Journal of Pediatric Academy

Original Article

Year: 2023 Volume: 4 Issue: 1
Doi: 10.4274/jpea.2023.213
J Pediatr Acad 2023; 4: 6-11

Vitamin D, Insulin Resistance and Cytokine Levels in Obese Pubertal Children

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Article Information

Article Type: Original Articles
Article Group: Pediatric Endocrinology

Received: 13.01.2023
Accepted: 07.03.2023
Available Online: 31.03.2023

Cite this article as: *Kirkiz Kayalı S, Cinaz P, Bideci A. Vitamin D, Insulin Resistance and Cytokine Levels in Obese Pubertal Children. J Pediatr Acad 2023; 4: 6-11*

Abstract

Insulin resistance (IR) develops in obese children because of low vitamin levels and increased pro-inflammatory cytokine levels. This study aimed to analyze the relation between vitamin D, insulin resistance, tumor necrosis factor-alpha and interleukin 6 (IL-6) levels at admission and after weight loss. This study included 84 obese and 28 healthy pubertal children. Patient group was divided into four: IR positive and negative; weight loss (WL) positive and negative. Baseline and follow-up (6th month) values of serum 25-hydroxyvitamin D and other parameters were evaluated. The prevalence of serum vitamin D deficiency and insufficiency were 3.6% and 21.4% in the control group, 15.2% and 10.9% and 7.9% and 15.8% in the obese insulin positive and negative group; respectively. There was no relationship between vitamin D and IRand IL-6 levels, whereas cytokine levels were lower in obese children. As WL increased, vitamin D level and IR improved. No significant difference was found between vitamin D levels of obese and control subjects. In obese children with weight loss, an insignificant increase was observed in vitamin D, cytokines, quantitative insulin sensitivity check index values and an insignificant decrease was noted in homeostatic model assessment for IR value. Further longitudinal studies with larger patient series with greater WL are warranted.

Keywords: Insulin resistance, interleukin 6, obesity, tumor necrosis factor alpha, vitamin D

Introduction

Obesity is an important health problem, the frequency of which increase in all age groups. There are more than 43 million overweight or obese children in the world.[1] According to the Turkish Statistical Institute, obese individuals over 15 years constituted 19.6% of the population in Turkey in 2016.[2] Numerous etiologic factors such as genetic predisposition, nutritional habits, hormones, sociocultural factors, sedentary life, and drug use could cause obesity. Furthermore, childhood obesity leads to diabetes, hypertension, cardiac diseases, and respiratory system problems in adulthood.[1,3]

Vitamin D deficiency is a prevalent health issue causing rickets and osteopenia in children. It has an essential role in calcium (Ca) metabolism, cell differentiation and replication, glucose homeostasis, insulin secretion, immunological response, and inflammation-related obesity.[4] Recent studies have revealed a correlation between childhood obesity and low vitamin D levels.[5,6] Deficiency of vitamin D results when parathyroid hormone (PTH) concentration, the transformation of serum 1,25 hydroxy vitamin D

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[1,25(OH)2D] and Ca migration to adipose tissue increase. These lead to increased inhibition of lipolysis mediated through the stimulation of phosphodiesterase 3B and increased lipogenesis through the stimulation of fatty acid synthase. Additionally, increased PTH directly suppresses lipid oxidation in the muscle tissue.[7]

Insulin resistance (IR) has also been related to vitamin D deficiency.[8,9] Moreover, excess body weight (BW) increases adipose tissue, impairs its distribution and functions, promotes macrophage migration and transformation, increases the release of proinflammatory cytokines, and impairs insulin sensitivity.[5]

Highlights

- Obesity is a growing worldwide health problem with an increasing prevalence in children.
- Obese children have a higher risk of low 25(OH)D.
- Hypovitaminosis D is associated with abnormal glucose homeostasis and inflammatory cytokines.

The prospective study aimed to explore levels of vitamin D, proinflammatory cytokines, and IR measured at the initial diagnosis and a six-month follow-up in obese children with or without weight loss (WL).

Material and Method

This prospective study included 84 pubertal obese children who were admitted to the pediatric endocrinology outpatient clinic between January 2009 and June 2009. The patients presented either with a complaint of obesity or they had been referred from another outpatient clinic because of a body mass index (BMI) ≥95th percentile. Pubertal development was defined as the development of the breast in females and the testicular volume of 4 mL in males (Tanner stage 2).

