Original Article

Protective Effect of Vitamin D Supplementation on Some Hemogram Derived Inflammatory Indices in Normal and High-Fat Diet Fed Male Wistar Rats

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Abstract

Background: Hematological inflammatory indices are currently suggested to assess systemic inflammation. This study aims to investigate a vitamin D supplementation effect on hematological indices of inflammation in rats. **Method:** Forty-eight middle-aged male rats were allocated into a normal diet (ND) group (10% fat) and a high-fat diet (HFD) group (60% fat). The animals were fed for 26 weeks. After this period, each group was randomly divided into three subgroups, each of 8 rats: Group (1): animals were fed the ND and HFD containing 1 IU/g vitamin D for 4 months, group (2): animals were fed the ND and HFD containing 6 IU/g vitamin D for 4 months and group (3): animals were euthanized to evaluate the HFD effect. Serum 25-hydroxyvitamin D level, white blood cell count (WBCs), platelet count, platelet crit (PCT), mean platelet volume (MPV), platelet distribution width (PDW), platelet-to-lymphocyte ratio (PLR), neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR) were measured. **Results:** The HFD, significantly increased body weight, PCT, PDW, PLR, NLR, and MLR and significantly reduced serum vitamin D levels compared to the ND ($P < 0.05$). There was a significant decrease in food intake, MPV, PDW, and NLR after vitamin D supplementation in the ND-fed group ($P < 0.05$). A significant reduction in platelet count, PCT, and MLR was observed after vitamin D supplementation in HFD-fed rats ($P < 0.05$). **Conclusions:** In our study, some hemogram-derived inflammatory indices were higher in the HFD-fed group, and vitamin D supplementation lowering effects on some hematological indices were seen in both ND and HFD groups.

Keywords: *Blood cell count, high-fat diet, inflammation, vitamin D*

Introduction

The population average age is rising in developed and developing countries.[1] As the population ages, the risk of developing age-related disorders also increases.[2] So, healthy aging has become the most important global public health issue in the last few years. "Inflammaging", low-grade, chronic, and systemic inflammation", is one of the contributory factors in aging and age-related diseases.[3,4]

Some novel and inexpensive hematological inflammatory indicators are currently suggested to assess systemic inflammation such as neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), and platelet-to-lymphocyte ratio (PLR) in different medical disorders.[5-7] With increasing age, NLR, and PLR values increase.[8,9] Elevated NLR, MLR, PLR, and white blood cell count (WBCs) values have been associated with unpleasant clinical-pathologic characteristics of many disorders.[9-12]

Other hemogram-derived factors involved in inflammation are platelets and platelet indices.[13,14] Mean platelet volume (MPV), platelet distribution width (PDW) and platelet crit (PCT) are platelet indices that are potential markers of platelet activation.[13,14] With aging, platelet count and platelet crit decrease, and platelet reactivity increases.[14,15] Increased platelet activity is implicated in age-related inflammatory and neurological disorders.[15]

One of the associated factors with the inflammatory status of the elderly is the quality of the diet.[16] The western dietary pattern is positively correlated with NLR.[17] High-fat diet-induced obesity increases the number of WBC, neutrophils, and lymphocytes.[18] Elevated levels of

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platelet counts, PCT, PLR, and WBC have been reported in obese individuals.[19]

Low vitamin D status is a common and emerging health condition in older adults.[20] According to available data, the association between vitamin D correlation and also supplementation with hematological indices is inconsistent.[21-24] More recently, a study by Stojsic Vuksanovic and colleagues found that Vitamin D3 correction in vitamin D3 deficiency and diabetes type 2/ diabetic nephropathy has anti-inflammatory activity that is most pronounced on NLR and less on PLR.[25] According to an investigation by Tabatabaeizadeh and coworkers, high-dose vitamin D supplementation in adolescent girls reduces NLR.[26] Although there were several studies on the anti-inflammatory effect of vitamin D, few of them focused on hematological indices of inflammation. Therefore, in the present study, middle-aged rats were fed a normal diet (ND) and high-fat diet (HFD) and then received vitamin D supplements to be experimentally investigated concerning the HFD and vitamin D supplementation influence on WBCs, platelet count, PCT, MPV, PDW, PLR, NLR, and MLR.

