



## Association of Macronutrient Intake, Phytic Acid Consumption, and Menstrual Patterns with Hemoglobin Levels in Adolescent Girls

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### ABSTRACT

**Background:** Anemia among adolescent girls represents a critical public health concern and can negatively affect academic achievement and long-term health. The prevalence of anemia in adolescent girls is often higher than in other age groups. However, the factors influencing the incidence of anemia among adolescent girls remain understudied in the working area of Puskesmas Sukapakir, Bandung City. This study aims to analyze the relationship between macronutrient intake, consumption habits of phytic acid sources, as well as menstrual cycle and duration, with hemoglobin levels in adolescent girls.

**Research Methods:** This research is a quantitative study with a cross-sectional design. The study involved 75 adolescent girls selected through total sampling. The instruments used included questionnaires and digital hemoglobin meters. Data analysis was conducted using univariate and bivariate statistical tests, including Pearson correlation, Spearman correlation, and Independent-Samples T-Test.

**Research Result:** The study found significant relationships between anemia and protein intake ( $p = 0.030$ ), consumption habits of phytic acid sources ( $p = 0.024$ ), menstrual cycle regularity ( $p = 0.001$ ), and menstrual duration ( $p = 0.015$ ). Other factors, such as energy, fat, and carbohydrate intake, did not show significant associations.

**Conclusion:** Interventions focusing on improving protein intake, reducing phytic acid consumption, and managing menstrual irregularities are essential to reduce anemia risk among adolescent girls.

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## 1. INTRODUCTION

Adolescent girls are a vulnerable group for developing anemia because they are in a phase of rapid physical growth and sexual development, which increases their nutritional needs, particularly for iron (Putriwati et al., 2024). Anemia is a medical condition characterised by low blood hemoglobin (Hb) levels. Hemoglobin ensures an adequate oxygen supply to red blood cells, muscle cells, and brain cells. Therefore, anemia can lead to weakened immunity, reduced physical fitness, and impaired cognitive function, which may negatively affect academic performance and long-term health outcomes (Kemenkes, 2018).

In Indonesia, the prevalence of anemia increased from 21.7% in 2013 to 23.7% in 2018, with a higher prevalence among females (27.2%) than males (20.3%) (Riskesdas, 2018). Among adolescents aged 15–24, the prevalence of anemia rose significantly from 6.9% in 2007 to 32.0% in 2018 (Riskesdas, 2018). West Java Province has a higher anemia prevalence than the national average, at 41.5% (Putriwati et al., 2024), and the incidence among adolescent girls in Bandung City is also considered high, with a reported prevalence of 20.94% (Dinas Kesehatan Kota Bandung, 2023).

Several risk factors contributing to anemia in adolescent girls have been widely studied. According to WHO (2001), adolescent girls are at higher risk of developing anemia due to increased physiological demands, malabsorption, inadequate iron stores, insufficient dietary intake, and other factors such as health behaviors and lifestyle. Additional factors influencing hemoglobin levels include frequent consumption of iron absorption inhibitors and menstrual duration of  $\geq 5$  days (Berhe et al., 2022; Putriwati et al., 2024).

According to the Dinas Kesehatan Kota Bandung (2023), the prevalence of anemia among high school girls is higher than among junior high school girls. A preliminary study conducted at a secondary school in the working area of Puskesmas Sukapakir revealed that 57,69% of adolescent girls were anemic. However, the risk factors contributing to this high prevalence have not been comprehensively examined in a single study, particularly within the secondary schools in the working area of Puskesmas Sukapakir. Therefore, this study aims to analyze the risk factors associated with hemoglobin levels in adolescent girls in this region, to provide evidence-based recommendations for the effective management and prevention of anemia among adolescent girls.

## 2. METHODS

This study is a quantitative research with a cross-sectional design. The independent variables in this study are macronutrient intake (energy, protein, fat, carbohydrates), consumption habits of phytic acid sources, and menstrual cycle and duration. In contrast, the dependent variable is the hemoglobin level. The research was conducted in three secondary schools within the Puskesmas Sukapakir working area, covering all stages from proposal preparation, data collection, to final reporting. The entire research process took place from August 2024 to May 2025.

The population consisted of all female students in the three secondary schools within the Puskesmas Sukapakir working area, totaling 92 subjects. Total population sampling was employed, with 75 subjects meeting the inclusion criteria, while 17 were excluded for not meeting the predetermined criteria. Inclusion criteria included female students aged 15–19 years from junior and senior high schools, conscious and able to communicate well, willing to participate throughout the research process, and who had signed informed consent. Exclusion criteria were students absent during data collection, professional athletes, or those with a

history of infectious or chronic diseases such as leukemia, AIDS, malaria, cancer, kidney failure, or thalassemia.