The control group consisted of 28 pubertal healthy children, whose ages were similar. Patients with endocrine obesity and chronic liver or kidney disease, those using vitamin D supplementation, anticonvulsant drugs or steroids were excluded from the study. The local ethics committee approved the research (number 410, date:22.12.2008). Informed consent was obtained from the parents of the patients. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

In order to evaluate the nutritional status of patients, a three-day retrospective food consumption record was requested. According to the values of the World Health Organization, the daily energy required by the patients was calculated. The activity level of the patients was followed up with physical activity diaries. The daily intake of Ca was calculated using the Nutrition Information System computer application according to the food consumption record of the patients. The patients confirmed that they adhered to their diet protocol included in the study.

BW was assessed using an electronic scale while subjects were in a standing position in light clothes and bare feet. Body height was measured in a standing stance using a wall-mounted stadiometer (Harpenden, UK). BMI was calculated and evaluated as defined [Normal BMI: 85 kg/m^2, overweight BMI: 85-95 kg/m^2, obesity BMI over 95 kg/m^2.[10] The LMS approach was used to analyze BMI standard deviation (SD) scores (BMI-SDS).[11]

Fasting blood glucose (FBS), insulin, Ca, phosphorus (P), and alkaline phosphatase (ALP) levels were obtained from venous blood samples after a 12-hour fasting between January and June. PTH, tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6) samples were stored at -80 °C and measured using an immunoradiometric assay (BioSource, Nivelles, Belgium). The PTH, TNF-α, and IL-6 cutoff values were determined using the manufacturer's book, which were 6.87-64.87 pg/mL, 5 pg/mL, and 6-31 pg/mL, respectively. Radioimmunoassay (RIA) (BioSource, Nivelles, Belgium) was used to measure serum 25 Hydroxy vitamin D [25(OH)D] levels and it was evaluated as defined [adequate serum 25(OH)D: over 30 ng/mL, inadequate 20-29 ng/mL, deficiency less than 20 ng/mL].[12] Serum 1,25(OH)2D (range 19.6-54.5 pg/mL was also measured RIA.

The IR parameters including homeostatic model assessment for insulin resistance (HOMA-IR), fasting glucose/fasting insulin ratio (FGIR), and quantitative insulin sensitivity check index (QUICKI) were analyzed for each individual. Formulas applied for these calculations were described/reported in a different report.[13]

The patients were classified into four subgroups based on their IR status and the presence of WL at follow-up:

Group 1a: Obese pubertal children diagnosed with IR at the time of diagnosis,

Group 1b: Obese pubertal children diagnosed non-IR at the time of diagnosis,

Group 2a: Obese pubertal children without WL during follow-up,

Group 2b: Obese pubertal children with WL during follow-up,

Control group: Healthy pubertal children.

Statistical Analysis

All data was analyzed using Statistical Package for the Social Sciences version 22. The conformity of variables to normal distribution was assessed using the Shapiro-Wilk test. Categorical variables were expressed as frequencies (n) and percentages (%) and continuous variables were expressed as mean, SD, and minimum-maximum. The means of two independent groups were compared using Student's t-test and the means of more than three or more groups were compared using one-way ANOVA. Differences in the median values between groups were analyzed using Mann-Whitney U test for the comparison of two groups and Kruskal-Wallis H test for the comparison of more than two groups. Both One-Way ANOVA and Kruskal-Wallis H tests were followed by a post-hoc Tukey's test or non-parametric multiple comparison test, respectively, to identify the underlying reasons. In patients that had regular follow-up visits, baseline and follow-up laboratory parameters

were compared using Wilcoxon signed-rank test with Bonferroni correction. Categorical variables were compared using chi-square or Fisher's exact test. Correlations between continuous variables were assessed using Spearman's correlation coefficient. A p value of <0.05 was considered significant.

Results

Table 1 presents a comparison of the patient and control groups. The mean age of patients was 12.7 years (8.5-17.9).

While the patient group had more sun exposure and physical activity than the control group, no statistical difference was revealed. Although Ca consumption was below the recommended dose (1,300 mg/day for the 9-18 age group), Ca, P, ALP, 25OHD, 1,25(OH)2D and PTH measurements were within normal ranges for all groups, with no difference identified between the patient and control groups (**Table 1**).

