

Is Urinary Pyridinium Crosslinks Associated with Stunting in Stunting Children in Indonesia

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Is Urinary Pyridinium Crosslinks Associated with Stunting in Stunting Children in Indonesia

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Abstract

The objective of this study was to analyze the correlation between pyridinium crosslinks (Pyd) urine and stunting among children. We also determined the effect of nutritional intervention on the Pyd content in urine among stunting children. The study was a cross-sectional involving 173 children in Pekanbaru and Kabupaten Lima Puluh Kota, Indonesia in 2014 (children aged 0-3 days: n = 32), in 2017 (children aged 4-6 years: n = 80), in 2018 (children 4-6 years old: n = 25), and in 2020 (children 12-15 years old: n = 36). Height gauges, family socio-economic questionnaires, pot urine and Pyd kit were utilized to gather the data. As nutritional interventions, milk was given to children aged 4-6 years old for 4 months (as additional energy; 20% of the recommended dietary allowance); brunch meals and milk were given to children 12-15 years old for 34 days (as additional energy; 30% recommended dietary allowance). Pyd and height were used as parameter indicators in this study. Pearson correlation and t-test (significance $p < 0.05$ and $p < 0.01$) were applied for statistical analysis. The Pyd content of stunted children aged in 0-3 days, 3-5 years, 4-6 years, and 12-15 years were discovered to be 982, 16.4, 16.9 and 9.6 nmol/mmol creatinine, respectively. The Pyd content of stunted children aged 4-6 and 12-15 years before and after nutritional intervention were 16.9 vs 15.3 and 9.81 vs 5.33 nmol/mmol creatinine, respectively. Stunting neonatal urine Pyd content was found to be different from normal neonatal urine Pyd content ($p < 0.01$). There was a correlation revealed between urine Pyd content and height of children aged 4-6 years ($p < 0.05$) and $r = -0.242$. A difference was observed in the urine Pyd content of children 4-6 years before nutrition intervention ($p < 0.01$) as well as in urine Pyd content of children aged 12-15 years before and after nutritional intervention ($p < 0.05$), as many as 19.4% of the subjects increased their nutritional status from stunting to normal. Urine Pyd is expected to be a marker of the efficacy of nutritional care in bone growth disorders associated with bone resorption in stunting children.



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Introduction

Stunting is one of the major health problems in Indonesia and even in the world. The prevalence of stunting among children under five years of age in Indonesia are 36.8% in 2007,¹ 35.6% in 2010,² 37.2% in 2013³ and 30.87% in 2018.⁴ A review study in 36 countries found that the prevalence of stunting in children under one year was 40% and the prevalence of stunting for children under two years reached 54%.⁵

About 59.3% of children aged 3-5 years were included stunting.⁶ The prevalence of global stunting of children aged 13-15 years is around 35.1%.⁷ The results of Indonesia's basic health research in 2010 show that the prevalence of stunting in children aged 13-15 years is 35.2%, a prevalence is 36.6% in the Riau Province.⁸ Public health problems are considered severe if the prevalence of stunting is 30-39% and serious if the prevalence of stunting is $\geq 40\%$.⁷ World Health Organization (WHO) established stunting standards based on anthropometrics measurement with Height for Age (HAZ)-score < -2 SD.⁹

Stunting is associated with impaired linear growth processes.¹⁰ The linear growth retardation process begins at 2 or 3 months of age.¹¹ Growth retardation reflects a process of failure to achieve linear growth potential as a result of suboptimal health and/or nutritional conditions.⁷ One of the linear growths can be measured from bone growth. Bone growth increases with increasing height. Bone growth occurs when bone formation is greater than bone absorption. Pyridinium crosslinks are markers of bone resorption.¹² Urinary pyridinium crosslinks are removed during the release of mature collagen in bone. Pyridinium is constructed as an intramolecular crosslinker during collagen maturation.¹³

The biochemical markers of bone resorption can be analyzed clinically using conditions and treatments that affect bone metabolism. This bone formation marker is derived from type I collagen. About 90% of the bone organic matrix is made of collagen type I which is a helical protein, stabilized by cross-linking between the N terminal and C terminal in the formation of the base of bone tissue. The pyridin crosslinks (Pyd) are formed by hydroxyllysine or lysine residues at the C- and

