Research Article

Rahim Kocabaş* and Mehmet Aköz

The effects of vitamin D supplementation on healthy and hypercholesterolemic rabbits on levels of OSI and paraoxonase

Sağlıklı ve hiperkolesterolemik tavşanlara vitamin D ilavesinin OSI ve paraoksonaz düzeylerine etkileri

https://doi.org/10.1515/tjb-2017-0154
Received June 5, 2017; accepted November 25, 2017; previously published online October 15, 2018

Abstract

Objective: Conflicting data are available in literature regarding the effects of vitamin D (VitD) supplementation diet on lipid panel. Therefore, we had the purpose to evaluate the effects of VitD supplementation on lipid panel by a controlled experimental study, and those of VitD supplementation on oxidative stress index (OSI) and paraoxonase-1 (PON1) values in healthy and hypercholesterolemic male rabbits.
Methods: Thirty New Zealand rabbits were randomly separated into control, VD, HC + VD and HC groups. Control and VD groups were fed with standard chow, whereas HC + VD and HC groups were fed with 0.5% cholesterol chow a period of 8 weeks. During this period, VD and HC + VD groups were orally administered with 300 IU/kg/day VitD.
Results: The increase in serum total cholesterol (TC) and OSI level of HC group were significant compared to those in HC + VD group. Decreases in serum HDL-cholesterol (HDL-C) and TC levels of VD group were significant within the groups.
Conclusion: Without any doubt it is important that applied VitD level should be in the ideal range for healthy living. However, it is also necessary to increase the serum HDL-C level (and hence PON1), which is decreases as a result of VitD supplementation. Therefore, we believe that during VitD supplementation, regular physical activity should be performed to increases serum HDL-C.

***Corresponding author: Rahim Kocabaş,** Necmettin Erbakan University, Konüdam Experimental Medicine and Application Research Center, 42080 Meram, Konya, Turkey, Phone: +90 33 2223 7111, Fax: +90 33 2223 7124, e-mail: drrahim42@gmail.com.
http://orcid.org/0000-0001-8006-284X
Mehmet Aköz: Necmettin Erbakan University, Faculty of Medicine, Department of Biochemistry, Konya, Turkey, e-mail: mehmetakoz2003@yahoo.com

Keywords: Vitamin D; Paraoxonase-1; Oxidative stress index; HDL cholesterol; Hypercholesterolemia; Rabbit.

Özet

Amaç: Literatürde, diyete D vitamini (VitD) ilavesinin lipit paneli üzerine etkisi hakkında birbirinden farklı sonuçlar bulunmaktadır. Bu yüzden projemizde kontrollü deneysel bir çalışma ile, sağlıklı ve hiperkolesterolemik erkek tavşanlara VitD ilavesinin lipit paneli üzerine etkisine, ve yine VitD ilavesinin oksidatif stress indeksi (OSI) ve paraoksonaz-1 (PON-1) değerleri üzerine etkilerini araştırmayı amaçladık.
Metod: 30 adet yeni zellanda tavşanı rastgele control, VD, HC + VD ve HC gruplarına ayrıldı. Kontrol ve VD grupları normal tavşan pelet yemiyle, HC + VD ve HC grupları ise önceden hazırlanmış olan % 0.5 kolesterollü yem ile 8 hafta süresince beslenildi. Bu süreçte VD ve HC + VD gruplarına oral yoldan 300 IU/kg/gün VitD verildi.
Bulgular: HC + VD grubuna göre; HC grubu serum total kolesterol (TC) ve OSI seviyesindeki artış anlamlı bulundu. VD grubu, grup içi serum HDL kolesterol (HDL-C) ve TC seviyelerindeki azalışlar anlamlı bulundu.
Sonuç: Kuşkusuz, sağlıklı bir yaşam için kan VitD seviyemizin ideal aralıkta olması önemlidir. Ancak VitD takviyesi sonucunda azalan serum HDL-C (dolayısı ile PON1) seviyesini, artırmak da gerekmektedir. Dolayısı ile VitD takviyesi ile birlikte, serum HDL-C'yi artıran, düzenli fiziksel aktivite yapılması gerektiğini düşünüyoruz.

Anahtar Kelimeler: D vitamini; Paraoksonaz-1; Oksidatif Stres İndeksi; HDL Kolesterol; Hiperkolesterolemi; Tavşan.

Introduction

It is now generally accepted that VitD deficiency is a worldwide health problem that affects not only musculoskeletal

health but also a wide range of acute and chronic diseases. However, there remains cynicism about the lack of randomized controlled trials to support the association studies regarding the nonskeletal health benefits of VitD. There is potentially a great upside to increasing the VitD status of children and adults worldwide for improving musculoskeletal health and reducing the risk of chronic illnesses, including some cancers, autoimmune diseases, infectious diseases, type 2 diabetes mellitus, neurocognitive disorders and mortality [1].

