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Association between body weight and composition and plasma 25-hydroxyvitamin D level in the Diabetes Prevention Program

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Abstract

Objective—We examined associations between body weight and plasma 25-hydroxyvitamin D concentration (25OHD) in pre-diabetes and sought to estimate the impact of adiposity on these associations.

Methods—The study was conducted in the placebo (n=1,082) and intensive lifestyle (n=1,079) groups of the Diabetes Prevention Program (DPP), a multicenter trial to prevent type 2 diabetes in adults with pre-diabetes. Weight and 25OHD were measured at baseline, month 6, years 1 and 2. In a subset (n=584), visceral [VAT] and subcutaneous [SAT] adiposity were assessed by computed tomography at baseline and year 1.

Results—In cross-sectional analyses, baseline body weight, total fat, VAT, and SAT were inversely associated with plasma 25OHD concentration after multivariable adjustment. VAT accounted for 40% [95% CI 11, 69] of the association of body weight with plasma 25OHD

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concentration. There was no significant contribution by total fat or SAT. Two-year changes in plasma 25OHD concentration varied inversely with changes in body weight ($p<0.0001$). One-year changes in total fat, VAT, or SAT were not significant mediators of the association between change in plasma 25OHD concentration and body weight.

Conclusion—Our study found an inverse association between body weight and plasma 25OHD concentration at baseline and over a 2-year period in adults with pre-diabetes. These findings in the DPP, a weight loss intervention study, raise the possibility that weight loss increases plasma 25OHD concentration. Whether adiposity mediates this association remains inconclusive.

Keywords

body composition; diabetes prevention program; body weight; obesity; vitamin D; adipose tissue

Introduction

Over recent decades, obesity has risen to epidemic proportions while vitamin D status, assessed by blood 25-hydroxyvitamin D concentration (25OHD), has declined among US adults [1]. Cross-sectional studies consistently report an inverse association between body weight or body mass index (BMI) and circulating 25OHD concentration [2-6]. However, longitudinal data on this relationship are inconclusive and the causal direction of this relationship lacks consensus [7-14]. Some have suggested that weight influences plasma 25OHD concentration rather than the reverse [6, 9, 15-17]. There are several proposed mechanisms by which higher body weight could lead to lower plasma 25OHD concentration, including that obese individuals have 1) decreased vitamin D biosynthesis due to low sun exposure secondary to limited outdoor activity or concealing clothing [18]; 2) decreased circulating vitamin D levels due to sequestration of the vitamin in adipose tissue [16]; 3) greater tissue volume within which vitamin D is evenly distributed – a simple volumetric dilutional model [19]; or 4) increased vitamin D metabolism due to activity of 24-hydroxylase in adipose tissue [20]. Considering these potential mechanisms, reduction in body weight may influence plasma 25OHD concentration by way of changes in adipose tissue causing de-sequestration of vitamin D, reduced body volume and/or decreased vitamin D catabolism.

Using a large multi-racial cohort of U.S. adults with pre-diabetes who participated in the Diabetes Prevention Program (DPP), a multicenter randomized controlled trial comparing different treatment modalities to prevent incident type 2 diabetes [21], we examined the association between measures of body weight and plasma 25OHD concentration at baseline and over a 2-year period. We also sought to estimate the impact of baseline and 1-year changes in body fat (total, visceral, and subcutaneous) on this relationship.

Methods

Study participants/cohort

The DPP, a randomized controlled clinical trial conducted from 1996 to 2001 at 27 sites in the U.S., compared the effects of intensive lifestyle intervention, metformin, and placebo on the development of type 2 diabetes in adults with pre-diabetes. The eligibility criteria,

design, and methods of the DPP have been described in detail elsewhere [21]. Briefly, inclusion criteria were age ≥ 25 years, BMI ≥ 24 kg/m² (≥ 22 kg/m² in Asian Americans), fasting plasma glucose 95 to 125 mg/dL (multiply by 0.0555 for mmol/L) and 2-hour plasma glucose 140 to 199 mg/dL after a 75-gram oral glucose tolerance test. The primary exclusion criterion was use of any medication known to alter glucose tolerance. The intensive lifestyle intervention was unmasked. The Institutional Review Board at each site approved the protocol and all participants gave written informed consent. The Tufts University Institutional Review Board approved the current study.

The current observational study was conducted among participants randomized to two of the three arms, placebo (standard lifestyle, n=1,082) and intensive lifestyle (n=1,079). The metformin arm was excluded to minimize the cost associated with measurement of plasma 25OHD concentration. One hundred and sixty-one participants were also excluded because of lack of consent for ancillary studies (n=120) or an available plasma 25OHD measurement (n=41). Additionally, two participants with plasma 25OHD concentrations below the detectable limit of the assay (0.14 and 0.10 ng/mL) were excluded from all analyses. After exclusions, 1,998 participants were included in the unadjusted analyses for body weight. Participants with complete data for all covariates were included in the multivariate analyses with weight (n=1,958; **Figure 1**).

