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Vitamin D3 supplementation improves insulin sensitivity in subjects with impaired fasting glucose

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Abstract

Vitamin D has *in vitro* and *in vivo* effects on β -cells and insulin sensitivity. Vitamin D deficiency (VDD) has been associated with onset and progression of type 2 diabetes mellitus (DM-2). However, studies involving supplementation of vitamin D in subjects with previously established diabetes have demonstrated inconsistent effects on insulin sensitivity. The aim of this open-label study was to assess the effects of high dose vitamin D3 supplementation on insulin sensitivity in subjects with VDD and impaired fasting glucose. We studied 8 subjects with VDD and pre-diabetes with the modified frequently sampled intravenous glucose tolerance (mFSIGT) test before and after vitamin D supplementation. Vitamin D3 was administered as 10,000 IU daily for 4 weeks. The mFSIGT was analyzed with MinMod Millennium to obtain estimates of Acute Insulin Response to Glucose (AIRg), Insulin Sensitivity (SI), and Disposition Index (DI). We found that AIRg decreased ($p = 0.011$) and insulin sensitivity, expressed as SI, increased ($p = 0.012$) after a intervention with vitamin D. If these findings are repeated in a randomized, double-blind, study the results indicate that orally administered high dose vitamin D3 supplementation improves insulin sensitivity in subjects with impaired fasting glucose and suggests that high dose vitamin D3 supplementation might provide an inexpensive public health measure in preventing, or at least delaying, the progression from impaired fasting glucose to diabetes.

Introduction

Observational studies have linked vitamin D deficiency (VDD) to type-2 diabetes (1). Beneficial effects of vitamin D have been demonstrated on both beta cell function and insulin sensitivity in animal studies (2–10). However, published clinical trials involving supplementation of vitamin D in subjects with type-2 diabetes have demonstrated inconsistent results (6, 11–13). Results from these trials were confounded by low vitamin D replacement doses, variable definitions of vitamin D deficiency and variability in the duration of diabetes among the subjects studied. Subgroup analyses in these studies revealed

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a positive, yet non-significant trend, for improved insulin sensitivity in subjects with less than 3 years of diabetes (11, 12). In subjects with normal glucose tolerance, serum vitamin D concentration has been significantly and positively correlated with insulin sensitivity as measured by hyperinsulinemic clamp (14). To overcome some of the limitations in the previously described studies, we hypothesized that high dose vitamin D supplementation in VDD subjects with impaired fasting glucose would have a beneficial effect on insulin sensitivity.

Subjects and Methods

Study participants

Study participants were recruited through primary care and endocrinology clinics affiliated with Hennepin County Medical Center and the University of Minnesota (Minneapolis, MN). Participants included adults, ages 18 to 65, with fasting glucose levels ≥ 100 mg/dL but less than 126 mg/dL, and serum 25-OH vitamin D levels ≤ 30 ng/mL. We used this latter concentration as patients with values less than this are stated to be at increased risk for the development of type 2 diabetes (1). Exclusion criteria included liver or kidney disease, history of nephrolithiasis or hypercalcemia, current or anticipated pregnancy, and treatment with medications known to affect calcium or glucose metabolism. Eleven subjects were recruited and provided informed consent. Three of the eleven were not included in the analysis because their initial 25 OH vitamin D levels were greater than 30 ng/mL at confirmatory baseline testing..

Study design

This study was a prospective, open-label, within subject, before and after design. Once enrolled in the study, participants were asked not to vary their diet or activity from their normal routine. They were instructed not to take calcium or vitamin D supplementation other than what was given for the study. All subjects were asked to fast for 12 hours before presenting to the University of Minnesota General Clinical Research Center (GCRC) on both test days.

A sample size of 10 subjects was estimated to provide 80% power to detect a 20% change in the primary endpoint at $p < 0.05$.

Methods

The consent form and process were approved by the institutional review boards at the University of Minnesota and Hennepin County Medical Center. The research was carried out according to the principles of the Declaration of Helsinki. Qualified subjects presented to the GCRC after an overnight 12-hour fast. Two intravenous catheters were established, one for blood samples and another for administration of insulin and glucose. Baseline laboratories (performed at the University of Minnesota Clinical Laboratory) included serum 25-hydroxyvitamin D, parathyroid hormone, Ca^{++} , albumin, electrolytes, phosphorous, blood urea nitrogen, creatinine, and lipids. Serum calcium values were corrected to a serum albumin of 4.0 gm/dL. Serum vitamin D levels were determined by mass spectroscopy and included measurements of vitamin D₂, D₃ and total 25-hydroxyvitamin D. Blood samples for glucose, insulin, and C-peptide were collected at 22 pre-defined time points using the published modified Frequently Sampled Intravenous Glucose Tolerance (mFSIGT) protocol (15). Per protocol, supplemental insulin was administered at 20 minutes after protocol initiation to augment plasma insulin concentration and thus ensure accurate measurement of peripheral glucose utilization. Concentration-time profiles for glucose, insulin, and c-peptide were compiled and analyzed using the MinMod Millennium computer software (purchased from Dr. Richard Bergman, Keck School of Medicine of USC, Los Angeles, CA). Final

estimates were confirmed by direct calculation. Anthropometric data was collected at each visit, including height, weight, blood pressure and waist circumference. Body mass index (BMI) was calculated using weight in kg/(height in meters)².