Method

Experimental animals

This was an experimental intervention study. The research protocol was approved by the Ethics Committee of ?? University of Medical Sciences (??1397.472). Forty-eight healthy Wistar male rats, at 12-12.5 months, were purchased from Royan Institute. Animals were housed in animal care???. The rats were maintained at 22 ± 1°C under a 12-hour day-night cycle in a separate room.

Experimental design and procedures

After one week of acclimatization, the animals were randomized by a computer-generated randomization program into 2 groups (n = 24, each group) of ND and HFD. ND contains 70% carbohydrates, 10% fat, and 20% protein, and HFD consisted of 60% energy from fat, 20% from carbohydrates, and 20% from protein. The animals were on diets for 26 weeks. After this period, 24 rats in each group were randomly allocated into three sub-groups: Group (1): animals were fed the ND and HFD containing 1 IU/g vitamin D (Control groups; n = 8), group (2): animals were fed the ND and HFD containing 6 IU/g vitamin D (Intervention groups; n = 8) and group (3): animals were euthanized to determine the HFD effects (n = 8) [Figure 1]. The duration of the second phase was 4 months. All diets were purchased from Royan Institute for Biotechnology and met the national standard for rat feed. All animals were individually housed in cages. Food and water always were provided ad libitum during the intervention period for all rats. The animal experiments' health was monitored daily. Their body weight was assessed at baseline and weekly during the experimental period. Daily food intake was recorded and the average intake was measured weekly throughout the study.

Sample size

The sample size was determined according to the Resource Equation Method.[27]

Inclusion and exclusion criteria

Inclusion criteria were healthy male Wistar rats at 12 to 14 months of age. Lack of inclusion criteria at baseline as well as severe weight loss and morbidity during the study were exclusion criteria.

Blinding

In this study, full blinding was not possible because the researcher who conducted the experiment was not blind. However, the researcher who did the randomization, allocation of the groups, and data analysis was blind.

Outcomes

Primary outcomes were hematological indices of inflammation. Measurement of serum 25-hydroxyvitamin D 25(OH) D was a secondary outcome.

Sample collection

At the end of the study, rats were fasted overnight and received water ad libitum. Blood was collected from the heart of animals which were anesthetized by an intraperitoneal injection of xylazine hydrochloride and ketamine hydrochloride. Whole blood samples collected in EDTA tubes were used to determine the following blood indices: WBCs, platelet count, PCT, MPV, PDW, neutrophils, lymphocytes, and monocytes. All measurements were carried out using a veterinary blood cell counter. Rations were derived from complete blood count (CBC) including PLR, NLR, and MLR. The remaining blood samples were centrifuged at 1500 × g for 10 min to separate the serum for measurement of 25(OH) D. Serum 25(OH) D was assessed by High-Performance Liquid Chromatography (HPLC) method.

Statistical methods

First, the normality of the data was assessed using graphs and the Shapiro-Wilks test. Independent-Samples T-Test and Paired-Samples T-Test were used to analyze the variables with normal distribution while to analyze the non-normally distributed data Mann-Whitney U, and Wilcoxon tests were used. The experimental results were presented as means (SD) and Median (Q1-Q3). Data were analyzed by SPSS software. P value < 0.05 was regarded as a statistically significant difference.

Results

There were no significant differences in animal body weight at baseline between the ND [514.95 (13.83); n = 24] and HFD [515.66 (13.32); n = 24)] groups (P = 0.857).

