

RESEARCH STUDY

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Differences in Serum 25(OH)D Levels, Intake of Vitamin D, Magnesium, and Zinc in Obese and Normal Nutritional Status

Perbedaan Kadar 25(OH)D Serum, Asupan Vitamin D, Magnesium dan Zink pada Status Gizi Obesitas dan Normal

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ABSTRACT

Background: Vitamin D deficiency affects about 50% of people worldwide and 95% of Indonesian women of childbearing age. Micronutrient intake can impact serum 25(OH)D levels. Obesity is linked to serum 25(OH)D levels and micronutrients like zinc, magnesium, and vitamin D consumption.**Objectives:** This study aims to determine the differences in serum levels of 25(OH)D, vitamin D, magnesium, and zinc intakes in obese and normal students at the Faculty of Medicine, Andalas University, Padang.**Methods:** This research was observational with a comparative case-control design at the Faculty of Medicine and Biomedical Laboratory, Andalas University, from November 2021 to July 2022. Systematic random sampling was used to select the sample, obtaining 64 female students with obesity and normal nutritional status. Data were collected by blood sampling and questionnaires. Bivariate analysis used an independent t-test.**Results:** The results showed the mean serum levels of 25(OH)D, vitamin D, magnesium, and zinc intakes in the obese group were 10.98 ± 2.12 ng/mL; 5.07 ± 1.47 mcg; 166.04 ± 65.27 mcg and 6.88 ± 2.73 mcg, while the mean in the standard group were 13.94 ± 4.57 ng/mL; 5.92 ± 1.77 mcg; 205.88 ± 92.84 mcg; and 7.62 ± 2.67 mcg. The mean serum levels were 25(OH)D, vitamin D, magnesium, and zinc intakes were lower in the obese group (p -value = 0.001; 0.040; 0.044; 0.280).**Conclusions:** There are significant differences in serum levels of 25(OH)D, vitamin D, and magnesium in female students with obese and normal nutritional status. There is no significant difference in zinc intake in female students with obese and normal nutritional status.

INTRODUCTION

Vitamin D is a type of fat-soluble vitamin divided into ergocalciferol and cholecalciferol¹. The normal range of vitamin D levels in the body is 30 ng/mL - 100 ng/mL. Vitamin D deficiency is a condition in which serum 25(OH)D levels are less than 30 ng/mL. Serum 25(OH)D levels reflect the body's total vitamin D production, which can be the best biomarker to measure a person's vitamin D status. Nearly one billion people worldwide have vitamin D deficiency, and about half of the population has vitamin D insufficiency. Vitamin D deficiency also occurs in 80% of adults in South Asian countries^{2,3}. On average, more than 95% of pregnant women and women of childbearing age in Indonesia experience vitamin deficiencies⁴. Around 86.7% of pregnant women in West Sumatra province experience vitamin D deficiency⁵.

Vitamin D levels are influenced by outdoor physical activity and micronutrient intake. Outdoor physical activity exposes the body to direct sunlight,

which can increase vitamin D production under the skin. Research in Austria in 2020 stated that physical activity and the duration of direct sun exposure to the skin affect vitamin D levels⁶. Micronutrient intake is a nutrient that the body needs, even if only in small amounts. Micronutrient intake consists of vitamins and minerals needed in the process of hormone formation, enzyme activity, and regulation of immune function and the reproductive system⁷. Micronutrient intake influences vitamin D levels: vitamin D, magnesium, and zinc. Magnesium intake is involved in forming enzymes (25 α hydroxylases and one α hydroxylase) needed for vitamin D synthesis, and zinc intake is involved in activating the vitamin D receptor gene in target tissues^{8,9}.

Vitamin D levels and micronutrient intake, such as vitamin D, magnesium, and zinc, are related to the incidence of obesity. Obesity is a condition in the body caused by an imbalance between intake and energy (too much food but little energy expended)¹⁰. The incidence of

obesity in Indonesia in the age group over 18 years increases yearly; in 2013, it was 15.4%; in 2016, it was 20.7%, and the highest percentage was 21.8% in 2018. Obesity is more common in females (41.6%)¹¹.