Measurement of body weight and adiposity

Body weight was measured using a standard calibrated scale and standing height was measured with a standard stadiometer at the baseline, month 6, and years 1 and 2 follow-up visits. BMI was calculated as kg/m². Waist circumference was assessed in the standing position midway between the highest point of the iliac crest and the lowest point of the costal margin in the mid-axillary line. All anthropometric measures reflected the average of 2 measurements. In a subset of the cohort (18 out of 27 DPP sites), visceral (VAT) and subcutaneous (SAT) adipose tissue were measured at L2 to L3 and L4 to L5 lumbar disc spaces by computed tomography [22] at baseline (n=584 for unadjusted and 576 for adjusted analyses) and year 1 (n=400). As results at the two disc spaces did not differ significantly, we present the results at L4 to L5 disc space in this analysis. The reading center calculated the total adipose area on each scan, delineated VAT from SAT by circumscribing the transversalis fascia, and calculated SAT by subtracting the VAT from the total cross-sectional area for fat [22]. Total fat was calculated by adding the VAT and SAT measurements.

Measurement of plasma 25OHD concentration

Plasma 25OHD concentrations at baseline, month 6, and years 1 and 2 follow-up visits were measured in samples stored at -70°C since collection. Samples were analyzed in the fewest batches possible, and all samples from a single participant analyzed in the same run. Stability of vitamin D metabolites during transport and long-term freezing has been documented previously [23, 24]. Plasma 25OHD concentration was measured at the Metabolic Laboratory at Tufts Medical Center by liquid chromatography, tandem mass spectrometry (LC/MS/MS) (Waters Acquity UPLC with TQD triple quadrupole mass spectrometer), certified through the National Institute of Standards and Technology (NIST)

vitamin D quality assurance program [25]. In the most recent testing, correlation with the NIST external standard for total 25OHD concentration was 0.994.

Assessment of Potential Confounders and Laboratory Assessment

Self-reported level of leisure physical activity was assessed annually with the Modifiable Activity Questionnaire and expressed as the average metabolic equivalent (MET-hours) per week for the previous year [21]. Usual daily nutrient intake during the previous year was assessed at baseline and year 1 follow-up visits with the use of a modified version of the Block food-frequency questionnaire [26]. Data on general supplement use were available, but not specifically on vitamin D. Standardized interviewer-administered questionnaires were used annually to obtain self-reported data on personal medical history, smoking, medications, and alcohol use. Self-reported race/ethnicity was classified according to the 1990 U.S. Census questionnaire. Fasting blood was obtained and processed following standardized procedures. Measurement methods for creatinine have been described previously [21].

Statistical Analysis

Cross-Sectional Analyses—We compared the baseline characteristics between the lifestyle and placebo groups using independent samples t-tests for continuous variables and chi-square tests for categorical variables. Using data from the baseline visit, we conducted a cross-sectional analysis to evaluate the association between body weight, BMI, waist circumference, and adipose tissue distribution (total fat, VAT and SAT) as the predictors, and plasma 25OHD concentration as the outcome of interest using linear regression models. The baseline distribution of plasma 25OHD was log-transformed for regression analyses to improve the normal approximation. All models included potential confounders including clinical site location (categorical variable, 1 to 27); season of blood draw; age (years); gender (male or female); race (African American or non-African American), smoking status at baseline (never, past, or currently smoking); baseline alcohol and calcium intake (g/day and mg/day respectively); baseline glomerular filtration rate (GFR) using the modification of diet in renal disease estimation [27] (mL/min/1.73 m^2); and baseline physical activity (MET-hours per week). Additional covariates (baseline weight, VAT, or SAT) were incorporated in the adipose tissue analyses depending on the analysis. We tested for gender and race interactions with the predictors of interest (i.e. weight, BMI, waist circumference, VAT, SAT and total adiposity) and did not identify any interactions.

Longitudinal Analyses—Analyses were restricted to data obtained in the first 2 years of the DPP because all participants had the opportunity to complete two years of follow-up [28]. To investigate the association between change in 25OHD and change in weight during the 2-year period, we defined the change at each visit (both as absolute and as percent change) as change from the prior visit. We used linear mixed models estimated with maximum likelihood and an unstructured covariance matrix to account for correlation between repeated measures. We repeated these analyses using BMI and waist circumference as the predictor. For the body composition analyses over a 1-year period, we examined the association between a 1-year change in adipose tissue distribution (total, VAT, SAT) and 1-year change in plasma 25OHD concentration using linear regression models. Change in

plasma 25OHD from baseline to year 1 was calculated as the difference between log-transformed values, $\log(\text{year 1 25OHD}) - \log(\text{baseline 25OHD})$. All analyses were adjusted for potential confounders including clinical site location, season of baseline blood draw, age, gender, race, physical activity (average of baseline and year 1), baseline smoking status, alcohol and calcium intake (average of baseline and year 1), GFR (average of baseline, month 6 and year 1), and baseline 25OHD concentration. Additional covariates (1-year change in weight, VAT, or SAT) were incorporated in the adipose tissue analyses depending on the analysis. We conducted analyses for the entire cohort adjusting for the study intervention (lifestyle arm or placebo), and also separate analyses for each intervention group to investigate potential confounding by unmeasured factors associated with the intervention beyond weight loss and physical activity and to gain insight into the effect of the lifestyle intervention in addition to weight loss on plasma 25OHD concentration.