N-telopeptide terminals of the collagen molecule and released during matrix resorption, excreted in the urine. Pyd appears in urine that is characterized by peptide formation. There are several studies reported that the number of free crosslinks excreted in the urine is related to the rate of bone formation.¹⁴

The absorption takes around 7-10 days, whereas the formation takes 2-3 months. Overall, 10% of bone is replaced each year. The process of bone metabolism occurs in pairs (bone formation is related to bone resorption) in a balanced manner which indicates that the amount of bone removed will be completely replaced.¹² There are two types of cells responsible for bone metabolism, namely osteoblasts and osteoclasts.¹⁵ The function of osteoblast is influenced by calcium intake, which can cause low mineralization of the new bone deposit matrix, severe calcium deficiency in childhood can lead to stunting.¹⁶ Calcium forms complex bonds with phosphate which can provide strength to bones.¹⁷

Until now, there is no convincing stunting indicator reported in the literature. Anthropometric measurements of length or height to determine stunting have been inconclusive for many reasons. There are still many opportunities for errors in the measuring instruments used and the ability of the enumerator to measure whose value can vary with other enumerators.¹⁸

Radiological indicators are being debated to be used to measure children's bone density as biomarkers for their linear growth. Radiological results from the hospital can be used for medicinal purposes recommended by a doctor, but if only for research purposes it will not be permitted by the hospital. Biochemical indicators using blood are unethically carried out on children without any medical reason because they are invasive (painful).

Based on the aforementioned arguments, it is essential to study a convincing and noninvasive biomarker to determine stunting in children using urine. The aim of this study was to assess the correlation between urine Pyd levels, height and the effect of nutritional interventions on the stunting status of children aged 0-3 days, 4-6 years, and 12-15 years, respectively.

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Methods

Study Design

This cross-sectional study was carried in 2014, 2017, 2018 and 2020 and conducted in two Provinces namely Riau (Pekanbaru City) and West Sumatera (Lima Puluh Kota District) Province. The two research sites have the same characteristics which are located side by side on the same island in Indonesia, namely Sumatra Island. Subjects are different people in each year of the study.

Subject and Material

The study subjects consisted of neonates, children under the age of five and adolescents. The total number of study subjects was 173. In 2014, 32 neonates aged 0-3 days were selected. The subject was in *Andini* Mother and Child Hospital, Pekanbaru City, Riau Province.

In 2017, 80 children aged 4-6 years were selected. Subjects were children who attend As-Shofa Kindergarten and Hidayatullah Kindergarten in Pekanbaru City, Riau Province and Al-Falah PAUD (Early childhood education programs) in Lima Puluh Kota District, West Sumatra Province. In the 2018 study, 25 children aged 4-6 years were selected, who attended Al Falah PAUD, Lima Puluh Kota Kota District, West Sumatra Province. In 2020, 36 teenagers aged 12-15 years were selected. These teenagers attended SMP (Junior high school) Negeri 3 Pekanbaru in Riau Province.

The study obeyed the Helsinki-Ethical Principles for Medical Research Involving Human Subjects and approved by the university review board (University of Riau), Ministry of Education and Culture of Republic Indonesia. (certificate number 067/UN.19.1.28/UEPKK /2014, 351/UN.19.5.1.1.8/UEPKK/2017; 073 /UN.19.5.1.1.8/ UEPKK/2018, and 351/UN. 19.5.1.1/UEPKK/2020).

Data Collection and Instrument

All parent subjects gave written informed consent. At the time of informed parental consent, and race (Indonesian, expatriate). The urine was collected by using sterile pot, aliquot to 6 ml and stored in freezer at -20°C (GEA by Vestfrost-Denmark Type G.201 Serial No: 20021808005) until further analysis.

In this study, body height gauges (microtoa) (STATURE METER 2M GEA, Indonesia), pot urine sterile (MERAH 60ml, Indonesia), sanitizing wipe, and household socio-economic questionnaires for the children (name, gender, age, race, height parents) were used.

