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Is Urinary Pyridinium Crosslinks Associated with Stunting in Stunting Children in Indonesia in 2014-2020

Abstract

Objective: 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.
Methods: The study was a cross-sectional involving 173 children in Pekanbaru and Payakumbuh, 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; 25% 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.
Results: 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, 15.3, 9.81 and 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.

Key-words

Children, Height, Pyridinium Crosslinks Urine, Stunting

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 is 30.87 %[1]. 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% [2].

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About 59.3% of children aged 3-5 years were included stunting[3]. The prevalence of global stunting of children aged 13-15 years is around 35.1%[4]. 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%., the prevalence is 36.6% in the Riau Province[5]. Public health problems are considered severe if the prevalence of stunting is 30-39% and serious if the prevalence of stunting is ≥40%[4]. World Health Organization (WHO) established stunting standards based on anthropometrics measurement with Height for Age (HAZ)-score <-2 SD[6].

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 pyridinium crosslinks (Pyd) are formed by hydroxylline 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[7].

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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; occurs in a balanced manner which indicates that the amount of bone removed will be completely replaced)[8]. There are two types of cells responsible for bone metabolism, namely osteoblasts and osteoclasts[9]. 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[10]. Calcium forms complex bonds with phosphate which can provide strength to bones[11].

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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.

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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.

Materials and Methods

Study Design

This cross-sectional study was carried in 2014, 2017, 2018 and 2019 and conducted in two Provinces namely Riau (Pekanbaru City) and West Sumatera (50 Kota District) Province

It was a cross-sectional study conducted in 2014, 2017, 2018, 2020. The study was conducted in various Province including; Pekanbaru City, Riau Province and in 50 Kota district, West Sumatra Province, Indonesia.

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Subject and Urine Collection

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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 50 Kota District, West Sumatra Province. In the 2018 study, 25 children aged 4-6 years were selected, who attended Al Falah PAUD, 50 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

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

Equipment and Materials

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.

Research Procedure

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[12]

Pyd Urine Measurement and Standardization

Pyd measurements were performed with the use of MicroVue™ PYD EIA kit, USA. Pyd analysis was performed according to Hayati et al.[13] using a Spectrophotometer (Microplate Reader 680 series, Bio-Rad Laboratories, Inc, Hercules, CA 94547, USA).

Creatinine Urine Measurement and Standardization

Creatinine measurements were performed with the use of Jaffe reactions according to the method developed by Staden[14]. 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 are 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. 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.

Urine Pyd content according to the age 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 1).

Stunting children's urine Pyd content by sex

The Pyd content in urine of stunted girls was found to be higher than the Pyd content of stunted boys (Table 2). 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.

Urine Pyd levels according to stunting status

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The Pyd content of stunting neonates' urine was 982.92 ± 61.64, whereas normal neonates was 594.11 ± 266.16 nmol/mmol creatinine ($p < 0.01$). The Pyd content of urine in very 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 aged ($p < 0.05$) ($r = -0.242$).

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[8]. 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[15]. Pyd excretion for neonates was reported to be 642.7±281.3 nmol/mmol creatinine by Fujimoto et al[7].

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In another study, Pyd excretion among elementary school children was reported to be about 50-500 nmol/mmol creatinine[16]. The urine pyd of adults who have health problems is higher than normal adults. For example, Harvey et al.[17] used pyridinium 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 patients compared to controls ([edian 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[18].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 µmol / 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[20]. The increases in height occurred two years earlier in girls than boys. The peak height growth rate (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.

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Sex steroid hormones also affect bone maturation in the epiphyseal plate. At the end of puberty, the epiphyseal plate closes and height growth stops[21]. Relatively the same height at the age of 30-45 years. After 45 years there is a decrease in height[22].

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. 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.

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Urine pyd 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. Urine Pyd cross-link also shows great hope as a marker of therapeutic efficacy in bone disorders associated with accelerated bone resorption[23].

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.

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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.

Acknowledgements

Herewith we convey our thanks and best regard for financial support from Poltekkes Kemenkes Riau; the research facilities Andini Hospital, As Shofa Kindergarten and Hidayatullah Kindergarten, SMP Negeri 3 Pekanbaru and PAUD Al Falah, 50 Kota district; urine Pyd content analysis facility from Prodia Clinical Laboratory Pekanbaru and Jakarta, and milk assistance from PT Indolakto Jakarta.

Funding Sources

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Conflict of Interest

The authors declare no conflict of interest.

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Table 1. Pyd content in urine based on nutritional intervention (nmol /mmol creatinine)

	Year			
	2014	**2017**	**2018**	**2020**
Location	Andini Hospital Pekanbaru in Riau Province	Al Falah PAUD, 50 Kota District (in West Sumatra), As-Shofa Kindergarten and Hidayatullah Kindergarten Pekanbaru (in Riau Province)	Al Falah PAUD, 50 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)				
• **Verry 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 25% nutritional adequacy rate / RDA	Brunch meals and milk daily for 34 days. Additional energy, namely 30% nutritional adequacy rate / RDA.
Pyd urine (nmol/mmol creatinine)				
• **Verry 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
Conclusion	The Pyd content in the urine of stunted neonates was found to	There was a negative correlation observed between urine Pyd	There was a difference in Pyd content in the urine	There was a difference observed in Pyd content of subjects before and after

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

Table 2. Pyd content in urine of stunted children by sex

Year	Status nutritiona 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 nutritiona 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)

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Introduction

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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.

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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.

Materials and Methods

Study Design

This cross-sectional study was carried in 2014, 2017, 2018 and 2019 and conducted in two Provinces namely Riau (Pekanbaru City) and West Sumatera (50 Kota District) Province

It was a cross-sectional study conducted in 2014, 2017, 2018, 2020. The study was conducted in various Province including; Pekanbaru City, Riau Province and in 50 Kota district, West Sumatra Province, Indonesia.

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Subject and Urine Collection

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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 50 Kota District, West Sumatra Province. In the 2018 study, 25 children aged 4-6 years were selected, who attended Al Falah PAUD, 50 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

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

Equipment and Materials

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.

Research Procedure

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[12]

Pyd Urine Measurement and Standardization

Pyd measurements were performed with the use of MicroVue™ PYD EIA kit, USA. Pyd analysis was performed according to Hayati et al.[13] using a Spectrophotometer (Microplate Reader 680 series, Bio-Rad Laboratories, Inc, Hercules, CA 94547, USA).

Creatinine Urine Measurement and Standardization

Creatinine measurements were performed with the use of Jaffe reactions according to the method developed by Staden[14]. 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 are 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. 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.

Urine Pyd content according to the age 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 1).

Stunting children's urine Pyd content by sex

The Pyd content in urine of stunted girls was found to be higher than the Pyd content of stunted boys (Table 2). 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.

Urine Pyd levels according to stunting status

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The Pyd content of stunting neonates' urine was 982.92 ± 61.64, whereas normal neonates was 594.11 ± 266.16 nmol/mmol creatinine ($p < 0.01$). The Pyd content of urine in very 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 aged ($p < 0.05$) ($r = -0.242$).

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[8]. 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[15]. Pyd excretion for neonates was reported to be 642.7±281.3 nmol/mmol creatinine by Fujimoto et al[7].

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In another study, Pyd excretion among elementary school children was reported to be about 50-500 nmol/mmol creatinine[16]. The urine pyd of adults who have health problems is higher than normal adults. For example, Harvey et al.[17] used pyridinium 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 patients compared to controls ([edian 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[18].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 µmol / 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[20]. The increases in height occurred two years earlier in girls than boys. The peak height growth rate (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.

