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Vitamin D supplementation: Biochemical and inflammatory effects in non-pathological Wistar rats

Silvia Muller de Moura Sarmento [a], Elizandra Gomes Schmitt [a], Laura Smolski dos Santos [a], Gênifer Erminda Schreiner [a], Rafael Tamborena Malheiros [b], Clóvis Klock [c], Charline Casanova Petry [c], Itamar Luís Gonçalves [d], Vanusa Manfredini [a,b,*]

[a] Pós-Graduate Program in Biochemistry, Federal University of Pampa, BR 472, Km 585, Uruguaiana, RS, Brazil
[b] Programa de Pós-graduação Multicêntrico em Ciências Fisiológicas, Universidade Federal do Pampa, Campus Uruguaiana, Rio Grande do Sul, Brazil
[c] Grupo Infolaudo e Medicina Diagnóstica, Erechim, Rio Grande do Sul, Brazil
[d] Universidade Regional Integrada Alto-Uruguai e das Missões, Sete de Setembro Avenue, 1621, Erechim, Rio Grande do Sul, Brazil

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ABSTRACT

Vitamin D_3 (VD_3) is shown to be a biochemical and physiological modulator of the body. Debates about route of administration, prescribed dosage and serum levels have arisen, and thus the interaction of VD_3 with the body in overdose. Using an experimental model of Wistar rats of both sexes, rats were subdivided into 5 groups, which represents a control group, and 4 groups with VD_3 treatments (2.500, 7.000, 14.000 and 21.000 IU/kg/week) for one month. Thereafter biochemical, hormonal, inflammatory and histological analyses were performed. Regarding the biochemical findings, there was an increase in the levels of the AST in comparison of the control group with the treatments with higher doses (14.000 IU and 21.000 IU). Furthermore, changes in the inflammatory cytokine profile were identified at doses of 14,000 IU and 21,000 IU, with an increase in inflammatory cytokines (IL-1β, IL-6, IL-8 and TNF-α) and a decrease in the anti-inflammatory IL-10. Histological evaluation of the liver tissue also revealed changes at the highest doses. Finally, in the evaluation of a murine physiological model, it showed that the supplementation of VD_3 in overdoses there was an inflammatory exacerbation in the body, suggesting that the VD_3 supplementation should be administered with caution, and takes into account physiological factors of the individual.

1. Introduction

Vitamin D_3 (VD_3), a recently recognized important physiological modulator of metabolism, acts in various biochemical and metabolic reactions, exhibiting a pleiotropic character. It plays a prominent role in immune system function, growth modulation, cell differentiation, antioxidant activity, and protection against and control of chronic diseases such as heart disease, liver disease, and endocrinopathies [1,2].

Actually, numerous debates on the cut-off points of sufficiency and deficiency of serum VD_3, dosage and ideal concentration of $25(OH)D_3$ administered, have brought the discussion about the importance of these actions in the health of babies, children, adults and the elderly, in addition to physiological conditions like as pregnancy and menopause or pathological conditions such as metabolic syndromes, autoimmune diseases, cardiovascular, among others [3].

Prescriptions and administration of VD_3, mainly in the form of cholecalciferol, have been used to prevent and/or treat VD_3 deficiency, but the target of 20 ng/mL is considered difficult to achieve in obese individuals or those experiencing metabolic disorders [1]. Regarding administration, daily doses of 600 IU are recommended for individuals aged 1–70 years, and after the age of 70 years, due to bone weakness and difficulty in calcium absorption, these doses rise to 800 IU/day, with a limit of 4.000 IU/day for correction of more severe cases of deficiency [4].

However, several studies have focused on the safe levels of VD_3 supplementation in the body in the long term. Kaur et.al. (2015) demonstrated that its association with the overdose of other minerals, calcium, has been shown to be potentially deleterious to the body [5]. Tugcu et.al. (2019) reports that there is still no evidence that the ingestion of VD_3 in high doses can be toxic to the body [6] and Bouillon

* Corresponding author at: Pós-Graduate Program in Biochemistry, Federal University of Pampa, BR 472, Km 585, Uruguaiana, RS, Brazil.
E-mail address: vanusamanfredini@unipampa.edu.br (V. Manfredini).

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(2020) showed that high doses (>4000 IU) should be administered with caution, which can lead to hypercalcemia and hypercalciuria [7].

Upper limits of VD_3 intake require caution, numerous studies with variations in design and intervention, where excess VD_3 is seen as a serious clinical condition, which can persist for a prolonged time, and the main signs and symptoms described are hypercalcemia and hypercalciuria, in addition to neurological symptoms such as lethargy, confusion; gastrointestinal symptoms such as vomiting and nausea, among other pathophysiological manifestations [3,8].

The supplementation becomes a complex subject, and serum levels of $25(OH)D_3$ should be monitored by qualified professionals. With the increase in the availability of supplements, several cases of hypervitaminosis have been occurring and alerting to the consequences of bioaccumulation in the body and its effects in the short and long term [9,10].

The deficiency of VD_3 in the general population shows that the consumption of supplements has been increasing year after year, and the continuous prescription and/or in high doses also [11,12]. Therefore, there is a need to further study about the continuous administration of VD_3, expanding knowledge of possible benefits and harms of chronic supplementation [5,6]. Considering these aspects, this study aimed to evaluate the effects of various levels of VD_3 doses on biochemical, histological, and inflammatory parameters in male and female rats.

