

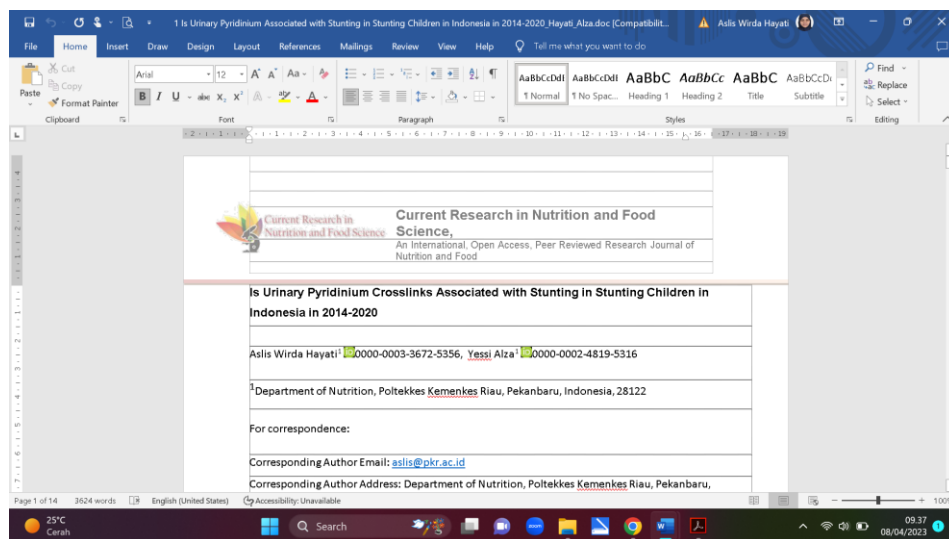
ARTICLE PUBLISHING HISTORY

The screenshot shows the article page for "Is Urinary Pyridinium Crosslinks Associated with Stunting in Stunting Children in Indonesia" on the Food and Nutrition Journal website. The article is by Aslis Wirda Hayati* and Yessi Alza. It was received on 01 Mar 2021, accepted on 07 Mar 2022, and published online on 18 Apr 2022. The article has 414 views and 488 PDF downloads. It is indexed in CABELLS and has a CiteScore of 2021: 1.7 and SJR 2021. The journal is a member of COPE and is part of the CABELLS Scholarly Analytics program. The abstract states: "The objective of this study was to analyze the correlation between pyridinium crosslinks (Pvd) urine and stunting among children. We also determined the effect of nutritional intervention on the Pvd content in urine among stunting children. The study was a cross-sectional involvins".



1. Received: 01 Mar 2021

The screenshot shows a Gmail inbox with an email from "Current Research in Nutrition and Food Science" dated Mon, Mar 1, 2021, 9:26 AM. The email subject is "Successful Manuscript submission for Current Research in Nutrition and Food Science Journal". The body of the email reads: "Dear Aslis Wirda Hayati, Thank you for submitting your manuscript 'Is Urinary Pyridinium Crosslinks Associated with Stunting in Stunting Children in Indonesia in 2014-2020' through Online Submission! Shortly our team will mail you the status of manuscript. Please feel free to contact us back at info@foodandnutritionjournal.org. Our team will always be glad to answer your inquiries!!! Editorial Assistant, Current Research in Nutrition and Food Science, www.foodandnutritionjournal.org".

Artikel yang dikirim sebagai berikut:



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

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Abstract

The objective of this study was to analyze the correlation between pyridinium crosslinks (Pyd) urine and stunting among children. We also determined the effect of nutritional intervention on the Pyd content in urine among stunting children. The study was a cross-

sectional involving 173 children in Pekanbaru and 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, spot 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. 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.

About 59.3% of children aged 3-5 years were included stunting³. 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 Province⁵. Public health problems are considered severe if the prevalence of stunting is 30-39% and serious if the prevalence of stunting is $\geq 40\%$ ⁴. World Health Organization (WHO) established stunting standards based on anthropometrics measurement with Height for Age (HAZ)-score < -2 SD⁶.

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 (Pyr) are formed by hydroxylysine or lysine residues at the C- and N-telopeptide terminals of the collagen molecule and released during matrix resorption, excreted in the urine. Pyr 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 formation⁷.

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 osteoclasts⁹. 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 stunting¹⁰. Calcium forms complex bonds with phosphate which can provide strength to bones¹¹.

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.

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 Sumatera Province, Indonesia.

Subject and Urine Collection

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

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 simultaneously¹²

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 Staden¹⁴. 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

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 adult⁸. 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 later¹⁵. Pyd excretion for neonates was reported to be 642.7 ± 281.3 nmol/mmol creatinine by Fujimoto et al⁷.

In another study, Pyd excretion among elementary school children was reported to be about 50-500 nmol/mmol creatinine¹⁶. 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 ([median 131 vs 26 nmol/mmol creatinine ($p < 0,001$); in postmenopausal women urinary Pyd excretion was raised in those taking T4 which is 40.0 ± 2.7 nmol/mmol creatinine ($p < 0,05$)].

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

Reference interval Premenopausal adult female and male urine contained Pyd around 15.3-33.6 and 10.3-20.0 nmol / mmol creatinine. The target value for treated postmenopausal adult female was the same as the premenopausal reference

interval¹⁸. The Pyd content of premenopausal women's urine ranged from 3.0 to 7.4, whereas their male peers ranged from 2.3 to 5.4 $\mu\text{mol} / \text{mol}$ of creatinine¹⁹.

The growth spurt among boys occurs 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 years²⁰. 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 stops²¹. Relatively the same height at the age of 30-45 years. After 45 years there is a decrease in height²².

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.

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 resorption²³.

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.

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	Brunch meals and milk daily for 34 days. Additional energy,

			months. Additional energy, namely 25% nutritional adequacy rate / RDA	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 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

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

^s average ± standard deviation (minimal: maximal)

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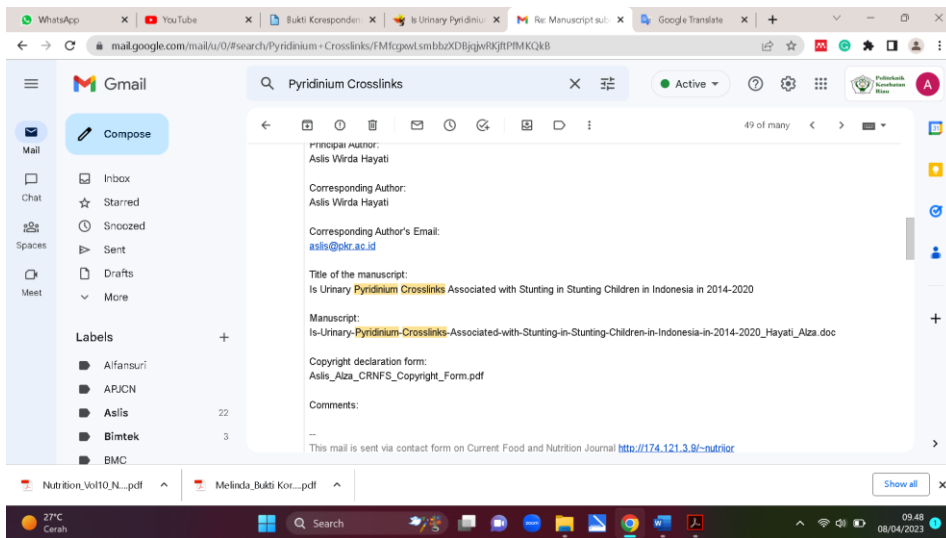
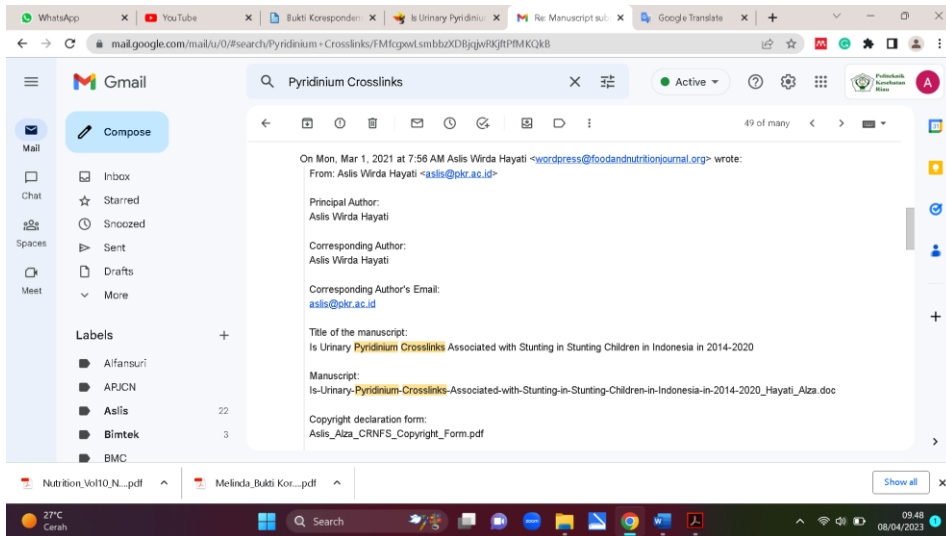
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Aslis Wirda Hayati -aslis@pkr.ac.id-
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Dear, Managing Editor

Thankyou for the warm welcome.

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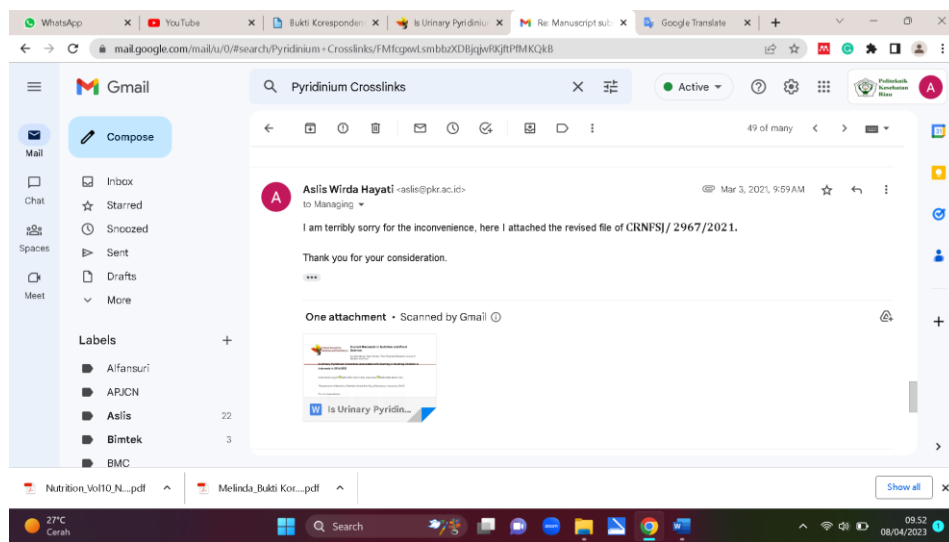
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Reply



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2. Revised: 03 Mar 2021



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

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Abstract

The objective of this study was to analyze the correlation between pyridinium crosslinks (Pyd) urine and stunting among children. We also determined the effect of nutritional intervention on the Pyd content in urine among stunting children. The study was a cross-sectional involving 173 children in Pekanbaru and 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. 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.

About 59.3% of children aged 3-5 years were included stunting³. 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 Province⁵. Public health problems are considered severe if the prevalence of stunting is 30-39% and serious if the prevalence of

stunting is $\geq 40\%$ ⁴. World Health Organization (WHO) established stunting standards based on anthropometrics measurement with Height for Age (HAZ)-score < -2 SD⁵.

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 (Pyc) are formed by hydroxylysine or lysine residues at the C- and N-telopeptide terminals of the collagen molecule and released during matrix resorption, excreted in the urine. Pyc 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 formation⁷.

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 osteoclasts⁹. 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 stunting¹⁰. Calcium forms complex bonds with phosphate which can provide strength to bones¹¹.

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.

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.

Subject and Urine Collection

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

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 simultaneously¹²

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 Staden¹⁴. 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

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 adult⁸. 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 later¹⁵. Pyd excretion for neonates was reported to be 642.7 ± 281.3 nmol/mmol creatinine by Fujimoto et al⁷.

In another study, Pyd excretion among elementary school children was reported to be about 50-500 nmol/mmol creatinine¹⁶. 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 interval¹⁸. The Pyd content of premenopausal women's urine ranged from 3.0 to 7.4, whereas their male peers ranged from 2.3 to 5.4 $\mu\text{mol} / \text{mol}$ of creatinine¹⁹.

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 years²⁰. 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 stops²¹. Relatively the same height at the age of 30-45 years. After 45 years there is a decrease in height²².

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.

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 P_{td} cross-link also shows great hope as a marker of therapeutic efficacy in bone disorders associated with accelerated bone resorption²³.

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

Conclusion

The P_{td} content of stunting children's urine was found to be different from normal children. There was a negative correlation observed between urine P_{td} content and children's height ($p < 0.05$). P_{td} content showed a weak correlation with height $r = -0.242$. There was a difference found in the P_{td} 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 P_{td} 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)	109±97(121:5)		

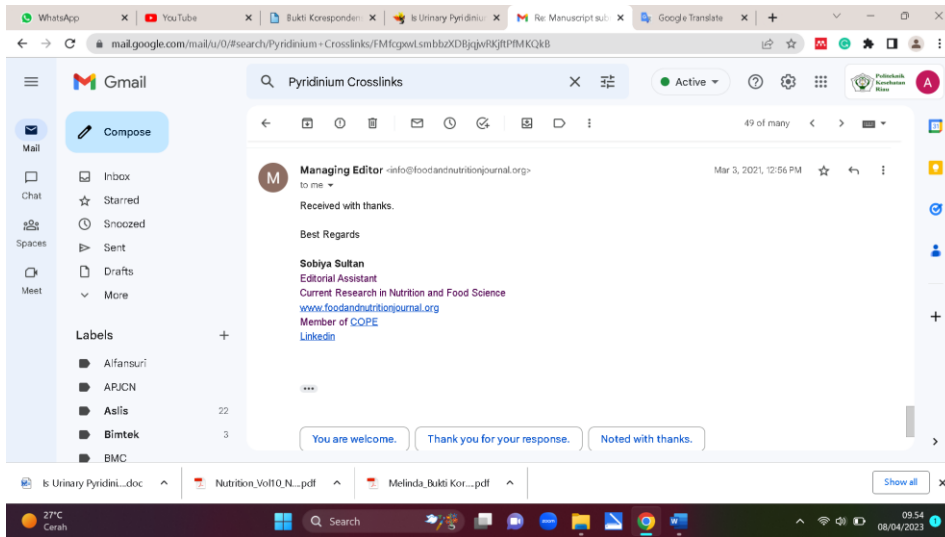
)b			
Nutritional status before intervention	Normal stunting	and	Normal stunting	and Stunting
Intervention	-		-	Milk every day and four eggs per week for 4 months. Additional energy, namely 25% nutritional adequacy rate / RDA
				Brunch meals and milk daily for 34 days. Additional energy, namely 30% 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
• After nutritional Intervention	-		-	15.3
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

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

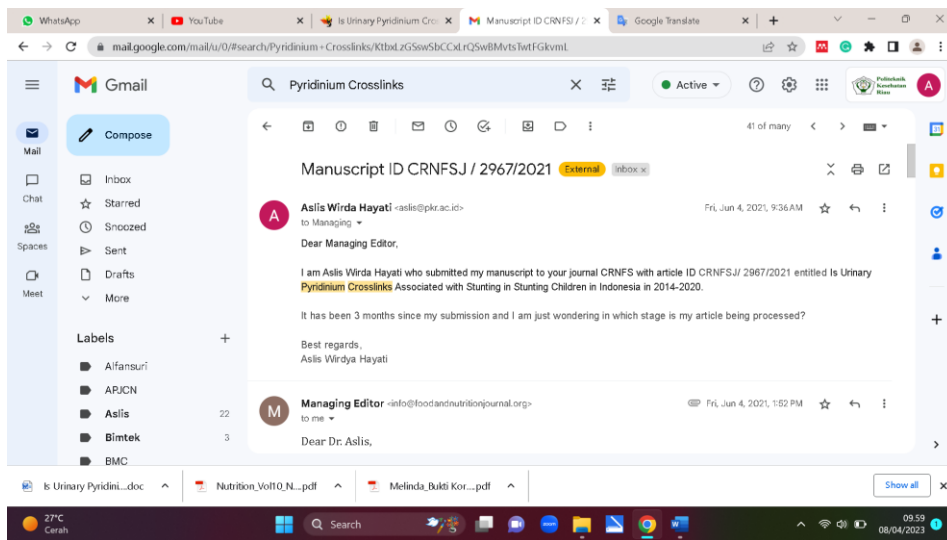
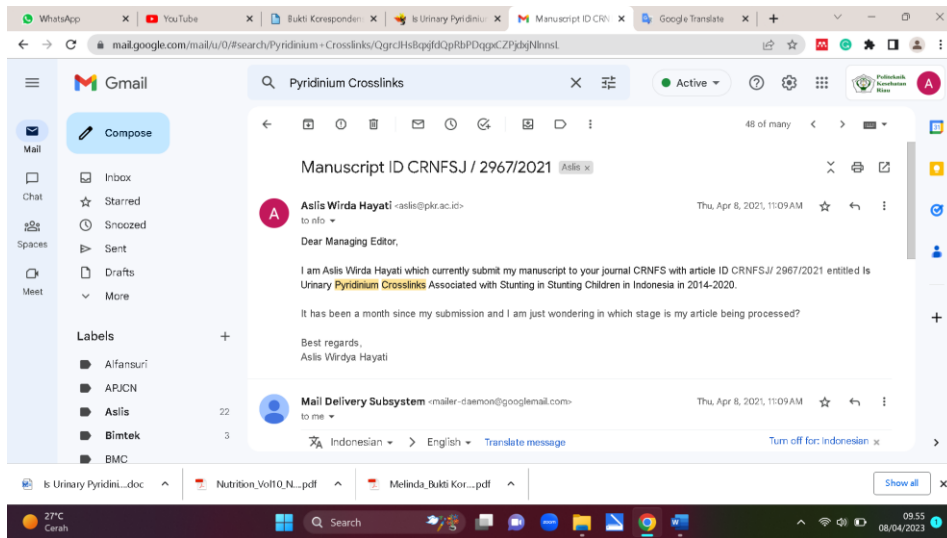
Year	Status nutritional intervention	Pyd (nmol/mmol creatinine)			
		n	Girl	n	Boy
2014	No intervention	2	988.45±29.20 (967.80: 1009.10)	7	641.40±257.73 (319.80: 1049.60)
2018	Before nutritional intervention	7	18.70±7.73(9.73: 29.79)	11	16.27±5.98(6.23:27.71)

