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Research article

Is Bioavailable Vitamin D Better Than Total Vitamin D to Evaluate Vitamin D Status in Obese Children?

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What is already known on this topic?
There are some studies reported that frequency of vitamin D deficiency is high in obese children, on the other hand some studies reported that it is not. In these studies, vitamin D deficiency was evaluated with the total vitamin D level. It is also known that VDBP level and polymorphism affect vitamin D level, however, this effect is contradictory.

What this study adds?
While there was no difference in terms of total vitamin D, it was detected that free and bioavailable vitamin D was lower in obese group in winter. Also VDBP and PTH were found to be higher. VDBP level and polymorphism had no effect on vitamin D level.

Abstract
Introduction: Free hormones are biologically more active in the target tissues. So we wanted to evaluate the vitamin D adequacy with bioavailable and free vitamin D. In order to calculate the bioavailable and free vitamin D according to the previously reported formula, VDBP level was measured. VDBP polymorphisms were also evaluated as they can affect the binding affinity.
Methods: Eighty-four obese and 78 healthy children included in the study. Anthropometry, calcium, phosphorus, alkaline-phosphatase, parathyroid hormone, 25 (OH) vitamin D, bioavailable-free vitamin D, VDBP level and polymorphism were evaluated in the whole group.
Results: When the girls compared within themselves, PTH values were found to be higher in the obese group (p=0.001). When we compared obese and control subjects without gender discrimination, VDBP and PTH levels were found to be statistically higher in the obese group(respectively p=0.008, p=0.002). When only the cases included in the winter period were analyzed, PTH and VDBP were found to be higher and bioavailable and free vitamin D lower in the obese group. There was no difference in terms of total vitamin D between groups during the winter season.
Discussion/Conclusion: While total, free, bioavailable vitamin D in obese group was similar to the control group in autumn, free and bioavailable vitamin D in the winter was lower than the control group. Also PTH was higher in obese group than the control group in both autumn and winter. Therefore, many more studies are needed to evaluate the variability of free, bioavailable vitamin D according to the seasons and its effects.
Keywords: 25- hydroxyvitamin D, bioavailable vitamin D, free vitamin D, vitamin D binding protein, polymorphism, obesity

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Introduction

Vitamin D is a pre-pro-hormone that regulates calcium metabolism and bone homeostasis. Obesity, the frequency of which has been increasing in recent years, is considered as a risk factor for vitamin D deficiency. Vitamin D deficiency in obesity is considered to be due to rapid metabolic clearance of vitamin D, large distribution volume and decreased bioavailability (1, 2). Vitamin D is a hormone that synthesis starts in the skin with sunlight exposure. Another reason for vitamin D deficiency in obese is decreased cutaneous synthesis (3). Therefore, a seasonal difference may be observed in vitamin D levels.
25 (OH) Vitamin D shows high lipophilic properties. It needs carrier serum protein to act in the target cell. 25 (OH) vitamin D circulates 85-90% vitamin D-binding protein (VDBP) and 10-15% albumin-bound. Less than 1% of the circulating hormone is in free form (4, 5). According to the free hormone hypothesis, only hormones separated from the binding protein can enter the cell and have a biological effect (6). It is thought that the bioavailable vitamin D, which is not bound to VDBP, is known to be biologically more active in the target tissues (7).

Vitamin D binding protein level and polymorphism can affect vitamin D sufficiency level as it will change the affinity of binding to vitamin D. There are also opinions that there may be a VDBP effect on 25 (OH) vitamin D related intacrine responses (8). In most of the studies investigating vitamin D deficiency, total vitamin D level has been measured to date. Some researchers hypothesized that the total vitamin D level does not reflect the biologically active vitamin D level in the organism. The evaluation of hormonal activity and sufficiency with bioavailable vitamin D will be much more reliable. In this study, we aimed to evaluate whether vitamin D levels was different from the control group in the obese group and to compare the factors affecting total, bioavailable, free vitamin D levels in both the obese and control groups.

