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Original Article

Effects of Paricalcitol on Body Composition in Vitamin D-Deficient Rats

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Abstract

Background: Paricalcitol has been proposed for the treatment of secondary hyperparathyroidism in patients with renal failure and vitamin D deficiency (VDD); however, VDD is related to a range of clinical complaints. We aimed to investigate the effects of paricalcitol on body composition in VDD rats.

Methods: Thirty adult male rats aged 10 weeks were randomly divided into three groups of 10, comprising control, VDD, and VDD plus paricalcitol32) ng/rat intraperitoneal injection) (VDD+P), at the Animal Lab of the Endocrinology and Metabolism Research Center, Shiraz, Iran, in 2020. Body composition was assessed after three weeks via serum biochemical tests and dual-energy X-ray absorptiometry. Finally, the data were analyzed by using the paired-sample *t* test, the one-way ANOVA, and the Tukey *post hoc* test.

Results: Global lean mass and fat mass were lower in the VDD and VDD+P groups than in the controls ($P<0.001$). Global fat percentage was reduced significantly in the VDD+P group ($P=0.029$).

Conclusion: Paricalcitol reduced global fat mass and fat percentage in a rat model with VDD. Evaluation of insulin and adiponectin levels is suggested to clarify the physiology of paricalcitol in VDD states.

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Keywords ● Body composition ● Vitamin D ● Calcium ● Rats

What's Known

- Paricalcitol has been proposed for the treatment of secondary hyperparathyroidism in patients with renal failure and vitamin D deficiency
- Few studies have evaluated the effects of paricalcitol on vitamin D-deficient rats.

What's New

- Paricalcitol reduced global fat mass and fat percentage in our rat model with vitamin D deficiency.
- Paricalcitol reduced tibia and spine fat mass in our rat model.

Introduction

In the past, calcitriol therapy was prescribed to treat vitamin D deficiency (VDD) and secondary hyperparathyroidism in patients with chronic kidney disease (CKD) by lowering the parathyroid hormone; its use was, however, limited due to the development of hypercalcemia and hyperphosphatemia, which could lead to bone disease.[1, 2]

Paricalcitol, which is a selective vitamin D-receptor activator, was first proposed in 1998 for the treatment of secondary hyperparathyroidism due to advanced CKD[3, 4] and patients on hemodialysis.[5] It can suppress the parathyroid hormone in secondary hyperparathyroidism without significant effects on calcium (Ca)-phosphorous (P) products in patients with CKD.[6]

Several studies on the effects of paricalcitol on bone mass showed that this therapy could prevent renal insufficiency associated with bone mineral loss.[6-10] Nonetheless, only a few studies have evaluated the effects of paricalcitol on body composition and fat mass.[11]

Since uremia and hemodialysis could affect body composition

in patients suffering from CKD,[12, 13] we evaluated the effects of paricalcitol on dual-energy X-ray absorptiometry (DXA)-derived body composition in a rat model of VDD. There is a paucity of information regarding the effects of paricalcitol on body composition among animals, or patients using paricalcitol. Commonly, paricalcitol is used in patients with CKD, who develop VDD and secondary hyperparathyroidism.[7] To evaluate the effects of paricalcitol on body composition, we used a rat model with VDD because, in patients with CKD, uremia, as a confounding factor, could affect the results of paricalcitol itself. Therefore, the present study aimed to investigate the effects of paricalcitol on body composition in VDD rats.

Materials and Methods

The study protocol was approved by the Ethics Committee of Shiraz University of Medical Sciences and its Vice-Chancellorship of Research (code: IR.SUMS.REC.1398.1114).

Chemicals

Paricalcitol (131918-61-1) was acquired from Sigma (Taufkirchen, Germany). A VDD diet was provided by Royan Institute (Isfahan, Iran). Enzyme-linked immunosorbent assay (ELISA) kits for examining 25-hydroxy vitamin D (25[OH] D) in rats (Catalog No.: ZB-11556C-H9648) were purchased from ZellBio GmbH, Germany. Serum Ca, P, and alkaline phosphatase (ALP) kits were purchased from Biosystems Company, Spain.

Thirty adult male Sprague-Dawley rats (age=10 weeks and weight=300±20 g) were included in this research, which was performed in the Shiraz Endocrinology and Metabolism Research Center, affiliated with Shiraz University of Medical Sciences, in 2020. The rats were obtained from the Animal Laboratory of the Shiraz Endocrinology and Metabolism Research Center.