Mediation Analyses—To investigate whether adiposity measures (total, VAT, SAT) act as potential mediators of the association between weight and 25OHD concentration, we conducted a formal mediation analysis [29, 30]. To qualify as mediators, regional adiposity measures had to satisfy the Baron and Kenny [31] criteria of mediation as follows: 1) weight is associated with the mediator (total fat, VAT, SAT), 2) the mediator is associated with 25OHD concentration, and 3) controlling for the mediator significantly alters the association between weight and plasma 25OHD concentration. Mediation was determined based on the method by MacKinnon [29]. The proportion mediated, a measure of the effect size of the mediator on the relationship between the predictor and outcome, is determined by the following equations:

$$\text{VAT} = \text{intercept} + a * \text{Weight} \quad (1)$$

$$\ln(25\text{OHD}) = \text{intercept} + c' * \text{Weight} + b * \text{VAT} \quad (2)$$

$$\text{proportion mediated} = ab / (ab + c') \quad (3)$$

The proportion mediated in equation 3 is the ratio of the mediation effect ($a*b$) and the sum of the mediation effect and c' , where c' is the beta coefficient for the predictor (e.g., weight) regressed on the outcome (25OHD) adjusted for the mediator (e.g., VAT) in equation 2. The mediation effect ($a*b$) is the product of the beta coefficient for the predictor regressed on the mediator (a in equation 1) and the beta coefficient for the mediator regressed on the outcome adjusted for the predictor (b in equation 2). The standard error was calculated using the delta method for the mediation effect and first order Taylor series approximation for proportion mediated [30].

Residual analyses were performed to assess the normality of residuals and the linearity of relationships between 25OHD and predictors and did not reveal any violations of these

assumptions. All p-values are two-sided. Statistical analyses were performed using SAS version 9.2 (SAS, Cary, NC).

Results

Participant Characteristics

At baseline, mean ($\pm$ SD) age of the combined lifestyle and placebo treatment groups was 51.0 ± 10.8 years, weight was 94.5 ± 20.5 kg, BMI was 34.0 ± 6.7 kg/m², and waist circumference was 105.1 ± 14.7 cm (**Table 1**). The study included 67% women. The racial distribution was 57% Caucasians, 20% African-Americans, and 23% Hispanic, Asian or American Indian. About 52% of participants reported regular use of multivitamins (**Table 1**). Mean baseline plasma 25OHD concentration was 21.6 ng/mL. The lifestyle and placebo arms did not differ significantly in any participant characteristic. The cohort with available data on adiposity (n=584) was statistically different from the entire cohort (n=1,998) in age (subset: 52.2 ± 11.0 years vs. entire cohort: 51.0 ± 10.8 years, $p=0.026$), weight (subset: 92.4 ± 17.8 kg vs. entire cohort: 94.5 ± 20.5 kg, $p=0.016$), BMI (subset: 33.1 ± 5.8 kg/m² vs. entire cohort: 34.0 ± 6.7 kg/m², $p=0.001$), and waist circumference (subset: 103.4 ± 13.2 cm vs. entire cohort: 105.1 ± 14.7 cm, $p=0.008$). However, these differences were small and do not indicate that the sub-cohort was clinically different from the entire cohort.

Cross-Sectional Analyses

Body weight, BMI, Waist Circumference—Unadjusted analyses at baseline were conducted in the entire cohort of 1,998 adults. Weight was inversely associated with log-transformed plasma 25OHD concentration (**Table 2**, $\beta = -0.0048$, $p < 0.001$). This relationship remained significant after multivariate adjustment ($\beta = -0.0040$, $p < 0.001$, $n=1,958$). The association did not vary by gender or race; however, African-Americans had significantly lower plasma 25OHD concentrations across the entire weight range (data not shown). Analyses with BMI and waist circumference as predictor variables showed similar negative associations with log-transformed plasma 25OHD concentration (BMI: $\beta = -0.0174$, $p < 0.001$; waist circumference $\beta = -0.0064$, $p < 0.001$). Multivariate adjustment did not significantly alter these associations (BMI: $\beta = -0.0125$, $p < 0.001$; waist circumference $\beta = -0.0055$, $p < 0.001$).

Measures of Regional Adipose Distribution—The association between regional adipose distribution and log-transformed plasma 25OHD concentration was performed in the subgroup of participants with available measures at baseline (n=584; **Table 2**). Baseline characteristics (mean age, BMI, 25OHD and gender distribution) did not differ between this subgroup and the entire cohort. Unadjusted analyses revealed that SAT and total fat were inversely associated with plasma 25OHD concentration; the inverse association with VAT was not statistically significant. As expected, after adjustment for body weight, the association with SAT was no longer significant. Conversely, after adjustment for multiple covariates including body weight and SAT, there was a significant inverse association between VAT and 25OHD concentration (**Table 2**).