Conflicting data are available in literature regarding the effects of VitD supplementation diet on lipid panel. Thus, we are of the idea that a controlled experimental study should be more informative than the others. We evaluated the effects of VitD supplementation on lipid panel with experimental studies. At the same time, this study aimed to evaluate the effects of VitD supplementation on TAS, TOS, OSI, MDA and PON1 values in healthy and hypercholesterolemic male rabbits.

Materials and methods

Experimental animals: All experiments were performed in adherence with the National Institutes of Health Guidelines on the Use of Laboratory Animals, and were approved by the Konya University, Experimental Medicine Application and Research Center and Experimental Animal Ethics Committee with number 2012-030.

A total of 30 male New Zealand rabbits (8–12 months old, 3.15 ± 0.65 kg weight) were obtained from Necmettin Erbakan University, Experimental Medicine Application and Research Center, Konya, Turkey. The animals were individually housed in cages with food and water available at all times, maintained under standard conditions of temperature (22 ± 1°C) and humidity (40 ± 10%), in an air conditioned room (12 full changes of air per 1 h), with regular 12 h light/dark cycle.

Chemicals: Cholesterol-C75209, Corn oil-C8267 (solvent of cholecalciferol) and Cholecalciferol-C9756 were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA).

Experimental chow: Hypercholesterolemic diet prepared by adding 0.5% cholesterol to standard chow diet (w/w), (Bilyem Food Industry Trade Ltd. Co., Ankara, Turkey).

Experimental design: After a 1-week acclimation period animals were randomly divided into four groups: 1st group as control (n:6), 2nd group VD was given VitD (n:8), 3rd group HC + VD was given 0.5% hypercholesterolemic + VitD (n:8), and 4th group HC was given 0.5% hypercholesterolemic (n:8). Control and VD groups were fed with standard chow whereas HC + VD and HC groups were fed with 0.5% cholesterol chows for a period of 8 weeks. During this period VD and HC + VD groups were orally administered with 300 IU/kg/day Cholecalciferol. In addition Control and HC groups were given corn oil. Cholecalciferol was dissolved in corn oil.

Determination of VitD dose: Although, 10–13 IU/kg/day VitD amount is enough for the continuation of life, especially for growing and suckling rabbits, 600–1000 IU/kg/day VitD amount could be sufficient. On the other hand, high VitD amount (3250 IU/kg) can resulted in calcification in soft tissues, aorta and kidney due to excess VitD [2]. Therefore, by considering body weight, age and amount of feeds consumed daily, in this study VitD level determined as 300 IU/kg/day.

Animal preparation and blood collection: Blood samples were collected just before the experiment and in 2 week intervals from all rabbits, and their weights were measured. Blood was withdrawn from the marginal ear veins of all the rabbits after an overnight fasting and the samples stored in both EDTA (1 mL blood/animal) and polymer gel containing tubes (1 mL blood/animal). Blood samples were collected in the same manner, and were allowed to clot for 30 min before centrifugation. Then all samples were centrifuged at 1800×*g* for 10 min at 8°C (NüveNF-800R), and the supernatant was transferred to micro-tubes and they were frozen at −80°C until the analysis (Sanyo MDF-U53865). Serum TC, triglyceride (TG), LDL cholesterol (LDL-C), HDL-C, calcium, and plasma 25(OH) D_3 vitamin levels were measured from collected blood samples. At the end of the 8 weeks, blood and liver were collected. The liver samples were collected under anesthesia with intramuscular injection of ketamine (35 mg/kg, Ketalar, Pfizer, Istanbul, Turkey) and xylazine (5 mg/kg, Rompun, Bayer, Turkey), and then all rabbits were euthanized with the intravenous administration of sodium pentobarbital (30 mg/kg, Pental Sodium, I. E. Ulagay, Istanbul, Turkey).

The levels of plasma 25(OH)D were measured with liquid chromatography-tandem mass spectrometry (LC-MS/MS) method (Zivak-Tandem LC-MS/MS, Istanbul, Turkey). Results were expressed in ng/mL. The levels of serum TC, TG, LDL-C, HDL-C, and calcium were measured using commercially available kits based on routine methods on Architect C 8000 System (Abbott Laboratories, Abbott Park, IL, USA).

TAS, TOS, PON1 and MDA were measured from both blood samples and liver tissues. Total antioxidant status assay kit (Rel Assay Diagnostics, prod. code RL0017, Gaziantep, Turkey) for TAS, total oxidant status assay kit

(Rel Assay Diagnostics, prod. code RL0024, Gaziantep, Turkey) for TOS, and paraoxonase 1 activity measurement kit (Rel Assay Diagnostics, prod. code RL0031, Gaziantep, Turkey) for PON1 activity were measured with analyzer in Architect C 8000 System according to the manufacturer's instructions (Abbott Laboratories, Abbott Park, IL, USA). Rabbit malondialdehyde elisa kit (Hangzhou Eastbiopharm Co., Ltd., prod. code E20131205010, Hangzhou, China) for MDA levels were spectrophotometrically analyzed according to the manufacturer's instructions.

