Effect of Vitamin D supplementation on Insulin Resistance in Vitamin D-Deficient Obese Saudi Females; Randomized Controlled Trial

Noura S. Al-Mulhim, MS, Rabia Latif, PhD, Lubna I. Al-Asoom, PhD, Ahmed A. Al-Sunni, PhD, and Tharwat G. Eldin, PhD

Department of Physiology, College of Medicine
University of Dammam, Dammam, Saudi Arabia

Correspondence
Dr. Rabia Latif
Department of Physiology, College of Medicine
University of Dammam
P.O. Box 1982
Dammam 31411, Saudi Arabia
e:M. rhussain@uod.edu.sa

Abstract
This study evaluates the effects of vitamin D supplementation on insulin resistance in vitamin D deficient obese Saudi females. Thirty vitamin D deficient obese female students were recruited from University of Dammam, Saudi Arabia and divided into a vitamin D group and a placebo group. Homeostasis model assessment of insulin resistance was compared between the groups and within the groups by two sample t test and paired t test, respectively. After treatment with 50,000 IU of vitamin D weekly for 8 weeks, the mean plasma levels of 25-hydroxy-vitamin D was significantly increased from 2.9 to 31.3 ng/ml in the vitamin D group. The results in the vitamin D supplemented group at baseline and at the end, for fasting plasma glucose were 97.90 ± 2.13 and 101.01 ± 1.67 mg/dl (P = 0.21), for insulin, 5.14 ± 3.22 and 4.68 ± 3.24 μU/ml (P = 0.15) and for Homeostasis model assessment of insulin resistance, 1.28 ± 0.81 and 1.23 ± 0.87 (P = 0.62), respectively. This data showed insignificant changes in insulin resistance after treatment with vitamin D, suggesting that vitamin D supplementation may not have any effect on insulin resistance in vitamin D deficient obese females.

Keywords
Insulin resistance, Obesity, Vitamin D, Randomized controlled trial.

Introduction
Type 2 diabetes mellitus accounts for approximately 90%–95% of all diabetes cases and has become increasingly prevalent worldwide. It is considered an “Epidemic of the 21st Century”, affecting approximately 382 million individuals worldwide and 3.5 million individuals in Saudi Arabia in 2013[11]. Recent evidence suggests that altered vitamin D (VD) homeostasis may play a role in the development of type 2 diabetes. Type 2 diabetes is characterized by impaired pancreatic β cell function and insulin resistance. Animal studies show that VD may influence both of these processes. In in vitro and in vivo studies, glucose-mediated insulin secretion was impaired in VD deficient subjects[2-6], whereas VD supplementation restored the insulin secretion[2,4-7]. The active form 1,25 dihydroxy-vitamin D binds to the VD receptor, which is expressed in pancreatic β cells[8,9]. The existence of the vitamin D response element in the human insulin gene promoter[10] and transcriptional
activation of the human insulin gene caused by 1,25 dihydroxy vitamin D\textsuperscript{11} further supported a direct effect of VD on insulin synthesis and secretion. Vitamin D has a direct effect on insulin sensitivity by stimulating the expression of insulin receptors in both muscle and adipose tissues\textsuperscript{12}. In addition, VD has anti-inflammatory and immuno-modulating effects, and might cause a decrease in insulin resistance and an increase in insulin secretion by modulating the immune system\textsuperscript{13,14}.

There is substantial evidence of alterations in the VD-endocrine system in obese subjects\textsuperscript{15}. Low levels of VD are common in association with obesity\textsuperscript{16,17}. Total body fat is inversely related to VD levels in women even after adjustment for age, lifestyle, and Parathyroid Hormone (PTH)\textsuperscript{18}. VD is stored in adipose tissue (AT), and released later on during the winter, when cutaneous production is low or absent\textsuperscript{19}. Suggested mechanisms underpinning the low levels of VD in obesity include; a decreased exposure to sun light\textsuperscript{20}, or a decreased hepatic activation of VD in obesity inhibited by elevated levels of 1,25-dihydroxy VD and PTH\textsuperscript{21}. The expression levels of VD-metabolizing enzymes have been shown to decrease in the adipose tissue of obese subjects\textsuperscript{22}, signifying that the ability of adipose tissue to metabolize VD can be dynamically altered during obesity and weight loss.

The above mentioned facts suggest a close relationship between plasma levels of VD, Obesity and Insulin resistance. Several VD–interventional trials have been conducted worldwide to explore the link among these three parameters; however the data from these trials are inconsistent and lack conclusive evidence\textsuperscript{23-29}. Moreover, none of the Vitamin D intervention studies have been conducted in KSA; a country where prevalence of VD deficiency in females has been found to be 99\%\textsuperscript{30}. Low levels of VD are more common in Saudi females and in the younger age groups; most likely due to the wearing of traditional clothes, limited exposure to sun, and inadequate dietary intake. The current study; first VD intervention trial in KSA; aimed to investigate the effects of oral vitamin D supplementation on glucose homeostasis in Vitamin D deficient Saudi obese adolescent females.