Material and Method

The study was performed at the pediatric endocrinology outpatient clinic of our hospital between September 2018 and March 2019. Informed consent form was taken from the families of volunteers participating in the study. The Ethics Review Board of Zekai Tahir Burak Women's Health Education and Research Hospital approved the study protocol (approval number 16/2018 dated 06.03.2018**).** The study was conducted as a University of Health Science' Scientific Research Coordination Unit (Project Number: 2018/040).

Eighty-four obese children (over BMI> 95 percentiles) and 78 healthy children (BMI = 15-85 percentiles) who are without additional systemic diseases and drug-free included in the study. The cases who received vitamin D in the last six months were not included in the study. Prepubertal cases were not included in the study. Pubertal staging was done according to Tanner in all cases included. In girls breast stage ≥ 2 and in males testicular volum ≥ 4 ml were defined as pubertal.

A SECA scale (SECA, Hamburg, Germany) and a Harpenden stadiometer (Holtain Ltd., Crymych, UK) were used to measure weight and height, respectively.Anthropometric data for the Turkish population, such as height, weight and body mass index (BMI), are available in an online database (www.ceddcozum.com) (9).

Calcium, phosphorus, alkaline phosphatase (ALP), parathyroid hormone (PTH), 25 (OH) vitamin D, VDBP level and polymorphism were examined in both obese and healthy groups. In addition, fasting blood glucose and insulin, total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride, AST, ALT levels were measured in the obese group. (Plasma glucose, insulin, lipid profile, HbA1c, 25 (OH) D, calcium, phosphorous, albumin, ALP, PTH levels were of all cases measured after a 10-hour fasting period. Glucose, HbA1c, total cholesterol, HDL cholesterol, LDL cholesterol and triglyceride levels were measured with Architect C16000 auto-analyzer system, insulin levels were measured by the chemiluminescence method (Advia Centaur XP). Calcium, phosphorous, ALP and PTH values were measured with the device SynchronDxC 800 pro Coulter Beckman.

Serum vitamin D measurement-Serum 25(OH) vitamin D concentrations were measured at hospital laboratory by chemiluminescence method with LC-MS/MS.

VDBP measurement- Blood samples taken from the patient and healthy control groups were centrifuged at 3000g for 10 minutes. Separated serums were stored at -80 ºC in ependorphic tubes until they were analyzed. All samples were dissolved simultaneously and studied using ready-made kits (Cloud-Clone Corp. (USCNK) (product number:SEB810HU)) by ELISA (an enzyme-bound immune-absorbent, Biotek, USA) method. This method uses polyclonal DBP antibodies. The intra-test coefficient variability of the kit was CV <10% and the inter-test coefficient variability was CV <12%.

Bioavailable vitamin D was calculated using the previously reported formula (4). The formula is a mathematical calculation model that includes VDBP and albumin binding constant. However, the most common polymorphisms of rs4588, rs7041 and rs587776830 were studied in the VDBP gene because genotypic differences in these proteins may cause significant variations in binding affinity and serum concentration.

Polymorphism genotyping- For VDBP polymorphism, samples separated into EDTA tubes were stored at -20 degrees. DNA was isolated with magnetic bead method (MagPurix- Zinexts, Taiwan). PCR amplification was done in-house designed primers. Amplicons were checked by 2% agarose gel electrophoresis. Sequencing was done by next generation sequencing method by Miseq-Illumina equipment (Illumina, San Diego, CA, USA) by following the instructions of the manufacturers. Data was evaluated by IGV 2.3 (Broad Institute) software.

$$\text{Free vitamin D} = \frac{\text{total 25 (OH)vitaminD}}{1+(6x10^5 xalbumin)+(7x10^8 xVDBP)}$$

Bioavailable Vitamin D = (D Free) + (D albumin) = (D-free). ($K_{albumin.}$ (Alb) +1)