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Sex steroid hormones also affect bone maturation in the epiphyseal plate. At the end of puberty, the epiphyseal plate closes and height growth stops[21]. Relatively the same height at the age of 30-45 years. After 45 years there is a decrease in height[22].

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. 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.

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Urine pyd 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. Urine Pyd cross-link also shows great hope as a marker of therapeutic efficacy in bone disorders associated with accelerated bone resorption[23].

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.

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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.

Acknowledgements

Herewith we convey our thanks and best regard for financial support from Poltekkes Kemenkes Riau; the research facilities Andini Hospital, As Shofa Kindergarten and Hidayatullah Kindergarten, SMP Negeri 3 Pekanbaru and PAUD Al Falah, 50 Kota district; urine Pyd content analysis facility from Prodia Clinical Laboratory Pekanbaru and Jakarta, and milk assistance from PT Indolakto Jakarta.

Funding Sources

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Conflict of Interest

The authors declare no conflict of interest.

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Table 1. Pyd content in urine based on nutritional intervention (nmol /mmol creatinine)

	Year			
	2014	**2017**	**2018**	**2020**
Location	Andini Hospital Pekanbaru in Riau Province	Al Falah PAUD, 50 Kota District (in West Sumatra), As-Shofa Kindergarten and Hidayatullah Kindergarten Pekanbaru (in Riau Province)	Al Falah PAUD, 50 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)				
• **Verry 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 25% nutritional adequacy rate / RDA	Brunch meals and milk daily for 34 days. Additional energy, namely 30% nutritional adequacy rate / RDA.
Pyd urine (nmol/mmol creatinine)				
• **Verry 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
Conclusion	The Pyd content in the urine of stunted neonates was found to	There was a negative correlation observed between urine Pyd	There was a difference in Pyd content in the urine	There was a difference observed in Pyd content of subjects before and after

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

Table 2. Pyd content in urine of stunted children by sex

Year	Status nutritiona 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 nutritiona 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)

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

Abstract

Objective: 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.
Methods: 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.
Results: 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. The 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.

Key-words

Children, Height, Pyridinium Crosslinks Urine, Stunting

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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, 35.6 % in 2010, 37.2 % in 2013 and 30.87 % in 2018[1]. 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%[2].

About 59.3% of children aged 3-5 years were included stunting[3]. The prevalence of global stunting of children aged 13-15 years is around 35.1%[4]. 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%., the prevalence is 36.6% in the Riau Province[5]. Public health problems are considered severe if the prevalence of stunting is 30-39% and serious if the prevalence of stunting is ≥40%[4]. World Health Organization (WHO) established stunting standards based on anthropometrics measurement with Height for Age (HAZ)-score <-2 SD[6].

Stunting is associated with impaired linear growth processes[7]. The linear growth retardation process begins at 2 or 3 months of age[8]. Growth retardation reflects a process of failure to achieve linear growth potential as a result of suboptimal health and/or nutritional conditions[4]. 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 [9]. Urinary pyridinium crosslinks are removed during the release of mature collagen in bone. Pyridinium is constructed as an intramolecular crosslinker during collagen maturation[10].

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 pyridinium crosslinks (Pyd) are formed by hydroxylline 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[11].

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; occurs in a balanced manner which indicates that the amount of bone removed will be completely replaced)[9]. There are two types of cells responsible for bone metabolism, namely osteoblasts and osteoclasts[12]. 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[13]. Calcium forms complex bonds with phosphate which can provide strength to bones[14].

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[15]

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).

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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.

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.
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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,

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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^{o}C (GEA by Vestfrost-Denmark Type G.201 Serial No: 20021808005) until further analysis.

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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[15].

Pyd measurements were performed with the use of MicroVue™ PYD EIA kit, USA. Pyd analysis was performed according to Hayati et al.[16] 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[17]. 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.

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).

The Pyd content in urine of stunted girls was found to be higher than the Pyd content of stunted boys (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 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[9]. 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[18]. Pyd excretion for neonates was reported to be 642.7±281.3 nmol/mmol creatinine[11].

In another study, Pyd excretion among elementary school children was reported to be about 50-500 nmol/mmol creatinine[19]. The urine pyd of adults who have health problems is higher than normal adults. For example, Harvey et al.[20] used pyridinium 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 patients compared to controls ([edian 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[21].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 µmol / 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[22]. The increases in height occurred two years earlier in girls than boys. The peak height growth rate (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[23]. Relatively the same height at the age of 30-45 years. After 45 years there is a decrease in height[24].

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.

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[25].

Urine pyd 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. Urine Pyd cross-link also shows great hope as a marker of therapeutic efficacy in bone disorders associated with accelerated bone resorption[26].

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[27]. 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 at al[28] 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 Sapulete[29] 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 the 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.

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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[30].

The research that the authors conducted in 2018 aims to determine the effect of milk and egg consumption on the content of Pyridinium Crosslinks (Pyd) urine of stunting children aged 4-6 years. The research was conducted in Early Childhood Education (PAUD) Al Falah, Lima Puluh 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[31], while their energy needs are 1,550 calories per day[32]; 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. 1,500 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 Ho 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 Research Health in 2007[33] and in 2010[34] 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[35]. 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 Pyd content of subjects before and after the intervention, but all subjects were still in the stunting category (Table 2).

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The research that the authors conducted in 2020 aimed to determine the difference in urine Pyd 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[36].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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Conflict of Interest

The authors declare no conflict of interest.

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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)				
• **Verry 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 normal neonatal urine ($p < 0.01$). The pattern of Pyd content in urine according to height was like the letter "U"	There was a negative correlation observed between urine Pyd content and the subject's height ($p < 0.05$). Urine Pyd content showed a weak correlation with height at $r = -0.242$	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	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

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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)
• **Verry 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)

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Author's Response to Reviewer's Comments

Reviewer number 1

Paper title: Is Urinary Pyridinium Crosslinks Associated with Stunting in Stunting Children in Indonesia in 2014-2020

Title	Reviewer's Comments	Author's Response
Abstract		
Keywords		
Introduction	The prevalence of stunting among children under five years of age in Indonesia is 30.87 %[1].	
	The prevalence of stunting among children under five years of age in Indonesia is 36.8 (2007), 35.6 (2010), 37.2 (2013), 30.87 %[1]. 30.87 (2018).	Telah ditunjukkan data lain dari Riset Kesehatan Dasar atau tren stunting di Indonesia yaitu data tahun 2007, 2010, dan 2013.

Commented [H1]: Maybe shown other data from Basic Health Research or trends stunting in Indonesia.

Commented [H2]: Maybe shown other data from Basic Health Research or trends stunting in Indonesia.

	The biochemical markers of bone resorption can be analyzed clinically using conditions and treatments that affect bone metabolism.	
	Stunting terkait dengan gangguan pada proses pertumbuhan linier (Frongillo 1999). Proses perlambatan pertumbuhan linier dimulai usia 2 atau 3 bulan (Waterlow dan Schürch 1994). Pertumbuhan terhambat mencerminkan proses kegagalan untuk mencapai potensi pertumbuhan linier sebagai hasil kesehatan tidak optimal dan/atau kondisi gizi (WHO 2010). Pertumbuhan linier salah satunya dapat diukur dari pertumbuhan tulang. Pertumbuhan tulang meningkat seiring dengan pertambahan tinggi badan. Pertumbuhan tulang terjadi apabila pembentukan tulang (bone formation) lebih banyak dibandingkan dengan penguraian tulang (bone absorbtion). Pyridinium crosslinks adalah marker dari resorpsi tulang (Robins 1994). Pyridinium crosslinks urin dibuang selama pelepasan kolagen matang pada tulang. Pyridinium dibangun sebagai pengikat silang (crosslinker) intramolekul selama pematangan kolagen (Shaw et al. 1995).	Telah ditambahkan satu paragraph untuk menghubungkan paragraph yang ditanyakan dengan paragraph yang sebelumnya.
	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.	