Mediation Analysis—Baseline VAT, but not SAT or total fat, met the formal criteria for a mediator of the relationship between body weight and plasma 25OHD. The direct effect of body weight in the model for log-transformed 25OHD was $\beta = -0.0040$, $p < 0.0001$. In the mediated outcome model, adding VAT to the model attenuated the association of body weight with plasma 25OHD ($\beta = -0.0025$, $p = 0.0401$). The proportion of the association mediated by VAT was 0.40 [95% CI 0.11, 0.69].

Longitudinal Analyses

Body Weight—The association between change in weight and change in plasma 25OHD concentration was evaluated during a 2-year follow-up period. Given the significant differences in weight change between treatment arms, mean value plots for weight and plasma 25OHD concentration are presented separately by treatment arm (**Figures 2a-b**). Mean percent change ($\pm$ SE) in body weight over the first 6 months was $-7.3 \pm 0.2\%$ in the lifestyle group and $-0.4 \pm 0.2\%$ in the placebo group ($p < 0.0001$ for lifestyle group and $p = 0.02$ for the placebo group; **Figure 2a**). There was relatively little change in mean weight in either arm between month 6 and year 1 ($-0.1 \pm 0.2\%$ in lifestyle group; $-0.1 \pm 0.2\%$ in placebo group). From year 1 to 2, the mean percent weight change was $1.7 \pm 0.2\%$ in the lifestyle group and $0.6 \pm 0.2\%$ in the placebo group ($p < 0.001$ for both groups).

The pattern of change in plasma 25OHD concentration was inverse to the weight change over the 2-year period (**Figure 2b**). In the first 6 months, the mean change in plasma 25OHD concentration was 0.9 ± 0.3 ng/ml in the lifestyle group and 0.1 ± 0.3 ng/ml in the placebo group ($p < 0.001$ for the lifestyle group). Between month 6 and year 1, the average change in plasma 25OHD was 0.2 ± 0.3 ng/ml in the lifestyle group and 0.0 ± 0.3 ng/ml in the placebo group ($p > 0.05$ for both groups). From year 1 to 2, plasma 25OHD concentration declined slightly in the lifestyle group but it was not statistically significant (-0.4 ± 0.3 ng/ml in lifestyle group; 0.5 ± 0.3 ng/ml in placebo group; $p > 0.05$ for both groups).

Measures of Regional Adipose Distribution—A subgroup of participants with regional adipose tissue measurements ($n = 400$) had repeat adiposity measurements at year 1. **Supplementary Figure 1 a-c** presents baseline and year 1 measurements in regional adiposity measures in the entire cohort and by intervention group. After adjusting for change in body weight, changes in SAT, VAT, or total fat were not associated with changes in plasma 25OHD concentration (**Table 3**). In addition, there was no interaction noted by treatment arm.

Mediation Analysis—One-year changes in total fat, VAT, or SAT were not statistically significant mediators of the relationship between change in body weight and change in plasma 25OHD concentration.

Discussion

Body Weight and Plasma 25OHD Concentration

Our study demonstrated a significant inverse cross-sectional association between body weight, BMI, and waist circumference and plasma 25OHD concentration after multivariate

adjustment in a cohort of adults with pre-diabetes. These findings are consistent with cross-sectional studies in populations with normal glucose tolerance [2-6]. As shown in studies in normoglycemic adults, our data indicated that race (defined as African American versus non-African American) influenced baseline 25OHD concentration [32], but did not affect the relationship between body weight and 25OHD concentration [33].

We also demonstrated that 2-year changes in body weight were inversely proportional to 2-year changes in plasma 25OHD concentration. The differential weight changes by treatment group appeared to be matched by small but proportional inverse fluctuations in plasma 25OHD concentration. Because the DPP was a non-surgical weight loss trial with similar starting body weight and plasma 25OHD concentration in the two intervention groups, this study lends support to the concept that weight change may be driving plasma 25OHD concentration. The possibility that the intensive lifestyle group altered dietary intake resulting in a change in plasma 25OHD concentration which, in turn, influenced weight loss seems less likely since very few food sources contain significant amounts of vitamin D [34]. A more plausible confounder to consider is greater sun exposure influencing the change in plasma 25OHD concentration in the intensive lifestyle group due to moderate intensity exercise performed outdoors. Counterarguments to this point would include 1) that this study obtained annual 25OHD levels controlling for season and more than 50% of the DPP sites were located north of latitude 37 degrees north, which are not affected by sunlight year-round. Furthermore, given the large percentage of study subjects with darker skin pigmentation in this multiracial study, sun exposure may not have had a large influence on the cutaneous production of vitamin D.

