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Protective roles of the red-dragon fruit peels (*Hylocereus costaricensis)* against the cigarette-smoke harmful effect in Wistar rats

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ABSTRACT

Background: Many people are exposed to cigarette smoke unintentionally in numerous places worldwide. Cigarette smoke contains carbon monoxide, nicotine, and polycyclic aromatic hydrocarbons, which are toxic and can trigger the production of free radicals in the body.

Objective: To study the impact of cigarette-smoke exposure twice daily for 30 days on 4-5 weeks Rattus norvegicus L. without or with a daily intake of the juice or ethanol extract of the red-dragon fruit peels H. costaricensis.

Materials and Methods: Twenty-eight 4-5 weeks old male Wistar rats were randomly allocated into Control (not exposed to cigarette-smoke), exposed to cigarette-smoke only (C_{smoke}), exposed to cigarette-smoke and H. Costaricensis-peel juice (Jc_{Hc}), exposed to cigarette-smoke and had H. Costaricensis-peel extract (Ex_{Hc}). Cigarette-smoke exposure was given twice daily. The juice (3g/mL) and extract (3.15g/mL) were given for 30 days ad libitum. Feed and drink intake, body weight, and serum biochemistry (MDA, bilirubin, ALT and AST) were determined. Data were analyzed by ANOVA.

Results: The positive control group with cigarette-smoke exposure (C_{smoke}) had a significant elevation in serum malondialdehyde (MDA), alanine-transaminase (ALT), and aspartate-transaminase (AST) and drinking water intake ($p<0.05$) but reduced feed intake and body weight ($p<0.05$). The Jc_{Hc} and Ex_{Hc} groups had reduced serum MDA, ALT, and AST and higher body weight and feed intake than the $C_{smoke,}$ and the extract had a better reduction than the juice ($p<0.05$). Furthermore, the extract had a lower biochemical profile than the Control group ($p<0.05$).

Conclusion: The disturbance in serum MDA, ALT, AST, water and feed intake, and body weight by cigarette smoke was ameliorated by H. costaricensis peel juice or extract daily for 30 days. H. costaricensis peel juice or extract can be used to prevent the adverse effects of cigarette smoke exposure and has the potential to be developed into valuable products.

Keywords*: antioxidants; free radicals; MDA; smoking; tobacco*

BACKGROUND

Many people of all ages are exposed to cigarette smoke unintentionally or involuntarily in various places and across the globe.[1-3] These exposures threaten public health due to the increased mortality and morbidity of various non-communicable diseases at the population level.[4,5] In 2019, data from 204 countries suggested that 7.69 million deaths were attributable to smoking tobacco use, which accounts for 13.6% of deaths worldwide.[6] Encumbrance of this magnitude also negatively impacted healthcare and economic costs.[7]

Meanwhile, cigarette smoke contains more than 7,000 toxic chemicals, some of which are carbon monoxide (CO), nicotine, benzo (a) pyrene, nitrous oxide (NO), and polycyclic aromatic hydrocarbons (PAH), which are dangerous free radicals.[8] Radicals containing cigarette smoke are pro-oxidants, and a high amount of exposure to free radicals that enter the body reduces antioxidant enzymes such as Superoxide Dismutase (SOD), catalase, Glutathione Peroxidase (GSH-PX), and antioxidants such as glutathione, coenzyme Q10, and melatonin.[9-12] Moreover, cigarette smoke also causes lipid peroxidation, which damages the normal cell membrane of the liver, causing cell leakage that leads to an increase in serum alanine transaminase (ALT) and aspartate transaminase (AST).[13] Therefore, supplementary antioxidant intake possesses the potential to neutralise such radicals containing cigarette smoke exposure.

Fruits and vegetables are natural sources rich in antioxidants. Numerous studies have explored and reviewed the role of antioxidants acquired from daily intake in combating oxidative stress, with results suggesting some positive effects.[14-16] The red-purple dragon fruit (*H. Costaricensis*) has a high antioxidant content from the natural pigment's anthocyanins and phenolic compounds. The peels of *H. costaricensis* fruit retain the deep red-purple, which signifies its high pigment content and antioxidants. The total phenolic, antioxidant and antiproliferative activity of *H. costaricensis* fruit peels is better than the flesh, with no toxic compounds.[17] Ethanol extract of *H. costaricensis* peels has an average total anthocyanin 58.0720 ±

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0.0001mg/L and betacyanin 186.90 mg/100g dry weight, with antioxidant activity (IC50) 96.95 mg/L within 5 minutes incubation.[18] Another study also showed that red and white dragon peels have stronger antioxidant activity than pulps.[19] One mg/ml dragon fruit *H. polyrhizus* extract can inhibit free radicals 83.48 ± 1.02%, while the fruit flesh inhibits only 27.45 ± 5.03%.[19] Despite its potential as an antioxidant source, fruit peels have been neglected and wasted.

A study of total oxidant status and oxidative stress index levels of active, passive, and non-smoker university students found that smoking reduces the plasma total antioxidant capacity and status.[20] Another study showed that single cigarette smoking significantly decreased plasma antioxidant concentration.[21,22] Therefore, the underutilised peels of *H. costaricensis* fruit were studied to prevent the adverse effect of cigarette smoke exposure on serum biochemistry, i.e., MDA (oxidative stress indicator), bilirubin, ALT, and AST levels in Wistar rats, including body weight, food and drink intake. The Wistar rats were chosen as the standard and ideal laboratory animal for mammalian study.[23] The Wistar rats enable researchers to control variables such as temperature, humidity, light, diet, and the tested intervention, i.e., cigarette exposure and *H. costaricensis* administration in this study, which is otherwise problematic in humans.

MATERIALS AND METHODS

Preparation of juice or ethanol extract of *H. costaricensis* peels

H. costaricensis was collected from agro-tourism in Sleman Regency, Central Java. *H. costaricensis* fruit peels were obtained by peeling the fruit's skin. The peels were blanched using water steam for 2 minutes, and then, after cooled, it was immediately packed in plastic and stored in the freezer until used. The peel juice was prepared by blending 100 g of the peels in 50 ml of drinking water. The blends were filtered using a tea filter, and the juice was stored in a refrigerator. The juice was given at 3 g/mL drinking water. The juice drink has a pinkish colour, indicating more anthocyanin content.

The peel extract was obtained by weighing 1000 g of the peels and extracted by wet maceration technique in 1000 mL of 96% ethanol mixed with 1% HCl with a volume ratio of 9 to 1. The 96% or absolute ethanol was used as a fruit extraction solvent, which is the safest after water.[24] While water extracts more polar compounds, 96% ethanol extracts more of less polar compounds. After 24 hours of maceration, the filtrate was collected by filtration. The filtrate was evaporated using a rotary vacuum evaporator to obtain a thick ethanol extract. The resulting filtrate was collected in a beaker glass, while the pulp was extracted again similarly. Extraction was repeated until all anthocyanins in the peels were extracted completely (the solvent was clear/colourless). All extracts were combined and then centrifuged for 15 minutes at 2000 rpm to separate the sediment and the supernatant. The supernatant obtained is then concentrated using a rotary vacuum evaporator at 40°C to get 30 ml of concentrated extract from the peels of the red-purple dragon fruit. The concentration of concentrated ethanol extract given to mice is 3.15 gr/ml drinking water. The extract drink is brownish, indicating higher levels of polyphenolic compounds.

Animal Experimental Design

Twenty-eight four to five weeks old male Wistar rats were randomly selected and divided into four groups, namely: control groups that were not exposed to cigarette smoke (Control), exposed to cigarette smoke (Csmoke), exposed to cigarette smoke and had Hylocereus costaricensis juice (JcHc), exposed to cigarette smoke and had *H. costaricensis* peels extract (ExHc). Each group consisted of seven rats. Each experimental rat was housed in individual hollow aluminium cages of 42x21x20 cm (length x wide, height). Wistar rats were kept in animal rooms with automatic room temperature control of 25 ± 20C, 70-90% relative humidity and a dark cycle of 12 hours per day.

For cigarette smoke exposure daily for 30 days, the experimental rats in the cage were placed in an enclosure made of glass. The bottom of the enclosure was a wire screen. The enclosure has two spaces: the lower for a cigarette and the upper for smoke exposure. The cigarette was positioned in a cigarette holder in the lower space of the enclosure (Figure 1).

A local commercial cigarette was ignited to produce the cigarette smoke. The smoke entered the upper space where the rats were placed. The lit cigarette was left burning till it went out. One cigarette smoke was given twice daily at 09.00 a.m. and 03.00 p.m. Feed (AIN - 93G) was delivered every morning at 08.00 *ad libitum* before cigarette smoke exposure.[25] For the JcHc and ExHc groups, red-dragon fruit peel juice and extract, respectively, were provided via a drinking bottle that was freely accessible *(ad libitum).* The juice group received juice drinks only. The extract group received extract drinks only. No plain water bottle was given. Each group has only one type of drinking bottle according to the treatment (Figure 2). The volume of

the juice and extract in the drinking bottles for each rat was the same (50 mL). For the Control and C_{smoke} groups, a regular drinking bottle with plain water was given and replaced with a fresh one daily. The remaining feed and drink of individual rats in each group were measured and recorded daily before the new replacement.

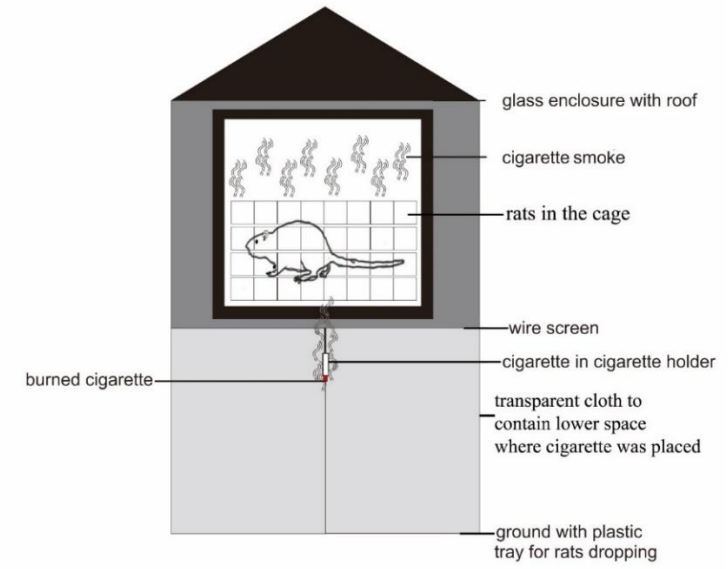

Figure 1. Cigarette smoke exposure method

The administration of *H. costaricensis* lasted for 30 days, considering our previous studies utilizing fruits and botanicals have shown positive effects.[26-28] After 30 days of treatment, blood sampling was carried out by administering ketamine anaesthesia at 50 mg/kg, and blood was withdrawn via the sinus orbitalis. Serum was collected and stored frozen until serum MDA, bilirubin, ALT and AST were determined. This study had ethical clearance from the preclinical health research ethics commission of the Integrated Research and Testing Laboratory, No.00054/04/LPPT/V/2018 Gadjah Mada University, where the animal study was conducted.