Deficiency in vitamin D levels is often found in cases of obesity because, in obesity, there is a buildup of fat tissue, which fails the conversion of pre-vitamin D into vitamin D under the skin. That excessive buildup of fat tissue also results in inflammation and degradation of vitamin D, so vitamin D levels are low, and production decreases¹². This is explained by research in 2019 in Iran, which stated that there was a significant relationship between 25(OH)D levels and obesity¹³. In line with previous research, in 2020, a meta-analysis study was also conducted, which stated that the nutritional status of obese people was mainly deficient in serum 25(OH)D levels¹⁴. Research in the United Arab Emirates in 2020 contrasts two previous studies, where the results stated no significant link between serum 25(OH)D levels and obesity¹⁵.

Low intake of micronutrients (vitamin D, magnesium, and zinc) is also typical in obesity. Individuals with obesity have poor eating habits where they consume more macronutrients and less micronutrients¹⁶. This is explained by Jiang S et al. in 2020 in the United States, who stated a significant link between obesity and BMI¹⁷. Similar to previous research, research was also conducted in 2019, which noted a meaningful relationship between the intake of vitamin D, magnesium, and zinc and the incidence of obesity¹⁸. Women aged 18 to 25 are early adult women at their peak fertility, so their reproductive health needs to be considered¹⁹. Vitamin D levels, micronutrient intake (vitamin D, magnesium, and zinc), and their relationship to obesity are closely related to the preconception reproductive health of early adult women. Low intake of micronutrients such as vitamin D, magnesium, and zinc in obesity can disrupt reproductive health²⁰. Lack of vitamin D levels in obesity can also disturb the folliculogenesis process, disrupting the ovulation process and thus damaging fertility^{1,19,20}. Andalas University's Faculty of Medicine has the most female students in West Sumatra²¹. Andalas University Faculty of Medicine students are in the group of early adult women whose preconception health needs to be a concern. There have been several studies that discuss this variable with young adult women. One of them is research by Lorensia et al. in 2022 in Surabaya. So far, this topic has yet to be researched in Padang City, so researchers are interested in exploring it. Based on the description above regarding serum 25(OH)D levels and micronutrient intake (vitamin D, magnesium, and zinc) in obesity and its effect on reproductive health in the preconception phase of early adult women, the research was carried out to examine differences in 25(OH)D levels, vitamin D, magnesium, and zinc intake in female students with obese and normal nutritional status at the Faculty of Medicine, Andalas University.

METHODS

This research is included in an observational study using a case-control design. The analysis was conducted from November 2021 to July 2022 at the

Faculty of Medicine and Biomedical Laboratory, Andalas University. The population in this study was all students from the Faculty of Medicine, Andalas University, for the 2020/2021 and 2021/2022 academic years. The research sample was female students with obese and normal nutritional status. The sample is calculated using the sample proportion formula in unpaired categorical comparative research as follows:

$$n1 = n2 = 2 \left[\frac{(z\alpha + z\beta)s}{x1 - x2} \right]^2$$

$$= 2 \left[\frac{(1.96 + 1.28)12.08}{10.5} \right]^2$$

$$= 27.796 = 28$$

Notes:

- n1 = n2 : the sample size
- zα : type I error (5% = 1.96)
- zβ : type I error (10% = 1.28)
- x1-x2 : differences that are considered significant (10.5)
- S : combined standard deviation (12.08)

To anticipate 10% of subjects dropping out, the sample size in this study was 32 people for each obese and normal group. All samples in this study met the predetermined inclusion criteria. The inclusion criteria were aged 18 to 25 years, unmarried, had a BMI ≥ 25.0 for female students with obese nutritional status, had a BMI of 18.50 to 22.99 for female students with normal nutritional status, and were willing to be research subjects.

