



Effect of Vitamin D Supplementation on Insulin Resistance Status in Pubertal Girls with Congenital Adrenal Hyperplasia

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Abstract

INTRODUCTION: Congenital adrenal hyperplasia (CAH) patients especially females are at increased risk for developing obesity and resistance to insulin (IR). Rising evidence indicates that insufficient Vitamin D levels are associated with impaired function of β -cells of islets of Langerhans and IR; however, this finding has not been investigated in patients with CAH.

AIM: The aim was to identify the Vitamin D levels and IR resistance status in pubertal females with CAH, following up at Diabetes Endocrine and Metabolism Pediatric Unit, Abou ElRish Children's Hospital, Cairo University. We also examined the association between Vitamin D level and IR resistance and the impact of cholecalciferol administration on metabolic abnormalities.

SUBJECTS AND METHODS: Sixteen pubertal CAH females with deficient or insufficient Vitamin D levels were recruited. Follow-up was done after a period of 6 months during which oral Vitamin D replacement therapy was prescribed.

RESULTS: The serum levels of 25-hydroxy Vitamin D (25(OH)D3) were significantly raised following replacement therapy. Significant correlation was detected between homeostasis model assessment of IR sensitivity (HOMA-IR) and serum levels of insulin, in addition to other IR indices both before and following Vitamin D supplementation. However, HOMA-IR did not correlate with any of the following: 25(OH)D3 levels, 17 hydroxyprogesterone (17-OHP), or steroid dose either before or following replacement therapy.

CONCLUSION: Vitamin D replacement therapy did not have favorable effects on IR in females with CAH. Future research using randomized controlled trials is required to examine the impact of Vitamin D on IR. This might influence the production of adrenal androgen, resulting in reduction of the therapeutic effect of glucocorticoids and evolution of metabolic derangements.

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Introduction

Lifelong steroid replacement has to be maintained in subjects with congenital adrenal hyperplasia (CAH), to decrease the overall excessive production of androgens by the adrenal gland, and to provide the everyday cortisol needs [1]. Optimizing steroid dosing is crucial in children to enhance growth outcome, bone health and hence improve quality of life. Patients with classic CAH have an increased frequency of growth suppression, obesity especially visceral adiposity, elevated insulin levels, Insulin resistance (IR), hyperandrogenism, and peripheral precocious puberty that might trigger activation of the hypothalamic-pituitary axis prematurely, compared to normal individuals [2]. Abnormal adrenomedullary function and periods of intermittent hypercortisolism, due to the use of supraphysiological doses of corticosteroid during treatment, may contribute to the development of these aberrations and increase the risk of developing metabolic derangements especially

metabolic syndrome (MetS) and atherosclerosis in those patients [3].

Vitamin D has a crucial impact on keeping calcium/phosphorus homeostasis. However, other possible extra-skeletal functions of 25-hydroxy Vitamin D (25(OH)D3) are emerging [3]. Numerous researchers have disclosed the immunomodulatory effects of 25(OH)D3 [4] and that 25(OH)D3 deficiency may be accompanied by a state of sub-inflammation, IR, and MetS. These conditions are all considered insulin-resistant states, in which different chemical substances such as hormones and cytokines are involved. However, all these pathologic conditions are characterized by of chronic sub-inflammatory state [5]. Sub-inflammation has a crucial role in the development of IR and obesity/MetS, and it has been hypothesized that 25(OH)D3 deficiency might contribute to it [6].

Both insulin action and secretion have been thought to be influenced by optimal 25(OH)D3 levels [7]. It might not have a direct effect on IR; however, it could be through its favorable effects on adiposity. Other mechanisms have been implicated including increasing the expression of insulin receptors,

regulating the calcium homeostasis, and adjusting cytokine expression and activity. All these mechanisms may account for the beneficial effect of 25(OH)D₃ on insulin action in peripheral tissues [6]. Data from studies including human participants also indicate that low levels of 25(OH)D₃ are associated with impaired function of β -cells of Islets of Langerhans, IR, and poor glucose tolerance [8].

Numerous studies also indicated a reversed association between serum levels of 25(OH)D₃ and fasting plasma glucose, IR, and lipid panel except for HDL-cholesterol [5] which demonstrate the beneficial effect of administering 25(OH)D₃. However, evidence is still contradictory regarding its influence [9]. A number of interventional trials proposed that we are still lacking proof concerning the favorable effect of 25(OH)D₃ on metabolic aberrations especially IR [10]. Others indicated the lack of favorable impact of Vitamin D intake on metabolic profile [11].

Many studies have assessed the levels of 25(OH)D₃ in patients with CAH and have detected low levels especially in pubertal females compared to healthy controls [12]. In addition, a Turkish study was conducted to detect the prevalence and association between 25(OH)D₃ deficiency and bone density in individuals with CAH. This study detected the presence of significantly lower 25(OH)D₃ in females than in males especially during puberty [12]. However, the impact of Vitamin D replacement therapy on metabolic derangements in CAH patients has not been explored. Therefore, this pilot aimed at identifying the serum 25(OH)D₃ status in female patients with CAH who are following up at Diabetes Endocrine and Metabolism Pediatric Unit (DEMPU) clinic at Abou EIRish Children's Hospital, Cairo University and to detect the presence of metabolic abnormalities in those patients. We also examined the association between both aspects and the impact of Vitamin D supplementation on metabolic abnormalities as a mean to enhance the quality of life in those girls.