Data collection was conducted through interviews using questionnaires and hemoglobin level measurements. The process began by obtaining the subjects' willingness to participate and written consent from their parents or guardians via informed consent forms. Subjects were then interviewed using research instruments covering menstrual cycle and duration, macronutrient intake assessed via a 2x24-hour food recall questionnaire, and consumption habits of phytic acid sources assessed through a Food Frequency Questionnaire (FFQ). Hemoglobin levels were measured by health centre staff using a digital Hb meter.

Data analysis stages included data entry, coding, editing, and cleaning using Microsoft Excel for Windows 2021, followed by analysis using IBM SPSS Statistics version 21. Univariate analysis was employed to describe the research variables frequency distributions, percentages, means, and standard deviations. Bivariate analysis used three methods: Pearson correlation test for the relationship between macronutrient intake and menstrual cycle with hemoglobin levels, Spearman correlation test for consumption habits of phytic acid sources with hemoglobin levels, and Independent-Samples T-Test for the relationship between menstrual duration and hemoglobin levels. This study received ethical approval from the Health Research Ethics Committee, Faculty of Medicine, Universitas Negeri Jember, under number 4475/UN25.1.10.2/KE/2024.

### 3. RESULTS AND DISCUSSION

#### 3.1. Macronutrient Intake and Adequacy

The macronutrient intake of the subjects was obtained through interviews using a 2 x 24-hour food recall form. Nutrient adequacy levels represent the daily nutrient intake compared to the Recommended Dietary Allowance (RDA), expressed as a percentage. The RDA for adolescents varies according to age group and gender. The subjects' macronutrient adequacy levels were calculated by comparing their macronutrient intake with their requirements. Macronutrient adequacy levels were then categorised into three groups: inadequate (<80% RDA), adequate (80–110% RDA), and excessive (>110% RDA). The macronutrient intake of the subjects is presented in Table 1, while the macronutrient adequacy levels are shown in Table 2.

Table 1. Distribution of Subjects Based on Macronutrient Intake

| Macronutrients | Intake           |              |
|----------------|------------------|--------------|
|                | Mean $\pm$ SD    | Min – Max    |
| Energy         | 1416 $\pm$ 395   | 688 - 2596   |
| Protein        | 45,6 $\pm$ 14,7  | 17,4 - 92,8  |
| Fat            | 53,2 $\pm$ 21,1  | 22,0 - 140,1 |
| Carbohydrates  | 167,9 $\pm$ 55,9 | 53,9 - 296,1 |

Based on Table 1, the average energy intake of the subjects was 1416  $\pm$  395 kcal, ranging from 688 to 2596 kcal. The average protein intake was 45,6  $\pm$  14,7 grams, ranging from 17,4 to 92,8 grams. The average fat intake was 53,2  $\pm$  21,1 grams, ranging from 22,0 to 140,1 grams. The average carbohydrate intake was 167,9  $\pm$  55,9 grams, ranging from 53,9 to 296,1 grams.

Table 2. Distribution of Subjects Based on Macronutrient Adequacy Levels

| Level of Macronutrient Adequacy | Frequency (n) | Percentage (%) |
|---------------------------------|---------------|----------------|
| Energy                          |               |                |
| Inadequate                      | 44            | 58,7           |
| Adequate                        | 25            | 33,3           |

| Level of Macronutrient Adequacy | Frequency (n) | Percentage (%) |
|---------------------------------|---------------|----------------|
| Excessive                       | 6             | 8,0            |
| Total                           | 75            | 100,0          |
| Mean ± SD (%)                   | 73,8 ± 23,7   |                |
| Min – Max (%)                   | 30 - 145      |                |
| Protein                         |               |                |
| Inadequate                      | 41            | 54,7           |
| Adequate                        | 27            | 36,0           |
| Excessive                       | 7             | 9,3            |
| Total                           | 75            | 100,0          |
| Mean ± SD (%)                   | 77,0 ± 27,3   |                |
| Min – Max (%)                   | 33 - 151      |                |
| Fat                             |               |                |
| Inadequate                      | 41            | 54,7           |
| Adequate                        | 21            | 28,0           |
| Excessive                       | 13            | 17,3           |
| Total                           | 75            | 100,0          |
| Mean ± SD (%)                   | 83,8 ± 36,1   |                |
| Min – Max (%)                   | 32 - 230      |                |
| Carbohydrates                   |               |                |
| Inadequate                      | 59            | 78,7           |
| Adequate                        | 14            | 18,7           |
| Excessive                       | 2             | 2,7            |
| Total                           | 75            | 100,0          |
| Mean ± SD (%)                   | 60,7 ± 22,9   |                |
| Min – Max (%)                   | 16 - 116      |                |