Figure 2. Animal Experimental Design. The grey square represents one rat housed individually

The coloured bottle represents the juice (pinkish) and the extract (brownish) drinks. The Control and Csmoke groups received plain water. All bottles were filled with 50 mL drinks.

Serum Malondialdehyde (MDA) as a marker of oxidative stress

Serum MDA was determined to assess oxidative stress using the TBRS (2-thiobarbituric acid reactive substance) approach. MDA in the serum combines with TBRS in an acidic medium for 60 minutes at a temperature of 95°C to produce a reactive thiobarbituric acid product that can be detected spectrophotometrically at 534 nm.[26]

Serum Bilirubin

Serum bilirubin was measured using a DiaSys kit (Diagnostic system GmBh) following the instruction manual. Diazotized Sulphanilic Acid (DSA) and bilirubin combine to generate a red-azo product that may be detected spectrophotometrically at 546 nm.

Serum ALT and AST

The serum alanine transaminase (ALT) and aspartate transaminase (AST) (previously designated as glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) were analysed following the instruction manual of DiaSys kit.[27-30]

Statistical Analysis

All data were analysed using Microsoft Excel 2019 version 2306 and SPSS Statistics 25 for Windows (Microsoft Corporation Redmond, WA, USA). The normality test was done using the Saphiro-Wilk test. Normal distribution was followed with one-way ANOVA and further analysed with a posthoc test when statistical significance was reached. When the data were not normally distributed, Kruskall-Wallis was used; and if the result was significant, further analysis with the Mann-Whitney test was performed.

RESULTS

In this experiment, cigarette smoke exposure to Wistar rats went well, as it could be observed from their behaviour, who were attracted to and approached cigarette smoke, sniffing and quietly enjoying it. The serum MDA, bilirubin, SGOT, AST, feed and drink intake, and body weight results are presented in Table 1.

Table 1. Serum MDA, Bilirubin, ALT, and AST, Feed and Drink Intake, and Body Weight of Wistar Rats Exposed to Cigarette Smoke for 30 Days Without or With A Daily Intake of *Hylocereus costaricensis* Peel Juice and Ethanol Extract

Variables	Control	Csmoke	Jc_{Hc}	Ex_{Hc}	p
MDA (mg/mL)	38.69 ± 14.47^{b}	59.47 ± 15.76^{c}	28.56 ± 11.03^{b}	15.19 ± 8.65^{a}	0.000^{*}
Bilirubin (mg/dL)	0.44±0.06	0.43±0.05	0.44±0.09	0.51±0.07	0.101
ALT (U/L)	48.64 ± 8.06^{b}	55.16 ± 9.48^{c}	43.11 ± 4.55^{b}	34.63 ± 8.67^{a}	0.000^{*}
AST (U/L)	98.49 ± 14.04^{b}	133.33 ± 30.31^{b}	96.17 ± 33.97^{b}	77.90 ± 18.02^{a}	0.000^{*}
Feed Intake (g)	19.23 ± 0.15^{c}	16.90 ± 0.28^{a}	17.11 ± 0.40^{b}	17.46 ± 0.30^{b}	0.000^{*}
Drink Intake (mL)	8.97 ± 0.39^{a}	10.20 ± 0.35^{b}	8.53 ± 0.31^{a}	10.30 ± 0.30^{b}	0.000^{*}
Body Weight (g)	218.71 ± 7.39^{c}	156.71 ± 11.66^{a}	192.00 ± 7.55^{b}	197.14 ± 12.09^{b}	0.000^{*}

(Control): not exposed to cigarette smoke, (C_{smoke}): exposed to cigarette smoke only, (Jc_{Hc}): exposed to cigarette smoke with the intake of *H. costaricensis* peel juice, (Ex_{Hc}): exposed to cigarette smoke with the intake of *H. costaricensis* peels extract. Each data is the average of seven replicate rats ± SD. Different letters within the same row mean significant at $p<0.05$. *significant $p<0.05$.

The rats exposed to cigarette smoke alone (C_{smoke}) had significantly (<0.05) higher MDA, SGOT, and AST, drink intake but lower feed intake and body weight than the control rats not exposed to cigarette smoke (Control). The rats in the Jc_{Hc} group had serum MDA, ALT, and AST similar to those in the control group. However, the rats in the Ex_{Hc} group had significantly (<0.05) the lowest serum MDA, ALT and AST than Control, C_{smoke} and Jc_{Hc} groups. The drink intake of the Ex_{Hc} group was similar to the C_{smoke} group and was significantly higher than the Control group. The juice and extract groups had significantly higher body weight than the C_{smoke} group but were still lower than the Control group.

DISCUSSION

Table 1 shows that exposure to cigarette smoke increased MDA, ALT, and AST levels. Serum ALT and AST are biomarkers of hepatocyte integrity or cholestasis.[31] These enzymes catalyse reactions crucial for gluconeogenesis and urea formation.[32] Although their concentration is high in the liver, they are also present in the kidney, heart, skeletal muscle, and, to some extent, in the spleen, small intestine, and brain. The slightest disruption in hepatocyte membrane permeability can cause these enzymes to leak and be detectable, which may indicate necrosis or inflammation. It has been established that smoking creates a pro-inflammatory environment with the increase of cytokines, which then causes tissue inflammation and cell death.[33,34] The rise in liver enzymes could also be due to harmful free radicals of cigarette smoke, including carbon monoxide

(CO), nicotine, and polycyclic aromatic hydrocarbon.[8] Nicotine exposure has been shown to increase oxidative stress and hepatocellular apoptosis through multiple signalling pathways.[35,36]

The increase in MDA levels correlates to producing reactive oxygen species (ROS), thus causing oxidative stress.[36] Formation of MDA can occur when hydroxyl free radicals react with fatty acid components from cell membranes so that a chain reaction known as lipid peroxidation occurs. Lipid peroxidation will cause the breakdown of fatty acid chains into various compounds such as hydrocarbons (pentane, ethane) and aldehydes such as MDA. MDA is a dialdehyde compound that is the end product of lipid peroxidation in the body, which is toxic.[37] High MDA levels can portray the process of liver damage, which, through the cell membrane oxidation process, can damage the cell membranes and lead to elevated liver enzymes. [38]

Radicals of cigarette smoke inhaled into the lungs and the bloodstream will be distributed throughout the body and cause damage to the membranes of the cells of various tissues, one of which is cells of the liver, a processing center of drugs and toxicants.[39-41] Damage to the liver cell membrane causes the liver enzymes in cells to leak and enter the blood circulation, resulting in increased serum levels.[41-43] The serum and salivary liver enzyme levels in male smokers who smoked at least ten cigarettes per day for 15 years or more than 100 over a lifetime increased sharply compared to non-smokers.[13, 44] Microsomal enzymes in the liver bind chemicals such as tar in cigarette smoke, transporting nicotine to the lungs and can cause cancer.[41, 45] Cigarette smoking enhances lipid peroxidation, and this peroxidation is responsible for the observed fatty degeneration in the liver.[46]

The rats treated with juice (JcHc) or extract (ExHc) had lower MDA, ALT, and AST levels than the group exposed to cigarette smoke only (Csmoke) and even lower than the Control group. It showed that the antioxidant content of *H.costaricensis* peels in juice or extract could reduce MDA, ALT, and AST levels in Wistar rats exposed to cigarette smoke. MDA, ALT, and AST levels in the ExHc group were the lowest compared to the other three groups of rats. Such lower levels, presumably due to the active substance in the extracts, namely anthocyanin and polyphenolic compounds, which function as antioxidants, are higher than in juice and more effective in reducing MDA, ALT, and AST. A higher antioxidant in extracts neutralizes cigarette smoke radicals and can also likely regenerate liver cells damaged by free radicals; therefore, the serum ALT and AST decrease in this group is the highest (lowest level of ALT and AST). All groups did not have differences in bilirubin levels, and this is because bilirubin is not a sensitive indicator to show the effect of antioxidant administration.

The control rats not exposed to cigarette smoke had the highest feed intake, manifested in the highest body weight among all groups. In the Csmoke group, the feed consumption and body weight were lowest among all groups, which proved that exposure to cigarette smoke decreased appetite in Wistar rats, whereas, in the group of *H.costaricensis* peel juice (JcHc) and extract (ExHc), the feed intake and body weight are higher than the group exposed to cigarette smoke only (Csmoke) even though it is still not equal to Control group. It also proved that supplying *H.costaricensis* peel juice or extracts as antioxidants improved cigarette smoke-decreased appetite in Wistar rats. The group exposed to cigarette smoke only (Csmoke) consumed more drinks than the rats not exposed to cigarette smoke (Control). These results showed that exposure to cigarette smoke increases drinking water consumption, possibly due to higher water requirements to neutralize or metabolize radicals in inhaled cigarette smoke. Such a mechanism was supported by increasing serum ALT and AST levels in the Csmoke group. Exposure to cigarette smoke radicals in vivo causes lipid peroxidation, which causes damage to normal liver cell membranes. The damage can cause leakage of intra-cellular transaminase enzymes into the blood resulting in increased levels of AST and ALT.[47] The rats receiving *H.costaricensis* peel juice (JcHc) consumed less drinks than the group given *H.costaricensis* peels extract (ExHc), which is probably an indication that the intake of *H.costaricensis* peels extract (ExHc) requires more water consumption to metabolize higher levels of the active substance (anthocyanin and polyphenol). This mechanism is in line with the metabolism of polyphenolic compounds *in vivo* by conversion into a more hydrophilic form and enabling their excretion via bile or urine.[48, 49]

CONCLUSIONS

Exposure to one cigarette smoke twice daily caused an increase in oxidative stress as indicated by elevated MDA levels, liver cell damage characterized by a marked increase in serum AST and ALT levels, water consumption, decreased feed consumption, and weight loss. Daily intake of the juice or ethanol extract of *H. costaricensis* fruit peels can restore the disrupted MDA, AST and ALT, feed and drink intake, and weight loss. Therefore, the underutilized peels of *H. costaricensis* fruit can be developed into a valuable product to prevent cigarette smoke-induced oxidative stress and reduce food waste.