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Current Research in
Nutrition and Food Science

	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. [12.]	Telah ditambahkan tulisan references yaitu nomor 12
	12.Ningsih S. W., Lubis N. A., Hayati A. W., Azis A. Is urinary creatinine associated with wasting in neonates. *Asian J Pharm Clin Res*. 2018;11(Special Issue 1):187-189. doi:10.22159/ajpcr.2018.v11s1.26603.	
Methodology	**Study Design** This cross-sectional study was carried in 2014, 2017, 2018 and 2019 and conducted in two Provinces namely Riau (Pekanbaru City) and West Sumatera (50 Kota District) Province It was a cross-sectional study conducted in 2014, 2017, 2018, 2020. The study was conducted in various Province including; Pekanbaru City, Riau Province and in 50 Kota district, West Sumatra Province, Indonesia.	
	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. Kedua lokasi penelitian berkarakteristik sama yang terletak berdampingan di pulau yang sama di Indonesia yaitu Pulau Sumatera. Subjek merupakan orang yang berbeda di setiap tahun penelitian.	Dua kalimat yang ditanyakan reviewer sudah penulis rubah menjadi satu kalimat yang pertama saja, sedangkan yang kalimat yang kedua dihapus karena

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		kedua kalimat bermakna sama.
		Penyebab pengambilan data di dua tempat yang berbeda adalah terkait dengan persyaratan yang diajukan oleh penyandang dana penelitian terkait dengan skema pembiayaan penelitian. Skema pembiayaan penelitian yang mendanai penelitian ini mensyaratkan bahwa salah satu persyaratan yang harus dipenuhi yaitu peneliti berasal dari minimal dua institusi di dua propinsi. Peneliti telah memilih dua tempat lokasi pengambilan data yang karakteristiknya sama.

		Subjek merupakan orang yang berbeda di setiap tahun penelitian.
	Subject and Urine Collection	
	Subject ~~and Urine Collection~~	The explain of urine collection in this sub section tidak ditemukan karena ternyata saya menuliskannya di bagian setelah itu dengan sub judul "**Research Procedure".** Oleha karena itu maka pada bagian ini saya menghapus tulisan **"and Urine Collection"** pada bagian ini.

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	Data Collection	
	Data Collection and Instrument	Saya telah mengedit artikel sesuai saran reviewer dengan menambahkan frase "**and Instrument"** di sub judul serta menghapus beberapa sub judul yang terkait dengan instrumen.
	~~**Equipment and Materials**~~	
	~~**Research Procedure**~~ ~~**Urine Collection**~~	
	~~**Pyd Urine Measurement and Standardization**~~	
	~~**Creatinine Urine Measurement and Standardization**~~	
Results		
Discussion	In another study, Pyd excretion among elementary school children was reported to be about 50-500 nmol/mmol creatinine[16].	
	In another study, Pyd excretion among elementary school children was reported to be about 50-500 nmol/mmol creatinine[16]. The urine pyd of adults who have health problems is	Topik paragraf ini yaitu untuk membandingkan kandungan Pyd urin

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	higher than normal adults. For example, Harvey et al.[17] used pyridinium 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 patients compared to controls ([edian 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)].	dari penelitian yang saya lakukan dengan kandungan Pyd dari peneliti terdahulu. Penelitian terdahulu yang menjelaskan tentang kandungan Pyd yang saya temukan selama ini yaitu seperti yang saya tulisakan di artikel ini. Jika kita hilangkan the explanation for adults in this section ini akan mengurangi kedalaman informasi.
	Pyd in urine is a marker of bone resorption. 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.	
	Pyd in urine is a marker of bone resorption (Seyedin, Kung, Daniloff, Hesley, Gomez, Nielsen, Rosen, Zuk 1993). This means that the Pyd content of urine in the age group of children and adolescents with normal	Sudah saya tambahkan reference

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	nutritional status is less than the Pyd content of urine in the age group of children with stunting nutritional status. S M Seyedin, V T Kung, Y N Daniloff, R P Hesley, B Gomez, L A Nielsen, H N Rosen, R F Zuk. Immunoassay for urinary pyridinoline: the new marker of bone resorption. J Bone Miner Res. 1993 May;8(5):635-41.doi: 10.1002/jbmr.5650080515. [https://pubmed.ncbi.nlm.nih.gov/8511991/ diakses 19 Juni 2021]	
	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.	
	Ada banyak penelitian tentang pengaruh gizi terhadap panjang badan (WHO 2001). Asupan energi merupakan prediktor paling kuat dari peningkatan pertumbuhan linier. Pemberian energi dari makanan (310 Kal/hari) pada anak-anak India yang kurang gizi dapat meningkatkan pertambahan tinggi badan. Protein diberikan dari susu skim dan sereal. Penelitian tersebut dilakukan oleh Bhandari et al. (2001) tentang pemberian intervensi pada masyarakat kota kumuh di Nehru, India. Penelitian dilakukan secara randomized controlled trial. Pada penelitian ini jumlah sampel yaitu 418 anak berusia 4-12 bulan. Anak-anak	Sudah ditambahka pembahsan tentan intervensi gizi dalam artikel ini.

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Commented [H17]: Overall, the discussion has not focused on research results, I have not found discussions related to interventions, properly. Even though this is important to become a practical study in Indonesia to solve stunting.
The limitation of research need to explain, so the suggestion for future research will prevent the limitation

	tersebut dibagi menjadi dua kelompok. Kelompok pertama yaitu diberi makanan tambahan yang berkualitas setiap hari dengan pengawasan agar konsumsi optimal. Kelompok kedua yaitu diberikan konseling gizi saja; kelompok ini menerima konseling 30- 45 menit setiap bulan oleh ahli gizi yang terlatih. Intervensi gizi diberikan selama 8 bulan. Penelitian tersebut menunjukkan bahwa $\mu 1 - \mu 2 = 0.4$ cm (capaian pertambahan panjang badan subjek), dan standar deviasi yaitu $\sigma = 1.6$ cm. Peneitian lain yang terkait dengan pemberian intervensi gizi dan pertumbuhan linier yaitu penelitian Matali, Wungouw dan Sapulete (2017) di Manado (Indonesia) yaitu melakukan pemberian intervensi berupa asupan 250 ml susu UHT low fat high calcium setiap hari selama 60 hari kepada anak sekolah dasar. Subyek penelitian yaitu 40 orang yang terdiri dari 20 orang kelompok intervensi dan 20 orang kelompok kontrol. Rerata tinggi badan kelompok intervensi pada pengukuran pertama ialah 133,23 cm dan pengukuran kedua 134,78 cm, sedangkan rerata tinggi badan kelompok kontrol pada pengukuran pertama adalah 131,52 cm dan pengukuran kedua 132,52 cm. Rerata kenaikan tinggi badan kelompok intervensi ialah 1,55 cm sedangkan rerata kenaikan tinggi badan kelompok kontrol 0,99 cm. Perbedaan kenaikan tinggi badan kelompok intervensi dengan kelompok kontrol yaitu 0,56 cm. Hasil uji t indepen menunjukkan terdapat perbedaan bermakna rerata	