Children' urine was collected by a nurse who was trained by researchers at the kindergarten/nursery school. The mothers were briefly explained about the implementation of the study as well as pot urine collection. Urine was collected between 7:00 and 10:00 am. The minimum amount of urine taken from the subject was 10 ml. The urine samples were then stored in the freezer at a temperature of -20 °C in Prodia Clinical Laboratory Pekanbaru Branch, and then sent to Prodia Center in Jakarta for analysis. The analysis was carried out simultaneously.¹⁸

Pyd measurements were performed with the use of MicroVue™ PYD EIA kit, USA. Pyd analysis was performed according to Hayati *et al.*¹⁹ using a Spectrophotometer (Microplate Reader 680 series, Bio-Rad Laboratories, Inc, Hercules, CA 94547, USA).

Creatinine measurements were performed with the use of Jaffe reactions according to the method developed by Staden.²⁰ Creatinine is reacted with picric acid under alkaline conditions to form a red-orange compound. The absorbance of the compound formed was detected at a wavelength of 490- 520 nm using Spectrophotometer (ADVIA 1800: ADVIA, Germany).

Statistical Analysis

Statistical data analysis is reported based on the complete data. Pearson correlation and t-test with significance *p < 0.05 and **p < 0.01 was applied for statistical analysis. The analysis was performed using IBM SPSS Statistics version.²⁰

Results

The Socioeconomic Characteristics of the Child's Family

All the respondents in this study were lived in cities (Table 1). The average income of the respondent's parents was IDR 3,000,000 per month.

The education of the respondents' parents was on average high school. Almost all respondent mothers were housewives (90%). The respondent father's job was usually entrepreneur, employee or laborer. The number of siblings of the respondent was around 1-3 peoples. Almost all of the respondent's parents' height was > 150 cm.

Table 1: The socioeconomic characteristics of the child's family

	Year of the research			
	2014	2017	2018	2020
Location	Andini Hospital Pekanbaru City in Riau Province	Al Falah PAUD study Lima Puluh Kota District (in West Sumatra), As-Shofa Kindergarten and Hidayatullah Kindergarten Pekanbaru (in Riau Province)	Al Falah PAUD, Lima Puluh Kota District (in West Sumatra),	SMP Negeri 3 Pekanbaru in Riau Province
Age	Neonatus 0 - 3 days	4-6 years	4-6 years	12-15 years
Number of subjects	32	80	25	36
Sex	Boys (n=26) & girls (n=9)	Boy	Boys (n=16) & Girls (n=9)	Boys (n=18) & Girls (n=18)
Height (cm)				
•Very Stunting		98±96(99:2)		
•Stunting before nutritional intervention	46.8±0.5 (46:47) ^a	102±97(108:3)	131.5	143,6±52(133,6:1549)
•Stunting after nutritional intervention			133.2	144,9±51(134,7:155,2)
•Normal	49.9±1.4(48.0:53.0) ^b	109±97(121:5)		
Nutritional status before intervention	Normal and stunting	Normal and stunting	Stunting	Stunting
Intervention	-	-	Milk every day and four eggs per week for 4 months. Additional energy, namely 20% nutritional adequacy rate/RDA	Brunch meals and milk daily for 34 days. Additional energy, namely 30% nutritional adequacy rate / RDA.
Conclusion	The Pyd content in the urine of stunted neonates was found to be different from the Pyd content in	There was a negative correlation observed between urine Pyd content and the subject's	There was a difference in Pyd content in the urine of subjects before and after nutri-	There was a difference observed in Pyd content of subjects before

normal neonatal urine (p <0.01). The pattern of Pyd content in urine according to height was like the letter "U" height (p <0.05). Urine Pyd content showed a weak correlation with height at r = -0.242 tional intervention (p <0.01). However, all subjects were still in the stunting category and after the nutritional intervention (p <0.05). Approximately, 19.4% of subjects increased their nutritional status from stunting to normal

Urine Pyd Content of the Stunting Child

The Pyd content of stunted children aged 0-3 days, 3-5 years, 4-6 years, and 12-15 years were found to be 982, 16.4, 16.9 and 9.81 nmol / mmol creatinine, respectively (Table 2).

