no significant association was found between 25(OH)D and CRP in the Framingham Offspring Study cohort (n=1,381) (69) and Multi-Ethnic Study of Atherosclerosis (70-72). This heterogeneity of findings may be due to baseline CRP level, supplemental dose of 25(OH)D, seasonal change or geographical location and intervention duration.

There are several possible mechanisms through which 25(OH)D may influence serum CRP. VDR are involved in the decreased activation of the pro-inflammatory transcription factor nuclear factor kappa B (NF-κB) (73, 74). This suggests that VDR plays an intrinsic inhibitory role in inflammation (73, 74). One important target of 25(OH)D is NF-κB, which is inhibited by 25(OH)D, and via NF-κβ downstream release of the pro-inflammatory cytokines. It is known that NF-κB activation participates in the endogenous induction of CRP (75). Studies have shown the active form of vitamin D (1,25-dihydroxyvitamin D3 [1,25(OH)2D] inhibits NF-κB activation by upregulating the inhibitor of NF-κB (IκB-α) and reducing IkB-α phosphorylation in lipopolysaccharide-stimulated murine macrophage cells (76, 77). 25(OH)D also inhibits synthesis of IL-6 by monocytes, which is the primary stimulant of CRP production in the liver (19, 78).

We also found an inverse association between 25(OH)D levels and adiposity factors after adjustment for demographic, SES, and lifestyle factors including physical activity. This inverse association between serum 25(OH)D concentration and adiposity could be explained by the increased storage of 25(OH)D in the adipose tissue of obese participants(79). Low 25(OH)D levels are associated with increased adiposity, possibly due to enhanced uptake by adipose tissue, thus decreasing the bioavailability of vitamin D3 from cutaneous and dietary sources because of its deposition in body fat compartments(8). Furthermore, evidence suggests that 1,25hydroxyvitamin D modulates adipogenesis through 25(OH)D receptor-dependent inhibition of critical molecular components of adipogenesis, such as peroxisome proliferator-activated receptor γ and C/EBP α(80). Therefore, depletion of 25(OH)D stores may lead to excess differentiation of pre-adipocytes to

adipocytes. However, it has been reported that serum concentrations and the expression of vitamin D dependent enzymes are not necessarily linked, especially in obese subjects (81).

The main strength of the present study was that we examined for potential mediator effects using a variety of markers of adiposity - BMI, apVAT and VAI - in the associations between serum 25(OH)D and markers of glucose/insulin metabolism and inflammation using causal mediation analyses. The analyses were conducted in a large sample size and nationally representative of population and were adjusted for key confounding variables.

Limitations of our study include the cross-sectional study design and inability to ascertain a causal and temporal relation between a serum 25(OH)D, adiposity, and markers of glucose/ insulin homeostasis and inflammation. Hence, prospective studies with long-term follow-up are warranted to confirm our results. The mediated effect of WC may be affected by BMI, or vice versa, because of the high correlation between WC and BMI. This could be addressed by adding the two mediators (BMI and WC) simultaneously in the model (45). However, this was not feasible in the conventional or causal mediation models with the use of the complex survey design in the present study. To address this point we have applied on another validated adiposity factors which is apVAT and VAI. Lastly, although BMI and WC are commonly used to estimate obesity, these indicators can be inaccurate and lead to bias in measuring adiposity. For example, BMI, an indirect measure of adiposity, is traditionally weaker than direct measures of adiposity such as DEXA, because it does not take age, sex, bone structure, fat distribution or muscle mass into consideration(82). Thus the association between serum 25(OH)D and overall adiposity can be underestimated when BMI is used as an estimate of adiposity. To overcome, we have applied for apVAT which is sensitive to age and sex and also for VAI which, given its calculations and parameters, is not only an index of adiposity but also more representative of both adiposity and lipid profile at the same time. The apVAT equation was derived using data collected in a white, European population and therefore estimates of visceral adiposity may be confounded by ethnicity. However, ethnicity was added to the models to account for the potential confounding effects on the association between vitamin D status, adiposity and markers of metabolic health.

In conclusion, greater serum 25(OH)D is associated with favourable plasma concentrations of inflammatory and glucose/ insulin metabolic biomarkers. Adiposity statistically accounted for a significant proportion of the associations between serum 25(OH) D and glucose and insulin metabolism suggesting a key role of adiposity in modulating the metabolic effects of 25(OH)D. These findings support the importance of optimizing 25(OH)D status in conditions with abnormal adiposity (i.e., obesity) and treatments for the prevention of cardio-metabolic diseases affecting adipose tissue metabolism (i.e., weight loss).