Groups 1a and 1b

The mean age of group 1b was significantly lower compared to both group 1a (+) and the control group (p 0.05) (**Table 1**).

There was a significant difference in BW, BMI and Body mass index standard deviation score (BMI SDS) between the control group and groups 1a, 1b (p<0.001) (**Table 1**).

TNF-α levels were different compared to the control group and groups 1a, 1b (p<0.001). However, a comparison of groups 1a and 1b showed no difference. In the control group and group 1b, only IL-6 was different (p<0.05) (**Table 1**).

A significant difference was found in insulin, IR parameters between the control group and groups 1a and 1b (p<0.001 for all). However, no difference in these values was reported between the control group and groups 1b (p>0.05 for all) (**Table 1**).

Groups 2a and 2b

There was a significant difference in BW, BMI and BMI SDS between the control group and groups 2a, 2b (p<0.001) (**Table 1**).

The only significant difference between the control group and group 2a were in HOMA-IR and QUICKI values (p<0.05 for all) (**Table 1**).

Insulin, HOMA-IR measurements were observed to be higher in groups 2b and group 2a IR (-) than in group 2a IR (+) group. However, the FGIR level was lower in groups 2b and 2a IR (-) (**Table 1**).

Discussion

Obesity is an important problem worldwide in all age groups. Hypovitaminosis D is associated with abnormal glucose homeostasis and inflammatory cytokines.[14] In our study, pubertal obese and control

Table 1.
Demographic characteristics and laboratory parameters of groups 1, 2 and the control group

	Control group (n=28)	Group 1a (n=38)	Group 1b (n=46)	Group 2a (n=17)	Group 2b (n=12)
Age (years) (mean ± SD) **Gender (Female/Male)**	13.6±2.5 (10/18)	13.3±2† (20/18)	11.7±1.9** (24/22)	12.2±1.6 (8/9)	13.8±2.4 (6/6)
Body weight (kg) (mean ± SD)	47.2±8.7	78.8±17.7**††	66.2±12**	71±12.6**	78±18.5**
Height (cm) (mean ± SD)	156.8±11	158.8±10	152.5±9.2	157±7.9	163±10.2
BMI (kg/m²) (mean ± SD)	19.1±2.2	30.7±4.2**†	28.2±2.6**	28.7±3.3**	29±5.4**
BMI-SDS (mean ± SD)	1.1±8.6	2.5±0.6**	2.3±0.4**	2.2±0.6**	2.1±1**
Ca (mg/dL) (mean ± SD)	9.6 ±0.3	9.6±0.3	9.6±0.3	9.6±0.3	9.7±0.2
P (mg/dL) (mean ± SD)	4.3±0.6	4.2±0.6	4.4±0.5	4.4±0.5	4.1±0.6
ALP (U/L) (mean ± SD)	183.8±89.9	207.3±99	240.2±91*	210.8±90.1	180.7±83
Ca intake (mg/day)	303.5 (113-988)	381 (66-828)	315.5 (90-1461)		
25 (OH) D (ng/L) (mean ± SD)	39.1±14.6	43±20.6	41.3±21.1	54.9±40.6	44.2±14.1
1,25(OH)$_2$ D (pg/mL) (mean ± SD)	30.3±15.3	31±14.3	27±13	40.1±32.1	49±27.7
PTH (pg/L) (mean ± SD)	14.7±10	15.7±15.6	18±16.3	15.3±10.5	17±11.7
FBS (mg/dL) (mean ± SD)	85.3±6.4	88.6±7.6	85±6.5	87.3±6.6	88.2±6.4
Insulin (µU/mL) (mean ± SD)	9.8±6.9	21±6.7**††	10.9±2.4	15.3±9	14.4±8.1
FGIR (mean ± SD)	12.2±7.6	4.7±2.2**††	8.2±2.1	9.5±12	7.4±2.7
HOMA-IR (mean ± SD)	2.1±1.6	5±1.7**††	2.3±0.5	3.3±2*	3.1±1.8
QUICKI (mean ± SD)	0.35	0.3**††	0.3	0.3*	0.32
TNF-α (pg/mL) mean ± SD **[a](minimum, maximum)**	69.3±126 30 (0-603.9)	17±19** 17 (0-101.2)	14.1±11.8** 15.1 (0-35.1)	25.8±32.1 15.9 (0-30.8)	42±42.2 16.2 (0-39.2)
IL-6 (pg/mL) mean ± SD **[a](minimum, maximum)**	33±43.1 22.1 (0- 222.1)	16.3±13.8 12.4 (0.7-72.1)	15.4±15* 9.5 (0-85.1)	22.4±15.7 12.9 (0.7-85.1)	99.5±251.3 9.5 (7.2-41.3)

