

## Association of nutritional status, protein and iron intake, and physical activity with hemoglobin levels in adolescent girls: a case-control study in Rural East Java, Indonesia

Kartika Pibriyanti<sup>1\*</sup>, Dono Indarto<sup>2</sup>, Desy Auliya Qudsi<sup>1</sup>, Lulu' Luthfiya<sup>1</sup>,  
Qotrunnadaa Fajr Rooiqoh<sup>1</sup>, Indahtul Mufidah<sup>1</sup>, Nur Amala<sup>1</sup>, Ladyamayu Pinasti<sup>1</sup>,  
Ivena Claresta<sup>1</sup>

<sup>1</sup>Department of Nutrition Sciences, Faculty of Health Science, Universitas Darussalam Gontor, Ponorogo, East Java, Indonesia.

<sup>2</sup>Department of Physiology and Biomedical Laboratory, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia.

**Corresponding Author:** Kartika Pibriyanti **Email:** [dkartika.02@unida.gontor.ac.id](mailto:dkartika.02@unida.gontor.ac.id)

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### ABSTRACT

Anemia among adolescent girls is a significant public health concern, negatively affecting physical development, cognitive function, and quality of life. This study aims to analyze the impact of nutritional status, dietary intake, and physical activity on hemoglobin levels. A case-control study design was employed on 116 adolescent girls aged 12-18 years. Data were collected through structured interviews. Statistical tests were used to determine the association between nutritional status, protein and iron intake, and physical activity with hemoglobin levels. Adolescent girls with good nutritional status had a significantly lower risk of anemia compared to those with poor nutritional status ( $p < 0.05$ ). Low protein intake, both in quantity and frequency, increased the risk of anemia nearly sixfold (OR = 5.884, 95% CI = 2.634–13.146;  $p < 0.05$ ). This finding underscores the critical role of sufficient protein intake in supporting hemoglobin synthesis and preventing anemia. Adequate consumption of iron-rich foods was significantly associated with higher hemoglobin levels. Regular physical activity was also found to have a protective effect, with physically active adolescent girls demonstrating better hemoglobin levels than their less active counterparts. This suggests that an active lifestyle not only improves circulation and red blood cell production but also helps maintain optimal hemoglobin levels. In conclusion, good nutritional status, sufficient protein and iron intake, and regular physical activity are key factors in preventing anemia among adolescent girls. These findings emphasize the importance of holistic nutritional interventions, including improved dietary quality and promotion of physical activity, to reduce anemia prevalence.

### Keywords:

anemia; nutritional status; protein intake; iron intake; physical activity; adolescent girls

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## INTRODUCTION

Anemia is a significant public health issue worldwide, characterized by low hemoglobin levels in the blood, leading to insufficient oxygen supply for optimal body function. The World Health Organization (WHO) estimates that around 30% of the global population suffers from anemia, with a significant number of cases occurring among adolescents. In low- and middle-income countries (LMICs), anemia prevalence can reach as high as 62.95%, underscoring the urgent need for targeted interventions.<sup>1</sup> In Indonesia, the prevalence of anemia among adolescent girls is concerning, with factors such as dietary habits, nutritional status, and physical activity playing critical roles.<sup>2,3</sup> Among adolescent girls, anemia is particularly concerning due to increased iron demands during rapid growth and menstruation.<sup>4</sup> Diets in Indonesia often rely heavily on staple foods such as rice, which are low in bioavailable iron, while the consumption of iron-rich foods like red meat, green leafy vegetables, and fortified foods remains inadequate.<sup>5</sup> Additionally, fast food and nutrient-poor snacks are widely consumed by adolescents, contributing to an unbalanced diet and insufficient iron intake. This pattern is compounded by cultural and socioeconomic factors, such as limited access to diverse foods and a lack of awareness about proper nutrition.<sup>6</sup> These conditions disproportionately affect rural and urban populations differently; for instance, rural adolescent girls may experience high levels of physical labor with inadequate dietary compensation, while urban counterparts often face sedentary lifestyles coupled with poor diet quality. Many adolescent girls in rural areas engage in strenuous physical labor or high levels of activity without adequate dietary compensation, increasing the risk of iron deficiency. Conversely, urban adolescents may adopt more sedentary lifestyles, which