Figure legend:

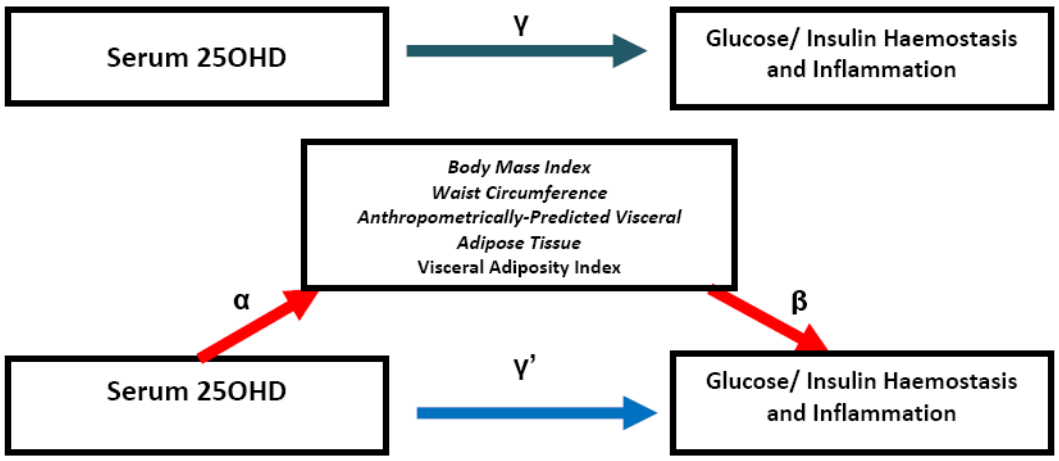

FIGURE 1. Mediation model for the association between serum 25OHD with glucose/ insulin homeostasis and inflammation; with *body mass index (BMI)*, waist circumference (WC) and *anthropometrically-predicted visceral adipose tissue* (apVAT) and Visceral Adiposity Index (VAI) as mediators. Path α represents the regression coefficient for the association of serum 25OHD with BMI, WC, apVAT and VAI. Path β represents the regression coefficient for the association of BMI, WC, apVAT and VAI with glucose/ insulin homeostasis and inflammation. The product of regression coefficients α and β represents the mediated effect (indirect effect) of BMI, WC, apVAT or VAI (α#β). Path £' represents the direct effect of serum 25OHD with glucose/ insulin homeostasis and inflammation, after adjustment for BMI, WC, apVAT or VAI. Path £ represents the simple total effect of serum 25OHD on glucose/ insulin homeostasis and inflammation, without adjustment for BMI, WC, apVAT or VAI.

References:

1. Holick MF. Vitamin D: evolutionary, physiological and health perspectives. Current drug targets. 2011;12(1):4-18.
2. Holick MF. Vitamin D deficiency. New England Journal of Medicine. 2007;357(3):266-81.
3. Grammatiki M, Rapti E, Karras S, Ajjan RA, Kotsa K. Vitamin D and diabetes mellitus: Causal or casual association? Rev Endocr Metab Disord. 2017:1-15.
4. Schnatz PF, Jiang X, Aragaki AK, Nudy M, O'sullivan DM, Williams M, et al. Effects of Calcium, Vitamin D, and Hormone Therapy on Cardiovascular Disease Risk Factors in the Women's Health Initiative. Obstetrics & Gynecology. 2017;129(1):121-9.
5. McGill AT, Stewart JM, Lithander FE, Strik CM, Poppitt SD. Relationships of low serum vitamin D3 with anthropometry and markers of the metabolic syndrome and diabetes in overweight and obesity. Nutr J. 2008;7:4.
6. Yanoff LB, Parikh SJ, Spitalnik A, Denkinger B, Sebring NG, Slaughter P, et al. The prevalence of hypovitaminosis D and secondary hyperparathyroidism in obese Black Americans. Clinical endocrinology. 2006;64(5):523-9.
7. Gonzalez L, Ramos-Trautmann G, Diaz-Luquis GM, Perez CM, Palacios C. Vitamin D status is inversely associated with obesity in a clinic-based sample in Puerto Rico. Nutr Res. 2015;35(4):287-93.
8. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr. 2000;72(3):690-3.
9. Abbasi F, Blasey C, Feldman D, Caulfield MP, Hantash FM, Reaven GM. Low circulating 25-hydroxyvitamin D concentrations are associated with defects in insulin action and insulin secretion in persons with prediabetes. The Journal of nutrition. 2015;145(4):714-9.
10. Cheng S, Massaro JM, Fox CS, Larson MG, Keyes MJ, McCabe EL, et al. Adiposity, cardiometabolic risk, and vitamin D status: the Framingham Heart Study. Diabetes. 2010;59(1):242-8.
11. Wright CS, Weinheimer-Haus EM, Fleet JC, Peacock M, Campbell WW. The apparent relation between plasma 25-hydroxyvitamin D and insulin resistance is largely attributable to central adiposity in overweight and obese adults. The Journal of nutrition. 2015;145(12):2683-9.
12. Kostoglou-Athanassiou I, Athanassiou P, Gkountouvas A, Kaldrymides P. Vitamin D and glycemic control in diabetes mellitus type 2. Therapeutic advances in endocrinology and metabolism. 2013;4(4):122-8.
13. Gagnon C, Lu ZX, Magliano DJ, Dunstan DW, Shaw JE, Zimmet PZ, et al. Serum 25-hydroxyvitamin D, calcium intake, and risk of type 2 diabetes after 5 years. Diabetes care. 2011;34(5):1133-8.
14. Marques-Vidal P, Vollenweider P, Guessous I, Henry H, Boulat O, Waeber G, et al. Serum vitamin D concentrations are not associated with insulin resistance in Swiss adults. The Journal of nutrition. 2015;145(9):2117-22.
15. Canning MO, Grotenhuis K, de Wit H, Ruwhof C, Drexhage HA. 1-alpha, 25-Dihydroxyvitamin D3 (1, 25 (OH)(2) D (3)) hampers the maturation of fully active immature dendritic cells from monocytes. European Journal of Endocrinology. 2001;145(3):351-7.
16. McCarty MF. Secondary hyperparathyroidism promotes the acute phase response–a rationale for supplemental vitamin D in prevention of vascular events in the elderly. Medical hypotheses. 2005;64(5):1022-6.
17. Zhu Y, Mahon BD, Froicu M, Cantorna MT. Calcium and 1α, 25-dihydroxyvitamin D3 target the TNF-α pathway to suppress experimental inflammatory bowel disease. European journal of immunology. 2005;35(1):217-24.
18. Gepner AD, Ramamurthy R, Krueger DC, Korcarz CE, Binkley N, Stein JH. A prospective randomized controlled trial of the effects of vitamin D supplementation on cardiovascular disease risk. PLoS One. 2012;7(5):e36617.