**Materials and Methods**

Approval of this randomized, placebo controlled study was granted by Research and Ethics Committee of University of Damman. All Saudi females studying at health colleges in the university (N = 698) were assessed for eligibility criteria. The inclusion criteria were 18-23 years old, obese (BMI > 30 kg/m\textsuperscript{2}), VD deficient (serum 25 (OH) VD < 20 ng/ml (< 50 nmol/L\textsuperscript{24}) Saudi females willing to participate. Students with any history of systemic illness, regularly taking multivitamins especially VD, pregnancy or lactation were excluded from the study. Out of 44 students meeting the inclusion criteria, 10 students withdrew consent for personal reasons and 4 students were excluded due to recent major weight loss.

Study participants were randomly assigned into VD group (received 50,000 IU/wk of vitamin D3 drops) or placebo group (received normal saline drops/wk) for 8 weeks duration. A block randomization procedure with serial entry in blocks was used\textsuperscript{28}. Four participants were included in each block, ensuring that within each block two participants were allocated to VD group and two were allocated to placebo group. All participants from both groups received their dose under supervision in the Physiology Department Laboratory, College of Medicine, University of Damman. Participants were advised to maintain their usual diets and avoid taking VD supplementation on their own throughout the study period.

VD status was assessed before randomization to involve only VD deficient subjects. VD level was measured by serum 25 (OH) ELISA instead of gold standard methods; high performance liquid chromatography (HPLC) and Liquid Chromatography: Tandem Mass Spectrometry Method (LC-TM) because ELISA is relatively cheap and has sensitivity comparable to HPLC and LC-TM (31). Fasting blood samples were obtained at the baseline and at the end of 8-weeks supplementation period and then centrifuged to separate the serum.

Fasting glucose was automatically measured by using glucometer (ACC-Chek G, Roche Diagnostics GmbH, Mannhein, Germany) which has been reported to perform well and give accurate results\textsuperscript{32,33}. Since whole blood glucose values are typically lower than the plasma values, those were subsequently converted to equivalent plasma glucose, after multiplication with a constant factor 1.11\textsuperscript{34}.

Serum insulin was measured by insulin (human) ELISA kit\textsuperscript{28}. (Phoenix Pharmaceuticals Inc., Mannhein, Germany) (Cat. No. EK-035-06) with intra- and inter-assay variations < 10\% and < 15\%, respectively.

Insulin sensitivity was estimated by homeostasis model assessment of insulin resistance (HOMA-IR) using the following formula \textsuperscript{35}:

$$\text{HOMA-IR} = \frac{\text{Fasting Plasma Glucose} \times \text{Fasting Insulin}}{405}$$

Fasting insulin and fasting glucose were measured in (\textmu U/ml) and (mg/dl)\textsuperscript{36}.

HOMA-IR is the most frequently employed simple technique both in clinical practice and in epidemiological studies. HOMA-IR has a high correlation with measures of insulin sensitivity obtained from the euglycemic clamp procedure\textsuperscript{37}. High HOMA-IR scores denote low insulin sensitivity (increased insulin resistance).
Statistical Analysis

The data were analyzed using Microsoft Excel (Microsoft Inc., Redmond, WA USA) version 2010 and the Statistical Package of Social Science (IBM SPSS Statistics, Armonk, New York, USA) version 20. Data are presented as means ± Standard Error of Mean (SEM). Differences between groups were assessed by a two-sample t test. Paired sample t test was used to compare changes within each group (pre- and post-treatment) of all variables. A ‘p’ value of < 0.05 was taken as statistically significant.

Results

The baseline characteristics of all participants are represented in Table 1 and Figure 1. There were no significant differences in age (20.26 years vs. 19.67; P = 0.25), Body Mass Index (32.19 vs. 34.53; P = 0.09) or plasma 25-hydroxy-vitamin D (25(OH)D) (3.089 ± 1.06 ng/ml vs. 2.9 ± 0.93; P = 0.7) between groups at baseline. After treatment with 50,000 IU of cholecalciferol weekly for 8 weeks, the mean plasma levels of 25(OH)D was significantly increased from 2.9 to 31.3 ± 9.2 ng/ml in vitamin D group (Table 2). Fasting plasma glucose significantly decreased in Vitamin D group compared with placebo (two-sample t test) and within the placebo group (paired t test). Comparison within groups as done by paired t test revealed statistically insignificant differences in HOMA-IR in both groups (Table 3).