are often associated with poor diet quality and increased reliance on fast foods, further exacerbating micronutrient deficiencies.<sup>7,8</sup> Dietary habits significantly impact nutritional status and hemoglobin production. Adolescent girls with poor diets, low in iron and essential micronutrients, are at greater risk of anemia. Diets lacking in meat, leafy greens, and fruits, while high in refined carbs and fast food, often fail to provide adequate iron in an easily absorbable form. Unhealthy eating behaviors, such as skipping meals or consuming nutrient-poor snacks, further contribute to this risk. Excessive physical activity without sufficient nutrition can also lower hemoglobin levels, particularly in active girls who may lose more iron. Conversely, a sedentary lifestyle is linked to poor diet quality and increased risk of anemia due to micronutrient deficiencies.<sup>9</sup>

Previous studies have identified significant links between nutritional status, diet, and physical activity with hemoglobin levels in adolescent girls.<sup>10</sup> However, research focusing specifically on the Indonesian context remains limited. Research specific to Indonesia highlights stark disparities in anemia prevalence between rural and urban regions, with urban areas like Jakarta reporting anemia prevalence associated with poor dietary diversity and socioeconomic challenges. These studies often focus on specific populations and do not fully explore the interactions between these factors. Household education and income levels also influence dietary choices, access to health services, and awareness of anemia prevention strategies. A relationship between diet, body composition, and health, while higher physical activity levels correlate with better protein intake and normal body mass.<sup>11,12</sup> A systematic review linked dietary diversity to reduced malnutrition risk, which affects hemoglobin levels.<sup>13</sup> Fast-food consumption, prevalent among teenagers, is a risk factor for anemia.<sup>14</sup>

In the Indonesian context, the prevalence of anemia in adolescent girls is still quite high, especially in areas with limited access to nutritious food and health services. Results from previous studies found that anemia among adolescent girls in rural areas of West Java was influenced by inadequate nutritional intake, low awareness of anemia prevention, and low levels of physical activity.<sup>2,15</sup> Another study showed that the prevalence of anemia in children and adolescents in poor urban areas was 14% in Jakarta, with a higher prevalence in women.<sup>16</sup> The prevalence of anemia among young women (15-24 years) in low- and middle-income countries is very high.<sup>1</sup> Understanding the specific dietary and activity patterns of Indonesian adolescent girls is critical for addressing anemia in this population. Poor nutritional intake, coupled with either excessive physical activity or sedentary behaviors, can significantly influence hemoglobin levels. These factors underscore the urgency of investigating the interrelationships between nutritional status, diet, and physical activity within the Indonesian context.

Therefore, the purpose of this study is to determine and analyze the relationship between nutritional status, diet, and physical activity with hemoglobin levels in adolescent girls. This study uses a case-control study design, where adolescent girls with normal hemoglobin levels will be compared with adolescent girls who experience anemia. By analyzing the relationships between nutritional status, dietary intake, and physical activity, this study provides a comprehensive understanding of factors contributing to anemia in this population and supports the design of effective interventions that can be carried out to prevent and overcome anemia in adolescent girls in Indonesia.

## MATERIALS AND METODS

### *Study design*

This observational case-control study aims to identify factors associated with anemia in adolescents aged 16-18 years at a high school in Ngawi, East Java, Indonesia, conducted from December 2023 to March 2024. The design compares a case group with a control group to analyze the prevalence of anemia and its contributing factors. Cross-sectional analysis is employed to assess relationships between variables simultaneously, making it effective for this epidemiological study.

### *Participant approach and recruitment*

Participants were initially approached through collaboration with school authorities and homeroom teachers. Information about the study was disseminated through in-person meetings and by distributing information sheets to students and parents. These sessions aimed to explain the study's objectives, procedures, potential risks, and benefits, ensuring that participants fully understood their involvement before consenting.