Table 2 shows that most subjects (58,7%) had inadequate energy adequacy levels, averaging  $73,8 \pm 23,7\%$ . Protein adequacy was also insufficient in most subjects (54,7%), averaging  $77,0 \pm 27,3\%$ . Similarly, fat adequacy was inadequate in 54,7% of the subjects, averaging  $83,8 \pm 36,1\%$ . Carbohydrate adequacy was insufficient in most subjects (78,7%), with an average level of only  $60,7 \pm 22,9\%$ .

### 3.2. Consumption Habits of Phytic Acid Sources

A Food Frequency Questionnaire (FFQ) can assess an individual's consumption habits. The FFQ used in this study focused on the subjects' consumption habits of phytic acid sources over the past month. The frequency of phytic acid source consumption was categorised into two groups: infrequent (<3 times per week) and frequent ( $\geq 3$  times per week). Table 3 presents the types of phytic acid sources most frequently consumed by the subjects, while Table 4 shows the frequency of consumption of these sources.

Table 3. Types of Phytic Acid Sources

| Phytic Acid Sources | Frequency (times/week) |           |
|---------------------|------------------------|-----------|
|                     | Mean $\pm$ SD          | Min - Max |
| Tempe               | 2,8 $\pm$ 3,6          | 0 - 21    |
| Tahu                | 3,4 $\pm$ 4,0          | 0 - 21    |

Based on Table 3, the phytic acid sources most frequently consumed by the subjects were tofu and tempeh. The average consumption of tofu was  $3.4 \pm 4.0$  times per week, while tempeh was consumed on average  $2,8 \pm 3,6$  times per week.

Table 4. Distribution of Subjects Based on Frequency of Phytic Acid Source Consumption

| Frequency of Phytic Acid Source Consumption | Frequency (n) | Percentage (%) |
|---|---------------|----------------|
| Infrequent                                  | 26            | 34,7           |
| Frequent                                    | 49            | 65,3           |
| Total                                       | 75            | 100,0          |
| Mean $\pm$ SD                               | 6,5 $\pm$ 6,7 |                |
| Min - Max                                   | 0 - 28        |                |

Table 4 shows that the majority of subjects (65,3%) consumed phytic acid sources frequently, an average of 6,5  $\pm$  6,7 times per week.

### 3.3. Menstrual Cycle and Duration

The menstrual variables studied in this research were the subjects' cycles and duration. A menstrual cycle was considered infrequent if it exceeded 35 days, normal between 25 and 31 days, and frequent if less than 21 days. Menstrual duration was considered abnormal if less than 2 days or more than 6 days, and normal if between 2 and 6 days. The distribution of subjects based on menstrual cycle and duration is presented in Table 5.

Table 5. Distribution of Subjects Based on Menstrual Cycle and Duration

| Menstrual Characteristics | Frequency (n) | Percentage (%) |
|---------------------------|---------------|----------------|
| Menstrual Cycle           |               |                |
| Infrequent                | 14            | 18,7           |
| Normal                    | 55            | 73,3           |
| Frequent                  | 6             | 8,0            |
| Mean ± SD                 | 31,0 ± 7,3    |                |
| Min – Max                 | 14 - 61       |                |
| Menstrual Duration        |               |                |
| Abnormal                  | 28            | 37,3           |
| Normal                    | 47            | 62,7           |
| Mean ± SD                 | 5,8 ± 1,3     |                |
| Min – Max                 | 3 - 10        |                |

Based on Table 5, the menstrual cycle and duration for most subjects were within the normal range. Most subjects (73,3%) had a normal menstrual cycle, with an average cycle length of 31,0  $\pm$  7,3 days. Similarly, most subjects (62,7%) had a normal menstrual duration, averaging 5,8  $\pm$  1,3 days.

### 3.4. Relationship Between Macronutrient Intake and Hemoglobin Levels

The bivariate analysis used to examine the relationship between macronutrient intake (energy, protein, fat, and carbohydrates) and hemoglobin levels was the Pearson correlation test. The results of this analysis can be seen in Table 6.