	kenaikan tinggi badan pada kelompok intervensi dan kelompok kontrol. Penelitian yang dilakukan oleh I.A. Agdeppa., Emilita M.O., Julian F.G., Mario V.C (2019) tentang memberikan pendidikan dan pengetahuan gizi untuk orang tua, dan tambahan makan bergizi untuk anak-anak di Sekolah Cavite di di Taguig City, Philippines. Subjek terdiri dari 146 orang dan pemberian intervensi selama 120 hari di dua sekolah. Kelompok pertama yaitu di sekolah 1 menerima beras yang diperkaya zat besi dan sayur dan kelompok kedua yaitu di sekolah 2 diberikan beras biasa dan sayur. Tinggi badan subjek mengalami peningkatan yang signifikan di Sekolah 1 dan Sekolah 2 dari awal ke titik akhir. Namun, peningkatan rata-rata berat di Sekolah 1 secara signifikan lebih tinggi (1,33 ± 0,72, p = 0,0134) dibandingkan di Sekolah 2 (0,84 ± 0,59) cm. Penelitian yang penulis lakukan tahun 2018 bertujuan untuk mengetahui pengaruh konsumsi susu dan telur terhadap kandungan Pyridinium Crosslinks (Pyd) urin anak stunting usia 4 – 6 tahun. Penelitian dilakukan di Pendidikan Anak Usia Dini (PAUD) Al Falah Kabupaten Lima Puluh Kota Propinsi Sumatera Barat. Penelitian ini merupakan penelitian quasi eksperimen. Jumlah subjek penelitian sebanyak 25 orang berjenis kelamin laki-laki. Subjek tersebut diberi intervensi gizi yaitu diberi susu setiap hari dan telur sebanyak empat butir dalam satu	

	minggu. Rancangan penelitian ini adalah pre dan post test. Lama pemberian intervensi gizi yaitu 4 bulan. Morbiditas dan kepatuhan mengkonsumsi susu dan telur dicatat setiap hari oleh guru PAUD yang sudah dilatih. Hanya ada satu kelompok dalam penelitian ini yaitu kelompok yang diberi susu dan telur; tidak ada kelompok control (kelompok yang tidak diberi ntervensi gizi untuk perbandingan hasil intervensi gizi). Hayati dkk (2017) melaporkan bahwa rata-rata konsumsi energi anak usia 4 – 6 tahun yaitu 1.048 Kalori per hari, adapun kebutuhan energi mereka yaitu 1.550 Kalori per hari (Hardinsyah, Hadi Riyadi dan Victor Napitupulu 2004); dengan demikian tingkat kecukupan konsumsi energi rata-rata anak stunting tersebut 67.6%. Oleh karena itu untuk memenuhi kebutuhan energi anak stunting perlu dilakukan pemberian intervensi gizi berupa makanan tambahan. Berdasarkan data konsumsi di atas, diketahui bahwa terjadi kekurangan konsumsi energi anak stunting sebanyak 502 Kalori. Bahan intervensi gizi yang diberikan kepada anak stunting yaitu susu dan telur ayam. Susu diberikan sebanyak 7 kotak untuk dikonsumsi satu kotak per hari. Telur ayam diberikan 4 butir dalam seminggu untuk dikonsumsi pada hari Senin, Rabu, Kamis, dan Jumat. Susu dan telur ayam diberikan oleh peneliti kepada	

	orang tua anak ketika orang tua menjemput anak sepulang sekolah seminggu sekali setiap hari Jumat. Energi 1 kotak susu dan 1 butir telur yaitu 323 Kalori. Telur ayam dimasak oleh orang tua subjek di rumah masing-masing. Telur ayam tersebut dikonsumsi bersama nasi oleh subjek. Peneliti mencatat tentang konsumsi susu dan telur oleh subjek melalui bertanya kepada orang tua subjek di sekolah setiap hari. Jika ada makanan intervensi bersisa maka diperkirakan jumlah sisa tersebut dan dicatat. Harga susu Bendera UHT 70 ml 1 kotak kecil Rp 1.500 dan harga telur 1 butir Rp 1.500. Hasil Uji-t Berpasangan menunjukkan bahwa rata-rata perbedaan antara tinggi badan sebelum dengan tinggi badan setelah intervensi adalah sebesar 1,91 cm. Artinya ada peningkatan tinggi badan sesudah intervensi dengan rata-rata peningkatan sebesar 1,91 cm. Hasil perhitungan nilai "t" adalah sebesar 5,133 dengan p-value 0.000 dapat ditulis 0,001 (uji 2-arah). Hal ini berarti kita menolak Ho dan menyimpulkan bahwa secara statistik ada perbedaan yang bermakna antara rata-rata tinggi badan sebelum dengan sudah intervensi. Rata-rata perbedaan antara tinggi badan subjek sebelum dan setelah intervensi adalah sebesar 1,91 cm. Kekurangan tinggi badan subjek dibandingkan tinggi badan rata-rata nasional menurut hasil Riskesdas	

	2007 (Kemenkes RI 2008) dan 2010 (Kemenekes RI 2010) yaitu 4,82 cm. Median tinggi badan subjek sebelum intervensi yaitu 106,40 cm dan setelah intervensi yaitu 107,95 cm. Median tinggi badan anak usia 4 – 6 tahun berdasarkan hasil Riskesdas 2007 dan 2010 menurut AsDI, IDAI, PERSAGI (2015) yaitu 112 cm. Selisih median tinggi badan subjek jika dibandingkan dengan median tinggi badan tersebut berturut-turut yaitu 5,60 cm sebelum intervensi dan 4,05 cm setelah intervensi. Peningkatan tinggi badan subjek setelah intervensi yaitu 1,91 cm. Kandungan pyridinium crosslinks urin subjek sebelum dan setelah intervensi berturut-turut yaitu 16,9 ± 6,7 (5,1 : 29,8) dan 15,9 ± 7,0 (9,1 : 43,6). Ada perbedaan yang bermakna antara rata-rata tinggi badan dan kandungan Pyd subjek sebelum dengan sudah intervensi, namun semua subjek masih dalam kategori stunting (Tabel 2). Penelitian yang penulis lakukan pada tahun 2020 mengetahui perbedaan kandungan Pyd urin pada remaja sebelum dan setelah diberi brunch selama 34 hari. Penelitian dilakukan di Sekolah Menengah Pertama Negeri 3 Pekanbaru dengan subjek berjumlah 36 orang yang terdiri laki-laki dan perempuan. Brunch merupakan singkatan dari *breakfast* dan *lunch.* Brunch adalah hidangan yang disajikan antara makan pagi dan makan siang, biasanya brunch dihidangkan antara	

	pukul 10.00 hingga pukul 11.00. Brunch disediakan bagi seseorang yang tidak sempat makan pagi. Menu *brunch* biasanya tidak terlalu berat seperti makanan utama, tapi juga tidak terlalu ringan seperti camilan. Karena itulah, *brunch* adalah solusi tepat untuk mengisi energi saat melewatkan sarapan namun belum sampai waktu makan siang (Trisna, 2014). Sebelum dilakukannya penelitian maka subjek diseleksi terhadap alergi telur dan susu. Hal ini dilakukan untuk menghindari kejadian yang tidak diinginkan terkait selama pelaksanaan peneltian ini. Susu yang disediakan peneliti yaitu susu kotak UHT. Harga susu UHT full cream 115 ml per kotak yaitu Rp 3.000. Disediakan 3 kotak susu per hari bersamaan dengan pemberian brunch. Lama simpan susu UHT yaitu 9 bulan. Pemberian brunch setiap hari selama 35 hari dari hari Senin sampai Minggu. Teknis pemberian brunch yaitu susu diberikan 1 kotak pukul 08.00 pagi sebelum siswa masuk jam pelajaran pertama, setelah itu 1 kotak susu diberikan pada jam istirahat pertama yaitu pukul 10.00 bersamaan dengan brunch dan 1 kotak susu untuk diminum pukul 12.00 siang. Pada hari Minggu brunch diberikan dengan cara yang berbeda yaitu diantar ke rumah masing-masing oleh tenaga volentir yang sudah dilatih yaitu terdiri dari 24 mahasiswa Jurusan Gizi Poltekkes Kemenkes Riau. Selama pemberian brunch, tim peneliti mendampingi subjek sampai selesai mengkonsumsinya. The brunch menu was changed	