(Table 3). The Pyd content of stunted neonates' urine for men and women were 988.45 and 641.40 nmol / mmol creatinine, respectively. Moreover, Pyd content of urine for stunted girls and boys aged 4-6 years were 18.70 and 16.27 and nmol / mmol creatinine, respectively.

The Pyd content in urine of stunted girls was found to be higher than the Pyd content of stunted boys

Table 2: Urine Pyd content based on nutritional intervention

Status nutritional	Urine Pyd content (nmol/mmol creatinine)			
	Neonates 0 - 3 days (2014)	Children 4-6 years (2017)	Children 4-6 years (2018)	Children 12-15 years (2020)
•Very Stunting	-	18.1	-	-
•Stunting	982	16.4	-	-
•Normal	594	15.5	-	-
•Before nutritional Intervention	-	-	16.9	9.81
•After nutritional Intervention	-	-	15.3	5.33

Table 3: Urine Pyd content based on sex

Year	Status nutritional intervention	Pyd (nmol/mmol creatinine)			
		n	Girl	n	Boy
2014	No intervention	2	988.45±29.20 (967.80: 1009.10)	7	641.40±257.73 (319.80: 1049.60)
2018	Before nutritional intervention	7	18.70±7.73 (9.73: 29.79)	11	16.27±5.98 (6.23:27.71)
2018	After nutritional intervention	7	18.55±10.91 (9.84: 43.56)	11	14.64±3.63 (9.07:20.80)

[§]average ± standard deviation (minimal: maximal)

The Pyd content of stunting neonates' urine was 982.92 ± 61.64 , whereas normal neonates were 594.11 ± 266.16 nmol/mmol creatinine ($p < 0.01$). The Pyd content of urine in very stunting, stunting and normal children aged 4-6 years were found to be 18.4, 16.4 and 15.5 nmol / mmol creatinine. There was a negative correlation found between urine Pyd content and height of children ($p < 0.05$) ($r = -0.242$).

Urine Pyd Content Based on Nutritional Intervention

The Pyd content of stunted children aged 4-6 years before and after nutritional intervention were found to be 16.9 and 15.3 nmol / mmol creatinine, respectively. The same results were also observed among the older age group. Moreover, Pyd content in urine of stunting children aged 12-15 years before and after nutritional intervention were 9.81 and 5.33 nmol/mmol creatinine, respectively. A decreasing trend in the amount of urine Pyd indicated an increased in the linear growth of the child. The results also indicated that by providing nutritional interventions to stunting children reduced urine Pyd content (Table 2).

Discussion

In this study, it was found that the urine Pyd content of stunting children decreased with increasing age. The same trend was also observed in the previous studies where Pyd urine excretion of children aged 0-3 days (neonates) was 10-100 times higher than that of children aged 3-16 years. The crosslink excretion in children was reported to be 20 times higher than in adult.¹² This was because of the day as we took neonates urine, the condition of neonates in dehydration. When the neonates just born, they were separated from their mother for hours without milk whether breastfeeding or formula. They were given formula milk (10-30 ml) then breastfeeding practice about 2-6 hours later.²¹ Pyd excretion for neonates was reported to be 642.7 ± 281.3 nmol/mmol creatinine.¹⁴

In another study, Pyd excretion among elementary school children was reported to be about 50-500 nmol/mmol creatinine.²² The urine pyd of adults who have health problems is higher than normal adults. For example, Harvey *et al.*²³ used pyridinurium cross-links as specific urinary markers for the measurement of bone collagen degradation in hyperthyroidism

and during thyroxine replacement therapy. They reported that the urinary Pyd excretion was higher among postmenopausal female thyrotoxic subjects compared to controls (median 131 vs 26 nmol/mmol creatinine ($p < 0.001$)), in postmenopausal women urinary Pyd excretion was raised in those taking T4 which is 40.0 ± 2.7 nmol/mmol creatinine ($p < 0.05$).

Urine Pyd content has been used to determine the severity of osteoporosis in the elderly. The more urine Pyd content in the elderly group, the higher the level of osteoporosis. This means that more bone resorption occurs in this group of elderly people.