Table 6. Results of the Analysis of the Relationship Between Macronutrient Intake and Hemoglobin Levels

| Variable            | r     | p-value       |
|---------------------|-------|---------------|
| Energy Intake       | 0,157 | 0,178         |
| Protein Intake      | 0,251 | <b>0,030*</b> |
| Fat Intake          | 0,114 | 0,331         |
| Carbohydrate Intake | 0,093 | 0,428         |

\*significant at  $p < 0,05$

The statistical test results showed a significant relationship between protein intake and hemoglobin levels ( $p = 0,030$ ;  $r = 0,251$ ). This positive correlation indicates lower protein intake is associated with lower hemoglobin levels. This finding aligns with the studies by [Farinendya et al. \(2019\)](#) and [Junengsih & Yuliasari \(2017\)](#), which reported a higher prevalence of anemia among adolescent girls with insufficient protein intake than those with adequate intake.

The subjects' average protein adequacy level was below 80% of the Recommended Dietary Allowance (RDA) based on the 2 x 24-hour food recall, indicating inadequate intake. Protein deficiency in the subjects may be attributed to a diet predominantly consisting of plant-based protein sources such as tempeh and tofu, which contain non-heme iron. Non-heme iron has low absorption and bioavailability, making it insufficient to meet the body's daily iron requirements and potentially leading to anemia ([Arima et al., 2019](#)).

Physiologically, protein plays an essential role in forming hemoglobin and various components of red blood cells. Additionally, some iron in the body conjugates with protein to form transferrin, which functions to transport iron through the bloodstream. When protein intake is inadequate, hemoglobin production, iron transport, and red blood cell formation are disrupted, affecting red blood cell count and increasing anemia risk ([Sediaoetama, 2008](#)). Therefore, attention to protein intake is necessary to indicate anemia risk in adolescent girls, and nutrition education, particularly regarding adequate protein consumption, is essential for optimal hemoglobin and red blood cell production.

Conversely, the test results showed no significant relationship between energy, fat, and carbohydrate intake and hemoglobin levels ( $p > 0.05$ ). This may be due to the generally low intake of these macronutrients among most subjects. According to the 2 x 24-hour recall data, the leading causes were small portions and low meal frequency. Furthermore, not consuming vegetables as a source of fiber and complex carbohydrates also contributed to the imbalance in daily nutrient intake.

Other factors suspected to affect hemoglobin levels include low consumption of animal protein sources, high intake of iron absorption inhibitors, and menstrual disorders. According to [Berhe et al. \(2022\)](#) and [Putriwati et al. \(2024\)](#), these factors can increase anemia risk even when macronutrient intake is generally adequate. Additionally, [Fatie et al. \(2021\)](#) noted that bias in food recall data may occur due to subjects' dishonesty or inaccuracy when reporting consumed foods.

These findings highlight the importance of monitoring protein intake as a key indicator of anemia risk among adolescent girls. To effectively prevent anemia, appropriate nutritional interventions, including education on the importance of animal protein consumption and balanced diets, should be reinforced.

### 3.5. Relationship Between Consumption Habits of Phytic Acid Sources and Hemoglobin Levels

The bivariate analysis used to examine the relationship between consumption habits of phytic acid sources and hemoglobin levels employed the Spearman correlation test. The results of this analysis are shown in Table 7.

Table 7. Results of the Analysis of the Relationship Between Consumption Habits of Phytic Acid Sources and Hemoglobin Levels

| Variable            | r      | p-value |
|---------------------|--------|---------|
| Phytic Acid Sources | -0,260 | 0,024*  |

\*significant at  $p < 0,05$



The analysis showed a significant relationship between the consumption habits of phytic acid sources and hemoglobin levels ( $p = 0,024$ ;  $r = -0,260$ ). This negative correlation indicates that the more frequently subjects consume foods high in phytic acid, the lower their hemoglobin levels tend to be. According to the Food Frequency Questionnaire (FFQ), the average frequency of phytic acid source consumption among subjects was 6,5 times per week, indicating a relatively high consumption habit.

The most frequently consumed foods were tofu and tempeh, two processed products made from soybeans known as primary sources of phytic acid. This finding aligns with the study by Mokoginta et al. (2016), which reported that adolescents often consume tofu and tempeh more than once a day. Phytic acid is an antinutrient compound that binds essential minerals such as iron, zinc, and calcium in the digestive tract, inhibiting their absorption (Putriwati et al., 2024). According to Hallberg et al. (1989), as cited in Marina et al. (2015), phytic acid in food can inhibit iron absorption by up to 82%.

This study is also consistent with Marina et al. (2015), who found a significant relationship between the frequency of phytic acid consumption and low hemoglobin levels in adolescent girls. These findings strengthen the evidence that a high intake of phytic acid-rich foods may contribute to anemia risk, especially if not accompanied by iron absorption enhancers.