Statistical analyses- Descriptive statistics for the continuous variables (characteristics) were presented as mean and standard deviation, while count and percent for the categorical variables. Normality assumption of the continuous variables was tested with Kolmogov-Simirnov test. After normality test, ANOVA or Student t test was used for the comparison of means in normally distributed characteristics. However, for the non-normal distributed characteristics, Kruskal – Wallis or Mann-Whitney U test was performed. For determination linear relationships among the continuous variables, Pearson correlation analysis was carried out in each group. In addition, Chi-square test was also performed to determine the relationships between categorical variables. Statistical significance level was considered as 5% and SPSS (IBM SPSS version 22.0; IBM, New York, N.Y., USA) statistical program was used for all statistical computations.

Results

There was no differences between the groups in terms of age and gender (respectively p=0.886, p=0.309). There was no difference between the two groups in terms of pubertal stages (p= 0.051). In the obese group, height sds, weight, weight sds, BMI, BMI sds were significantly higher than the control group (Table-1). When we compared obese and control subjects without gender discrimination, VDBP and PTH levels were found to be statistically higher in the obese group. Total, bioavailable and free vitamin D levels were similar in both groups. When the girls compared within themselves, PTH values were found to be higher in the obese group (Table-1). Also VDBP values were found to be higher in the obese girls, but were not statistically significant (p=0.057). No differences was detected in men in these parameters. In addition, when VDBP level was compared according to the stages of puberty, there was no difference (p=0.180).

In 34.5% (n= 29) of the obese group and 39.7% (n= 31) of the control group, 25 (OH) vitamin D level was below 12 ng/mL

and was considered as deficient. When the obese cases is evaluated within themselves, PTH and HDL cholesterol were higher, phosphorus, free and bioavailable vitamin D were lower in deficient group (Table-2). The comparison of the whole obese group and the subgroup of control group with normal vitamin D (25 (OH) vitamin D> 20 ng/mL and PTH< 65 pgl/mL) (n=11) is indicated in the Table-3. In this comparison, total, free, bioavailable vitamin D and phosphorus were lower and PTH was higher in the obese group.
65 of the obese cases were included in the study in the autumn season and 19 in the winter season. 26 of the control cases were included in the study in the autumn season and 52 in the winter season. When only the cases included in the winter period were analyzed, PTH and VDBP were found to be higher and bioavailable and free vitamin D lower in the obese group (Table-4). There was no difference in terms of total vitamin D between groups during the winter season. In autumun, phosphorus and ALP were found lower while PTH was higher in the obese group. When groups are divided by seasons within themselves; total, bioavailable and free vitamin D were lower, VDBP were higher in the obese group in winter. In the control group p, ALP, total, bioavailable and free vitamin D were found to be lower in the winter.
VDBP polymorphisms were similar in obese and control groups (Table-5). Also calcium metabolism parameters were compared each rs587776830, rs7041, rs4588 genotype subgroups. There were 12 subgroups in rs587776830 genotype group and there was no difference between the groups in terms of Ca, P, ALP, PTH, total vitamin D, free vitamin D, bioavailable vitamin D and VDBP (respectively p=0.457, p=0.786, p=0.706, p=0.897, p=0.125, p=0.200, p=0.239, p=0.722). Also, as indicated in Table-6, no difference was found in the rs7041 and rs4588 group in terms of between calcium parameters, especially VDBP.
In the whole group, there was positive correlation between VDBP and PTH (p = 0.009; r = 0.20), negative correlation between VDBP rs4588 polymorphism and PTH (p = 0.04; r = -0.15), negative correlation between PTH (p = 0.01; r = -0.20) and total 25 (OH) vitamin D, negative correlation between bioavailable vitamin D and PTH (p = 0.001; r = -0.24). Free vitamin D positively correlated with P, ALP, total (25) OH vitamin D and bioavailable vitamin D (p = 0,000; r = 0.30; p = 0.008; r = 0.20, p = 0,000, r = 0, 86; p = 0,000; r = 0.99, respectively), and negatively correlated with PTH and VDBP (p = 0.001; r = -0.25; p = 0,000; r = -0.47). In addition, negative correlation between bioavailable vitamin D and HDL cholesterol (p = 0.04; r = -0.21), positive correlation between PTH and fasting blood glucose and fasting insulin (p = 0.00; r = 0, 39; p = 0.04; r = 0.22, respectively) were detected in the obese group.