2. Materials and methods

2.1. Ethical aspects

All procedures carried out in this study were approved by the Ethics Committee on the Use of Animals of the Federal University of Pampa (CEUA-Unipampa) under protocol number 016/2020.

2.2. Animals

A total of 50 Wistar rats (25 males and 25 females) were used, aged 45 days. From BIOPAMPA of the Federal University of Pampa (UNIPAMPA), the animals were kept under controlled environmental conditions (12 h light-dark cycle, temperature and humidity) after weaning, with commercial feed and water ad libitum until the end of the experiment. The work was carried out in both sexes, and the procedures were performed in an equivalent and concomitant manner after 60 days of life of the animals.

This way, the animals were randomly divided, according to sex, into five groups (n = 5/sex/group). Group 1 (Control) – 0.5 mL of saline, Group 2 – 2.500IU/Kg/week VD_3, Group 3 – 7.000IU/Kg/week VD_3, Group 4 – 14.000IU/Kg/week VD_3, Group 5 – 21.000IU/Kg/week VD_3. The supplementation was administered according to the weight of the animal on the day, once a week, for 4 weeks, by gavage, the same timetable was observed in all administrations.

After 4 doses of supplementation, the animals fasted for 12 hours, and were anesthetized intraperitoneally with ketamine (80 mg/Kg) and xylazine (10 mg/Kg), and the cardiac puncture was performed to remove whole blood for biochemical and inflammatory analyses, and liver collection for histological analysis.

2.3. Vitamin supplement

The cholecalciferol - VD_3 suspension was formulated in a certified compounding pharmacy (Purifarma brand - code. 081300.000005, Lot: B-151-M201124), with a dosage of 40.576.000 IU/g, on an anhydrous basis. The concentrations manipulated were 500IU, 1000IU, 2000IU and 3000IU of VD_3 in each 300 μL of solution.

2.4. Analysis of laboratory parameters

The reagents were purchased from Sigma Chemical Corp (St. Louis, MO, USA). Glycemic and lipid profiles, as well as biochemical markers of hepatic function (ALT, AST, and alkaline phosphatase), renal function (creatinine and urea), and bone metabolism (calcium and phosphorus) were assessed in serum using commercial kits from Labtest® and Bioclin®. The measurements of $25(OH)D_3$, leptin, and adiponectin were performed by electrochemiluminescence using Abbott Architect Kits®. For the interleukins IL-1β, IL-6, IL-8, IL-10, and TNF-α, as well as ultrasensitive C-reactive protein (C-RP) determination, the samples were stored at −80°C and was used a kit from Thermo Fisher® [13].

For the liver histological analysis, the organ was stored in 1 % formalin for further processing and staining in Hematoxyl & Eosin [14]. The slides were examined and photographed using an optical microscope (Leicaº DM50).

2.5. Statistical analysis

Data were expressed as mean ± standard deviation. Comparisons between groups were performed after homogeneity (Levene) and normality (Shapiro-Wilk) tests, with subsequent unidirectional analysis of variance (1-way ANOVA), followed by Bonferroni post-hoc testing for multiple comparison. Furthermore, Principal Component Analysis (PCA) was conducted to identify correlations among the variables and to assess the clustering of the experimental animals. The results were considered statistically significant when $p < 0.05$. Statistical analysis was performed using GraphPad Prism 9.2 software.

3. Results

To evaluate the hormonal effects of vitamin D on the quantified endpoints, we conducted a separate experimental design in which the levels of biochemical, hormonal, and inflammatory parameters were investigated. In general, the profile of the dose-effect curves produced were very similar.

Regarding the biochemical and hormonal parameters in male rats, all doses of vitamin D produced changes only in some of the quantified parameters. A significant increase in LDL cholesterol ($p = 0.0403$) was observed at 14.000 IU/kg/week compared to the dose of 2.500 IU/kg/week (Fig. 1d). The two highest doses produced a decrease in triglycerides compared to the control group ($p = 0.0085$ and $p = 0.0132$), the 2.500 IU/kg/week group ($p = 0.0032$ and $p = 0.0050$), and the 7.000 IU/kg/week group ($p = 0.0054$ and $p = 0.0085$) - Fig. 1e. Additionally, doses of 2.500, 7.000, and 21.000 IU/kg/week produced an increase in glutamic oxalacetic transaminase compared to the control group ($p = 0.0119$, 0.0429, and 0.049, respectively), as observed in Fig. 1i. Plasmatic vitamin D increased proportionally with the dose (Fig. 1m). As shown in Fig. 1, the other parameters monitored were statistically unchanged across all doses.

For female rats (Fig. 2), the two highest doses of vitamin D produced a decrease in triglyceride levels compared to the control group ($p = 0.0013$ and $p = 0.0165$) and the 2.500 IU/kg/week group ($p = 0.0220$ and $p = 0.0273$). These same high doses produced an increase in LDL cholesterol ($p = 0.0183$ and $p = 0.0183$) compared with 2.500 IU/kg/week, as represented in Fig. 2d. Glutamic oxalacetic transaminase increased compared to the control group in a consistent manner across all doses, including the lowest dose, with p-values of 0.0010, 0.0034, 0.0065, and 0.0034, respectively, as observed in Fig. 2i. Interestingly, serum calcium levels decreased in relation to the control only in female rats, starting from the dose of 7.000 IU/kg/week, with p-values of 0.0489 (7.000 IU/kg/week compared to control), 0.0035 (14.000 IU/kg/week compared to control), and 0.0075 (21.000 IU/kg/week compared to control).