Intervention

Each subject was issued a four week supply of 5,000 IU cholecalciferol capsules (BIO-TECH Pharmacal, Inc., Fayetteville, AR). Formulation purity and cholecalciferol content were verified by independent testing (Covance Laboratories Inc., Madison, WI). Following the baseline mFSIGT, subjects were instructed to take two capsules (10,000 IU) per day for 4 weeks (28 days). This was chosen as the optimal vitamin D replacement dose after review of the literature based on adequate serum levels and safe parameters for rapid replacement (16–19). Periodic telephone communication with each subject suggested proper compliance during the 4 week period.

Outcomes measures

With each mFSIGT test the glucose and insulin levels were analyzed to determine the insulin sensitivity index (SI), our primary outcome measure. Serum calcium, serum albumin and intact parathyroid hormone (PTH) were also measured. Calcium was corrected for the serum albumin ($((4 - \text{albumin}) * 0.8) + \text{calcium}$). Estimates of SI were determined from Minimal Model fits of insulin and glucose concentration-time profiles.

Statistics

Statistical significance of differences was determined using paired-t test analyses. When necessary, data were log transformed for parametric testing. Correlations between SI and other measured parameters were determined by general linear regression modeling. Unless otherwise stated, data are presented as mean $\pm$ SD.

Results

Eleven subjects were consented and completed pre- and post-intervention assessment with the mFSIGT. However, Vitamin D measurements were reported only after completion of all testing. Consequently, three subjects were excluded from analysis as they were not VDD at baseline. Therefore, eight eligible subjects (3 men, 5 women) who completed the study were used for the final analysis.

Table 1 gives the patient characteristics, pre- and post-25-hydroxyvitamin D levels, pre- and post-PTH levels, and pre- and post-SI values of these 8 patients. On average our subjects were obese; the range of BMI varied from 22.6 to 45 and with a BMI >30 in 50% of the subjects. However, the subjects did not have other markers of the metabolic syndrome based on normal lipids and blood pressure values (not shown). After one month of Vitamin D supplementation, there was no significant change in any of these parameters, including the weight..

25-hydroxyvitamin D levels increased after the intervention from 20 ± 7 ng/mL pre-intervention to 45 ± 8 ng/mL post-intervention, $p < 0.01$. This shows both the adequacy of the chosen replacement dose and excellent subject compliance. While there was no significant change in corrected serum Ca^{++} , as expected the parathyroid hormone (PTH) levels decreased ($p < 0.05$). (Table 1 and Figure 1). The decline in PTH documents that the rise in Vitamin D had significant physiologic effects after one month of Vitamin D supplementation.

We used the mFSIGT along with the MINMOD program to obtain parameters of insulin kinetics and glucose disposal. We noted that seven of the eight subjects had improvement in their insulin sensitivity. We found that the acute insulin response to glucose (AIRg) was decreased (364 ± 347 mU/L $\times$ 10 min pre-treatment to 293 ± 402 post-treatment, $p = 0.011$) suggesting that less insulin was released after Vitamin D treatment. More importantly, the insulin sensitivity index (Si) increased after Vitamin D treatment (from $4.57 \pm 3.57 \times 10^4$ min⁻¹/mU·ml pre-treatment to 6.50 ± 4.83 post-treatment, $p = 0.0124$) (Table 1 and Figure 2). We also noted a trend toward a positive correlation between the initial Si and the increment in Si after treatment (Figure 3, $p = 0.09$). Since the Disposition Index is calculated by the product of the Si and the AIRg, and these two parameters responded in opposite directions, we did not find a statistically significant change in the disposition index (DI) ($1653 \pm 2103 \times$ min⁻¹ pre-treatment to 1993 ± 2706 post-treatment).

Discussion

The influence of vitamin D on beta cell function and insulin sensitivity has been demonstrated in animal and *in vitro* studies. In human studies, a strong correlation exists between vitamin D deficiency and reduced insulin sensitivity (14). However, the therapeutic usefulness of these findings has been difficult to demonstrate. Interventional trials using vitamin D supplementation in subjects with existing type 2 diabetes have shown no overall improvement in beta cell function or insulin resistance (6, 11, 12). These trials were limited by a low therapeutic exposure to vitamin D and variable duration of type 2 diabetes in the study populations. With increasing severity or duration of diabetes the therapeutic benefit of vitamin D was limited. Interestingly, subset analyses from two of these trials revealed trends toward improved insulin sensitivity in subjects with more recent onset of type 2 diabetes. These findings led to our study question focusing on vitamin D deficient subjects with impaired fasting glucose (IFG) using an aggressive vitamin D replacement regimen. The success of our approach is evidenced by the 37% increase in insulin sensitivity. Because of this dramatic increase we were able to demonstrate a highly significant increase in SI with fewer patients than predicted by our initial power analysis.