Reference interval Premenopausal adult female and male urine contained Pyd around 15.3-33.6 and 10.3-20.0 nmol / mmol creatinine. The target value for treated postmenopausal adult female was the same as the premenopausal reference interval.²⁴ The Pyd content of premenopausal women's urine ranged from 3.0 to 7.4, whereas their male peers ranged from 2.3 to 5.4 $\mu\text{mol} / \text{mol}$ of creatinine.¹⁹

The growth spurt among boys occur more slowly than girls. Growth spurt in boys began to occur at the age of 10.5 years, whereas in girls it began to occur at the age of 9.5 years.²⁵ The increases in height occurred two years earlier in girls than boys. The peak height growth (peak height velocity) in girls occurs around the age of 12 years, whereas in boys at the age of 14 years. In girls, growth will end at the age of 16 years while in boys in 18 years. After that age, in general, height gain is almost complete.

Sex steroid hormones also affect bone maturation in the epiphyseal plate. At the end of puberty, the epiphyseal plate closes and height growth stops.²⁶ Relatively the same height at the age of 30-45 years. After 45 years there is a decrease in height.²⁷

In the age group of children and adolescents with normal nutritional status, there was more bone formation observed than bone resorption. Pyd in urine is a marker of bone resorption.²⁸ This means that the Pyd content of urine in the age group of children and adolescents with normal nutritional status is less than the Pyd content of urine in the age group of children with stunting nutritional status.²⁸

Urine pyridinols is a specific constituent of skeletal collagen, released into the circulation and excreted in the urine. Their measurement in urine is a sensitive index of the ongoing rate of bone resorption. The clinical applications of urinary Pyd markers include many metabolic disorders of bone such as osteoporosis, primary hyperparathyroidism and metastatic bone diseases. Urinary Pyd cross-link also shows great hope as a marker of therapeutic efficacy in bone disorders associated with accelerated bone resorption.²⁹

There was a decrease in the subject's Pyd content before and after the provision of nutritional interventions and the provision of nutritional interventions in the form of food with additional energy, namely 30% nutritional adequacy rate / RDA that could change the nutritional status of respondents from stunting to normal (Table 1). The provision of nutritional intervention to stunting children in this study was carried out in two studies, the first in the 2018 study and the second in the 2020 study. In the first study, milk was given every day and four eggs per week for 4 months; additional energy, namely 20% nutritional adequacy rate / RDA. In the second study, they were given brunch meals and milk daily for 34 days; additional energy, namely 30% nutritional adequacy rate / RDA. The results of the first study were there was a difference in Pyd content in the urine of subjects before and after nutritional intervention ($p < 0.01$), however, all subjects were still in the stunting category. In the second study, there was a difference observed in Pyd content of subjects before and after the nutritional intervention ($p < 0.05$). Approximately, 19.4% of subjects increased their nutritional status from stunting to normal.

There are many studies on the effect of nutrition on body length.³⁰ Energy intake was the strongest predictor of increased linear growth. Providing energy from food (310 Cal/day) in malnourished Indian children can increase height gain. Protein is provided from skim milk and cereals. The research was conducted by Bhandari et al³¹ on providing interventions to slum communities in Nehru, India. The study was conducted in a randomized controlled trial. In this study, the number of samples was 418 children aged 4-12 months. The children were divided into two groups. The first group is given quality supplementary food every day

with supervision so that consumption is optimal. The second group is given nutrition counseling only this group received 30-45 minutes of counseling monthly by a trained dietitian. Nutritional intervention was given for 8 months. The study showed that $1 - 2 = 0.4$ cm (attainment of the subject's body length increase), and the standard deviation was = 1.6 cm.

Another research related to the provision of nutrition and linear growth interventions, namely the research of Matali, Wungouw and Sapulete³² in Manado (Indonesia) which carried out an intervention in the form of intake of 250 ml of low fat high calcium UHT milk every day for 60 days to elementary school children. The research subjects were 40 people consisting of 20 people in the intervention group and 20 people in the control group. The average height of the intervention group in the first measurement was 133.23 cm and the second measurement was 134.78 cm, while the average height of the control group in the first measurement was 131.52 cm and second measurement was 132.52 cm. The average height increase in the intervention group was 1.55 cm while the average height increase in the control group was 0.99 cm. The difference in height increase in the intervention group and the control group was 0.56 cm. The results of the independent t test showed that there was a significant difference in the mean height gain in the intervention group and the control group.