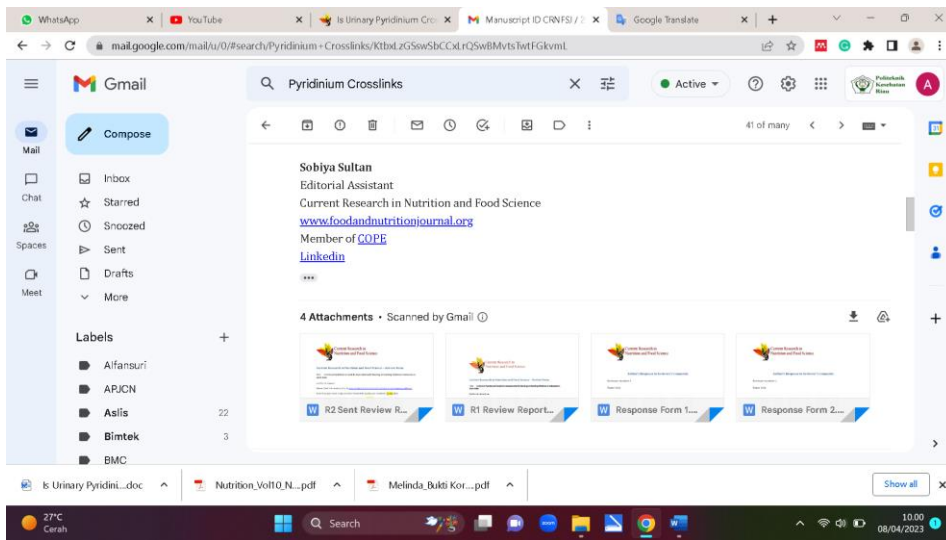
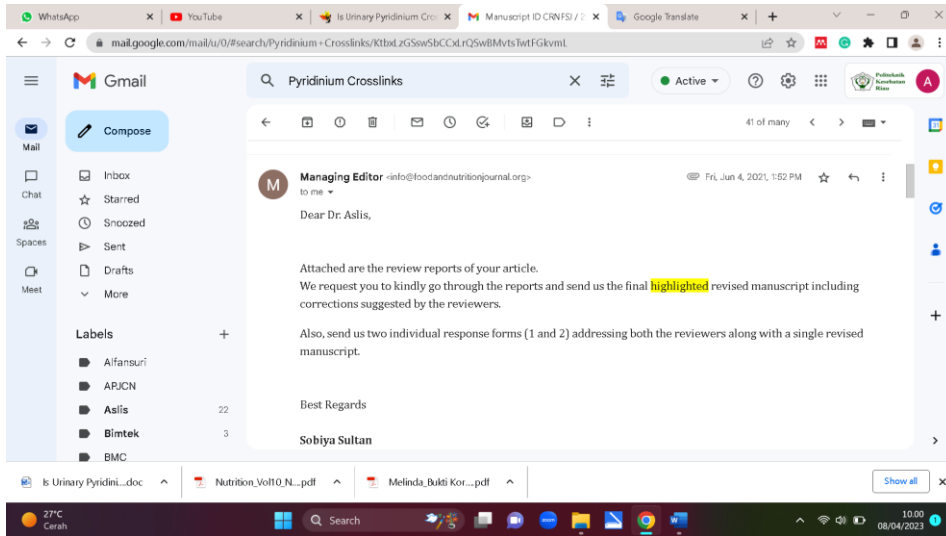
2018	After nutritional intervention	7	18.55±10.91 (9.84: 43.56)	11	14.64±3.63(9.07:20.80)
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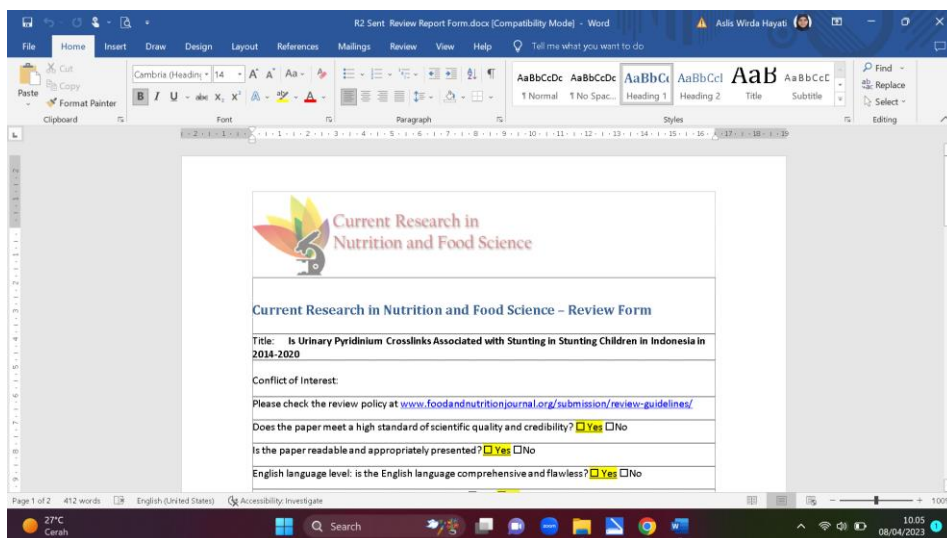
^saverage ± standard deviation (minimal: maximal)



3. Revised Manuscript: 04 Jun 2021







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Other Comments?

- Good addition to the scientific research
- The duration of the study ,2014-2020, could be omitted from the title

Comments per section of manuscript:

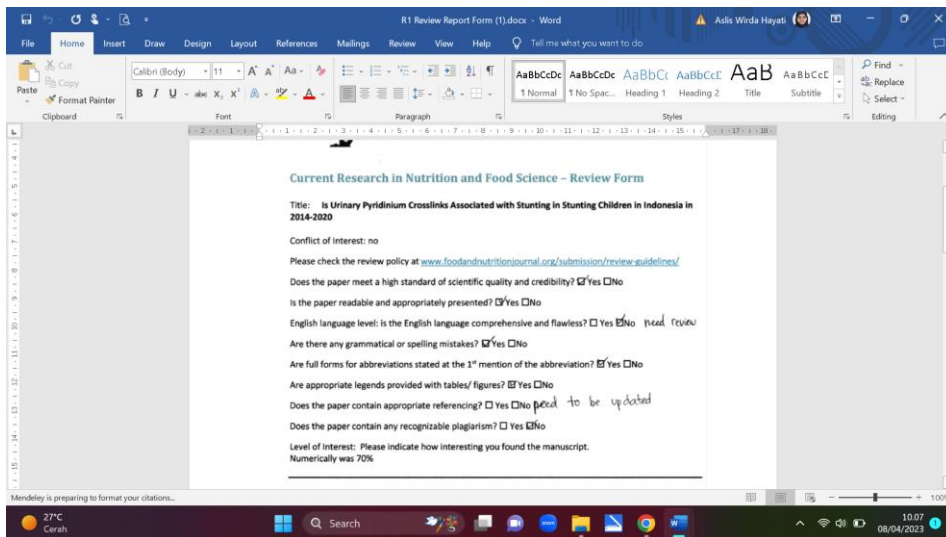
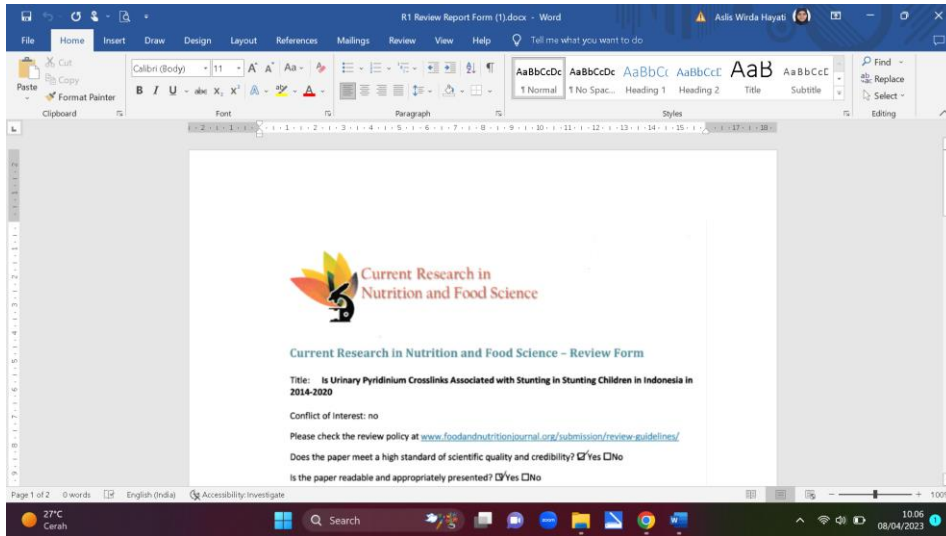
Abstract	Excellent, but needs a closing sentence as a recommendation
Introduction	Excellent
Methodology	Very good, but has some note such as: <ul style="list-style-type: none">- Equipment and Materials: better to be changed to Subjects and Materials
Results and Discussion	Results: <ul style="list-style-type: none">- The title of the first table not compatible with the content.- 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. . Also, the results section needs more explanation (elaboration) concerning the obtained results.
References (Appropriateness)	Vey appropriate

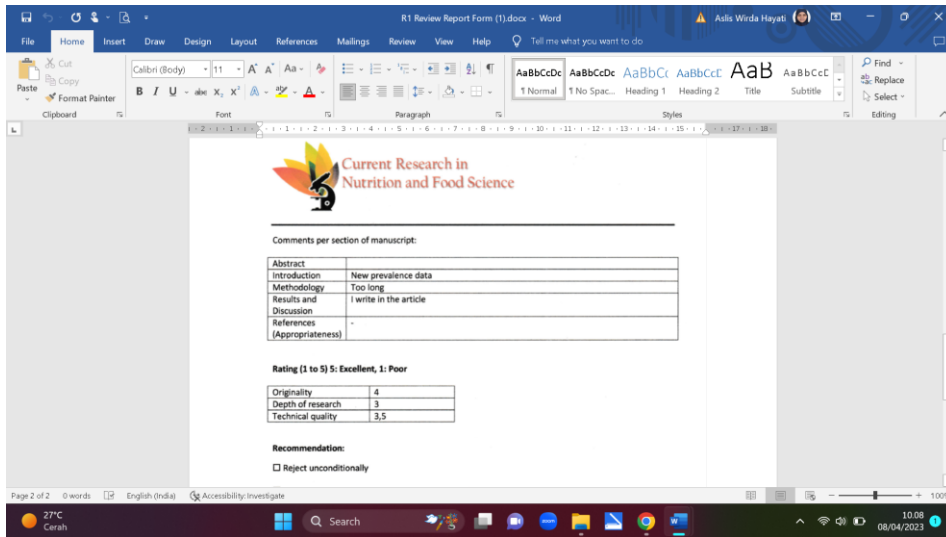
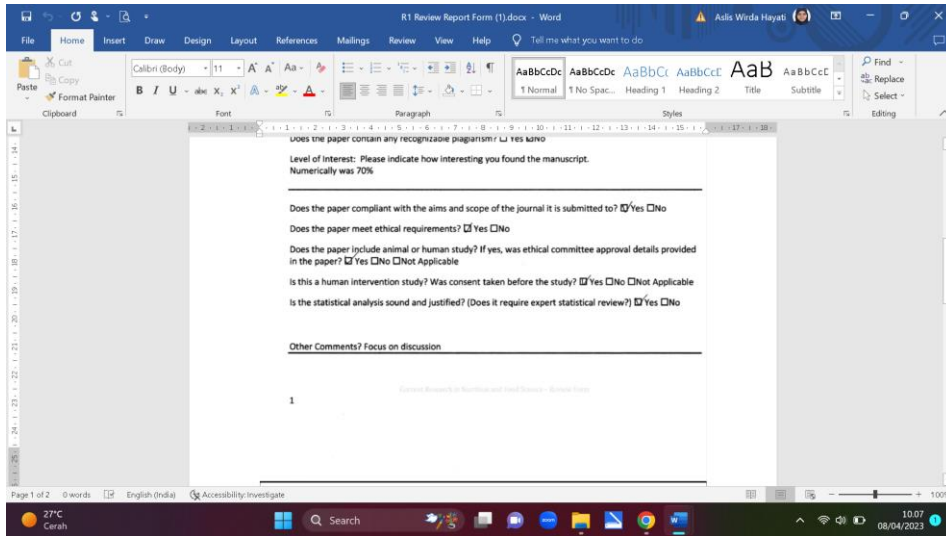
Rating (1 to 5) 5: Excellent, 1: Poor

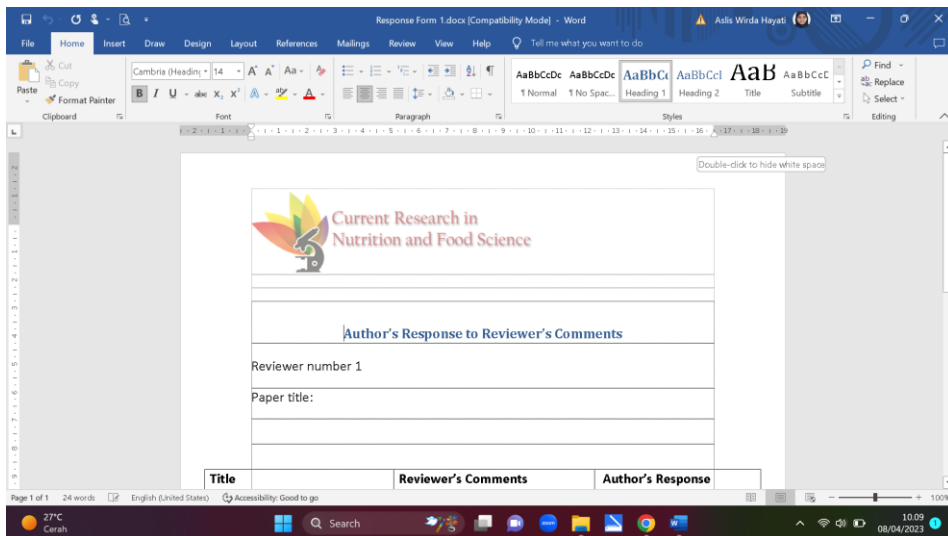
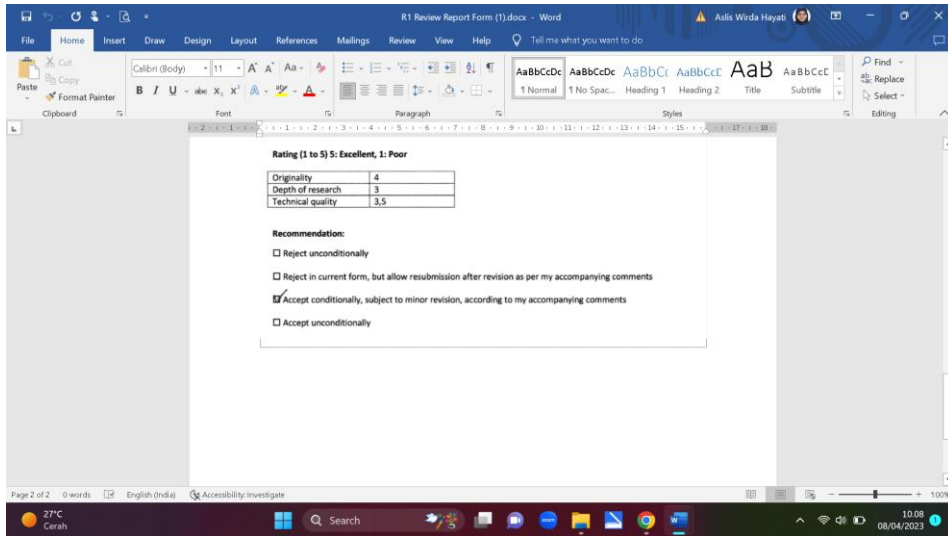
Originality	5
Depth of research	4
Technical quality	5

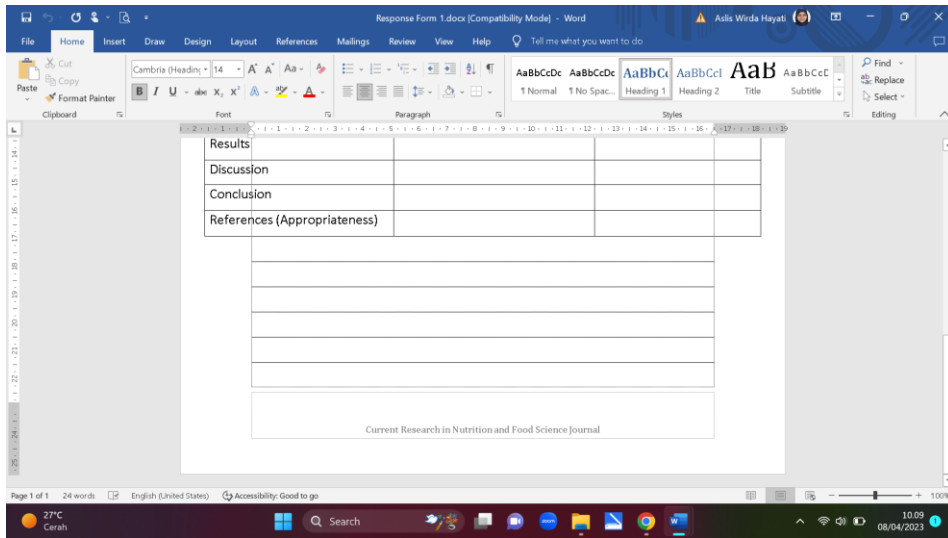
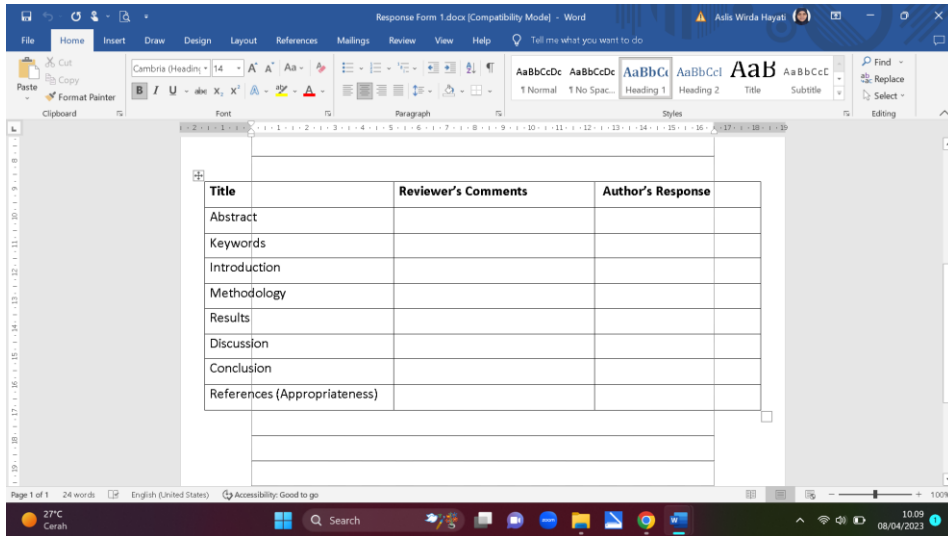
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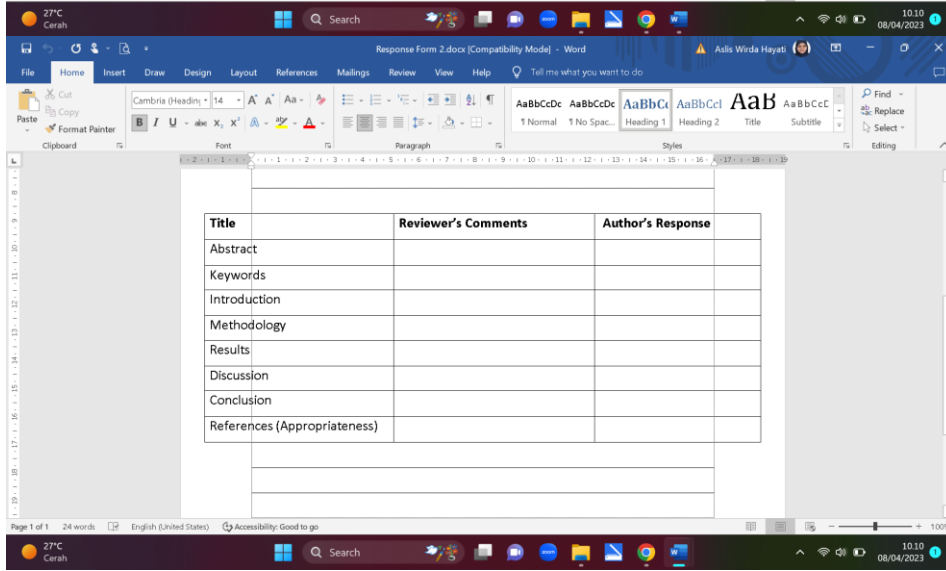
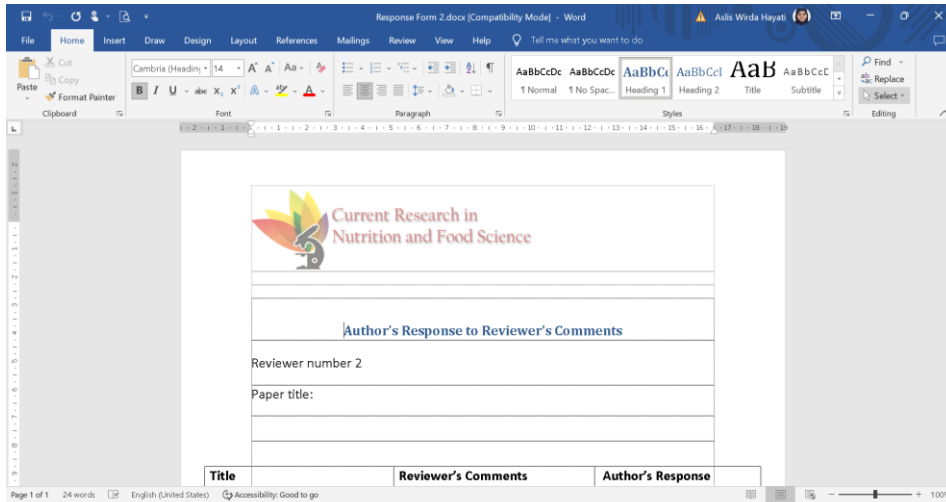
- Reject unconditionally
- Reject in current form, but allow resubmission after revision as per my accompanying comments
- Accept conditionally, subject to minor revision, according to my accompanying comments
- Accept unconditionally