19. Zhang Y, Leung DY, Richers BN, Liu Y, Remigio LK, Riches DW, et al. Vitamin D inhibits monocyte/macrophage proinflammatory cytokine production by targeting MAPK phosphatase-1. The Journal of Immunology. 2012;188(5):2127-35.
20. Baron RM, Kenny DA. The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. Journal of personality and social psychology. 1986;51(6):1173-82.
21. Fairchild AJ, McDaniel HL. Best (but oft-forgotten) practices: mediation analysis. Am J Clin Nutr. 2017.
22. MacKinnon DP, Fairchild AJ, Fritz MS. Mediation analysis. Annual review of psychology. 2007;58:593-614.
23. Richiardi L, Bellocco R, Zugna D. Mediation analysis in epidemiology: methods, interpretation and bias. Int J Epidemiol. 2013;42(5):1511-9.
24. Robins JM, Greenland S. Identifiability and exchangeability for direct and indirect effects. Epidemiology. 1992;3(2):143-55.
25. Wang Y, Rimm EB, Stampfer MJ, Willett WC, Hu FB. Comparison of abdominal adiposity and overall obesity in predicting risk of type 2 diabetes among men. The American journal of clinical nutrition. 2005;81(3):555-63.
26. Videla LA, Rodrigo R, Orellana M, Fernandez V, Tapia G, Quinones L, et al. Oxidative stress-related parameters in the liver of non-alcoholic fatty liver disease patients. Clinical science (London, England : 1979). 2004;106(3):261-8.
27. http://www.cdc.gov/NCHS/data/nhanes/nhanes_09_10/CRP_F_met.pdf. [accessed 19.08.13].
28. Needham BL, Adler N, Gregorich S, Rehkopf D, Lin J, Blackburn EH, et al. Socioeconomic status, health behavior, and leukocyte telomere length in the National Health and Nutrition Examination Survey, 1999-2002. Social science & medicine (1982). 2013;85:1-8.
29. Tighe P, Duthie G, Vaughan N, Brittenden J, Simpson WG, Duthie S, et al. Effect of increased consumption of whole-grain foods on blood pressure and other cardiovascular risk markers in healthy middle-aged persons: a randomized controlled trial. The American journal of clinical nutrition. 2010;92(4):733-40.
30. Mohsen Mazidi EDM, Maciej Banach. The association of telomere length and serum 25-hydroxyvitamin D levels in US adults: the National Health and Nutrition Examination Survey. Arch Med Sci. 2017;13(1):61–5.
31. National Center for Health Statistics CfDCaPNHaNESA, http://www.cdc.gov/nchs/nhanes.htm f.
32. Tai SS, Bedner M, Phinney KW. Development of a candidate reference measurement procedure for the determination of 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 in human serum using isotope-dilution liquid chromatography-tandem mass spectrometry. Analytical chemistry. 2010;82(5):1942-8.
33. Musso G, Gambino R, Bo S, Uberti B, Biroli G, Pagano G, et al. Should nonalcoholic fatty liver disease be included in the definition of metabolic syndrome? A cross-sectional comparison with Adult Treatment Panel III criteria in nonobese nondiabetic subjects. Diabetes Care. 2008;31(3):562-8.
34. Samouda H, Dutour A, Chaumoitre K, Panuel M, Dutour O, Dadoun F. VAT=TAAT-SAAT: innovative anthropometric model to predict visceral adipose tissue without resort to CT-Scan or DXA. Obesity (Silver Spring). 2013;21(1):E41-50.
35. Amato MC, Giordano C, Galia M, Criscimanna A, Vitabile S, Midiri M, et al. Visceral Adiposity Index: a reliable indicator of visceral fat function associated with cardiometabolic risk. Diabetes Care. 2010;33(4):920-2.
36. Liu Y. The relationship between lifestyle and self-reported oral health among American adults. International dental journal. 2014;64(1):46-51.
37. Statistics. NCfH. ANALYTIC AND REPORTING GUIDELINES http://www.cdc.gov/nchs/data/nhanes/ nhanes 03 04/nhanes analytic guidelines dec 2005.pdf. .