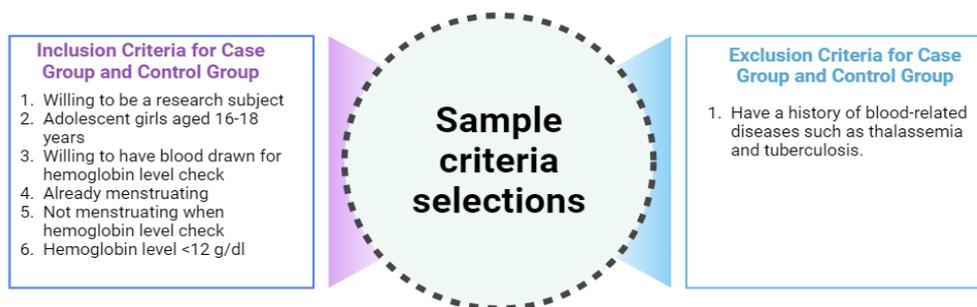
### *Population and sample*

The study population comprised 261 female adolescents from SMA Negeri 1 Kendal, Ngawi, East Java, Indonesia. A purposive non-probability sampling technique was used to select 104 respondents, with 52 in the case group (anemia) and 52 in the control group (non-anemia). The sample size was calculated using the Lemeshow formula, with  $Z_{\alpha} = 1.645$  for a 90% significance level and  $Z_{\beta} = 1.282$  for a 90% test power. To account for potential dropouts, the sample size was increased by 10%, resulting in 116 respondents, with 58 in each group.

$$n_1 = n_2 = \left( \frac{z_{\alpha}\sqrt{2.PQ} + z_{\beta}\sqrt{p_1Q_1 + p_2Q_2}}{p_1 - p_2} \right)^2 \quad (1)$$

Purposive non-probability sampling, while suitable for the context of this study, may introduce selection bias because it does not guarantee that the sample is representative of the broader adolescent population. This limitation should be acknowledged when interpreting the study's findings. The inclusion and exclusion criteria ensured data accuracy and relevance. Inclusion criteria required participants to meet certain characteristics, such as being within a specific age range to ensure uniformity in physical and hormonal development and having a hemoglobin level  $<12$  g/dl, as defined by WHO for anemia in non-pregnant women. The menstrual cycle was

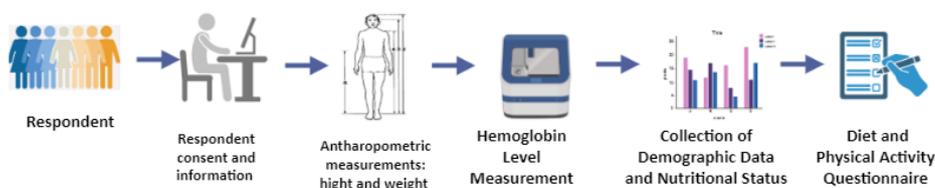
also considered an inclusion factor due to its potential impact on hemoglobin levels. Exclusion criteria included a history of blood-related diseases like Thalassemia and Tuberculosis, which can significantly affect hemoglobin levels through mechanisms such as reduced red blood cell production, increased destruction, or chronic inflammation. These conditions were determined through self-reported medical history and verified by school health records. In detail, the inclusion and exclusion criteria used for selecting samples in this study can be seen in Figure 1.



**Figure 1.** Inclusion and exclusion criteria in sample selection.

### **Data collection**

This study utilized various techniques and instruments to ensure the accuracy and completeness of the data. The data collection flow can be seen in Figure 2.



**Figure 2.** Data collection flow

The details of the data collection techniques used are as follows:

#### **Respondent Consent and Information**

Before data collection, participants signed an Informed Consent Sheet after being provided with detailed information about the study's objectives, procedures, benefits, and risks. Consent for participants

under 18 years of age was obtained from parents or guardians after a thorough explanation from the research team. Participant consent was given by parents or homeroom teachers after receiving an explanation from the research team regarding the consideration of the

advantages and disadvantages of participating in the research.