Our longitudinal findings are consistent with shorter-term clinical trials of non-surgical weight loss [7, 9]. Some have hypothesized that weight loss is accompanied by an increase in plasma 25OHD concentration due to liberation of stored vitamin D from adipose tissue and reduced sequestration of newly-acquired vitamin D (either via cutaneous synthesis or intake) [16]. Others have suggested a dilutional model in which weight loss, regardless of loss in adipose tissue, reduces the body volume and concentrates existing vitamin D in remaining tissues [19]. Although this study does not provide definitive evidence for a causal effect of weight change on vitamin D, our data show a consistent inverse relationship over a 2-year period in a large cohort of obese adults with pre-diabetes. Our results also suggest that the overall fluctuation in plasma 25OHD concentration with change in body weight is modest.

Adipose Tissue as a Mediator

Since vitamin D is fat soluble, one leading hypothesis has been that it is sequestered and stored in fat tissues [16]. Accordingly, greater loss of body adipose tissue, rather than body weight of which adipose tissue is only one component, would be hypothesized to result in a greater release of vitamin D in the circulation. Another hypothesis has been that adipose tissue increases vitamin D metabolism due to activity of 24-hydroxylase in adipocytes [20]; thus, the selective loss of adipocytes would drive changes in circulating 25OHD concentration by reducing metabolism.

Based on these potential mechanisms, one objective of our study was to examine whether the relationship between body weight and plasma 25OHD concentration could be accounted for by amount and type of adiposity. Thus, in a subset of participants who underwent regional adipose tissue measurements, we found that total fat and its compartments were inversely associated with 25OHD concentration, as with body weight. At baseline, only VAT was noted to be a partial mediator of the relationship between body weight and plasma 25OHD, suggesting that the association between weight and plasma 25OHD was significantly reduced in magnitude when VAT was included in the model. The results indicated that higher VAT was associated with lower plasma 25OHD concentration. This result appears to be consistent with findings in the Framingham cohort [35] and others [33]. The VAT-vitamin D association may be driving the relationship between total fat and plasma 25OHD concentration as SAT was not important in determining plasma 25OHD in this population. Visceral fat is known to have some different characteristics than subcutaneous fat. For example, it is more strongly associated with impaired fasting glucose levels [36, 37], type 2 diabetes [38, 39], and insulin resistance [36] than subcutaneous depots. In addition, the secretory component of visceral fat is rich in proinflammatory and low in anti-inflammatory adipokines, hormones secreted by adipocytes. How these factors may have an impact on blood 25OHD concentration is unclear; however, a few reports have suggested that inflammation may lower circulating 25OHD levels potentially by effects on vitamin D-binding proteins [40, 41].

In the subset of participants with available data, 1-year changes in total fat or regional adipose tissue compartments did not predict 1-year changes in plasma 25OHD concentration independent of changes in body weight. This null finding may, in part, be explained by the smaller sample size which may have precluded detecting statistically significant mediation effects of body fat or its compartments in this study. Another potential explanation is over-adjustment of intercorrelated factors.

Strengths and Limitations

The strengths of the present study include the study design, the large and multiracial sample size, the long-term follow-up, and the validated measurements of the predictor and outcome variables and various covariates, which were repeatedly measured during the follow-up period. A limitation of our study was that vitamin D intake from supplements was not available. We did, however, adjust for calcium intake (from food and supplements) and general use of supplements, which can provide a good proxy for vitamin D intake, given shared sources of calcium and vitamin D intake. We also did not have data on female hormone therapy, which may affect measurement of 25OHD. An additional limitation was that the subset of participants with body fat measurements was small and may in part account for the null findings in our longitudinal mediation analysis.

Conclusion

Our analysis in the DPP is consistent with studies showing an inverse association between weight and vitamin D status in normoglycemic populations. Furthermore, because the DPP was a weight loss intervention study, the findings raise the possibility that weight loss increases plasma 25OHD concentration. The clinical significance of the modest changes in

plasma 25OHD concentration is not clear. VAT was a potential mediator of the association between plasma 25OHD and body weight at baseline; however, longitudinal analyses did not corroborate this finding. Given that the analyses were post-hoc, further interventional studies on the effect of weight and body fat fluctuations on vitamin D status are needed to elucidate this relationship.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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LC, JW, KDA, GAB, AGP were responsible for the study design. CG conducted the 25OHD assay analysis. JN was responsible for the statistical analysis. All authors were involved in the interpretation of the data. All authors and the DPP Publications and Ancillary Studies Committees were involved in the critical revision of the manuscript for intellectual content.