Control Group vs. Group 1a/1b, Control Group vs. Group 2a/2b *p<0.05 **p<0.001
Group 1a vs. 1b, Group 2a vs. 2b †p<0.05 ††p<0.001
[a]non-normally distributed data
IR; Insulin resistance, WL; Weight loss, SD; Standard deviation, BMI; Body mass index, BMI-SDS; Body mass index standard deviation scores, FBS; Fasting blood glucose, FGIR; Fasting glucose/fasting insulin ratio, HOMA-IR; Homeostasis model assessment-estimated insulin resistance, QUICKI; Quantitative insulin sensitivity check index, Ca; Calcium, P; Phosphorus, ALP; Alkaline phosphatase, PTH; Parathyroid hormone, TNF-α;Tumor necrosis factor-alpha, IL-6; Interleukin 6

groups were compared for age, BMI, 25(OH) D,1,25(OH)2D, PTH, IR, and inflammatory status. Age differences between the control and obese groups 1a and 1b were significant; however, we did not take this into account as they had all entered puberty.

Vitamin D insufficiency is mostly caused by inadequate sun exposure, limited dietary vitamin D intake, and gastrointestinal malabsorption. Some studies suggest that vitamin D is deficient in obesity due to its accumulation in adipose tissue and low bioactivity. On the other hand, some other studies have reported conflicting results on this issue. While some studies showed a reciprocal relation between obesity and vitamin D deficiency.[15,16] Oommen and Al-Zahrani[17] did not find any relation between vitamin deficiency and obesity. Thereby Çizmecioğlu et al.[18] found that vitamin D deficiency and insufficiency did not differ between normal, obese or overweight groups. In our study, deficiency of vitamin D along with insufficiency were found to be 3.6% and 21.4% in the control groups and 11.9% and 13.1% in the patient groups. However, unlike the study by Torun et al.[9], no difference was found between these groups ($p>0.05$), which implies that normal-weight children in Turkey should also be carefully followed for vitamin D insufficiency. Additionally, although both groups had lower Ca intakes for their ages, the patient group had higher levels of physical activity and winter sun exposure than the control group, which could explain the lower ratio of vitamin D deficiency in the patient group than expected.

Insufficient vitamin D consumption and inadequate Ca levels are associated with hyperparathyroidism and weight gain. PTH promotes the hydroxylation of serum 25(OH)D in the kidney, converting it to serum 1,25(OH)2D. In obesity, increased serum PTH enhances lipogenesis, thus promoting Ca^{+2} influx into adipocytes, ultimately impeding catecholamine-induced lipolysis implications.[19] Bolland et al.[20] showed that patients with primary and secondary hyperparathyroidism have excess BW and fat mass. Tzotzas et al.[21] reported that the 25(OH) D level was lower in obese patients than the control groups. However, PTH levels were not different. They investigated the effects of a low-calorie diet on PTH and 25(OH)D levels in obese patients and although there was a negative correlation between 25(OH)D and PTH no significant change was seen in PTH levels after the diet. Furthermore, Reinehr et al[7] followed 133 obese children for an intervention program lasting one year. Initially, PTH levels were higher in obese children, whereas 25 (OH) D levels were higher in the control group. A slight WL resulted in considerable alterations in 25 (OH) D and PTH. In our research, the serum 25 (OH)D, 1,25 (OH)2D, and PTH levels between the patient and control groups before and after WL were not different ($p>0.05$). No significant variance in PTH concentrations between the two groups and the normal Ca^{+2} and P levels of patient groups suggests that the Ca homeostasis of the patient groups was unaffected. This could be a result of the fact that our study had only a limited number of participants and a short (6-month) period of observation.