All the rats underwent one week's adaptation in the animal laboratory facilities before the study commencement. The animals were kept in standard cages (five in each cage), with 12/12 hours light/dark cycles at 23±2 °C.[14] Simple randomization method was used to divide the rats into three groups of 10 rats each: (1) the control group, which was given a normal standard rodent chow diet and free access to tap water; (2) the VDD group, which was given the standard VDD diet (TD. 87095 Brown C.C.), containing 20% lactose, 2% Ca, and 1.25% phosphate for three weeks; and (3) the VDD+paricalcitol (VDD+P) group, which was given the VDD diet plus intraperitoneal injections of 32 ng of 9-nor-1,25-dihydroxy-vitamin D2 (paricalcitol; Zemplar, Germany) on days 1, 3, 5, 8, 10, and 12 of the study, according to the Stavenuiter protocol.[15]

At the end of the experiment, all the rats were anesthetized with ketamine 10% (100 mg/kg, Alfasan, the Netherlands) and xylazine 2% (10 m/kg, Alfasan, the Netherlands) solutions injected intraperitoneally and sacrificed by using thiopental (100 mg/kg).

Biochemical Studies

Serum Ca, P, ALP, and 25(OH)D were checked for all the rats. On day 22 of the study, serum Ca (mg/dL), P (mg/dL), and ALP (Iu/L) were evaluated via the colorimetric assay with the AutoAnalyzer (Biosystems, S.A., Spain). Serum 25(OH)D (ng/mL) was measured via the ELISA method (ZellBio GmbH, Germany) with a 3.3% intra-assay coefficient of variation and a 5.1% inter-assay coefficient of variation.

Body Composition Measurement

Body composition was assessed using the Hologic system DXA (Discovery W[S/N84107], USA] with small animal software in Shiraz Endocrinology and Metabolism Research Center. First, the Rat Step Phantom (Hologic P/N 010-0758 Rev.004) scan was set up (figure 1 A). In this method, when the system motion is

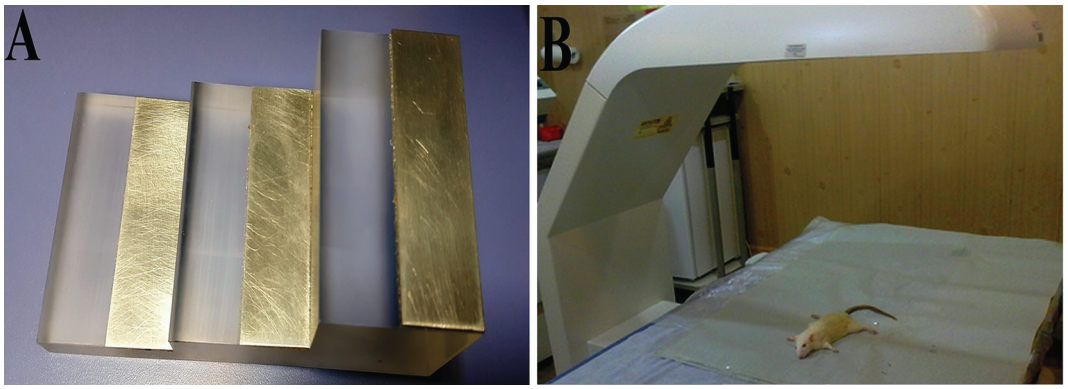

Figure 1: A, The Rat Step Phantom (Hologic P/N 010-0758 Rev.004) scan was set up. B, The Step Phantom is centered.

complete, the Step Phantom is centered on the table along the long axis of the laser, with the laser crosshair placed at 3/4″ (2 cm) of the right edge of the thinnest step (figure 1 B). Thereafter, the Continue button is pressed to start the scan. After the Hologic system was set up, the rats were anesthetized with 50 mg/kg of body weight of ketamine hydrochloride and 5 mg/kg of body weight of xylazine hydrochloride injected intramuscularly in the gluteus region. Finally, the animals were placed in the prone position under the Hologic system and scanned for the whole body, lumbar spine, tibia, and femur.

Statistical Analysis

The data were analyzed with SPSS Statistics, version 23, (SPSS, Chicago, IL., U.S.A.), and they were described as mean±SD. The paired-sample *t* test was used to analyze values within the same group at baseline and the end of the study. The one-way analysis of variance (ANOVA) with the Tukey *post hoc* test was utilized to compare biochemical data between the groups. Statistical significance was defined as a P value of less than 0.05.