For serum TAS, TOS, MDA and PON1 activity, the results were expressed in mmoltrolox equivalent/L, μmol H_2O_2 equivalent/L, nmol/mL, U/L; sequentially. For liver TAS, TOS, MDA and PON1 activity, the results were expressed in μmol trolox equivalent/mg protein, μmol H_2O_2 equivalent/mg protein, nmol/mg protein, U/mg protein; sequentially. OSI determined using the formula OSI = [(TOS, μmol H_2O_2 equivalent/L)/(TAS, μmoltrolox equivalent/L) × 100] [3].

Preparation of tissue homogenate: Liver tissues, for biochemical analysis, 10% (w/v) homogenate was prepared in sodium phosphate buffer (50 mM PBS, pH 7.4). Tissues in ice bath (4–8°C) were homogenized for 20 s (Ultra-Turrax T10, Germany), and then they were sonicated for 30 s (Bandelin Sonopuls UW 2070, Germany). Homogenates were centrifuged at 10.000×*g* for 15 min at 4°C (Nüve NF-800R), and supernatant was transferred to micro-tubes and they were frozen at –80°C until the analysis (Sanyo MDF-U53865).

Statistical analysis

All of the data were analyzed using Statistical Package for the Social Sciences (SPSSv16.0, Chicago, IL, USA) statistical software. Tests of normality were performed on response variables using the Shapiro-Wilk statistic. All results were given as means ± standard deviation. Multiple groups were compared using a one-way ANOVA and repeated measures ANOVA which was followed by a Bonferroni test. Differences within groups were analyzed by analysis of paired T. $p < 0.05$ was considered statistically significant.

Results

The body weights, serum TC, TG, LDL-C, HDL-C, calcium and plasma 25(OH)D_3 levels were evaluated at the beginning and end of the 8th week within the each group.

The results showed that the average rabbit body weight of each group was not statistically significant between the groups. At the end of study, there was a statistically

Table 1: The effects of VitD supplementation on experimental animals on body weight and levels of lipid panel.

	Groups	Baseline	End[a]	Within-group "p" values
Body weight (g)	Control	3137 ± 415	3363 ± 433	*0.021*
	VD	3159 ± 531	3227 ± 335	0.571
	HC + VD	3249 ± 422	3091 ± 388	*0.022*
	HC	3088 ± 405	2960 ± 484	0.227
TC (mg/dL)	Control	39.2 ± 15.1	46.0 ± 17.1	0.323
	VD	54.8 ± 13.7	35.0 ± 12.4	*0.040*
	HC + VD	49.3 ± 16.4	**725.6 ± 245.8**[a2,b2]	*0.000*
	HC	52.4 ± 20.5	**1312.4 ± 648.8**[a3,b3,c1]	*0.001*
TG (mg/dL)	Control	70.7 ± 20.2	49.7 ± 21.9	0.244
	VD	54.9 ± 13.1	75.3 ± 14.0	*0.051*
	HC + VD	54.1 ± 21.3	**199.0 ± 94.5**[a2,b2]	*0.007*
	HC	72.8 ± 23.0	**183.8 ± 64.1**[c2,b2]	*0.001*
LDL-C (mg/dL)	Control	6.83 ± 3.55	6.83 ± 4.45	1.000
	VD	8.00 ± 2.89	9.13 ± 8.11	0.737
	HC + VD	6.00 ± 2.93	**372.6 ± 141.57**[a3,b3]	*0.000*
	HC	7.88 ± 3.40	**438.9 ± 209.21**[a3,b3]	*0.001*
HDL-C (mg/dL)	Control	27.50 ± 15.83	24.33 ± 14.04	0.615
	VD	32.75 ± 9.85	17.50 ± 8.05	*0.028*
	HC + VD	23.25 ± 12.14	34.50 ± 9.12	0.066
	HC	20.63 ± 10.74	21.38 ± 4.53	0.844

[a]Between groups compared to (control $p < 0.01$: a2, $p < 0.001$: a3); (VD $p < 0.01$: b2, $p < 0.001$: b3); (HC + VD $p < 0.05$: c1, $p < 0.01$: c2) significant values. Between-group evaluations are given in bold and within-group comparisons are given in italic.

Table 2: The effects of VitD supplementation on experimental animals on levels of serum calcium and plasma $25(OH)D_3$.

	Groups	Baseline	End[a]	Within-group "p" values
Calcium (mg/dL)	Control	15.03 ± 1.13	15.67 ± 1.03	0.369
	VD	15.59 ± 0.94	13.62 ± 0.92	*0.002*
	HC + VD	14.84 ± 0.57	**14.38 ± 1.85**[a3,b3]	0.529
	HC	15.05 ± 1.05	**12.25 ± 0.89**[a3,b3]	*0.000*
$25(OH)D_3$ (ng/mL)	Control	88.67 ± 10.31	86.17 ± 15.77	0.793
	VD	85.23 ± 12.95	128.71 ± 43.25	*0.027*
	HC + VD	78.40 ± 17.18	85.13 ± 49.60	0.740
	HC	81.40 ± 16.34	**24.53 ± 13.14**[a1,b3,c1]	*0.000*

[a]Between groups compared to (control $p < 0.05$: a1, $p < 0.001$: a3); (VD $p < 0.001$: b3) significant values. Between-group evaluations are given in bold and within-group comparisons are given in italic.

significant increase ($p < 0.05$) in the control group, and in the HC + VD group, there was a statistically significant decrease ($p < 0.05$). Moreover, this kind of change was not observed within the other groups (Table 1).