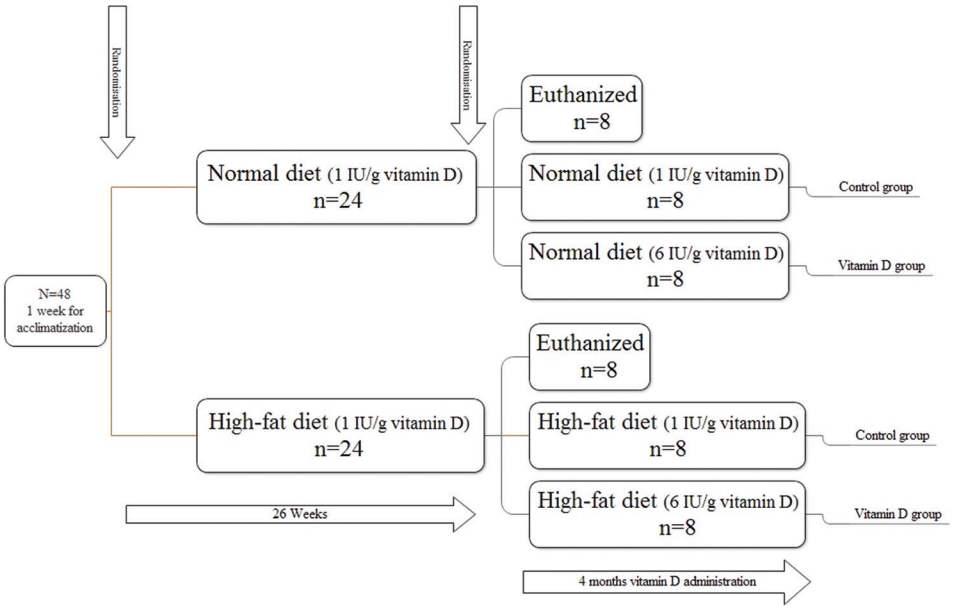

Figure 1: Study method

Effect of a high-fat diet on body weight

The baseline body weight did not differ among the ND and HFD groups (P = 0.597). Rats maintained on the HFD showed a significantly increased body weight gain compared to the ND group after 26 weeks of feeding regimen (P = 0.007) [Table 1].

Effect of a high-fat diet on food and vitamin D intake

Rats maintained on HFD consumed significantly less food and subsequently less vitamin D than the ND group (P < 0.001) [Table 2].

Effect of a high-fat diet on serum vitamin D and some hematological parameters

Serum 25(OH) D level was significantly lower in the HFD group in comparison with the ND group (P = 0.048). A significant increase in PDW, PCT, PLR, NLR, and MLR was observed in rats maintained on HFD as compared to animals fed on ND (All $P < 0.05$). Whereas, an increase in platelet count, WBCs, and MPV in the HFD-fed rats in comparison with the ND-fed rats was not statistically significant (All $P > 0.05$) [Table 2].

Effects of vitamin D supplementation on body weight

Vitamin D supplementation reduced the body weight of ND and HFD-fed rats; however, this decrease was not significantly different from relevant control groups (P > 0.05). In HFD + vitamin D group, the difference between the baseline and final body weight was statistically significant (P = 0.002) [Tables 3 and 4].

Effects of vitamin D supplementation on Food and vitamin D intake

Vitamin D supplementation significantly decreased the food consumption of the intervention group versus the control group (P < 0.05). The average daily vitamin D intake was significantly increased in both vitamin D-treated groups (P < 0.05) [Tables 3 and 4].

Effects of vitamin D supplementation on serum vitamin D and some hematological parameters

As expected, Vitamin D supplementation gives rise to higher serum 25 (OH) D levels in both vitamin D-treated groups. Vitamin D supplements in ND + vitamin D group significantly decreased the MPV, PDW, and NLR compared with the relevant control group (All $P < 0.05$). However, after vitamin D supplementation, a non-significant increase in platelet count, PCT, and WBC and an insignificant decrease in PLR and MLR values were observed in ND + vitamin D group versus the relevant control group (All $P > 0.05$). Vitamin D supplementation significantly reduced platelet count, PCT, and MLR in the HFD + vitamin D group (All $P < 0.05$). In HFD + vitamin D group versus the HFD group the observed decrease in MPV, PLR, and NLR was not statistically significant (All $P > 0.05$) [Tables 5 and 6].