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Determinants of stunting in children under five: a scoping review

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ABSTRACT

Background: Inadequate diet, socioeconomic condition, and maternal and child characteristics can damage stunted children under five's mental and physical development. As a result, they have difficulty developing physically and cognitively, have low intellectual abilities, are more susceptible to disease, and have less creativity and innovation.
Objective: This study seeks to investigate and summarize the determinants of stunted children under five.
Materials and Methods: The study used a scoping review method. The literature search was carried out on indexed databases of Scopus, PubMed, Google Scholar, Crossref, and Pro-Quest in English and Indonesian. There were 720 research articles, and 18 of them met the inclusion criteria. From the 18 journals, information was collected from the publication year 2015-2021. Relevant study articles related to the topic were analyzed qualitatively using NVIVO-12 Plus.
Results: Our findings identify that maternal education, low birth weight (LBW), gender, exclusive breastfeeding, parental income, parental age, and child age are the dominant determinants of stunting among under-five children.
Conclusion: Higher risk factors of stunting among children are parents' lack of knowledge, low family income, low nutrition, low level of mother's education, and lack of parents' supervision and parenting skills.

Keywords*: children under five; determinants of stunting; scoping review*

BACKGROUND

Stunting becomes a nutritional problem in children, especially for children under five. Stunting is a condition in which a child under the age of 5 has a shorter length or height than his or her age.[1] Children under five who experience stunting will harm the children under five during their growth and development period.[2] The impact children under five who experience stunting such as they struggle to achieve adequate physical and cognitive development, have lower levels of intelligence, are more susceptible to disease, and reduce productivity.[3] Stunting causes a decrease in the immune system of children under five. This condition make they get sick more easy and have higher risk to develope diseases.[4] Factors that cause stunting both in the world and in Indonesia include lack of knowledge about stunting, food insecurity, premature birth or LBW, exclusive breastfeeding, management of complementary foods for children, sanitation of the environment, and low socioeconomic status of the family.[5] Other factors that cause stunting are not receiving antenatal care during pregnancy and having a smoking habit during pregnancy.[6] A study from United Nations International Children's Emergency Fund (UNICEF) in 2018 estimated there were one in 4 children under five worldwide suffered from stunting with the affected children 149 million.[7] The World Health Organization (WHO) 2022 estimated the prevalence of stunting under five worldwide at 22.3%.[8]

In recent years, a large amount of literature has developed about the causes of stunting among children. One of them is that infants without exclusive breastfeeding will be at greater risk of causing them to experience stunting because exclusive breastfeeding is critical in children's growth to reduce and prevent stunting.[9] Bad quality of environmental hygiene and sanitation is related to stunting whereas the incidence of children being stunted is smaller in households with access to good sanitation facilities.[10] The mother's education factor is also a factor that has a close relation with the incidence of stunting among children under five. Highly educated mothers will decide to increase their children's nutritional intake and good health.[11] The problem of stunting in children must be appropriately handled. However, previous research is still inconsistent, and there is still a lot of uncertainty about the dominant risk factors for stunting. Although studies of various previous findings have supported it, it is important to analyze and summarize the results of previous studies to find out what factors influence the incidence of stunting in children under five. Therefore, this study is expected to contribute to a deeper understanding of the incidence of stunting in children under five. This research aims to analyze and summarize the evidence for the determinants of stunting in children under five so that it can complete the research that has been done previously, and aims to answer research

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questions: 1) What are the dominant risk factors impacting the incidence of stunting from previous studies?; and 2) What are the research designs and sampling techniques used in the previous studies?

MATERIALS AND METHODS

This study uses the methodological framework by Xiao & Watson[12] to conduct a scoping review. Several steps were taken to identify articles, such as formulating problems, developing and validating reviews, searching the literature, detecting relevant literature, extracting data, analyzing and synthesizing data using NVIVO-12 Plus, and making data reports.

This study used a search strategy to find relevant articles on the determinants of stunted children under five. Search articles using indexed Journal databases Scopus, PubMed, Google Scholar, Crossref, Pro-Quest and search strategies with English keywords ("determinant" OR "risk factors" OR "factors associated") AND ("stunting" OR "undernutrition" OR "underweight" OR "nutrition status") AND ("children" OR "child" OR "baby"). On the other hand, search strategy with Indonesian keywords, namely ("*determinan*" OR "*faktor risiko*" OR "*faktor yang mempengaruhi*" AND ("stunting" OR "*kekurangan gizi*" OR "*berat badan kurang*" OR "*status gizi*") AND ("*anak-anak*" OR "*anak*" OR "*bayi*"). This study's inclusion criteria were papers published between 2015 and 2021, full-text journals in English and Indonesian languages, and study designs in case-control, cross-sectional, and retrospective cohorts. Of the 720 journals that have been identified from various databases, 18 journals meet the inclusion criteria. Journals that are not full text and journals that are preprinted and not peer-reviewed in the background are excluded. Then the journal manuscripts in the proofreading stage are not included because there is a possibility of changes occurring in the research reporting results.

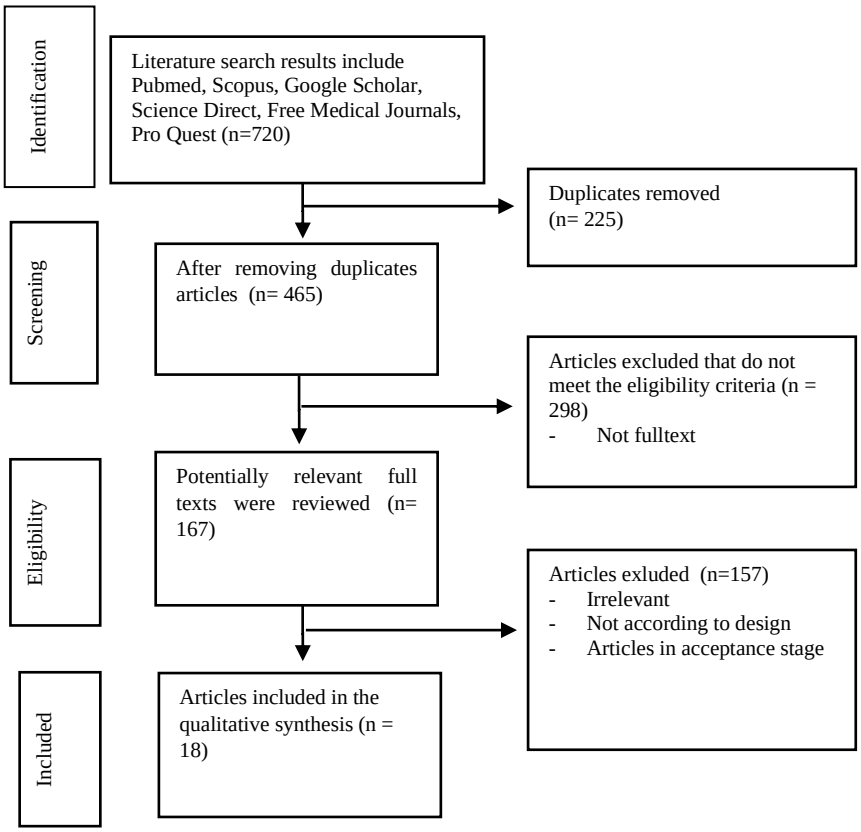

Figure 1. PRISMA Flow Diagram for Database Search of Studies

Relevant study articles related to the identified topics were systematically reviewed and analyzed using NVIVO-12 Plus.

RESULTS

Table 1 shows all the articles included in this research and meet the inclusion criteria published in the 2015-2021 range from various journal databases.

Table 1. Results of Data Extraction

Author and Year	Country	Participant	Study Design	Results	Database
Parenreng et al., 2020[13]	Indonesia	858 (6-23 months)	*Cross-Sectional*	In this study, they found that the presence of a family member who smokes, washes hands and breastfeeding alone become factors associated with locus stunted growth, while in non-locus areas there was a history of diarrhea and hand washing.	*Crossref*
Kahssay et al., 2020[14]	Ethiopia	322 (6 to 59 months)	*Case-control study*	The determinants of stunting obtained from the results of this study were illiterate mothers and the previous birth spacing was < 24 months with AORs of 4.92 and 4.94, respectively. Other factors related to the incidence of stunting in children were no follow-up ANC, no access to the toilet, short mother's baby < 150 cm, no first breast milk or breast milk < 24 months, and nonbreast milk only.	*Pubmed*
Cruz et al., 2017[15]	Spain	282 (0–59 months)	*Case-control study*	Significantly, there were several risk factors of stunting such as birth weight, mother's occupation, education, family size, number of children, charcoal cooking, in wooden or thatched roof houses or apartments without suitable floors, length of life and complete breastfeeding, and exclusive breastfeeding.	*Scopus*
Berhanu et al., 2018[16]	Ethiopia	1039 (24-59 months)	*Cross-sectional*	The prevalence of stunting in preschool children in the research was 39.3% and of the number of stunted children, the incidence was higher in families with food insecurity (42.8%) compared to food security (35.9%).	*Scopus*
Lobo et al., 2019[17]	Indonesia	82 (6-59 months)	*Case-control study*	This study discovered various variables that impact the incidence of stunting, including mother's education, nutrition awareness, and household income, eating habits, family size, environmental hygiene and hygiene practices, parenting style, energy validity level and protein validity level.	Google Scholar
Nkurunziza et al., 2017[18]	Burundi	6199 (6 to 23 months)	*cluster-randomized controlled trial design*	The prevalence of stunting and severe stunting in children were 53% and 20.9%, respectively, whereas children aged 12-17 and 18-23 months had an increased risk of stunting compared to children 6-11 months with OR: 2.1 and 3.2.	*Scopus*
Rakotomanana et al., 2017[19]	USA	4774 (0 to 59 months)	*Cross-Sectional*	Girls were at higher risk to experience stunting compared to boys with AOR was 0.69 and p-value <0.01.	*Scopus*
Abeway et al., 2018[20]	Ethiopia	410 (6-59 months)	*Cross-sectional*	The general magnitude of stunting was 52.4. Females included the aged between 25–59 months and birth weight <2.5 kg, lack of maternal ANC visits, and the improper start of complementary foods were positively associated with stunted growth in children.	*Pubmed*