There was a statistically significant increase in serum TC, TG, LDL-C levels at the end of the 8th week in the HC + VD and HC groups, which were fed with hypercholesterolemic diet. Compared to the control group, alteration in serum TC, HDL-C, LDL-C, TG levels of VD group was not statistically significant. Furthermore, compared to the HC + VD group, the increase in serum LDL-C levels of HC group and the decrease in serum TG, HDL-C levels of this group were not statistically significant. However, compared to the HC + VD group, the increase in serum TC levels of HC group was a statistically significant ($p < 0.05$). In the VD group there was a statistically significant decrease ($p < 0.05$) in serum TC, HDL-C levels within each group. The results belonging to other lipid parameter values were given in the Table 1.

In our study, there was no change in the HC + VD group, but in the VD group, there was a statistically significant increase ($p < 0.05$) and in the HC group, there was a statistically significant decrease ($p < 0.001$) in plasma $25(OH)D_3$ levels within each group. Serum calcium and plasma $25(OH)D_3$ levels parameter results were presented in the Table 2.

Alteration in serum TAS, TOS, MDA, OSI and PON1 levels of VD group compared to the control group, and TAS, TOS, MDA levels of HC group compared to the HC + VD group were not statistically significant. Similarly, in the liver tissue, change in TAS, TOS, MDA, PON1, OSI levels of VD group compared to the control group and TAS, TOS, OSI and MDA, PON1 levels of HC group compared to the HC + VD group was not statistically significant. On the other hand, compared to the HC + VD group, the increase in serum OSI levels ($p < 0.05$), the decrease in serum PON1 levels ($p < 0.05$) of HC group was a statistically significant (Table 3).

Table 3: The effects of VitD supplementation on experimental animals on levels of TAS, TOS, MDA and PON1 at the end of study.

	Groups	Serum[a]	Liver[a]
TAS	Control	1.78 ± 0.25	15.17 ± 3.24
Serum: mmol/L	VD	1.81 ± 0.36	11.84 ± 2.10
Liver: µmol/mg	HC + VD	1.87 ± 0.33	14.03 ± 1.76
protein	HC	1.64 ± 0.14	**16.06 ± 3.60**[b1]
	Control	15.43 ± 10.18	1.04 ± 0.16
TOS	VD	10.47 ± 5.43	0.95 ± 0.22
Serum: µmol/L	HC + VD	**40.43 ± 16.58**[b1]	1.65 ± 0.40
Liver: µmol/mg	HC	**61.02 ± 31.31**[a3, b3]	**2.21 ± 1.04**[a2,b2]
protein			
OSI	Control	0.86 ± 0.57	7.07 ± 1.44
	VD	0.55 ± 0.23	8.20 ± 2.33
	HC + VD	**2.11 ± 0.71**[b1]	11.85 ± 2.83
	HC	**3.75 ± 1.96**[a3,b3,c1]	**15.30 ± 10.41**[a1]
MDA	Control	6.28 ± 0.42	9.49 ± 3.07
Serum: nmol/	VD	6.44 ± 1.24	7.33 ± 2.53
mL	HC + VD	7.57 ± 0.68	9.64 ± 1.74
Liver: nmol/mg	HC	**8.83 ± 1.37**[a3,b2]	**11.34 ± 2.53**[b1]
protein			
PON1	Control	232.83 ± 32.9	8.98 ± 0.92
Serum: U/L	VD	210.7 ± 56.2	8.15 ± 1.35
Liver: U/mg	HC + VD	218.1 ± 56.5	**7.68 ± 0.45**[a1]
protein	HC	**147.85 ± 40.0**[a2,c1]	**6.43 ± 0.52**[a3,b1]

[a]Between groups compared to (control $p < 0.05$: a1, $p < 0.01$: a2, $p < 0.001$: a3); (VD $p < 0.05$: b1, $p < 0.01$: b2, $p < 0.001$: b3); (HC + VD $p < 0.05$: c1) significant values. Between-group evaluations are given in bold.

Discussion

In this study, we evaluated the effects of VitD supplementation on TAS, TOS, MDA and PON1 levels in serum and liver from normal and hypercholesterolemic rabbits, and furthermore, we evaluated TC, TG, LDL-C, HDL-C, calcium and VitD(plasma) values in serum.