HE. Agdeppa., Emilita M.O., Julian F.G., Mario V.C in 2019 conducted a study on providing nutrition education and knowledge for parents, and nutritional supplements for children at the Cavite School in Taguig City, Philippines. The subjects consisted of 146 people and the intervention was given for 120 days in two schools. The first group, namely in school 1, received iron-fortified rice and vegetables and the second group, namely in school 2, was given plain rice and vegetables. The subject's height experienced a significant increase in School 1 and School 2 from the start to the end point. However, the increase in mean weight in School 1 was significantly higher (1.33 ± 0.72 , $p = 0.0134$) than in School 2 (0.84 ± 0.59) cm.³³

The research that the authors conducted in 2018 aims to determine the effect of milk and egg consumption on the content of Pyridinium Crosslinks

(P_{vd}) urine of stunting children aged 4-6 years. The research was conducted in Early Childhood Education (PAUD) Al Falah, Limapuluh Kota District, West Sumatra Province. This research was a quasi-experimental research. The number of research subjects was 25 men. The subject was given a nutritional intervention that was given milk every day and four eggs in one week. The design of this research is pre and posttest. The duration of the nutrition intervention was 4 months. Morbidity and adherence to consuming milk and eggs were recorded daily by trained PAUD teachers. There was only one group in this study, namely the group that was given milk and eggs; there was no control group (the group that was not given nutritional intervention for comparison of nutritional intervention results). Hayati et al in 2017 reported that the average energy consumption of children aged 4-6 years is 1,048 calories per day,³⁴ while their energy needs are 1,550 calories per day,³⁵ Thus, the average level of energy consumption for stunting children is 67.6%. Therefore, to meet the energy needs of stunting children, it is necessary to provide nutritional interventions in the form of additional food.

Based on the consumption data above, it is known that there is a lack of energy consumption for stunting children as much as 502 calories. Nutritional intervention materials given to stunting children are milk and chicken eggs. Milk is given as much as 7 boxes to be consumed one box per day. Chicken eggs are given 4 eggs a week to be consumed on Monday, Wednesday, Thursday, and Friday. Milk and chicken eggs were given by researchers to their children's parents when parents picked up their children after school once a week every Friday. Energy 1 box of milk and 1 egg is 323 Calories. Chicken eggs were cooked by the subject's parents in their respective homes. The chicken eggs were consumed with rice by the subject.

The researcher recorded the consumption of milk and eggs by the subjects by asking the subject's parents at school every day. If any intervention food is left over, it is estimated that the remaining amount is and recorded. The price of 70 ml UHT flag milk for 1 small box is Rp. 5300 and the price of 1 egg is Rp. 1,500. Paired t-test results showed that the average difference between the height before and

after the intervention was 1.91 cm. This means that there is an increase in height after the intervention with an average increase of 1.91 cm. The result of calculating the "t" value is 5.133 with a p-value of 0.000 which can be written as 0.001 (2-way test). This means that we reject H₀ and conclude that there is a statistically significant difference between the mean height before and after the intervention.

The average difference between the height of the subjects before and after the intervention was 1.91 cm. The lack of the subject's height compared to the national average height according to the results of Basic Health Research in 2007¹ and in 2010² is 4.82 cm. The median height of the subjects before the intervention was 106.40 cm and after the intervention was 107.95 cm. The median height of children aged 4-6 years based on the results of Basic Research Health in 2007 and 2010 according to AsDI, IDAI, PERSAGI in 2015 is 112 cm.³⁶ The difference in the median height of the subjects when compared with the median height was 5.60 cm before the intervention and 4.05 cm after the intervention, respectively. The increase in the subject's height after the intervention was 1.91 cm. The content of pyridinium crosslinks in the urine of the subjects before and after the intervention were 16.9 ± 6.7 (5.1: 29.8) and 15.9 ± 7.0 (9.1: 43.6). There was a significant difference between the average height and P_{vd} content of subjects before and after the intervention, but all subjects were still in the stunting category (Table 2).