	daily, which may include *gado-gado*, egg noodle, *batagor*, *lontong medan*, sandwich, chicken porridge, fried rice teri and bread. The total amount of energy of the meals and milk was 600 calories (30% of RDA). Terjadi peningkatan percepatan tinggi badan subjek setelah intervensi. Rata-rata tinggi badan subjek sebelum intervensi gizi yaitu 143,6±5,2(133,6:154,9) dan setelah diberi intervensi gizi yaitu 144,9±5,1(134,7:155,2) cm. Kandungan Pyd sebelum intervensi 9.81±7.02 dan kandungan Pyd setelah intervensi 5.33±2.89 nmol/mmol creatinine (Tabel 2). Terjadi penurunan kandungan Pyd remaja setelah pemberian intervensi gizi selama 34 hari. 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. Kekurangan penelitian tahun 2020 ini yaitu subjek terdiri dari perempuan dan laki-laki. Untuk masa yang akan datang maka sebaiknya dibedakan antara subjek laki-laki dan perempuan. Keunggulan penelitian ini yaitu brunch dibeli di warung yang berada di sekitar sekolah tempat penelitian dilakukan. Dengan demikian diharapkan siswa-siswa di sekolah dapat membeli makanan tersebut dengan jenis, jumlah dan waktu yang tepat sehingga dapat memenuhi kebutuhan gizi mereka walaupun pemberian inervensi gizi yang diberikan oleh peneliti sudah selesai dilakukan seiiing	

	dengan berkahirnya waktu penelitian. Artinya walaupun penelitian ini sudah selesai dilakukan diharapkan kebiasaan jajan mereka dapat berlanjut sesuai dengan pola yang diterapkan ketika penelitian dilakukan. Uang jajan mereka mencukupi untuk membeli makanan seperti yang dilakukan ketika intervensi gizi dalam penelitian. Penyuluhan tentang pentingnya brunch yang benar agar remaja mengetahui jenis, jumlah dan waktu jajan yang dapat mencukupi kebutuhan gizi untuk mencapai pertumbuhan linier yang optimal perlu dilakukan. Selama ini jajan mereka tidak tepat sehingga tidak dapat memenuhi kebutuhan gizi yang dapat menimbulkan stunting. The limitation of research yaitu pada penelitian intervensi gizi ini yaitu tidak ada kelompok control; lama pemberian intervensi gizi hanya 1 bulan dari yang seharusnya minimal tiga bulan. The suggestion for future research will prevent the limitation yaitu perlu ada kelompok control dan ditambah durasi pemeberian intervensi menjadi minimal selama 3 bulan, sangat bagus jika sampai 8 atau 12 bulan, bahkan 24 bulan. 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.	

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The limitation of research need to explain, so the suggestion for future research will prevent the limitation

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Conclusion		
References (Appropriateness)		

Current Research in Nutrition and Food Science

Author's Response to Reviewer's Comments

Reviewer number 2

Paper title: Is Urinary Pyridinium Crosslinks Associated with Stunting in Stunting Children in Indonesia in 2014-2020

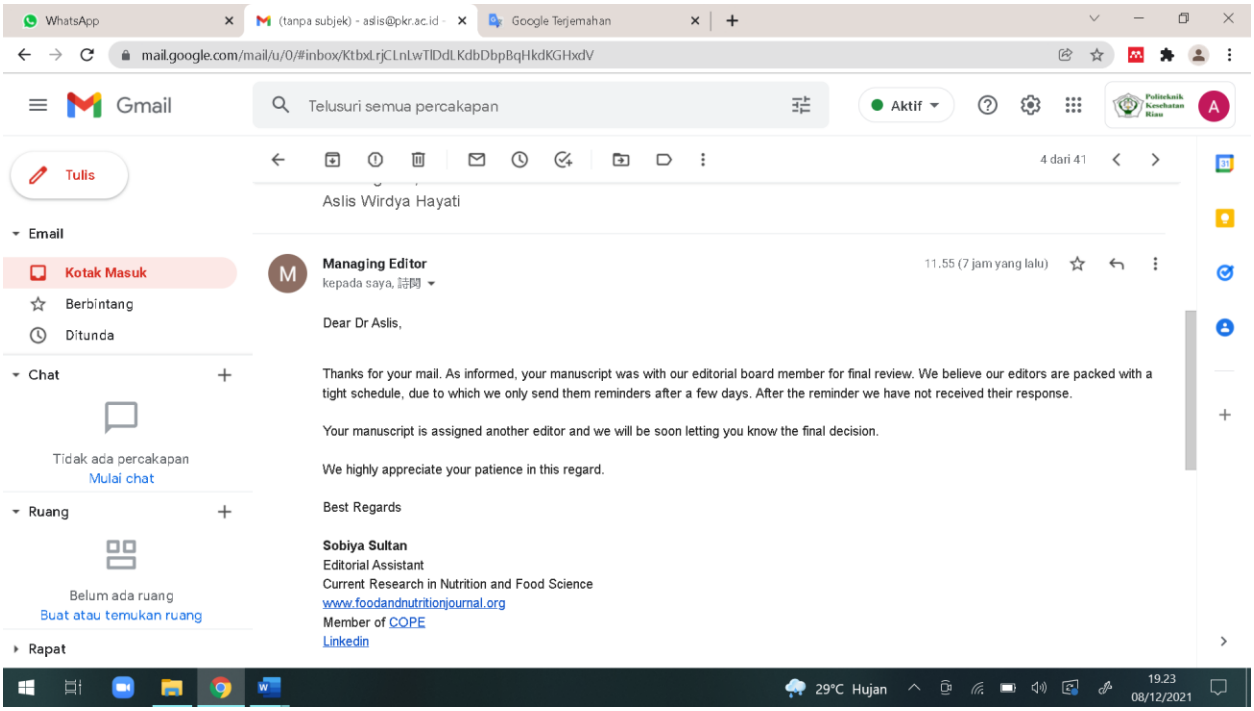
Title	Reviewer's Comments	Author's Response
	The duration of the study **,2014-2020,** could be omitted from the title	Saya sudah omitted the duration of the study**, 2014-2020,** from the title
	Is Urinary Pyridinium Crosslinks Associated with Stunting in Stunting Children in Indonesia ~~in 2014-2020~~	Is Urinary Pyridinium Crosslinks Associated with Stunting in Stunting Children in Indonesia
Abstract	Excellent, **but** needs a closing sentence as a recommendation	Sudah ditambahkan closing sentence as a recommendation
	Results: 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	**Results**: 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,

	years before and after nutritional intervention were 16.9, 15.3, 9.81 and 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.	respectively. The Pyd content of stunted children aged 4-6 and 12-15 years before and after nutritional intervention were 16.9, 15.3, 9.81 and 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. The 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.
Keywords		
Introduction		
Methodology	Very good, **but** has some note such as: **Equipment** and Materials: better to be changed to **Subjects** and Materials	Sudah diperbaiki sesuai saran reviewer
	Materials and Methods	**Subject and Material**
		~~**Equipment and Materials**~~
		~~**Research Procedure**~~ ~~**Urine Collection**~~
		~~**Pyd Urine Measurement and Standardization**~~
		~~**Creatinine Urine Measurement and Standardization**~~