Patients and Methods

Patients

Our study was conducted between May 2016 and January 2017 (9 months duration), 16 female patients with classic CAH who were managed at DEMPU, Abou EIRish Children's Hospital, Cairo University were recruited, and verbal consent was obtained. Patients with clinical and laboratory diagnosis of classic CAH (salt-wasting) and age ≥ 8 and < 18 years were recruited. The study included only subjects with Vitamin D insufficiency or deficiency. All participants had a stable condition and were not receiving any medications other than steroids that might affect the secretion of androgen or bone metabolism. Patients who had sufficient Vitamin D or on

Vitamin D therapy were excluded from the study. The present study was approved by the bioethical research committee, Faculty of medicine, Kasr Alainy University Hospitals, Cairo, Egypt (approval number: I-100315).

After explanation of the methodology and obtaining verbal informed consent or assent from the patient and/or the patient's legal guardians, all included subjects were evaluated as follows:

- Thorough clinical examination to detect any associated conditions or complications, including anthropometric assessment and growth velocity during the study period (GV). Weight (kg) was measured using a Seca Scale balance and approximated to the nearest 0.1 kg. Standing height (cm) was measured using Harpenden stadiometer and approximated to the nearest 0.1 cm. Weight and height readings were then plotted against the Egyptian growth curves [13]. Standard deviation scores (SDS) for the above measurements were assessed by the growth vision computer software provided by Novo Nordisk.
- Pubertal staging was assessed using Tanner maturity scale [14].
- Patients were examined for signs of hyperandrogenism including hirsutism, acne vulgaris, weight gain, and menstrual irregularities [15]. Hirsutism was scored using Modified Ferriman–Gallwey score of ≥ 8 is considered hirsutism [16].
- The dose of glucocorticoids was expressed as mg/m²/day and the mean dose was calculated over the 1 year before recruitment. Overtreatment or the use of supraphysiological doses was defined as the use of hydrocortisone of more than 15 mg/m²/day.

Laboratory investigations

Venous blood samples were obtained from all patients following an overnight fast of 8–12 h before 25(OH)D₃ supplementation to measure serum levels of 25(OH)D₃, fasting serum insulin, fasting serum glucose, and 17 hydroxyprogesterone (17-OHP). Serum 25(OH)D₃ levels were assessed using enzyme-linked immunosorbent assay (ELISA) based on the principle of competitive binding. Vitamin D status of patients was determined according to the 25(OH)D₃ levels. Levels of serum 25(OH)D₃ levels above 30 ng/mL were considered normal. Levels of 10–29 ng/mL were insufficient and levels < 10 ng/mL were considered deficient [17]. Fasting serum insulin was assessed using Tosoh Bioscience AIA-360 System, AIA-360 Immunoassay System Diagnostic test. Reference range < 17 uU/ml according to the kit was used by Tosoh Bioscience, Inc. Fasting blood glucose (mg/dl) was assayed by GOD-POD method which is an *in vitro* test for the quantitative assay of glucose concentration

in serum by photometric system [18]. 17-OHP (ng/mL) was measured by ELISA using competitive method [19]. Serum 17-OHP levels of 1–10 ng/ml were acceptable [20]. Therefore, in this study, we considered patients with serum 17-OHP levels ≤ 10 were controlled and > 10 were uncontrolled cases.

The following IR sensitivity and resistance indices were calculated:

- HOMA-IR was calculated according to the following equation [21]:
(Fasting plasma IR level [uU/ml] \times fasting plasma glucose level [mmol/l]) divided by 22.5
HOMA-IR cutoff value ≥ 2.5 for adolescents, the highest for adolescent girls = 3.8 [22].
- HOMA-S (Homeostasis model assessment of IR sensitivity) was equal to 1/HOMA-IR.
- HOMA-B (Homeostasis model assessment of beta cell function) was determined as follows [23]:
(Fasting IR [in milliunit/l] \times 20/fasting glucose [in millimolar] – 3.5)
- IR sensitivity was measured using the quantitative Insulin sensitivity check index (QUICKI; $1/[\log I0 - \log G0]$, where I0 is fasting Insulin [in mIU/L] and G0 is fasting glucose [mg/dl]) [24]. It assesses Insulin sensitivity, which is the inverse of Insulin; a value of < 0.339 indicates Insulin [25].

Then, cholecalciferol was given to all patients in the form of oral daily doses (Decal B12 syrup) for 6 months (4000 IU for patients aged from 8 to 12 years, 6000 IU for patients aged more than 12 years old) [26].

Follow-up was done after 6 months of cholecalciferol therapy, physical examination was repeated including anthropometric measurements, clinical assessment was done to detect any change in Tanner staging, signs of hyperandrogenism, and hirsutism scoring. Laboratory investigations were done including 17-OHP, 25(OH)D3, fasting Insulin, fasting glucose with calculation of the HOMA-IR, HOMA-B, HOMA-S, and QUICKI.

Statistical methods

Statistical analysis was done using SPSS 22 (IBM Corp, Armonk, NY, USA). Categorical data were compared using Chi-square test. Independent sample t-tests or Mann–Whitney U tests were used for measured data when appropriate. Within group, comparison of numerical variables was done using Wilcoxon signed rank test for paired (matched) samples. Exact test was used instead when the expected frequency is < 5 . McNemar test was used to compare between pre- and post-treatment, correlation between various variables was done using Spearman rank correlation equation. $P < 0.05$ was considered statistically significant.