Results

At the onset of the study, the three groups were not statistically significantly different in terms of body weight, as well as serum Ca, P, ALP, and 25(OH)D (P=0.786, P=0.397, P=0.643, P=0.112, and P=0.240, respectively). All the data are summarized in table 1. Daily food intake was approximately 22±2.9 g, without any significant difference between the three groups (P=0.194). During the study, there were no noticeable changes in the rats' behavior. Two rats, one in the VDD group and one in the VDD+P group, died. The mean body weight on the first day of the study was similar in all the groups (P=0.394), whereas body weight was lower in the VDD and VDD+P groups than in the control group at the end of the study (P<0.001) (figure 2 D). Global lean mass in the VDD and VDD+P groups was lower than that in the controls (P<0.001) (figure 2 A). Global fat mass was lower in the VDD and the VDD+P groups than in the control group (P=0.014 and P=0.003, respectively), with a more pronounced decline in the VDD+P group (figure 2 B). Global fat percentage was reduced significantly in the VDD+P group (P=0.029) (figure 2 C). Spine lean mass was not different between the three groups (figure 3 A), while spine fat mass, spine fat percentage, and total spine mass were lower in the VDD+P group than in the other two groups (P=0.016, P=0.010, and P=0.001, respectively) (figure 3 B, C, and D). Femur lean mass was decreased in the VDD and VDD+P groups (P=0.049 and P=0.033) (figure 4 A). Still, there was no significant decrease in fat mass and fat percentage in the three groups (figure 4 B and C). Total femur mass was lower in the VDD+P group than in the control group (P=0.039) (figure 4 D). There was no significant decrease in tibia lean mass and total tibia mass in all the groups (figure 5 A and D). Tibia fat mass and fat percentage were decreased in both VDD and VDD+P groups (P=0.024 and P=0.013, respectively) (figure 5 B and C). After the experiments, the animals were kept under the aforementioned conditions until they were sacrificed. Figure 6 illustrates three sample pictures of DXA reports in our three study groups.

Table 1: Comparisons of bodyweight, serum minerals, and vitamin D (mean±SD) on the first and last days of the study in all three groups

Studied Parameters	Time	Control	P value (first day *vs.* final day)	Vitamin D-Deficient Group	P value (first day *vs.* final day)	Vitamin D-Deficient Group Treated with Paricalcitol	P value (first day *vs.* final day)	P value of the Comparisons between the Three Groups
Bodyweight (g)	First day	300.10±6.23[a]	0.027	305.40±1.88[a]	0.284	301.80±6.91[a]	0.446	0.786
	Day 22	325.55±7.42[a]		300.00±5.36[b]		295.20±6.84[b]		0.007
25(OH)D (nmol/mL)	First day	89.25±3.95[a]	0.460	79.7±4.25[a]	0.000	82.37±3.95[a]	0.000	0.240
	Day 22	82.35±3.94[a]		19.03±1.59[b]		14.27±0.71[c]		<0.001
Calcium (mg/dL)	First day	9.76±0.13[a]	0.762	9.26±0.38[a]	0.449	9.01±0.56[a]	0.408	0.397
	Day 22	9.80±0.18[a]		9.47±0.07[a]		9.57±0.21[a]		0.419
Phosphorous (mg/dL)	First day	6.10±0.18[a]	0.779	5.88±0.29[a]	0.381	6.34±0.51[a]	0.499	0.643
	Day 22	6.37±.065[a]		6.23±0.19[a]		6.02±0.20[a]		0.813
Alkaline phosphatase (IU/L)	First day	482.80±31.3[a]	0.414	388.80±24.68[a]	0.553	410.55±40.66[a]	0.904	0.112
	Day 22	457.11±44.6[a]		360.22±37.84[a]		393.30±21.95[a]		0.173

[a,b,c] The same letter shows no significant difference between the experimental groups (P>0.05), whereas different letters show significant differences. The one-way ANOVA with the Tukey post hoc test was used for the comparison between the groups, and the paired sample *t* test was applied to evaluate differences before and after the intervention in each group. VDD: Vitamin D-deficient group; VDD+P: VDD+paricalcitol group