Although not statistically significant, an interesting trend of decreasing triglycerides (Figs. 1e and 2e) and increasing alkaline phosphatase (Figs. 1j and 2j) was observed in both male and female rats with increasing doses of vitamin D. Another point to highlight is that at all doses of supplementation, vitamin D3 was safe for renal function, as

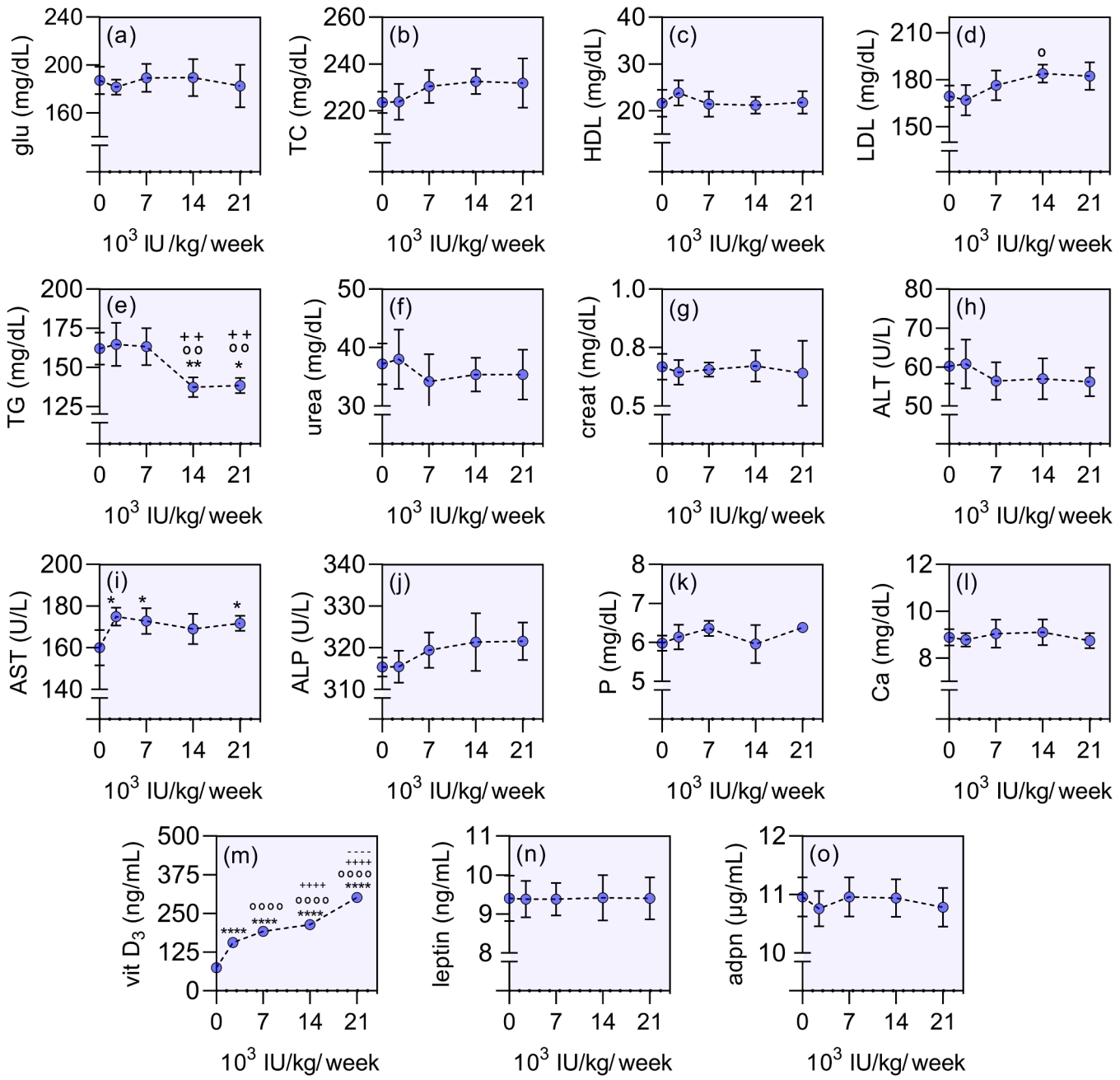

Fig. 1. Dose-effect curves of biochemical and hormonal parameters in male rats treated with increasing doses of vitamin D. * different in relation to the group 0 day; 0 IU/kg/week; ° different in relation to the group 7 IU/kg/week; + different in relation to the group 14 IU/kg/week; - different in relation to the group 21 IU/kg/week. The number of characters is proportional to the p value, according: $p < 0.05$; $p < 0.01$; $p < 0.001$ and $p < 0.0001$. Abbreviations: glu: glucose; TC: total cholesterol; HDL: high density lipoprotein; LDL: low density lipoprotein; TG: triglycerides; creat: creatinine; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; P: phosphorus; Ca: calcium; vit D_3: vitamin D_3; adpn: adiponectin. The data are presented as mean ± standard deviation.

indicated by the unchanged values of urea (Figs. 1f and 2f) and creatinine (Fig. 1 g and 2 g). Regarding the other parameters, were not found difference for the glucose levels (Figs. 1a and 2a), total cholesterol (Figs. 1b and 2b), HDL cholesterol (Figs. 1c and 2c), ALT (Fig. 1 h and 2 h), phosphorous (Figs. 1k and 2k), leptin (Fig. 1 n and 2 n) and adiponectin (Figs. 1o and 2o). For male, the absence of VD_3 effect was identified for calcium, as may be observed in the Fig. 1 l.