38. VanderWeele TJ. Mediation and mechanism. Eur J Epidemiol. 2009;24(5):217-24.
39. VanderWeele TJ. A three-way decomposition of a total effect into direct, indirect, and interactive effects. Epidemiology. 2013;24(2):224-32.
40. Lockwood CM, DeFrancesco CA, Elliot DL, Beresford SA, Toobert DJ. Mediation analyses: applications in nutrition research and reading the literature. Journal of the American Dietetic Association. 2010;110(5):753-62.
41. Mackinnon DP, Fairchild AJ. Current Directions in Mediation Analysis. Current directions in psychological science. 2009;18(1):16.
42. Preacher KJ. Advances in mediation analysis: a survey and synthesis of new developments. Annual review of psychology. 2015;66:825-52.
43. Chao A, Grilo CM, White MA, Sinha R. Food cravings mediate the relationship between chronic stress and body mass index. Journal of health psychology. 2015;20(6):721-9.
44. Park YM, Zhang J, Steck SE, Fung TT, Hazlett LJ, Han K, et al. Obesity Mediates the Association between Mediterranean Diet Consumption and Insulin Resistance and Inflammation in US Adults. The Journal of nutrition. 2017;147(4):563-71.
45. Preacher KJ, Hayes AF. Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. Behavior research methods. 2008;40(3):879-91.
46. Mohsen Mazidi, NK, DPM, MB. The link between insulin resistance parameters and serum uric acid is mediated by adiposity. Atherosclerosis. 2018.
47. Scragg R, Holdaway I, Singh V, Metcalf P, Baker J, Dryson E. Serum 25-hydroxyvitamin D3 levels decreased in impaired glucose tolerance and diabetes mellitus. Diabetes research and clinical practice. 1995;27(3):181-8.
48. Boucher B, Mannan N, Noonan K, Hales C, Evans S. Glucose intolerance and impairment of insulin secretion in relation to vitamin D deficiency in east London Asians. Diabetologia. 1995;38(10):1239-45.
49. Baynes K, Boucher B, Feskens E, Kromhout D. Vitamin D, glucose tolerance and insulinaemia in elderly men. Diabetologia. 1997;40(3):344-7.
50. Chiu KC, Chu A, Go VLW, Saad MF. Hypovitaminosis D is associated with insulin resistance and β cell dysfunction. The American journal of clinical nutrition. 2004;79(5):820-5.
51. Kamycheva E, Jorde R, Figenschau Y, Haug E. Insulin sensitivity in subjects with secondary hyperparathyroidism and the effect of a low serum 25-hydroxyvitamin D level on insulin sensitivity. Journal of endocrinological investigation. 2007;30(2):126-32.
52. Forouhi NG, Luan Ja, Cooper A, Boucher BJ, Wareham NJ. Baseline serum 25-hydroxy vitamin D is predictive of future glycemic status and insulin resistance. Diabetes. 2008;57(10):2619-25.
53. Orwoll E, Riddle M, Prince M. Effects of vitamin D on insulin and glucagon secretion in non-insulin-dependent diabetes mellitus. The American journal of clinical nutrition. 1994;59(5):1083-7.
54. Jorde R, Sollid ST, Svartberg J, Schirmer H, Joakimsen RM, Njolstad I, et al. Vitamin D 20,000 IU per Week for Five Years Does Not Prevent Progression From Prediabetes to Diabetes. J Clin Endocrinol Metab. 2016;101(4):1647-55.
55. Nigil Haroon N, Anton A, John J, Mittal M. Effect of vitamin D supplementation on glycemic control in patients with type 2 diabetes: a systematic review of interventional studies. Journal of diabetes and metabolic disorders. 2015;14:3.
56. Mohsen Mazidi PRaHV. The effect of vitamin D supplementation on C -reactive protein; fresults from a systematic review and meta-analysis of randomized controlled trials. BMC Nutrition. 2018.
57. Bland R, Markovic D, Hills CE, Hughes SV, Chan SL, Squires PE, et al. Expression of 25-hydroxyvitamin D 3-1α-hydroxylase in pancreatic islets. The Journal of steroid biochemistry and molecular biology. 2004;89:121-5.
58. Johnson JA, Grande JP, Roche PC, Kumar R. Immunohistochemical localization of the 1, 25 (OH) 2D3 receptor and calbindin D28k in human and rat pancreas. American Journal of Physiology-Endocrinology and Metabolism. 1994;267(3):E356-E60.