### ***Nutritional Status consists of height and weight***

Respondents' height was measured using a Stadiometer with the Gea Medical brand. Measurements were taken by ensuring that respondents stood upright without shoes, with their backs straight, and their heels touching the measuring wall. Measurement results were recorded in centimeters. Meanwhile, respondents' weight was measured using a Gea Medical digital scale. Measurements were taken in the morning with respondents wearing light clothing and without shoes. The results were recorded in kilograms. These weight and height measurements were used to calculate the Body Mass Index (BMI), which was then categorized according to WHO standards to determine the nutritional status of respondents.

### ***Hemoglobin Level***

Hemoglobin levels were measured using the EasyTouch Hemoglobin Meter with capillary blood samples. The EasyTouch Hemoglobin Meter has been validated in similar populations, demonstrating accuracy and reliability for field studies. Levels below 12 g/dL were

classified as anemia per WHO guidelines for non-pregnant women.

### ***Dietary patterns***

Dietary patterns were assessed using the Semi-Quantitative Food Frequency Questionnaire (SQ-FFQ), focusing on protein and iron intake. Protein sources were classified into animal-based (e.g., meat, eggs, fish) and plant-based (e.g., legumes, tofu, tempeh). Iron sources were categorized as heme iron (from meat and fish) and non-heme iron (from plant-based foods). Consumption frequency was grouped as low (<2 times per week), moderate (2–4 times per week), or high (≥5 times per week). Demographic and nutritional data, including BMI, were collected using a Data Collection Sheet during interviews.

### ***Physical activity***

Physical activity was measured using the Global Physical Activity Questionnaire (GPAQ), which evaluates activity levels based on the duration and frequency of daily activities. The GPAQ includes 16 questions covering work, recreational activities, and commuting, with answers calculated using the Metabolic Equivalent of Task (MET). The calculation of MET can be seen in Equation 2.

$$MET = [P2.P3.8) + (P5.P6.4) + (P8.P9.4) + (P11.P12.8) + (P14.P15.4)] \quad (2)$$

The MET results are presented in predetermined scale units and categorized to determine whether the respondent's physical activity is low, moderate, or high. Activities were categorized as follows:

- Light: <600 MET-min/week
- Moderate: 600–2999 MET-min/week
- Heavy: ≥3000 MET-min/week

The classification aimed to provide a nuanced understanding of physical activity's impact on hemoglobin levels.

### ***Statistical Analysis***

Data analysis employed univariate, bivariate, and multivariate techniques to explore relationships between nutritional status, dietary intake, physical activity, and hemoglobin levels. Univariate analysis described the characteristics of each

variable (e.g., mean BMI, MET values), while bivariate analysis assessed the relationships between pairs of variables, such as nutritional status and hemoglobin levels. Multivariate analysis was then used to examine multiple variables simultaneously, identifying the significant factors influencing hemoglobin levels after accounting for the effects of other variables. Data analysis was conducted using IBM SPSS Statistics for Windows version 16.0.

## RESULTS

### *General profile of respondents*

Anemia is a common issue among adolescents, negatively impacting their health and quality of life. This study analyzed factors related to anemia in female adolescents, including age, extracurricular activities, age of menstruation onset, nutritional status, protein and iron intake, and physical activity. As shown in Table 1, these factors significantly influence anemia incidence in adolescent girls.