The most significant changes occurred in the inflammatory profile at the two highest doses of vitamin D (14.000 and 21.000 IU/kg/week) in both males (Fig. 3) and females (Fig. 4). These doses produced substantial increases ($p < 0.0001$) compared to the control, 2.500 IU/kg/week, and 7.000 IU/kg/week groups in IL-1β (Figs. 3a and 4a), IL-6 (Figs. 3b and 4b), IL-8 (Figs. 3c and 4c), TNF-α (Figs. 3d and 4d), and C-reactive protein (Fig. 3 f and 4 f). The two highest doses also resulted in a decrease in the anti-inflammatory cytokine IL-10. Regarding the inflammatory profile, vitamin D appears to be safe up to the dose of 7.000 IU/kg/week.

To better understand the structure of our data and the relationships among all the variables, along with their effect on animal clustering, we performed a principal component analysis (PCA). Due to the similar profiles observed between males and females, the measurements from both male and female rats were combined for this analysis. Additionally,

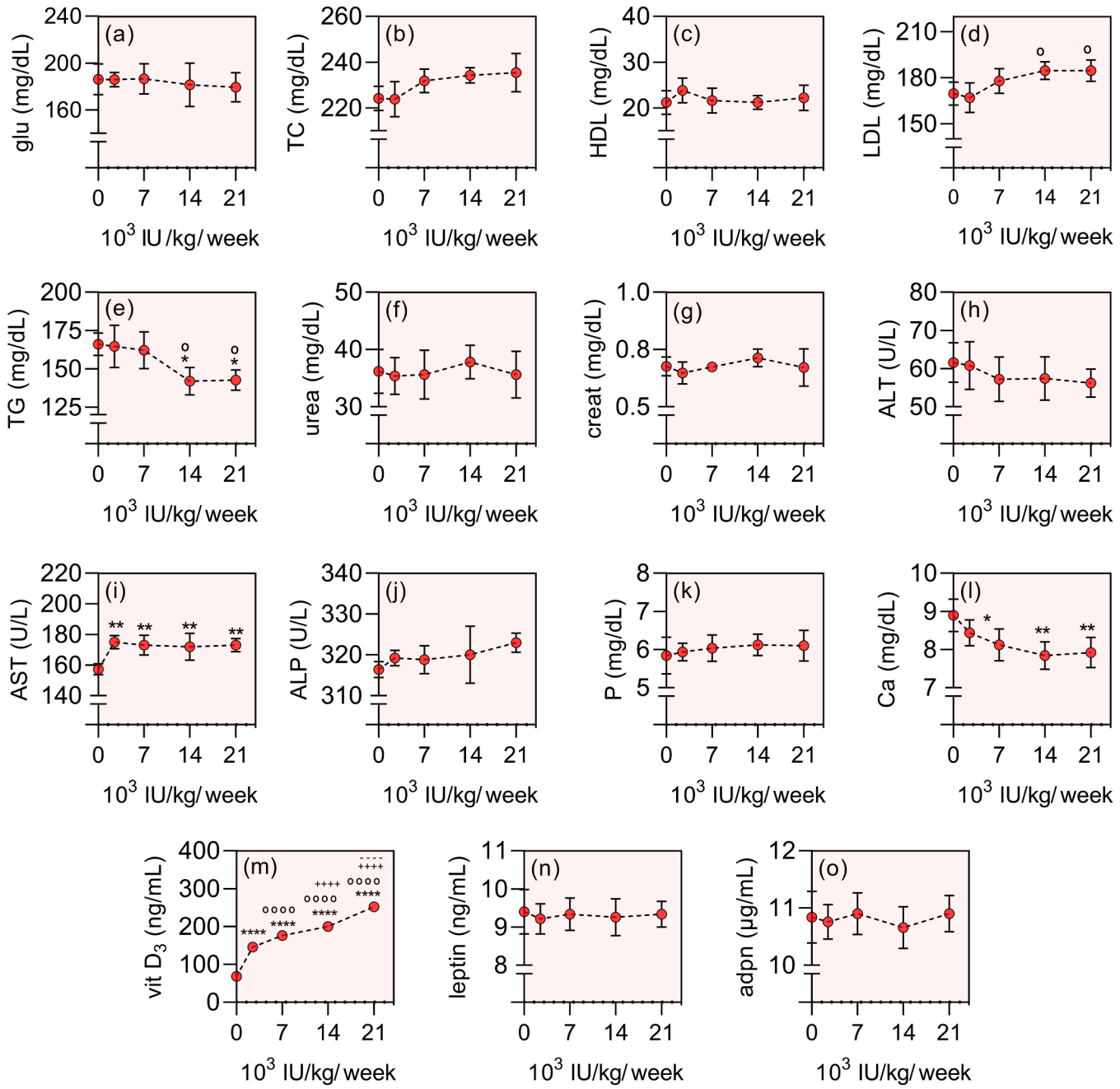

Fig. 2. Dose-effect curves of biochemical and hormonal parameters in female rats treated with increasing doses of vitamin D. * different in relation to the group 0 IU/kg/week; ° different in relation to the group 7 IU/kg/week; + different in relation to the group 14 IU/kg/week; - different in relation to the group 21 IU/kg/week. The number of characters is proportional to the p value, according: $p < 0.05$; $p < 0.01$; $p < 0.001$ and $p < 0.0001$. Abbreviations: glu: glucose; TC: total cholesterol; HDL: high density lipoprotein; LDL: low density lipoprotein; TG: triglycerides; creat: creatinine; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; P: phosphorus; Ca: calcium; vit D_3: vitamin D_3; adpn: adiponectin. The data are presented as mean ± standard deviation.

no clustering was identified when sex was used as a categorical variable to color the points distributed in the hyperspace of PC1 against PC2 (data not shown).