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Table 1. Results of Data Extraction (Continue…)

Author and Year	Country	Participant	Study Design	Results	Database
Titaley et al., 2019[21]	Indonesia	24.657 under two years	*Cross-sectional*	Stunting is for households with 3 or more children under five, households with 5 to 7 household members, mothers who used less than 4 antenatal care services during pregnancy, boys, 12–23 months old children, and children's weight was less than 2500 g at birth.	*Google Scholar*
Masereka et al., 2020[5]	Western Uganda	372 6 - 59 Months	*Cross-sectional*	Food storage used during the dry and child anthelmintics is significantly associated with absenteeism being stunted.	*Pubmed*
Semali et al., 2015[22]	Tanzania	678 households with under-five children	*Cross-sectional*	This study showed the relationship between the head of the family is young or <35 years old, the young mother, and the economy of the family to the incidence of stunting. The AORs were 0.67, 1.54, and 0.66, respectively.	*Scopus*
Wali et al., 2020[23]	South Africa	564,518 children aged 0–59 months	*Cross-Sectional*	The factors that have a strong possibility of causing stunting in children in the 3 different age groups of children evaluated by the researcher found low maternal education with an AOR in the 0-23 month age group 1.65, the 24-59 month age group 1.46 and the 0-59 month age group. 1.34.	*Pubmed*
Chirande et al., 2015[24]	Tanzania	7324 children aged 0-59 months	*Cross-Sectional*	Risk factors were evaluated for the incidence of stunting and severe stunting in children aged 0-23 and 0-59 months. This study found that mothers who had no education, perceived small size infants at birth, and water sources or unsafe drinking were the close factors of incidence of stunting.	*Scopus*
Rahmawati et al., 2018[25]	Indonesia	174 children < 5 years	*Retrospective cohort study*	The risk of stunting went down with birth and the mother's height. On the other hand, the risk of stunting went up with the mother's age < 20 years. Birth length went up with maternal height (b = 1.07) and higher family income (b = 0.93). Birth length went down with the mother's age <20 years or 35 years old during pregnancy (b = -0.74).	*Crossef*
Derso et al., 2017[26]	Ethiopia	587 mother-child	*Cross-Sectional*	In this study, several factors that have the possibility of increasing the incidence of stunting were low income of the family with AOR = 2.20, unavailability of toilets AOR = 1.76, children aged 12-24 months AOR = 3.24, did not receive vitamin A supplementation postnatal mother AOR = 1.541 and poor family food sources with AOR = 1.71.	*Pubmed*
Hendraswari et al., 2021[27]	Indonesia	60 children aged 24–59 months	*Case-control*	There is a significant relationship between stunted children and energy intake factor (p-value = 0.030; = 0.05; 95% confidence interval = 95%).	*Google scholar*

Table 1. Results of Data Extraction (Continue…)

Author and Year	Country	Participant	Study Design	Results	Database
Khan et al., 2019[28]	Pakistan	3071 aged 0–59 months	*Cross-Sectional*	About 44.4% of children under the age of 5 years of age are underdeveloped, 29.4% are underweight, and 10.7% are affected by waste. A mother who lives in a rural area, marriage in young age, and has been to a maternity hospital three or more times during pregnancy was less likely to cause growth retardation.	*Pro Quest*
Habimana & Biracyaza, 2019[29]	Rwanda, Central Africa	1905 children aged 6–59 months	*Cross-sectional*	Mother's education, age of the mother, mother's occupation, income, child's gender, and fortification feeding patterns statistically have a strong relationship to the incidence of stunting.	*Pro Quest*

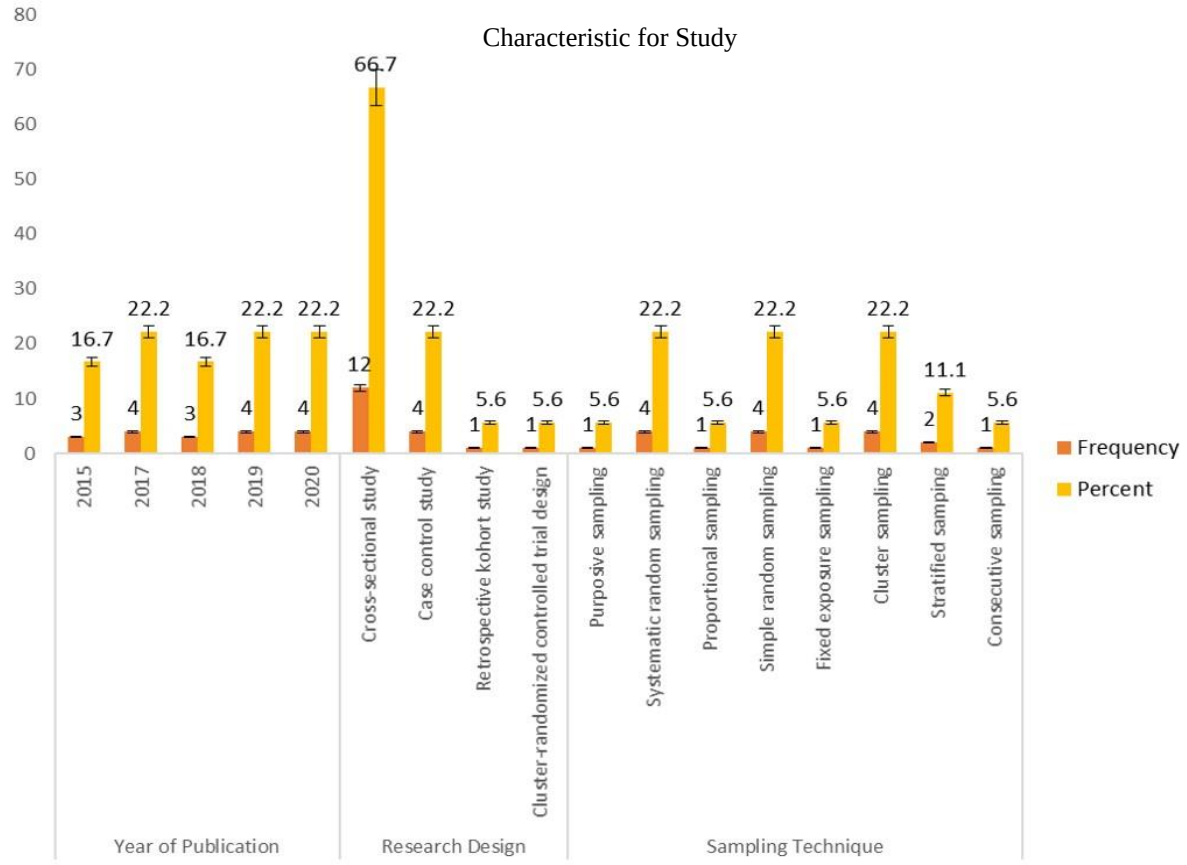

Figure 2. General Characteristics for Study Selection (n=18)

Table 2 shows the factors influencing the incidence of stunting from numerous reference sources. The research of Parenreng et al.[30] and Cruz et al.[15] indicates the incidence of children who experience stunting caused by nonexclusive breastfeeding. Infants who were not exclusively breastfed are 6.6 times higher to be stunted. This can happen because breast milk that is given exclusively contains colostrum, which is very good for children under five' health.[14] Energy and protein adequacy levels also affect the incidence of stunted children. If the energy and protein adequacy level is less, the risk of stunting is 4,319 times compared to children whose protein adequacy level is sufficient.[31] Birth weight also has a very close relation to the incidence of stunting.[20,21,24,28] Low birth-weight babies are born with deficient nutritional reserves.[15] This can

be attributed to the incorrect assessment of the nutritional status of the mother before and during the mother's pregnancy.[18,31] Not receiving vitamin A supplementation after delivery and attending less than four antenatal care services will affect the nutritional health of children.[26]

Table 2. The Related Factors Affecting Stunting in Children Under Five

Related Factors	Significant Risk Factors	Main Empirical Sources
Smoking family members, Exclusive breastfeeding, duration of complementary feeding and hand washing practice.	exclusive breastfeeding (p = 0.001), family members smoking (p = 0.005)	Parenreng et al.[30]
Mother's education, birth spacing, no follow-up ANC, children born to short mothers < 150 cm, not given colostrum, breastfeeding below 24 months, and non-exclusive breastfeeding.	education of mother (AOR = 4.92, 95%CI (1.94, 12.4), not fed colostrum (AOR = 4.45, 95% CI (1.68, 11.8), non-exclusive breast feeding (AOR = 6.68, 95% (3.1, 14.52), preceding birth interval less than 24 months (AOR = 4.94, 95% (2.17, 11.2),	Kahssay et al.[14]
Birth weight, mother's education, mother's occupation, residential area, number of children under the age of five in the family, charcoal cooking.	birth weight [AOR = 19.99, 95% CI = (5.8–68.85), p < 0.001], urban areas [AOR = 138.0, 95% CI = (32.38–587.80), p < 0.001]	Cruz et al.[15]
Uneducated mother, Number of family members, and gender.	education of mother (AOR= 5.24, 95% CI; 2.30-11.91) and (AOR= 4.2, 95% CI; 1.77-9.97),	Berhanu et al.[16]
Mother education, parent income, maternal nutrition knowledge, number of families, feeding practice, environment sanitation, energy adequacy rate, and protein adequacy rate.	Mother with low education (AOR= 3.07, 95%CI; 1.40-6.75), have a big number of family (AOR= 3.47, 95% CI; 2.62, 4.60)	Lobo et al.[17]
Uneducated mothers, incorrect assessment of the nutritional status of their children, giving birth at home.	mother without education (AOR=1.6; 95% CI: 1.2-2.1), delivering at home (AOR=1.4; 95% CI: 1.2-1.6)	Nkurunziza et al.[18]
Gender, and region of residence.	girls have higher risk than boys (p < 0.01)	Rakotomanana et al.[19]
Birth weight, gender, older age, duration of breastfeeding, and lack of maternal ANC visits.	female (AOR: 2.8, 95% CI: 1.503–5.099), age group of > 25 months (AOR: 4, 95% CI: 1.881–8.424) , weight of <2.5 kg (AOR: 5, 95% CI: 1.450–17.309), mothers' lack of ANC visits (AOR: 3.2 95% CI: 1.40–7.10)	Abeway et al.[20]
Children whose mothers attended < 4 antenatal care services during pregnancy, boy, children aged 12–23 months, and children weighing <2500 g at birth.	mothers used less than 4 antenatal care services during pregnancy (AOR = 1.22, 95% CI: 1.08–1.39), children's weight < 2,5 kg at birth (AOR = 2.55; 95% CI: 2.05–3.15)	Titaley et al.[21]
Children aged 6-59 months, boys, insufficient food in the household.	Food storage (AOR = 0.23, CI = 0.08-0.62, p = 0.004)	Masereka et al.[5]
Households where the head of the family is young (<35 years), the mother's age is still young, and family income.	Economic (AOR = 0.66, 96% CI 0.46–0.94, p = 0.023)	Semali et al.[22]
Mother who does not go to school, mother is short (height < 150 cm).	uneducated mother and maternal stature < 150 cm (p < 0.001)	Wali et al.[23]
Low education levels of mothers, sons, small babies, and households with unsafe drinking water.	aged 0-23 months = 1.37; 95% CI: (1.05, 2.14)], aged 0-59 months = 1.42	Chirande et al.[24]
The age of mother, body length at birth, the education of mother, family income.	education of mother (b= 1.08; 95% CI= 0.41 to 1.75; p= 0.001), mother's age <20 years b= 0.73; 95% CI= -0.03 to 1.46; p=0.051)	Rahmawati et al.[32]

