Vitamin D Levels in Subjects With and Without Type 1 Diabetes Residing in a Solar Rich Environment

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**Objective**- Previous studies, largely in Northern Europe, have suggested an association between type 1 diabetes (T1D) and reduced serum 25(OH)-vitamin D levels; a concept we tested in individuals residing in a solar rich region (Florida, USA).

**Research design and methods**- Serum samples from 415 individuals residing were cross-sectionally analyzed: 153 controls, 46 new-onset T1D patients, 110 established T1D patients (samples > 5 months from diagnosis), and 106 of their first-degree relatives.

**Results**- 25(OH)-vitamin D levels (median ng/ml; range; IQR) were similar amongst controls (20.1; below detection (bd)-163.5; 13.0-37.4), new-onset T1D patients (21.2; bd-48.6; 12.2-30.2), established T1D patients (23.2; bd-263.8; 13.8-33.9), and first-degree relatives (22.15; bd-59.9; 12.7-33.1) ($P=0.87$). Mean 25(OH)-vitamin D levels were less than the optimal WHO level of 30 ng/ml in all study groups.

**Conclusions**- Reduced serum 25(OH)-vitamin D levels were not specifically associated with T1D. The uniform suboptimal 25(OH)-vitamin D levels, despite residence in a zone with abundant sunshine, support additional dietary vitamin D fortification practices.
The role for environment in the development of type 1 diabetes (T1D) has remained elusive, with multiple factors purported to modulate risk for this disease (e.g., viruses, breastfeeding, age for cereal introduction, childhood immunizations) (1,2). Further to this list is vitamin D (3), with previous studies suggesting T1D patients had lower serum concentrations of this metabolite than healthy controls (4-6), as well as disease associated polymorphisms in a vitamin D metabolism gene (7). While certainly intriguing, we noted the aforementioned studies were largely undertaken in northern European countries (4,5), while the one study performed in the United States failed to provide values amongst healthy controls and hence, did not identify disease specificity (6). Therefore, we measured serum 25(OH)-vitamin D levels from T1D patients, their first-degree relatives, and healthy controls; all residing in a solar rich region (Florida, USA).

**RESEARCH DESIGN AND METHODS**

Serum from 415 individuals were obtained from healthy controls (T1D autoantibody negative; no family history of T1D; median age 22.0 yr; range 5.0-65.1 yr; 84 females (F); 153 total), new-onset T1D, ≤ 5 months duration (12.2 yr; 5.9-35.0 yr; 23F; 46 total), established T1D beyond 5 months duration (16.0 yr; 5.1-62.6 yr; 50F; 110 total) and relatives of those with T1D (21.0 yr; 1.0-62.6 yr; 54F; 106). All samples were collected under informed consent with University of Florida IRB approval. As a retrospective study of de-identified samples, no information regarding sun avoidance routines or dietary practices (including vitamin D fortification) were available, nor were methods for case matching permissible.

25(OH)-vitamin D levels were quantified in duplicate with a commercial EIA kit (ALPCO; Salem, NH); an analyte shown previously as stable in storage (8). This assay measures both D2 and D3 forms of 25(OH)-vitamin D. The intra- and inter-assay coefficients of variation for this assay were 10.7% and 13.2% respectively. The lower limit of detection was 2.56 ng/ml. 25(OH)-vitamin D deficiency was defined as less than or equal to 20ng/ml, insufficiency as 21-30ng/ml and sufficiency as >30ng/ml (9,10).

Analysis of multiple, unpaired group comparisons were achieved using the non-parametric Kruskal-Wallis test with Dunn’s post-test to correct for multiple comparisons (11). Age and 25(OH)-vitamin D relationships were analyzed by linear regression, with Fisher’s exact test (two-tailed) used to compare proportions of subjects deemed insufficient. Power calculations were performed post hoc with GraphPad StatMate (GraphPad Software Inc., San Diego, CA, www.graphpad.com) version 2.00, revealing an 80% power to detect a 10.5 ng/ml difference in 25(OH)-vitamin D levels.

**RESULTS**

25(OH)-vitamin D levels (median ng/ml; range; IQR.) were: healthy controls (20.1; below detection (bd)-163.5; 13.0-37.4), new-onset T1D (21.2; bd-48.6; 12.2-30.2), established T1D (23.2; bd-263.8), and first-degree relatives (22.2; bd-59.9; 12.7-33.1) (Fig.1A). The medians were not different amongst individuals in these cohorts (P=0.87). Suboptimal 25(OH)-vitamin D levels (≤ 30ng/ml) were observed in 70.1% of
controls, 76.1% of new-onset T1D subjects, 68.5% of established T1D patients, and 68.8% of relatives; values that while low, were not significantly different from each other ($P=0.46$).

Comparison of age with serum 25(OH)-vitamin D levels indicated that for all groups combined, $r^2 = 0.004; P=0.22$. For individual groups: healthy controls, $r^2 = 0.010, P=0.21$; new-onset T1D, $r^2 = 0.0001, P=0.96$; established T1D, $r^2 = 0.013, P=0.24$; and relatives, $r^2 = 0.075, P=0.005$. Hence, regression analysis revealed no trend in 25(OH)-vitamin D levels as it pertained to overall age.