Lipid panel levels: In the literature, there are conflicts about the effect of the supplementation of VitD on serum TC level. That is, some studies [4–6] showed that

VitD supplementation had no effect on serum TC level and some studies [7, 8] declared that there was an decrease in serum TC level after VitD supplementation but not statistically significant. Besides, some other studies [9–11] pointing out statistically significant decrease in serum TC level after VitD supplementation similar to our study. In literature, some reasons influencing the response of individuals to the VitD supplementation are indicated. These reasons are; being obese or overweight [6, 10], race [4], physiological [4, 6, 7, 10] and physiopathological condition [9, 11, 12], sex [10, 11], age [6, 7, 10], insufficient increase of blood VitD levels [9], and other individual differences [8].

In our study, the effect of VitD in decreasing serum TC is minimal [11] and in high levels of cholesterol, its effect would be more apparent, therefore a significant difference between VD group and control group might not occur. Most target genes of liver X receptors play a regulatory role in the synthesis pathway of fatty acid metabolism that also contains cholesterol. These receptors form heterodimer with retinoid X receptor and just as it acts as a cholesterol sensor, it also provides cholesterol hemostasis by acting as a gene regulator in the transportation of lipid and movement of cholesterol from the cells [11]. With the increase of $25(OH)D_3$ vitamin levels in blood, one of the metabolites of calcitriol or VitD affect liver X receptors α or/and liver X receptors β, and thus, the synthesis or absorption of cholesterol might decrease and/or cholesterol out flow [11, 13], clearance [14] might increase.

Serum TG levels were evaluated with regard to VitD supplementation, and there was not statistically significant difference between the groups (Table 1). Conversely, evaluations were done within the group even though the increase in TG levels from the start to end of the study in VD group was not statistically significant, and here p-value was found to be 0.051. The effects of VitD supplementation on TG levels are evaluated in studies some researchers reported that serum TG levels decreased significantly with supplementation of VitD ($p < 0.05$) [6, 7], and in diabetic rats there was also a significant decrease ($p < 0.05$) [12]. Others reported that there was a decrease but insignificant [10]. At the same time there are studies reporting that long periods of VitD supplementation tend to cause decrease [8], and some even have reported that it has no effect [4, 5, 9]. It is well known that coprophagy is seen in rabbits. In other words, they eat their soft feces at night. In the reports, the importance of microbial digestion in rabbits and other herbivorous animals and also microbial fermentation in cecum are still not very known. Digestion of foods with microbial fermentation is related to the isomerisation and hydrogenation of unsaturated fatty acids. This results in the production of considerable amounts of several trans fatty acid, conjugated linolenic acids and stearic acid [15]. These kinds of fatty acids, vitamins and nutrients are retaken into the body by coprophagy in rabbits. In our study even though we did not feed the rabbits in the evening when blood was withdrawn, the rabbits fed themselves through coprophagy, and hence serum TG levels might have been affected in this situation. It has been concluded from the results of our study that the tendency of serum TG levels to increase in the VD and HC + VD groups compared to their own control groups might be as a result of coprophagy or coprophagy and VitD interaction or VitD supplementation, even though evaluation among the groups is not significant.

In various studies different results are obtained from the effects of VitD supplementation on serum LDL-C. In one of these studies, there was a significant increase in serum LDL-C ($p < 0.05$) [8]. In another, there was no effect [6]. There was a significant decrease ($p < 0.05$) [4, 9, 12], in yet other study. There are results showing insignificant decrease [7, 10] like those in our study. We think that these differences in outcome may be due to the same reasons explained in serum TC levels.

Just like in the other lipid panel results there were differences in effects of VitD supplementation on serum HDL-C levels when the recent years studies were reviewed. Regarding these results, the researchers found out that there was a significant increase ($p < 0.05$) [10, 12], no effect [4, 6, 9], an insignificant decrease [7], and that continuous VitD supplementation would decrease in serum HDL-C [8]. Another study reported results supporting our study in that HDL-C levels was significantly high and that HDL-C's main protein apolipoprotein A-I (apoA-I) was also high in VDR knock-out rats [11]. In our study the differences in serum HDL-C levels among the groups were not statistically significant. But serum HDL-C levels were found to be significantly decreased in the VD group when compared to the beginning and end of the study (Table 1). In addition, it has been reported that $24,25(OH)_2D_3$ suppresses the expression of HDL-C's main protein apoA-I gene [16]. With the increase of $25(OH)D_3$ vitamin levels in blood, one of the metabolites of calcitriol or VitD affected apoA-I [17], liver X receptors α [13] and/or liver X receptors β [11], and therefore it was concluded that the decrease in cholesterol would result in the decrease of HDL-C at the same time.

Plasma $25(OH)D_3$ and serum calcium levels: It is well known that 25(OH)D vitamin is the storage form of VitD in the body, and when required it can change to calcitriol or the other metabolites of VitD [1]. Therefore, plasma 25(OH)D vitamin levels were monitored in the all groups. While there was no increase in the levels of $25(OH)D_3$ vitamin in the control group, plasma $25(OH)D_3$ vitamin

levels significantly increased in the VD group. As for the hypercholesterolemia groups, plasma 25(OH)D_3 vitamin levels decreased towards the end of the study in the HC group and in the HC + VD group plasma 25(OH)D_3 vitamin levels was maintained at the same levels as that in the beginning (Table 2).

