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Effect of Vitamin D Supplementation During Pregnancy on Birth Weight: An Experimental Study in Rats

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ABSTRACT

Introduction. Vitamin D deficiency, commonly observed during pregnancy, can affect fetal growth, which can be evaluated by birth weight. Vitamin D supplementation during pregnancy is anticipated to beneficially affect birth weight, a crucial factor for neonatal health and long-term health outcomes. **Methods.** This research was conducted using an *in vivo* experimental design on *Rattus norvegicus* rats as animal models. This study involved 24 rat pups from four groups of pregnant rats, each provided with standard feed and varying doses of vitamin D supplementation: Group A received 62 IU/kgBW, Group B received 415 IU/kgBW, Group C received 663 IU/kgBW, and Group D received no vitamin D supplementation. Vitamin D supplementation was provided through oral gavage from the first day of gestation until parturition. At the time of delivery, six pups from each group were randomly chosen to measure their birth weights. **Results.** The finding from this research indicated that vitamin D supplementation during pregnancy positively influenced birth weight ($p<0.001$). The birth weights of pups in Group C were significantly higher compared to those in group D ($p<0.001$) and Group A ($p<0.001$). Pups in Group B also had significantly higher birth weights compared to Group D ($p=0.002$) and Group A ($p=0.006$). **Conclusion.** Based on these findings it can be concluded that vitamin D supplementation at doses 415 IU/kgBW and 663 IU/kgBW can increase birth weight.

1. Introduction

Birth weight is one of the initial evaluations conducted on newborns, offering a glimpse into the conditions that the fetus experiences in the intrauterine environment.[1,2] Birth weight is a crucial determinant for predicting newborn health, since it directly affects the risk of neonatal morbidity and mortality, along with long-term health consequences. Newborn with low birth weight (LBW) are at higher risk of experiencing growth disorders, neurocognitive development issues, asthma, and metabolic or degenerative disease in adulthood.[3,4] Several variables can affect birth weight, such as gestational age, maternal age, parity, maternal nutrition status, and the number of antenatal care visits.[5] Maternal nutrition intake during pregnancy is also an important factor affecting the birth weight of the newborns. A study by Wubetu et al. (2021) demonstrated a correlation between maternal nutritional factors during pregnancy and birth weight.[3]

Vitamin D is a crucial micronutrient during pregnancy. Besides its involvement in bone health, vitamin D is essential for cell proliferation, differentiation, and maturation, as well as for placental function, calcium homeostasis, and bone mineralization, all of which are crucial for fetal growth and development.[6,7] Several studies indicate that adequate maternal vitamin D levels are correlated with optimal fetal growth, while deficiencies in vitamin D during pregnancy is associated with preterm birth, small-for-gestational-age, impaired bone growth, and neurodevelopmental disorders.[8]

Insufficient vitamin D levels in pregnant women are a prevalent problem globally.[6] It is estimated that 18-84% of pregnant women have insufficient vitamin D levels.[9] A study by Aurora et al. (2018) revealed that over 86% of pregnant women suffered from vitamin d deficiency, while only 4.5% maintained adequate vitamin D levels. This study also identified a significant association between vitamin D deficiency during pregnancy and low birth weight.[10] Additionally, Rolexza and Meirina (2022) found a

significant correlation between the amount of vitamin D consumed by mothers during pregnancy and the birth weight of their babies.[9]

Supplementing with vitamin D during pregnancy may be an effective approach to reduce the incidence of hypovitaminosis in pregnant women, which may also positively impact fetal health.[6] Despite extensive research on the advantages of vitamin D supplementation during pregnancy, which indicated elevated vitamin D levels, its effect on birth weight remains inconsistent. Some studies report an increase in birth weight with vitamin D supplementation, while others find no significant association.[6,11] Therefore, further research is needed to explore the optimal dose of vitamin D supplementation and its effect on birth weight.

This research aims to evaluate the effects of vitamin D supplementation at specific doses in pregnant women on the birth weight of their babies. The results of this research are expected to contribute to better understanding of the role of vitamin D supplementation during pregnancy and provide recommendations for nutritional interventions strategies during pregnancy to prevent low birth weight.