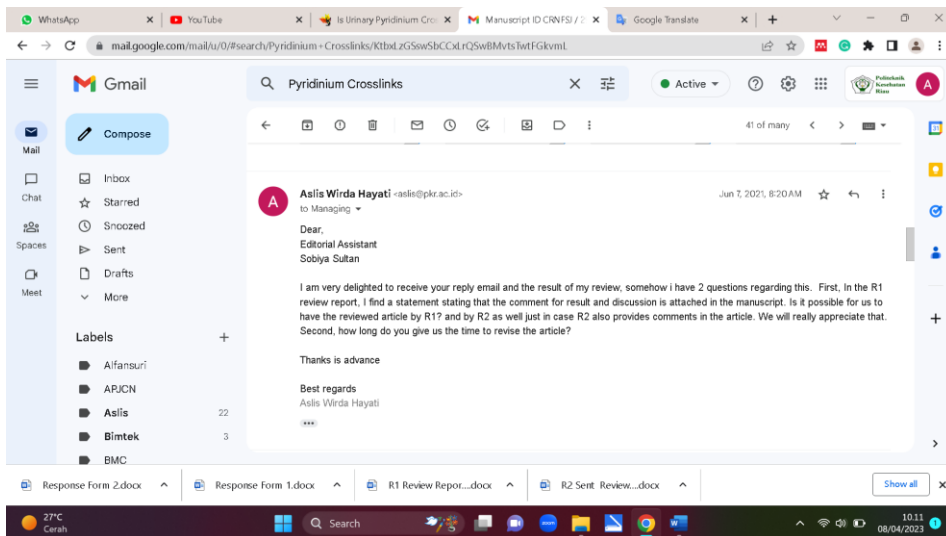
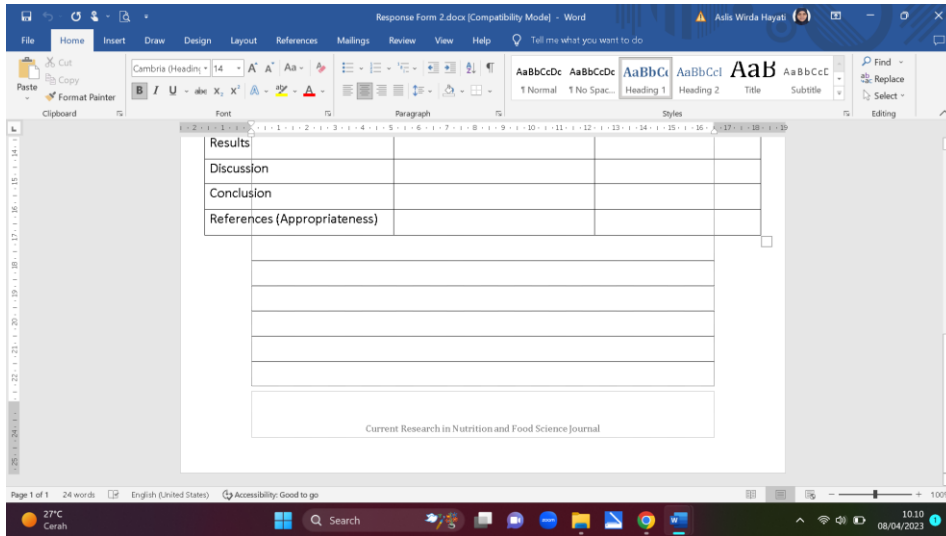


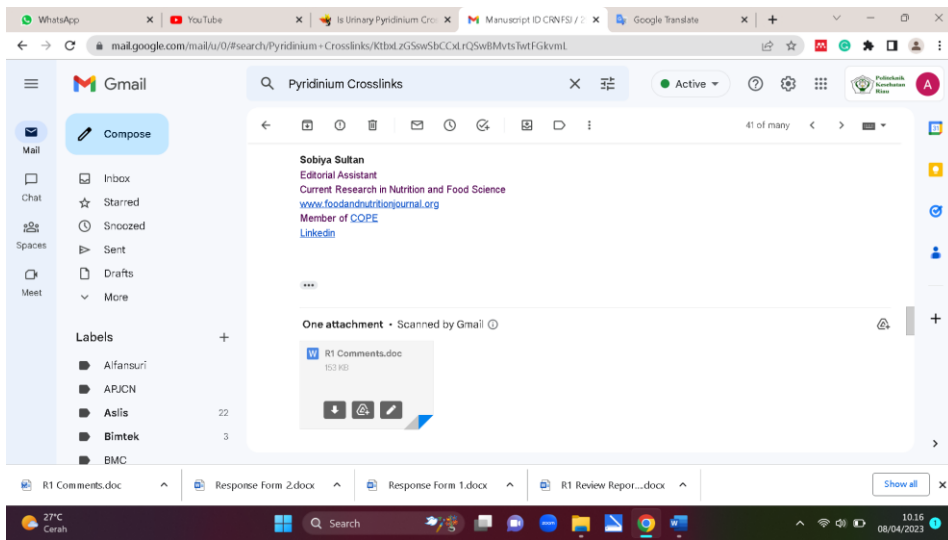
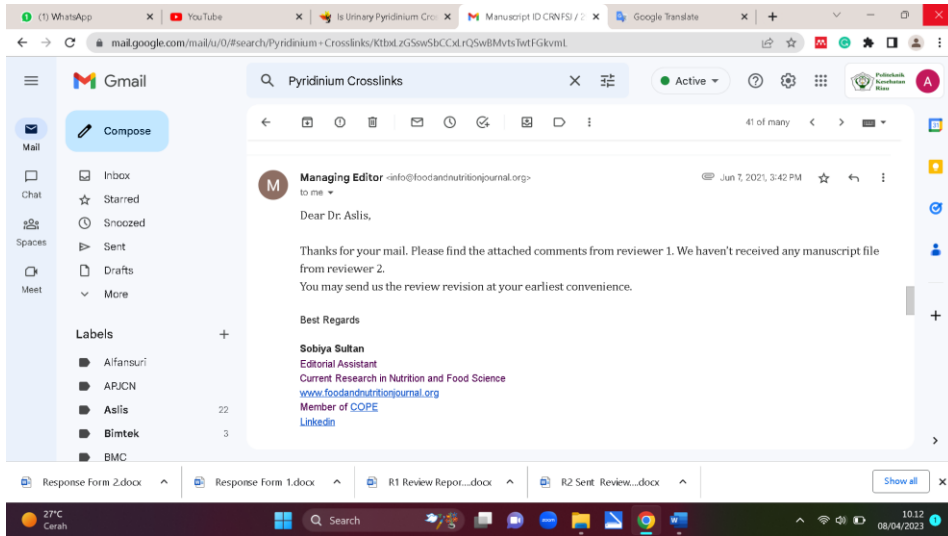


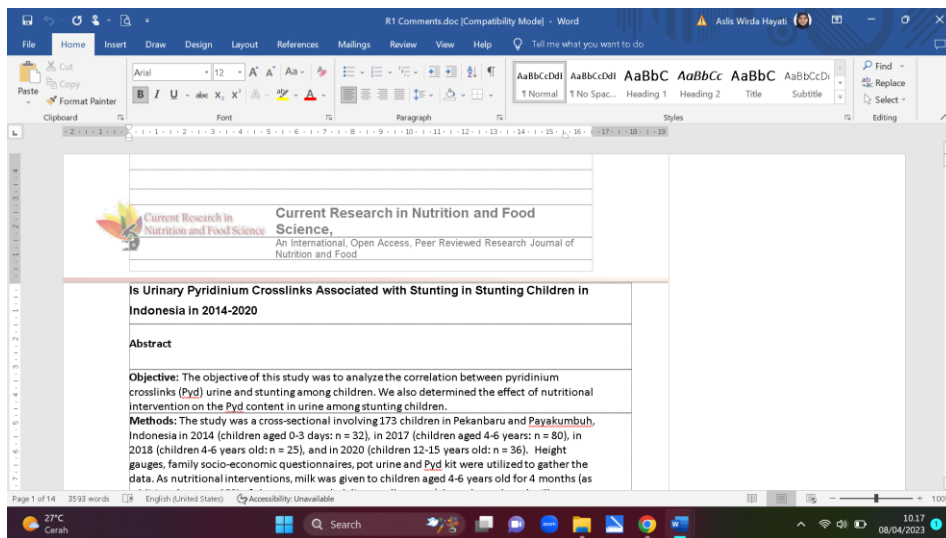












Is Urinary Pyridinium Crosslinks Associated with Stunting in Stunting Children in Indonesia in 2014-2020

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.

About 59.3% of children aged 3-5 years were included stunting³. 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 Province⁵. Public health problems are considered severe if the prevalence of stunting is 30-39% and serious if the prevalence of stunting is $\geq 40\%$ 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 hydroxylysine 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

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the number of free crosslinks excreted in the urine is related to the rate of bone formation⁷.

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 osteoclasts⁹. 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 stunting¹⁰. Calcium forms complex bonds with phosphate which can provide strength to bones¹¹.

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 Sumatera Province, Indonesia.

Subject and Urine Collection

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

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Data Collection

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 simultaneously¹²

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

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Creatinine measurements were performed with the use of Jaffe reactions according to the method developed by Staden¹⁴. 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

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 adult⁸. 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 later¹⁵. Pvd excretion for neonates was reported to be 642.7 ± 281.3 nmol/mmol creatinine by Fujimoto et al⁷.

In another study, Pvd excretion among elementary school children was reported to be about 50-500 nmol/mmol creatinine¹⁶. The urine pvd 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 Pvd 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 Pvd excretion was raised in those taking T4 which is 40.0 ± 2.7 nmol/mmol creatinine ($p < 0.05$]).

Urine Pvd content has been used to determine the severity of osteoporosis in the elderly. The more urine Pvd 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 Pvd 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 interval¹⁸. The Pvd content of premenopausal women's urine ranged from 3.0 to 7.4, whereas their male peers ranged from 2.3 to 5.4 $\mu\text{mol} / \text{mol}$ of creatinine¹⁹.

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 years²⁰. 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

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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 stops²¹. Relatively the same height at the age of 30-45 years. After 45 years there is a decrease in height²².

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 resorption²³.

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 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

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

^saverage ± standard deviation (minimal: maximal)

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Pyridinium Crosslinks

41 of many

Aslis Wirda Hayati <aslis@pkr.ac.id> to Managing Editor Jul 15, 2021, 11:45 AM

Dear, Managing Editor

Thank you for your patience in waiting for our revised manuscript. Along with this email, I attach the three files including the full revised manuscript and two individual response forms from R1 and R2.

I am looking forward to your feedback.

sincerely,
Aslis WH

3 Attachments - Scanned by Gmail

R1 Comments.doc Response Form 2.docx Response Form 1.docx R1 Review Report.docx

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mail.google.com/mail/u/0/#search/Pyridinium+Crosslinks/Ktbl.ZGSowSbcCxl.rQSwBMvtsIwFGkvmI

Pyridinium Crosslinks

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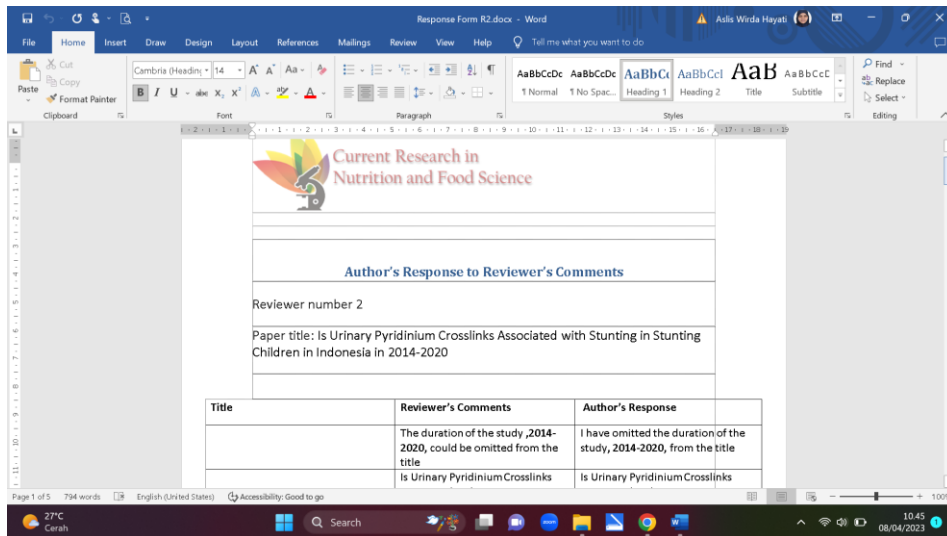
sincerely,
Aslis WH

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Response Form R... Response Form R... Is Urinary Pyridin...

R1 Comments.doc Response Form 2.docx Response Form 1.docx R1 Review Report.docx

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Author's Response to Reviewer's Comments

Reviewer number 2

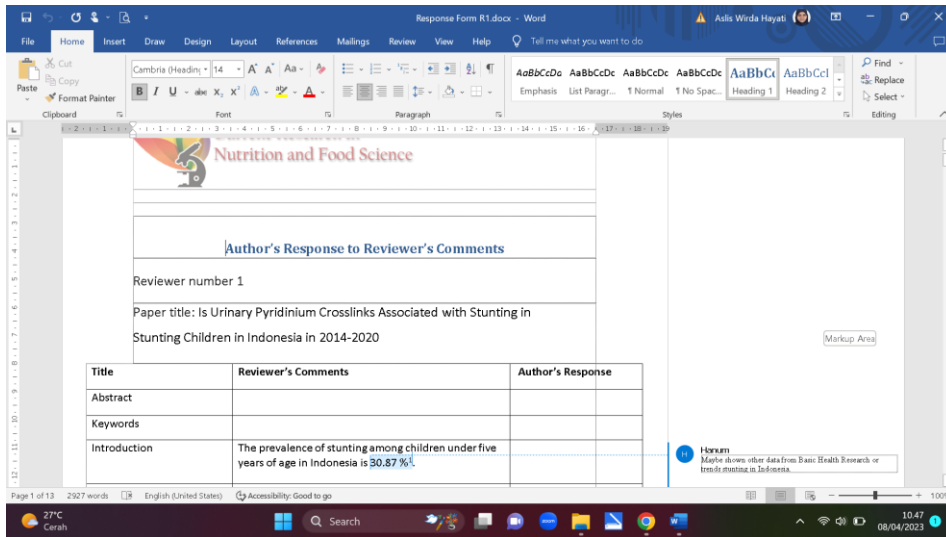
Paper title: Is Urinary Pyridinium Crosslinks Associated with Stunting in Stunting Children in Indonesia in 2014-2020

Title	Reviewer's Comments	Author's Response
	The duration of the study ,2014-2020, could be omitted from the title	I have 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	A closing sentence has been added as a recommendation
	Results: The Pyd content of stunted children aged in 0-3	Results: The Pyd content of stunted children aged in 0-3

	<p>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 Pvd 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 Pvd content was found to be different from normal neonatal urine Pvd content ($p < 0.01$). There was a correlation revealed between urine Pvd content and height of children aged 4-6 years ($p < 0.05$) and $r = -0.242$. A difference was observed in the urine Pvd content of children 4-6 years before nutrition intervention ($p < 0.01$) as well as in urine Pvd 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.</p>	<p>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 Pvd 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 Pvd content was found to be different from normal neonatal urine Pvd content ($p < 0.01$). There was a correlation revealed between urine Pvd content and height of children aged 4-6 years ($p < 0.05$) and $r = -0.242$. A difference was observed in the urine Pvd content of children 4-6 years before nutrition intervention ($p < 0.01$) as well as in urine Pvd 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.</p> <p>The urine Pvd is expected to be a marker of the efficacy of nutritional care in bone growth disorders associated with bone resorption in stunting children.</p>
Keywords		
Introduction		
Methodology	<p>Very good, <u>but</u> has some note such as: Equipment and Materials: better</p>	<p>It has been fixed according to the reviewer's suggestion</p>

	to be changed to Subjects and Materials	
	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.	It has been fixed according to the reviewer's suggestion
	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	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

		<p>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 Pvd 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 Pvd 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.</p>
Discussion		
Conclusion		
References (Appropriateness)		



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).	Other data from Basic Health Research about trend of stunting has been shown, name, from 2007, 2010 and

Commented [H10]: Maybe shown other data from Basic Health Research or trends stunting in Indonesia.

Commented [H11]: Maybe shown other data from Basic Health Research or trends stunting in Indonesia.

		2013.
	The biochemical markers of bone resorption can be analyzed clinically using conditions and treatments that affect bone metabolism.	
	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 .	A paragraph has been added to connect the paragraph in question with the previous paragraph.
	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.	
	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.]	References have been added, namely number 18
	18. Ningsih SW, Lubis NA, Hayati AW, Azis A. Is urinary creatinine associated with wasting in neonates. <i>Asian J Pharm Clin Res.</i> 2018;11(Special Issue 1):187-189. doi:10.22159/ajpcr.2018.v11s1.26603	

Commented [H12]: What is the link between this paragraph and before?

Commented [H13]: References?

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Methodology	<p>Study Design</p> <p>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</p> <p>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 Lima Puluh Kota district, West Sumatra Province, Indonesia.</p>	
	<p>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.</p> <p>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.</p>	<p>The two sentences asked by the reviewer have been changed by the author into only the first sentence, while the second sentence has been deleted because the two sentences have the same meaning.</p>
		<p>The cause of data collection in two different places is related to the requirements proposed by research funders related to research financing schemes. The research financing scheme that funds this research requires that one of the requirements that must be met is that researchers come from at least two institutions in two provinces. Researchers have chosen two locations for data collection with the same characteristics.</p>

Commented [H15]: why is it taken in a different place? Were the subject same or difference person?

		Subjects are different people in each year of the study.
	Subject and Urine Collection	
	Subject and Urine Collection	The explanation of urine collection in this sub section was not found because it turned out that I wrote it in the next section with the subtitle "Research Procedure". Therefore, in this section I delete the words "and Urine Collection" in this section.
	Data Collection	
	Data Collection and Instrument	I have edited the article according to the reviewer's suggestion by adding the phrase "and Instrument" in the subtitles and removing some subtitles related to the instrument.
	Equipment and Materials	
	Research Procedure Urine Collection	
	Pyd Urine Measurement and Standardization	
	Creatinine Urine Measurement and Standardization	

Commented [H16]: I didn't found the explain of urine collection in this sub section

Commented [H17]: I didn't found the explain of urine collection in this sub section

Commented [H18]: It's better explain data collection and instrument in one sub section so the method not too long

Commented [H19]: It's better explain data collection and instrument in one sub section so the method not too long

Commented [H20]: I didn't found the explain of urine collection in this sub section

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 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)].	The topic of this paragraph is to compare the Pyd content of urine from the research I did with the Pyd content of previous researchers. Previous research that describes the content of Pyd that I have found so far is as I wrote in this article. If we omit the explanation for adults in this section this will reduce the depth of information.
	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 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:	I have added a reference

Commented [H21]: I don't know what is the topic of this paragraph. If you want to explain to elementary students, please connect with related research. In my opinion, the explanation for adults is not precisely conveyed in this section.