Results	The title of the first table not compatible with the content.	Sudah saya perbaiki sesui saran Reviewer
	Table 1. Pyd content in urine based on nutritional intervention (nmol /mmol creatinine)	**Table 1. The socioeconomic characteristics of the child's family**
	Table (1) is very huge table, so better to be divided into more than one table to be simple (for example: one with a title of **The socioeconomic characteristics of the child's family**, other one titled**, Urine Pyd content based on nutritional intervention……..etc…**	**Table 2. Urine Pyd content based on nutritional intervention**
		Table 3. Urine Pyd content based on sex
	Also, the results section needs more explanation (elaboration) concerning the obtained results	Ada penurunan kandungan Pyd subjek sebelum dan setelah pemberian intervensi gizi dan pemberian intervensi gizi berupa makanan dengan additional energy, namely 30% nutritional adequacy rate / RDA dapat merubah status gizi responden dari stunting menjadi normal (Tabel 1). Pemberian intervensi gizi kepada anak stunting dalam penelitian ini ada dua kali penelitian yaitu pertama pada

		penelitian tahun 2018 dan kedua yaitu pada penelitian tahun 2020. Pada penelitian pertama, diberikan milk every day and four eggs per week for 4 months; additional energy, namely 20% nutritional adequacy rate / RDA. Pada peneltian kedua, diberikan brunch meals and milk daily for 34 days; additional energy, namely 30% nutritional adequacy rate / RDA. Hasil penelitian pertama yaitu 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. Pada penelitian kedua, 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.
Discussion		
Conclusion		
References (Appropriateness)		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Berbintang
Ditunda
Chat
Tidak ada percakapan
Mulai chat
Ruang
Belum ada ruang
Buat atau temukan ruang
Rapat
Managing Editor
kepada saya, 詩岡
11.55 (7 jam yang lalu)
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Thanks for your mail. As informed, your manuscript was with our editorial board member for final review. We believe our editors are packed with a tight schedule, due to which we only send them reminders after a few days. After the reminder we have not received their response.
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Current Research in Nutrition and Food Science,
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Is Urinary Pyridinium Crosslinks Associated with Stunting in Stunting Children in Indonesia?

Abstract

Objective: The objective of this study was to analyze the correlation between pyridinium crosslinks (Pyd) in urine and stunting among children. We also determined the effect of nutritional intervention on the Pyd content in urine among stunting children.
Methods: 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.
Results: The Pyd content obtained for stunted children aged in 0-3 days, 3-5 years, 4-6 years, and 12-15 years were 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 was 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 an inverse 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. The 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.

Key-words

Children, Height, Pyridinium Crosslinks Urine, Stunting

Comment [A1]: Need to indicate if "spot urine" samples or 24-hour urine samples were used.

Comment [A2]: What is Height "guage"?

Comment [A3]: What is "pot" urine?

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Comment [A4]: What was the value for the "Normal neonatal" urine Pyd content?

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Comment [A5]: This sentence makes no sense. Need to rephrase for clarity. Why "expected" to be a marker……?

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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 was 36.8% in 2007[1], 35.6% in 2010[2], 37.2% in 2013[3] 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 in Indonesia were stunted[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 was 35.2%., the prevalence was 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 ≥40%[7]. World Health Organization (WHO) established stunting standards based on anthropometric 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

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Comment [A6]: The sentence need to be rephrased, because it is not urinary pyridinium crosslinks that are removed. Need to delete "Urinary".
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protein, stabilized by cross-linking between the N terminal and C terminal in the formation of the base of bone tissue. The pyridinium crosslinks (Pyd) are formed by hydroxylline 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; occurs 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).

Comment [A7]: This sentence makes no sense.

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Comment [A8]: Why until now?

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Comment [A9]: It is not clear what biochemical parameters are referred to in this sentence. Need to give reference(s) to support this statement or delete.

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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.

GENERAL COMMENTS: This section is too long and repetitive. The author(s) need to reduce this section by about 25%.

Methods

Study Design

This cross-sectional study was carried out 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 neonates were in *Andini* Mother and Child Hospital, Pekanbaru City, Riau Province. In 2017, 80 children aged 4-6 years were selected. They 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; they 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.

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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

The parents of all the 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^{o}C (GEA by Vestfrost-Denmark Type G.201 Serial No: 20021808005) until further analysis.

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Comment [A10]: Need to rephrase this sentence because it makes no sense.

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 collected 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].

Comment [A11]: How was 10ml of urine collected from a neonate 0-3 day of age? Give the reference.

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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).

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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.

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).

The Pyd content in urine of stunted girls was found to be higher than the Pyd content of stunted boys (Table 3). The Pyd content of stunted neonates' urine for men and women were 988.45 and

Comment [A12]: This is not the acceptable format for presenting results in a scientific paper.
Need to give the average height including the Standard Deviation for the parents in the various groups.

Comment [A13]: The information presented in this section is contradictory.
What was the parameter used for classification of the children in the various groups as "Very stunted", "Stunted" "Normal"?
The information is not presented in the method section.
In the various groups, how many were "very stunted", "stunted" and "normal"?
Such data is not presented in the result section.

Comment [A14]: Which men and women are you referring to?????

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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 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 ($p < 0.05$) ($r = -0.242$).

Comment [A15]: How did you get the "normal" neonates? The indication was that all the neonates were stunted.

Comment [A16]: How many were "very stunted", "stunted" and "normal"? What was the basis for the classification, it is not stated in the method section.

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).

Comment [A17]: This is a contradictions of the data presented in Table 2.

Comment [A18]: Need to give the p-value to indicate statistical significance of the result.

General comment: This section is very poorly written for publication in a scientific journal. The author(s) need to review the data presented to clearly indicate the parameter used for classification of the children as very stunted, stunted and normal. Table 1 is poorly presented. Table 2 is also poorly presented. Table 3 is not clear, because a total of 173 children participated in the study (32, 80, 25, 36), however the numbers presented in the table do not add up to 173. The total number presented in Table 3 is 45. What is the justification for such a significant change in numbers? The author(s) need to account for the intial number of 173 and the final number of 45 presented in Table 3. In my view, this manuscript cannot be recommended for publication in the present form because of the poor presentation of the results.

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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 pyridinium 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 patients compared to controls ([edian 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

Comment [A19]: This statement is not true because different groups of children participated in the study.
You can say that the Pyd content is different in children in different age groups.

Comment [A20]: What previous studies, need to give the references of the studies.

Comment [A21]: Need to focus on teenagers not adults. Your study did not include adults.

Field Code Changed

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Comment [A22]: This is completely irrelevant to your study design. Need to focus your discussion on the findings in you present study and compare with other studies with similar design.

Comment [A23]: Completely irrelevant to your present study design.

Comment [A24]: Your study design included children 12 to 15 years of age.

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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 µmol / mol of creatinine[19].

Comment [A25]: Irrelevant to this study.

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 rate (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.

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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].

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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 pyd 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. Urine Pyd cross-link also shows great hope as a marker of therapeutic efficacy in bone disorders associated with accelerated bone resorption[29].

Comment [A26]: Need to transfer to the Introduction section. No need to repeat this information in the discussion section.

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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.

Comment [A27]: The categories are not presented in the result section. Therefore this information is not clearly presented in the result section.

Comment [A28]: Not clearly presented in the result section. Thus it seems like just a speculation.

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 at al[31] 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.

Comment [A29]: Need to transfer such details to the Introduction section. For a scientific paper, you only need to give the reference for the study.