Results

Our study included 16 adolescent females with classic CAH. Anthropometric, clinical, and laboratory data of the studied cases prior to and after Vitamin D therapy are shown in (Tables 1 and 2) and (Figure 1). Mean 25(OH)D3 levels before and after treatment were 16.35 ± 5.24 ng/ml and 30.8 ± 10.6 ng/ml, respectively ($p = 0.0001$; Table 2). The whole group (16 participants) had 25(OH)D3 levels below 30 ng/ml; 13 patients (81.3%) had insufficient 25(OH)D3 (levels: 10–29 ng/ml); and 3 patients (18.7%) had deficient 25(OH)D3 (levels < 10 ng/ml) before treatment, and after the treatment, none of the participants had 25(OH)D3 deficiency, 8 patients (50%) had 25(OH)D3 insufficiency, and 8 patients (50%) had normal 25(OH)D3 levels (Table 1). Regarding the anthropometric data, there were statistically significant differences between BMI, BMI SDS, weight, weight SDS, height, and height SDS before and after Vitamin D ($p = 0.009, 0.024, 0.005, 0.048, 0.019,$ and 0.019 respectively). There was no significant difference regarding other laboratory data and steroid doses before and after Vitamin D (Table 2). Vitamin D levels correlated negatively with each of the weight SDS before and height SDS after Vitamin D therapy reaching statistical significance ($p = 0.02$ and 0.027 , respectively) (Table 3). Low height SDS despite of improvement of 25(OH)D3 level might be due to the negative impact of

Table 1: Anthropometric, clinical, and laboratory data of the study group before and after Vitamin D therapy

	Before	After
	Frequency (%)	
BMI SDS		
< 2 SDS	10 (62.5%)	9 (56.3%)
> 2 SDS	6 (37.5%)	7 (43.8%)
Weight SDS		
< 2 SDS	11 (68.7%)	9 (56.3%)
> 2 SDS	5 (31.2%)	7 (43.8%)
Height SDS		
< 2 SDS	15 (93.7%)	16 (100%)
> 2 SDS	1 (6.2%)	0
Hirsutism Score		
< 8	13 (81.3%)	13 (81.3%)
≥ 8	3 (18.8%)	3 (18.8%)
Symptoms of hyperandrogenism		
Yes	5 (31.3%)	5 (31.3%)
No	11 (68.8%)	11 (68.8%)
Tanner Staging		
T2	3 (18.8%)	3 (18.8%)
T3	3 (18.8%)	2 (12.5%)
T4	0	1 (6.3%)
T5	10 (62.7%)	10 (62.5%)
Vitamin D (ng/ml)		
< 10	3 (18.7%)	0 (0.0%)
October-29	13 (81.3%)	8 (50%)
≥ 30	0	8 (50%)
HOMA-IR		
< 2.5	10 (62.5%)	9 (56.3%)
> 2.5	6 (37.5%)	7 (43.8%)
< 3.8	12 (75%)	10 (62.5%)
> 3.8	4 (25%)	6 (37.5%)
Glucose (mg/dl)		
< 100	12 (75%)	13 (81.3%)
> 100	4 (25%)	3 (18.8%)
Insulin (uU/ml)		
< 17	11 (68.7%)	10 (62.5%)
> 17	5 (31.2%)	6 (37.5%)
17 OH Progesterone (ng/ml)		
< 10	3 (18.8%)	7 (43.8%)
> 10	13 (81.3%)	9 (56.3%)

Data are expressed as frequency and percent (%). BMI SDS: body mass index standard deviation score, Hirsutism Score: according to Modified Ferriman–Gallwey (MFG), which grades 9 body areas from 0 (no hair) to 4 (frankly virile), including the upper lip, chin, chest, upper abdomen, lower abdomen, thighs, back, arm, and buttocks with a score of ≥ 8 is considered hirsutism (19), HOMA-IR: homeostatic model assessment-insulin resistance, 17 OH Progesterone: 17 hydroxyprogesterone.

prolonged administration of supraphysiological doses of steroids on height especially during puberty which is a long-term effect. In addition, statistically significant positive correlation was found between HOMA-IR and IR level and HOMA-B before and after Vitamin D supplementation ($p = 0.0001$ for each) (Table 4). When correlating HOMA-IR with the other parameters, a statistically significant negative correlation was detected between HOMA-IR and each of HOMA-S and QUICKI both before and following Vitamin D therapy ($p = 0.0001$ for each) (Table 4). No statistically significant correlation between the steroid dose and all laboratory and anthropometric data was found before Vitamin D therapy.