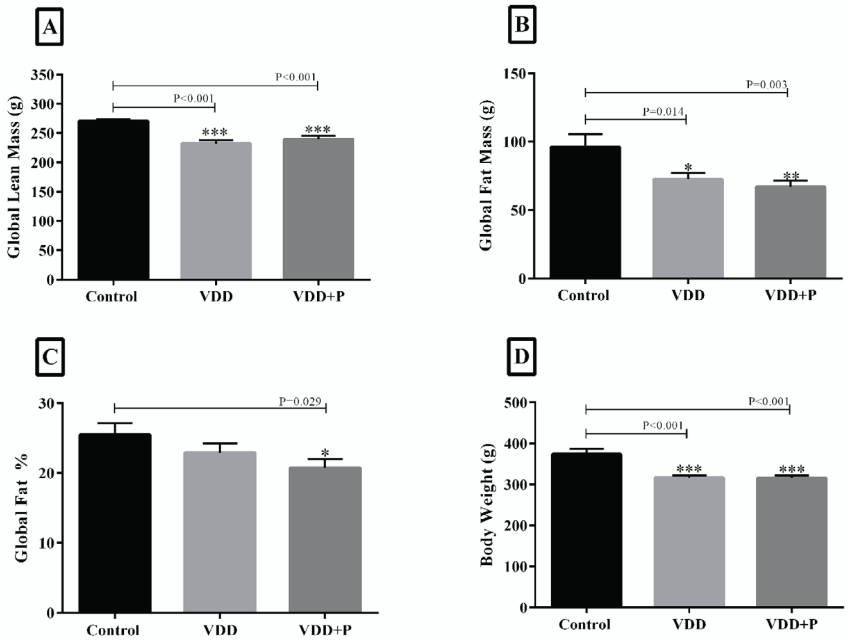

Figure 2: The global lean mass, global fat mass, global fat percentage, and body weight of the rats at the end of the study are compared. The data were analyzed by using the one-way ANOVA with the Tukey post hoc test. VDD: Vitamin D-deficient group; VDD+P: VDD+paricalcitol group; *, **, and *** Columns with different counts of stars have significant differences. *P<0.05, **P<0.01, and ***P<0.001, Control *vs.* VDD, or VDD+P

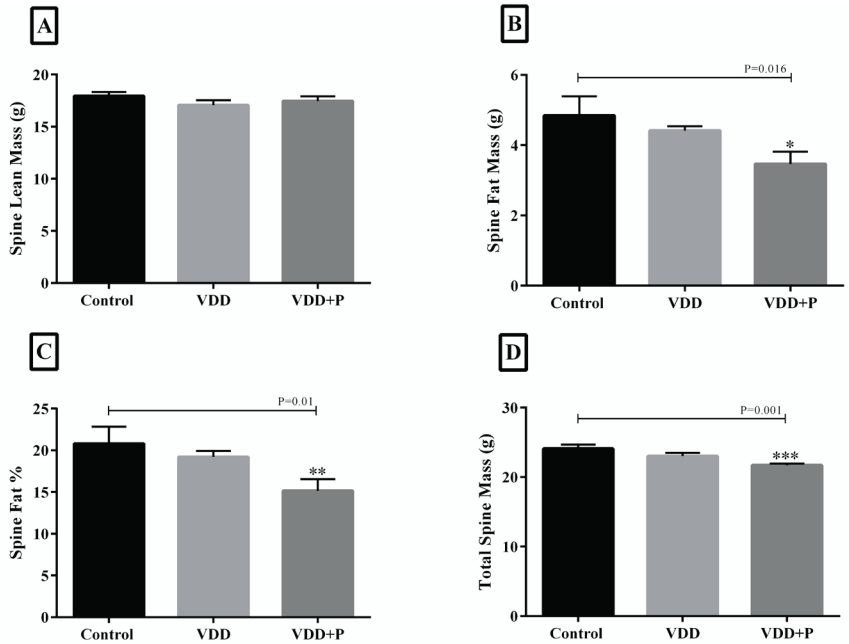

Figure 3: The spine lean mass, spine fat mass, spine fat percentage, and total spine mass of the rats at the end of the study are compared. The data were analyzed by using the one-way ANOVA with the Tukey post hoc test. VDD: Vitamin D-deficient group; VDD+P: VDD+paricalcitol group; *, **, and *** Columns with different counts of stars have significant differences. *P<0.05, **P<0.01, and ***P<0.001, Control *vs.* VDD, or VDD+P

It appears that the rats in the VDD and VDD+P groups were thinner than the control rats.

