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Journal

Hormone and Metabolic Research, 47(04)

ISSN

0018-5043

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Publication Date

2015-04-01

DOI

10.1055/s-0034-1383652

Peer reviewed

HHS Public Access

Author manuscript

Horm Metab Res. Author manuscript; available in PMC 2015 November 17.

Published in final edited form as:

Horm Metab Res. 2015 April ; 47(4): 280–283. doi:10.1055/s-0034-1383652.

Effect of Long Term Vitamin D Supplementation on Biomarkers of Inflammation in Latino and African-American Subjects with Pre-Diabetes and Hypovitaminosis D

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Abstract

Background—Low vitamin D levels are associated with minority subjects, the metabolic syndrome and inflammation. The effect of vitamin D supplementation on markers of inflammation has not been well studied.

Objective—To evaluate the effects of high doses of vitamin D supplementation for 1 year on serum biomarkers of inflammation in Latino and African-American subjects with pre-diabetes and hypovitaminosis D.

Participants and methods—Latino (N =69) and African-American (N = 11) subjects who had both pre-diabetes and hypovitaminosis D with a mean age of 52.0 years, a BMI of 32.7 kg/m^2 and 70% of whom were female were randomized to receive weekly doses (mean ± SD) of vitamin D (85,300 IU ± 16,000) or placebo oil for 1 year. Serum levels of interleukin-6, tumor necrosis factor, highly sensitive C-reactive protein), plasminogen activator inhibitor 1, and insulin-like growth factor - 1 were measured at baseline, 6 and 12 months.

Results—Serum 25-OH vitamin D levels of 22 ng/ml at baseline quickly rose to nearly 70 ng/ml in subjects receiving vitamin D and did not change in the placebo group. Two-way repeated measures ANOVA showed no differences between the 2 groups in any of the five selected parameters.

Conclusion—High dose vitamin D supplementation for 1 year in minority subjects with pre-diabetes and hypovitaminosis D failed to affect serum biomarkers of inflammation.

Keywords

Vitamin D supplementation; inflammation; pre-diabetes; hypovitaminosis D

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Clinical trial reg. no. NCT00876928, clinicaltrials.gov

Introduction

Individuals with pre-diabetes have lower vitamin D levels than those with normal glucose tolerance tests [1-3]. Lower vitamin D levels are also seen in people with the metabolic syndrome [4-6], in which pre-diabetes is one of the factors. High concentrations of vitamin D were associated with a significantly lower rate of the development of diabetes (hazard ratio of 0.52), but when adjustments were made for highly sensitive C-reactive protein (hsCRP) and interleukin-6 (IL-6), the hazard rate was attenuated by 18% suggesting that incident diabetes may be partially mediated by subclinical inflammation [7]. Inflammation characterizes the dysglycemic state [8] and vitamin D has been implicated in the inflammatory process [9]. Vitamin D and hsCRP [5,10-12], tumor necrosis factor-α (TNF-α) [13,14] and IL-6 [12,14] concentrations are inversely related in most, but not all [15,16], studies. Furthermore, 1,25 OH vitamin D limits the production of the pro-inflammatory cytokines TNF-α and IL-6 in human macrophages [17,18] and plasminogen activator inhibitor-1 (PAI-1) in human coronary artery smooth muscle cells [19]. Finally, insulin-like growth factor-1 (IGF-1) levels are lower in people with the metabolic syndrome and correlate positively with vitamin D levels [4].

Since association does not mean causation, intervention studies are necessary to prove the latter. Pro-inflammatory cytokines increase after an acute myocardial infarction. In a randomized control trial in such patients, a short course of treatment with vitamin D significantly attenuated the rise of hsCRP and IL-6 (but not TNF-α) [20]. To our knowledge, the effect of vitamin D supplementation on non-stressed concentrations of pro-inflammatory cytokines has not been studied. We recently published the results of a randomized control trial of a year-long supplementation with very high doses of vitamin D in 99 Latino and African American subjects with pre-diabetes and hypovitaminois D [21]. Since the screening characteristics for the trial included central obesity and hypertension, it is very likely that most of the subjects fulfilled the criteria for the metabolic syndrome. Here we examine the effect of vitamin D supplementation on non-stressed serum concentrations of hsCRP, IL-6, PAI-1, TNF-α and IGF-1 in 80 of these subjects.

Material and Methods

Charles R. Drew University (CDU) is sited in South Central Los Angeles in which almost all of the inhabitants are Latino or African American. The CDU IRB approved the study and determined that an informed consent was not necessary for individuals screened with a point-of-care HbA1c test because screening for diabetes often takes place at community sites. An informed consent was obtained for those undergoing an oral glucose tolerance test (OGTT). A separate informed consent describing the study was obtained for subjects before randomization.