The 21 variables in our dataset, when reduced to PC1 and PC2, explained 48.08 % of the data variability. PC1 and PC2 are linear combinations of all the variables under investigation, and the dimensionality reduction allows to represent all the variables in a bi-dimensional plane. An interesting clustering pattern can be identified in the plot represented as Fig. 5a: the control group is placed near the two lowest doses on the left side of the plot, characterized by high values of triglycerides and IL-10 (the two vectors with the most intense projection on the PC1 axis). In contrast, the two highest doses (14.000 and 21.000 IU/kg/week) are placed on the right side of the plot as depicted in Fig. 5a. These two groups are characterized by high values of inflammatory markers (IL-1β, TNF-α, IL-6, IL-8, and C-reactive protein), serum vitamin D_3 (as expected), LDL cholesterol, and alkaline phosphatase. Generally, the rats were uniformly distributed along the PC2 axis. An interesting finding that validates our results is the inverse correlation between IL-10 and the inflammatory cytokines, which can be verified by the vectors placed in opposite regions of the vector plot shown in Fig. 5b. In addition, an inverse correlation may be observed between leptin and adiponectin (Fig. 5b).

Histological analysis shows a difference between the control (Fig. 6a, f) and the vitamin D gavage supplementation in both male and female

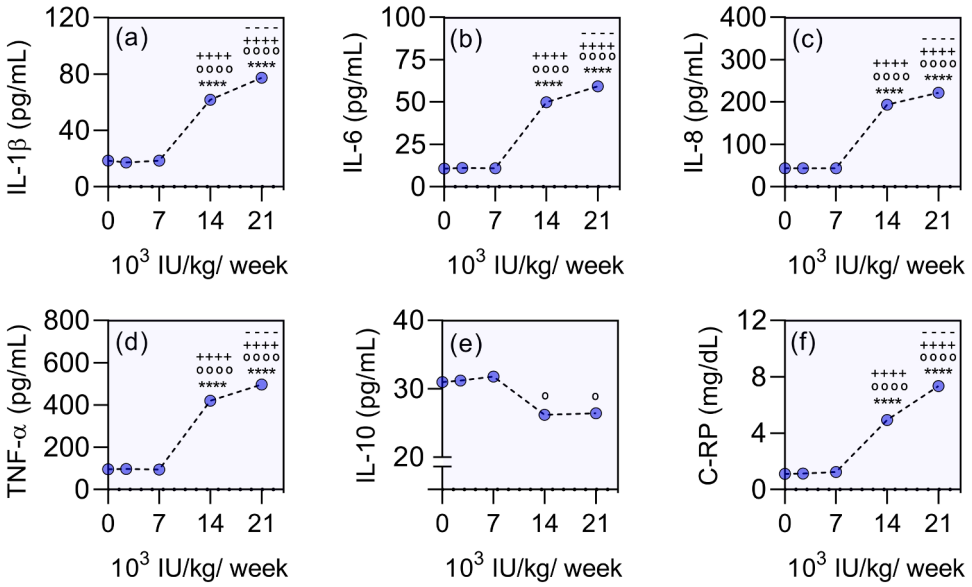

Fig. 3. Dose effect curves of inflammatory parameters in male rats treated with increasing doses of vitamin D. * different in relation to the group 0 IU/kg/week; º different in relation to the group 7 IU/kg/week; + different in relation to the group 14 IU/kg/week; - different in relation to the group 21 IU/kg/week. The number of characters is proportional to the p value, according: $p < 0.05$; $p < 0.01$; $p < 0.001$ and $p < 0.0001$. The data are presented as mean ± standard deviation.