Discussion

In the present study, the HFD harmful effect and some protective impact of vitamin D supplementation on some CBC-derived inflammatory indices in aging rats were shown. Considering that the animals were middle-aged at the baseline, and middle age is a high-risk period for weight gain[28] in our study, body weight gradually increased as they got older, regardless of the diet type, but the rats maintained on the HFD showed significantly more body weight than ND-fed rats.

In the current research, vitamin D supplementation in elderly rats resulted in non-significant weight loss in both

Table 1: The effect of a high-fat diet on body weight gain in middle-aged male Wistar rats

Variable	Groups		*P*
	ND (*n*=8)	HFD (*n*=8)	
Baseline body weight (g)[#]	515 (501-524.5)	519.5 (515-520)	0.597*
Final body weight (g)[#]	525 (521-547.5)	583.50 (544.25-638.25)	0.007*
*P***	0.012	0.012	
Mean difference[#]	13.5 (6-27.5)	64 (33.75-119.7)	0.003*

ND, normal diet; HFD, high fat diet, *Mann-Whitney U, **Wilcoxon, [#]Median (Q1-Q3)

Table 2: The effect of a high-fat diet on food intake, vitamin D intake, serum vitamin, D and some hematological parameters in middle-aged male Wistar rats

Variable	Groups		*P*
	ND (*n*=8)	HFD (*n*=8)	
Food intake (g/day)[#]	21.37 (0.91)	17.14 (1.03)	<0.001*
Vitamin D intake (IU/day)[#]	21.37 (0.91)	17.14 (1.03)	<0.001*
Serum 25(OH) D level (ng/ml)[#]	16.65 (3.06)	13.79 (0.56)	0.048*
WBCs (10^3/UL)[#]	3.70 (1.25)	4.18 (1.53)	0.497*
Platelet count (10^3/UL)[##]	576 (480.50-607.00)	612 (593.25-644.75)	0.059**
PCT%[#]	0.23 (0.05)	0.29 (0.02)	0.027*
MPV (fL)[#]	4.46 (0.35)	4.76 (0.17)	0.055*
PDW%[#]	14.25 (0.53)	15.20 (0.20)	<0.001*
PLR[#]	196.72 (71.63)	359.08 (125.04)	0.017*
NLR[##]	0.51 (0.40-0.64)	0.91 (0.88-1.42)	0.003**
MLR[##]	0.03 (0.02-0.06)	0.11 (0.07-0.12)	0.046**

ND, normal diet; HFD, high fat diet; 25(OH) D, 25-hydroxy vitamin D; WBCs, white blood cells; PCT, plateletcrit; MPV, mean platelet volume; PDW, platelet distribution width; PLR, platelet-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; MLR, monocyte-to-lymphocyte ratio, *Independent-Samples *T*-Test, **Mann-Whitney U, [#]Mean (SD), [##]Median (Q1-Q3)

Table 3: The effect of vitamin D supplementation on body weight, food intake, and vitamin D intake in normal diet fed rats

Variable	Groups		*P*
	Control (*n*=8)	Vitamin D (*n*=8)	
Baseline body weight (g)[#]	531.75 (31.44)	531.25 (16/33)	0.969*
Final body weight (g)[#]	527.87 (54.56)	512.12 (29.74)	0.485*
*P***	0.729	0.069	
Baseline Food intake (g/day)[##]	21.10 (20.42-21.32)	20.77 (20.01-21.89)	0.834***
Final Food intake (g/day)[##]	20. 26 (19.87-21.03)	19.14 (17.89-19.81)	0.005***
*P****	0.012	0.012	
Baseline Vitamin D intake (IU/day)[##]	21.10 (20.42-21.32)	20.77 (20.01-21.89)	0.834***
Final Vitamin D intake (IU/day) [#]	20.52 (0.87)	113.19 (6.70)	0.001*