2. Methods

This research applied an in vivo experimental design including white rats (*Rattus norvegicus*) of the Sprague Dawley strain, conducted at the animal Laboratory of the Faculty of Medicine, Universitas Baiturrahmah, Padang, West Sumatera, Indonesia. The study protocol has been reviewed and approved by the Ethics Committee of the Faculty of Medicine, Universitas Baiturrahmah, under ethical clearance number 040/ETIK-FK-UNBRAH/03/07/2024.

The research was conducted on 24 rat pups taken *from* four groups of pregnant rats. Six rat pups were randomly selected from each group. One group was fed a standard diet (Supplementation 1) without vitamin D supplementation and served as the control group, while the other three groups of pregnant rats were fed a standard diet with vitamin D supplementation at the different doses during pregnancy, as detailed below:

a) Group A: Standard diet with vitamin D supplementation at a dose of 62 IU/kgBW
b) Group B: Standard diet with vitamin D supplementation at a dose of 415 IU/kgBW
c) Group C: Standard diet with vitamin D supplementation at a dose of 663 IU/kgBW
d) Group D: Standard diet without vitamin D supplementation

The doses of vitamin D supplementation used in this research were adjusted to dose suitable for experimental rats. A dose of 62 IU/kgBW in rats is equivalent to 600 IU/day in humans,[12] while a dose of 415 IU/kgBW in rats is equivalent to 4.000 IU/day in humans[13], and a dose of 663 IU/kgBW in rats is equivalent to 6.400 IU/day in humans[12], using the following human-to-rat dose conversion formula: AED (mg/kg) = human dose (mg/kg) x Km ratio, while rat's Km ratio is 6,2 and then results convert to IU/kgBW.[14]

The standard diet was provided at 30 grams per day with *ad libitum* access, and vitamin D was administered once daily via oral gavage from the first day of pregnancy (mark by copulatory plug at female rats) until parturition (at 19th-21st day of pregnancy). The leftover feed was weighed daily to calculate the average daily intake. When the rats in each group gave birth, six pups were randomly selected from each group, and their birth weights were measured using a blinded method to ensure objective assessment.

The data from this research will be analyzed using the Statistical Product and Service Solution (SPSS) software version 27. The birth weight data will first be tested for normality using the Shapiro-Wilk test and for homogeneity using Levene's test. If the data are normally distributed and homogeneous, further analysis will be conducted using a One-Way ANOVA with the post hoc Least Significant Difference (LSD). The data will be presented as mean ± standard deviation. Data were considered statistically significant when the P-value was less than 0.05 ($p<0.05$).

3. Results

As reported in this research, as shown in Table 1, it was found that rat pups born to pregnant rats supplemented with 663 IU/kgBW of vitamin D (Group C) had the highest birth weight, averaging 7.67 ± 0.82 grams, while pups from the control group (Group D) had the lowest birth weight, with an average of 4.67 ± 0.82 grams.

Table 1. Average birth weight of rat pups in each group

Group	Birth Weight (gram)	*p*
Group A (Vit. D 62 IU/kgBW)	4.80 ± 0.84	<0.05*
Group B (Vit. D 415 IU/kgBW)	6.60 ± 1.14	
Group C (Vit. D 663 IU/kgBW)	7.67 ± 0.82	
Group D (Control)	4.67 ± 0,82	