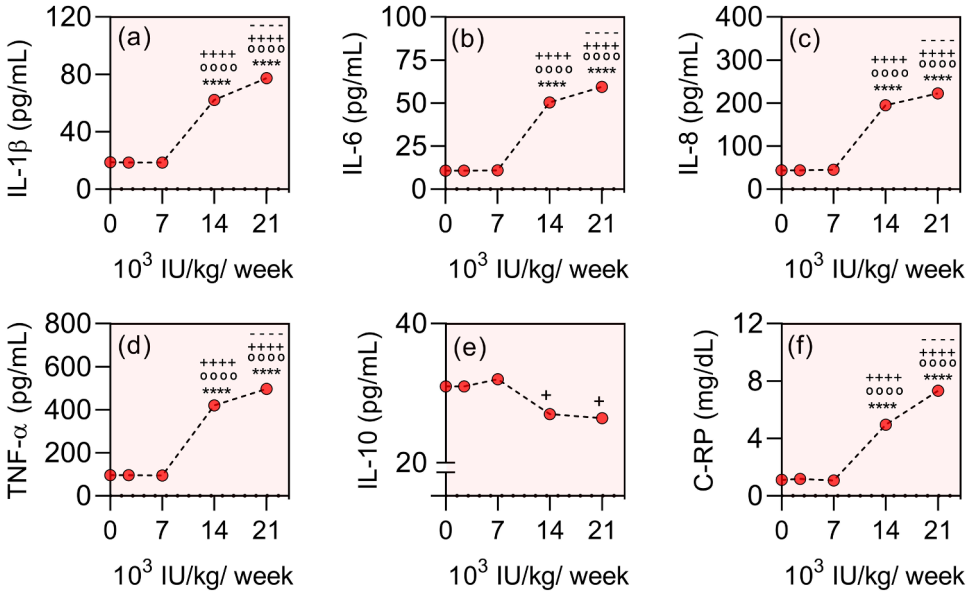

Fig. 4. Dose effect curves of inflammatory parameters in female rats treated with increasing doses of vitamin D. * different in relation to the group 0 IU/kg/week; º different in relation to the group 7 IU/kg/week; + different in relation to the group 14 IU/kg/week; - different in relation to the group 21 IU/kg/week. The number of characters is proportional to the p value, according: $p < 0.05$; $p < 0.01$; $p < 0.001$ and $p < 0.0001$. The data are presented as mean ± standard deviation.

Wistar rats, showed a higher degree of structural and nuclear disorder at the dosages of 14.000 IU and 21.000 IU of VD_3 (Fig. 2d, i, e and j).

4. Discussion

Studies show that serum levels above 150 ng/mL of VD_3 are associated with toxicity and should be avoided [15]. Figs. 1 and 2 show that VD_3 levels had a dose-dependent gradual increase, reaching 301.2 ± 2.7 ng/mL and 254,1 ± 3,7 ng/mL in the supplementation of 21.000 IU VD_3 of males and females, respectively. It is noted that the increase was higher in male Wistar rats than in female Wistar rats. It should be noted that serum level of $25(OH)D_3$ is usually higher in men than in women [16,17].

About the lipid profile, the levels of total cholesterol and triglycerides (Figs. 1 and 2) showed a significant reduction in males and in females; this relationship was between groups control, 2.500 IU and 7.000IU, for the highest treatments 14.000IU and 21.000 IU. There was no difference in the cholesterol fractions tested (HDL and LDL). Nimitphong et.al. (2012), *in vitro* study, it has been shown that VD_3 favors adipogenesis with accumulation of triglycerides in human fat cells [18].

Dibaba (2019) in his meta-analysis, showed that VD_3 supplementation has beneficial effects in patients with hypercholesterolemia, however it did not show changes in HDL cholesterol dosages [19]. Findings such as this are present in the research databases, and in women with polycystic ovary syndrome or healthy premenopausal women, no changes in the lipidogram profile were observed [20,21]. However,

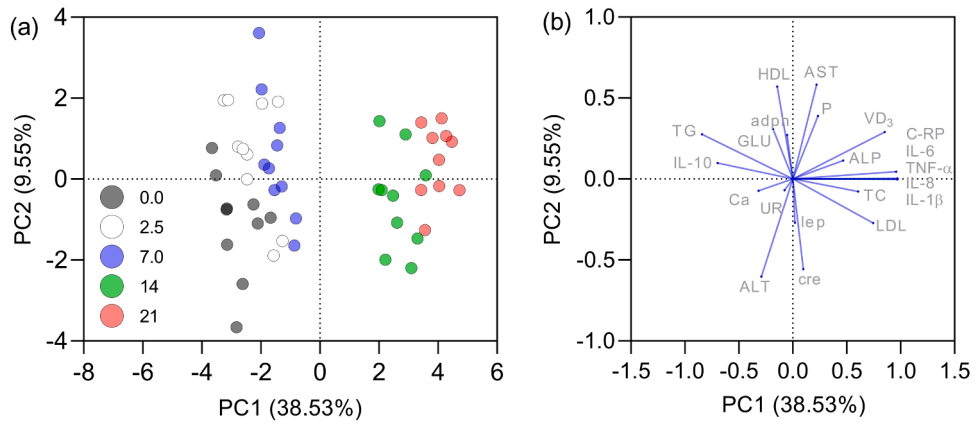

Fig. 5. Principal Component Analysis for the biochemical, hormonal, and inflammatory parameters in male and female rats supplemented with different doses of vitamin D. In (a), the distribution of animals according to their PC1 and PC2 values is shown. In (b), the vector plot shows the correlations among all the variables and their effects on the clustering of the animals. Abbreviations: glu: glucose; TC: total cholesterol; HDL: high density lipoprotein; LDL: low density lipoprotein; TG: triglycerides; UR: urea; cre: creatinine; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; P: phosphorus; Ca: calcium; vit D_3: vitamin D_3; lep: leptin; adpn: adiponectin; CRP: C-reactive protein.