Table 2: Comparison between anthropometric and laboratory data before and after Vitamin D therapy

n=16 cases	Before Vitamin D therapy	After Vitamin D therapy	P value
	Mean \pm SD	Mean \pm SD	
Vitamin D (ng/ml)	16.35 \pm 5.24	30.8 \pm 10.6	0.0001
Insulin (uU/ml)	12.83 \pm 9.39	14.8 \pm 11.49	0.301
Glucose (mg/dl)	94.2 \pm 11.5	89.6 \pm 9.3	0.093
HOMA-IR	3.05 \pm 2.5	3.24 \pm 2.74	0.518
HOMA-S	0.52 \pm 0.34	0.7 \pm 0.8	0.569
HOMA-B	2.86 \pm 1.86	3.39 \pm 2.5	0.326
QUICKI	0.33 \pm 0.03	0.34 \pm 0.04	0.731
BMI	23.8 \pm 5.6	25.5 \pm 5.5	0.009
BMI SDS	1.40 \pm 1.18	1.74 \pm 1.05	0.024
Weight (kg)	51.13 \pm 16.5	55.8 \pm 16.4	0.005
Weight SDS	0.96 \pm 1.65	1.3 \pm 1.68	0.048
Height (cm)	144.9 \pm 12.5	146.4 \pm 11.5	0.019
Height SDS	-0.98 \pm 1.45	-1.18 \pm 1.3	0.019
Growth velocity SDS	-0.46 \pm 1.18	-1.24 \pm 1.82	0.059
Steroid dose (mg/m ²)	18.05 \pm 2.82	17.99 \pm 4.67	0.691

Data are expressed as mean \pm SD. BMI SDS: Body mass index standard deviation score, 17 OH Progesterone: 17 hydroxyprogesterone, HOMA-IR: Homeostatic model assessment-insulin resistance and is equal to fasting insulin level (uU/ml) \times the fasting glucose level (mmol/l)/22.5, HOMA-B: homeostasis model assessment of beta cell function and is equal to fasting insulin (mU/l) \times 20/fasting glucose (mmol/l-3.5), HOMA-S: homeostasis model assessment of insulin sensitivity and is equal to 1/HOMA-IR, QUICKI: quantitative insulin sensitivity check index and is equal to 1/(log I0-log G0), where I0 is fasting insulin (mU/l) and G0 is fasting glucose (mg/dl). Significant if $P < 0.05$.

Discussion

Researchers have been studying the effect of 25(OH)D3 status on Insulin resistance (IR) for years [27]. CAH patients, compared with normal individuals with the same BMI, are more prone for the development of IR and greater insulin levels. This fact may be related to the long-term disturbance of adrenomedullary function, higher androgen concentrations, and glucocorticoid replacement therapy [28].

To the best of our knowledge, the impact of Vitamin D administration on IR in pubertal females with CAH was not investigated before. However, its effect on IR in populations other than CAH, such as polycystic ovarian syndrome that has higher than normal levels of androgens and IR, has been well investigated. Additionally, in populations with Type 2 diabetes mellitus, it has been well explored.

In the present study, a remarkable rise in 25(OH)D3 levels was observed after administration ($p = 0.0001$); however, this was not associated with notable changes in insulin, glucose, HOMA-S, HOMA-IR, HOMA-B, and QUICKI. These findings were similar to that observed in a randomized controlled trial (RCT) conducted in 2015 including adult T2DM patients indicating that despite the significant rise in serum levels of 25(OH)D3 ($p \leq 0.01$) detected, there were no statistically significant differences observed in other aspects including HOMA-IR, HOMA-B, QUICKI, fasting glucose, and fasting insulin [29]. Similarly, another RCT conducted in patients with T2DM and 25(OH)D3