Table 1. Baseline characteristics of study participants.

<table>
<thead>
<tr>
<th></th>
<th>Placebo (Mean ± SEM)</th>
<th>Vitamin D (Mean ± SEM)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D (ng/ml)</td>
<td>3.089 ± 1.06</td>
<td>2.9 ± 0.93</td>
<td>0.7</td>
</tr>
<tr>
<td>FPG (mg/dl)</td>
<td>94.79 ± 2.52</td>
<td>97.90 ± 2.13</td>
<td>0.354</td>
</tr>
<tr>
<td>Insulin (µIU/mL)</td>
<td>6.25 ± 3.29</td>
<td>5.14 ± 3.22</td>
<td>0.811</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.32 ± 0.65</td>
<td>1.28 ± 0.81</td>
<td>0.9</td>
</tr>
</tbody>
</table>

*Differences between groups were compared using a two sample t test at P < 0.05.

Table 2. Post-treatment characteristics of study participants.

<table>
<thead>
<tr>
<th></th>
<th>Placebo (Mean ± SEM)</th>
<th>Vitamin D (Mean ± SEM)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D (ng/ml)</td>
<td>5.7 ± 1.6</td>
<td>31.3 ± 9.2</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>FPG (mg/dl)</td>
<td>106.56 ± 1.49</td>
<td>101.01 ± 1.67</td>
<td>0.020</td>
</tr>
<tr>
<td>Insulin (µIU/mL)</td>
<td>6.28 ± 3.22</td>
<td>4.68 ± 3.24</td>
<td>0.728</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.73 ± 0.90</td>
<td>1.23 ± 0.87</td>
<td>0.694</td>
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</table>

*Differences between groups were compared using a two sample t test at P < 0.05.
P < 0.05; †P < 0.001

Table 3. Comparison of Pre and Post-treatment values in vitamin D and placebo groups.

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Vitamin D</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre Test</td>
<td>Post Test</td>
<td>P-values</td>
<td>Pre Test</td>
</tr>
<tr>
<td>25 - OHD (ng/ml)</td>
<td>3.089 ± 1.06</td>
<td>5.52 ± 1.71</td>
<td>0.001</td>
</tr>
<tr>
<td>FPG (mg/dl)</td>
<td>94.79 ± 2.52</td>
<td>106.56 ± 1.49</td>
<td>0.01*</td>
</tr>
<tr>
<td>Insulin (µIU/mL)</td>
<td>6.25 ± 3.29</td>
<td>6.28 ± 3.22</td>
<td>0.96</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.32 ± 0.65</td>
<td>1.73 ± 0.90</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Values are reported as Mean ± SEM. Comparison within groups was done by paired t test.
P < 0.001

Figure 1. Baseline characteristics of study participants.
**Discussion**

Out of 698 students screened, only 6.59% were found to be obese. This prevalence of obesity is much less than the prevalence reported in previous studies. In a community-based national epidemiological health survey conducted in 2005, the prevalence of obesity was found to be 35.5%[17]. Another survey involving only the eastern province found the overall prevalence of obesity to be 43.8%[18]. The reason for low obesity prevalence in these study subjects could be that these students at health colleges were more educated about the health issues relating to weight and so were actively managing their weight.

Prevalence of VD deficiency found in these subjects was 94%. It is in accordance with previous studies. Al Erq[19] found VD deficiency prevalence to be 99% in female medical students in Eastern province of KSA. Alfawaz[20] stated the over-all prevalence of vitamin D deficiency as 78.1% in Saudi females; significantly associated with increasing weight. Ardawi et al.[21] reported prevalence to be 80% in Saudi females; largely attributed to obesity and poor exposure to sunlight due to their traditional clothing.

In the present study vitamin D3 treatment (50,000 IU/Wk) for 8 weeks led to increased mean plasma levels of 25(OH)D from deficient levels (< 20 ng/ml) to sufficient levels (> 30 ng/ml) and this finding seems to be consistent with a previous study which found that high doses of cholecalciferol supplementation for 8 weeks would be enough to increase the mean plasma levels of 25(OH)D to sufficient range[22].

The significant difference between placebo and vitamin D- treated groups in plasma glucose levels was probably due to significantly increased glucose in placebo group (pre vs. post). The reason for this is not clear but it may be due to the effect of exam stress which led to excess secretion of cortisol and catecholamines since the blood collection from both groups was just before the exams. Lack of significant increase in blood sugar in vitamin D supplemented group might be because of cortisol-antagonizing effects of vitamin D which needs further exploration.