**Table 1.** Respondent characteristics

No	Variables	Anemia		Non-Anemia	
		Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)
1.	Teenage Girls Age				
	16 Years	30	51.7	24	41.4
	17 Years	18	31.0	24	41.4
	18 Years	10	17.2	10	17.2
2.	Age of onset of menstruation				
	5 Elementary School	7	12.1	6	10.3
	6 Elementary School	26	44.8	22	37.9
	1 Junior High School	13	22.4	18	31.0
	2 Junior High School	12	20.7	12	20.7
3.	Extracurricular Activities				
	Scouts	24	41.4	24	41.4
	PMR	11	19.0	12	20.7
	Volleyball	8	13.8	13	22.4
	Basketball	5	8.6	1	1.7
	Running	6	10.3	8	13.8
	Band	4	6.9	0	0
4.	Nutritional Status				
	Abnormal	41	70.7	28	48.3
	Normal	17	29.3	30	51.7
5.	Physical Activities				
	heavy activity	40	69.0	25	43.1
	light activity	18	31.0	33	56.9
6.	Amount of Food Sources of Protein				
	not good	43	74.1	19	32.8
	good	15	25.9	39	67.2

No	Variables	Anemia		Non-Anemia	
		Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)
7.	Amount of Food Sources of Iron	44	75.9	32	55.2
	not enough	14	24.1	26	44.8
	enough				

**The impact of nutritional status and amount of protein on hemoglobin levels**

The impact of nutritional status and amount of protein on hemoglobin levels in adolescent girls highlight key risk factors for anemia. As shown in Table 2, of the 116 girls studied, 69 (59.5%) had abnormal nutritional status, with 41 (70.7%) of them suffering from anemia. Conversely, among the 47 girls (40.5%) with normal nutritional status, only 17 (29.3%) had anemia. The chi-square analysis revealed a significant association between nutritional status and hemoglobin levels ( $p = 0.002$ ). The Odds Ratio (OR) of 2.584 indicates that girls with abnormal nutritional status are 2.5 times more likely to experience anemia compared

to those with normal nutritional status. This study assessed the impact of protein intake on hemoglobin levels in adolescent girls. Among the 62 girls (53.4%) with insufficient protein intake, 43 (74.1%) had anemia, compared to only 15 (25.9%) of the 54 girls (46.6%) with sufficient protein intake. Chi-square analysis revealed a significant relationship between protein intake and hemoglobin levels ( $p = 0.001$ ). The Odds Ratio (OR) of 5.884 indicates that girls with insufficient protein intake are nearly six times more likely to develop anemia compared to those with adequate protein intake. The 95% confidence interval for the OR ranges from 2.634 to 13.146.

**Table 2.** The impact of nutritional status and amount of protein on hemoglobin levels

nutritional status										
Nutritional status	Anemia		Non-Anemia		Total		OR	95% CI		p-value
	n	%	n	%	n	%		Min	Max	
Abnormal	41	70.7	28	48.3	69	59.5	2.584	1.203	5.552	0.023
Normal	17	29.3	30	51.7	47	40.5				
Total	58	100	58	100	116	100				
Amount of Protein										
Amount of Protein Food Intake	Anemia		Non-Anemia		Total		OR	95% CI		p-value
	n	%	n	%	n	%		Min	Max	
Not Good	43	74.1	19	32.8	62	53.4	5.884	2.634	13.146	0.001
Good	15	25.9	39	67.2	54	46.6				
Total	58	100	58	100	116	100				

**The impact of the amount of iron and physical activity on hemoglobin**

Table 3 highlights the impact of the amount of Iron and Physical Activity on

Hemoglobin Levels in adolescent girls. The data show that 75.9% of girls with insufficient iron intake had anemia, compared to 24.1% with adequate intake. In

the non-anemic group, 44.8% had good iron intake, while 55.2% had insufficient intake. The Odds Ratio of 2.554 (95% CI: 1.874 - 9.185,  $p = 0.032$ ) confirms this significant relationship. These findings emphasize the importance of adequate iron intake in preventing anemia, as iron is crucial for hemoglobin production, directly impacting cognitive function, productivity, and overall health in adolescents. Physical activity significantly impacts hemoglobin levels in adolescent girls. Hemoglobin, crucial for oxygen transport, is vital for

health and physical performance, that can be seen in Table 5. The study found that 69% of girls engaged in heavy physical activity experienced anemia, while 56.9% of those with light activity did not. The statistical test revealed a significant relationship between heavy physical activity and anemia ( $p = 0.009$ ). With an Odds Ratio of 2.933, girls involved in heavy activity are 2.9 times more likely to develop anemia than those with light activity, as confirmed by a 95% confidence interval (1.279 - 5.847).