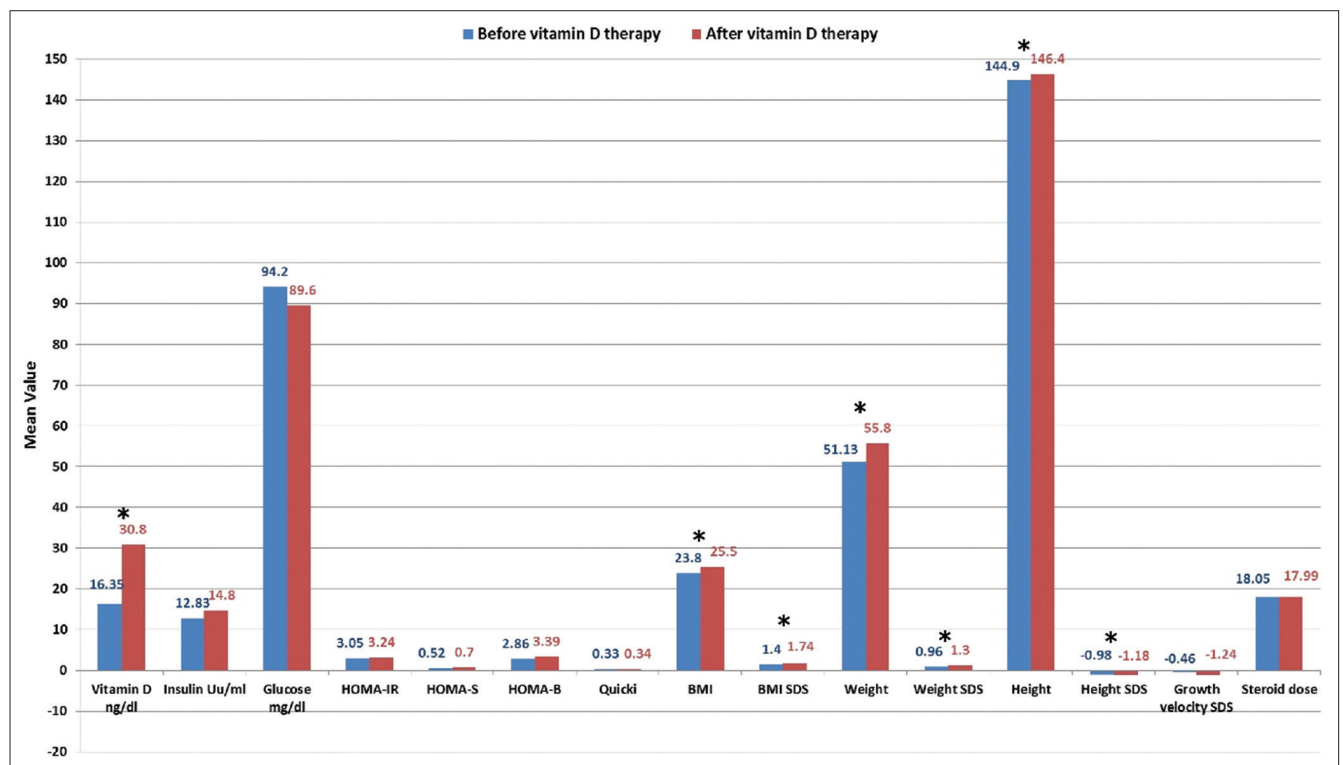


Figure 1: Comparison between anthropometric and laboratory data before and after Vitamin D therapy

Table 3: Correlations between Vitamin D and other laboratory and anthropometric data before and after supplementation

n = 16 cases	Before		After	
	Correlation	p value	Correlation	p value
Insulin (uU/ml)	-0.14	0.601	-0.35	0.178
Glucose (mg/dl)	0.117	0.667	0.15	0.571
HOMA-IR	-0.14	0.593	-0.35	0.18
HOMA-S	0.14	0.593	0.35	0.18
HOMA-B	-0.11	0.676	-0.32	0.217
QUICKI	0.065	0.81	0.29	0.266
17OH Progesterone (ng/ml)	0.035	0.898	-0.06	0.801
BMI SDS	-0.42	0.1	-0.075	0.782
Weight SDS	-0.54	0.02	-0.26	0.32
Height SDS	-0.26	0.315	-0.55	0.027

BMI SDS: Body mass index standard deviation score, 17 OH Progesterone: 17 hydroxyprogesterone, HOMA-IR: Homeostatic model assessment-insulin resistance and is equal to fasting insulin level (uU/ml) × the fasting glucose level (mmol/l)/22.5, HOMA-B: Homeostasis model assessment of beta cell function and is equal to fasting insulin (mU/l) × 20/fasting glucose (mmol/l-3.5), HOMA-S: Homeostasis model assessment of insulin sensitivity and is equal to 1/HOMA-IR, QUICKI: Quantitative insulin sensitivity check index and is equal to 1/(log I0-log G0), where I0 is fasting insulin (mU/l) and G0 is fasting glucose (mg/dl). Significant if P < 0.05.

deficiency, mentioned that no difference was detected between the two study groups (Vitamin D and placebo) regarding the HOMA-IR to which participants were randomly assigned, meanwhile, there was remarkable rise in the serum levels of 25(OH)D3 in the group who received Vitamin D supplementation versus the placebo group (p < 0.001) [30].

Table 4: Correlations between HOMA-IR and all laboratory and anthropometric data before and after Vitamin D therapy

n = 16 cases	Before		After	
	Correlation	p value	Correlation	p value
Vitamin D (ng/ml)	-0.145	0.593	-0.018	0.948
Insulin (uU/ml)	0.99	0.0001	0.989	0.0001
Glucose (mg/dl)	0.162	0.548	0.204	0.45
HOMA-S	-1	0.0001	-1	0.0001
HOMA-B	0.947	0.0001	0.968	0.0001
QUICKI	-0.994	0.0001	-0.995	0.0001
17OH Progesterone (ng/ml)	0.209	0.437	0.342	0.195
Steroid dose (mg/m ²)	-0.05	0.84	0.194	0.471
BMI	0.186	0.49	0.209	0.438
BMI SDS	0.275	0.303	0.265	0.321
Weight (kg)	0.143	0.598	0.047	0.862
Weight SDS	0.364	0.165	0.322	0.224
Height (cm)	0.196	0.466	-0.088	0.745
Height SDS	0.366	0.163	0.283	0.289

BMI SDS: Body mass index standard deviation score, 17 OH Progesterone: 17 Hydroxyprogesterone, HOMA-IR: Homeostatic model assessment-insulin resistance and is equal to fasting insulin level (uU/ml) × the fasting glucose level (mmol/l)/22.5, HOMA-B: homeostasis model assessment of beta cell function and is equal to fasting insulin (mU/l) × 20/fasting glucose (mmol/l-3.5), HOMA-S: homeostasis model assessment of insulin sensitivity and is equal to 1/HOMA-IR, QUICKI: quantitative insulin sensitivity check index and is equal to 1/(log I0-log G0), where I0 is fasting insulin (mU/l) and G0 is fasting glucose (mg/dl). Significant if P < 0.05.