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References

1. Looker AC, et al. Serum 25-hydroxyvitamin D status of the US population: 1988-1994 compared with 2000-2004. *Am J Clin Nutr*. 2008; 88(6):1519–27. [PubMed: 19064511]
2. Snijder MB, et al. Adiposity in relation to vitamin D status and parathyroid hormone levels: a population-based study in older men and women. *J Clin Endocrinol Metab*. 2005; 90(7):4119–23. [PubMed: 15855256]
3. Bell NH, et al. Evidence for alteration of the vitamin D-endocrine system in obese subjects. *J Clin Invest*. 1985; 76(1):370–3. [PubMed: 2991340]
4. Liel Y, et al. Low circulating vitamin D in obesity. *Calcif Tissue Int*. 1988; 43(4):199–201. [PubMed: 3145124]
5. Parikh SJ, et al. The relationship between obesity and serum 1,25-dihydroxy vitamin D concentrations in healthy adults. *J Clin Endocrinol Metab*. 2004; 89(3):1196–9. [PubMed: 15001609]
6. Arunabh S, et al. Body fat content and 25-hydroxyvitamin D levels in healthy women. *J Clin Endocrinol Metab*. 2003; 88(1):157–61. [PubMed: 12519845]
7. Mason C, et al. Effects of weight loss on serum vitamin D in postmenopausal women. *Am J Clin Nutr*. 2011; 94(1):95–103. [PubMed: 21613554]
8. Jorde R, et al. Cross-sectional and longitudinal relation between serum 25-hydroxyvitamin D and body mass index: the Tromso study. *Eur J Nutr*. 2010; 49(7):401–7. [PubMed: 20204652]

9. Tzotzas T, et al. Rising serum 25-hydroxy-vitamin D levels after weight loss in obese women correlate with improvement in insulin resistance. *J Clin Endocrinol Metab.* 2010; 95(9):4251–7. [PubMed: 20534751]
10. Newbury L, et al. Calcium and vitamin D depletion and elevated parathyroid hormone following biliopancreatic diversion. *Obesity surgery.* 2003; 13(6):893–5. [PubMed: 14738677]
11. Rickers H, et al. Impairment of vitamin D metabolism and bone mineral content after intestinal bypass for obesity. A longitudinal study. *Scandinavian journal of gastroenterology.* 1984; 19(2): 184–9. [PubMed: 6609420]
12. Pramyothin P, et al. Vitamin D in adipose tissue and serum 25-hydroxyvitamin D after roux-en-Y gastric bypass. *Obesity (Silver Spring).* 2011; 19(11):2228–34. [PubMed: 21701564]
13. Reinehr T, et al. Vitamin D status and parathyroid hormone in obese children before and after weight loss. *Eur J Endocrinol.* 2007; 157(2):225–32. [PubMed: 17656603]
14. Vimalaswaran KS, et al. Causal relationship between obesity and vitamin D status: bi-directional Mendelian randomization analysis of multiple cohorts. *PLoS Med.* 2013. 10(2): p. e1001383.
15. Carlin AM, et al. Effect of gastric bypass surgery on vitamin D nutritional status. *Surg Obes Relat Dis.* 2006; 2(6):638–42. [PubMed: 17138235]
16. Wortsman J, et al. Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr.* 2000; 72(3): 690–3. [PubMed: 10966885]
17. Alemzadeh R, et al. Hypovitaminosis D in obese children and adolescents: relationship with adiposity, insulin sensitivity, ethnicity, and season. *Metabolism.* 2008; 57(2):183–91. [PubMed: 18191047]
18. van der Wielen RP, et al. Serum vitamin D concentrations among elderly people in Europe. *Lancet.* 1995; 346(8969):207–10. [PubMed: 7616799]
19. Drincic AT, et al. Volumetric Dilution, Rather Than Sequestration Best Explains the Low Vitamin D Status of Obesity. *Obesity (Silver Spring)*, 2012.
20. Li J, et al. 1 α ,25-Dihydroxyvitamin D hydroxylase in adipocytes. *J Steroid Biochem Mol Biol.* 2008; 112(1-3):122–6. [PubMed: 18840526]
21. Knowler WC, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med.* 2002; 346(6):393–403. [PubMed: 11832527]
22. Bray GA, et al. Relation of central adiposity and body mass index to the development of diabetes in the Diabetes Prevention Program. *Am J Clin Nutr.* 2008; 87(5):1212–8. [PubMed: 18469241]
23. Lissner D, Mason RS, Posen S. Stability of vitamin D metabolites in human blood serum and plasma. *Clin Chem.* 1981; 27(5):773–4. [PubMed: 7226510]
24. Hankinson SE, et al. Effect of transport conditions on the stability of biochemical markers in blood. *Clin Chem.* 1989; 35(12):2313–6. [PubMed: 2591049]
25. National Institute of Standards and Technology, Standard Reference Materials. 11/3/2010; Available from: <http://www.nist.gov/srm/>
26. Mayer-Davis EJ, et al. Dietary intake in the diabetes prevention program cohort: baseline and 1-year post randomization. *Ann Epidemiol.* 2004; 14(10):763–72. [PubMed: 15573453]
27. Poggio ED, et al. Performance of the modification of diet in renal disease and Cockcroft-Gault equations in the estimation of GFR in health and in chronic kidney disease. *Journal of the American Society of Nephrology : JASN.* 2005; 16(2):459–66. [PubMed: 15615823]
28. Diabetes Prevention Program Research, G. Long-term safety, tolerability, and weight loss associated with metformin in the Diabetes Prevention Program Outcomes Study. *Diabetes Care.* 2012; 35(4):731–7. [PubMed: 22442396]
29. MacKinnon DP, Fairchild AJ, Fritz MS. Mediation analysis. *Annu Rev Psychol.* 2007; 58:593–614. [PubMed: 16968208]
30. Mackinnon DP, Warsi G, Dwyer JH. A Simulation Study of Mediated Effect Measures. *Multivariate Behav Res.* 1995; 30(1):41. [PubMed: 20157641]
31. Baron RM, Kenny DA. The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. *J Pers Soc Psychol.* 1986; 51(6): 1173–82. [PubMed: 3806354]
32. Harris SS. Vitamin D and African Americans. *J Nutr.* 2006; 136(4):1126–9. [PubMed: 16549493]