The research that the authors conducted in 2020 aimed to determine the difference in urine P_{vd} content in adolescents before and after being given brunch for 34 days. The research was conducted at State Junior High School 3 Pekanbaru with 36 subjects consisting of boys and girls. Brunch is an acronym for breakfast and lunch which is a dish served between breakfast and lunch, usually brunch is served between 10:00 and 11:00. Brunch is provided for someone who doesn't have time to eat breakfast. The brunch menu is usually not too heavy like a main meal, but also not too light like a snack. For this reason, brunch is the right solution to fill energy when skipping breakfast but not until lunch time.³⁷ Prior to the study, the subjects were selected for egg and milk allergies. This is done to avoid unwanted incidents related during the

implementation of this research. The milk provided by the researcher is UHT box milk. The price of 115 ml full cream UHT milk per box is IDR 3,000. 3 boxes of milk are provided per day along with the provision of brunch where the shelf life of UHT milk is 9 months.

Daily brunch was provided for 35 days from Monday to Sunday. The technique for giving brunch was that 1 box of milk was given at 08.00 am before students enter the first lesson, after that 1 box of milk was given during the first break at 10.00 at the same time as brunch and 1 box of milk to drink at 12.00 noon. On Sunday brunch was given in a different way, namely delivered to their homes by trained volunteers, consisting of 24 students from the Department of Nutrition, Health Polytechnic, Ministry of Health, Riau. During the provision of brunch, the research team accompanied the subjects until they finished consuming it.

The brunch menu was changed daily, which may include *gado-gado*, egg noodle, *batagor*, *lontong* Medan, sandwich, chicken porridge, and fried rice anchovies. The total amount of energy of the meals and milk was 600 calories (30% of RDA). There was an increase in the subject's height acceleration after the intervention. The average height of the subjects before the nutrition intervention was 143.6±5.2(133,6:154,9) and after the nutrition intervention was 144.9±5.1(134,7:155,2) cm. The Pyd content before the intervention was 9.81±7.02 and the Pyd content after the intervention was 5.33±2.89 nmol/mmol creatinine (Table 2). There was a decrease in adolescent Pyd content after the provision of nutritional intervention for 34 days. There was a difference observed in Pyd content of subjects before and after the nutritional intervention ($p < 0.05$). Approximately, 19.4% of subjects increased their nutritional status from stunting to normal.

The lack of research in 2020 is that subjects between women and men are still combined. For the future, it is better if the same research with male and female subjects can be distinguished. The advantage of this research is that the brunch provided can be purchased at stalls around the school where the research was conducted. Thus, it is hoped that students at the school can buy the food in the right type, quantity and time so that

it can meet their nutritional needs even though the nutritional intervention provided by the researcher has been completed by the end of the research period.

This means that even though this research has been completed, it is hoped that their snack habits can continue according to the pattern that was applied when the research was conducted. Their pocket money is sufficient to buy food as was done during the nutrition intervention in the study. Counseling on the importance of the right brunch so that teenagers know the type, amount and time of snacks that can meet their nutritional needs to achieve optimal linear growth needs to be done. So far, their snacks are not appropriate so they cannot meet the nutritional needs which can lead to stunting.

The limitation of research is that in this nutritional intervention research, there is no control group; the duration of the nutrition intervention was only 1 month from what should have been a minimum of three months. The suggestion for future research to prevent the limitation is that there needs to be a control group and added the duration of the intervention to a minimum of 3 months, it is better if it is up to 8 or 12 months, even 24 months.

In this study, urine Pyd is expected to be a marker of the efficacy of nutritional care in bone growth disorders associated with bone resorption.

Conclusion

The Pyd content of stunting children's urine was found to be different from normal children. There was a negative correlation observed between urine Pyd content and children's height ($p < 0.05$). Pyd content showed a weak correlation with height $r = -0.242$. There was a difference found in the Pyd content of children's urine before the nutritional intervention. The data is in accordance with the foundation theory.

It is necessary to do further research with more subjects in certain sex and age groups by providing nutritional interventions between the treatment and control groups at the same time and location.

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1 Conflict of Interest

The authors declare no conflict of interest.

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