Our findings also may help to explain the apparent discrepancy in the efficacy of vitamin D supplementation previously reported. We found a positive correlation between the initial SI and the increase in SI after Vitamin D supplementation (Figure 3). This observation lends support to the hypothesis that Vitamin D is beneficial only when given before the onset or early in the course of development of Type 2 diabetes.

We chose to define VDD in our subjects by a serum 25-hydroxyvitamin D of less than 30 ng/ml. We realize that this value is controversial because the optimal concentration for bone health is reported to be anywhere from 20 to 30 ng/ml (20). It is possible that if we selected subjects with significantly lower levels we may have seen even more improvement in SI. However, it is also possible that the high dose we used to raise the 25-hydroxyvitamin D to 45 ng/ml led to improvement in SI that wouldn't have been seen at lower doses. The optimum concentration of 25-hydroxyvitamin D for non-bone responses has to be determined in future studies.

Our study is the first to demonstrate a significant improvement in insulin sensitivity in subjects with impaired fasting glucose after oral vitamin D supplementation. It also demonstrated that use of high dose cholecalciferol, 10,000 IU per day, is safe and not associated with hypercalcemia, nephrolithiasis, or other recognizable side effects in our study.

Potential confounding variables after vitamin D supplementation include effects on calcium and parathyroid hormone. Calcium is known to influence insulin secretion and insulin sensitivity. In our study there was no change in mean corrected calcium concentration after the intervention and, thus, it is not likely to have been a factor in the improved SI. There are conflicting data regarding the effects of PTH on insulin sensitivity (21, 22). We did not find a significant correlation by linear regression between PTH values and the improved SI suggesting that this is not a contributing factor in the improved SI demonstrated in our study.

The mechanism of improvement in SI was not evaluated in this study. It would be of interest to determine whether high dose Vitamin D in the pre-diabetic subjects influenced systemic inflammatory markers as this has been suggested to also play a role in the association of VDD with type 2 diabetes (23, 24). Additionally, we wish to point out some of the limitations of our study. Firstly, the study population was only 8 patients. The relatively small sample size limits the overall conclusions we can draw from this study. For example, potentially interesting correlations could not be analyzed with confidence due to the small sample size. Secondly, the study design was an open label study which lends itself to potential bias including subtle changes in diet. Clearly a much larger, randomized, double-blind, placebo controlled study is required to confirm and extend our observations.

In summary, our data show that insulin sensitivity in vitamin D deficient subjects with IFG improves after one month of treatment with cholecalciferol 10,000 IU/day. This result was independent of serum calcium, PTH, and BMI.

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Abbreviations

AIRg	Acute Insulin Response to Glucose
BMI	body mass index
DI	Disposition Index
DM-2	type 2 diabetes mellitus
GCRC	general clinical research center
mFSIGT	modified frequently sampled intravenous glucose tolerance test
SI	Insulin Sensitivity
VDD	vitamin D deficiency
Vitamin D	cholecalciferol

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Figure 1. Corrected Calcium and PTH Before and After Vitamin D Supplementation. Corrected calcium remained unchanged after vitamin D supplementation. PTH decreased significantly ($p < 0.05$) after vitamin D supplementation. Each value is the mean $\pm$ SEM of 8 subjects.

Figure 2. Acute insulin response to glucose (AIRg) and Insulin sensitivity (SI) before and after Vitamin D supplementation. Each point represents an individual subject. The line and bars are the median and interquartile range.

Figure 3. Relationship between the initial insulin sensitivity and the increment in insulin sensitivity. Each point represents an individual subject. The line is the least squares regression equation with the correlation coefficient = 0.619 and $p=0.09$.

Table 1

Subject	Age	Weight	Ethnicity	Pre-D	Post-D	Pre-PTH	Post-PTH	Pre-SI	Post-SI
1	62	74	AI	20	57	32	37	1.0703	1.9500
2	20	90	AI	19	38	78	56	3.3824	3.2214
3	62	72	AI	22	54	50	56	1.8190	5.0846
4	52	99	C	14	41	68	37	3.4515	4.3265
5	41	118	H	9	33	92	51	5.6908	6.1444
6	44	123	C	29	41	60	37	12.085	16.900
7	58	101	C	24	43	32	32	2.4910	4.4635
8	34	63	C	28	51	65	41	6.5524	9.7751

Age is in years and weight is in Kg. Ethnicity abbreviations are: AI = African Immigrant, C = Caucasian, H = Hispanic. 25-hydroxyvitamin D levels are in ng/ml, PTH levels are pg/ml, and SI is insulin sensitivity in $1/(mU*ml/(min*(10^{-4})))$