The following 4 criteria were used to identify Latino and African American subjects ≥40 years old who may have pre-diabetes: a) waist circumference measured at the umbilicus of 40 inches or greater in men and 35 inches or greater in women; b) family history of diabetes in first degree relatives; c) hypertension, either being treated or newly diagnosed at screening of ≥140/90 mm Hg; and d) history of gestational diabetes. A point-of-care

fingerstick HbA1c level was measured and those with values of 40 mmol/mol through 52 mmol/mol were invited to undergo an OGTT. Pre-diabetes was diagnosed by a 2 hr glucose concentration of 140-199 mg/dl and/or a fasting plasma glucose (FPG) concentration of 110-125 mg/dl. Vitamin D concentrations were measured in those subjects in whom pre-diabetes was diagnosed. The criterion for hypovitaminosis D was a vitamin D concentration of <30 ng/ml. Ninety-nine subjects with pre-diabetes and hypovitaminois D, randomized to receive either high weekly doses of vitamin D or placebo in oil completed the year-long study. Samples from 80 of these subjects (40 in each group) were randomly selected for the current study. Fasting blood samples were collected at baseline, 6, and 12 months and serum separated and frozen for subsequent measurements of the pro-inflammatory cytokines, IL-6, TNF-α, PAI-1, and hsCRP and IGF-1 (the latter not measured at 6 months) in each group. Subjects in the original study were not counseled regarding diet and exercise as we wanted to specifically examine the effects of vitamin D supplementation on glycemic parameters.

Vitamin D Dosing

Subjects were given a blinded, identical-appearing and smelling solution in a pre-filled syringe containing the weekly dose of either placebo (medium-chain triglycerides) or vitamin D3 dissolved in the triglyceride at a concentration of 1,000 IU/drop or 35,714 IU/ml. The formula for vitamin D supplementation was (100-baseline vitamin D) x kg weight x 15.7 = IU per week with a target level of 65-90 ng/ml. The dose was decreased by 25% when the serum level in the vitamin D group reached ≥80 ng/ml. The average dose (± SD) administered was 85,300 IU ± 16,000 per week (range 64,700-134,000). According to the number of empty syringes returned at each visit, there was 100% compliance, i.e., none were lost and subjects claimed that they took the contents of each one. No increase in serum or urinary calcium levels was noted.

Assay Kits

ELISA kits (Invitrogen, Carlsbad, CA) were used to measure IL-6 (normal range 1.3-6.8 pg/ml, intra-assay variation - 8.3%, inter-assay variation - 10.0%), TNF-α (normal range 0-3.8 pg/ml, intra-assay variation – 5.8%, inter-assay variation – 8.7%), and PAI-1 (normal range 320-8560 pg/ml, intra-assay variation - 5.0%, inter-assay variation – 9.0%). hsCRP was measured (normal range, 1.6-3.0 mg/L, intra-assay variation – 7.5%, inter-assay variation – 4.1%) using a kit from Cayman Chemical (Ann Arbor, MI). IGF-1 (normal range 60-298 ng/ml, intra-assay variation – 5.9%, inter-assay variation – 6.7%) was measured using a kit from Alpco Diagnostics (Salem, NH). Serum 25-OH vitamin D levels were measured by a high performance liquid chromatography/tandem spectrophotometry (Quest Diagnostics, San Juan Capistrano, CA).

Statistical Analyses

Since these measurements were not part of the original study, a power calculation was not performed. The primary statistical analyses for these biomarkers were performed using NCSS 8 (NCSS LLC, Kaysville, Utah, 2012). Data are presented as mean ± SD. A 2-way repeated measures analysis of variance model on each of the 5 measurements was used to

identify group differences across the three time points. The main statistical measure of group difference (vitamin D versus placebo) was the group X time interaction.

Results

All subjects had pre-diabetes; 73 with impaired glucose tolerance and 7 with impaired fasting glucose only. Twenty-one (27%) had both central obesity and hypertension fulfilling the criteria for the metabolic syndrome. Another 49 (61%) had central obesity without hypertension and 5 (6%) had hypertension without central obesity. Only 5 (6%) had neither central obesity nor hypertension. Although fasting lipids were not measured, it is very likely that many of the 66% with one other risk factor had elevated triglyceride and/or decreased HDL cholesterol levels that would also define the metabolic syndrome in them.

There were no differences in the baseline characteristics between the placebo and vitamin D groups Table 1). Serum 25-OH vitamin D levels quickly rose to nearly 70 ng/ml in those receiving vitamin D and did not change in the placebo group. The values of the biomarkers at baseline, six months (except IGF-1) and twelve months are summarized in Table 1. There were no differences between the 2 groups. There was also no difference in the responses between the two sexes (data not shown).