*Independent-Samples *T*-Test, **Paired-Samples *T* Test, ***Mann-Whitney U, ****Wilcoxon, [#]Mean (SD), [##]Median (Q1-Q3)

vitamin D treated groups compared to control groups. Our results are in line with earlier literature that found vitamin D administration had a significant influence on increased serum 25(OH) D levels[29] and also reduced food intake in animals maintained on both ND and HFD.[30] This finding also supports Park's study which concluded that serum 25(OH) D level has a dose-response correlation with dietary vitamin D intake.[31]

Our data revealed that high-fat diet consumption in middle age can affect platelet parameters. The observed increase in PCT and PDW was significant. ÇEÇEN found that there is a significant association between PLT activation (PLT and PCT increase) and body fat amount[32] which is in agreement with the results of this study. In the ND group, vitamin D supplementation significantly decreased some platelet indices such as PDW and MPV.

We found that HFD consumption elevates the PLR value, and vitamin D in the elderly experiments alleviated this inflammatory biomarker level; however, this decrease was not statistically significant. In line with our findings, Vuksanovic and colleagues showed that vitamin D3 correction has less effect on PLR.[25] Assuming that a higher PLR value has been recommended as a risk factor for age-related disorders such as sarcopenia,[7] eating a healthy diet, and correcting and preventing micronutrient deficiencies such as vitamin D can help healthy aging.

Table 4: The effect of vitamin D supplementation on body weight, food intake, and vitamin D intake in high-fat diet fed rats

Variable	Groups		*P*
	Control (*n*=8)	Vitamin D (*n*=8)	
Baseline body weight (g)[#]	592.12 (42.10)	591.5 (49.52)	0.979*
Final body weight (g)[#]	588.25 (37.61)	550.62 (38.12)	0.067*
*P***	0.183	0.002	
Baseline Food intake (g/day)[#]	16.91 (1.05)	16.73 (1.10)	0.754*
Final Food intake (g/day)[##]	15.71 (15.13-16.60)	14.68 (13.99-14.74)	0.005***
*P****	0.093	0.012	
Baseline Vitamin D intake (IU/day)[#]	16.91 (1.05)	16.73 (1.1)	0.754*
Final Vitamin D intake (IU/day)[##]	15.71 (15.13-16.60)	88.11 (83.95-88.46)	0.001***

*Independent-Samples *T*-Test, **Paired-Samples *T* Test, ***Mann-Whitney U, ****Wilcoxon, [#]Mean (SD), [##]Median (Q1-Q3)

Table 5: The effect of vitamin D supplementation on serum vitamin D and some hematological parameters in normal diet fed rats

Variable	Groups		*P*
	Control (*n*=8)	Vitamin D (*n*=8)	
Serum 25(OH) D level (ng/ml)[#]	11.86 (1.13)	27.78 (1.30)	<0.001*
WBCs (10^3/UL)[#]	3.63 (0.62)	4.05 (0.79)	0.268*
Platelet count (10^3/UL)[#]	522.25 (55.40)	630.62 (139.17)	0.060*
PCT %[#]	0.24 (0.03)	0.27 (0.08)	0.350*
MPV (fL)[#]	4.75 (0.24)	4.37 (0.42)	0.048*
PDW %[#]	15.12 (0.38)	14.47 (0.27)	0.002*
PLR[##]	233.14 (206.44-286.35)	228.12 (221.79-239.28)	0.908**
NLR[#]	0.63 (0.05)	0.55 (0.07)	0.029*
MLR[#]	0.02 (0.02)	0.01 (0.01)	0.378*

25(OH) D, 25-hydroxy vitamin D; WBCs, white blood cells; PCT, plateletcrit; MPV, mean platelet volume; PDW, platelet distribution width; PLR, platelet-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; MLR, monocyte-to lymphocyte ratio, *Independent-Samples *T*-Test, **Mann-Whitney U, [#]Mean (SD), [##]Median (Q1-Q3)