Serum calcium levels were monitored because of the risk of developing hypercalcemia due to VitD. Serum calcium levels were significantly decreased in the VD ($p < 0.01$) and HC groups ($p < 0.001$) at the end of the study. On the other hand, there was no significant difference in the other groups (Table 2). Different in the other mammals, absorption of calcium in rabbits is independent of VitD. Serum total calcium concentration in blood changes according to the amount of calcium in the diet. Intestinal absorption of calcium occurs by means of passive diffusion. An excess is cleared away with urine [18]. Even though normal blood calcium levels in rabbits depends on the diet they consume, according to Jekl and Redrobe it is around 5.96–14.8 mg/dL. It has also been reported that even when rabbits are fed with high calcium diet, calcium levels can quickly reach over 18 mg/dL [18]. The average levels of serum calcium at the beginning of the study decreased from 15.59 ± 0.94 to 13.62 ± 0.92 in the VD group. Furthermore, according to the results of the measurements taken every 2 weeks, the average maximum levels of calcium in the control and VD groups were close to each other. Even though there is a significant decrease in calcium levels, according to the literature [18] this reduction is within normal limits. The aim of monitoring calcium levels stems from hypercalcemia that can develope due to hypervitaminosis of VitD. When the individual high calcium levels that can develope due to daily diet consumed by rabbits is considered, the average calcium levels in the control group did not exceed that of VD group and therefore it can be concluded that hypervitaminosis due to VitD did not occur.

TAS, TOS, MDA and PON1 levels: Considering the results of VitD supplementation or normal VitD levels, TAS levels were found to be significantly higher compared to those of the groups that were deficient/insufficient of VitD ($p < 0.01$, $p = 0.03$, $p < 0.001$; sequentially) [19–21]; whereas Asemi et al. and Sharifi et al. did not find any differences in the levels of TAS between the VD group and controls [9, 22]. Asemi performed the study on pregnant women and Sharifi on individuals with fatty liver and in these studies, VitD levels did not increase enough. This may cause the fact that TAS levels may not have increased. There is a positive correlation between VitD levels and TAS levels [21, 23] and for this reason those that consumed normal diet did not have low VitD and therefore no significant difference between TAS levels which might have occurred. Even though the VD group did not have a significant increase in TAS levels, it was higher than that in the control group (Table 3). In light of these findings, we have come to the conclusion that addition of VitD to the diet can cause a partial increase in TAS levels.

With regard to TOS levels, Baser et al. [21] reported that TOS levels are significantly higher ($p < 0.05$) in people with deficiency VitD levels compared to control group. de Medeiros Cavalcante et al. [20] reported that high sensitivity C reactive protein (hs-CRP) which is a sensitive inflammation indicator decreased significantly in the VD group compared to the group that was deficient of VitD ($p = 0.007$). Foroozanfard et al. [19] and Sharifi et al. [22] reported that hs-CRP levels decreased but not significantly in the VD group compared to control group. Sharifi et al. [22] reported that there was no effect on the levels of TNFα which is an inflammation indicator in the VD group compared to control group. Başer et al. [21] reported that there was not statistically significant change in hs-CRP and Ox-LDL levels between people with deficient VitD levels and those with normal VitD levels. In this study, TOS levels were higher especially in HC group compared to the other groups (control, VD and HC + VD). However, when comparison is done between the groups (VD and control, HC + VD and HC), they were not significant. This is because just like those in TAS levels, VitD levels are not very different between rabbits that are fed with normal diet and those that are given VitD supplements. For this reason, their responses to oxidative stress are quite close, and so their protective effects against oxidant stress [24–26], DNA injury [25] are close to each other. OSI which is an oxidative stress index indicator was not found to be significant in serum and tissue samples in the VD group compared to control group. However, in the HC + VD group compared to HC group serum OSI levels were significantly lower ($p < 0.05$). This result supports that VitD supplementation has an effect of decreasing OSI index against oxidant stress increasing factors. This effect may be increased by an increase in the relative VitD levels (Table 3).

There was not statistically significant difference in MDA levels between the groups in terms of VitD supplementation. Our findings were consistent with some studies [19, 20, 24]. However, MDA levels had a tendency to decrease in the group that was given VitD (Table 3). VitD's protective effect against oxidative stress would be at a low levels [24–26] or there may not be significant differences in plasma 25(OH)D_3 between the groups that were given VitD and the group that were not given VitD. Because of this there may have been no significant difference in MDA levels.