*One-way ANOVA

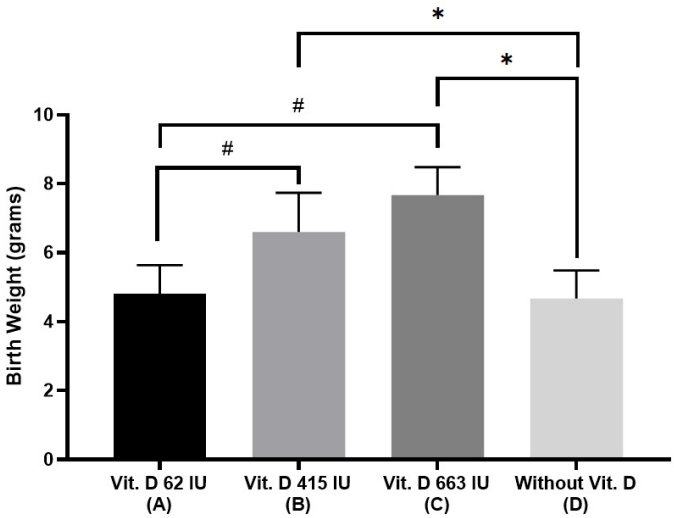

Figure 1. The effect of vitamin D supplementation during pregnancy on birth weight. Results are presented as the mean ± SD. Statistically significant differences: * $p < 0.05$ compared to control group (D); # $p < 0.05$ compared to group with Vit. D 62 IU/kgBW (A)

The data analysis results from this study showed the significant effect of vitamin D supplementation during pregnancy on the birth weight of rat pups ($p<0.05$). The research indicated that pups born to rats receiving 663 IU/kgBW of vitamin D (Group C) during pregnancy had significantly higher birth weights compared to those in the control group (Group D; $p<0.05$) and the group supplemented with 62 IU/kgBW of vitamin D (Group A; $p<0.05$). Rat pups born to mothers supplemented with 415 IU/kgBW of vitamin D (Group B) during pregnancy also had significantly higher birth weights compared to pups in the control group (Group D, $p<0.05$) and the group supplemented with 62 IU/kgBW of vitamin D (Group A; $p<0.05$). However, the birth weight of pups in Group B was not significantly different from that pups in Groups C ($p>0.05$) (Figure 1).

4. Discussion

The selection of doses in this research was based on previous research and established recommendation.[12,13] The choice of 600 IU/day dose for human, equivalent to 63 IU/kgBW in experimental rats, is based on the recommendations of the Institute of Medicine (IOM), which suggest vitamin D supplementation for pregnant and lactating mothers in the range 400-600 IU/day. However, a study demonstrated that supplementation at this dose does not provide adequate vitamin D levels. The selection of a maximum dose of 6,400 IU/day, equivalent to 663 IU/kgBW in experimental rats, is also based on the findings that vitamin D supplementation at 6,400 IU/day meets the adequate vitamin D requirements for breastfeeding mothers without causing adverse effects.[12] For the selection of a 4,000 IU/day dose for humans, equivalent to 415 IU/kgBW in experimental rats, it is based on the study which showed that vitamin D supplementation at 4,000 IU/day also fulfills adequate vitamin D requirements for breastfeeding mothers.[13]

In this study, we found a significant effect on vitamin D supplementation during pregnancy on birth weight. All rat pups were delivered within a gestational period of 19 to 21 days, indicating aterm births. The findings revealed that the birth weight of rat pups from group supplemented with 415 IU/kgBW and 663 IU/kgBW of vitamin D was significantly higher compared to the control group, which did not receive vitamin D supplementation. Although the group supplemented with 62 IU/kgBW of vitamin D did not show a significant difference in birth weight compared to the control group, vitamin D supplementation, particularly at doses of 415 IU/kgBW and 663 IU/kgBW, was shown to increase birth weight in neonates.

The findings of this research align with the systematic review and meta-analysis study, which indicated that birth weight among pregnant women receiving vitamin D supplementation was significantly higher than the control group. The study indicated that the incidence of low birth weight was reduced in the vitamin D treatment group compared to the control group. Several studies suggest that adequate maternal vitamin D concentration are associated with a reduced risk of preterm birth. A case control study in Sudan, reported that women with preterm birth had significantly lower median 25(OH)D concentrations than those with aterm deliveries.[15]

The majority of vitamin D supplementation doses varied between 200 IU to 4.000 IU. The recommended dose of vitamin D supplementation during pregnancy is 600 IU/day; however, supplementation can be provided up to a maximum dose of 4.000 IU/day during pregnancy, although the potential side effects of higher doses are not yet well understood.[11] The

range of doses administered in the analyzed studies aligns with the dose used in our study.