The sample was selected using a systematic random sampling method, and the sample frame was created based on the results of student health screening at Andalas University Hospital. From these results, data were obtained on 559 female students with details of 114 (20.39%) people with obesity and 274 (49.02%) people with normal nutritional status. The sample of female obese students was selected using an interval of 3, and female students with average dietary rates were chosen using a gap 9.

The selected samples were contacted via the WhatsApp application and asked to complete a questionnaire regarding research sample screening in a Google form. Samples willing to attend and meet the specified inclusion criteria were collected in one place to collect research data. Selected samples were collected in the Nutrition Department of the Faculty of Medicine, Andalas University, Limau Manis Campus, on 25 June and 1 July 2022. Data was collected by carrying out anthropometric measurements, taking blood samples, and interviewing the SQ-FFQ questionnaire. Anthropometric measurements include measurements of height, weight, and fat percentage. Body height (in cm) was measured using a microtome attached to a flat wall. A BIA device on a flat surface measured body weight (kg) and fat percentage. Enumerators from the Pramita Laboratory carried out blood collection. Serum 25(OH)D levels were examined using the ELISA method. Data on vitamin D, magnesium, and zinc intake was obtained

through an interview process by enumerators (nutrition experts) using the SQ-FFQ questionnaire. The SQ-FFQ questionnaire was filled out through an interview process, and the data will be processed using the Nutrisurvey application. After processing the data with this application, data was obtained regarding the amount of the research subjects' vitamin D, magnesium, and zinc intake.

The data obtained then goes through a process of data checking, data coding, data entry, data cleaning, and data tabulation. Next, the data is processed or analyzed statistically using the SPSS application (version 25.0). The

number of samples in each group in the study was 32, which is smaller than 50, so the normality test used was Shapiro Wilk. All variables in this study have normally distributed data, so the mean value and standard deviation are displayed in the univariate analysis. In this study, bivariate analysis used the independent t-test. Research was carried out in compliance with research ethical principles. This research has received ethical permission from the Faculty of Medicine Ethics Committee, Andalas University, with number 675/UN.16.2/KEP-FK/2022.

RESULTS AND DISCUSSION

Table 1. Average characteristics of female students with obese and normal nutritional status at the Faculty of Medicine, Andalas University, Padang City

Respondent Characteristics	Obesity	Normal
	Mean ± SD	Mean ± SD
Age (years)	19.59 ± 0.837	20 ± 0.880
Body Fat Percentage (%)	42.61 ± 3.51	30.41 ± 2.1

Respondents in this study were early adult women divided into obese and normal groups. The average age of respondents with obese nutritional status was 19.59 ± 0.837, and regular dietary grade was 20 ± 0.880. Respondents with obese and normal healthy levels had an age corresponding to the age range for early adult

women, namely 18-25 years¹⁹. The body fat percentage in research respondents with obese nutritional status was 42.61 ± 3.51%, higher than those with normal nutritional status, 30.41 ± 2.1%. The high rate of fat in the obese group can disrupt the process of vitamin D formation in the skin, resulting in low vitamin D levels¹⁶.

Table 2. Differences in mean serum 25(OH)D levels in female students with obese and normal nutritional status at the Faculty of Medicine, Andalas University, Padang City

Respondent Characteristics	Obesity	Normal	p-value
	Mean ± SD	Mean ± SD	
Serum 25(OH)D Levels (ng/mL)	10.98 ± 2.12	13.94 ± 4.57	0.001*

T-test independent; *p-value is significant if < 0.05

Vitamin D is classified as a type of fat-soluble vitamin that comes from food intake and can be produced under the skin with the help of exposure to direct sunlight. The best biomarker for measuring body vitamin status is serum 25 dihydroxy vitamin D levels³. According to the Endocrine Society, 25(OH)D levels are normal if they range from 30 to 100 ng/mL²².