Table 2. The Related Factors Affecting Stunting in Children Under Five (Continue…)

Related Factors	Related Factors	Related Factors
Family income, unavailability of latrines, children's age: 12-24, did not receive maternal vitamin A supplementation after giving birth.	family income AOR= 2.20; 95%, CI (1.42, 3.40)	Derso et al.[26]
Energy intake and protein intake, patients with ARI and diarrhea.	energy intake factor (p= 0.030; α = 0.05; CI = 95%)	Hendraswari et al.[27]
The mother's low status of education, the size of the baby at birth, and the mother's BMI.	low education of mother (AOR = 3.61, 95%CI 1.33–9.82), BMI < 18.5 (AOR = 1.78, 95%CI 1.00–3.17)	Khan et al.[28]
Mother's education, age, work, child's gender, fortifying feeding, and prenatal care.	gender of child [OR=1.08; 95% CI (1.057–1.093), p=0.008], household wealth index [OR=0.386; 95% CI (0.357–0.414)] and breastfeeding [OR=0.02; 95% CI (0.004–0.036), p=0.013]	Habimana & Biracyaza[29]

Gender is another factor of stunting in children less than 5 years. Habimana & Biracyaza[29] said boys were more prone to stunting (53.3%) compared to girls (32.2%). This is thought to be a consequence of the enlarged vulnerability of boys to infectious diseases that can interfere with the growth of children under five.[21] Thus, boys were more likely to be stunted [AOR] = 0.84 [0.72–0.97] and p < 0.01) 0.69 [0.55– 0.88] compared to girls.[19]

Parental age also affects the incidence of stunting among children.[22,29,32] If the parent's age is less than 25 years, the child is prone to be stunted.[22] Because the mother's age at pregnancy was less than 20 years, the stunting logit score increased by 0.73 (b = 0.73; 95% CI = -0.03 to 1.46; p = 0.051).[29] Children with age 12-23 months have a higher likelihood of experiencing stunting than those children aged <12 months.[21] This research supported with another study by Derso et al.[26] which states the probability of stunting in children aged 12-24 months [AOR = 3.24; 95% CI: 2.24, 4.69] is bigger than infants aged 6-11 months.

Parental income is also very influential on the incidence of stunting.[22,26,31] The level of family income will affect household purchasing power, impacting food consumption. If the level of family income is very low due to the work of parents who are still low[15,29] then there is a shortage or insufficient food in the household, causing stunted growth and development of children. Children with high family incomes are 0.34 times smaller to experience stunting compare to families with low incomes.[32]

The area of residence is also related to the incidence of stunted children.[15,19] Families living in rural areas are more likely to experience stunting. This is because places and access to health services in villages are more difficult to reach than in cities.[31] So that there are still many pregnant women giving birth alone at home or elsewhere without medical assistance.[18] Using charcoal for cooking [AOR = 3.10, 95% CI = (1.53–6.26), p = 0.002] was also associated with stunting.

The number of the family number who smoked also influence the incidence of stunting (p = 0.005) with the frequency of complementary feeding (p = 0.027), and the practice to wash hand (p = 0.001).[30] The study of Kahssay et al.[14] also said that the previous birth spacing was less than 24 months, no follow-up, no access to latrine, not given colostrum, and non-exclusive breastfeeding (are very influential on the incidence of stunting in children under five. Cleanliness and environmental sanitation also affect the incidence of stunting in children among children less than 5 years.[31] Poor environmental sanitation will increase the incidence of infectious diseases in children under five, such as ARI and diarrhea.[24,27]

Mother's educational status also has an important influence on stunted children.[15,16,18,23,24,28,29,31,32] If a child is born to a mother who is not highly educated, the baby is 4.9 times risk to experience stunting compared to a child born from a mother who has a high education (AOR = 4.92, 95%CI (1.94, 12 ,4).[14]

DISCUSSION

All studies included in this review assessed the factors influencing the incidence of stunting among children under five. One of them is maternal education, low birth weight (LBW), gender, exclusive breastfeeding, parental income, the parents' age, and the child's age. This risk factors reflected in the millions

of children worldwide resulting the children not achieve their full potential of growth due to suboptimal health conditions and insufficient nutrition and childcare.

Maternal Education

Among the 18 journal articles in the literature review, ten articles show that maternal education factors greatly influence the incidence of under-five child stunting.[15,16,18,23,24,28,29,31,32] A mother's education level will affect the absorption of nutritional information, impacting the process of choosing and providing nutritious food to children under five. The selection and provision of nutritious food will affect children under five's growth and nutritional status. This should be used as a precautionary measure for mothers to routinely care for children under the age of five.[33] Mothers with better knowledge about nutrition, supportive attitudes, and good behavior will affect the growth and development of children to achieve good health status. One of the efforts that can be made is to increase the mother's knowledge under five about stunting and nutrition.[34]

Birth Weight

Among the 18 journal articles in the literature review, five articles showed the birth weight affects stunted children.[16,20,21,24,29] From each of these articles, most infants with low birth weight are related to the incidence of stunting. Low birth weight (LBW) is a birth weight that is less than 2500 grams, and usually, babies with low birth weight have a very thin body and look very small and different from babies whose bodies are normal. Babies with low birth weight experience developmental disorders while in the womb, such as high blood pressure, malnutrition, infection during pregnancy, genetic disorders or birth defects in the baby, born to mothers with low body weight during pregnancy, maternal age during pregnancy is less of 17 years or over 35 years and multiple pregnancies. If the baby fails to thrive at an early age within 2 months, the risk of failure to thrive in the next period will be even greater. This research is supported by previous research conducted by Rukmana et al.[35] that babies with low birth weight below 2,500 grams will be at risk of 4.192 times stunting compared to children with normal birth weight, which is above or equal to 2,500 grams. Low birth weight is caused by consuming less nutritious food during pregnancy, infection during pregnancy, genetic disorders or birth defects in the baby, inadequate health services, and being born to mothers with low body weight during pregnancy. Thus, birth weight is an important measurement in newborns and is the best indicator to measure children's nutritional status and growth and development. The impact of LBW in general is closely related to fetal death, death in infants aged 0-28 days (neonatal), death in infants after the age of 1 month to 1 year (post-neonatal), as well as long-term growth and development in children. If children under five have a record of low birth weight, then they have a greater risk to be stunted than children with normal body weight. So, birth weight cannot be ignored and should be an important thing in the health and survival of the baby.

Gender

Among the 18 journal articles in the literature review, 6 articles show that gender has an effect on the incidence of stunting among children less than 5 years.[5,16,19,20,24,29] From several literature reviews, it is explained that boys are more prone to be stunted compared to girls. This happens maybe as a result of the high vulnerability of boys to get an infection of disease. Thus, boys showed an effect of chronic malnutrition, especially in environments of playing groups, such as repeated infections and exposure to toxins and air pollutants.[24,36] In addition, boys tend to have more active than girls, so a lot of energy comes out.[37] If it is show the non-balancing with adequate nutrition and food intake, it can cause children to become stunted. The prevalence of stunting is higher in boys under the age of 5 due to the high risk of malnutrition in boys due to their high protein energy requirements.[38]

Exclusive Breasfeeding

From the literature review of 18 journal articles, 3 articles explained that exclusive breastfeeding affected the incidence of stunting.[14,15,30] The study showed that 57.1% of children not get an exclusive breastfeeding experience stunting. On the other hand, infants who are exclusively breastfed are lower to experience stunting. The success of exclusive breastfeeding has a very positive impact on the growth and development of children because breast milk can meet the nutritional needs of infants.[39,40] This is due to the presence of calcium in breast milk which is more and more easily absorbed by the body than the calcium found in formula milk.[41] The study by Parenreng et al[30] showed that children aged 6 to 24 months who do not receive breast milk alone have a 1,282 times higher risk of stunting compared to children who receive breast milk alone.

Family Income

There are 4 from 18 journal articles in the literature review that apply the family income factor that greatly influences the incidence of stunting among children less than 5 years old namely.[22,26,31] Low family income will certainly affect the level of consumption expenditure for lower food needs. Low family incomes have more difficulty meeting their daily needs and balanced nutritional needs. Children under five from families with less per capita income have a 5.385 times risk of experiencing stunting than children under five from families with sufficient income. Stunting that occurs in families with low incomes is due to the family's low understanding of nutrition and management of family diets and hygiene practices.[42] Family income related to fulfilling energy and protein intake for children can be an indirect factor related to stunting. Low family income will affect the purchasing power of food so spending on food is also low.[43]

Mother's Age

Among the 18 journal articles in the literature review, there are 3 articles apply the parental age factor which greatly influences the incidence of stunting among children less than 5 years old.[22,29,32] In the literature review, more stunting came from the group of mothers aged < 20 years. Women at the age <20 years still need adequate nutrition to grow to adulthood and not ready to be a mother. This is because adolescent mothers may have limited access to face multifaceted socioeconomic and difficult to meet nutritional needs during pregnancy. Mothers who are still teenagers (<20 years) during pregnancy have a higher risk to have a stunted child compared to mothers aged 20-24 years.[32] Pregnancy at the age of less than 20 years and above 35 years can cause anemia, because at fewer than 20 years of pregnancy, biologically, their emotions are not optimal, they tend to be unstable, and mentally immature so that they are easily shaken which results in a lack of attention to the fulfillment of nutritional needs during pregnancy.