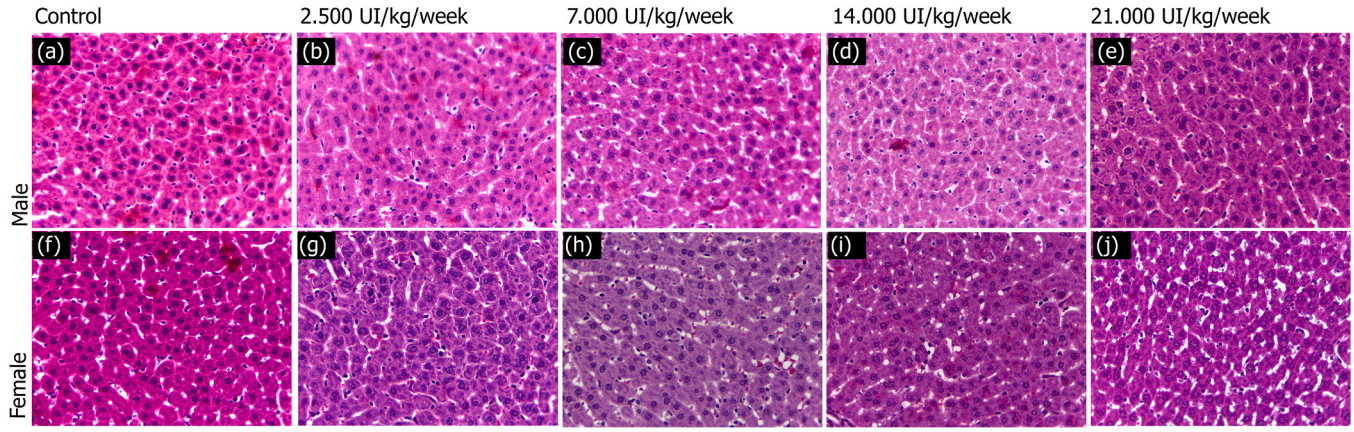

Fig. 6. Histology images of the liver tissue of the control group (a) and (f) and the rats treated with increasing doses of vitamin D. The first line (a-e) shown the data for males in increasing doses and the second line shown the data for females in increasing doses (f-j). Each column represents a different concentration. All the images were obtained with 400X of magnification.

other studies show that VD_3 can chemically downregulate sterol regulatory agent binding proteins (SREBPs), regulating lipid formation in the body and explaining its interaction in the progression of metabolic diseases [22].

The relationship of the renal profile shows an increase in creatinine, without statistical evidence in this experimental model. VD_3 supplementation in renal pathologies is widely discussed, especially in children and adolescents, where the therapeutic range between vitamin sufficiency and vitamin intoxication is short, which can lead to cardiovascular complications [23].

The literature shows that moderate VD_3 ingestion for long periods has several deleterious effects, including calcium and phosphorus metabolism, but they still remain unexplored [10]. In the present study, there was only alteration in the serum calcium of female Wistar rats, with a significant reduction, when comparing control with higher doses (14.000 IU and 21.000 IU). However, Dudenkov et.al. (2015), with a prospective study of 10 years, showed that VD_3 levels between 51 and 218 ng/mL are not related to hypercalcemia in adults or children, this lack of association was extended to age progression and female gender [11].

Lima et.al. (2022), in experimental model to evaluate brain and heart waves in male rats with an acute dosage of 25.000 IU/Kg, showed that VD_3 provided an increase in calcium and creatinine in serum, when compared to the control. In healthy individuals supplemented with 2.000IU of VD_3 for 12 weeks, an increase in serum 25(OH)D_3 levels and a reduction in parathyroid hormone were observed, yet, serum and ionic calcium levels did not change [24]. In our findings, we saw a reduction in serum calcium only in female rats, however the maximum dosages used in this study was 21.000IU/Kg.

The liver is one of the main organs of VD_3 biotransformation, being responsible for its first hydroxylation (CYP2R1). The analysis of VD_3 supplementation with the healthy liver showed that there was a significant increase in AST enzyme and a non-significant reduction in ALT in both sexes (Fig. 1 and Fig. 2). Analyses show that VD_3 is linked to the progression of metabolic diseases such as non-alcoholic fatty liver disease and type 2 diabetes mellitus, and may be a therapeutic target, however there is no evidence of liver enzyme alterations with vitamin supplementation [25–27]. Otto-Slusarczyk et.al. (2016) shows the important biochemical pathway that the AST enzyme is involved in, especially in the urea cycle and in the formation of purines and pyrimidines in various tissues of the human body, but especially in liver tissue. This increase in AST dosages leads to the questioning of possible liver damage, especially in the dosages of 14.000IU and 21.000IU of VD_3 [28].

Studies show that many of the VD_3 hydroxylation enzymes are present in adipose tissue (CYP2R1, CYP27A1, CYP2J2), matching the

results of Nimitphong et.al. (2012), who showed that VD_3 has the ability to regulate growth and model adipose tissue [18]. Thus, VD_3 supplementation has different effects on the balance of adipokines in the body, correlating serum $25(OH)D_3$ levels with plasma levels of adiponectin and leptin. In the analysis of possible therapeutic targets, it was demonstrated that the interaction between intestine, white adipose tissue and enterogastric hormones and adipokines (adiponectin and leptin) participate in humoral and cellular immune modulation, in addition to being disparate between lean and obese individuals [29,30].