Commented [H22]: I don't know what is the topic of this paragraph. If you want to explain to elementary students, please connect with related research. In my opinion, the explanation for adults is not precisely conveyed in this section.

Commented [H23]: Reference?

Commented [H24]: Reference?

	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.	
	<p>There are many studies on the effect of nutrition on body length³⁰. Energy intake was the strongest predictor of increased linear growth. Providing energy from food (310 Cal/day) in malnourished Indian children can increase height gain. Protein is provided from skim milk and cereals. The research was conducted by Bhandari et al³¹ on providing interventions to slum communities in Nehru, India. The study was conducted in a randomized controlled trial. In this study, the number of samples was 418 children aged 4-12 months. The children were divided into two groups. The first group is given quality supplementary food every day with supervision so that consumption is optimal. The second group is given nutrition counseling only; this group received 30-45 minutes of counseling monthly by a trained dietitian. Nutritional intervention was given for 8 months. The study showed that $1 - 2 = 0.4$ cm (attainment of the subject's body length increase), and the standard deviation was = 1.6 cm.</p> <p>Another research related to the provision of nutrition and linear growth interventions, namely the research of Matali, Wungouw and Sapulete³² in Manado (Indonesia) which carried out an intervention in the form of intake of 250 ml of low fat high calcium UHT milk every day for 60 days to elementary school children. The research subjects were 40 people consisting of 20 people in the intervention group and 20 people in the control group. The average height of the intervention group in the first measurement was</p>	<p>A discussion nutritional intervention has been added to the article.</p>

Commented [H25]: 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

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.

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) cm³³.

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 day³⁴, while their energy needs are 1,550 calories per day³⁵; 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 H_0 and conclude that there is a statistically significant difference between the mean height before and after the intervention.

The average difference between the height of the subjects before and after the intervention was 1.91 cm. The lack of the subject's height compared to the national average height according to the results of Basic Health Research in 2007¹ and in 2010² is 4.82 cm. The median height of the subjects before the intervention was 106.40 cm and after the intervention was 107.95 cm. The median height of children aged 4-6 years based on the results of Basic Research Health in 2007 and 2010 according to AsDI, IDAI, PERSAGI in 2015 is 112 cm³⁶. 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 Pvd 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 Pvd 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 time³⁷. 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 Pvd content before the intervention was 9.81 ± 7.02 and the Pvd 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.

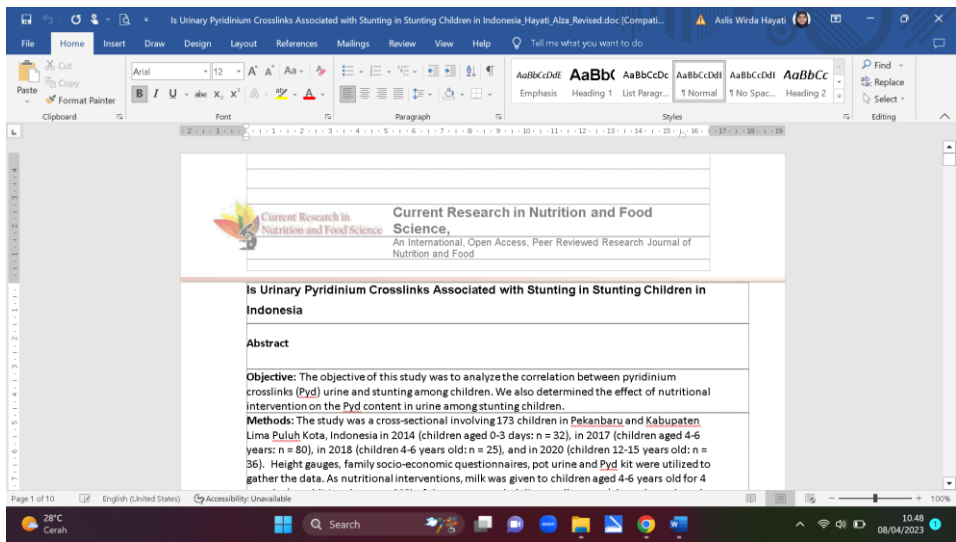
The lack of research in 2020 is that subjects between women and men are still combined. For the future, it is better if the same research with male and female subjects can be distinguished. The advantage of this research is that the brunch provided can be purchased at stalls around the school where the 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

	<p>to a minimum of 3 months, it is better if it is up to 8 or 12 months, even 24 months.</p> <p>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.</p>	
Conclusion		
References (Appropriateness)		

Commented [H26]: 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



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

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 2007¹, 35.6% in 2010², 37.2% in 2013³ and 30.87% in 2018⁴. A review study in 36 countries found that the prevalence of stunting in children under one year was 40% and the prevalence of stunting for children under two years reached 54%⁵.

About 59.3% of children aged 3-5 years were included stunting⁶. The prevalence of global stunting of children aged 13-15 years is around 35.1%⁷. The results of Indonesia's basic health research in 2010 show that the prevalence of stunting in children aged 13-15 years is 35.2%, the prevalence is 36.6% in the Riau Province⁸. Public health problems are considered severe if the prevalence of stunting is 30-39% and serious if the prevalence of stunting is $\geq 40\%$ ⁷. World Health Organization (WHO) established stunting standards based on anthropometrics measurement with Height for Age (HAZ)-score < -2 SD⁹.

Stunting is associated with impaired linear growth processes¹⁰. The linear growth retardation process begins at 2 or 3 months of age¹¹. Growth retardation reflects a process of failure to achieve linear growth potential as a result of suboptimal health and/or nutritional conditions⁷. 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 resorption¹². Urinary pyridinium crosslinks are removed during the release of mature collagen in bone. Pyridinium is constructed as an intramolecular crosslinker during collagen maturation¹³.

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

hydroxylysine 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 formation¹⁴.

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 osteoclasts¹⁵. 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 stunting¹⁶. Calcium forms complex bonds with phosphate which can provide strength to bones¹⁷.

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 enumerators¹⁸.

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.

Methods

Study Design

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

Subject and Material

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

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

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

Data Collection and Instrument

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

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

Children' urine was collected by a nurse who was trained by researchers at the kindergarten/nursery school. The mothers were briefly explained about the implementation of the study as well as pot urine collection. Urine was collected between 7:00 and 10:00 am. The minimum amount of urine taken from the subject was 10 ml. The urine samples were then stored in the freezer at a temperature of -20 °C in Prodia Clinical Laboratory Pekanbaru Branch, and then sent to Prodia Center in Jakarta for analysis. The analysis was carried out simultaneously¹⁸.

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

Creatinine measurements were performed with the use of Jaffe reactions according to the method developed by Staden²⁰. 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 adult¹². 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 later²¹. Pyd excretion for neonates was reported to be 642.7 ± 281.3 nmol/mmol creatinine¹⁴.

In another study, Pyd excretion among elementary school children was reported to be about 50-500 nmol/mmol creatinine²². 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 was the same as the premenopausal reference interval²⁴. The Pyd content of premenopausal women's urine ranged from 3.0 to 7.4, whereas their male peers ranged from 2.3 to 5.4 $\mu\text{mol} / \text{mol}$ of creatinine¹⁹.

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 years²⁵. 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 stops²⁶. Relatively the same height at the age of 30-45 years. After 45 years there is a decrease in height²⁷.

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 resorption²⁸. 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 status²⁸.

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 resorption²⁹.

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 length³⁰. Energy intake was the strongest predictor of increased linear growth. Providing energy from food (310 Cal/day) in malnourished Indian children can increase height gain. Protein is provided from skim milk and cereals. The research was conducted by Bhandari et al³¹ on providing interventions to slum communities in Nehru, India. The study was conducted in a randomized controlled trial. In this study, the number of samples was 418 children aged 4-12 months. The children were divided into two groups. The first group is given quality supplementary food every day with supervision so that consumption is optimal. The second group is given nutrition counseling only; this group received 30-45 minutes of counseling monthly by a trained dietitian. Nutritional intervention was given for 8

months. The study showed that $1 - 2 = 0.4$ cm (attainment of the subject's body length increase), and the standard deviation was = 1.6 cm.

Another research related to the provision of nutrition and linear growth interventions, namely the research of Matali, Wungouw and Sapulete³² in Manado (Indonesia) which carried out an intervention in the form of intake of 250 ml of low fat high calcium UHT milk every day for 60 days to elementary school children. The research subjects were 40 people consisting of 20 people in the intervention group and 20 people in the control group. The average height of the intervention group in the first measurement was 133.23 cm and the second measurement was 134.78 cm, while the average height of the control group in the first measurement was 131.52 cm and 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.

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) cm³³.

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 day³⁴, while their energy needs are 1,550 calories per day³⁵; 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 H_0 and conclude that there is a statistically significant difference between the mean height before and after the intervention.

The average difference between the height of the subjects before and after the intervention was 1.91 cm. The lack of the subject's height compared to the national average height according to the results of Basic Health Research in 2007¹ and in 2010² is 4.82 cm. The median height of the subjects before the intervention was 106.40 cm and after the intervention was 107.95 cm. The median height of children aged 4-6 years based on the results of Basic Research Health in 2007 and 2010 according to AsDI, IDAI, PERSAGI in 2015 is 112 cm³⁶. 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 time³⁷. 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 Pvd content before the intervention was 9.81 ± 7.02 and the Pvd content after the intervention was 5.33 ± 2.89 nmol/mmol creatinine (Table 2). There was a decrease in adolescent Pvd content after the provision of nutritional intervention for 34 days. There was a difference observed in Pvd 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 Pvd is expected to be a marker of the efficacy of nutritional care in bone growth disorders associated with bone resorption.

Conclusion

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

	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"	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	Pyd content in the urine of subjects before and after nutritional intervention (p <0.01). However, all subjects were still in the stunting category	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	n	Pyd (nmol/mmol creatinine)			
			Girl		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)	

^saverage ± standard deviation (minimal: maximal)

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Pyridinium Crosslinks

41 of many

Managing Editor -info@foodandnutritionjournal.org
to me

Jul 16, 2021, 12:29 PM

Dear Dr Aslis,

Thanks for the revised file. We have sent the manuscript to the reviewers again for the reassessment process. We will soon update you if they require any changes.

Best Regards

Sobiya Sultan
Editorial Assistant
Current Research in Nutrition and Food Science
www.foodandnutritionjournal.org
Member of COPE
[LinkedIn](#)

xxx

Labels: Afiansuri, ARJCN, Aslis (22), Bimtek (3), BMC

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Pyridinium Crosslinks

41 of many

Aslis Winda Hayati -aslis@pkr.ac.id
to Managing

Aug 29, 2021, 11:17 AM

Dear, Managing Editor

Please let me know the result of the reassessment process of the revised file. I am looking forward for this information.

Sincerely,
Aslis WH

xxx

Managing Editor -info@foodandnutritionjournal.org
to me

Aug 30, 2021, 12:01 PM

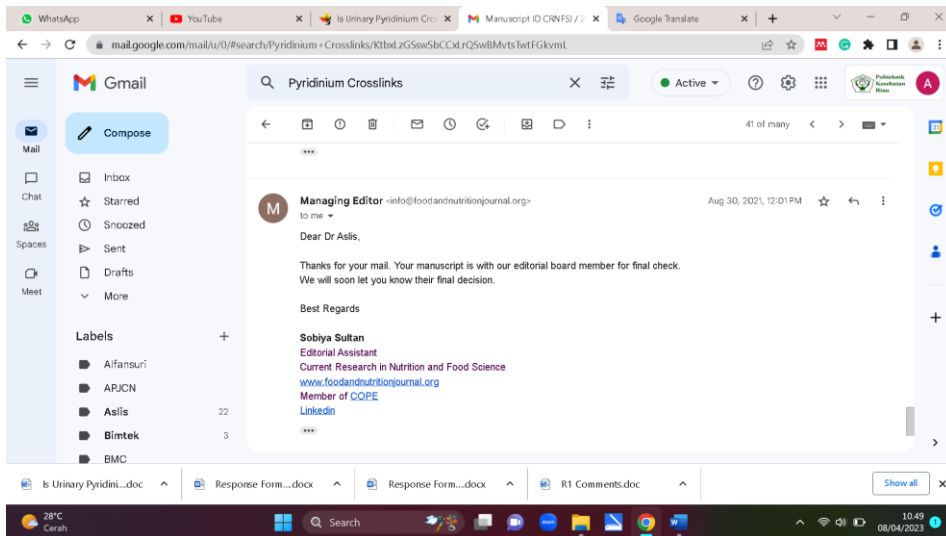
Dear Dr Aslis,

Thanks for your mail. Your manuscript is with our editorial board member for final check. We will soon let you know their final decision.

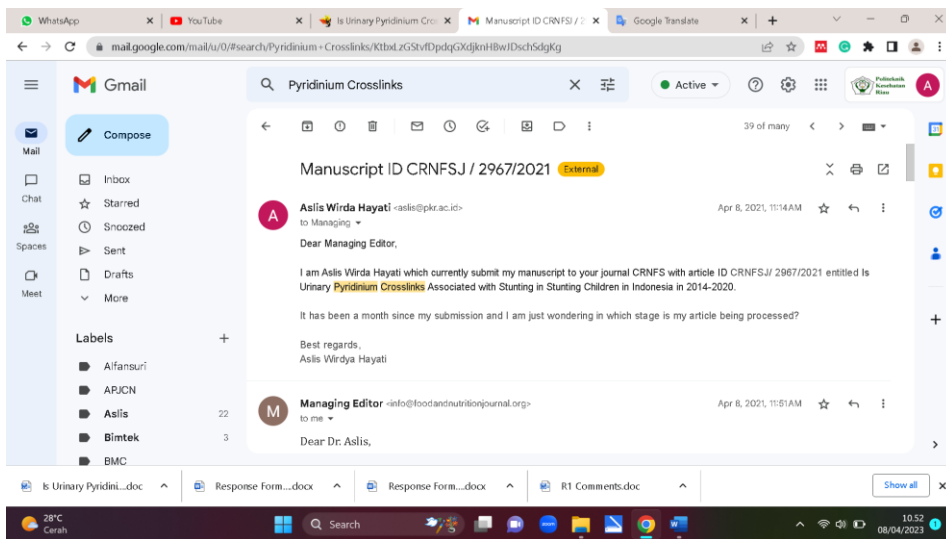
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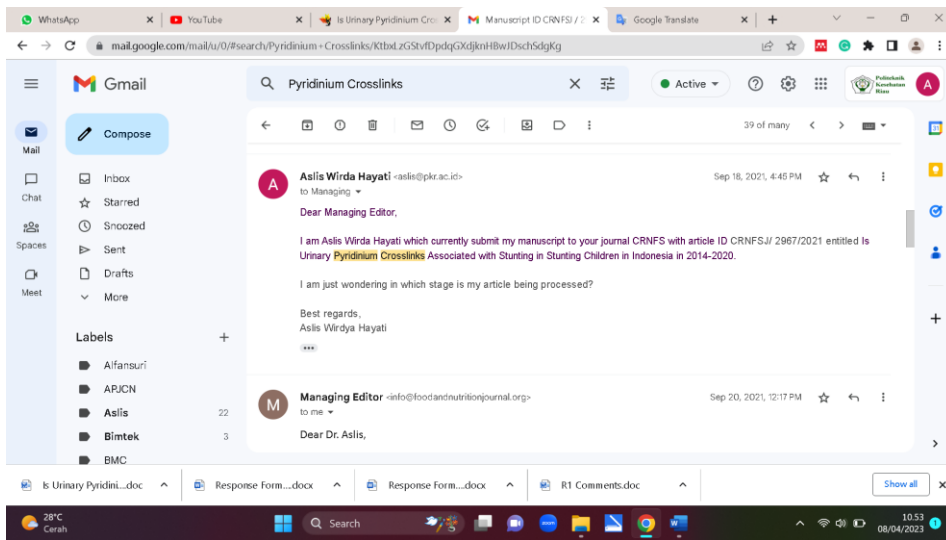
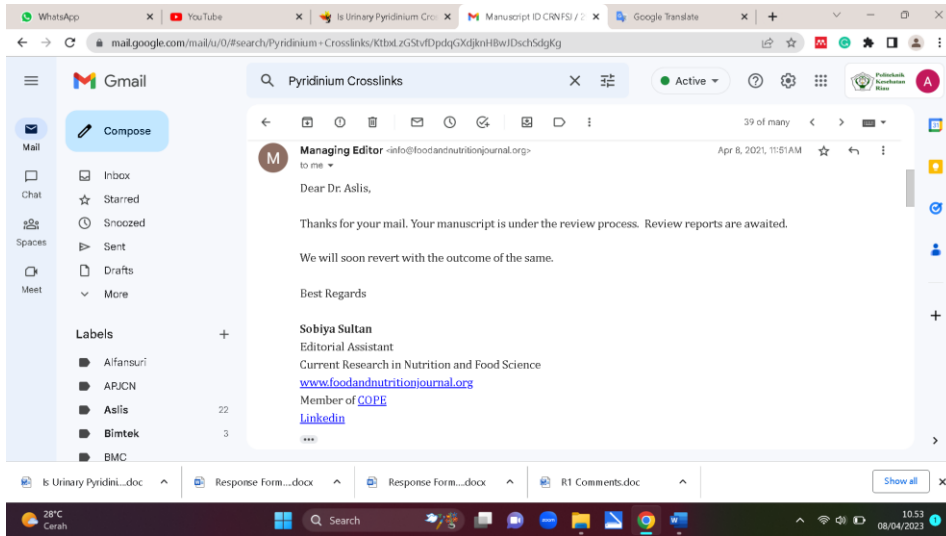
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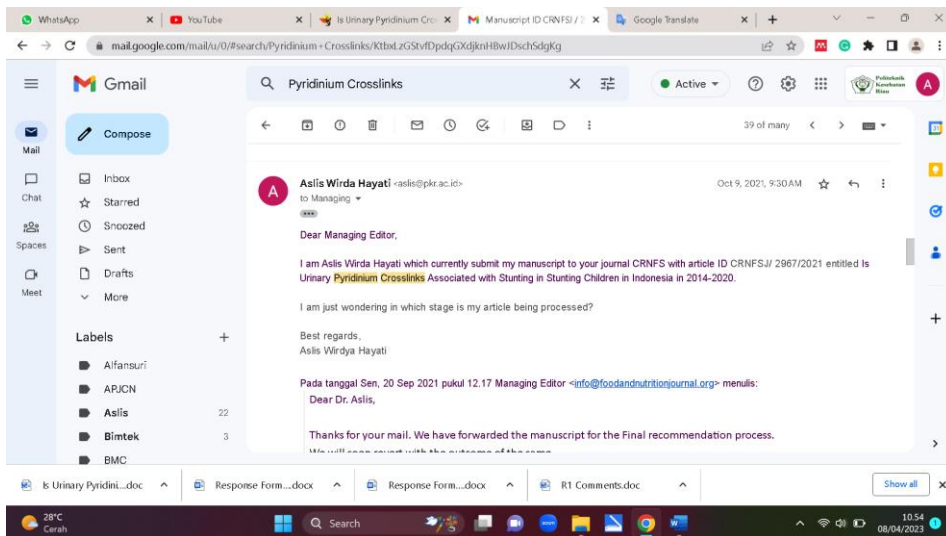
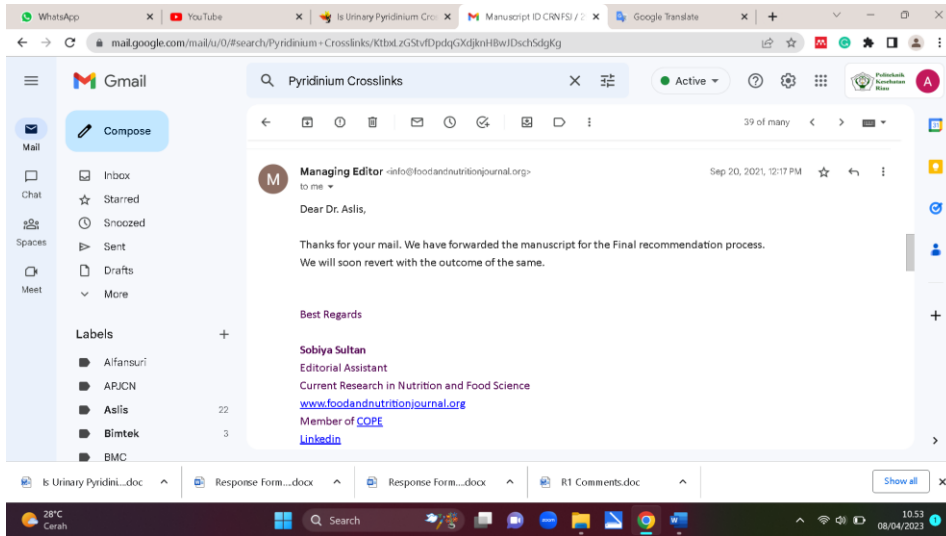
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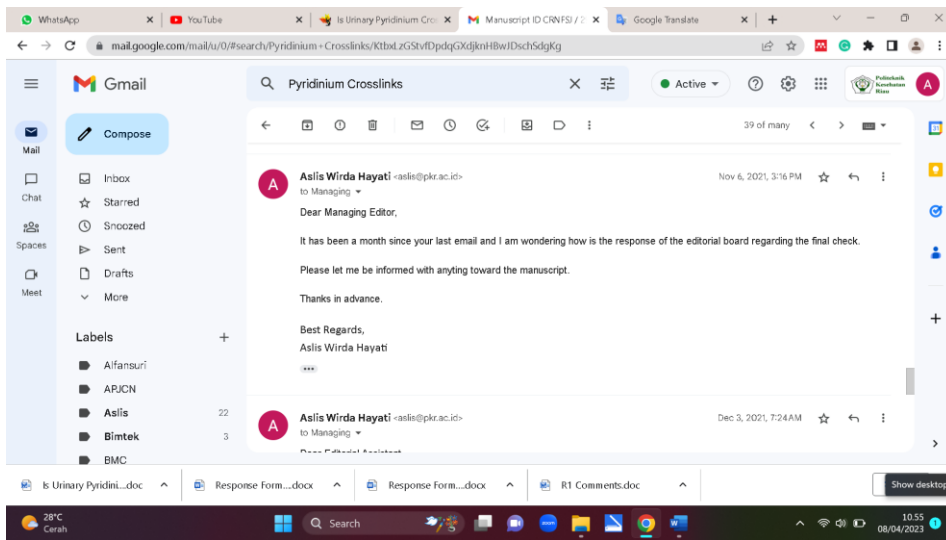
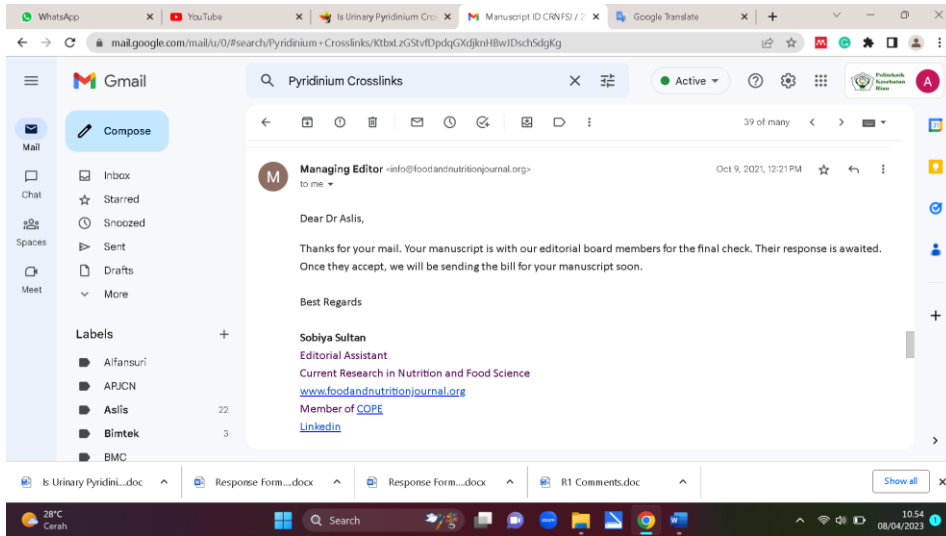