Discussion and Conclusion

In spite of the inverse relationship between the concentrations of vitamin D and hsCRP, IL-6 and TNF-α [5,10-14] and the inhibition of PAI-1 by 1,25 OH vitamin D in human aortic smooth muscle cells [19], we were unable to demonstrate any effect of vitamin D supplementation on these non-stressed serum levels of these inflammatory biomarkers nor on IGF-1. These negative results with hsCRP and IL-6 are consistent with several prior vitamin D supplementation reports [10,22,23] although one group did find decreases in hsCRP after ingestion of yogurt that had been fortified with vitamin D [24]. Regarding the effect of vitamin D supplementation on cellular immunity [17,18], cell culture supernatants from human macrophages harvested from subjects receiving vitamin D showed a decrease in IL-6 but no change in TNF-α. The latter is consistent with no changes seen in serum soluble TNF-α receptor type 2 levels after vitamin D supplementation [10]. To our knowledge, there has been one published report of the effect of vitamin D supplementation on IGF-1 levels and another reported in abstract form only. Kamycheva et al [25] found that vitamin D supplementation decreased the IGF-I/IGFBP-3 ratio in those without severe obesity but did not affect GH, IGF-I, IGFBP-3, or IGF-I/IGFBP-3 ratio when all subjects were examined. In an abstract presented at the 2009 Endocrine Society meeting Woodworth and Woodworth reported that vitamin D supplementation increased IGF-1 levels [26].

To our knowledge, there are 4 possible explanations for negative studies regarding vitamin D supplementation: a) some subjects had normal baseline levels of vitamin D; b) the dose of vitamin D was too small; c) high enough levels of 25-OH vitamin D were not achieved; and d) duration of supplementation of vitamin D was too short. None of these could explain our negative results. All subjects had vitamin D levels at baseline <30 ng/ml, very high doses were used achieving levels of nearly 70 ng/ml and supplementation was carried out for a

year in all subjects. Thus, we conclude that these cardiovascular markers are not causally related to vitamin D status. In our recently published study [20], we could also not show any effect on the development of diabetes, insulin secretion or insulin sensitivity in these minority subjects with pre-diabetes and hypovitaminosis D.

Although low levels of vitamin D are associated with many disease conditions, the 2011 report on dietary requirements for vitamin D from the Institute of Medicine concluded that the evidence that vitamin D reduces risks of non-skeletal chronic disease outcomes, including cancer, cardiovascular disease, diabetes, and autoimmune disorders was inconsistent, inconclusive, and did not meet criteria for establishing cause-and-effect relationships [27]. A recent meta-analysis on the effect of vitamin D supplementation on myocardial infarctions, strokes, fractures or mortality could find no beneficial effects and concluded that future trials were unlikely to change these negative findings [28]. The lack of an effect of prolonged high dose vitamin D supplementation in Latinos and African Americans with pre-diabetes and hypovitaminosis D on these biomarkers of inflammation would support the conclusions of these two papers. Perhaps the speculation that low 25-OH vitamin D levels are simply a marker for morbidity, including the amount and duration of obesity, staying indoors too much, not enough exercise and overall ill health, may have merit [29].

Acknowledgments

This work was supported by a grant from the National Institutes of Health grant U54MD007598 (formerly U54RR026138; J. Vadgama), and ADA research grant 1-09CR-15. T.C.F. received salary support from R24DA017298. M.B.D. received salary support from UCLA CTSI grant UL1TR000124.

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Table 1

Baseline Characteristics

	Placebo (N = 40)	**Vitamin D (N = 40)**
Age (years ± SD)	52.4 ± 6.7	51.6 ± 7.7
Female	70%	70%
Latino/African American	80%/20%	93%/7%
BMI (kg/m^2 ± SD)	32.9 ± 4.5	32.5 ± 4.4
FPG (mg/dl ± SD)	98.9 ± 9.5	99.0 ± 8.0
2-hr glucose/OGTT (mg/dl ± SD)	161 ± 18	158 ± 21
HbA1c (mmol/mol ± SD)	43 ± 3	44 ± 2
25-OH Vitamin D (ng/ml ± SD)	22.1 ± 4.7	21.9 ± 4.7

BMI – body mass index; FPG – fasting plasma glucose; OGTT – oral glucose tolerance test

Table 2

Effect of Vitamin D Supplementation

	Baseline	Six Months	Twelve Months
IL-6 (1.3-6.8 pg/ml) P = 0.67			
Placebo	3.2 ± 2.0	2.9 ± 1.9	3.3 ± 2.0
Vitamin D	3.4 ± 2.0	3.3 ± 1.9	3.7 ± 2.4
TNF-α (0-3.8 pg/ml) P = 0.43			
Placebo	1.1 ± 0.4	1.2 ± 0.4	0.9 ± 0.3
Vitamin D	1.2 ± 0.4	1.2 ± 0.5	1.1 ± 0.4
hsCRP (1.6-3.0 mg/l) P = 0.43			
Placebo	5.6 ± 2.7	5.9 ± 2.9	5.6 ± 2.9
Vitamin D	5.6 ± 2.4	5.6 ± 2.5	5.8 ± 2.9
PAI-1 (320-8560 pg/ml) P = 0.14			
Placebo	3700 ± 1800	3460 ± 1800	3230 ± 1740
Vitamin D	3010 ± 1800	3120 ± 1400	3030 ± 1470
IGF-1 (60-298 ng/ml) P = 0.88			
Placebo	224 ± 120	ND	207 ± 130
Vitamin D	221 ± 96	ND	210 ± 120

Units and normal ranges are provided in parentheses; values are means ± SD; ND – not done