Since sunlight plays a major role in vitamin D synthesis, we then examined 25(OH)-vitamin D levels as a function of the month the sample was drawn, as a surrogate marker of UV-B exposure (Fig. 1C). Comparison of the 25(OH)-vitamin D levels between each three-month block showed no significant difference ($P=0.78$). Further analysis revealed no significant differences on a monthly exposure basis when examining controls versus T1D new-onset, established T1D or first-degree relatives ($P=0.71$)

**CONCLUSIONS**

Our study did not find significant differences in 25(OH)-vitamin D levels between healthy controls, subjects with T1D and their first-degree relatives in samples obtained in a solar rich region of the United States. However, more surprisingly, we identified that within each group, there exists a high frequency of vitamin D insufficiency even, in the sun rich environment of Florida.

For analysis of UV exposure, we elected to group samples by similarity in UVI (in order to increase statistical power). In addition, we performed analysis in a month-by-month fashion. By either method, no difference amongst the study groups was observed. While we may have introduced an ecological fallacy bias in assigning UVI aggregate data to individual subjects, other factors such as sun avoidance practices to inadequate supplementation may also account for the low 25(OH)-vitamin D levels observed in this cross sectional study. These biases may also be present in studies examining geographical distribution (12). Another possibility is that at Florida’s latitude, the duration of sunlight hours/day does not vary as dramatically as that which occurs closer to the geographical poles; thereby providing a mechanism to explain our lack in seasonal variation for 25(OH)-vitamin D levels.

With respect to race and ethnicity, a variable that has previously been noted to influence 25(OH)-vitamin D levels (13), our study was reflective of the prevalence of T1D amongst all races and given the predominance for this disease in Caucasians, it was not of sufficient power to analyze such a question. That said, the frequency of samples from non-Caucasians did not differ significantly amongst the study groups (data not shown). Indeed, the issue of sample size is one worth noting. As mentioned previously, the study herein was of a size larger than those reporting a negative association between serum 25(OH)-vitamin D levels and T1D, with our current study (based on its size) having a 20% chance of reporting a type 2 error. Hence, future efforts, that are larger, prospective, and take into account analysis of additional factors (e.g., skin type, vitamin D fortification practices, photoprotection behaviors, time spent outdoors, etc.) that may influence serum 25(OH)-vitamin D, would be beneficial to perform. This, as well as the question addressing 25(OH)-vitamin D levels in a
Northern U.S. state, should be addressed in the future.

Given the amount of UV available to those residing in Florida and the fortification of milk products with vitamin D, the low serum levels of 25(OH)-vitamin D found add credence to the recent recommendation by the American Academy of Pediatrics to double the amount of vitamin D supplementation provided to children (14). Given our results contrast with those of several other efforts (4-6), additional studies utilizing a prospective cohort design to further define the role of vitamin D in the pathogenesis of T1D are urgently needed, as trials using active forms of this metabolite for T1D prevention are actively being considered.

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**Figure Legend**

**Figure 1** - 25(OH)-vitamin D levels in cohorts based on parameters of disease, age or estimated solar exposure. For disease status (A), values are presented as a function of study group, with definitions of insufficiency (orange line) and deficiency (red line) provided. With respect to age (B), shown are the values for all study participants independent of cohort; with the definitions of insufficiency and deficiency as defined in (A), along with age correlation (blue line). In (C), estimated average UV-B exposure for the entire study population is presented. UV Index (UVI) climatological data was obtained from the National Weather Service (NWS) and United States Environmental Protection Agency (EPA) websites (http://www.cpc.ncep.noaa.gov and http://www.epa.gov) to determine relative UV exposure. Based on the previous five yr worth of data, for the proximate city of Jacksonville, FL we established UV exposure monthly means: January: 3.215, February: 4.08, March: 5.96, April: 7.68, May: 8.238, June: 8.578, July: 8.976, August: 8.254, September: 6.902, October: 5.11, November: 3.694, and December: 2.79. The numbers correspond to the UVI scale (1-11+) developed by the NWS and EPA and implemented by the World Health Organization. The samples were grouped according to month drawn, and placed into one of four possible 3-month blocks, each block formed on the basis of similar UV-B indices. The 25(OH)-vitamin D levels (reported as median ng/ml; range; IQR) for the November/December/January group of 112 samples (20.7; bd-263.8; 12.7-33.6) with an average estimated UV exposure of 3.23. The October/February/March group of 113 samples (20.8; bd-146.8; 12.7-31.5) with an average estimated UV exposure of 5.05. The September/April/May group of 84 samples (19.3; bd-163.5; 14.0-36.9) with an average estimated UV exposure of 7.61. The June/July/August group of 106 samples (23.9; bd-82.9; 13.4-35.6) with an average estimated UV exposure of 8.60.
REFERENCES