**Table 3.** The impact of the Amount of Iron and Physical Activity on Hemoglobin Levels

The Amount of Iron										
Amount of Iron Food Intake	Anemia		Non-Anemia		Total		OR	95% CI		<i>p-value</i>
	n	%	n	%	n	%		Min	Max	
Not Good	44	75.9	32	55.2	76	65.5	2.554	1.874	9.185	0.032
Good	14	24.1	26	44.8	40	34.5				
Total	58	100	58	100	116	100				
Physical Activity										
Physical Activity	Anemia		Non-Anemia		Total		OR	95% CI		<i>p-value</i>
	n	%	n	%	n	%		Min	Max	
Heavy	40	69.0	25	43.1	65	56.0	2.933	1.279	5.847	0.009
Light	18	31.0	33	56.9	51	44.0				
Total	58	100	58	100	116	100				

### Multivariate analysis

The multivariate analysis showed that the model explained 39.2% of the variability in outcomes ( $R\text{-Square} = 0.392$ ). Physical activity had a significant positive effect on outcomes ( $B = 1.077$ ,  $p = 0.026$ ,  $OR = 2.935$ ), indicating that increased physical activity was strongly associated with improved outcomes. Other variables, such as nutritional status, protein intake,

and iron intake, showed no significant effect ( $p > 0.05$ ). The significant negative constant ( $B = -2.700$ ,  $p = 0.001$ ) suggests outcomes decrease significantly without the influence of the independent variables. Physical activity appears to be the dominant factor, while other variables may require further investigation. The complete multivariate analysis can be seen in Table 4.

**Table 4.** Multivariate analysis

Variable	B	Wald	Sig	Exp (B)	95% CI For EXP (B)		R Square
					Min	Max	
Nutritional Status	0.413	0.757	0.384	1.511	0.596	3.831	0.392
Amount of protein	0.369	0.329	0.567	1.446	0.410	5.099	
Amount of iron	0.538	0.724	0.395	1.712	0.496	5.914	
Physical Activity	1.077	4.973	0.026	2.935	1.139	7.559	
<b>Constant</b>	<b>-2.700</b>	<b>18.013</b>	<b>0.000</b>	<b>0.067</b>			

## DISCUSSION

Based on Table 1, this study found that most anemic respondents were 16 years old (51.7%), which was also the largest age group among non-anemic respondents (41.4%). This suggests that age 16 is critical for anemia risk, likely due to increased nutritional needs during rapid growth. Adolescent girls aged 15-19 are at high risk of anemia due to increased iron demands during growth and menstruation.<sup>17</sup> Many respondents participated in extracurricular activities like scouting, which could influence their lifestyle and nutritional needs.<sup>18</sup> Most respondents began menstruating in grade 6, with early menstruation increasing iron needs and anemia risk if dietary intake is insufficient.<sup>19</sup> Additionally, more non-anemic respondents had normal nutritional status (51.7%), while the anemic group was largely malnourished (70.7%), highlighting the importance of good nutrition in preventing anemia.<sup>20</sup>

### *Nutritional Status*

Table 2 shows the impact of nutritional status and amount of protein intake on hemoglobin levels in adolescent girls. This study found that adolescent girls with abnormal nutritional status were more than twice as likely to experience anemia compared to those with normal nutritional status (OR = 2.584,  $p = 0.023$ ). Iron deficiency and inadequate nutritional intake