59. Zeitz U, Weber K, Soegiarto DW, Wolf E, Balling R, Erben RG. Impaired insulin secretory capacity in mice lacking a functional vitamin D receptor. The FASEB journal. 2003;17(3):509-11.
60. Maestro B, Molero S, Bajo S, Davila N, Calle C. Transcriptional activation of the human insulin receptor gene by 1, 25-dihydroxyvitamin D3. Cell biochemistry and function. 2002;20(3):227-32.
61. Bland R, Markovic D, Hills CE, Hughes SV, Chan SL, Squires PE, et al. Expression of 25-hydroxyvitamin D3-1alpha-hydroxylase in pancreatic islets. J Steroid Biochem Mol Biol. 2004;89-90(1-5):121-5.
62. Calle C, Maestro B, Garcia-Arencibia M. Genomic actions of 1,25-dihydroxyvitamin D3 on insulin receptor gene expression, insulin receptor number and insulin activity in the kidney, liver and adipose tissue of streptozotocin-induced diabetic rats. BMC molecular biology. 2008;9:65.
63. Kawashima H, Castro A. Effect of 1 alpha-hydroxyvitamin D3 on the glucose and calcium metabolism in genetic obese mice. Research communications in chemical pathology and pharmacology. 1981;33(1):155.
64. Zhou QG, Hou FF, Guo ZJ, Liang M, Wang GB, Zhang X. 1, 25-Dihydroxyvitamin D improved the free fatty-acid-induced insulin resistance in cultured C2C12 cells. Diabetes/metabolism research and reviews. 2008;24(6):459-64.
65. MAESTRO B, CAMPIÓN J, DÁVILA N, CALLE C. Stimulation by 1, 25-dihydroxyvitamin D3 of insulin receptor expression and insulin responsiveness for glucose transport in U-937 human promonocytic cells. Endocrine journal. 2000;47(4):383-91.
66. Amer M, Qayyum R. Relation between serum 25-hydroxyvitamin D and C-reactive protein in asymptomatic adults (from the continuous National Health and Nutrition Examination Survey 2001 to 2006). The American journal of cardiology. 2012;109(2):226-30.
67. Ngo DT, Sverdlov AL, McNeil JJ, Horowitz JD. Does vitamin D modulate asymmetric dimethylarginine and C-reactive protein concentrations? The American journal of medicine. 2010;123(4):335-41.
68. Bellia A, Garcovich C, D'Adamo M, Lombardo M, Tesauro M, Donadel G, et al. Serum 25-hydroxyvitamin D levels are inversely associated with systemic inflammation in severe obese subjects. Internal and emergency medicine. 2013;8(1):33-40.
69. Shea MK, Booth SL, Massaro JM, Jacques PF, D'Agostino RB, Dawson-Hughes B, et al. Vitamin K and vitamin D status: associations with inflammatory markers in the Framingham Offspring Study. American journal of epidemiology. 2008;167(3):313-20.
70. Blondon M, Cushman M, Jenny N, Michos ED, Smith NL, Kestenbaum B, et al. Associations of Serum 25-Hydroxyvitamin D With Hemostatic and Inflammatory Biomarkers in the Multi-Ethnic Study of Atherosclerosis. J Clin Endocrinol Metab. 2016;101(6):2348-57.
71. Shea M, Booth SL, Massaro JM, Jacques PF, D'agostino RB, Dawson-Hughes B, et al. Vitamin K and vitamin D status: associations with inflammatory markers in the Framingham Offspring Study. American journal of epidemiology. 2008;167(3):313-20.
72. Michos ED, Streeten EA, Ryan KA, Rampersaud E, Peyser PA, Bielak LF, et al. Serum 25-hydroxyvitamin d levels are not associated with subclinical vascular disease or C-reactive protein in the old order amish. Calcified tissue international. 2009;84(3):195-202.
73. Wu S, Liao AP, Xia Y, Li YC, Li J-D, Sartor RB, et al. Vitamin D receptor negatively regulates bacterial-stimulated NF-κB activity in intestine. The American journal of pathology. 2010;177(2):686-97.
74. Chen Y, Zhang J, Ge X, Du J, Deb DK, Li YC. Vitamin D receptor inhibits nuclear factor κB activation by interacting with IκB kinase β protein. Journal of Biological Chemistry. 2013;288(27):19450-8.
75. Agrawal A, Cha-Molstad H, Samols D, Kushner I. Overexpressed nuclear factor-κB can participate in endogenous C-reactive protein induction, and enhances the effects of C/EBPβ and signal transducer and activator of transcription-3. Immunology. 2003;108(4):539-47.
76. Cohen-Lahav M, Shany S, Tobvin D, Chaimovitz C, Douvdevani A. Vitamin D decreases NFκB activity by increasing IκBα levels. Nephrology Dialysis Transplantation. 2006;21(4):889-97.