In the literature, data on the effects of VitD on PON1 activity are limited. With regards to our study, serum PON1

enzyme activity was higher in the HC + VD group compared to HC group. In the HC + VD group, the presence of PON1 activity which is usually localized on HDL-C [27], may be due to an increase in serum HDL-C levels. However, there was no increase in serum PON1 activity in the HC group, and the increase in the HC + VD group was thought to be due to an increase in serum HDL-C levels rather than VitD supplement (Tables 1 and 3). This is because the value of PON1 activity (even though not significant) in serum and tissue decreased in VD group compared to control group (Table 3). Eren et al. in their study reported that value of PON1 activity decreased with proportion in the decrease in VitD level. However, VitD was not supplemented in this study [28]. Ferretti et al. [29] related to the decrease in value of PON1 activity with cardiovascular diseases in their study. This situation raises the question whether this disease state is as a result of the decrease in value of PON1 activity, if not then is it because of the presence of cardiovascular diseases or is it the precipitating factors of the disease that cause decrease in the value of PON1 activity. Serum HDL-C levels were seen to decrease significantly in the VD group at the end of the study (Table 1). Both the value of PON1 activity and value of HDL-C decreased in the VD group compared to control group, and for this reason value of PON1 activity was thought to have decreased due to VitD supplementation. PON1 activity which has very important functions, can decrease as a result of VitD supplementation.

In conclusion, serum TC, HDL-C, OSI decreased significantly in rabbits (male) that were given VitD compared to their own control group in our study. Furthermore, as a result of decreased levels of HDL-C, the value of PON1 activity which is usually located on HDL-C also decreased but this was not statistically significant. We share the belief that when VitD that can be obtained naturally from sunlight is to be supplemented, caution should be taken according to the daily dose, period, age, and sex. Without any doubt it is important that applied VitD level should be in the ideal range for healthy living. However, it is also necessary to increase the serum HDL-C level (and hence PON1), which is decreases as a result of VitD supplementation. Therefore, we believe that during VitD supplementation, regular physical activity should be performed to increases serum HDL-C [30, 31].

Acknowledgment: This thesis study was supported by from Necmettin Erbakan University Scientific Research Projects Commission (project no: 131418001).

Conflict of interest statement: The authors have no conflict of interest.

References

1. Hossein-Nezhad A, Holick MF. Vitamin D for health: a global perspective. Mayo Clin Proc 2013;88:720–55.
2. Lebas F. Vitamins in rabbit nutrition: literature review and recommendations. World Rabbit Sci 2000;8:185–92.
3. Kosecik M, Erel O, Sevinc E, Selek S. Increased oxidative stress in children exposed to passive smoking. Int J Cardiol 2005;100:61–4.
4. Schnatz PF, Jiang X, Vila-wright S, Aragaki AK, Nudy M, O'Sullivan DM, et al. Calcium/vitamin D supplementation, serum 25-hydroxyvitamin D concentrations, and cholesterol profiles in the Women's Health Initiative calcium/vitamin D randomized trial. Menopause J North Am Menopause Soc 2014;21:1–11.
5. Al-Asmari AK, Ullah Z, Al-Sabaan F, Tariq M, Al-Eid A, Al-Omani SF. Effect of vitamin D on bioavailability and lipid lowering efficacy of simvastatin. Eur J Drug Metab Pharmacokinet 2015;40:87–94.
6. Muñoz-Aguirre P, Flores M, Macias N, Quezada AD, Denova-Gutiérrez E, Salmerón J. The effect of vitamin D supplementation on serum lipids in postmenopausal women with diabetes: a randomized controlled trial. Clin Nutr 2015;34:799–804.
7. Islam MZ, Shamim AA, Akhtaruzzaman M, Kärkkäinen M, Lamberg-Allardt C. Effect of vitamin D, calcium and multiple micronutrients supplementation on lipid profile in pre-menopausal Bangladeshi garment factory workers with hypovitaminosis D. J Heal Popul Nutr 2014;32:687–95.
8. Wang H, Xia N, Yang Y, Peng D-Q. Influence of vitamin D supplementation on plasma lipid profiles: a meta-analysis of randomized controlled trials. Lipids Health Dis 2012;11:42.
9. Asemi Z, Hashemi T, Karamali M, Samimi M, Esmaillzadeh A. Effects of vitamin D supplementation on glucose metabolism, lipid concentrations, inflammation, and oxidative stress in gestational diabetes : a double-blind randomized controlled clinical trial. Am J Clin Nutr 2013;98:1425–32.
10. Maki KC, Rubin MR, Wong LG, McManus JF, Jensen CD, Lawless A. Effects of vitamin D supplementation on 25-hydroxyvitamin D, high-density lipoprotein cholesterol, and other cardiovascular disease risk markers in subjects with elevated waist circumference. Int J Food Sci Nutr 2011;62:318–27.
11. Wang J-H, Keisala T, Solakivi T, Minasyan A, Kalueff AV, Tuohimaa P. Serum cholesterol and expression of ApoAI, LXRbeta and SREBP2 in vitamin D receptor knock-out mice. J Steroid Biochem Mol Biol 2009;113:222–6.
12. Calgaroto NS, da Costa P, Cardoso AM, Pereira LB, Vieira JM, Dalenogare D, et al. Vitamin D3 prevents the increase in ectonucleotidase activities and ameliorates lipid profile in type 1 diabetic rats. Mol Cell Biochem 2015;405:11–21.
13. Jiang W, Miyamoto T, Kakizawa T, Nishio SI, Oiwa A, Takeda T, et al. Inhibition of LXRalpha signaling by vitamin D receptor: possible role of VDR in bile acid synthesis. Biochem Biophys Res Commun 2006;351:176–84.
14. Wójcicka G, Jamroz-wiśniewska A, Horoszewicz K. Liver X receptors (LXRs). Part I: structure, function, regulation of activity, and role in lipid metabolism. Postep Hig Dosw 2007;61:736–59.
15. Leiber F, Meier JS, Burger B, Wettstein HR, Kreuzer M, Hatt JM, et al. Significance of coprophagy for the fatty acid profile in body tissues of rabbits fed different diets. Lipids 2008;43:853–65.