Discussion

Our study showed that paricalcitol could reduce tibia fat mass and spine fat mass in a rat model with VDD. Data are scarce regarding the effects of paricalcitol on the body composition among animals, or patients using paricalcitol. Commonly, paricalcitol is prescribed in patients with CKD, who develop VDD and secondary hyperparathyroidism.[7] To evaluate the effects of paricalcitol on the body composition, we used a rat model with VDD, because, in patients with CKD, uremia, as a confounding factor, could affect the results of paricalcitol itself. In this study, VDD rats received an adequate amount

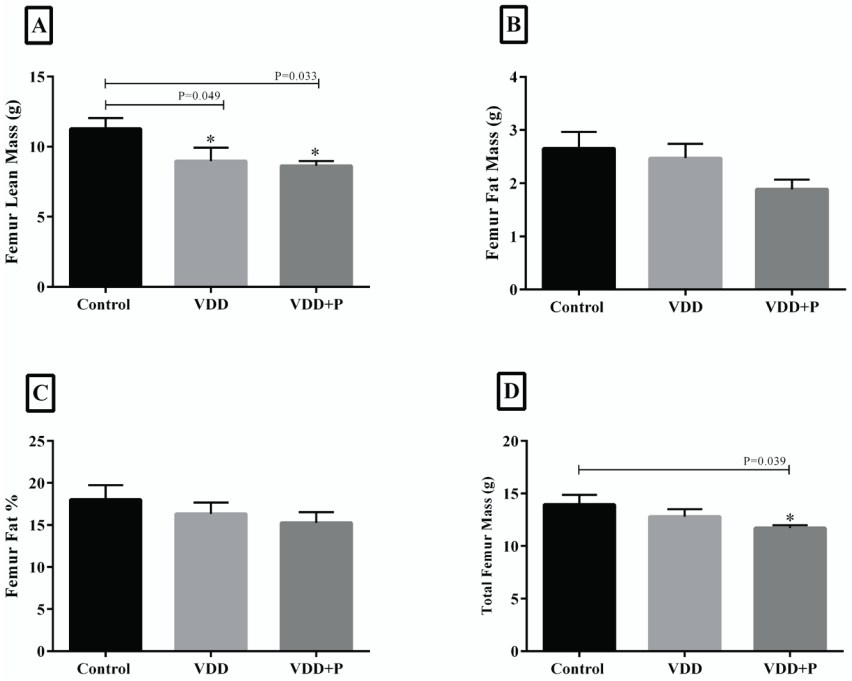

Figure 4: The femur lean mass, femur fat mass, femur fat percentage, and total femur mass of the rats at the end of the study are compared. The data were analyzed by using the one-way ANOVA with the Tukey post hoc test. VDD: Vitamin D-deficient group; VDD+P: VDD+paricalcitol group; *, **, and *** Columns with different counts of stars have significant differences. *P<0.05, **P<0.01, and ***P<0.001, Control *vs.* VDD, or VDD+P

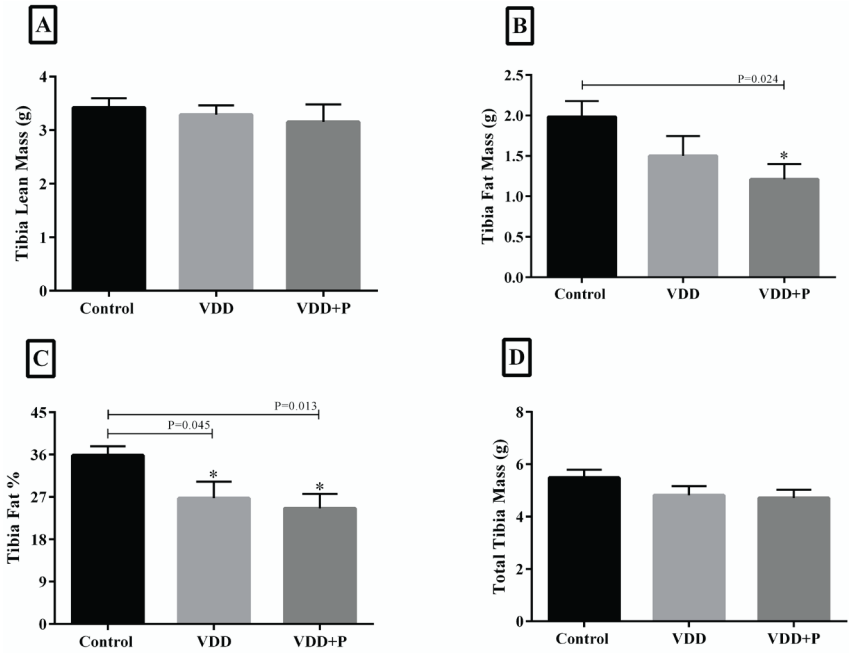

Figure 5: The tibia lean mass, tibia fat mass, tibia fat percentage, and total tibia mass of the rats at the end of the study are compared. The data were analyzed by using the one-way ANOVA with the Tukey post hoc test. VDD: Vitamin D-deficient group; VDD+P: VDD+paricalcitol group; *, Columns with different counts of stars have significant differences. *P<0.05, Control *vs.* VDD, or VDD+P