Another RCT conducted including 28 women with PCOS. They were randomly allocated in one of two groups; one group was receiving oral Vitamin D3 and the other was consuming placebo for 3 months. A significant rise in 25(OH)D3 level was detected in the Vitamin D group (p ≤ 0.001) but no statistically significant changes have been observed in the fasting plasma glucose and IR levels, HOMA-S, HOMA-IR, and QUICKI in the Vitamin D group after supplementation. However, a significant rise in HOMA-B in this group after treatment was noted (p = 0.01) [31]. Another study reported that supplementation of PCOS females who had 25(OH) D3 deficiency significantly increased the serum levels of 25(OH)D3 and HOMA-B (p = 0.001 and 0.014, respectively), whereas fasting plasma glucose was significantly decreased (p = 0.001) [32]. In the present study, an increase in HOMA-B following Vitamin D supplementation was detected, but it did not reach statistical significance (p = 0.326).

On the other hand, several studies reported that Vitamin D administration decreased Insulin as in the one carried out to detect the impact of Vitamin D replacement on indices of IR in females aged 20–40 years diagnosed with PCOS and deficient in 25(OH)D3 where it was reported that a single injection (300,000 IU) leads to a significant elevation in 25(OH)D3 (p < 0.001) associated with a remarkable decline in fasting plasma glucose levels, fasting insulin levels, and HOMA-IR (p = 0.003, 0.019, and 0.004, respectively) [33]. Similarly, a significant decline of serum fasting plasma glucose levels and HOMA-IR (p < 0.01 for both) was associated with a significant elevation in serum 25(OH)D3 following supplementation of healthy Japanese participants with Vitamin D in a double-blind RCT conducted in 2016. Moreover, fasting serum insulin level was reduced but did not reach statistical significance [34]. Another study carried out including 100 participants with T2DM, showed that administration of Vitamin D supplements reduced fasting blood glucose, insulin, and HOMA-IR significantly (p = 0.05, 0.028, and 0.008, respectively) [35].

Similarly, a reduction in HOMA-IR was noticed following supplementation with Vitamin D (one dose of 300,000 IU) for 3 weeks in a study conducted on females diagnosed with PCOS and IR, although a non-significant decline of glucose and insulin levels had been observed. In this study, 17-OHP was not significantly changed and there was no correlation detected between 25(OH)D3 and HOMA-IR, 17-OHP in addition to other hormones including androstenedione, testosterone, dehydroepiandrosterone (DHEA), estradiol, and sex hormone binding globulin [36] which comes in agreement with what was observed in the present study where no significant correlation was found between 25(OH)D3 with HOMA-IR and 17-OHP despite that there was an increase in the number of patients that had 17-OHP level < 10 ng/ml after the study (43.8% of cases) compared to only 18.8% before the study. Similarly, no significant correlation was detected between 25(OH)D3 level and fasting blood glucose and IR levels in addition to other hormonal parameters such as testosterone in Iranian females with PCOS when compared to healthy controls [37]. However, in a study carried out in 2016 including Chinese participants with T2DM, a negative correlation was noted between 25(OH)D3 and HOMA-IR (p = 0.001) [38].

While studying the correlation between HOMA-IR and various parameters in the present study, a statistically significant positive correlation was detected between HOMA-IR and insulin level and HOMA-B before and after Vitamin D supplementation (p = 0.0001 for each) while a statistically significant negative correlation was noted between HOMA-IR and each of HOMA-S and QUICKI before and following Vitamin D therapy (p = 0.0001 for each). However, there was no statistically significant correlation found between the steroid dose and all laboratory and anthropometric found before Vitamin D therapy. We were not able to

find studies that have investigated the effect of Vitamin D supplementation on the studied correlations.

Study limitations

The present study had some limitations including the small sample size with short duration of supplementation that may have contributed to the fact that only 50% of the participants reached sufficient 25(OH)D3 levels after supplementation, additionally, the serum calcium level before and after the study was not assessed and Vitamin D polymorphism could not be evaluated. During previous studies, various researchers contributed the lack of sufficient evidence of the impact of Vitamin D supplementation to the insufficient dose and duration of Vitamin D administration [39,40] in addition to the small sample size [41].

Conclusion

Vitamin D replacement therapy did not have favorable effects on IR and other insulin sensitivity indices in females with CAH. Future research using RCT is required to examine the impact of 25(OH)D3 on IR, which might influence the production of adrenal androgen, resulting in reduction of the therapeutic effect of glucocorticoids that might play a part in to the evolution of MetS.