contribute to low hemoglobin levels and an increased risk of anemia in adolescents.<sup>15</sup> Poor nutritional status, including micronutrient deficiencies, is directly related to the prevalence of anemia.<sup>21,22</sup> This finding is consistent with the literature stating that poor nutrition can affect red blood cell production and hemoglobin levels in the body.<sup>23</sup> Poor nutrition can affect the production of red blood cells and reduce hemoglobin levels in the body. Based on statistical analysis, the  $p$ -value of 0.023 indicates that the relationship between nutritional status and hemoglobin levels did not occur by chance but was statistically significant. The importance of interventions that focus on improving nutritional status to reduce the prevalence of anemia among adolescents is widely recognized. Therefore, addressing the nutritional needs of adolescent girls through interventions is crucial, as they are vulnerable to anemia. These results indicate the importance of monitoring nutritional status as part of efforts to prevent anemia in adolescent girls, as well as the need for nutritional intervention programs that can increase nutrient intake to support hemoglobin production.<sup>24</sup>

### *Protein Intake*

Low protein intake significantly increased the risk of anemia (OR = 5.884,  $p < 0.05$ ), highlighting its essential role in hemoglobin synthesis. Protein deficiencies impair the production of amino acids

necessary for hemoglobin formation, increasing anemia risk. Adequate protein consumption supports hemoglobin production, which is essential for oxygen transport in the body, and helps maintain healthy hemoglobin levels. Protein deficiencies can lead to inadequate amino acids required for hemoglobin synthesis, disrupting its production and elevating anemia risk. A lack of protein can impair hemoglobin synthesis, increasing the risk of anemia. These findings align with research indicating that sufficient protein intake is crucial for maintaining normal hemoglobin levels in women of childbearing age and preventing blood-related disorders.<sup>25</sup> Insufficient protein intake can lead to deficiencies of essential amino acids needed for hemoglobin synthesis, which can ultimately disrupt hemoglobin production and increase the risk of anemia.

### ***Iron Intake***

Table 3 emphasizes the importance of adequate iron intake during adolescence, a period of rapid growth and increased nutrient needs, particularly iron. Regular menstruation in adolescent girls also contributes to blood and iron loss, increasing the risk of anemia. Iron deficiency anemia is a widespread health issue, especially among adolescent girls.<sup>26</sup> Supplementation and education on iron-rich foods have been shown to improve hemoglobin levels and reduce anemia prevalence.<sup>27</sup> Enhancing iron absorption, especially from non-heme sources, through vitamin C intake, is crucial for preventing anemia. Table 3 highlights that excessive heavy physical activity, without adequate nutrition, can lead to a decrease in hemoglobin levels, increasing the risk of anemia. Intense exercise raises oxygen and energy demands, leading to red blood cell breakdown (hemolysis) and higher iron requirements.<sup>28,29</sup> Anemia resulting from heavy physical activity often stems from an imbalance between the body's needs and nutrient intake, especially iron. In contrast,

light to moderate physical activity helps maintain stable hemoglobin levels by stimulating red blood cell production and improving circulation.<sup>30</sup> Adequate nutritional intake, particularly iron, folate, and vitamin B12, is crucial for adolescent girls engaged in heavy physical activity to support optimal hemoglobin production. Moderate physical activity plays a role in maintaining the balance between the production and breakdown of red blood cells, as well as improving blood circulation.

### ***Physical activity***

Physical activity was the only variable with a statistically significant impact on hemoglobin levels (OR = 2.935). Regular physical activity has a protective effect, likely by improving circulation and red blood cell production. However, excessive heavy activity without adequate nutrition increased anemia risk due to heightened iron demands and red blood cell breakdown (hemolysis). These findings align with studies demonstrating the balance between physical activity and nutritional intake in maintaining hemoglobin levels.<sup>31</sup> Other variables, like protein and iron intake, were not statistically significant, suggesting that factors beyond nutrient intake, such as iron absorption influenced by its chemical form and vitamin C intake, may play a larger role,<sup>32</sup> suggest that sampling variability and unmeasured factors may obscure true effects. Further research with more detailed measurements is needed to clarify protein's role in anemia.