4. Final recommendation process: 20 Sep 2021









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Mail: Inbox, Starred, Snoozed, Sent, Drafts, More

Labels: Alfansuri, APJCN, Aslis (22), Bimtek (3), BMC

Aslis Wirda Hayati -aslis@pkr.ac.id> to Managing
Dec 3, 2021, 7:24AM

Dear Editorial Assistant,
Sobiya Sultan

It's been two months since your last mail regarding my manuscript. I wonder how is the process so far. How long it usually takes for the editorial board member to do the final check?

Please let me be informed

Best Regards,
Aslis WH

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Compose

Mail: Inbox, Starred, Snoozed, Sent, Drafts, More

Labels: Alfansuri, APJCN, Aslis (22), Bimtek (3), BMC

Aslis Wirda Hayati -aslis@pkr.ac.id> to Managing
Dec 8, 2021, 8:56AM

Dear Editorial Assistant,
Sobiya Sultan

I am Aslis Wirda Hayati which currently submit my manuscript to your journal CRNFS with article ID CRNFS/J/2967/2021 entitled Is Urinary **Pyridinium Crosslinks** Associated with Stunting in Stunting Children in Indonesia in 2014-2020.

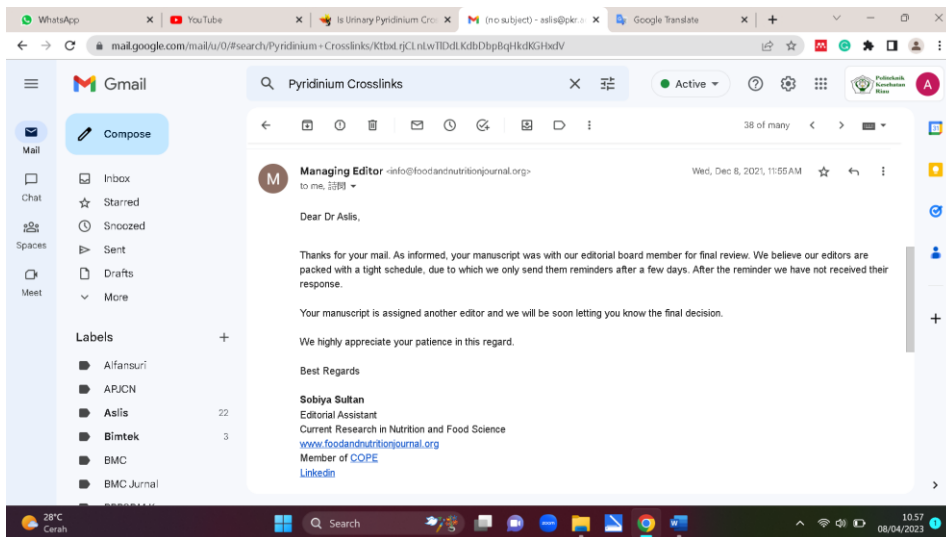
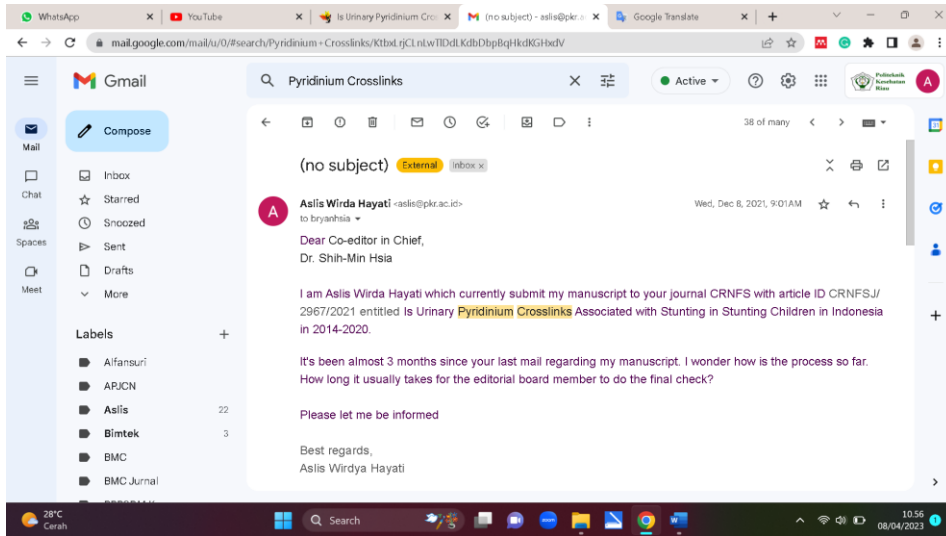
It's been almost 3 months since your last mail regarding my manuscript. I wonder how is the process so far. How long it usually takes for the editorial board member to do the final check?

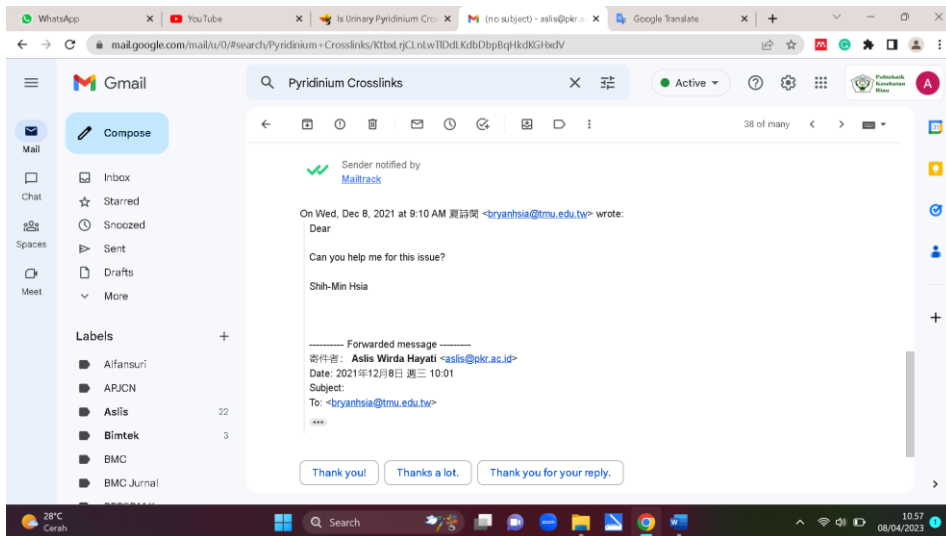
Please let me be informed

Best regards,
Aslis Wirdaya Hayati

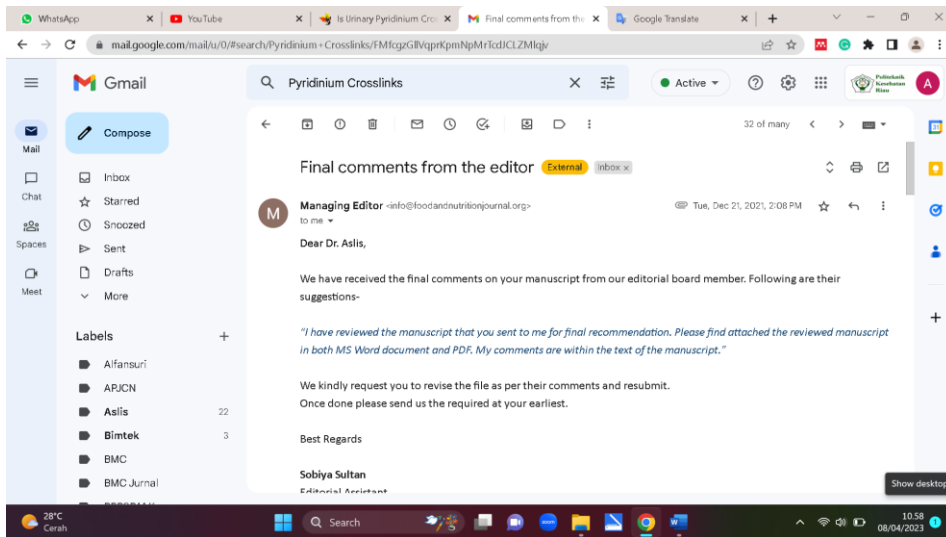
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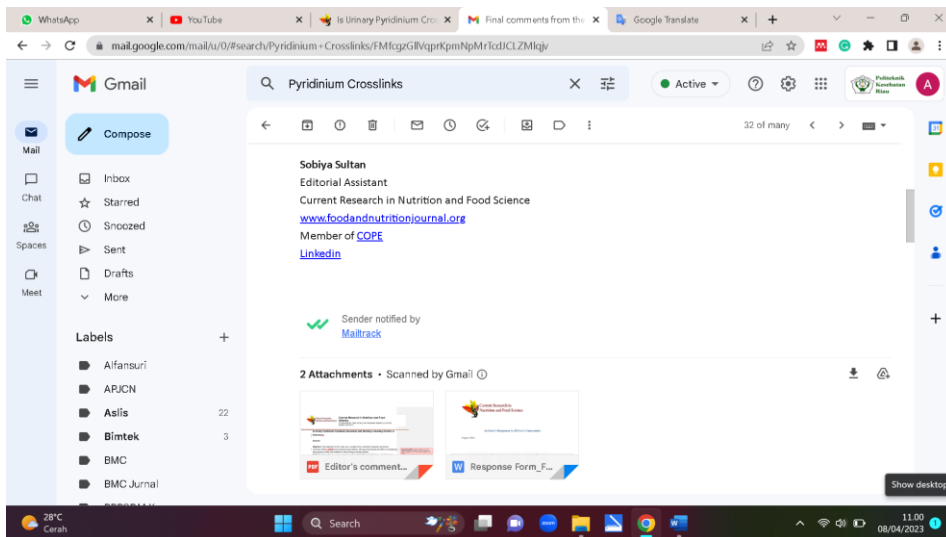
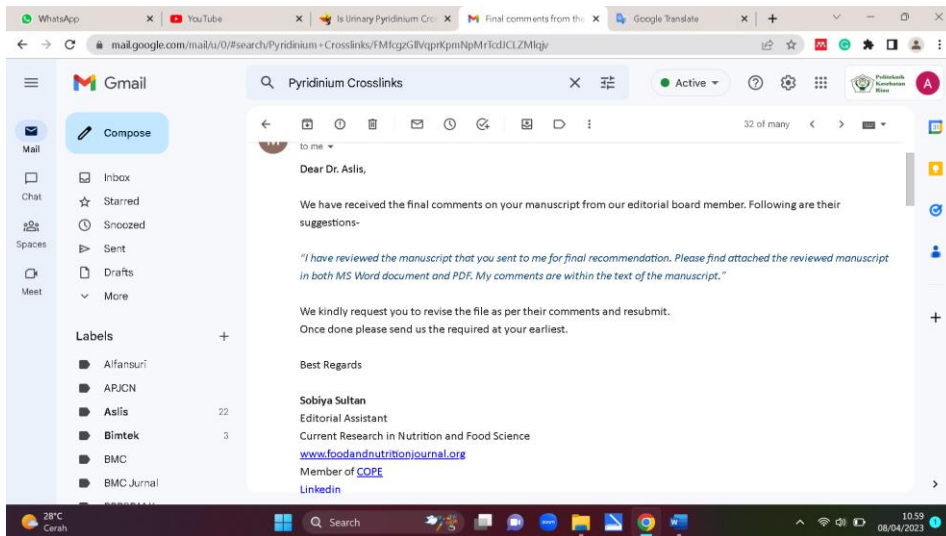
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5. Final recommendation from editor: 21 Dec 2021





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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 (Pyl) and stunting among children. We also determined the effect of nutritional intervention on the Pyl content in urine among stunting children.

Methods: The study was a cross-sectional involving 175 children in Kabupaten and Kabupaten Lima Puluh Kota, Indonesia in 2014 (children aged 3-5 years, n = 51), in 2017 (children aged 4-6 years, n = 65), in 2018 (children 4-6 years old, n = 25), and in 2020 (children 12-15 years old, n = 34). Height/length, family socio-economic questionnaire, urine and Pyl in urine were utilized to gain the data. A nutritional intervention, with was given to children aged 4-6 years old for 4 months an additional energy 20% of the recommended daily allowance, protein meals and milk were given to children 12-15 years old for 30 days (a additional energy 20% recommended daily allowance). Pyl and height were used as parameter indicator in this study. Pearson correlation and t test (significance p < 0.05 and post-t) were applied for statistical analysis.

Results: The Pyl content (ng/ml) in stunting children aged 0-5 years, 3-5 years, 4-6 years, and 12-15 years were 262.34, 4.3, 9 and 7.6 nmol/mmol creatinine, respectively. The Pyl content of stunting children aged 4 and 12-15 years before and after nutritional intervention was 153.14, 15.3 and 9.83 vs 53.5 nmol/mmol creatinine, respectively. Stunting remained after Pyl content was found to be different from non-stunting children (p < 0.05). There was a significant correlation revealed between urine Pyl content and height of children aged 4-6 years (r = 0.55) and r = 0.26. A difference was observed in the urine Pyl content of children 4-6 years before nutritional intervention (p < 0.05) as well as in urine Pyl content of children aged 12-15 years before and after nutritional intervention (p < 0.05), in 83.8% of the subjects improved their nutritional status from stunting to normal. The urine Pyl 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 was 36.8% in 2007, 35.8% in 2012, 37.2% in 2013, and 30.87% in 2018. 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%.

About 56.3% of children aged 3-5 years were stunted. The prevalence of global stunting of children aged 12-15 years is around 35.2%. The results of Indonesian basic health research in 2010 show that the prevalence of stunting in children aged 12-15 years was 35.2%, the prevalence was 36.4% in the Riau Province. Public health problems are considered serious if health Organization (WHO) established stunting threshold based on anthropometry, measurement with height for Age (HAZ) score < 2 SD.

Stunting is associated with impaired linear growth processes. The linear growth retardation process begins at 2 or 3 months of age. Growth retardation reflects a process of failure to achieve linear growth potential as a result of suboptimal health and/or nutritional conditions. One of the linear growth 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. Urinary pyridinium crosslinks are removed during the release of mature collagen in bone. Pyridinuronic acid is an extracellular crosslinker during collagen maturation.

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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The study obeyed the Helsinki-Clinical 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.15.1.2K/SPK/2014, 05/UN.15.1.1.1.1/SPK/2017, 07/ UN.15.1.1.1/SPK/2018, and 05/UN.15.1.1.1/SPK/2020).

Data Collection and Instrument

70 percent of 120 subjects gave written informed consent. In the time of informed parental consent, urine was collected (Indonesian: *keperluan*) The urine was collected by using sterile pot, aliquot to 5 ml and stored in freezer at -20°C (GEA by Vestfrost Denmark Type G.201 Serial No: 2002380800) until further analysis.

In this study, body height gauges (Incrostat) (STATURE METER JM GEA, Indonesia), pot urine meter (Mettler DM, Indonesia), weighing scale, and household socio-economic questionnaire for the children (age, gender, age, race, weight parents) were used.

Children's urine was collected by a nurse who was trained by researchers at the kindergarten/homery 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 maximum amount of urine collected from the children was 30 ml. The urine samples were then stored in the freezer at a temperature of -20 °C in Probes Clinical Laboratory Pediatrics Branch, and then sent to Probes Center in Jakarta for analysis. The analysis was carried out simultaneously.

Pylt measurements were performed with the use of Microtiter[®] P.YD 614 MI, USA. Pylt analysis was performed according to Hayati et al.,¹⁷ using a Spectrophotometer (Microplate Reader 680 series, Bio-Rad Laboratories, Inc, Hercules, CA 94547, USA).

Comment 1418: There is no need to use the word "keperluan" because it is not a noun.

Comment 1419: There is no need to use the word "collected" because it is not a verb.

Comment 1420: There is no need to use the word "collected" because it is not a verb.

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Crystalline measurements were performed with the use of λ 636 reactions according to the method developed by Staben¹⁸. 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 492-520 nm using spectrophotometer (ADNA 1800 ADNA, Germany).