This study discovered that the mean serum 25(OH)D level in female students with obese nutritional status was 10.98 ± 2.12 ng/mL lower than in female students with normal dietary status of 13.94 ± 4.5 ng/mL. Further statistical tests were carried out, where the p-value = 0.001 was obtained to conclude that there was a significant difference in the mean serum 25(OH)D levels in female students with obese and normal nutritional status.

These results align with research by Khodabakshsi A. et al. in 2022, which stated that a lower mean serum 25(OH)D level was found in the obese group, where the mean for obese and normal nutritional status was 26.5 ± 14.5 ng/mL and 29.5 ± 16.3 ng/m with a p-value of 0.016²³. Different results were obtained from research by MAVIŞ, M. E. et al. in 2020, which showed higher mean serum 25(OH)D levels were found in the obese group. The age of the respondents likely causes this difference in results²⁴.

Even though there were significant differences, young adult women aged 18-25 who were sampled in this study had 25(OH)D levels below the standard limit in both the obese and normal nutritional status. This deficiency in vitamin D levels is due to insufficient vitamin D intake and low exposure to direct sunlight. These results are strengthened by research by Jang et al. in 2019, which stated that 87.39% of young adult women experienced vitamin D deficiency, where vitamin D deficiency was more common in the obese group²⁵.

Low serum 25(OH)D levels in groups with obese nutritional status can be caused by fat accumulation, which causes an increase in adipose tissue, thus preventing the formation of vitamin D3 under the skin. Fat accumulation also causes inflammation in adipose tissue, resulting in vitamin D degradation and a decrease in serum 25(OH)D levels in the body^{12,16}. Another factor that causes low serum 25(OH)D levels in obesity is the tendency for lower outdoor physical activity carried out by the obese group. This statement is supported by research in 2019 in Surabaya, which showed that the obese group did fewer outdoor activities than the standard group, where the p-value obtained was 0.047²⁶. Poor eating habits in the obese group can also be another factor causing low serum 25(OH)D levels, even though intake only affects 20% of the body's vitamin D levels¹⁶.

According to the researchers' assumptions, there is a significant difference in the mean serum 25(OH)D levels in groups with obese and normal nutritional status due to excessive fat accumulation in the obese group, which causes low levels of 25(OH)D in the body. The above conditions are further worsened by lower vitamin D intake and outdoor physical activity in the obese group, resulting in lower vitamin D levels in the obese group.

The respondents of this study were early adult women in the preconception period. Low levels of vitamin D in female students, especially in the obese group, can harm a woman's preconception health.

Vitamin D deficiency can increase the possibility of disturbances in the folliculogenesis process, which can interfere with fertility¹. It is necessary to carry out health promotion and screening so that preconception problems experienced can be detected quickly and appropriate treatment can be given. To normalize vitamin D levels, you can do outdoor activities, take vitamin D, and provide supplementation if necessary. For obese students, it is essential to undergo nutritional consultation and adopt a healthy lifestyle so that their body weight can be brought back to normal.

Table 3. Differences in mean intake of vitamin D, Magnesium, and zinc among female students with obesity and normal nutritional status at the Faculty of Medicine, Andalas University, Padang City

Types of Micronutrient Intake	Obesity	Normal	p-value
	Mean ± SD	Mean ± SD	
Vitamin D (mcg)	5.07 ± 1.47	5.92 ± 1.77	0.040*
Magnesium (mcg)	166.04 ± 65.27	205.88 ± 92.84	0.044*
Zink (mcg)	6.88 ± 2.73	7.62 ± 2.,67	0.280