33. Young KA, et al. Association of plasma vitamin D levels with adiposity in Hispanic and African Americans. *J Clin Endocrinol Metab.* 2009; 94(9):3306–13. [PubMed: 19549738]
34. Holick MF. Vitamin D deficiency. *N Engl J Med.* 2007; 357(3):266–81. [PubMed: 17634462]
35. Cheng S, et al. Adiposity, cardiometabolic risk, and vitamin D status: the Framingham Heart Study. *Diabetes.* 2010; 59(1):242–8. [PubMed: 19833894]
36. Goodpaster BH, et al. Association between regional adipose tissue distribution and both type 2 diabetes and impaired glucose tolerance in elderly men and women. *Diabetes Care.* 2003; 26(2): 372–9. [PubMed: 12547865]
37. Hayashi T, et al. Visceral adiposity and the risk of impaired glucose tolerance: a prospective study among Japanese Americans. *Diabetes Care.* 2003; 26(3):650–5. [PubMed: 12610016]
38. Kanaya AM, et al. Adipocytokines attenuate the association between visceral adiposity and diabetes in older adults. *Diabetes Care.* 2004; 27(6):1375–80. [PubMed: 15161791]
39. Boyko EJ, et al. Visceral adiposity and risk of type 2 diabetes: a prospective study among Japanese Americans. *Diabetes Care.* 2000; 23(4):465–71. [PubMed: 10857936]
40. Thurnham DI. Plasma 25-Hydroxy-Cholecalciferol (Vitamin D) is Depressed by Inflammation: Implications and Parallels with Other Micronutrients. *Sight and Life.* 2011; 25(2):38–47.
41. Duncan A, et al. Quantitative data on the magnitude of the systemic inflammatory response and its effect on micronutrient status based on plasma measurements. *Am J Clin Nutr.* 2012; 95(1):64–71. [PubMed: 22158726]

Figure 1.
CONSORT Flow Diagram

Figure 2.
Mean weight and 25OHD by treatment group during follow-up

Table 1

Baseline characteristics of the DPP cohort included in current study.

Characteristic	Overall cohort	Lifestyle ¹	Placebo ¹
Number of participants	1998	1002	996
Age, mean (SD), years	51.0 (10.8)	51.1 (11.3)	51.0 (10.4)
Gender, No. (%) women	1335 (66.8)	668 (67.1)	667 (66.6)
Race, No. (%)			
White	1140 (57.1)	567 (56.6)	573 (57.5)
African-American	405 (20.3)	200 (20.0)	205 (20.6)
Other (Hispanic, Asian, American Indian)	453 (22.7)	235 (23.5)	218 (21.9)
Weight, mean (SD), kg	94.5 (20.5)	94.4 (20.8)	94.6 (20.3)
Body mass index, mean (SD), kg/m ²	34.0 (6.7)	33.9 (6.7)	34.1 (6.7)
Waist circumference, mean (SD), cm	105.1 (14.7)	105.1 (14.9)	105.1 (14.4)
Body composition variables			
Visceral at Lumbar disc 4-5, mean (SD), cm ²	161.3 (64.9)	162.8 (64.1)	159.8 (65.7)
Subcutaneous at Lumbar disc 4-5, mean (SD), cm ²	443.5 (152.2)	439.6 (152.4)	447.5 (152.1)
Total fat (visceral and subcutaneous), mean (SD)	604.8 (174.8)	602.4 (173.1)	607.2 (176.7)
Season of blood sampling			
December-January-February, No. (%)	492 (24.6)	248 (24.8)	244 (24.5)
March-April-May, No. (%)	474 (23.7)	253 (25.3)	221 (22.2)
June-July-August, No. (%)	437 (21.9)	216 (21.6)	221 (22.2)
September-October-November, No. (%)	595 (29.8)	285 (28.4)	310 (31.1)
Physical Activity MET, mean (SD), hours ²	15.9 (25.7)	15.2 (21.5)	16.6 (29.3)
Smoking status, No. (%)			
Never	1143 (57.2)	569 (56.8)	574 (57.6)
Past	715 (35.8)	369 (36.8)	346 (34.7)
Current	140 (7.0)	64 (6.4)	76 (7.6)
Alcohol consumption, mean (SD), g/day	2.2 (5.6)	2.4 (6.2)	2.1 (4.9)
Calcium intake, mean (SD), mg/day	1102.7 (724.5)	1095.3 (700.7)	1110.2 (747.9)
Vitamin regular use, No. (%)	1032 (51.7)	526 (54.6)	506 (52.3)
Glomerular Filtration Rate, mL/min/1.73 m ²	100.6 (30.2)	100.2 (25.0)	100.9 (34.7)
25-hydroxyvitamin D, mean (SD), ng/mL	21.6 (9.7)	21.9 (9.9)	21.3 (9.5)