Children Age

There are 4 articles that discuss the child's age factor in the incidence of stunting among children <5 years of age from the 18 journal articles in the literature review.[5,20,21,26] In a literature review, children are more prone to stunting at the age of 13-24 months. Another study found that children aged 6-11 months experienced less stunting than children aged 12-24 months.[44] This can happen because the Basal Metabolic Rate (BMR) is higher in older children compared to the younger ones.[45] Research in Ethiopia also showed the same results where stunting was more dominant in the 6-11 month age group.[46] This can be explained because this period is a transition period from infancy and there are many changes in lifestyle, diet (exclusive breastfeeding to solid food), and social or environmental interactions. Boys under two years of age experienced stunting more in boys than girls. Male children have a higher BMR than female children, so they need more energy which can affect stunting nutritional status. Usually, children aged <11 months are more susceptible to getting an infection. compared to children aged > 11 months. This must be considered immediately by providing regular immunizations and nutritious food for children to have strong immunity.

CONCLUSIONS

The negative impact on children under five with stunting will be shown in their impaired growth and development due to the result of long-term inadequate nutrient intakes. This will lead the child's height to be shorter than the standard age. Several journals that meet the eligibility criteria, there are several causes or risk factors of stunting, including maternal education, low birth weight (LBW), gender, exclusive breastfeeding, parental income, mother's age and child age. Lack of mother's knowledge, low family income, inadequate fulfillment of family nutrition, low maternal education, and lack of supervision and parenting of children become the risk factors linked with stunting. There are 5 articles discuss about the prevalence of stunting in Indonesia indicates that Indonesia still have a lot of cases of stunting. It is important to support healthcare providers related to stunting prevention for increasing knowledge and meet the information needs of families. Thus, with the support of health care workers, public understanding of stunting will be better. It is crucial to provide education about the nutritional status to increase the mother's knowledge regarding the fulfillment of nutrition for their families in preventing stunting in children under five. These factors should be addressed within the scope of health or across sectors so that stunting events in Indonesia and the world do not occur again so that the future of children is not hampered because of nutritional problems.

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Association of food consumption and physical activity with metabolic syndrome according to central obesity status in Indonesian adults: A cross-sectional study

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ABSTRACT

Background: Previous studies have reported that the risk of metabolic syndrome differs between obese and non-obese individuals based on food consumption and physical activity.

Objective: This study aims to analyze differences in the association of food consumption and physical activity with the incidence of metabolic syndrome in individuals with and without central obesity.

Materials and Methods: This cross-sectional study examined individuals aged 19 to 64 years using Riskesdas 2018 data. Sample characteristics, including smoking habits, alcohol consumption, food consumption, physical activity, anthropometric data, clinical data, and biochemical data were collected for univariate, bivariate, and multivariate analyses. Logistic regression was used as a multivariate analysis to investigate the association of food consumption and physical activity with the risk of metabolic syndrome in individuals with and without central obesity.

Results: In this study, individuals with and without central obesity significantly increased risk of metabolic syndrome ($p<0.05$) due to consuming nearly all kinds of unhealthy foods (sweet foods, savory foods, fatty/cholesterol-rich/fried foods, grilled foods, processed meat/chicken/fish, soft drinks/carbonated drinks, energy drinks, and instant noodles/other instant foods). However, only individuals without central obesity who frequently consume seasonings (OR=1.519, 95% CI: 1.241-1.859) have a significant association with an increased risk of metabolic syndrome. Meanwhile, only individuals with central obesity who often consume sugary drinks (OR=1.315, 95% CI: 1.132-1.529) are significantly associated with an increased risk of metabolic syndrome. In addition, inadequate consumption of fruits and vegetables as well as lack of physical activity also significantly increase the risk of metabolic syndrome in individuals with and without central obesity ($p<0.05$).

Conclusion: Only the consumption of seasonings and sugary drinks shows a different relationship to the risk of metabolic syndrome in individuals with and without central obesity.

Keywords: *central obesity; food consumption; metabolic syndrome; physical activity*

BACKGROUND

As a serious global health problem, the global prevalence of central obesity reaches 41.5%.[1] The prevalence of central obesity is also high in some countries, such as southern China (10.2%),[2] southwestern Iran (28.6%),[3] and north-eastern Ethiopia (16.5%).[4] In Indonesia, the prevalence of central obesity also increased by 4.4% between 2013 and 2018.[5,6] The increase in cases of central obesity is influenced by several factors, including age, gender, place of residence,[2] education level, Body Mass Index (BMI), smoking habit, alcohol consumption,[7] food consumption,[8–11] and physical activity.[12]

Central obesity-induced metabolic syndrome risk,[13] a group of risk factors, i.e., increased waist circumference, blood glucose level, triglycerides level, and blood pressure, as well as decreased HDL level, which has a close relation to cardiometabolic diseases.[14] Increases in visceral adipose tissue volume and waist circumference reduce insulin sensitivity,[15] which eventually lead to an increased risk of metabolic syndrome in individuals with central obesity.[13]

Several factors, such as food consumption and physical activity, affect the incidence of metabolic syndrome in individuals with central obesity. The incidence of metabolic syndrome is more common in individuals with central obesity who often consume unhealthy foods and rarely consume fruits and vegetables compared to individuals with central obesity who rarely consume unhealthy foods and often consume fruits and vegetables.[15–21] Furthermore, individuals with central obesity who undertake low-intensity physical activity are more likely to develop metabolic syndrome compared to individuals with central obesity who engage in higher-intensity physical activity.[17,22,23] Not only individuals with central obesity but also individuals without central obesity have metabolic syndrome. In this regard, individuals without central obesity who consume large amounts of unhealthy foods and do not engage in physical

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activity may have an increased risk of metabolic syndrome compared to individuals without central obesity who consume less unhealthy food and do physical activity.[24–26]

However, previous studies have not analyzed whether there a differences or similarities in association between food consumption and physical activity on the incidence of metabolic syndrome in individuals with and without central obesity. Leite et al. (2009) and Suliga et al. (2018) explained that the risk of metabolic syndrome differs between obese individuals and those with normal weight based on food consumption and physical activity.[27,28] Individuals with central obesity and without central obesity may differ in their risk of metabolic syndrome based on food consumption and physical activity levels. Therefore, we aimed to analyze the differences in the association of food consumption and physical activity with metabolic syndrome risk in individuals with and without central obesity in Indonesia.

MATERIALS AND METHODS

This cross-sectional study employed Riset Kesehatan Dasar (Riskesdas) data in 2018 The Riskesdas survey covered all households in Indonesia using a stratified sampling method. The sample interviewed are a sample of selected households. Furthermore, the sample for the biochemical measurements is a sub-sample that represents the selected population from 26 provinces.[6] In this study, the selected samples from the Riskesdas data were filtered using a consecutive sampling method. Individuals aged 19-64 years with anthropometric, clinical, and biochemical measurements were included in this study. Meanwhile, the exclusion criteria were missing and extreme data. The sample size of this study was 14,302. This study has been approved by Badan Kebijakan Pembangunan Kesehatan Kementerian Kesehatan Republik Indonesia No. IR.03.01/8/300/2023.

Riskesdas collected data on sample characteristics (age, gender, education level, and place of residence), alcohol consumption, and smoking habit through interviews using a standardized questionnaire[6]. Meanwhile, data regarding consumption of unhealthy foods (sweet foods, sugary drinks, savory foods, fatty/cholesterol-rich/fried foods, grilled foods, processed meat/chicken/fish, seasonings, soft drinks/carbonated drinks, energy drinks, and instant noodles/other instant foods), fruits, and vegetables were obtained through interviews using questionnaires and food image models. The frequency of unhealthy food consumption was classified into often (≥1 time per day or 1-6 times per week) and rare (≤3 times per month or never), whereas the consumption of fruits and vegetables was considered adequate if individuals consumed ≥5 servings of fruits and vegetables per day. Furthermore, data on physical activity were acquired from interviews using the GPAC questionnaire and physical activity models. Physical activity was categorized as sufficient if moderate and vigorous physical activity was done for ≥150 minutes/week.

The anthropometric data used this study cover body weight, height, and waist circumference. Body weight was measured using a body weight scale, while height was measured with a stadiometer. Measurements of body weight and height were used to calculate the Body Mass Index (BMI). Furthermore, waist circumference was measured with a measuring tape to determine central obesity and metabolic syndrome. Blood pressure was measured using a tensimeter, while blood glucose data (fasting blood glucose and postprandial blood glucose level) were obtained via capillary blood sampling and measured by Accuchek Performa. Lastly, blood lipid biochemical data (cholesterol, LDL, HDL, and triglyceride level) were gained through venous blood sampling and analyzed with a chemical autoanalyzer.[6]

In this study, waist circumference, blood pressure, and biochemical data (including fasting blood glucose, triglycerides, and HDL level) were used to determine metabolic syndrome status. An individual was considered to have metabolic syndrome if there were at least 3 of the following 5 risk factors: increase in waist circumference >90 cm in males and >80 cm in females, increase in triglyceride level ≥150 mg/dL, decrease in HDL level <40 mg/dL in males and <50 mg/dL in females, increase in systolic blood pressure ≥130 and/or diastolic blood pressure ≥85 mmHg, and increase in fasting blood glucose level ≥100 mg/dL.14

The data collected by Riskesdas were analyzed using Microsoft Excel and SPSS 25 software with a significance level of a *p*-value ≤0.05. Univariate analysis was performed to determine the distribution of values for each variable. Meanwhile, bivariate analyses were carried out to examine the association between sample characteristics and central obesity using the chi-square test (for categorical variables) and t-test (for numerical variables). The chi-square test was also done to find the association between independent variables (food consumption and physical activity) and metabolic syndrome according to central obesity status. Furthermore, the multivariate analysis used logistic regression to investigate the association of food consumption and physical activity with the risk of metabolic syndrome in individuals with and without central obesity. Logistic regression analysis was performed to control confounding variables, such as age,

gender, education level, place of residence, alcohol consumption, smoking habit, Body Mass Index (BMI), cholesterol level, and Low Density Lipoprotein (LDL) level.