For public health implications, these findings have direct implications for public health interventions in Indonesia. The high prevalence of anemia among adolescent girls underscores the need for targeted nutritional programs in schools, focusing on iron and protein intake through dietary diversity and supplementation. Educational campaigns promoting the consumption of locally available iron-rich

foods, such as tempeh, fish, and leafy greens, combined with vitamin C sources to enhance iron absorption, are crucial. Incorporating moderate physical activities into school curriculums can help maintain stable hemoglobin levels while reducing the risk of anemia. Collaborating with local governments to provide cost-effective supplementation programs and improving access to fortified foods can significantly reduce anemia prevalence in adolescent girls.

In addition, this study has several limitations. First, the use of purposive non-probability sampling may introduce selection bias, limiting the generalizability of findings to the broader adolescent population. The sample was drawn from a single school in East Java, which may not represent adolescents from other regions with differing dietary habits and socioeconomic conditions. Second, this study did not comprehensively assess participants' socioeconomic status, which may influence dietary intake, access to healthcare, and physical activity levels. Third, while this study focused on dietary protein and iron intake, it did not evaluate factors like bioavailability or concurrent intake of inhibitors (e.g., phytates) that may affect nutrient absorption.

## RECOMMENDATIONS

Based on the findings of this study, several recommendations can be made to address anemia in adolescent girls. First, there is a need to intensify nutrition education among adolescents through school and community programs that emphasize the importance of iron and protein intake to prevent anemia. These programs can be integrated into health-related curricula or extracurricular activities. Second, the government should prioritize the distribution of iron supplements to high-risk groups and

promote the fortification of staple foods such as rice and flour with iron and other essential micronutrients. This should be a priority for government health programs to achieve wider coverage. Third, promoting balanced physical activity should be encouraged so that adolescents remain active without overexertion, as intense physical activity can increase the risk of anemia. Physical activity programs that facilitate light to moderate exercises can support stable hemoglobin levels.

Furthermore, regular health monitoring of adolescent girls in schools is essential for the early detection of anemia risks. Hemoglobin and nutritional status checks should be conducted routinely, especially for adolescents experiencing rapid growth or engaging in strenuous physical activities. Additionally, the development of an integrated nutrition program involving the government, schools, and healthcare providers should be implemented. This program includes education, health monitoring, and adjustments to school meal menus to ensure adolescents receive adequate nutrition. Finally, campaigns on menstrual health are also important, as menstruation increases iron requirements in adolescent girls. These campaigns can help raise awareness among adolescents about the importance of iron intake during menstruation to prevent anemia.

## CONCLUSION

This study shows that there is a significant relationship between nutritional status, diet, and physical activity with hemoglobin levels in adolescent girls. Adolescents with good nutritional status tend to have higher hemoglobin levels, while a diet rich in protein and iron, both in terms of quantity, type, and frequency, also contributes positively to increasing hemoglobin levels. Additionally, regular

physical activity helps maintain the balance of body metabolism and supports optimal nutrient absorption, which ultimately affects hemoglobin levels. The results of this study emphasize the importance of maintaining a balanced diet and regular physical activity as an effort to prevent anemia in adolescent girls, as well as the need for more intensive education regarding the importance of proper nutritional intake and a healthy lifestyle.

## **AUTHOR CONTRIBUTIONS**

KP: Project Administration, Conceptualization, and Methodology, DI: Supervisor, and Validation. DAQ, led data collection; LT, led the data analysis and interpretation, and visualization,; QFR, data analysis and interpretation, assisted in the drafting of the manuscript; IM and NA, led the investigation and managed resources; LP and IC, led data collection, investigation, and original draft preparation.

## **ETHICAL CONSIDERATION**

This research was registered with the Research Ethics Committee of the Faculty of Medicine, Sebelas Maret University, under approval number 36/UN27.06.11/KEP/EC/2024 which was approved on 12 February 2024. All procedures involving participants were reviewed and approved. Informed consent was obtained from the participants' parents or guardians, who were fully informed of the potential benefits and risks of participation prior to providing consent.

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## **CONFLICT OF INTEREST**

The author(s) stated that there are no potential conflicts of interest regarding the research, authorship, or publication of this article.

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