Discussion

Although there have been many studies examining the relationship between obesity and vitamin D in children and adults, there are very few studies evaluating bioavailable vitamin D, free vitamin D and VDBP levels and polymorphism together in the obese and healthy control group (2, 10-14). Our study is the first study to evaluate total, bioavailable, free vitamin D and VDBP levels and polymorphism in obese and healthy children.
In our study, total 25 (OH) vitamin D, bioavailable vitamin D and free vitamin D levels were similar in both obese and control group. However, considering the seasons separately, when obese group was compared to the control group, total vitamin D was similar, bioavailable and free vitamin D levels were low, and VDBP and PTH levels were high in winter. While there was no difference in vitamin D and VDBP in autumn, PTH levels were higher in obese group. Also in the obese group, while total, free, bioavailable vitamin D was lower in winter compared to autumn, VDBP was higher and there was no difference in PTH level. Similarly, in the control group, total, bioavailable and free vitamin D were lower in winter than in autumn. VDBP and PTH were not different in the control group according to the seasons. We observed that all forms of vitamin D in obese and healthy children were lower in winter than in autumn.
In a study investigating the seasonal variability of vitamin D and PTH in obese children, vitamin D was found to be higher in summer than in autumn and winter in obese and control group. In addition, seasonal variability was not detected in obese group in terms of PTH, while it was found to be high in the control group in autumn and spring months (3). Seasonal variation in vitamin D was evaluated in a study conducted in the UK which included 223 obese, overweight and normal weight adults (2). The level of vitamin D in obese and overweight group was found to be lower than in normal weight in autumn and spring, similar to those in normal weight in winter. Also it has been concluded that the synthesis of vitamin D in the skin is similar in obese and normal weight individuals. One study reported in 2015, with 63 obese and 21 healthy children aged 4-15 years (11). In this study total 25 (OH) vitamin D was found lower in the obese group than in the non-obese group, but no difference was found between bioavailable vitamin D and the PTH levels regardless of the season. In our study, in the obese group vitamin D levels were similar to the control group and PTH was higher than the control group in autumn. In winter, the total vitamin D level in the obese group was similar to the control group, free and bioavailable vitamin D was lower than the control group, and VDBP and PTH were higher than the control group.
In our study, calcium, phosphorus, alkaline phosphatase and albumin levels were similar in the obese and healthy control group, whereas PTH levels were higher in the obese group. Vitamin D deficiency is known to be associated with decreased calcium absorption and increased PTH (5, 15). PTH is also thought to be a useful indicator of the biological significance of low vitamin D level (16). In a study involving 595 female patients in adults, 25 (OH) vitamin D, PTH and VDBP levels were measured and VDBP Gc phenotyping was performed. Similar to our study, an inverse relationship was found between 25(OH) vitamin D and PTH. The patients with Gc1-1 phenotype and 25 (OH) vitamin D was less than 40 nmol/L had higher PTH levels but when all patients were included in the evaluation, in terms of PTH, it has been reported that there was no difference according to Gc phenotypes (17). In our study, negative correlation between VDBP rs4588 polymorphism and PTH suggested that VDBP polymorphisms may affect PTH levels.
In the present study, the VDBP level was significantly higher in the obese group than in the control group. VDBP is the major serum transport protein of vitamin D (18). VDBP also facilitates the transport of 25 (OH) D to tissues and regulates its bioavailability (19). In our study, total 25 (OH) vitamin D, bioavailable and free vitamin D levels were similar between the two groups, while PTH and VDBP were increased in the obese group**.** There was no difference between the groups in terms of pubertal stages. In the study conducted in 15 obese and 15 normal weight adults between the ages of 20-35, the VDBP level was found higher in the obese group. In a study performed in 2014, comparing 43 obese and 43 normal-weight women