of paricalcitol to reach normalized levels of serum parathyroid hormone. We compared the body composition between these rats and two groups of normal rats and VDD rats. Our results showed that VDD was associated with low body weight, low global lean mass, and low-fat mass. However, paricalcitol aggravated low global fat percentage in VDD rats. It seems that paricalcitol could decrease body weight by reducing global fat percentage.

Fujii and others investigated the effects of paricalcitol on the glycemic status in diabetic rats. They showed that weight gain was less in diabetic rats that were given paricalcitol than in

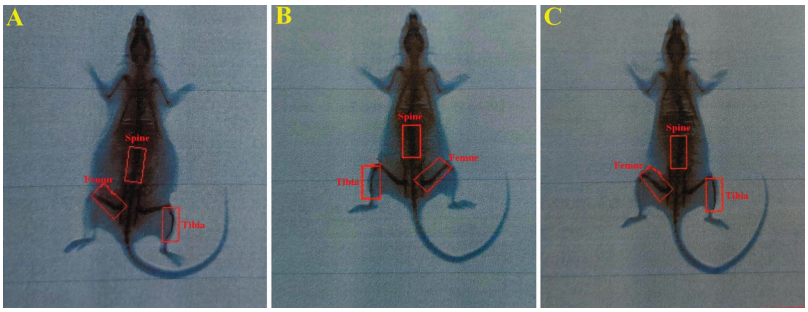

Figure 6: Images of whole body, spine, tibia, and femur scans in the experimental groups are presented. A: Control group, B: Vitamin D-deficient group, C: Vitamin D-deficient+paricalcitol group

the control group. The former group also had higher insulin and C-peptide levels than diabetic rats that did not receive paricalcitol.[10] In another investigation, diabetic rats receiving paricalcitol had a low Homeostatic Model Assessment-Insulin resistance (HOMA-IR) index compared with other diabetic rats, and paricalcitol was associated with higher adiponectin, interleukin 1-beta, and tumor necrosis factor-alpha. Hence, the authors concluded that paricalcitol could lower glycemia, insulin resistance, and inflammatory cytokines.[11] Yamauchi and colleagues suggested that along with the use of adiponectin, as a new therapy for obesity and insulin resistance, paricalcitol and glibenclamide could be prescribed, as we did in our present study.[16] In an animal study on atherosclerosis-prone apolipoprotein E-deficient mice, Bozic and colleagues showed that the vitamin D receptor was upregulated in hepatocytes in nonalcoholic fatty liver disease, which could activate the fat accumulation gene pathway. They also revealed that specific vitamin D-receptor antagonists could be novel drugs to reverse steatosis in early nonalcoholic fatty liver disease.[17] Our results showed that paricalcitol could decrease global fat percentage in VDD rats, which might be due to its role in increasing adiponectin, interleukin 1-beta, and insulin secretion. These findings should be fully investigated in future research.

The salient strength of the present study is that we assessed the effects of paricalcitol on the body composition in a rat model of VDD. Nonetheless, our results should be interpreted taking into account some limitations. Firstly, an evaluation of insulin and adiponectin levels, alongside body composition, would bolster our results. Secondly, the fact that our investigation was an animal study precluded an evaluation of body composition at baseline; we would, therefore, suggest human studies with baseline and follow-up measurements of body composition. Thirdly, what could further elucidate the effects of VDD versus paricalcitol on the body composition is a prolonged duration of a low vitamin D diet to achieve severe vitamin D deficiency in rats, given that some of the differences observed between our VDD and VDD+P groups could be due to differences in serum vitamin D levels. Future studies should seek to determine whether such changes are beneficial or detrimental to humans.

Conclusion

Our results indicated that paricalcitol could decrease the global fat mass and fat percentage in a rat model with VDD. Accordingly, we recommend that insulin and adiponectin levels be evaluated to clarify the physiology of paricalcitol in VDD states.

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Conflict of Interest: None declared.

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