Statistical Analysis

Statistical data analysis is reported based on the complete data. Pearson correlation and t test with significance $P < 0.05$ and $P < 0.02$ 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 found to cities (Table 3). The average income of the respondent's parents was IDR 3,000,000 per month. The education of the respondent's 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 persons. Almost all of the respondent's parents' height (men = 150 cm).

Urine Pylt content of the standing child

The Pylt content of standing children aged 3-5 days, 3-5 years, 6-8 years, and 12-15 years were found to be 982, 26.4, 24.9 and 3.85 mmol / normal creatinine, respectively (Table 2).

The Pylt content in urine of standing girls was found to be higher than the Pylt content of standing boys (Table 3). The Pylt content of standing females' urine for men and women were 386.45 and

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Comment 1424: There is no need to use the word "collected" because it is not a verb.

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641.40 mmol / mmol creatinine, respectively. Moreover, Pyl content of urine for stunted girls and boys aged 4-6 years were 18.70 and 15.27 and mmol / mmol creatinine, respectively.

The Pyl content of stunted neonates' urine was 182.52 ± 61.14, whereas normal neonates were 194.1 ± 266.16 mmol/mol creatinine (p < 0.01). The Pyl content of urine in girls (stunted, stunted and normal children aged 4-6 years) were found to be 18.4, 18.6 and 15.5 mmol / mmol creatinine. There was a negative correlation found between urine Pyl content and height of children (r = -0.49) (p = 0.242).

Urine Pyl content based on nutritional intervention

The Pyl content of stunted children aged 4-6 years before and after nutritional intervention were found to be 18.9 and 15.3 mmol / mmol creatinine, respectively. The same results were also obtained among the older age group (teenagers). Pyl content in urine of stunted children aged 12-15 years before and after nutritional intervention were 3.82 and 5.33 mmol/mol creatinine, respectively. A decreasing trend in the amount of urine Pyl indicated an increase in the linear growth of the child. The results also indicated that by providing nutritional interventions to stunted children reduced urine Pyl content (Table 2).

General comment: This section is very poorly written for publication in a scientific journal. The authors need to revise the data presented to clearly indicate the accuracy used for identification of the children as very malnourished and normal. Table 3 is poorly presented. Table 3 is a table presented in Table 3 is not clear, because a total of 213 children participated in the study (12, 40, 26, 26), between the numbers presented in the table do not add up to 213. The total number presented in Table 3 is 41. What is the justification for such a significant change in number? The authors need to account for the other number of 213 and the final number of 20 presented in Table 3. In my view, this manuscript cannot be recommended for publication in its present form because of the poor presentation of the results.

Comment (A11): This is not a peer-reviewed journal. The authors should submit their work to a peer-reviewed journal.

Comment (A12): This is a review of the literature. The authors should submit their work to a peer-reviewed journal.

Comment (A13): This is a review of the literature. The authors should submit their work to a peer-reviewed journal.

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Discussion

In this study, it was found that the urine Pyl content of stunted children decreased with increasing age.

The same trend was also observed in the previous studies where Pyl urine excretion of children aged 0-3 days (neonates) was 18-200 times higher than that of children aged 2-15 years. The creatinine excretion in children was reported to be 30 times higher than in adults. 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) after breastfeeding practice about 2-4 hours later. Pyl excretion for neonates was reported to be 642.7 (281.3 mmol/mol creatinine).

In another study, Pyl excretion among elementary school children was reported to be about 50-500 mmol/mol creatinine. The urine Pyl of adults who have health problems is higher than normal adults. For example, urinary Pyl excretion in patients with specific urinary markers for the measurement of bone collagen degradation in hyperparathyroidism and during the normal replacement therapy. They reported that the urinary Pyl excretion was higher among postmenopausal female thyroid patients compared to controls (median 1.31 vs 26 mmol/mol creatinine (p < 0.001)). In postmenopausal women urinary Pyl excretion was raised in those taking 1g which is 40.9 ± 2.7 mmol/mol creatinine (p < 0.05).

Urine Pyl content has been used to determine the severity of osteoporosis in the elderly. The more urine Pyl 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 Postmenopausal adult female and male urine contained Pyl around 15.3 ± 3.6 and 18.3 ± 20.0 mmol / mmol creatinine. The target value for treated postmenopausal adult female

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Peer Code Change

Peer Code Change

Comment (A12): This is a review of the literature. The authors should submit their work to a peer-reviewed journal.

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been the same as the premenstrual reference interval²⁷. The Pyl content of premenstrual women's urine ranged from 3.0 to 7.4, whereas their male peers ranged from 2.3 to 5.4 (pmol/²⁸ mol of creatinine²⁹).

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 years³⁰. The increase 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 ceases³¹. Relatively the same height at the age of 30-45 years. After 45 years there is a decrease in height³².

In the age group of children and adolescents with normal nutritional status, there was more bone formation observed than bone resorption. Pyl in urine is a marker of bone resorption³³. This means that the Pyl content of urine in the age group of children and adolescents with normal nutritional status is less than the Pyl content of urine in the age group of children with stunting nutritional status³⁴.

Urine pyl 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 Pyl markers include many metabolic disorders of bone such as osteoporosis, primary hyperparathyroidism and metastatic bone disease. Urine Pyl concentration is also a good biological marker of therapeutic efficacy in bone disorders associated with accelerated bone resorption³⁵.

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There was a decrease in the subject's Pyl 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 only / FGA that could change the nutritional status of independent 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 2008 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 only / FGA. In the second study, they were given brunch meals and milk daily for 34 days, additional energy, namely 30% nutritional adequacy only / FGA. The results of the first study were there was a difference in Pyl content in the urine of subjects before and after nutritional intervention ($p < 0.05$) however, all subjects below the fasting (during) in the second study, there was a difference observed in Pyl content of subjects before and after the nutritional intervention ($p < 0.05$). Approximately, 55.4% of subjects increased their nutritional status from stunting to normal.

There are many studies on the effect of nutrition on body length³⁶. Energy intake was the strongest predictor of increased linear growth. Providing energy from food (1000 Cal/day) in malnourished Indian children can increase height gain. Protein is provided from plain milk and cereals. The research was conducted by Bhanderi et al³⁷ on providing interventions to slum communities in Mumbai, India. The study was conducted in a randomized controlled trial. In this study, the number of samples was 438 children aged 12 months. The children were divided into two groups: the first group is given quality supplementary food every day with supervision in their 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 increased, and the standard deviation was 3.6 cm³⁸.

Another research related to the provision of nutrition and linear growth interventions, namely the research of Mahal, Wangsom and Saputro³⁹ in Manado (Indonesia) which carried out an

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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 134.76 cm, while the average height of the control group in the first measurement was 133.33 cm and the second measurement was 132.52 cm. The average height increase in the intervention group was 1.53 cm while the average height increase in the control group was 0.91 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 (A43) (11/11/2023) should you put the number of the study, not the date.

Hi Agapeta, Emilia M D, Julan F D, Marini V C in 2019 conducted a study on providing nutrition education and knowledge for parents, and nutrition empowerment for children at the Cendek School in Taping City, Philippines. The subjects consisted of 140 people and the intervention was given for 120 days in two schools. The first group, namely in school 1, received enriched 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 (3.33 ± 0.72 g) or (0.0334) than in School 2 (0.84 ± 0.58 cm)¹⁷.

Comment (A42) (11/11/2023) should you put the number of the study, not the date.

The research that the authors conducted in 2018 aims to determine the effect of milk and egg consumption on the content of Pyridinium Oxalate (PyO) in urine of stunting children aged 4-6 years. This research was conducted in Early Childhood Education (PAUD) of Park Liris Park Hill 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 nutritional intervention was 4 months. Reliability and adherence to consuming milk and eggs were recorded daily by trained PAUD teachers. There was only one group in this study.

Comment (A41) (11/11/2023) should you put the number of the study, not the date.

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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). Inapat et al in 2021 reported that the average energy consumption of children aged 4-6 years is 1,048 calories per day¹⁶, while their energy needs are 1,550 calories per day¹⁶. 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 bowls 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 123 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 not recorded. The price of 70 ml UHT high 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 5.118 with a p-value of 0.000 which can be written as 0.000 (3-way test). This means that we reject H₀ and conclude that there is a statistically significant difference between the mean height before and after the intervention.

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The average difference between the height of the subjects before and after the intervention was 1.91 cm. The lack of the subject's height compared to the national average height according to the results of Basic Health Research in 2007¹ and in 2007² is 4.82 cm. The median height of the subjects before the intervention was 136.40 cm and after the intervention was 137.31 cm. The median height of children aged 8-9 years based on the results of Basic Health Research in 2007 and 2010 according to ARI, GAL, PERAGA is 132 cm³. The difference in the median height of the subjects when compared with the median height was 5.40 cm before the intervention and 5.31 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 39.9 ± 4.7 (5.1-29.8) and 15.9 ± 7.0 (3.1-43.6). There was a significant difference between the average height and Pyl content of subjects before and after the intervention, but all subjects were still in the existing category (Table 2).

The research that the authors conducted in 2020 aimed to determine the difference in urine Pyl 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 12:00 and 1:00. 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 time⁴. 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 the 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 cost of 30 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

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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 Education, 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, boteng, boteng Medan, sandwich, chicken porridge, and fried rice anchovies. The total amount of energy of the meals and milk was 400 calories (100% of BMR). 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)131.6 (124.6) and after the nutrition intervention was 144.5(5.1)134.7 (125.2) cm. The Pyl content before the intervention was 9.8(1.7-12) and the Pyl content after the intervention was 5.1(3.2-28) respectively (Table 2). There was a decrease in adolescent Pyl content after the provision of nutritional intervention for 34 days. There was a difference observed in Pyl content of subjects before and after the nutritional intervention ($p < 0.05$). Approximately, 59.4% 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 problems at the school can be fixed in the right form, 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 well as during the nutrition intervention in the study.

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Commenting 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 needs 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 3 months 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 add 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
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 authors need to consult with senior colleagues for guidance on how to write a conclusion for publication in a scientific journal. This section should be reduced by 75%, if the authors 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 ($r = -0.28$). Pyd content showed a weak correlation with height ($r = 0.24$). 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.

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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.

Acknowledgments
Herein we convey our thanks and best regard for financial support from Health Politechnic, Ministry of Health, Riau, the research facilities Andri Hospital, Asyik 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 Indokita Jakarta.

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Conflict of Interest
The authors declare no conflict of interest.

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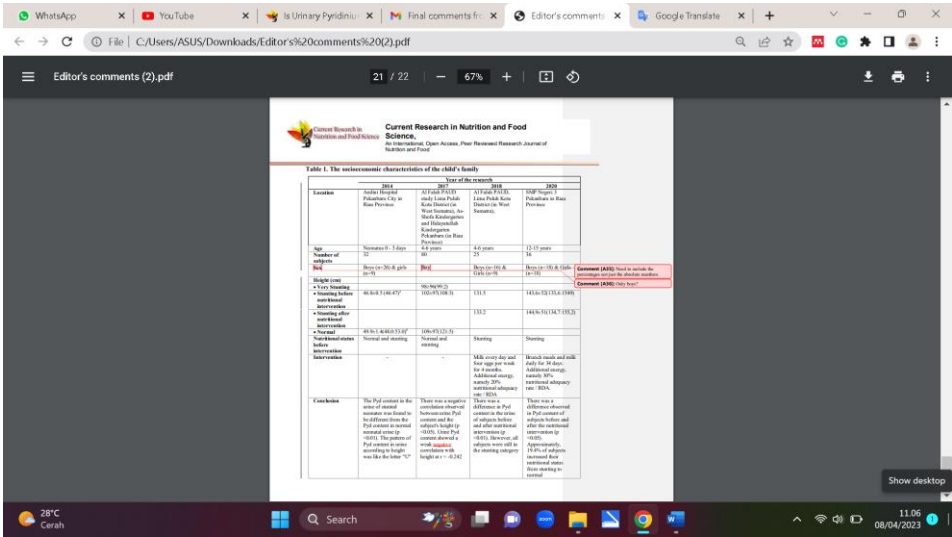
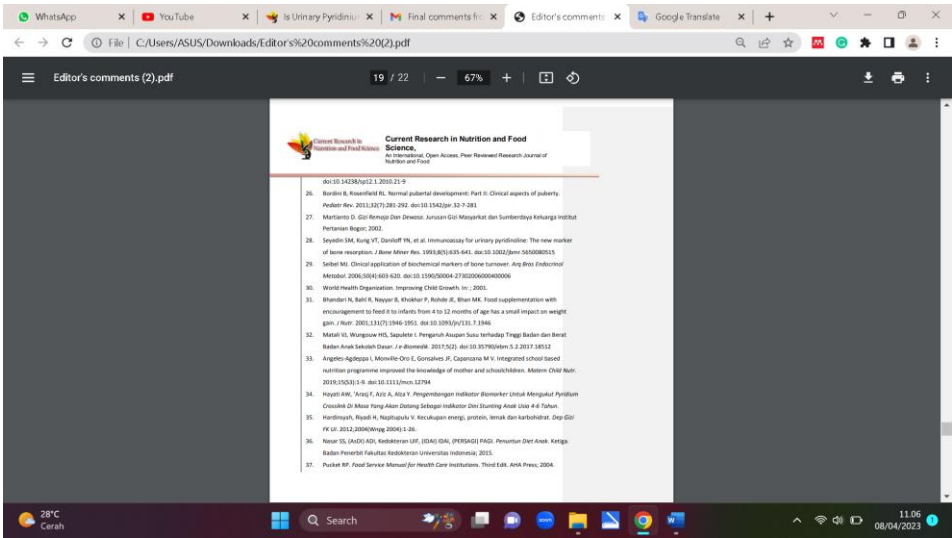
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Table 2. Value Pdf content based on nutritional intervention

Value	Standard Deviation	Standard Error	Confidence Interval	Significance
1. Total Energy	1000	100	800 - 1200	0.001
2. Protein	50	5	40 - 60	0.001
3. Fat	100	10	80 - 120	0.001
4. Carbohydrate	150	15	120 - 180	0.001
5. Fiber	20	2	16 - 24	0.001
6. Vitamin C	10	1	8 - 12	0.001
7. Vitamin E	5	0.5	4 - 6	0.001

Table 3. Value Pdf content based on sex

Value	Standard Deviation	Standard Error	Confidence Interval	Significance
1. Total Energy	1000	100	800 - 1200	0.001
2. Protein	50	5	40 - 60	0.001
3. Fat	100	10	80 - 120	0.001
4. Carbohydrate	150	15	120 - 180	0.001
5. Fiber	20	2	16 - 24	0.001
6. Vitamin C	10	1	8 - 12	0.001
7. Vitamin E	5	0.5	4 - 6	0.001

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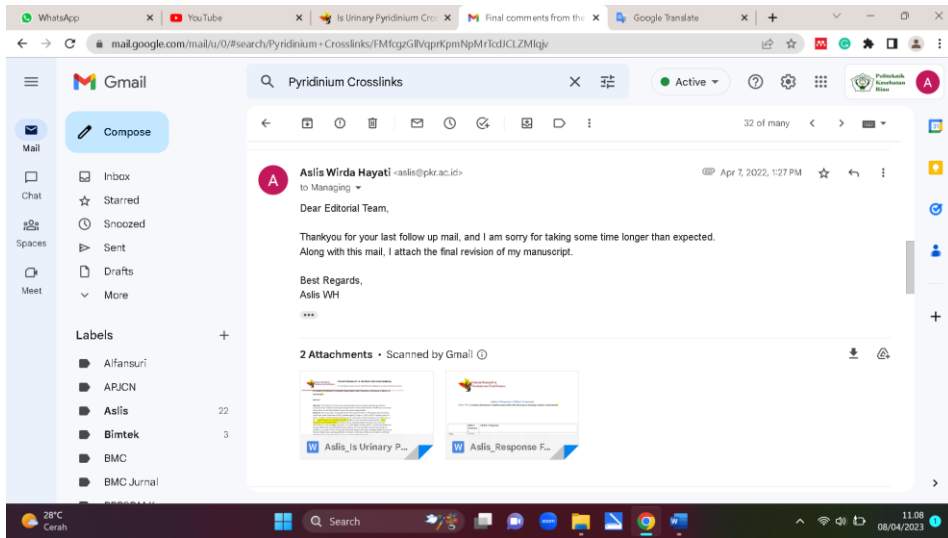
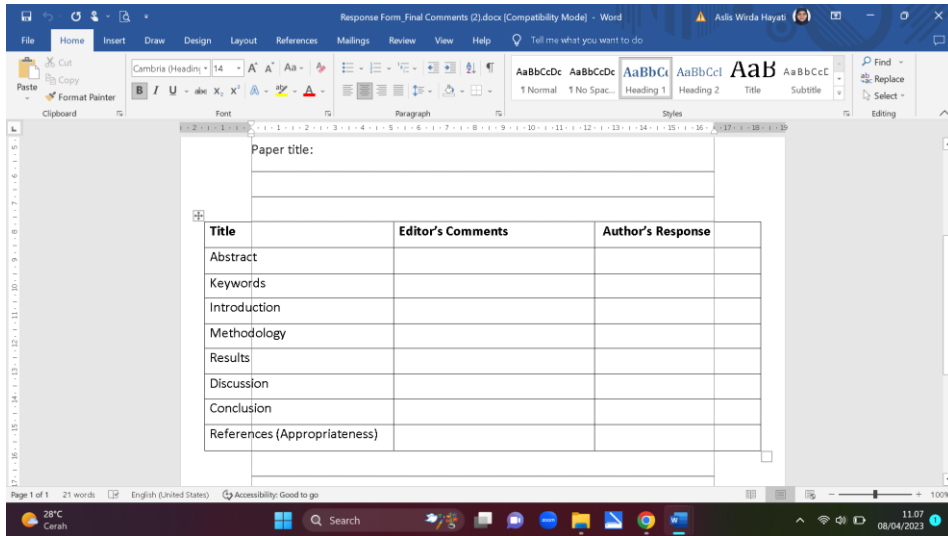
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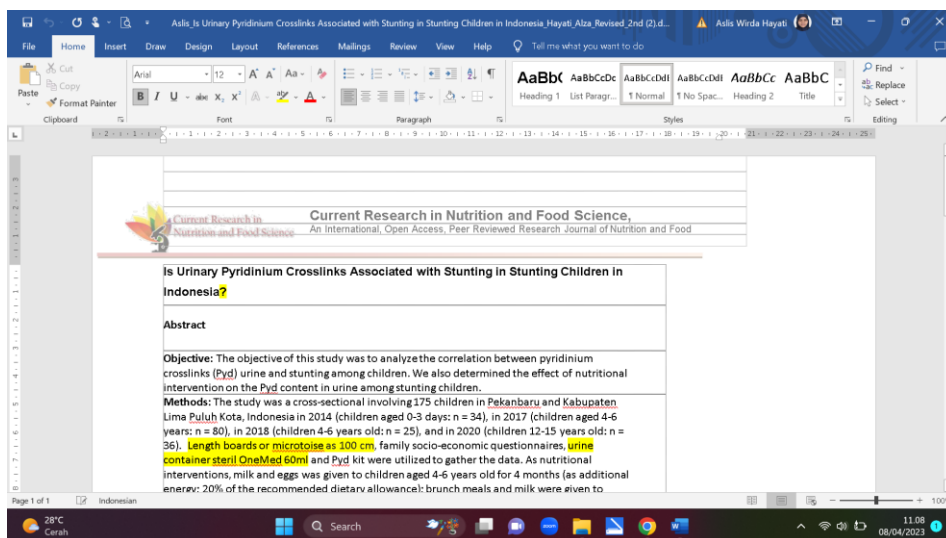
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Is Urinary Pyridinium Crosslinks Associated with Stunting in Stunting Children in Indonesia?

Abstract

Objective: The objective of this study was to analyze the correlation between pyridinium crosslinks (Pvd) urine and stunting among children. We also determined the effect of nutritional intervention on the Pvd content in urine among stunting children.