References

- Nimkarn S, Lin-Su K, New MI. Steroid 21 hydroxylase deficiency congenital adrenal hyperplasia. *Pediatr Clin North Am*. 2011;58(5):1281-300. <https://doi.org/10.1016/j.pcl.2011.07.012> PMID:21981961
- Nermoen I, Husebye ES, Myhre AG, Løvås K. Classic congenital adrenal hyperplasia. *Tidsskr Nor Lægeforen* 2017;137(7):540-3. <https://doi.org/10.4045/tidsskr.16.0376>
- Speiser PW, Arlt W, Auchus RJ. Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: An endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. 2018;103(11):4043-88. <https://doi.org/10.1210/jc.2018-01865>
- Rak K, Bronkowska M. Immunomodulatory effect of vitamin d and its potential role in the prevention and treatment of type 1 diabetes mellitus-a narrative review. *Molecules*. 2018;24(1):53. <https://doi.org/10.3390/molecules24010053> PMID:30586887
- Garbossa SG, Folli F. Vitamin D, sub inflammation and insulin resistance. A window on a potential role for the interaction between bone and glucose metabolism. *Rev Endocr Metab Disord*. 2017;18(2):243-58. <https://doi.org/10.1007/s11154-017-9423-2> PMID:28409320
- Szymczak-Pajor I, Śliwińska A. Analysis of association between Vitamin D deficiency and insulin resistance. *Nutrients*. 2019;11(4):794. <https://doi.org/10.3390/nu11040794> PMID:30959886
- Pittas AG, Lau J, Hu FB, Dawson-Hughes B. The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. *J Clin Endocrinol Metab*. 2007;92(6):2017-29. <https://doi.org/10.1210/jc.2007-0298> PMID:17389701
- Song Y, Wang L. Vitamin D and cardiometabolic disease: From observation to intervention. *Curr Nutr Rep*. 2012;1:55-63. <https://doi.org/10.1007/s13668-012-0014-2>
- Al-Daghri NM, Mohammed AK, Al-Attas OS, Ansari MG, Wani K, Hussain SD, et al. Vitamin D receptor gene polymorphisms modify cardiometabolic response to Vitamin D supplementation in T2DM patients. *Sci Rep*. 2017;7(1):8280. <https://doi.org/10.1038/s41598-017-08621-7>
- Seida JC, Mitri J, Colmers IN, Majumdar SR, Davidson MB, Edwards AL, et al. Effect of vitamin D3 supplementation on improving glucose homeostasis and preventing diabetes: A systematic review and meta-analysis. *J Clin Endocrinol Metab* 2014;99(10):3551-60. <https://doi.org/10.1210/jc.2014-2136> PMID:25062463
- Wang H, Xia N, Yang Y, Peng DQ. Influence of vitamin D supplementation on plasma lipid profiles: A meta-analysis of randomized controlled trials. *Lipids Health Dis*. 2012;11:1. <https://doi.org/10.1186/1476-511X-11-1> PMID:22433171
- Demirel F, Kara O, Tepe D, Esen I. Bone mineral density and Vitamin D status in children and adolescents with congenital adrenal hyperplasia. *Turk J Med Sci*. 2014;44(1):109-14. <https://doi.org/10.3906/sag-1301-114> PMID:25558569
- Ghali I, Salah N, Hussien F, Erfan M, El-Ruby M, Mazen I, et al. In: Satorio A, Buckler JMH and Marazzi N, editors. *Egyptian Growth Curves for Infants, Children and Adolescents*. Crecere nel mondo. Italy: Ferring Publisher; 2002.
- Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. *Arch Dis Child*. 1969;44(235):291-303. <https://doi.org/10.1136/ad.44.235.291> PMID:5785179
- Karnath BM. Signs of hyperandrogenism in women. *Hos Physician*. 2008;44:25-30.
- Aswini R, Jayapalan S. Modified ferriman-gallwey score in hirsutism and its association with metabolic syndrome. *Int J Trichol*. 2017;9(1):7-13. https://doi.org/10.4103/ijt.ijt_93_16 PMID:28761258
- Holick M. Vitamin D the underappreciated D-lightful hormone that is important for skeletal and cellular health. *Curr Opin Endocrinol Diabetes*. 2002;9(1):87-98. <https://doi.org/10.1097/00060793-200202000-00011>
- Tietz NW, editor. *Clinical Guide to Laboratory Tests*. 3rd ed. Philadelphia, PA: WB Saunders Company; 1995. p. 268-73.
- Abraham GE, Swerdloff RS, Tulchinsky D, Hopper K, Odell WD. Radioimmunoassay of plasma 17 hydroxyprogesterone. *J Clin Endocrinol Metab*. 1971;33:42.
- Dauber A, Kellogg M, Majzoub JA. Monitoring of therapy in congenital adrenal hyperplasia. *Clin Chem*. 2010;56(8):1245-51. <https://doi.org/10.1373/clinchem.2010.146035> PMID:20558634
- Selimoglu H, Duran C, Kiyici S, Ersoy C, Guclu M, Ozkaya G, et al. The effect of vitamin D replacement therapy on insulin resistance and androgen levels in women with polycystic ovary syndrome. *J Endocrinol Invest*. 2010;33(4):234-8. <https://doi.org/10.1007/BF03345785> PMID:19820295