aged 22-45, VDBP levels were found to be higher in the obese group (20). It has been stated that high estrogen levels in obese women increase the hepatic production of VDBP. However, in our study, there was no difference in VDBP by gender in the whole group. Similarly, in another study conducted in adults, VDBP and PTH were found higher in obese group (14). In our study, the positive correlation between VDBP and PTH in the whole group, independent of vitamin D, suggested that these two variables may be related to each other.

As is known, according to VDBP polymorphism, the binding affinity of 25 (OH) vitamin D to VDBP can change and its level may be affected. In our study, the distribution of VDBP polymorphism in the obese group was similar to the control group. According to polymorphisms, there was no difference in terms of 25 (OH) vitamin D, bioavailable vitamin D, free vitamin D and VDBP. Contrary to our results, in many studies, it has been shown that total vitamin D, free vitamin D and VDBP levels are lower in the Gc2 genotype (21-26).

In our study, a negative correlation was found between total 25 (OH), bioavailable, free vitamin D and PTH. Similarly in the literature, it was reported that a negative correlation was found between PTH and total 25 (OH) vitamin D and bioavailable vitamin D in a study investigating cases with chronic renal failure between the ages of 5-21 (26). In another study examining 94 dialysis patients in adulthood, a negative correlation was found between bioavailable vitamin D and PTH, while no correlation was found between total 25 (OH) vitamin D and PTH (28). In another study of adults, it was reported that a negative correlation was found between total vitamin D and free vitamin D and PTH in women and men, also negative correlation was found between bioavailable vitamin D and PTH only in women (14).

There are publications showing that vitamin D is related to many diseases such as obesity, insulin resistance, diabetes, dyslipidemia, atherosclerosis and cancer, other than regulating intestinal calcium absorption and bone homeostasis. In our study, negative correlations between bioavailable vitamin D and HDL cholesterol, and positive correlations between fasting blood glucose, fasting insulin and PTH were found in the obese group. In the literature, there are studies reporting negative correlation between total vitamin D level and fasting glucose levels (12), positive correlation between total and free vitamin D and insulin sensitivity, and negative correlation with HOMA-IR (29), negative correlation between free vitamin D and fasting blood glucose (30). There are also studies reporting positive correlation between VDBP and total cholesterol, LDL cholesterol and triglycerides (31). In light of all these data, it can be said that vitamin D and PTH are effective on metabolic balance.

Conclusion

As a result, it was found that total, bioavailable and free vitamin D levels in obese group were similar to the control group. Our results indicated that VDBP level and polymorphism may have a direct effect on parathyroid hormone regulation. However, when compared to the obese and control groups in winter, there was no difference in total vitamin D, while free and bioavailable vitamin D was lower and PTH and VDBP was higher in obese children. Many more studies are needed to explain the variability of total, free and bioavailable vitamin D according to seasons.

Study Limitations

The dietary calcium and vitamin D intakes could not be evaluated.

The clothing characteristics, skin pigmentation and the time spent outside of cases were not questioned.

Statements:

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Conflict of Interest Statement

There is no conflict of interest in this study.