Vitamin D affects insulin secretion and sensitivity of the adipose tissue in obesity. It is also known to cause vitamin D receptor (VDR) activation in pancreatic beta cells, 1α-hydroxylase expression, and local synthesis of serum 1,25 (OH) 2D either directly or through its paracrine effects. Additionally, it has a role in intracellular Ca^{+2} concentration and Ca^{+2} transport across cellular membranes. Insulin secretion and tissue insulin sensitivity are Ca-dependent mechanisms and vitamin D positively affects insulin receptor expression in peripheral cells.[5] Therefore, it is expected that vitamin D administration would enhance insulin sensitivity and function. Numerous studies have demonstrated that inadequate vitamin D results in impaired glucose homeostasis.[22,23] In contrast, Javed et al[24] reported no improvements in insulin action or beta-cell function after vitamin D treatment. Besides a placebo-controlled clinical study in obese adolescents showed IR or secretion parameters were not improved by vitamin D administration.[25] Although there was no significant difference in FBS levels between our control and obese groups, a significant difference was found in IR parameters (FGIR, HOMA-IR, and QUICKI). However, vitamin D deficiency or insufficiency did not differ between the obese IR (+) and IR (-) groups.

There are several studies on the changes in vitamin D levels and IR parameters after weight loss. and Tüfekçi[26] found that there was no significant improvement in IR parameters in obese women with vitamin D deficiency after weight loss. In our study at 6-months, although no significant difference was found between groups 2a and 2b in IR parameters, these parameters were closer to normal ranges in group 2b than group 2a. After WL with a short-term diet, IR parameters were improved, however statistical analysis could not be performed due to small number of patients of the six IR (+) patients with WL, five had adequate vitamin D levels, whereas one patient needed vitamin D supplementation. In one out of the five subjects who had normal vitamin D levels, vitamin D levels decreased to 20-30 ng/mL during the follow-up stage. Moreover, in three of these patients, IR parameters improved following WL.

Adipose tissue is contemplated as an endocrine organ that actively secretes cytokines known as adipokines. In obese individuals, cytokines secreted from dysfunctional adipose tissue (TNF-α, and IL-6) cause chronic low-grade inflammation. TNF-α and IL-6 have been associated with obesity and the development of type 2 diabetes. These cytokines also play an important role in energy balance, lipid and carbohydrate metabolism and control of inflammatory and immune responses.[19,27] Voltage dependent resistor receptors are expressed in immune cells such as antigen-presenting cells and active T lymphocytes. On the other hand, 1,25(OH) D treatment inhibits T lymphocytes, thereby altering their cytokine secretion profiles. Although some studies have reported a negative correlation between serum 25(OH) D and TNF-α, others have reported no significant relationship between serum 25(OH) D and IL-6.[24,28-30] In our study, TNF and IL-6 levels in the control group were higher than vitamin D deficient obese individuals ($p<0.001$

and p<0.05, respectively). Yet, while some studies found increased IL-6 levels in obese children, some others reported increased IL-6 levels in healthy children which were attributed to the discrepancy of the age, sex, daily intensive exercise, and diet in a recent study.[31-35]

Our findings might have been affected by several factors including limited number of patients, compliance of pediatric patients, and extra more time for WL. However whether vitamin D deficiency is a cause or consequence of obesity still remains controversial. Further longitudinal studies with large patient series are needed to substantiate our findings.

Conclusions

In conclusion, obesity is a complex disease, and the possible mechanisms associated with hypovitaminosis D, and IR are not fully described. In this present study, as expected, IR parameters were higher in the obese group (p<0.001). While there was no difference between the obese and control groups regarding vitamin D deficiency, we think that, in Turkey, not only obese children but also normal-weight healthy children could be at risk for vitamin D deficiency.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version. Kirkiz Kayalı S: Concept, Design, Data collection or processing, Analysis or interpretation, Literature search, Writing.; Cinaz P: Design, Analysis or interpretation, Writing; Bideci A: Design, Analysis or interpretation, Literature search, Writing.

Conflict of Interest: There are no conflicts of interest in connection with this paper, and the material described is not under publication or consideration for publication elsewhere.

Ethics Committee Approval: The study was carried out with the permission of Gazi University Ethics Committee (date: 22.12.2008, decision No: 2008/410).

Financial Disclosure: The authors have no conflicts of interest to declare.

Informed Consent: Informed consent was obtained from the parents of the patients.

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