22. Andrade MI, Oliveira JS, Leal VS, da Silva Lima NM, Costa EC, de Aquino NB, *et al.* Identification of cutoff points for homeostatic model assessment for insulin resistance index in adolescents: Systematic review. *Rev Paul Pediatr.* 2016;34(2):234-42. <https://doi.org/10.1016/j.rpped.2015.08.006>
PMid:26559605
23. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* 1985;28(7):412-9. <https://doi.org/10.1007/BF00280883>
PMid:3899825
24. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, *et al.* Quantitative insulin sensitivity check index: A simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab.* 2000;85(7):2402-10. <https://doi.org/10.1210/jcem.85.7.6661>
PMid:10902785
25. Mari A, Ahrén B, Pacini G. Assessment of insulin secretion in relation to insulin resistance. *Curr Opin Clin Nutr Metab Care.* 2005;8(5):529-33. <https://doi.org/10.1097/01.mco.0000171130.23441.59>
PMid:16079624
26. Munns CF, Shaw N, Kiely M, Specker BL, Thacher TD, Ozono K, *et al.* Global consensus recommendations on prevention and management of nutritional rickets. *Horm Res Paediatr.* 2016;85(2):83-106. <https://doi.org/10.1159/000443136>
PMid:26745253
27. Thomas N, Kalani A, Vincent R, Lam ML, Bahtiyar G, Borenszteind A, *et al.* Effect of Vitamin D in a patient with classical adrenal hyperplasia due to 11-hydroxylase deficiency. *J Med Cases.* 2013;4(8):569-75. <https://doi.org/10.4021/jmc1352w>
28. Charmandari E, Weise M, Bornstein SR, Eisenhofer G, Keil MF, Chrousos GP, *et al.* Children with classic congenital adrenal hyperplasia have elevated serum leptin concentrations and insulin resistance: Potential clinical implications. *J Clin Endocrinol Metab.* 2002;87(5):2114-20. <https://doi.org/10.1210/jcem.87.5.8456>
PMid:11994350
29. Krul-Poel YH, Westra S, ten Boekel E, ter Wee MM, van Schoor NM, van Wijland H, *et al.* Effect of Vitamin D supplementation on glycemic control in patients with type 2 diabetes (SUNNY Trial): A randomized placebo-controlled trial. *Diabetes Care.* 2015;38(8):1420-6. <https://doi.org/10.2337/dc15-0323>
PMid:25972575
30. Ryu OH, Chung W, Lee S, Hong KS, Choi MG, Yoo HJ. The effect of high-dose vitamin D supplementation on insulin resistance and arterial stiffness in patients with type 2 diabetes. *Korean J Intern Med.* 2014;29(5):620-9. <https://doi.org/10.3904/kjim.2014.29.5.620>
PMid:25228838
31. Raja-Khan N, Shah J, Stetter CM, Lott ME, Kunselman AR, Dodson WC, *et al.* High-dose vitamin D supplementation and measures of insulin sensitivity in polycystic ovary syndrome: A randomized, controlled pilot trial. *Fertil Steril.* 2014;101(6):1740-6. <https://doi.org/10.1016/j.fertnstert.2014.02.021>
PMid:24636395
32. Abootorabi MS, Ayremlou P, Behroozi-Lak T, Nourisaeidlou S. The effect of vitamin D supplementation on insulin resistance, visceral fat and adiponectin in vitamin D deficient women with polycystic ovary syndrome: a randomized placebo-controlled trial. *Gynecol Endocrinol.* 2018;34(6):489-94. <https://doi.org/10.1080/09513590.2017.1418311>
PMid:29271278
33. Salehpour S, Hosseini S, Nazari L, Hosseini M, Saharkhiz N. The effect of Vitamin D supplementation on insulin resistance among women with polycystic ovary syndrome. *JBRA Assist Reprod.* 2019;23(3):235-238. <https://doi.org/10.5935/1518-0557.20190032>
PMid:31091067
34. Sun X, Cao ZB, Tanisawa K, Ito T, Oshima S, Higuchi M. Vitamin D supplementation reduces insulin resistance in Japanese adults: A secondary analysis of a double-blind, randomized, placebo-controlled trial. *Nutr Res.* 2016;36(10):1121-9. <https://doi.org/10.1016/j.nutres.2016.07.006>
PMid:27865354
35. Talaei A, Mohamadi M, Adgi Z. The effect of vitamin D on insulin resistance in patients with type 2 diabetes. *Diabetol Metab Syndr.* 2013;5(1):8. <https://doi.org/10.1186/1758-5996-5-8>
PMid:23443033
36. Selimoglu H, Duran C, Kiyici S, Ersoy C, Guclu M, Ozkaya G, *et al.* The effect of vitamin D replacement therapy on insulin resistance and androgen levels in women with polycystic ovary syndrome. *J Endocrinol Invest.* 2010;33(4):234-8. <https://doi.org/10.1007/BF03345785>
PMid:19820295
37. Ghadimi R, Esmaeilzadeh S, Firoozpour M, Ahmadi A. Does vitamin D status correlate with clinical and biochemical features of polycystic ovary syndrome in high school girls. *Caspian J Intern Med* 2014;5(4):202-8.
PMid:25489430
38. Zhang J, Ye J, Guo G, Lan Z, Li X, Pan Z, *et al.* Vitamin D status is negatively correlated with insulin resistance in Chinese type 2 diabetes. *Int J Endocrinol.* 2016;2016:1794894. <https://doi.org/10.1155/2016/1794894>
PMid:27413370
39. Breslavsky A, Frand J, Matas Z, Boaz M, Barnea Z, Shargorodsky M. Effect of high doses of vitamin D on arterial properties, adiponectin, leptin and glucose homeostasis in type 2 diabetic patients. *Clin Nutr.* 2013;32(6):970-5. <https://doi.org/10.1016/j.clnu.2013.01.020>
PMid:23561637
40. Yiu YF, Yiu KH, Siu CW, Chan YH, Li SW, Wong LY, *et al.* Randomized controlled trial of vitamin D supplement on endothelial function in patients with type 2 diabetes. *Atherosclerosis.* 2012;227(1):140-6. <https://doi.org/10.1016/j.atherosclerosis.2012.12.013>
PMid:23298824
41. Jorde R, Figenschau Y. Supplementation with cholecalciferol does not improve glycaemic control in diabetic subjects with normal serum 25-hydroxyvitamin D levels. *Eur J Nutr.* 2009;48(6):349-54. <https://doi.org/10.1007/s00394-009-0020-3>
PMid:19370371