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Table-1: Antropometric and laboratory characteristics based on gender in the obese and control group

	Female			**Male**			
	Obese(n=54)	**Control(n=54)**	***p value***	**Obese(n=30)**	**Control(n=24)**	***p value***	***p* value***
Age (years) Median (Min- Max)	13.3 (9-17.8)	12.3 (9-17.9)	*0.265*[#]	13.0 (9.9-17.8)	14.4 (11-17.8)	*0.096*	*0.886*[#]
Height (cm)	158.8□7.6	152.2□10.2	***0.001***[†]	159.2□21.7	165.2□12.5	*0.236*	*0.208*[†]
Height sds	0.6□1.08	-0.1□1.08	***0.001***[†]	0.42□0.85	0.05□1.13	*0.193*	***0.001***[†]
Weight (kg)	77.9□15.2	45.2□9.4	***0.001***[†]	85.9□20.5	57.8□12	***0.001***	***0.011***[†]
Weight sds	3□0.98	-0.2□0.8	***0.001***[†]	2.4□0.8	-0.05□0.78	***0.001***	***0.001***[†]
BMI (kg/m^2)	30.7□4.5	19.2□2.1	***0.001***[†]	32.1□5.1	20.9□2.1	***0.001***	***0.001***[†]
BMI sds	2.7□0.6	-0.1□0.6	***0.001***[†]	2.4□0.6	-0.04□0.64	***0.001***	***0.001***[†]
Calcium	10.02□0.29	10.01□0.37	*0.885*[†]	10.05□0.32	10.14□0.28	*0.281*	*0.804*[†]

(mg/dL)							
Phosphorus (mg/dL)	4.3□0.64	4.4□0.7	*0.597*†	4.3□0.5	4.4□0.8	*0.636*	*0.579*†
Alkaline phosphatase (U/L)	178□90.1	209.3□125.6	*0.141*†	225.3□80.5	220.2□118.7	*0.858*	*0.687*†
Albumin (g/dL)	4.6□0.2	4.5□0.2	*0.689*†	4.6□0.24	4.7□0.32	*0.202*	*0.766*†
Parathyroid hormone (pg/mL)	71□29.5	54.3□19.6	***0.001***†	69.8□31.8	59.3□28.5	*0.207*	***0.002***†
25(OH) vitamin D (ng/mL)	16□8.5	14.5□7	*0.303*†	18.3□7.4	15□7.1	*0.103*	*0.083*†
Free vitamin D (pg/ml)	10.1□6.4	9.6□4.8	*0.616*†	11.5□6.9	9.9□4.6	*0.355*	*0.705*†
Bioavailable vitamin D (ng/ml)	4.4□2.8	4.1□2.1	*0.630*†	4.9□2.9	4.3□1.9	*0.414*	*0.743*†
VDBP(mg/L) Median (Min- Max)	61.1 (43.1-228.2)	52.8 (43.2-181.9)	*0.057*#	67.0 (43.2-161.4)	49.7 (43.2127.9)	*0.077*	***0.008***#

BMI: Body mass index, SD: Standard Deviation

†***:*** Student-t test was used and Mean□SD values were given.

#: Mann-Whitney U test was used and median (minimum-maximum) values were given.

*Comparison of obese and control group regardless of gender.

Table-2: Comparison of metabolic parameters of patients with vitamin D deficient and sufficient in obese group

	25(OH) vitamin D <12 ng/mL (n=29)	**25(OH) vitamin D □12 ng/mL (n=55)**	***p value***
Calcium (mg/dL)	10.0□0.3	10.0□0.3	*0.487*†
Phosphorus (mg/dL)	4.0□0.6	4.4□0.6	***0.022***†
Alkaline phosphatase (U/L)	168.9□96.8	208.7□82.7	*0.066*†
Albumin (g/dL)	4.6□0.2	4.5□0.2	*0.249*†
Parathyroid hormone (pg/mL)	79.7□32.0	65.8□28.2	***0.043***†
Free vitamin D (pg/ml)	4.9□2.0	13.6□6.2	***0.000***†
Bioavailable vitamin D (ng/ml)	2.1□0.9	5.8□2.7	***0.000***†
VDBP (mg/L) Median(Min- Max)	71.2 (43.2-197.5)	60.2 (43.1-228.2)	*0.202*#
Glucose (mg/dL)	90.3□8.8	92.1□10.4	*0.415*†
Insulin (□IU/mL)	23.0□13.5	22.9□15.3	*0.968*†
Cholesterol (mg/dL)	165.9□28.8	157.9□30.0	*0.240*†
LDL cholesterol (mg/dL)	109.1□25.9	93.9□25.1	*0.298*†
HDL cholesterol (mg/dL)	44.2□8.3	40.2□6.6	***0.030***†
Triglycerides (mg/dL)	107.5□44.0	118.6□48.9	*0.296*†