77. Song Y, Hong J, Liu D, Lin Q, Lai G. 1, 25-Dihydroxyvitamin D3 Inhibits Nuclear Factor Kappa B Activation by Stabilizing Inhibitor IκBα via mRNA Stability and Reduced Phosphorylation in Passively Sensitized Human Airway Smooth Muscle Cells. Scandinavian journal of immunology. 2013;77(2):109-16.
78. Dickie LJ, Church LD, Coulthard LR, Mathews RJ, Emery P, McDermott MF. Vitamin D3 down-regulates intracellular Toll-like receptor 9 expression and Toll-like receptor 9-induced IL-6 production in human monocytes. Rheumatology. 2010;49(8):1466-71.
79. McCarty MF, Thomas CA. PTH excess may promote weight gain by impeding catecholamine-induced lipolysis-implications for the impact of calcium, vitamin D, and alcohol on body weight. Medical hypotheses. 2003;61(5-6):535-42.
80. Wood RJ. Vitamin D and adipogenesis: new molecular insights. Nutrition reviews. 2008;66(1):40-6.
81. Carlberg C. The physiology of vitamin D-far more than calcium and bone. Frontiers in physiology. 2014;5:335.
82. Rothman KJ. BMI-related errors in the measurement of obesity. International journal of obesity (2005). 2008;32 Suppl 3:S56-9.

Table 1. Demographic and clinical characteristics of participants (N=16,621)			
Characteristics		**Overall**	**P-value**
Sex	**Men (%)**	48.3%	<0.001
	Women (%)	51.7%	
Age (Years)		47.1±1.1	
Race/Ethnicity	**White (non-Hispanic) (%)**	69.4%	<0.001
	Non-Hispanic Black (%)	11.5%	
	Mexican-American (%)	8.4%	
	Other Hispanic (%)	4.5%	
	Other (%)	6.2%	
Marital Status	**Married (%)**	56.1%	<0.001
	Widowed (%)	61.1%	
	Divorced (%)	10.1%	
	Never married (%)	17.9%	
Education Status	**Less than high school (%)**	19.1%	<0.001
	Completed high school (%)	24.4%	
	More than high school (%)	56.4%	
Body mass index (kg/m2)		28.7±0.1	
Waist circumference (cm)		98.2±0.1	
Anthropometrically predicted visceral adipose tissue		179.3±1.2	
Serum CRP (mg/dl)		0.432±0.001	
Serum Apolipoprotein (B) (mg/dL)		94.2±0.2	
Fasting blood glucose (mg/dl)		100.28±0.02	
Plasma Insulin (uU/mL)		2.318±0.008	
HOMA_IR		0.898±0.008	
HOMA_B		4.785±0.002	
HbA1c (%)		5.662±0.004	
2-hour blood glucose(mg/dL)		120.2±1.3	
Visceral Adiposity Index		2.53±0.02	
Lipid Accumulation Product		68.6±0.6	
Value expressed as a mean and SEM or percent. Abbreviation= Abbreviations: HOMA_IR, Homeostatic model assessment of insulin resistance ; HOMA_B, Homeostatic model assessment of β-cell function , CRP; C-reactive protein, ; HbA1c: Glycated haemoglobin, apVAT: anthropometrically predicted visceral adipose tissue.			

Table 2. Age-, sex-,season and race-adjusted mean levels of markers of insulin resistance and inflammation across quartiles of serum 25(OH)D

Variables	Quartiles of Serum 25OH				p – value [a]
	1	2	3	4	
N	4153	4158	4166	4144	
Median(25th–75th percentiles), 25(OH)D, nmol/L	30.6 (24.8-35.2)	47.4 (43.7-51.2)	63.2 (59.8-67.1)	85.2 (75.4-97.6)	
Serum CRP (mg/dl)	0.50±0.01	0.40±0.02	0.37±0.02	0.30±0.01	<0.001
Serum Apolipoprotein (B) (mg/dL)	96.6±0.9	95.4±1.0	94.8±0.8	92.1±0.9	<0.001
Fasting blood glucose (mg/dl)	104.2±0.8	102.2±0.6	100.6±0.3	97.7±0.1	<0.001
Plasma Insulin (uU/mL)	2.48±0.01	2.38±0.02	2.12±0.03	1.86±0.04	<0.001
HOMA_IR	1.07±0.02	1.01±0.01	0.83±0.01	0.65±0.05	<0.001
HOMA_B	4.83±0.03	4.73±0.02	4.62±0.01	4.35±0.03	<0.001
HbA1c (%)	5.77±0.02	5.70±0.01	5.63±0.01	5.49±0.02	<0.001
2-hour blood glucose(mg/dL)	128.5±1.3	120.8±2.3	116.8±1.9	112.1±1.0	<0.001

Abbreviations: *HOMA_IR, Homeostatic model assessment of insulin resistance ; HOMA_B, Homeostatic model assessment of β-cell function , CRP; C-reactive protein; HbA1c Glycated haemoglobin.* Values expressed as estimated mean and standard error.

. [a] p-values for linear trend across quartiles of hs-CRP. Variables were compared across quartiles of CRP using analysis of co-variance (ANCOVA) test. Value expressed as mean and standard error mean.