Methods: The study was a cross-sectional involving 175 children in Pekanbaru and Kabupaten Lima Puluh Kota, Indonesia in 2014 (children aged 0-3 days: n = 34), 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). Length boards or microtoise as 100 cm, family socio-economic questionnaires, urine container steril OneMed 60ml and Pvd kit were utilized to gather the data. As nutritional interventions, milk and eggs 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). Pvd 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 Pvd 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 Pvd 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 ($p < 0.01$). The contents of Pyd in the urine of stunted neonates were 982.9 ± 61.6 and normal neonates was 594.1 ± 266.1 nmol/mmol creatinine. 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 could 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

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 2007¹, 35.6% in 2010², 37.2% in 2013³ and 30.87% in 2018⁴. 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 stunted⁶. The prevalence of global stunting of children aged 13-15 years is around 35.1%⁶. 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 is 36.6% in the Riau Province⁷. Public health problems are considered severe if the prevalence of stunting is 30-39% and serious if the prevalence of stunting is $\geq 40\%$ ⁶. World Health Organization (WHO) established stunting standards based on anthropometric measurement with Height for Age (HAZ)-score < -2 SD⁸.

Stunting is associated with impaired linear growth processes⁹. The linear growth retardation process begins at 2 or 3 months of age¹⁰. Growth retardation reflects a process of failure to achieve linear growth potential as a result of suboptimal health and/or nutritional conditions⁶. 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 resorption¹¹. Pyridinium crosslinks are removed during the release of mature collagen in bone. Pyridinium is constructed as an intramolecular crosslinker during collagen maturation¹².

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 are formed by

hydroxylysine or lysine residues at the C- and N-telopeptide terminals of the collagen molecule and released during matrix resorption, excreted in the urine. There are several studies reported that the number of free crosslinks excreted in the urine is related to the rate of bone formation¹³.

Urine pyridinium crosslinks 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 pyridinium crosslinks markers include many metabolic disorders of bone such as osteoporosis, primary hyperparathyroidism and metastatic bone diseases. Urine pyridinium crosslinks also shows great hope as a marker of therapeutic efficacy in bone disorders associated with accelerated bone resorption¹⁴.

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)¹¹. There are two types of cells responsible for bone metabolism, namely osteoblasts and osteoclasts¹⁵. 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 stunting¹⁶. Calcium forms complex bonds with phosphate which can provide strength to bones¹⁷.

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 enumerators¹⁸.

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.

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 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 175. 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 Sumatera Province. In the 2018 study, 25 children aged 4-6 years were selected; they attended Al Falah PAUD, Lima Puluh Kota Kota District, West Sumatera Province. In 2020, 36 teenagers aged 12-15 years were selected. These teenagers attended SMP (Junior high school) Negeri 3 Pekanbaru in Riau Province.

There was no nutritional intervention given to the subject in the 2014 and 2017 studies but there was the provision of nutritional intervention to the subject in the 2018 and 2020 research. As nutritional interventions, milk and eggs was given to children aged 4-6 years old for 4 months (as additional energy; 20% of the recommended dietary allowance) in 2018; brunch and milk were given to children 12-15 years old for 34 days (as additional energy; 30% recommended dietary allowance) in 2020. 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 115 ml Indomilk Kids Choco milk for 1 small box is Rp. 2,500 and the price of 1 egg is Rp. 1,500.

The parameter used for classification of the children in the various groups was very stunting if height for age z-score (HAZ) < -3 standard deviation (SD), stunting if $-3 \text{ SD} \leq \text{HAZ} < -2 \text{ SD}$, normal if $-2 \text{ SD} \leq \text{HAZ} < +3 \text{ SD}$ ¹⁹.

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. The urine was collected by using urine container steril OneMed 60ml, aliquot to 6 ml and stored in freezer at -20°C (GEA by Vestfrost-Denmark Type G.201 Serial No: 20021808005) until further analysis.

In this study, body height gauges (microtoa) (STATURE METER 2M GEA, Indonesia), urine container steril (OneMed 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 urine collection. Urine was collected between 7:00 and 10:00 am. A two hour urine sampling between 07:00 and 10:00 am correlates well with a 24h urine²⁰. The minimum amount of urine collected from the subject was 10 ml. Urine collected from a neonate 0-3 day of age was used pediatric urine collector (<https://www.youtube.com/watch?v=mXJkjavH2Js>). 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 analysis²¹. The analysis was carried out simultaneously¹⁸.

Pyd measurements were performed with the use of MicroVue™ PYD EIA kit, USA. Pyd analysis was performed according to Hayati et al.²² using a Spectrophotometer (Microplate Reader 680 series, Bio-Rad Laboratories, Inc, Hercules, CA 94547, USA). The MicroVue PYD assay can be carried out using preservative free second morning void urine collections²³

Creatinine measurements were performed with the use of Jaffe reactions according to the method developed by Staden²⁴. 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. The respondent's parents' height was $160,3 \pm 6,7$ ($147,0 \pm 172,0$) 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 **girl** and **boy** 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 ($p < 0.01$) (Hayati, Aziz, Ahmad, Ningsih 2019). 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 creatinine¹⁹. 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 decrease after 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 ($p<0.01$); 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 ($p<0.05$), 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 Pyd content is different in children in different age groups. The Pyd urine excretion of children aged 0-3 days (neonates) was 10-100 times higher than that of children aged 3-16 years. 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 later²⁵. Pyd excretion for neonates was reported to be 642.7 ± 281.3 nmol/mmol creatinine¹³. In another study, Pyd excretion among elementary school children was reported to be about 50-500 nmol/mmol creatinine²⁶.

The growth spurt among boys occurs 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 years²⁷. 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 stops²⁸. Relatively the same height at the age of 30-45 years. After 45 years there is a decrease in height²⁹.

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

resorption³⁰. This means that the Pvd content of urine in the age group of children and adolescents with normal nutritional status is less than the Pvd content of urine in the age group of children with stunting nutritional status³⁰.

There was a decrease in the subject's Pvd 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 Pvd 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 Pvd 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 length³⁰. 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 cereals³¹.

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 group³²

The research that the authors conducted in 2018 aims to determine the effect of milk and egg consumption on the content of Pyridinium Crosslinks (Pvd) urine of stunting children

aged 4-6 years. 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 day³³, while their energy needs are 1,550 calories per day³⁴; 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.

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 H_0 and conclude that there is a statistically significant difference between the mean height before and after the intervention.

The average difference between the height of the subjects before and after the intervention was 1.91 cm. The lack of the subject's height compared to the national average height according to the results of Basic Health Research in 2007¹ and in 2010² is 4.82 cm. The median height of the subjects before the intervention was 106.40 cm and after the intervention was 107.95 cm. The median height of children aged 4-6 years based

on the results of Basic Research Health in 2007 and 2010 according to AsDI, IDAI, PERSAGI in 2015 is 112 cm³⁵. 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 time³⁶. 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

Variables	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	34	80	25	36
Sex	Boys: 74.3% (n=26); Girls: 25.7% (n=9)	Boy: 100.0% (n=80); Girls: 0.0% (n=0)	Boys: 64.0% (n=16); Girls: 36.0% (n=9)	Boys :50.0% (n=18); Girls: 50.0% (n=18)
Height (cm)		98±96(99:2)		
<ul style="list-style-type: none"> • Verry Stunting: height for age z-score (HAZ) <-3 standard deviation (SD) • Stunting: -3 SD ≤ HAZ <- 2 SD 				
- 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)
<ul style="list-style-type: none"> • Normal: -2 SD ≤ HAZ <+3 SD 	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.
Variables	Year of the research			
	2014	2017	2018	2020
Height for age z-score (HAZ)				
<ul style="list-style-type: none"> • Verry Stunting: height for age z-score (HAZ) <-3 standard deviation (SD) - Verry stunting before 				-3.22±0.09(-3. 34: -

nutritional intervention				3.11) The number of study subjects: 11.1% (n = 4)
- Verry stunting after nutritional intervention				-3.11±0.05(-3.15: -3.04) The number of study subjects: 11.1% (n = 4)
• Stunting: -3 SD ≤ HAZ < -2 SD				
- Stunting before nutritional intervention	-2,15±0.27(-2.95: -2.31) The number of study subjects: 100.0% (n = 34)	-2.52±0.43(-2.85: -2.01) The number of study subjects: 100.0% (n = 80)	-2.32±0.42(-2.96: -2.69) The number of study subjects: 100.0% (n = 25)	-2.41±0.28(-2.97: -2.02) The number of study subjects: 69.4% (n = 25)
- Stunting after nutritional intervention	No nutritional intervention	No nutritional intervention	-2.24±0.44(-2.98: -1.59) The number of study subjects: 100.0% (n = 25)	-2.37±0.27(-2.90: -2.01) The number of study subjects: 69.4% (n = 25)
• Normal: -2 SD ≤ HAZ < +3 SD (n = 63)				
Normal before nutritional intervention	-0,10±0.69(-1.00: 1.97) The number of study subjects: 100.0% (n = 34)	0.87±0.87(-1.99: 1.70) The number of study subjects: 100.0% (n = 80)		The number of study subjects: 0.0% (n = 0)
Normal after nutritional intervention	No nutritional intervention	No nutritional intervention		-1.71±0.31(-1.93: -1.19) The number of study subjects: 19.4% (n = 7)
Variables	Year of the research			
	2014	2017	2018	2020
Conclusion	The Pyd content in the urine of stunted neonates was found to be different from the Pyd content in normal	There was a negative correlation observed between urine Pyd content and the subject's height (p	There was a difference in Pyd content in the urine of subjects before and after nutritional intervention (p <0.01).	There was a difference observed in Pyd content of subjects before and after the nutritional

	neonatal urine (p <0.01). The pattern of Pyd content in urine according to height was like the letter "U"	<0.05). Urine Pyd content showed a weak negative correlation with height at r = - 0.242	However, all subjects were still in the stunting category	intervention (p <0.05). There were 19.4% of subjects increased their nutritional status from stunting to normal
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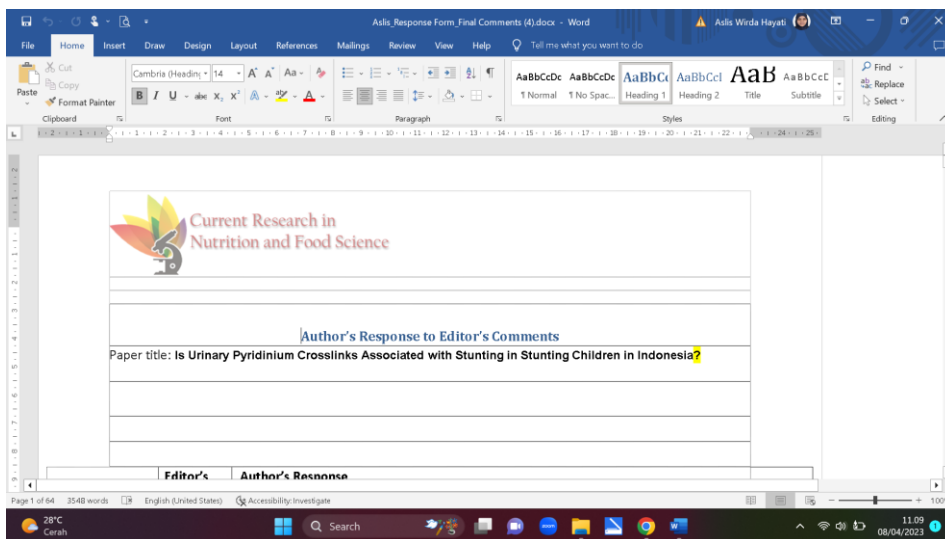
Table 2. Urine Pyd content based on nutritional intervention

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)
• Very Stunting	-	18.1 (n = 2)	-	-
• Stunting	982 (n = 9)	16.4 (n = 15)	-	-
• Normal	594 (n = 25)	15.5 (n = 63)	-	-
• Before nutritional Intervention	-	-	16.9 (n = 25)	9.81 (n = 36)
• After nutritional Intervention	-	-	15.3 (n = 25)	5.33 (n = 36)

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)

^saverage ± standard deviation (minimal: maximal)



Author's Response to Editor's Comments

Paper title: **Is Urinary Pyridinium Crosslinks Associated with Stunting in Stunting Children in Indonesia?**

	Editor's Comments	Author's Response
Title	Is Urinary Pyridinium Crosslinks Associated with Stunting in Stunting Children in Indonesia?	
		Before
		Is Urinary Pyridinium Crosslinks Associated with Stunting in Stunting Children in Indonesia
		After

		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.	
		Before
		The objective of this study was to analyze the correlation between pyridinium crosslinks (Pyd) urine and stunting among children.
		After
		The objective of this study was to analyze the correlation between pyridinium crosslinks (Pyd) in urine and stunting among children. We

	<p>Comment [A1]: Need to indicate if “spot urine” samples or 24-hour urine samples were used.</p>	<p>Urine samples were used yaitu “spot urine”</p>
		<p>Literature on “spot urine”:</p>
		<p>Jonathan CY, Tang, Dutton JJ, Piec I, Green D, Fisher E, Washbourne CJ , Fraser WD. LC–MS/MS application for urine free pyridinoline and free deoxypyridinoline: Urine markers of collagen and bone degradation. Clinical Mass Spectrometry 1 (2016) 11–18 https://doi.org/10.1016/j.clinms.2016.08.001</p>
		<p>Method Random second morning void fasting urines were collected into sterile containers stored at 20°C and protected from light prior to analysis</p>
		<p>Quidel Corporation. 2009. An enzyme immunoassay for the quantitation of pyridinium crosslinks. (PYD) in human urine. San Diego: Quidel Corporation https://www.quidel.com/sites/default/files/product/documents/8010_microvue_pyd_english_0.pdf</p>
		<p>SPECIMEN COLLECTION AND STORAGE The MicroVue PYD assay can be carried out using preservative free First Morning Void (FMV) or Second Morning Void (SMV) urine collections. Longitudinal collections (e.g. when assessing changes in resorption) should be collected at approximately the same time each day. Keep the urine sample refrigerated (2–8°C) for storage of less than 7 days, or freeze the sample at ≤ -20°C for longer storage. Do not subject sample to</p>

		more than 3 freeze/thaw cycles. Avoid prolonged exposure to light, especially sunlight. During routine processing, samples are not affected by normal, artificial laboratory lighting.
		Eagle Biosciences, Inc. 2019. Hydroxypyridinium Crosslinks HPLC Assay. Nashua: Eagle Biosciences, Inc. www.EagleBio.com
		<p>Specimen</p> <ul style="list-style-type: none"> ☑ Urine could be used in this test system. A two hour urine sampling between 07:00 and 10:00 am correlates well with a 24h urine. ☑ Samples are stable for 24h at room temperature and up to one week at 2-8 °C. For longer storage the samples should be kept at -20°C.
		The quote that I wrote in the article:
		<p>Data Collection and Instrument</p> <p>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. A two hour urine sampling between 07:00 and 10:00 am correlates well with a 24h urine (Eagle Biosciences Inc 2019). 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 (Jonathan, Tang, Dutton, Picc, Green, Fisher, Washbourne, Fraser 2016). The analysis was carried out simultaneously¹⁸.</p>



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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). The MicroVue PYD assay can be carried out using preservative free second morning void urine collections (Quidel Corporation 2009).
	Comment [A2]: What is Height “guage’?	Height “guage’ = length boards for measuring length of infants or microtoie for measuring height of children
		Before
		Height gauges , family socio-economic questionnaires, pot urine and Pyd kit were utilized to gather the data.
		After
		Length boards or microtoise as 100 cm , family socio-economic questionnaires, pot urine and Pyd kit were utilized to gather the data.



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	Comment [A3]: What is "pot" urine?	"Pot" urine is urine container steril OneMed 60ml
		Before
		Length boards or microtoise as 100 cm, family socio-economic questionnaires, pot urine and Pyd kit were utilized to gather the data.
		After
		Length boards or microtoise as 100 cm, family socio-economic questionnaires, urine container steril OneMed 60ml and Pyd kit were utilized to gather the data.
	Deleted: of	
		Before
		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.
		After
		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.



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	Deleted: discovered to be	
		Before
		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.
		After
		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
	Deleted: were	
		Before
		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.



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		After
		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.
	Comment [A4]: What was the value for the "Normal neonatal" urine Pyd content?	
		Before
		Stunting neonatal urine Pyd content was found to be different from normal neonatal urine Pyd content ($p < 0.01$).
		After
		Stunting neonatal urine Pyd content was found to be different from normal neonatal urine Pyd content ($p < 0.01$). The contents of Pyd in the urine of stunted neonates were 982.9 ± 61.6 and normal neonates was 594.1 ± 266.1 nmol/mmol.

	Deleted: a	
		Before
		There was a inverse correlation revealed between urine Pyd content and height of children aged 4-6 years ($p < 0.05$) and $r = -0.242$.
		After
		There was an inverse correlation revealed between urine Pyd content and height of children aged 4-6 years ($p < 0.05$) and $r = -0.242$.
	Comment [A5]: This sentence makes no sense. Need to rephrase for clarity. Why "expected" to be a marker.....?	
		Before
		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

		After
		The urine Pyd could be a marker of the efficacy of nutritional care in bone growth disorders associated with bone resorption in stunting children.
Keywords		
Introduction	Deleted: are	
		Before
		The prevalence of stunting among children under five years of age in Indonesia are 36.8% in 2007 ¹ , 35.6% in 2010 ² , 37.2% in 2013 ³ and 30.87% in 2018 ⁴ .
		After
		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 .

	Deleted: included stunting	
		Before
		About 59.3% of children aged 3-5 years were included stunting ⁶
		After
		About 59.3% of children aged 3-5 years in Indonesia were stunted ⁶ .
	Deleted: is	
		Before
		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 ⁸ .
		After

		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 Province8.
	Deleted: s	
		Before
		World Health Organization (WHO) established stunting standards based on anthropometrics measurement with Height for Age (HAZ)-score <-2 SD ⁹ .
		After
		World Health Organization (WHO) established stunting standards based on anthropometric measurement with Height for Age (HAZ)-score <-2 SD ⁹ .
	Comment [A6]: The sentence need to be rephrased, because it is not urinary pyridinium	



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	crosslinks that are removed. Need to delete "Urinary".	
		Before
		Urinary pyridinium crosslinks are removed during the release of mature collagen in bone.
		After
		Pyridinium crosslinks are removed during the release of mature collagen in bone.
	Comment [A7]: This sentence makes no sense.	
		Before
		Pyd appears in urine that is characterized by peptide formation.



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		After
		The sentence has been deleted.
	Deleted: .	
		Before
		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 .
		After
		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 .
	Comment [A8]: Why until now?	
		Before



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		Until now, there is no convincing stunting indicator reported in the literature
		After
		There is no convincing stunting indicator reported in the literature.
	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.	
		Before
		Biochemical indicators using blood are unethically carried out on children without any medical reason because they are invasive (painful).

		After
		This statement was deleted.
	<p>GENERAL COMMENTS: This section is too long and repetitive. The author(s) need to reduce this section by about 25%.</p>	<p>Belum ibu dilakukan untuk meningkatnya. Akan disingkat jika sudah selesai nanti diedit bagian bawahnya.</p>
Methodology		
		Before
		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.
		After

Commented [AWH1]: Belum ibu perbaiki.

		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.
	Deleted: subject	
	Deleted: was	
		Before
		The subject was in <i>Andini</i> Mother and Child Hospital, Pekanbaru City, Riau Province.
		After
		The neonates were in <i>Andini</i> Mother and Child Hospital, Pekanbaru City, Riau Province.
	Deleted: Subjects	
		Before
		Subjects were children who attend As-Shofa Kindergarten and Hidayatullah Kindergarten in Pekanbaru City, Riau Province and Al-Falah PAUD (Early childhood education programs) in Lima Puluh Kota District, West Sumatra Province.



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		After
		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.
	Deleted: ,	
		Before
		In the 2018 study, 25 children aged 4-6 years were selected , who attended Al Falah PAUD, Lima Puluh Kota Kota District, West Sumatra Province. In 2020, 36 teenagers aged 12-15 years were selected.
		After
		In the 2018 study, 25 children aged 4-6 years were selected , who attended Al Falah PAUD, Lima Puluh Kota Kota District, West Sumatra Province. In 2020, 36 teenagers aged 12-15 years were selected.
	Deleted: who	



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		Before
		In the 2018 study, 25 children aged 4-6 years were selected; who attended Al Falah PAUD, Lima Puluh Kota Kota District, West Sumatra Province.
		After
		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.
	Deleted: All	
		Before
		All parent subjects gave written informed consent.
		After
		The parents of all the subjects gave written informed consent.