This study did not show any significant improvement in insulin sensitivity after high dose of VD supplementation for 8 weeks. This finding is in agreement with Pittas et al. (2007)[23] who showed no significant difference in a 3 year clinical trial in insulin sensitivity between placebo and VD groups in subjects with normal fasting glucose. Also, the present finding seems to be consistent with that of Nagpal et al.[24] who found that short term oral supplementation with VD for 6 weeks in healthy, centrally obese men had no effect on HOMA-IR. Recently in a double blind, placebo controlled randomized clinical trial, Wamberg et al.,[25] showed lack of effect of 26 weeks vitamin D supplementation on insulin resistance in vitamin D deficient obese (BMI > 30 kg/m²) adults. In another double-blind, randomized, control study[26] with prediabetic subjects; a high dosage of vitamin D supplementation (88,865 IU/week) for 1 year had no effect on insulin sensitivity. Interventional trials using vitamin D supplementation in subjects with existing type 2 diabetes have also shown no overall improvement in insulin resistance[43-48]. Similarly, two other randomized trials conducted on obese adults showed that VD supplementation had no effects on glycemia, insulin resistance, and insulin secretion[27,28].

On the other hand, few studies have shown an increase in insulin sensitivity after VD supplementation. Nazarian et al.[46] showed that insulin sensitivity improved in subjects with VD supplementation compared with placebo. In contrast to this study, they used an aggressive VD replacement regimen (10,000 IU/day vs. 50,000 IU/week), and their subjects were having impaired fasting glucose vice normal fasting glucose. Similarly von Hurst et al.,[47] showed that administration of 4000 IUVD daily for 6 months to insulin resistant South Asian women, aged 23–68 years resulted in attenuation of insulin resistance. Their VD administration protocol (daily dosage, longer study duration) is different from this study’s (weekly doses, shorter study duration). While the less frequent doses may be beneficial from a compliance viewpoint; daily doses have been shown to be more effective than weekly or monthly doses as measured by serum 25(OH)D[48]. It seems that this study’s intervention did not raise VD concentration to a level at which insulin resistance is attenuated. Moreover, subjects participating in von Hurst et al. research[48] were insulin resistant in contrast to this study’s subjects who were not insulin resistant (in obese adolescents, HOMA-IR values greater than 3.2 are considered insulin resistant)[49]. There is a possibility that VD supplementation may affect insulin sensitivity differently in those with impaired insulin resistance.

**Conclusion**

Correction of VD deficiency seems to have insignificant effects on blood glucose levels, insulin concentration, and insulin sensitivity in young obese female subjects with normal baseline insulin resistance index HOMA-IR.

**Limitations**

The limitations of this study include the small sample size and the short duration of the study.

**Acknowledgment**

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References


تأثير مكملات فيتامين (د) على مقاومة الأنسولين للسعوديات اللاتى يعانين من السمنة ونقص فيتامين "د" : تجربة عشوائية ضابطة

نوره الملحم، ورابة لطيف، وليلى العسوم، وأحمد السني، وثروت جمال الدين

قسم علم وظائف الأعضاء، كلية الطب، جامعة الدمام

الدمام - المملكة العربية السعودية

الملخص: أقيمت الدراسة على عدد 30 طالبة سعودية في جامعة الدمام مصابات بالسمنة وليدين نقص فيتامين "د" تم تقسيمهم إلى مجموعتين كالتالي: 1) مجموعة فيتامين "د" (تناول جرعة واحدة من قطرات كوليكالسيفيرول (5000 وحدة دولية) كل أسبوع لمدة 8 أسابيع) 2) المجموعة الضابطة: تناول جرعة واحدة من قطرات محلول ملحي. تم مقارنة مقاومة الأنسولين عن طريق تقييم نموذج التوزان من مقاومة الأنسولين بين المجموعتين وداخل المجموعتين وتم استخدام اختبار (t) للتحليل الإحصائي. متوسط مستويات البلازما 25-هيدروكسي فيتامين "د" زادت بشكل ملحوظ بعد العلاج بجرعة واحدة أسبوعية من 2.0 إلى 3.13 نانوغرام/مل في مجموعة فيتامين "د". نتائج مجموعة فيتامين "د" قبل العلاج وبعده بالنسبة لمستوى السكر في الدم أثناء الصيام كانت 2.04±0.16 ملغ/ديسيتر (P=0.01) للانسولين 4.22±0.24 ميكرو وحدة دولية/مل (P=0.01) ومقاومة الأنسولين 0.19±0.07 (P=0.01). أظهرت نتائج الدراسة تغييرات غير معترضة إحصائيا بالنسبة لمقاومة الأنسولين بعد العلاج بفيتامين "د" لدى النساء المصابات بالسمنة مع نقص فيتامين "د". "

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