T-test independent; *p-value is significant if < 0.05

This research, based on Table 3, shows that the average intake of vitamin D in female students with obese nutritional status is 5.07 ± 1.47 mcg, which is lower in female students with normal nutritional status, which is 5.92 ± 1.77 mcg, where the p-value = 0.040, meaning that there is a significant difference in vitamin D intake between female students with obese and normal nutritional status. The results obtained are by research by Lorensia A. et al. in 2022, which explains that vitamin D has a significant relationship with obesity with a p-value = 0.001²⁶. This research also obtained the same results as Nikolova M & Penkov A, which was carried out in 2020 and showed that the obese group had a lower vitamin D intake (6 mcg) than the regular nutritional status group (7.6 mcg)²⁷. This result is inversely proportional to research by Shatwan & Almorai in 2022, where the p-value in that study was 0.26²⁸. Another study with different results was conducted by Farhat G et al. in 2019, which stated no significant difference in vitamin D intake in the obese and normal groups with a p-value = 0.33²⁹.

There was a significant difference in vitamin D intake between groups with obese and normal nutritional status due to poorer diet in the obese group. This is reinforced by the results of research by Vranić L et al., which stated that the obese group tended to have a poor diet where this group consumed a lot of macronutrients and little micronutrients. Hence, they had a lower vitamin D intake than the standard group. Even though it only affects 20%, low vitamin D intake in the obese group will further exacerbate vitamin D deficiency in the body¹⁶. More in-depth research is needed to find out eating patterns in more detail.

In Table 3, it can be explained that in this study, the average magnesium intake of female students with obese nutritional status was 166.04 + 65.27 mcg lower than female students with an average dietary rate of 205.88 + 92.84 mcg, where the p-value value = 0.044, meaning that there is a significant difference in magnesium intake between female students with obese and normal nutritional status. The same results were also obtained through research by Dai H et al. in 2018, which

stated that the obese group had a lower intake than the regular nutritional status group, where the average magnesium intake in the two groups was 333.4 mcg and 438.8 mcg³⁰. Different results were obtained from research by Shatwan and Almorai in 2022, which stated no significant difference in magnesium intake between groups with obese and normal nutritional status with a p-value of²⁸.

Magnesium is one of the second most abundant micronutrients in intracellular fluid and is needed for health. Magnesium regulates body metabolism, acid-base balance, protein synthesis, nerve impulse transmission, and muscle relaxation. Magnesium intake can be sourced from meat, milk, chocolate, green vegetables, nuts, and seafood. The amount of magnesium intake that must be met daily for early adult women is 330 mcg^{31,32}.

Magnesium intake is related to obesity, where increasing magnesium intake by 10 mg/1000 kcal per day can reduce BMI by 20%³³. Magnesium intake also influences the formation of enzymes involved in synthesizing vitamin D levels. Magnesium acts as an essential cofactor for the synthesis and activation of vitamin D. Vitamin D and Vitamin D Binding Protein (VDBP), which bind to each other in blood circulation, is also influenced by magnesium⁸. The significant difference in magnesium intake between groups with obese and normal nutritional status could be caused by poor diet in the obese group. These things could also be caused by the obese group consuming less green vegetables (one source of magnesium intake) than the standard group. This result is supported by Jesser and Santoso's 2021 research stating that as many as 84% of the group with obese nutritional status had low vegetable intake³⁴. The same results were found in a study by Nouri M et al. in 2022, which stated that consumption of vegetables in obese and overweight people was lower when compared to groups with normal nutritional status³⁵.

The results of this study based on Table 3 show that the average zinc intake of female students with obese nutritional status was 6.88 ± 2.73 mcg lower than

female students with an intermediate dietary level of 7.62 ± 2.67 mcg, where the p-value = 0.280, meaning that no significant differences were found between female students with obese and normal nutritional status. These results are by research by Ju et al. in 2022 in China, which stated that the group with obese dietary quality had a lower average zinc intake than the regular nutritional status group, where the average zinc intake in the group with obese and normal nutritional status was 8.3. mcg and 8.6 mcg³⁶. The same results were also obtained from research by Shatwan & Almoraie in 2022, which showed that the average zinc intake in obese nutritional status was 4.5 mcg lower than normal nutritional status of 5.1 mcg with a p-value = 0.06²⁸. The results of this study are inversely proportional to research by Farhat et al. in 2019, which stated that there was a difference in zinc intake in the obese and normal nutritional status where the p-value = 0.05²⁹.