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	Comment [A10]: Need to rephrase this sentence because it makes no sense.	
		Before
		At the time of informed parental consent, and race (Indonesian, expatriate).
		After
		The sentence has been deleted.
	Comment [A11]: How was 10ml of urine collected from a neonate 0-3 day of age? Give the reference.	



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		Before
		The minimum amount of urine collected from the subject was 10 ml.
		After
		The minimum amount of urine collected from the subject was 10 ml. Urine collected from a neonate 0-3 day of age was used pediatric urine collector (https://www.youtube.com/watch?v=mXJkjavH2Js).
	Deleted: taken	
		Before
		The minimum amount of urine taken from the subject was 10 ml.
		After
		The minimum amount of urine collected from the subject was 10 ml.
Results		
	Comment [A12]: This is not the	



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	<p>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.</p>	
		Before
		Almost all of the respondent's parents' height was > 150 cm.
		After
		The respondent's parents' height was $160,3 \pm 6,7$ ($147,0 \pm 172,0$) cm.
	Comment [A13]: The	



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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.

		The parameter used for classification of the children in the various groups was very stunting if height for age z-score (HAZ) <-3 standard deviation (SD), stunting if $-3 \text{ SD} \leq \text{HAZ} < -2 \text{ SD}$, normal if $-2 \text{ SD} \leq \text{HAZ} < +3 \text{ SD}$.
		Peraturan Menteri Kesehatan Republik Indonesia Nomor 2 Tahun 2020. http://hukor.kemkes.go.id/uploads/produk_hukum/PMK_No_2_Th_2020_ttg_Standar_Antropometri_Anak.pdf
	Comment [A14]: Which men and women are you referring to?????	
		Before
		The Pyd content of stunted neonates' urine for men and women were 988.45 and 641.40 nmol / mmol creatinine, respectively.
		After
		The Pyd content of stunted neonates' urine for girl and boy were 988.45 and 641.40 nmol / mmol creatinine, respectively.



	Comment [A15]: How did you get the "normal" neonates? The indication was that all the neonates were stunted.	
		Before
		The Pyd content of stunting neonates' urine was 982.92 ± 61.64 , whereas normal neonates were 594.11 ± 266.16 nmol/mmol creatinine ($p < 0.01$).
		After
		The Pyd content of stunting neonates' urine was 982.92 ± 61.64 , whereas normal neonates were 594.11 ± 266.16 nmol/mmol creatinine ($p < 0.01$) (Hayati, Aziz, Ahmad, Ningsih 2019).
		Hayati AW, A Aziz, SR Ahmad, SW Ningsih. 2019. Pyridinium Crosslinks (Pyd) in the Urine is Associated with Stunting in Neonates. Asian Journal of Research in Medical and Pharmaceutical Sciences; 7(1): 1-8.



	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.	
		Before
		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.
		After
		After
		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 (Peraturan Menteri Kesehatan Republik Indonesia Nomor 2 Tahun 2020).
		Hayati AW, A Aziz, SR Ahmad, SW Ningsih. 2019. Pyridinium Crosslinks (Pyd) in the Urine is Associated with Stunting in Neonates. Asian Journal of Research in Medical and Pharmaceutical Sciences; 7(1): 1-8.



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

Before

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.

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



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		After
		The Pyd content of stunted children decrease after 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; 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.
	Comment [A18]: Need to give the p-value to indicate statistical significance of the result.	
		Before



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.

After

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 ($p<0.01$); 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 ($p<0.05$), respectively.

General
comment



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 initial 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.

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.

		After
		Ditambahkan:
		The parameter used for classification of the children in the various groups was very stunting if height for age z-score (HAZ) <-3 standard deviation (SD), stunting if $-3 \text{ SD} \leq \text{HAZ} < -2 \text{ SD}$, normal if $-2 \text{ SD} \leq \text{HAZ} < +3 \text{ SD}$.
		Peraturan Menteri Kesehatan Republik Indonesia Nomor 2 Tahun 2020. http://hukor.kemkes.go.id/uploads/produk_hukum/PMK_No_2_Th_2020_ttg_Standar_Antropometri_Anak.pdf
		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 initial 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.
Discussion		
	Comment [A19]: This statement is	



	<p>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.</p>	
		<p>Before</p>
		<p>Discussion</p> <p>In this study, it was found that the urine Pyd content of stunting children decreased with increasing age.</p> <p>The same trend was also observed in the previous studies where Pyd urine excretion of children</p> <div data-bbox="1113 1015 1396 1096" style="border: 1px solid red; padding: 5px; margin-top: 10px;"> <p>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.</p> </div>
		<p>After</p>



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		In this study, it was found that the Pyd content is different in children in different age groups.
	<p>Comment [A20]: What previous studies, need to give the references of the studies.</p>	
		Before
		<p>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 adult¹². This was</p>
		<p>participated in the study. You can say that the Pyd content is different in children in different age groups.</p> <p>Comment [A20]: What previous studies, need to give the references of the studies.</p>
		After



		The Pyd urine excretion of children aged 0-3 days (neonates) was 10-100 times higher than that of children aged 3-16 years.
	Comment [A21]: Need to focus on teenagers not adults. Your study did not include adults.	
		Before
		<p>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 adult¹². 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 later²¹. Pyd excretion for neonates was reported to be 642.7±281.3 nmol/mmol creatinine¹⁴.</p>

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.



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		After
		The sentence "The crosslink excretion in children was reported to be 20 times higher than in adult ¹² . " was deleted.
	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.	
		Before



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In another study, Pyd excretion among elementary school children was reported to be about 50-500 nmol/mmol creatinine²². 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 (median 131 vs 26 nmol/mmol creatinine (p<0,001); in postmenopausal women urinary Pyd excretion was raised in those taking T4 which is 40.0 ± 2.7 nmol/mmol creatinine (p<0,05)).

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

Field Code Changed

Field Code Changed

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.

After



Discussion

In this study, it was found that the Pyd content is different in children in different age groups. The Pyd urine excretion of children aged 0-3 days (neonates) was 10-100 times higher than that of children aged 3-16 years. 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 later²¹. Pyd excretion for neonates was reported to be 642.7±281.3 nmol/mmol creatinine¹⁴. In another study, Pyd excretion among elementary school children was reported to be about 50-500 nmol/mmol creatinine²².

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

Before



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		<p>Urine Pvd content has been used to determine the severity of osteoporosis in the elderly. The more urine Pvd content in the elderly group, the higher the level of osteoporosis. This means that more bone resorption occurs in this group of elderly people.</p>	<p>your discussion on the findings in you present study and compare with other studies with similar design.</p> <p>Comment [A23]: Completely irrelevant to your present study design.</p>
		After	
		It was deleted	
	<p>Comment [A24]: Your study design included children 12 to 15 years of age.</p>		
		Before	



		<p>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</p>	<p>Comment [A24]: Your study design included children 12 to 15 years of age.</p>
		After	
		It was deleted	
	<p>Comment [A25]: Irrelevant to this study.</p>		
		Before	
		<p>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</p>	<p>Comment [A24]: Your study design included children 12 to 15 years of age.</p>



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		After
		It was deleted
	Comment [A26]: Need to transfer to the Introduction section. No need to repeat this information in the discussion section.	
		Before



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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.²⁹

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

Field Code Changed

After

It has been moved to the Introduction section.



protein, stabilized by cross-linking between the N terminal and C terminal in the formation of the base of bone tissue. The pyridinium crosslinks are formed by hydroxylysine or lysine residues at the C- and N-telopeptide terminals of the collagen molecule and released during matrix resorption, excreted in the urine. There are several studies reported that the number of free crosslinks excreted in the urine is related to the rate of bone formation¹⁴.

Urine pyridinium crosslinks 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 pyridinium crosslinks markers include many metabolic disorders of bone such as osteoporosis, primary hyperparathyroidism and metastatic bone diseases. Urine pyridinium crosslinks also shows great hope as a marker of therapeutic efficacy in bone disorders associated with accelerated bone resorption²⁹.

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

Comment [A27]: The categories are not presented in the result section.



	Therefore this information is not clearly presented in the result section.	
		Before
		<p>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%</p>

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



		After				
		Age	Neonatus 0 - 3 days	4-6 years	4-6 years	12-15 years
		Number of subjects	34	80	25	36
		Sex	Boys: 74.3% (n=26); Girls: 25.7% (n=9)	Boy: 100.0% (n=80); Girls: 0.0% (n=0)	Boys: 64.0% (n=16); Girls: 36.0% (n=9)	Boys :50.0% (n=18); Girls: 50.0% (n=18)
		Height (cm)		98±96(99:2)		
		• Very Stunting: height for age z- score (HAZ) <-3 standard deviation (SD)				
		• Stunting: -3 SD ≤ HAZ <- 2 SD				
		- 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: -2 SD ≤ HAZ <+3 SD	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



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Nutrition and Food Science

	<p>Comment [A28]: Not clearly presented in the result section. Thus it seems like just a speculation.</p>	
		<p>Before</p>
		<p>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.</p> <p>There are many studies on the effect of nutrition on body length³⁰. Energy intake was the</p>
		<p>After</p>

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.



	Year of the research			
	2014	2017	2018	2020
Height for age z-score (HAZ)				
<ul style="list-style-type: none"> • Very Stunting height for age z-score (HAZ) <-3 standard deviation (SD) 				
- Very stunting before nutritional intervention				-3.22±0.09(-3.34;-3.11) The number of study subjects: 11.1% (n = 4)
- Very stunting after nutritional intervention				-3.11±0.05(-3.15;-3.04) The number of study subjects: 11.1% (n = 4)
<ul style="list-style-type: none"> • Stunting -3 SD ≤ HAZ <- 2 SD 				
- Stunting before nutritional intervention	-2.15±0.27(-2.95;-2.31) The number of study subjects: 100.0% (n = 34)	-2.52±0.43(-2.85;-2.01) The number of study subjects: 100.0% (n = 85)	-2.32±0.42(-2.96;-2.69) The number of study subjects: 100.0% (n = 25)	-2.41±0.28(-2.97;-2.02) The number of study subjects: 69.4% (n = 25)
- Stunting after nutritional intervention	No nutritional intervention	No nutritional intervention	-2.24±0.44(-2.98;-1.59) The number of study subjects: 100.0% (n = 25)	-2.37±0.27(-2.90;-2.01) The number of study subjects: 69.4% (n = 25)
<ul style="list-style-type: none"> • Normal -2 SD ≤ HAZ <+3 SD (n = 63) 				
Normal before nutritional intervention	-0.10±0.69(-1.00;1.97) The number of study subjects: 100.0% (n = 34)	0.87±0.87(-1.99;1.70) The number of study subjects: 100.0% (n = 85)		The number of study subjects: 0.0% (n = 0)
Normal after nutritional intervention	No nutritional intervention	No nutritional intervention		-1.71±0.31(-1.93;-1.19) The number of study subjects: 19.4% (n = 7)



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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.

Before

There are many studies on the effect of nutrition on body length³⁰. Energy intake was the strongest predictor of increased linear growth. Providing energy from food (310 Cal/day) in malnourished Indian children can increase height gain. Protein is provided from skim milk and cereals. The research was conducted by Bhandari et al³¹ on providing interventions to slum communities in Nehru, India. The study was conducted in a randomized controlled trial. In this study, the number of samples was 418 children aged 4-12 months. The children were divided into two groups. The first group is given quality supplementary food every day with supervision so that consumption is optimal. The second group is given nutrition counseling only; this group received 30-45 minutes of counseling monthly by a trained dietitian. Nutritional intervention was given for 8 months. The study showed that $1 - 2 = 0.4$ cm (attainment of the subject's body length increase), and the standard deviation was ≈ 1.6 cm.

speculation.

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!



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		After
		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 ³¹ .
	Comment [A30]: All these are more for a student project report, not for a manuscript to be published in a scientific journal.	
		Before



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		<p>Another research related to the provision of nutrition and linear growth interventions, namely the research of Matali, Wungouw and Sapulete³² in Manado (Indonesia) which carried out an</p>	<p>reference for the study.</p> <p>Comment [A30]: All these are more for a student project report, not for a manuscript to be published in a scientific journal.</p>
		After	
		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 ³²	
	<p>Comment [A31]: ?????????? Need to give only the reference of this study, not the details.</p>		
		Before	



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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.

After

I have deleted it

Comment
[A32]: MORE
FOR A



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	STUDENT PROJECT REPORT!!!!!!	
		Before
		<p>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}.</p> <p>Comment [A32]: MORE FOR A STUDENT PROJECT REPORT!!!!!!</p>
		After
		I have deleted it



	<p>Comment [A33]: No need to repeat information already stated earlier.</p>	
		<p>After</p>
		<p>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.</p> <p>Comment [A33]: No need to repeat information already stated earlier.</p>
		<p>Before</p>
		<p>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. 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</p>

		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.
	Comment [A34]: Should have been included in the method section not the discussion section.	
		Before
		<p>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.</p>

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



		After
		<p>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 H_0 and conclude that there is a statistically significant difference between the mean height before and after the intervention.</p>
	GENERAL COMMENT:	



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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

References
(Appropriateness)

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



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	absolute numbers.	
		Before



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)		98±96(99.2)		
• Very Stunting				
• 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 Pvd content in the urine of stunted neonates was found to be different from the Pvd content in normal neonatal urine (p <0.01). The pattern of Pvd content in urine according to height was like the letter "U"	There was a negative correlation observed between urine Pvd content and the subject's height (p <0.05). Urine Pvd content showed a weak negative correlation with height at r = -0.242	There was a difference in Pvd 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 Pvd 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?



After

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	34	80	25	36
Sex	Boys: 74.3% (n=26); Girls: 25.7% (n=9)	Boy: 100.0% (n=80); Girls: 0.0% (n=0)	Boys: 64.0% (n=16); Girls: 36.0% (n=9)	Boys : 50.0% (n=18); Girls: 50.0% (n=18)

Comment
[A36]: Only
boys?



		Only boys?					
		Yes, they were					
		Before					
		<table border="1"> <tr> <td>Conclusion</td> <td>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"</td> <td>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 $r = -0.242$</td> <td>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</td> <td>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</td> </tr> </table>	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
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			
		After					



<p>Conclusion</p>	<p>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"</p>	<p>There was a negative correlation observed between urine Pyd content and the subject's height (p <0.05). Urine Pyd content showed a weak <u>negative</u> correlation with height at r = -0.242</p>	<p>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</p>	<p>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</p>
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Before



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 = 22)	-	18.1	-	-
• Stunting (n = 22)	982	16.4	-	-
• Normal (n = 22)	594	15.5	-	-
• Before nutritional Intervention (n = 22)	-	-	16.9	9.81
• After nutritional Intervention (n = 22)	-	-	15.3	5.33

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After

Table 2. Urine Pyd content based on nutritional intervention

Status nutritional	Urine Pyd content (nmol/mmol creatinine)			
	Neonates 0 - 3 days (2014)	Children 4-6 years (2017)	Children 4-6 years (2018)	Children 12-15 years (2020)
• Very Stunting	-	18.1 (n = 2)	-	-
• Stunting	982 (n = 9)	16.4 (n = 15)	-	-
• Normal	594 (n = 25)	15.5 (n = 63)	-	-
• Before nutritional Intervention	-	-	16.9 (n = 25)	9.81 (n = 36)
• After nutritional Intervention	-	-	15.3 (n = 25)	5.33 (n = 36)

6. Accepted: 7 Mar 2022

This screenshot shows a Gmail inbox on a desktop browser. The search filter is set to "Pyridinium Crosslinks". The selected email is from the Managing Editor of Food and Nutrition Journal, dated April 12, 2022, at 1:54 PM. The email content includes a greeting to Dr. Aslis, a confirmation of paper acceptance for the April 2022 issue, and banking details for a transfer. The sender's name is partially visible as "Politeknik Kesehatan Wita".

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Apr 12, 2022, 1:54 PM

Dear Dr. Aslis,

Attached is the Acceptance cum Bill for your Paper.

We would like to inform you that your paper was reviewed by our editorial Committee and is accepted for the issue of April 2022.

Kindly transfer the charges on the following account details below:

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This screenshot shows a Gmail inbox on a desktop browser, displaying a follow-up email from Sobiya Sultan. The search filter remains "Pyridinium Crosslinks". The email is dated 11:13 on 08/04/2023. It repeats the banking details from the previous email and includes contact information for Sobiya Sultan, an Editorial Assistant at Food and Nutrition Journal. A "Mailtrack" notification is visible at the bottom of the email.

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Department of Nutrition,
Poltekkes Kemenkes Riau, Pekanbaru,
Indonesia, 28122

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

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Aslis Wirda Hayati <aslis@pkr.ac.id>
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Wed, Apr 13, 2022, 6:38 PM

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M **Managing Editor** <info@foodandnutritionjournal.org> to me Apr 14, 2022, 2:26 PM

Dear Dr. Aslis

Thanks for your response. We have forwarded the manuscript for publication, soon it will appear in our coming issue link.

Please summarize in two lines what your paper is about and why it's essential in just one or two sentences. This will help in attracting readers and increasing the readership of your article on our social media channels.

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Aslis Wirda Hayati <aslis@pkr.ac.id> to Managing
Apr 16, 2022, 11:48 AM

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

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7. Published: 18 April 2022

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

Best Regards

Sobiya Sultan
Editorial Assistant

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

ASLIS WIRDA HAYATI* and YESSI ALZA

Department of Nutrition, Poltekkes Kemenkes Riau, Pekanbaru, Indonesia.

Abstract

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



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Introduction

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

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

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

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

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.

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

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

Data Collection and Instrument

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

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

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

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

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

Statistical Analysis

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

Results

The Socioeconomic Characteristics of the Child's Family

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

The education of the respondents' parents was on average high school. Almost all respondent mothers were housewives (90%). The respondent father's job was usually entrepreneur, employee

or laborer. The number of siblings of the respondent was around 1-3 peoples. Almost all of the respondent's parents' height was > 150 cm.

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

	Year of the research			
	2014	2017	2018	2020
Location	Andini Hospital Pekanbaru City in Riau Province	Al Falah PAUD study Lima Puluh Kota District (in West Sumatra), As-Shofa Kindergarten and Hidayatullah Kinder-garten 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	There was a negative correlation observed between urine Pyd content and the subject's	There was a difference in Pyd content in the urine of subjects before and after nutri-	There was a difference observed in Pyd content of subjects before

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

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)

^saverage ± standard deviation (minimal: maximal)

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

Urine Pyd Content Based on Nutritional Intervention

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

Discussion

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

In another study, Pyd excretion among elementary school children was reported to be about 50-500 nmol/mmol creatinine.²² The urine pyd of adults who have health problems is higher than normal adults. For example, Harvey *et al.*²³ used 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 ([median 131 vs 26 nmol/mmol creatinine ($p < 0.001$), in postmenopausal women urinary Pyd excretion was raised in those taking T4 which is 40.0 ± 2.7 nmol/mmol creatinine ($p < 0.05$)].

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

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

The growth spurt among boys occur more slowly than girls. Growth spurt in boys began to occur at the age of 10.5 years, whereas in girls it began to occur at the age of 9.5 years.²⁵ The increases in height occurred two years earlier in girls than boys. The peak height growth 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.²⁶ Relatively the same height at the age of 30-45 years. After 45 years there is a decrease in height.²⁷

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

Urine 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

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

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

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

Another research related to the provision of nutrition and linear growth interventions, namely the research of Matali, Wungouw and Sapulete³² in Manado (Indonesia) which carried out an intervention in the form of intake of 250 ml of low fat high calcium UHT milk every day for 60 days to elementary school children. The research subjects were 40 people consisting of 20 people in the intervention group and 20 people in the control group. The average height of the intervention group in the first measurement was 133.23 cm and the second measurement was 134.78 cm, while the average height of the control group in the first measurement was 131.52 cm and 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.

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

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

(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,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 H_0 and conclude that there is a statistically significant difference between the mean height before and after the intervention.

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