†*:* Student-t test was used and Mean□SD values were given.

#: Mann-Whitney U test was used and median (minimum-maximum) values were given.

Table-3: The evaluation of calcium metabolism parameters in obese and control group

	Obese Whole group (n=84)	**Control Vitamin D sufficient* (n=11)**	***p value*#**
Calcium (mg/dL)	10.05 (9,1-10.7)	10 (9.4-10.5)	*0.619*
Phosphorus (mg/dL)	4.2 (3.1-5.8)	4.8 (3.9-5.6)	***0.009***
Alkaline phosphatase (U/L)	198 (53-394)	222.5 (81-362)	*0.367*
Albumin (g/dL)	4.6 (4.1-5.3)	4.6 (4.3-4.9)	*0.324*
Parathyroid hormone (pg/mL)	64.6 (25-154.6)	48.4 (26.9-63.4)	***0.012***
25(OH) vitamin D (ng/mL)	16.4 (5.1-40.8)	25.9 (20.2-33.1)	***0.000***
Free vitamin D (pg/ml)	8.7 (1.2-35.3)	15.6 (7.8-24.5)	***0.011***
Bioavailable vitamin D (ng/ml)	3.7 (0.5-14.6)	6.8 (3.3-10.6)	***0.010***
VDBP (mg/L)	62.2 (43.1-228.1)	81.5 (43.2-181.9)	*0.935*

*The cases with 25 (OH) vitamin D> 20 ng/mL and PTH< 65 pgl/mL were defined as sufficient.

#: Mann-Whitney U test was used and median (minimum-maximum) values were given.

Table-4: Comparison of calcium metabolism parameters in obese and control groups by seasons

	Autumn			Winter				
	Obese (n=65)	**Control (n=26)**	***p value*#**	**Obese (n=19)**	**Control (n=52)**	***p value*#**	***p value*†**	***p valu***
Calcium (mg/dL)	10.1 (9.1-10.7)	10.2 (9.4-10.8)	*0,226*	9.9 (9.3-10.4)	10 (9.3-10.8)	*0,705*	*0,291*	*0,139*
Phosphorus (mg/dL)	4.3 (3.1-5.6)	4.8 (3.6-5.9)	***0,001***	4.1 (3.2-5.8)	4.1 (2.8-5.7)	*0,805*	*0,425*	***0,000***
Alkaline phosphatase (U/L)	198 (56-394)	274.5 (73-563)	***0,004***	194 (53-318)	139 (42-491)	*0,851*	*0,248*	***0,001***
Albumin (g/dL)	4.6 (4.1-5.3)	4.5 (4.2-5.1)	*0,561*	4.6 (4.2-4.9)	4.5 (4-5.2)	*0,829*	*0,552*	*0,685*
Parathyroid hormone (pg/mL)	61.7 (25-149.4)	45.8 (20.9-95.8)	***0,004***	85.8(26.7-154.6)	57.1 (4.9-153.6)	***0,007***	*0,060*	*0,059*
25(OH) vitamin D (ng/mL)	17.8 (5.6-40.8)	16.9 (11.3-36.3)	*0,477*	10.4 (5.1-24.5)	11.5 (4.8-30.7)	*0,483*	***0,000***	***0,000***
Free vitamin D (pg/ml)	11.2 (2.5-35.3)	12.8 (7.0-24.5))	*0,288*	4.4(1.2-14.0)	7.5 (2.2-17.2)	***0,008***	***0,000***	***0,000***
Bioavailable vitamin D (ng/ml)	4.6 (1.2-14.6)	5.4 (3.0-10.6)	*0,199*	1.92(0.5-6.2)	3.3 (0.94-7.1)	***0,006***	***0,000***	***0,000***
VDBP (mg/L)	57.3 (43.1-228.2)	54.2 (43.2-140.6)	*0,580*	86.9(46.6-197.5)	51.2 (43.3-181.9)	***0,000***	***0,001***	*0,672*