Values are means (standard deviation [SD]) for continuous variables or n (%) for categorical variables. To convert 25-hydroxyvitamin D (25OHD) concentration from ng/mL to nmol/L multiply by 2.459.

¹ P-values for differences between placebo and lifestyle were all >0.05. P-value for continuous variables are based on t-test. For categorical variables, the p value is based on chi-square tests.

² MET denotes metabolic equivalent. MET-hours represent the average amount of time engaged in specified physical activities multiplied by the MET value of each activity.

TABLE 2

Association between measures of weight/adiposity and natural log transformed plasma 25OHD concentration at baseline

Parameter	Beta coefficient	P value
Weight		
Unadjusted	−0.0048	<0.0001
Model 1 *	−0.004	<0.0001
Model 2 **	NA	NA
Model 3 ***^	−0.0009 ^	0.6314^
VAT		
Unadjusted	−0.0002	0.5198
Model 1 *	−0.0012	<0.0001
Model 2 **	−0.0008	0.0132
Model 3 ***	−0.0009	0.0076
SAT		
Unadjusted	−0.0007	<0.0001
Model 1 *	−0.0004	0.0004
Model 2 **	−0.0001	0.6054
Model 3 ***	−0.0002	0.2618
Total Fat (VAT+SAT)		
Unadjusted	−0.0006	<0.0001
Model 1 *	−0.0005	<0.0001
Model 2 **	−0.0004	0.0495
Model 3 ***	NA	NA

VAT, visceral adipose tissue measured by CT scan at lumbar disc area 4-5; SAT, subcutaneous adipose tissue measured by CT scan at lumbar disc area 4-5. For weight analyses, N=1,998 for unadjusted Model, N=1,958 for adjusted Model 1, N=576 for adjusted Model 3. For adiposity measures, N=584 for unadjusted Model, N=576 for adjusted Models.

* Model 1 adjusted for: age, gender, race (African-American vs. non-African-American), smoking status, alcohol, calcium intake, glomerular filtration rate, physical activity, clinical site, season of blood draw.

** Model 2 adjusted for: same as Model 1 and weight

^ Model 3 adjusted for: same as Model 2 and total fat

*** Model 3 adjusted for: same as Model 2 and opposite type of fat (VAT or SAT)

TABLE 3

Association between percent change in weight/adiposity and natural log transformed plasma 25OHD concentration over 1-year period

Parameter	Beta coefficient	P value
% Change in Weight		
Unadjusted	−0.0063	<0.0001
Model 1 *	−0.0068	<0.0001
Model 2 **	NA	NA
Model 3 ***	−0.0039	0.2635
% Change in VAT		
Unadjusted	−0.0013	0.0503
Model 1 *	−0.0007	0.3178
Model 2 **	0.0005	0.5867
Model 3 ***	0.0009	0.3606
% Change in SAT		
Unadjusted	−0.0031	0.0015
Model 1 *	−0.0003	0.0077
Model 2 **	−0.0022	0.1138
Model 3 ***	−0.0025	0.0815
% Change in Total Fat (VAT+SAT)		
Unadjusted	−0.0027	0.0033
Model 1 *	−0.0024	0.0276
Model 2 **	−0.0013	0.4065
Model 3 ***	NA	NA

VAT, visceral adipose tissue measured by CT scan at lumbar disc area 4-5; SAT, subcutaneous adipose tissue measured by CT scan at lumbar disc area 4-5. For weight analyses, N=1,682 for unadjusted Model, N=1,676 for adjusted Model 1; N=400 for adjusted Model 3. For adiposity measures, N=400 for all analyses.

* Model 1 adjusted for: baseline 25OHD, age, gender, race (African-American vs. non-African-American), baseline smoking status, alcohol (average of baseline and year 1 visits), calcium intake (average of baseline and year 1 visits), (average of baseline, month 6, and year 1 visits), physical activity (average of baseline and year 1 visits), clinical site, baseline season of blood draw, and intervention group.

** Model 2 adjusted for: same as Model 1 and weight change

^ Model 3 adjusted for: same as Model 2 and total fat

*** Model 3 adjusted for: same as Model 2 and opposite type of change in fat (VAT or SAT)