16. Wehmeier KR, Alamir AR, Sultan S, Haas MJ, Wong NC, Mooradian AD. 24,25-Dihydroxycholecalciferol but not 25-hydroxycholecalciferol suppresses apolipoprotein A-I gene expression. Life Sci 2011;88:110–6.
17. Wehmeier K, Beers A, Haas MJ, Wong NC, Steinmeyer A, Zugel U, et al. Inhibition of apolipoprotein AI gene expression by 1,25-dihydroxyvitamin D3. Biochim Biophys Acta 2005;1737:16–26.
18. Jekl V, Redrobe S. Rabbit dental disease and calcium metabolism – the science behind divided opinions. J Small Anim Pract 2013;54:481–90.
19. Foroozanfard F, Jamilian M, Bahmani F, Talaee R, Talaee N, Hashemi T, et al. Calcium plus vitamin D supplementation influences biomarkers of inflammation and oxidative stress in overweight and vitamin D-deficient women with polycystic ovary syndrome: a randomized double-blind placebo-controlled clinical trial. Clin Endocrinol (Oxf) 2015;83:888–94.
20. de Medeiros Cavalcante IG, Silva AS, Costa MJ, Persuhn DC, Issa CI, de Luna Freire TL, et al. Effect of vitamin D3 supplementation and influence of BsmI polymorphism of the VDR gene of the inflammatory profile and oxidative stress in elderly women with vitamin D insufficiency vitamin D3 megadose reduces inflammatory markers. Exp Gerontol 2015;66:10–6.
21. Baser H, Can U, Baser S, Taha B, Aslan U, Buyuktorun I, et al. Serum total oxidant/anti-oxidant status, ischemia-modified albumin and oxidized-low density lipoprotein levels in patients with vitamin D deficiency. Arch Endocrinol Metab 2015;59:318–24.
22. Sharifi N, Amani R, Hajiani E, Cheraghian B. Does vitamin D improve liver enzymes, oxidative stress, and inflammatory biomarkers in adults with non-alcoholic fatty liver disease? A randomized clinical trial. Endocrine 2014;47:70–80.
23. Saedisomeolia A, Taheri E, Djalali M, Djazayeri A, Qorbani M, Rajab A, et al. Vitamin D status and its association with antioxidant profiles in diabetic patients: a cross-sectional study in Iran. Indian J Med Sci 2013;67:29–37.
24. Eftekhari MH, Akbarzadeh M, Dabbaghmanesh MH, Hassanzadeh J. The effect of calcitriol on lipid profile and oxidative stress in hyperlipidemic patients with type 2 diabetes mellitus. ARYA Atheroscler 2014;10:82–8.
25. Lan N, Luo G, Yang X, Cheng Y, Zhang Y, Wang X, et al. 25-Hydroxyvitamin D3-deficiency enhances oxidative stress and corticosteroid resistance in severe asthma exacerbation. PLoS One 2014;9:e111599.
26. Bhat M, Ismail A. Vitamin D treatment protects against and reverses oxidative stress induced muscle proteolysis. J Steroid Biochem Mol Biol 2015;152:171–9.
27. van Himbergen TM, van Tits LJ, Roest M, Stalenhoef AF. The story of PON1 : how an organophosphate- hydrolysing enzyme is becoming a player in cardiovascular medicine. Neth J Med 2006;64:34–8.
28. Eren E, Ellidag HY, Yılmaz A, Aydın O, Yılmaz N. Acute phase response: implication in ST-segment elevation myocardial infarction. Open Biochem J 2014;8:44–51.
29. Ferretti G, Bacchetti T, Sahebkar A. Effect of statin therapy on paraoxonase-1 status: a systematic review and meta-analysis of 25 clinical trials. Prog Lipid Res 2015;60:50–73.
30. Ruppar TM, Conn VS, Chase JA, Phillips LJ. Lipid outcomes from supervised exercise interventions in healthy adults. Am J Health Behav 2014;38:823–30.
31. Mann S, Beedie C, Jimenez A. Differential effects of aerobic exercise, resistance training and combined exercise modalities on cholesterol and the lipid profile: review, synthesis and recommendations. Sport Med 2014;44:211–21.