RESULTS

The mean age of individuals with central obesity is 44.74 ± 10.71 years. In this study, central obesity is found to be more prevalent in females (82.3%), individuals living in urban areas (53.7%), individuals with low education level (71%), non-alcohol drinkers (99.3%), and non-smokers (84.7%). Most individuals with central obesity are overweight (80.8%) and have high blood pressure (systolic: 59.1%; diastolic: 63.7%), normal fasting blood glucose level (55%), high postprandial blood glucose level (54.7%), normal cholesterol level (59.7%), high LDL level (50.9%), normal triglycerides level (63.8%), and low HDL level (58.6%). The prevalence of metabolic syndrome in individuals with central obesity is 72.2%.

Table 1. Sample Characteristics

Variable	Central Obesity		Non Central Obesity		*p*	Sample Size	
	n=6,913	Mean±SD	n=7,389	Mean±SD		n =14,302	Mean±SD
Age (years)		44.74±10.7		43.51±12.4			44.11±11.7
Gender							
Male, n(%)	1,224 (17.7%)		4,021 (54.4%)		0.000*	5,245 (36.7%)	
Female, n(%)	5,689 (82.3%)		3,368 (45.6%)			9,057 (63.3%)	
Education Level							
High, n(%)	2,004 (29%)		1,929 (26.1%)		0.000*	3,933 (27.5%)	
Low, n(%)	4,909 (71%)		5,460 (73.9%)			10,369 (72.5%)	
Place of Residence							
Rural, n(%)	3,198 (46.3%)		5,042 (68.2%)		0.000*	8,240 (57.6%)	
Urban, n(%)	3,715 (53.7%)		2,347 (31.8%)			6,062 (42.4%)	
Alcohol Consumption							
No, n(%)	6,865 (99.3%)		7,271 (98.4%)		0.000*	14,136 (98.8%)	
Yes, n(%)	48 (0.7%)		118 (1.6%)			166 (1.2%)	
Smoking Habit							
No, n(%)	5,856 (84.7%)		4,138 (56%)		0.000*	9,994 (69.9%)	
Yes, n(%)	1,057 (15.3%)		3,251 (44%)			4,308 (30.1%)	
Body Mass Index (kg/m^2)		28.37±4.14		21.86±3.13			25.01±4.89
Underweight, n(%)	11 (0.2%)		889 (12%)		0.000*	900 (6.3%)	
Normal, n(%)	1,316 (19%)		5,436 (73.6%)			6,752 (47.2%)	
Overweight, n(%)	5,586 (80.8%)		1,064 (14.4%)			6,650 (46.5%)	
Systolic Blood Pressure (mmHg)		139.4 ± 24.7		129.8±22.3			134.4±23.9
Normal, n(%)	2,826 (40.9%)		4,401 (59.6%)		0.000*	7,227 (50.5%)	
High, n(%)	4.087 (59.1%)		2,988 (40.4%)			7,075 (49.5%)	
Diastolic Blood Pressure (mmHg)		89.9±13.1		82.4±12.2			86.08±13.2
Normal, n(%)	2,511 (36.3%)		4,575 (61.9%)		0.000*	7,086 (49.5%)	
High, n(%)	4,402 (63.7%)		2,814 (38.1%)			7,216 (50.5%)	
Fasting Blood Glucose Level (mg/dL)		108.9±39.4		101.4±30.6			105±35.3
Normal, n(%)	3,804 (55%)		4,829 (65.4%)		0.000*	8,633 (60.4%)	
High, n(%)	3,109 (45%)		2,560 (34.6%)			5,669 (39.6%)	

Table 1. Sample Characteristics (Continue…)

Variable	Central Obesity		Non Central Obesity		*p*	Sample Size	
	n=6,913	Mean±SD	n=7,389	Mean±SD		n=14,302	Mean±SD
Postprandial Blood Glucose Level (mg/dL)		159.4±60.2		142.9±52.5			150.9±56.9
Normal, n(%)	3,129 (45.3%)		4,394 (59.5%)		0.000*	7,523 (52.6%)	
High, n(%)	3,784 (54.7%)		2,995 (40.5%)			6,779 (47.4%)	
Cholesterol Level (mg/dL)		194.3±41.1		178±37.9			185.9±40.3
Normal, n(%)	4,128 (59.7%)		5,550 (75.1%)		0.000*	9,678 (67.7%)	
High, n(%)	2,785 (40.3%)		1,839 (24.9%)			4,624 (32.3%)	
Low-Density Lipoprotein (LDL) Level (mg/dL)		133.1±35.3		118.6±31.9			125.6±34.4
Normal, n(%)	3,391 (49.1%)		5,032 (68.1%)		0.000*	8,423 (58.9%)	
High, n(%)	3,522 (50.9%)		2,357 (31.9%)			5,879 (41.1%)	
High-Density Lipoprotein (HDL) Level (mg/dL)		48.37±11.6		46.53±10.3			47.48±11.1
Normal, n(%)	2,862 (41.4%)		4,627 (62.6%)		0.000*	7,489 (52.4%)	
Low, n(%)	4,051 (58.6%)		2,762 (37.4%)			6,813 (47.6%)	
Triglyceride Level (mg/dL)		145.1±105.4		120.3±87.1			132.3±97.2
Normal, n(%)	4,411 (63.8%)		5,678 (76.8%)		0.000*	10,089 (70.5%)	
High, n(%)	2,502 (36.2%)		1,711 (23.2%)			4,213 (29.5%)	
Metabolic Syndrome							
No, n(%)	1,923 (27.8%)		5,844 (79.1%)		0.000*	7,767 (54.3%)	
Yes, n(%)	4,990 (72.2%)		1,545 (20.9%)			6,535 (45.7%)	

The *p*-value was obtained from the chi-square[1] test (for categorical variables) and t-test[2] (for numerical variables). *significant ($p<0.05$)

Table 2 shows the prevalence of food consumption and physical activity according to central obesity status and metabolic syndrome. Individuals with central obesity who often consume fatty/cholesterol-rich/fried foods (88.6%) and seasonings (92.1%) are more likely to have metabolic syndrome than individuals without central obesity. Conversely, individuals without central obesity who often consume sugary drinks (86%), savory foods (75.9%), soft drinks/carbonated drinks (9.3%), and energy drinks (6.7%) are more likely to have metabolic syndrome than individuals with central obesity.

The results of the logistic regression analyses on the risk of metabolic syndrome in individuals with and without central obesity before and after controlling for confounding variables (age, gender, education level, place of residence, alcohol consumption, smoking habit, Body Mass Index, and postprandial glucose, cholesterol, and LDL level) are presented in Tables 3 and 4, respectively. Individuals with central obesity who often consume sweet foods (OR=1.272, 95%CI: 1.101-1.468), sugary drinks (OR=1.315, 95%CI: 1.132-1.529), savory foods (OR=1.397, 95%CI: 1.234-1.581), fatty/cholesterol-rich/fried foods (OR=1.270, 95%CI: 1.082-1.492), grilled food (OR=1.276, 95%CI: 1.118-1.475), processed meat/fish/chicken (OR=1.440, 95% CI: 1.238-1.674), soft drinks/carbonated drinks (OR=1.814, 95%CI: 1.360-2.420), energy drinks (OR=1.901, 95% CI: 1.266-2.854), and instant noodles/other instant foods (OR=1.330, 95% CI: 1.183-1.495) are significantly associated with an increased risk of metabolic syndrome after controlling for confounding variables. In addition, inadequate consumption of fruits and vegetables (OR=1.371, 95% CI: 1.014-1.853) and lack of physical activity (OR=1.163, 95% CI: 1.012-1.336) in individuals with central obesity have a significant association with an increased risk of metabolic syndrome after controlling for confounding variables.

Table 2. Prevalence of Food Consumption and Physical Activity According to Central Obesity Status and Metabolic Syndrome

Variable	Metabolic Syndrome		*p*	Sample Size
	Central Obesity	Non Central Obesity		
	n = 4,990	n = 1,545		n = 6,535
Sweet Foods				
Rare (n (%))	959 (19.2%)	297 (19.2%)	1.00	1,256 (19.2%)
Often (n (%))	4,031 (80.8%)	1,248 (80.8%)		5,279 (80.8%)
Sugary Drinks				
Rare (n (%))	806 (16.2%)	217 (14%)	0.05*	1,023 (15.7%)
Often (n (%))	4,184 (83.8%)	1,328 (86%)		5,512 (84.3%)
Savory Foods				
Rare (n (%))	1,347 (27%)	372 (24.1%)	0.03*	1,719 (26.3%)
Often (n (%))	3,643 (73%)	1,173 (75.9%)		4,816 (73.7%)
Fatty/Cholesterol-rich/Fried Foods				
Rare (n (%))	570 (11.4%)	213 (13.8%)	0.01*	783 (12%)
Often (n (%))	4,420 (88.6%)	1,332 (86.2%)		5752 (88%)
Grilled Foods				
Rare (n (%))	3,495 (70%)	1,055 (68.3%)	0.20	4,550 (69.6%)
Often (n (%))	1,495 (30%)	490 (31.7%)		1,985 (30.4%)
Processed Meat/Chicken/Fish				
Rare (n (%))	3,869 (77.5%)	1,217 (78.8%)	0.32	5,086 (77.8%)
Often (n (%))	1,121 (22.5%)	328 (21.2%)		1,449 (22.2%)
Seasonings				
Rare (n (%))	395 (7.9%)	147 (9.5%)	0.05*	542 (8.3%)
Often (n (%))	4,595 (92.1%)	1,398 (90.5%)		5,993 (91.7%)
Soft Drinks/Carbonated Drinks				
Rare (n (%))	4,609 (92.4%)	1,402 (90.7%)	0.05*	6,011 (92%)
Often (n (%))	381 (7.6%)	143 (9.3%)		524 (8%)
Energy Drinks				
Rare (n (%))	4,777 (95.7%)	1,442 (93.3%)	0.00*	6,219 (95.2%)
Often (n (%))	213 (4.3%)	103 (6.7%)		316 (4.8%)
Instant Noodles/Other Instant Foods				
Rare (n (%))	2,130 (42.7%)	617 (39.9%)	0.06	2,747 (42%)
Often (n (%))	2,860 (57.3%)	928 (60.1%)		3,788 (58%)
Fruit and Vegetable Consumption				
Adequate (n (%))	143 (2.9%)	47 (3%)	0.78	190 (2.9%)
Inadequate (n (%))	4,847 (97.1%)	1,498 (97%)		6,345 (97.1%)
Physical Activity				
Adequate (n (%))	3,827 (76.7%)	1,185 (76.7%)	1.00	5,012 (76.7%)
Inadequate (n (%))	1,163 (23.3%)	360 (23.3%)		1,523 (23.3%)

The *p*-value was obtained from the chi-square test. *significant ($p<0.05$).