Table 3. Estimates of regression coefficients (95% CIs) for the association between serum 25(OH)D, BMI, WC, apVAT, VAI (action theory), and markers of insulin resistance and inflammation (total effect) among US adults in NHANES

Mediator	Estimate	95% CI	P
BMI	-3.10	(-3.33 to -2.85)	<0.001
WC	-5.59	(-6.14 to -5.65)	<0.001
apVAT	-22.14	(-26.17 to -17.62)	<0.001
VAI	-0.025	(-0.06- 0.01)	0.191
LAP	-0.10	(-0.15 – 0.05)	<0.001
Outcome			
Serum CRP (mg/dl)	-0.31	(-0.35 to -0.26)	<0.001
Fasting blood glucose (mg/dl)	-8.89	(-10.26 to -7.65)	<0.001
Plasma Insulin (uU/mL)	-0.27	(-0.31 to -0.23)	<0.001
HOMA_IR	-0.34	(-0.38 to -0.30)	<0.001
HOMA_B	-0.09	(-0.13 to -0.54)	<0.001
HbA1c (%)	-0.30	(-0.33 to -0.26)	<0.001
2-hour blood glucose(mg/dL)	-13.76	(-16.10 to -10.32)	<0.001
Serum apolipoprotein (B) (mg/dL)	-0.83	(-0.47 to 2.15)	0.212

Abbreviations: *BMI: body mass index, WC, waist circumference, apVAT, Anthropometrically-predicted visceral adipose tissue , HOMA_IR, Homeostatic model assessment of insulin resistance ; HOMA_B, Homeostatic model assessment of β-cell function HOMA_S; Homeostatic model assessment of insulin sensitivity, CRP; C-reactive protein, HbA1c Glycated haemoglobin. All estimates were adjusted for age, sex, race/ethnicity, educational, smoking and level of physical activity. Estimates for mediator and outcomes correspond to the regression coefficients α and £, respectively, in Figure 1.*

Table 4. **Estimates of regression coefficients (95% CIs) for the association between BMI, WC, apVAT, VAI with markers of insulin resistance and inflammation (conceptual theory) among US adults**

Outcomes	BMI		WC		apVAT		VAI		LAP	
	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
Serum CRP (mg/dl)	0.082	0.080-0.085	0.037	0.036-0.038	0.0093	0.0089-0.0096	0.402	0.38-0.42	0.561	0.52-0.56
Serum Apolipoprotein (B) (mg/dL)	0.552	0.423-0.623	0.232	0.212-0.332	0.092	0.074-0.106	14.256	13.9-15.2	13.623	12.5-14.6
Fasting blood glucose (mg/dl)	0.722	0.625-0.832	0.332	0.301-0.492	0.073	0.06-0.09	8.232	7.6-9.1	7.423	6.352-8.532
Plasma Insulin(µU/mL)	0.056	0.054-0.059	0.025	0.024-0.026	0.006	0.005-0.007	0.402	0.38-0.42	0.442	0.4312-0.4662
HOMA_IR	0.063	0.061-0.065	0.029	0.028-0.030	0.007	0.006-0.008	0.471	0.44-0.49	0.512	0.491-0.521
HOMA_B	0.036	0.034-0.039	0.017	0.016-0.018	0.004	0.003-0.005	0.231	0.21-0.25	0.282	0.261-0.303
HbA1c (%)	0.025	0.023-0.027	0.011	0.010-0.012	0.002	0.001-0.003	0.212	0.19-0.23	0.203	0.195-0.228
2-hour blood glucose (mg/dL)	1.422	1.233-1.612	0.673	0.5932-0.751	0.192	0.164-0.233	17.45	15.62-18.32	15.26	14.32-16.92

Abbreviations: *BMI: body mass index, WC, waist circumference, apVAT, Anthropometrically-predicted visceral adipose tissue , HOMA_IR, Homeostatic model assessment of insulin resistance; HOMA_B, Homeostatic model assessment of β-cell function HOMA_S; Homeostatic model assessment of insulin sensitivity, , CRP; C-reactive protein. All estimates were adjusted for age, sex, race/ethnicity, educational, smoking and level of physical activity.* Regression coefficient β is shown in Figure 1.

Table5. Direct and indirect effects of serum 25OHD on markers of insulin resistance and inflammation with BMI, WC, apVAT, VAI and LAP as mediators among US adults