Another research related to the provision of nutrition and linear growth interventions, namely the research of Matali, Wungouw and Sapulete[32] in Manado (Indonesia) which carried out an

Comment [A30]: All these are more for a student project report, not for a manuscript to be published in a scientific journal.

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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 the 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.

Comment [A31]: ?????????? Need to give only the reference of this study, not the details.

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].

Comment [A32]: MORE FOR A STUDENT PROJECT REPORT!!!!!!

The research that the authors conducted in 2018 aims to determine the effect of milk and egg consumption on the content of Pyridinium Crosslinks (Pyd) urine of stunting children aged 4-6 years. The research was conducted in Early Childhood Education (PAUD) Al Falah, Lima Puluh 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,

Comment [A33]: No need to repeat information already stated earlier.

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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. 1,500 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 Ho and conclude that there is a statistically significant difference between the mean height before and after the intervention.

Comment [A34]: Should have been included in the method section not the discussion section.

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 2007[1] and in 2010[2] 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 Pyd 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 Pyd 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.

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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.

GENERAL COMMENT: This section is extremely poorly written. It is for the final project report of a final year student, not for publication in a scientific journal. The author(s) need to consult with senior colleagues for guidance on how to write a manuscript for publication in a scientific journal. This section should be reduced by 75%, if the author(s) wish to correct it and resubmit for further review. The manuscript CANNOT BE RECOMMENDED FOR PUBLICATION IN THE PRESENT FORM.

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.

Acknowledgements

Herewith we convey our thanks and best regard for financial support from Health Polytechnic, Ministry of Health, Riau; the research facilities Andini Hospital, As Shofa Kindergarten and Hidayatullah Kindergarten, SMP Negeri 3 Pekanbaru and PAUD Al Falah, Lima Puluh Kota Kota district; urine Pyd content analysis facility from Prodia Clinical Laboratory Pekanbaru and Jakarta, and milk assistance from PT Indolakto Jakarta.

Funding Sources

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Conflict of Interest

The authors declare no conflict of interest.

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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 normal neonatal urine ($p < 0.01$). The pattern of Pyd content in urine according to height was like the letter "U"	There was a negative correlation observed between urine Pyd content and the subject's height ($p < 0.05$). Urine Pyd content showed a weak negative correlation with height at $r = -0.242$	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	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

Comment [A35]: Need to include the percentages not just the absolute numbers.

Comment [A36]: Only boys?

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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 (n = ??)**	-	18.1	-	-
• **Stunting (n = ??)**	982	16.4	-	-
• **Normal (n =??)**	594	15.5	-	-
• **Before nutritional Intervention (n = ??)**	-	-	16.9	9.81
• **After nutritional Intervention (n = ??)**	-	-	15.3	5.33

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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)

Politeknik Kesehatan Riau

Aslis Wirda Hayati <aslis@pkr.ac.id>

Final comments from the editor

7 messages

Managing Editor <info@foodandnutritionjournal.org> Tue, Dec 21, 2021 at 2:08 PM
To: Aslis Wirda Hayati <aslis@pkr.ac.id>

Dear Dr. Aslis,

We have received the final comments on your manuscript from our editorial board member. Following are their suggestions-

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Editor's comments.pdf
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Aslis Wirda Hayati <aslis@pkr.ac.id> Thu, Apr 7, 2022 at 1:27 PM
To: Managing Editor <info@foodandnutritionjournal.org>

Dear Editorial Team,

Thankyou for your last follow up mail, and I am sorry for taking some time longer than expected.
Along with this mail, I attach the final revision of my manuscript.

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Aslis WH

Pada tanggal Sel, 21 Des 2021 pukul 14.08 Managing Editor <info@foodandnutritionjournal.org> menulis:

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Managing Editor <info@foodandnutritionjournal.org> Tue, Apr 12, 2022 at 1:54 PM
To: Aslis Wirda Hayati <aslis@pkr.ac.id>

Dear Dr. Aslis,

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On Thu, Apr 7, 2022 at 11:57 AM Aslis Wirda Hayati <aslis@pkr.ac.id> wrote:

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Aslis Wirda Hayati <aslis@pkr.ac.id> Wed, Apr 13, 2022 at 1:00 PM
To: amany.akhyar@gmail.com

---------- Forwarded message ---------
Dari: **Managing Editor** <info@foodandnutritionjournal.org>
Date: Sel, 12 Apr 2022 pukul 13.54
Subject: Re: Final comments from the editor
To: Aslis Wirda Hayati <aslis@pkr.ac.id>

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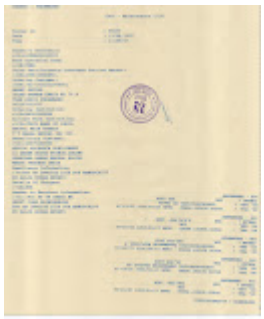

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Aslis Wirda Hayati <aslis@pkr.ac.id> Sat, Apr 16, 2022 at 11:48 AM
To: Managing Editor <info@foodandnutritionjournal.org>

Pyd is a convincing and noninvasive biomarker to determine stunting in children using urine.

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On Thu, Apr 7, 2022 at 11:57 AM Aslis Wirda Hayati <aslis@pkr.ac.id> wrote:

> Dear Editorial Team,
>
> Thankyou for your last follow up mail, and I am sorry for taking some time longer than expected.
> Along with this mail, I attach the final revision of my manuscript.
>
> Best Regards,
> Aslis WH
>
> Pada tanggal Sel, 21 Des 2021 pukul 14.08 Managing Editor <info@foodandnutritionjournal.org> menulis:
>
> > Dear Dr. Aslis,
> >
> > We have received the final comments on your manuscript from our editorial board member. Following are their suggestions-
> >
> > *"I have reviewed the manuscript that you sent to me for final recommendation. Please find attached the reviewed manuscript in both MS Word document and PDF. My comments are within the text of the manuscript."*
> >
> > We kindly request you to revise the file as per their comments and resubmit.
> > Once done please send us the required at your earliest.
> >
> > Best Regards
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> > **Sobiya Sultan**

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

Abstract

Objective: The objective of this study was to analyze the correlation between pyridinium crosslinks (Pyd) in urine and stunting among children. We also determined the effect of nutritional intervention on the Pyd content in urine among stunting children.
Methods: 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.
Results: The Pyd content obtained for stunted children aged in 0-3 days, 3-5 years, 4-6 years, and 12-15 years were 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 was 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 an inverse 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. The 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.

Key-words

Children, Height, Pyridinium Crosslinks Urine, Stunting

Comment [A1]: Need to indicate if "spot urine" samples or 24-hour urine samples were used.

Comment [A2]: What is Height "guage"?

Comment [A3]: What is "pot" urine?

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Comment [A4]: What was the value for the "Normal neonatal" urine Pyd content?

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Comment [A5]: This sentence makes no sense. Need to rephrase for clarity. Why "expected" to be a marker……?

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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 was 36.8% in 2007[1], 35.6% in 2010[2], 37.2% in 2013[3] 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 in Indonesia were stunted[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 was 35.2%., the prevalence was 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 ≥40%[7]. World Health Organization (WHO) established stunting standards based on anthropometric 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

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Comment [A6]: The sentence need to be rephrased, because it is not urinary pyridinium crosslinks that are removed. Need to delete "Urinary".
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protein, stabilized by cross-linking between the N terminal and C terminal in the formation of the base of bone tissue. The pyridinium crosslinks (Pyd) are formed by hydroxylline 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; occurs 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).

Comment [A7]: This sentence makes no sense.

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Comment [A8]: Why until now?

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Comment [A9]: It is not clear what biochemical parameters are referred to in this sentence. Need to give reference(s) to support this statement or delete.

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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.