A systematic review and meta-analysis also demonstrated that birth weight was significantly higher in children whose mothers received vitamin D supplementation. This study revealed that vitamin D supplementation initiated after 20 weeks of gestation may enhance birth weight, while supplementation beginning before to 20 weeks of gestation did not influence birth weight.[6] Similarly, another study also showed that vitamin D supplementation significantly increased birth weight compared to the control group.[16]

Unlike the previously referenced research, another systematic review and meta-analysis revealed no significant difference in birth weight between newborns born to mothers who got vitamin D supplementation during pregnancy. That study additionally discovered no significant correlation between vitamin D supplementation and the incidence of low birth weight the primary effect of vitamin D supplementation on newborn anthropometry was more pronounced in infant length, while its effect on birth weight was less significant.[8]

Vitamin D levels during pregnancy are essential for skeletal growth, enamel formation, and overall fetal growth and development.[17,18] Vitamin D supplementation during pregnancy may affect birth weight by influencing fetal cell mass and function, skeletal mineralization, and fetal metabolism.[11] Vitamin D influences cellular proliferation, a critical factor for fetal growth. This effect arises from the modulation of multiple processes related to cellular proliferation, such as apoptosis, cell cycle progression, and differentiation into specialized cells. Vitamin D acts through its binding to vitamin d receptors (VDR), after which the vitamin D-VDR complex binds with response elements in genes that regulate cell growth. Vitamin D can influence signaling molecules that are involved in apoptosis, cell cycle regulation, and cell differentiation.[19]

Vitamin D may contribute to increased birth weight primarily through its role in calcium and phosphate homeostasis, which affects bone mineralization and fetal development during pregnancy.[6,11] Additionally, maternal vitamin D levels during pregnancy positively influence essential factors for fetal growth and development, such as fetal bone formation, skeletal muscle development, and adipocyte maturation.[6,18,20,21]

The interaction of vitamin D with its receptor regulates genes associated with placental implantation, thereby facilitating proper implantation and adequate nutrient supply for fetal development. Vitamin D influences maternal immune response, the expression of human chorionic gonadotropin, and sex steroid hormone via the placenta, all of which affect fetal growth.[6,11,19]. The role of vitamin D in glucose and insulin metabolism impacts energy availability for the fetus, subsequently influencing musculoskeletal growth.[11]

Vitamin D supplementation dosage recommendations differ among various organizations and countries. The World Health Organization (WHO) recommends a daily dose of 200 IU, the United Kingdom suggests 400 IU, the United States advises 600 IU, and Germany recommends 800 IU. Research shows that a daily supplementation of 600 IU for pregnant women can decrease the risk of vitamin D insufficiency and deficiency by 83.4%. However, this dose has a lesser effect on newborn birth weight.[8]

It is hoped that the findings if this study, which show that vitamin D supplementation at 415 IU/kgBW/day (equivalent to 4.000 IU/day in humas) and 663 IU/kgBW/day (equivalent to 6.400 IU/day in humans) can increase birth weight, may serve as a consideration for recommendations on vitamin D supplementation in pregnant women. However, this study has several limitations. It only assessed birth weight at birth without conducting long-term follow-up to evaluate the effect of vitamin D supplementation on postnatal growth and developmental outcomes. Beside that, the study did not measure serum vitamin D levels in the mother rats, so it remains unclear whether the supplementation effectively increased circulating vitamin D levels and how those levels may have influenced birth weight. Future studies are recommended to evaluate the long-term effects of vitamin D supplementation on the offspring, including growth, development, and physiological outcomes beyond birth weight.

5. Conclusion

Vitamin D supplementation in rats during pregnancy significantly increased the birth weight for rat pups, with the dose of 663 IU/kgBW/day showing the most favorable results.

6. Author Contribution

W.S., G.K., and K.M.H. conceived the original idea. W.S. and K.M.H. carried put the experiment. W.S., G.K., and K.M.H. wrote the manuscript. L.Z. helped supervise the project.

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