Mediator and outcomes	Direct effect (£′)		Indirect effect (α#β)3		Proportion of mediation, %
	Estimate	P	Estimate	Sobel test statistic	
BMI					
Serum CRP (mg/dl)	**-0.054**	**0.012**	**-0.25**	**<0.001**	**82.1**
Serum Apolipoprotein (B) (mg/dL)	**2.54**	**<0.001**	**-1.73**	**<0.001**	**21.1%**
Fasting blood glucose (mg/dl)	**-6.47**	**<0.001**	**-2.41**	**<0.001**	**27.2%**
Plasma Insulin (uU/mL)	**-0.092**	**<0.001**	**-0.17**	**<0.001**	**66.9%**
HOMA_IR	**-0.14**	**<0.001**	**-0.20**	**<0.001**	**58.1%**
HOMA_B	**-0.023**	**0.211**	**-0.11**	**<0.001**	**12.3%**
HbA1c (%)	**-0.22**	**<0.001**	**-0.078**	**<0.001**	**26.1%**
2-hour blood glucose(mg/dL)	**-9.36**	**<0.001**	**-4.32**	**<0.001**	**31.1%**
WC					
Serum CRP (mg/dl)	**0.093**	**<0.001**	**0.20**	**<0.001**	**69.1%**
Serum Apolipoprotein (B) (mg/dL)	**2.34**	**<0.001**	**1.64**	**<0.001**	**22.3%**
Fasting blood glucose (mg/dl)	**-6.81**	**<0.001**	**-2.05**	**<0.001**	**23.1%**
Plasma Insulin (uU/mL)	**-0.13**	**<0.001**	**-0.14**	**<0.001**	**52.1**

HOMA_IR	**-0.18**	**<0.001**	**-0.16**	**<0.001**	**46.5%**
HOMA_B	**-0.003**	**0.84**	**-0.09**	**<0.001**	**4.2%**
HbA1c (%)	**-0.24**	**<0.001**	**-0.062**	**<0.001**	**20.4%**
2-hour blood glucose(mg/dL)	**-9.82**	**<0.001**	**-3.80**	**<0.001**	**27.4%**
apVAT					
Serum CRP (mg/dl)	**-0.009**	**0.838**	**-0.20**	**<0.001**	**95.1%**
Serum Apolipoprotein (B) (mg/dL)	**5.23**	**<0.001**	**-2.19**	**<0.001**	**72.1%**
Fasting blood glucose (mg/dl)	**-9.10**	**<0.001**	**-1.77**	**<0.001**	**16.3%**
Plasma Insulin (uU/mL)	**-0.22**	**<0.001**	**-0.15**	**<0.001**	**40.6%**
HOMA_IR	**-0.30**	**<0.001**	**-0.17**	**<0.001**	**36.1%**
HOMA_B	**0.028**	**0.48**	**-0.11**	**<0.001**	**33.1%**
HbA1c (%)	**-0.36**	**<0.001**	**-0.051**	**<0.001**	**13.1%**
2-hour blood glucose(mg/dL)	**-12.25**	**<0.001**	**-5.45**	**<0.001**	**32.4%**
VAI					
Serum Hs-CRP (mg/dl)	**-0.30**	**<0.001**	**-0.001**	**<0.001**	**6.2%**
Serum Apolipoprotein (B) (mg/dL)	**1.23**	**0.402**	**-0.37**	**0.201**	**43.1%**
Fasting blood glucose (mg/dl)	**-8.86**	**<0.001**	**-0.036**	**0.772**	**4.1%**

Plasma Insulin (uU/mL)	**-0.26**	**<0.001**	**-0.010**	**0.182**	**3.7%**
HOMA_IR	**-0.33**	**<0.001**	**-0.012**	**0.193**	**3.4%**
HOMA_B	**-0.085**	**<0.001**	**-0.006**	**0.185**	**6.2%**
HbA1c (%)	**-0.30**	**<0.001**	**-0.009**	**0.774**	**2.9%**
2-hour blood glucose (mg/dL)	**-12.82**	**<0.001**	**-0.67**	**0.092**	**4.9%**
LAP					
Serum CRP (mg/dl)	**-0.25**	**<0.001**	**-0.050**	**<0.001**	**16.2%**
Serum Apolipoprotein (B) (mg/dL)	**2.18**	**<0.001**	**-1.34**	**<0.001**	**16.2%**
Fasting blood glucose (mg/dl)	**-8.17**	**<0.001**	**-0.72**	**<0.001**	**8.2%**
Plasma Insulin (uU/mL)	**-0.23**	**<0.001**	**-0.045**	**<0.001**	**18.6%**
HOMA_IR	**-0.29**	**<0.001**	**-0.052**	**<0.001**	**14.8%**
HOMA_B	**-0.063**	**<0.001**	**-0.28**	**<0.001**	**31.2%**
HbA1c (%)	**-0.28**	**<0.001**	**-0.019**	**<0.001**	**6.3%**
2-hour blood glucose (mg/dL)	**-11.53**	**<0.001**	**-2.36**	**<0.001**	**15.6%**

Abbreviations: *BMI: body mass index, WC, waist circumference, apVAT, Anthropometrically-predicted visceral adipose tissue , HOMA_IR, Homeostatic model assessment of insulin resistance ; HOMA_B, Homeostatic model assessment of β-cell function HOMA_S; Homeostatic model assessment of insulin sensitivity, CRP; C-reactive protein. All estimates were adjusted for age, sex, race/ethnicity, educational, smoking and level of physical activity. Regression coefficients α, β, and £ are shown in Figure 1.*