In this experimental model, with VD_3 overdose for one month, there was no plasma variation of adiponectin (Figs. 1o and 2o). Dinca et.al. (2016), in a meta-analysis, showed that VD_3 supplementation did not suggest a correlation between the plasma level of VD_3, leptin and adiponectin, however the study does not rule out a relationship between time and the action of VD_3 supplementation, due to the expression of CYP27B1 genes in adipocytes [30]. This fact is based on studies that demonstrate an effect on the elevation of the level of leptin mRNA transcription, in a manner dependent on VDR expression and VD_3 plasma levels [31]. On the other hand, the relationship with adiponectin may be related to its gene expression of VD_3 hydroxylases in adipocytes or be linked to the renin-angiotensin-aldosterone system, in which active VD_3 has the ability to negatively inhibit [32,33].

About the inflammatory profile, the literature is conflicting [29], in a cross-sectional study, Wamberg et.al. [34], demonstrated that 7.000IU VD_3 per day, for 26 weeks, did not alter the levels of IL-6, IL-8 and adiponectin, even though there was evidence of this modulation in *vitro*, in human adipose cell cultures. Beilfuss et.al. [35], on the other hand, in a one-year randomized clinical trial, with weekly doses of 4.000IU, showed a decrease in IL-6 and an increase in serum PCR [35].

The relationship between the level of VD_3 in a pathological experimental model and a non-pathological one should be considered, as the increase in NF-κB in systemic pathologies increases oxidative stress and, consequently, the inflammatory response [36]. Thus, the immunomodulatory capacity and the effect on pro-inflammatory cytokines of VD_3 interactions have been studied, such as the regulation of negative NF-κB mRNA*, and Toll-Like* 2 and 4, and consequently IL-6 and TNF-α [37,38]. In addition to the direct long-term VD_3 effect on T lymphocyte culture, and the increased expression of VDR in these cells, reducing the levels of INF-γ and IL-10, in an *in vitro* experimental model [39].

Nevertheless, our findings show an increase in pro-inflammatory cytokines, especially in the comparison of groups control, 2.500IU VD_3 and 7.000IU VD_3, with the highest supplementation of 14.000IU and 21.000IU VD_3 (Figs. 3 and 4), and a reduction in the anti-inflammatory cytokine IL-10, in both sexes, however, the evaluation of IL-10 in female rats, an isolated increase in 7.000IU VD_3 group (32.2 $\pm$ 2.7 pg/mL) is observed, with a significant reduction in 14.000 IU VD_3 group with a dosage of 27.0 $\pm$ 3.3 pg/mL, and in 21.000 IU VD_3 group, with a dosage of 27.4 $\pm$ 0.5 pg/mL.

Histological analysis shows a difference between the control (Fig. 6a and f) and the VD_3 gavage supplementation in both male and female Wistar rats, showing a higher degree of structural and nuclear disorder at 14.000IU and 21.000IU of VD_3 (Fig. 6d, e male and Fig. 6i, j female). In isolation, Schwalfenberg et.al. (2015) show that VD_3 acts by improving the absorption of numerous minerals and metals, including essential elements for the body (iron, calcium, magnesium, phosphate) and others that can be toxic (lead, cadmium, aluminum) [40]. Still, the clinical recommendation, based on the literature, is that the pleiotropic action of VD_3 is important for numerous biochemical and physiological functions of the body. On the other hand, in pathological models, VD_3 has been shown to be beneficial, reducing the hepatic steatosis score in an experimental model of NAFLD [41].

Finally, serum VD_3 levels should be measured according to individual physiology, and the medical management should be taken in this way for each condition, whether pathological or not. Likewise, its supplementation and form of administration, taking into account that each physiological microenvironment has a need, and the vast role of this hormone in both skeletal and non-skeletal systems must be respected.

5. Conclusion

When proposing the oral overdose of VD_3 in a physiological model, it was evaluated that VD_3 did not alter the biochemical and inflammatory parameters at the doses of 2.500IU and 7.000IU. However, when evaluating the dosages of 14.000IU and 21.000IU of VD_3, it was noted that the inflammatory effects overlapped. For both the sex, an overlapping profile was identified, so that for the most of the investigated parameters, the changes were similar in male and female Wistar rats. In summary, oral VD_3 supplementation should be done with consideration, taking into account the physiological microenvironment of each individual. Prolonged VD_3 supplementation in the presence or absence of metabolic pathologies should be further discussed in relation to gender, age group, genetics and future needs

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CRediT authorship contribution statement

Chaline Casnova Petry: Methodology. **Clóvis Klock:** Methodology. **Rafael Tamborena Malheiros:** Methodology, Investigation. **Gênifer Erminda Schreiner:** Methodology, Investigation. **Vanusa Manfredini:** Writing – review & editing, Writing – original draft, Project administration, Funding acquisition, Conceptualization. **Itamar Luís Gonçalves:** Writing – review & editing, Writing – original draft, Software. **Laura Smolski dos Santos:** Methodology, Investigation. **Elizandra Gomes Schmitt:** Methodology, Investigation. **Silvia Muller de Moura Sarmento:** Writing – original draft, Software, Methodology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability

Data will be made available on request.

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