GENERAL COMMENTS: This section is too long and repetitive. The author(s) need to reduce this section by about 25%.

Methods

Study Design

This cross-sectional study was carried out 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 neonates were in *Andini* Mother and Child Hospital, Pekanbaru City, Riau Province. In 2017, 80 children aged 4-6 years were selected. They 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; they 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.

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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

The parents of all the 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^{o}C (GEA by Vestfrost-Denmark Type G.201 Serial No: 20021808005) until further analysis.

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Comment [A10]: Need to rephrase this sentence because it makes no sense.

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 collected 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].

Comment [A11]: How was 10ml of urine collected from a neonate 0-3 day of age? Give the reference.

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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).

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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.

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).

The Pyd content in urine of stunted girls was found to be higher than the Pyd content of stunted boys (Table 3). The Pyd content of stunted neonates' urine for men and women were 988.45 and

Comment [A12]: This is not the acceptable format for presenting results in a scientific paper.
Need to give the average height including the Standard Deviation for the parents in the various groups.

Comment [A13]: The information presented in this section is contradictory.
What was the parameter used for classification of the children in the various groups as "Very stunted", "Stunted" "Normal"?
The information is not presented in the method section.
In the various groups, how many were "very stunted", "stunted" and "normal"?
Such data is not presented in the result section.

Comment [A14]: Which men and women are you referring to?????

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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 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 ($p < 0.05$) ($r = -0.242$).

Comment [A15]: How did you get the "normal" neonates? The indication was that all the neonates were stunted.

Comment [A16]: How many were "very stunted", "stunted" and "normal"? What was the basis for the classification, it is not stated in the method section.

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).

Comment [A17]: This is a contradictions of the data presented in Table 2.

Comment [A18]: Need to give the p-value to indicate statistical significance of the result.

General comment: This section is very poorly written for publication in a scientific journal. The author(s) need to review the data presented to clearly indicate the parameter used for classification of the children as very stunted, stunted and normal. Table 1 is poorly presented. Table 2 is also poorly presented. Table 3 is not clear, because a total of 173 children participated in the study (32, 80, 25, 36), however the numbers presented in the table do not add up to 173. The total number presented in Table 3 is 45. What is the justification for such a significant change in numbers? The author(s) need to account for the intial number of 173 and the final number of 45 presented in Table 3. In my view, this manuscript cannot be recommended for publication in the present form because of the poor presentation of the results.

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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 pyridinium 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 patients compared to controls ([edian 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

Comment [A19]: This statement is not true because different groups of children participated in the study.
You can say that the Pyd content is different in children in different age groups.

Comment [A20]: What previous studies, need to give the references of the studies.

Comment [A21]: Need to focus on teenagers not adults. Your study did not include adults.

Field Code Changed

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Comment [A22]: This is completely irrelevant to your study design. Need to focus your discussion on the findings in you present study and compare with other studies with similar design.

Comment [A23]: Completely irrelevant to your present study design.

Comment [A24]: Your study design included children 12 to 15 years of age.

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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 µmol / mol of creatinine[19].

Comment [A25]: Irrelevant to this study.

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 rate (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.

Field Code Changed

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].

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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 pyd 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. Urine Pyd cross-link also shows great hope as a marker of therapeutic efficacy in bone disorders associated with accelerated bone resorption[29].

Comment [A26]: Need to transfer to the Introduction section. No need to repeat this information in the discussion section.

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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.

Comment [A27]: The categories are not presented in the result section. Therefore this information is not clearly presented in the result section.

Comment [A28]: Not clearly presented in the result section. Thus it seems like just a speculation.

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 at al[31] 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.

Comment [A29]: Need to transfer such details to the Introduction section. For a scientific paper, you only need to give the reference for the study.

Another research related to the provision of nutrition and linear growth interventions, namely the research of Matali, Wungouw and Sapulete[32] in Manado (Indonesia) which carried out an

Comment [A30]: All these are more for a student project report, not for a manuscript to be published in a scientific journal.

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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 the 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.

Comment [A31]: ?????????? Need to give only the reference of this study, not the details.

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].

Comment [A32]: MORE FOR A STUDENT PROJECT REPORT!!!!!!

The research that the authors conducted in 2018 aims to determine the effect of milk and egg consumption on the content of Pyridinium Crosslinks (Pyd) urine of stunting children aged 4-6 years. The research was conducted in Early Childhood Education (PAUD) Al Falah, Lima Puluh 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,

Comment [A33]: No need to repeat information already stated earlier.

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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. 1,500 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 Ho and conclude that there is a statistically significant difference between the mean height before and after the intervention.

Comment [A34]: Should have been included in the method section not the discussion section.

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 2007[1] and in 2010[2] 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 Pyd 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 Pyd 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.

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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.

GENERAL COMMENT: This section is extremely poorly written. It is for the final project report of a final year student, not for publication in a scientific journal. The author(s) need to consult with senior colleagues for guidance on how to write a manuscript for publication in a scientific journal. This section should be reduced by 75%, if the author(s) wish to correct it and resubmit for further review. The manuscript CANNOT BE RECOMMENDED FOR PUBLICATION IN THE PRESENT FORM.

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.

Acknowledgements

Herewith we convey our thanks and best regard for financial support from Health Polytechnic, Ministry of Health, Riau; the research facilities Andini Hospital, As Shofa Kindergarten and Hidayatullah Kindergarten, SMP Negeri 3 Pekanbaru and PAUD Al Falah, Lima Puluh Kota Kota district; urine Pyd content analysis facility from Prodia Clinical Laboratory Pekanbaru and Jakarta, and milk assistance from PT Indolakto Jakarta.

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Conflict of Interest

The authors declare no conflict of interest.

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Field Code Changed

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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 normal neonatal urine ($p < 0.01$). The pattern of Pyd content in urine according to height was like the letter "U"	There was a negative correlation observed between urine Pyd content and the subject's height ($p < 0.05$). Urine Pyd content showed a weak negative correlation with height at $r = -0.242$	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	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

Comment [A35]: Need to include the percentages not just the absolute numbers.

Comment [A36]: Only boys?

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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 (n = ??)**	-	18.1	-	-
• **Stunting (n = ??)**	982	16.4	-	-
• **Normal (n =??)**	594	15.5	-	-
• **Before nutritional Intervention (n = ??)**	-	-	16.9	9.81
• **After nutritional Intervention (n = ??)**	-	-	15.3	5.33

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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)

Politeknik Kesehatan Riau

Aslis Wirda Hayati <aslis@pkr.ac.id>

Article is online

6 messages

Managing Editor <info@foodandnutritionjournal.org> Fri, Apr 22, 2022 at 5:04 PM
To: Aslis Wirda Hayati <aslis@pkr.ac.id>

Dear Author,

We are glad to inform you that your article has been published online.
Please check the link below:
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Also, attached is the PDF proof of your article. Kindly go through and let us know if any corrections are needed at the earliest.

You are requested to proofread and send us if any corrections are required in the manuscript by 25th April.

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Dear Dr Warda,

We would like to inform you that the DOI will be assigned once the issue gets online.
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Yanha Ahmed

Editorial Assistant

Current Research in Nutrition and Food Science

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April Issue Volume 10 Number 1 is online

4 messages

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Have a great weekend!

Best Regards

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Aslis Wirda Hayati <aslis@pkr.ac.id> Sat, Apr 30, 2022 at 8:59 AM
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Subject: Fwd: April Issue Volume 10 Number 1 is online
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Dear **Sobiya Sultan**
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Current Research in Nutrition and Food Science

Thank you for informing us.

Best Regards
Aslis Wirda Hayati
[Quoted text hidden]