Table 6: The effect of vitamin D supplementation on serum vitamin D and some hematological parameters in high-fat diet fed rats

Variable	Groups		*P*
	Control (*n*=8)	Vitamin D (*n*=8)	
Serum 25(OH) D level (ng/ml)[#]	10.30 (10.00-11.22)	23.05 (22.55-23.12)	0.002*
WBCs (10^3/UL)[##]	3.87 (1.59)	3.58 (1.4)	0.708**
Platelet count (10^3/UL)[##]	612.87 (31.89)	533.87 (35.14)	<0.001**
PCT %[##]	0.28 (0.03)	0.23 (0.02)	0.006**
MPV (fL)[##]	4.62 (0.51)	4.46 (0.33)	0.468**
PDW %[##]	14.60 (0.47)	14.63 (0.46)	0.875**
PLR[##]	314.71 (100.22)	263.44 (72.10)	0.333**
NLR[##]	1.02 (0.19)	0.80 (0.24)	0.117**
MLR[##]	0.04 (0.006)	0.02 (0.01)	0.005**

25(OH) D, 25-hydroxy vitamin D; WBCs, white blood cells; PCT, plateletcrit; MPV, mean platelet volume; PDW, platelet distribution width; PLR, platelet-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; MLR, monocyte-to lymphocyte ratio, *Mann-Whitney U, **Independent-Samples *T*-Test, [#]Median (Q1-Q3), [##]Mean (SD)

The NLR and MLR values increased in animals from the HFD group. These enhancements suggest that inflammation may be augmented in HFD-fed middle-aged rats. Consistent with the findings of Tabatabaeizadeh[26] our findings showed that vitamin D can have a lowering impact on NLR as an inflammatory factor. This improving the effect of vitamin D supplementation was significant in the ND group while in HFD-fed rats, it was not statistically significant. The reason for the intervention's different effect is probably low vitamin D intake and accordingly lower 25(OH) D levels in the HFD group owing to low food consumption. Our result is in line with earlier literature that found improvement in lifestyle behaviors (diet and physical activity) can significantly lower NLR values.[33]

Considering that in the elderly, elevated NLR value is correlated with an increased risk of cognitive impairment[34] vitamin D supplementation in older adults might help slow down age-related health problems by reducing NLR values. In this study, a significant decline in platelet count, PCT, and MLR was observed following the vitamin D supplementation of the HFD-feed rats. This shows that vitamin D supplementation ameliorates inflammations caused by HFD in older animals.

The inverse association of vitamin D and inflammatory markers reported in previous studies,[35,36] in agreement with our results, suggests anti-inflammatory impacts of vitamin D on some CBC-derived inflammatory indices in aging rats. The results of the second phase are consistent with the findings of previous studies by Al-Nimer, which showed that vitamin D3 supplement as an add-on-therapy with iron sulfate in participants with iron deficiency anemia significantly increased the WBCs and decreased significantly the platelet count, PCT, MPV.[22]

We were unable to detect a significant reducing impact of vitamin D supplementation on MLR in ND-fed rats and MPV values in the HFD group. Some studies have reported beneficial and lowering effects and another report suggests the in-effectivity of vitamin D in the modulation of obesity-related inflammation; however, in the present study, we observed the ameliorating effects of vitamin D on some HFD-related hematological systemic inflammatory factors. Additionally, a high-fat diet consumption in middle age might alter the CBC parameters, and some of these alterations can be modified by vitamin D supplementation. However, further studies are needed to support this beneficial effect on elderly people in a clinical trial.

Conclusions

Vitamin D could ameliorate HFD-related side effects and also age-related problems. The main findings can be summarized as follows: 1. A HFD consumption significantly increases PCT, PDW, PLR, NLR, and MLR values. 2. Platelet count, PCT, and MLR values decrease significantly after vitamin D supplementation in HFD-fed rats. 3. Vitamin D supplementation reduces the MPV, PDW, and NLR in ND-fed rats.

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Conflicts of interest

There are no conflicts of interest.

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