#: Mann-Whitney U test was used and median (minimum-maximum) values were given.
†: Comparison of the obese group by seasons
*: Comparison of the control group by seasons

Table-5: VDBP polymorphism distributions in obese and control groups

SNP ID	**Genotype**	**Obese (n. %)**	**Control (n. %)**	***p value*** #
***VDBP rs*587776830**				*0.543*
VDBP *rs7041*				*0.363*
	TT	12 (% 14)	16 (% 20)	
	TG	42 (% 50)	31 (% 40)	
	GG	30 (% 36)	31 (% 40)	
	T allel	66 (% 39)	63 (% 40)	
	G allel	102 (% 61)	93 (% 60)	
VDBP *rs4588*				*0.256*
	CC	56 (% 67)	46 (% 59)	
	CA	22 (% 26)	29 (% 37)	
	AA	6 (% 7)	3 (% 4)	
	C allel	134 (%80)	121 (% 78)	
	A allel	34 (% 20)	35 (% 22)	

SNP: single nucleotide polymorphism
#: *Pearson* χ^2 test was used.

Table-6: The evaluation of calcium metabolism parameters according to the genotypes in whole group

	rs7041				***rs4588***		
	GG(n=61)	***TT(n=28)***	***TG(n=73)***	***p value***	***CC(n=102)***	***CA(n=51)***	***p value***
Calcium (mg/dL)	10.05□0.36	10.1□0.27	10□0.31	*0.273*†	10.03□0.33	10.05□0.32	*0.967*▫
Phosphorus (mg/dL)	4.3□0.6	4.3□0.7	4.3□0.7	*0.796*†	4.3□0.7	4.2□0.6	*0.283*▫
Alkaline phosphatase (U/L)	206.1□103.7	223.9□117.8	193.4□105.2	*0.416*†	201.7□104.9	195.3□104.5	*0.747*▫
Albumin (g/dL)	4.5□0.3	4.6□0.2	4.6□0.21	*0.455*†	4.5□0.2	4.6□0.2	*0.304*▫
Parathyroid hormone	66.43□31.1	61.88□27	61.66□24.9	*0.568*†	65.75□27.6	57.46□25.4	*0.248*▫

(pg/mL)							
25(OH) vitamin D (ng/mL)	15.8□7.9	15.8□7.9	15.8□7.5	*0.992*†	16.5□8.2	14.9□6.7	*0.381*▫
Free vitamin D (pg/ml)	10.4□5.8	9.3□5	10.3□6	*0.808*†	10.7□6.1	9.5□5.2	*0.337*▫
Bioavailable vitamin D (ng/ml)	4.43□2.47	4□2.2	4.4□2.6	*0.856*†	4.6□2.6	4.13□2.3	*0.367*▫
VDBP (mg/L) Median (Min- Max)	52.8 (43.2-181.2)	71.0 (43.2-153.2)	57.3 (43.1-228.2)	*0.111*#	56.9 (43.1-228.2)	56.5 (43.1-197.4)	*0.422*♂

SD: Standard Deviation

†: One way ANOVA was used and Mean□SD values were given.

#: Kruskal Wallis test was used and median (minimum-maximum) values were given.

▫: Student-t test was used and Mean□SD values were given.

♂ Mann-Whitney U test was used and median (minimum-maximum) values were given.