Zinc is one of the micronutrient minerals involved in the immune response process, gene expression, and body metabolism; apart from that, zinc also helps the growth and development of reproductive organs and the digestion of carbohydrates. Zinc intake comes from nuts, chicken gizzards, eggs, leafy and root vegetables, beef, chicken, milk, and cereals^{7,31}. The daily zinc intake needed by the body is based on the Nutritional Adequacy Rate set for early adult women, namely eight mcg³⁷.

Vitamin D has many target tissues in the human body. Vitamin D in active form will go to the target tissue to perform its function. Activated Vitamin D will carry out tasks according to its target tissue. In the target tissue, vitamin D's reproductive organs (ovaries) are involved in the folliculogenesis process¹. Activation of the vitamin D receptor gene in target tissues is influenced by zinc intake⁹. Zinc and vitamin D influence each other's absorption and desorption; this can be explained by Shams et al.'s 2016 research on experimental animals, which shows that increasing zinc intake can positively affect vitamin D levels³⁸.

Zinc intake affects the body's vitamin D levels. Zinc is an essential cofactor in vitamin D activity. Vitamin D receptors bind to zinc via zinc finger regions, and gene activity for vitamin D is influenced by zinc. Zinc helps the work of vitamin D receptor cells in target tissues. Zinc modulates the structure and DNA binding of the 1.25-dihydroxycholecalciferol DNA response element binding domain; zinc deficiency will result in the correct structural conformation of the vitamin D receptor not being formed, so this may affect vitamin D levels in the body. This statement was found in Shams et al. research conducted in Iran in 2016, which explained a significant relationship between low zinc status and vitamin D levels³⁸. Another study in Spain in 2021 led by Vázquez-Lorente H et al. showed an increase in vitamin D levels after being given zinc supplementation for eight weeks³⁹. Further mechanisms regarding the link between zinc intake and vitamin D levels are not yet known.

There was no significant difference in zinc intake between female students with obese and normal status due to several things, such as the choice of food sources of zinc, which were quite similar between the obese and normal groups. The way food is processed and anti-nutrients can also be other factors that influence

research results. Research by Sundari et al. explains this, where it is stated that the processing of chicken, beef, tofu, and tempeh, which are sources of zinc, affects the amount of zinc intake consumed, where processing by frying and boiling causes a decrease in the information of zinc consumed⁴⁰.

The body needs to take in vitamin D, magnesium, and zinc as part of its micronutrient intake. Overall, the average intake of vitamin D, magnesium, and zinc among female students who were respondents in this study was below the established Nutritional Adequacy Rate. Female students' generally irregular eating habits and the condition of female students who mostly live without their parents could be the cause. Continuous deficiencies in vitamin D, zinc, and magnesium intake can disrupt the body's working systems, such as enzyme activity, the endocrine system, the reproductive system, and the body's immune system⁷.

CONCLUSIONS

This study explains significant differences in serum 25(OH)D levels, vitamin D, and magnesium intake in female students with obese and normal nutritional status. There was no significant difference in zinc intake between female students with obese and normal nutritional status. For a more in-depth explanation regarding physical activity and the amount of sun exposure on vitamin D levels in the obese and regular groups, this could be used as a research topic for future researchers. The results of this research can become a reference and provide input for implementing preconception health programs related to reproductive system health. This input can be in the form of improving health promotion, early detection through routine screening, and providing quick treatment if problems occur.

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Author Contributions

SPR: conceptualization, investigation, methodology, formal analysis, writing—original draft, writing—review and editing; DD: conceptualization, supervision, methodology, writing—original draft, writing—review and editing; HRK: writing—review and editing.

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