Adolescent girls should consume foods containing vitamin C and animal protein simultaneously to counteract the negative effects of phytic acid on iron absorption. Vitamin C is an enhancer for non-heme iron absorption, while animal protein contains heme iron, which has a higher absorption rate (Rahayu et al., 2019). Therefore, nutrition education emphasizing combining iron-rich foods with absorption enhancers is essential in anemia prevention strategies for adolescent girls.

### 3.6. Relationship Between Menstrual Cycle and Duration with Hemoglobin Levels

The bivariate analysis used to examine the relationship between menstruation and hemoglobin levels employed the Pearson correlation test for menstrual cycle and the Independent-Samples T-Test for menstrual duration. The results of this analysis can be seen in Table 8.

Table 8. Results of the Analysis of the Relationship Between Menstrual Cycle and Duration with Hemoglobin Levels

| Variable           | r     | p-value              |
|--------------------|-------|----------------------|
| Menstrual Cycle    | 0,374 | 0,001 <sup>*a)</sup> |
| Menstrual Duration |       | 0,015 <sup>*c)</sup> |

<sup>\*</sup>significant at  $p < 0,05$ ; a) Uji Pearson c) Uji Independent-Samples T-Test

A significant relationship was found between menstruation and hemoglobin levels ( $p = 0,001$ ). Among the subjects, 83% with menstrual cycles shorter than 28 days were recorded as anemic. This condition can be explained by the increased frequency of menstruation due to a shorter cycle, resulting in greater blood loss. Repeated and significant blood loss leads to depletion of the body's iron stores, mainly if not compensated by adequate iron intake and optimal absorption (Suhariyati et al., 2020).

Adolescent girls with normal menstrual cycles generally have sufficient iron stores to replenish losses during menstruation, reducing their risk of anemia (Shariff & Akbar, 2018). Menstrual cycles in adolescent girls are influenced by various factors, including nutritional status, macronutrient intake, stress levels, body mass index (BMI), hemoglobin levels, and physical activity (Fauziah, 2022). This finding aligns with the study by Alfianingsih & Purwito (2024), which also found a significant relationship between menstrual cycle and hemoglobin levels.

Menstrual duration also significantly correlated with hemoglobin levels ( $p = 0,015$ ). Among the subjects, 54% with menstrual durations longer than six days experienced anemia. The longer the menstrual duration, the greater the blood volume lost, leading to a decrease in hemoglobin levels. Normal menstrual duration helps maintain hemoglobin within a reasonable range because blood loss is controlled (Rahimah et al., 2025). Conversely, longer menstrual durations increase the risk of anemia due to greater blood loss (Mochamad, 2017 in Yunita et al., (2023).

The estimated amount of blood lost per menstrual cycle ranges from 20 to 25 cc, equivalent to an iron loss of 12,5 to 15 mg per month or about 04 to 0,5 mg per day. Combined with basal iron loss, total loss can reach 1,25 mg daily (Yulivantina & Dwihestie, 2016). This finding is supported by research from Arisani et al. (2024), which also demonstrated a significant relationship between menstrual duration and hemoglobin levels.

However, this result differs from the study by Yunita et al. (2023), which stated that the length of menstrual duration does not necessarily reflect the volume of blood lost. Sometimes, blood volume may be small despite a long duration, or vice versa. Therefore, anemia risk interpretation should not be based solely on menstrual duration but also consider blood volume lost and other factors such as nutritional intake and the general health of the adolescent.

These findings emphasize the importance of regularly monitoring menstrual cycle and duration as indicators of anemia risk in adolescent girls. Nutrition education that includes iron requirements and routine iron supplementation is strongly recommended. Supplementation is crucial for adolescents with abnormal menstrual patterns, as the body often cannot replenish lost iron through daily intake alone, especially if iron absorption is impaired (Brien, 2018; Pibriyanti et al., 2021).

#### 4. CONCLUSION

This study revealed that most subjects had inadequate macronutrient intake, frequent consumption habits of iron absorption inhibitors, normal menstrual cycle and duration. Statistical analysis showed significant relationships between protein intake, consumption habits of phytic acid sources, menstrual cycle and duration with hemoglobin levels. Meanwhile, no significant associations were found between other factors such as energy intake, fat intake, and carbohydrate intake with hemoglobin levels. Interventions focusing on improving protein intake, reducing phytic acid consumption, and managing menstrual irregularities are essential to reduce anemia risk among adolescent girls. Education on proper dietary patterns and management of menstrual cycle and duration can reduce anemia risk and improve the health quality of adolescent girls.

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