As seen in Table 4, individuals without central obesity who often consume sweet foods (OR=1.426, 95%CI: 1.218-1.669), savory foods (OR=1.193, 95%CI: 1.035-1.374), fatty/cholesterol-rich/fried food (OR=1.398, 95%CI: 1.178-1.659), grilled foods (OR=1.197, 95%CI: 1.044-1.374), processed meat/fish/chicken (OR=1.614, 95%CI: 1.374-1.896), seasoning (OR=1.519, 95%CI: 1.241-1.859), soft drinks/carbonated drinks (OR=1.670, 95%CI: 1.310-2.129), energy drinks (OR=1.527, 95%CI: 1.158-2.012), and instant noodles/other instant foods (OR=1.746, 95%CI: 1.542-1.978) were significantly associated with an increased risk of metabolic syndrome after controlling for confounding variables. Furthermore, inadequate consumption of fruit and vegetable (OR=2.144, 95%CI: 1.538-2.988) and lack of physical activity (OR=1,295, 95%CI: 1.117-1,502) also had a significant correlation with an increased risk of metabolic syndrome in individuals without central obesity after controlling for confounding variables.

Table 3. Risk Analysis of Metabolic Syndrome Based on Food Consumption and Physical Activity in Individuals with Central Obesity

Variable	Model 1[a]			Model 2[b]		
	OR	95%CI	*p*	OR	95%CI	*p*
Sweet Foods						
Rare	ref			ref		
Often	1.183	1.031-1.357	0.017*	1.272	1.101-1.468	0.001*
Sugary Drinks						
Rare	ref			ref		
Often	1.235	1.070-1.426	0.004*	1.315	1.132-1.529	0.000*
Savory Foods						
Rare	ref			ref		
Often	1.313	1.166-1.478	0.000*	1.397	1.234-1.581	0.000*
Fatty/Cholesterol-Rich/Fried Foods						
Rare	ref			ref		
Often	1.337	1.146-1.560	0.000*	1.270	1.082-1.492	0.003*
Grilled Foods						
Rare	ref			ref		
Often	1.254	1.104-1.423	0.000*	1. 276	1.118-1.475	0.000*
Processed Meat/Chicken/Fish						
Rare	ref			ref		
Often	1.342	1.161-1.551	0.000*	1.440	1.238-1.674	0.000*
Seasonings						
Rare	ref			ref		
Often	1.090	0.903-1.316	0.370	1.096	0.900-1.333	0.362
Soft Drinks/Carbonated Drinks						
Rare	ref			ref		
Often	1.677	1.268-2.218	0.000*	1.814	1.360-2.420	0.000*
Energy Drinks						
Rare	ref			ref		
Often	1.907	1.282-2.837	0.001*	1.901	1.266-2.854	0.002*
Instant Noodles/Other Instant Foods						
Rare	ref			ref		
Often	1.195	1.071-1.335	0.002*	1.330	1.183-1.495	0.000*
Fruit and Vegetable Consumption						
Adequate	ref			ref		
Inadequate	1.377	1.031-1.841	0.030*	1.371	1.014-1.853	0.040*
Physical Activity						
Adequate	ref			ref		
Inadequate	1.215	1.065-1.386	0.004*	1.163	1.012-1.336	0.033*

Reference group: consumption of unhealthy foods: rarely; fruit and vegetable consumption and physical activity: adequate
[a]Unadjusted; [b]Confounding variables: age, gender, place of residence, education level, Body Mass Index, smoking habit, alcohol consumption, postprandial blood glucose level, cholesterol level, and LDL level. *significant (p<0.05).

Table 4. Risk Analysis of Metabolic Syndrome Based on Food Consumption and Physical Activity in Individuals without Central Obesity

Variable	Model 1[a]			Model 2[b]		
	OR	95%CI	*p*	OR	95%CI	*p*
Sweet Foods						
Rare	ref			ref		
Often	1.332	1.146-1.548	0.000*	1.426	1.218-1.669	0.000*
Sugary Drinks						
Rare	ref			ref		
Often	1.010	0.852-1.198	0.904	1.117	0.932-1.338	0.230
Savory Foods						
Rare	ref			ref		
Often	1.204	1.052-1.378	0.007*	1.193	1.035-1.374	0.015*
Fatty/Cholesterol-Rich/Fried Foods						
Rare	ref			ref		
Often	1.284	1.090-1.511	0.003*	1.398	1.178-1.659	0.000*
Grilled Foods						
Rare	ref			ref		
Often	1.189	1.044-1.353	0.009*	1.197	1.044-1.374	0.010*
Processed Meat/Chicken/Fish						
Rare	ref			ref		
Often	1.600	1.377-1.860	0.000*	1.614	1.374-1.896	0.000*

Table 4. Risk Analysis of Metabolic Syndrome Based on Food Consumption and Physical Activity in Individuals without Central Obesity (Continue…)

Variable	Model 1[a]			Model 2[b]		
	OR	95%CI	*p**	OR	95%CI	*p**
Seasonings						
Rare	ref			ref		
Often	1.382	1.141-1.674	0.001*	1.519	1.241-1.859	0.000*
Soft Drinks/Carbonated Drinks						
Rare	ref			ref		
Often	1.678	1.342-2.099	0.000*	1.670	1.310-2.129	0.000*
Energy Drinks						
Rare	ref			ref		
Often	1.307	1.013-1.688	0.040*	1.527	1.158-2.012	0.003*
Instant Noodles/Other Instant Food						
Rare	ref			ref		
Often	1.522	1.355-1.711	0.000*	1.746	1.542-1.978	0.000*
Fruit and Vegetable Consumption						
Adequate	ref			ref		
Inadequate	1.997	1.450-2.750	0.000*	2.144	1.538-2.988	0.000*
Physical Activity						
Adequate	ref			ref		
Inadequate	1.298	1.131-1.491	0.000*	1.295	1.117-1.502	0.001*

Reference group: consumption of unhealthy foods: rarely; fruit and vegetable consumption and physical activity: adequate
[a]Unadjusted; [b]Confounding variables: age, gender, place of residence, education level, Body Mass Index, smoking habit, alcohol consumption, postprandial blood glucose level, cholesterol level, and LDL level. *significant (p<0.05).

DISCUSSION

The prevalence of metabolic syndrome is higher in individuals with central obesity. Individuals with central obesity have increased visceral adipose tissue volume and waist circumference,[13] Increased visceral adipose tissue volume and waist circumference decrease insulin sensitivity, which triggers the risk of metabolic syndrome.[15,16,29,30] Individuals with and without central obesity who frequently consume almost all unhealthy foods (sweet foods, savory foods, fatty/cholesterol-rich/fried foods, grilled foods, processed meat/chicken/fish, soft drinks/carbonated drinks, energy drinks, and instant noodles/other instant foods) are at a higher risk of metabolic syndrome. Unhealthy foods are generally energy dense and high in sodium which can reduce insulin sensitivity Individuals with central obesity and without central obesity who have decreased insulin sensitivity resulting in an increased risk of metabolic syndrome.[15,16,35,20,21,29–34]

This study also shows that there is a difference in the association of unhealthy food consumption with the risk of metabolic syndrome in individuals with and without central obesity. In individuals with central obesity, the consumption of seasonings does not increase the risk of metabolic syndrome. In contrary, individuals without central obesity who frequently consume seasonings have an increased risk of metabolic syndrome. This is because the consumption of seasonings may reduce insulin sensitivity in individuals without central obesity,[36] but not in individuals with central obesity.[37,38] Individuals with central obesity who do not have decreased insulin sensitivity may lower risk of metabolic syndrome, whereas individuals without central obesity who have decreased insulin sensitivity may have an increased risk of metabolic syndrome.[15]

The association between the consumption of sugary drinks and the risk of metabolic syndrome also differs in individuals with and without central obesity. In individuals with central obesity, frequent consumption of sugary drinks may increase the risk of metabolic syndrome. On the other hand, individuals without central obesity who often consume sugary drinks do not have an increased risk of metabolic syndrome. Individuals with central obesity had an increase in waist circumference, which is associated with decreased insulin sensitivity that increases the risk of metabolic syndrome. Meanwhile, individuals without central obesity do not have an increased waist circumference,[39] which is related to improved insulin sensitivity, thus resulting in a lower risk of metabolic syndrome.[15,30]

Fruits and vegetables are high-fiber foods,[40] while low fiber may increase metabolic syndrome risk.[15,41] Therefore, individuals with and without central obesity who consume less fruits and vegetables are at a greater risk of metabolic syndrome. In addition, individuals with and without central obesity who lack physical activity also have an increased risk of metabolic syndrome. Lack of physical activity decreases insulin sensitivity,[26] which can increase the risk of metabolic syndrome.[15]

CONCLUSION

Individuals with and without central obesity who frequently consume almost all unhealthy foods (sweet foods, savory foods, fatty/cholesterol-rich/fried foods, grilled foods, processed meat/chicken/fish, soft drinks/carbonated drinks, energy drinks, and instant noodles/other instant foods) are significantly related to an increased risk of metabolic syndrome. Meanwhile, in terms of frequent consumption of seasonings, only individuals without central obesity are significantly correlated with an increased risk of metabolic syndrome. Furthermore, only individuals with central obesity who often consume sugary drinks have a significant correlation with an increased risk of metabolic syndrome. In individuals with and without central obesity, inadequate consumption of fruits and vegetables and lack of physical activity are significantly associated with an increased risk of metabolic syndrome. Therefore, future studies are highly recommended to include analysis of dietary patterns using an a priori or posteriori approach as well as examination on food consumption that includes nutritional values.

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Difference of thrombocyte profile between obesity and central obesity in women

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ABSTRACT

Background: Obesity considered as a low-grade inflammation. Increased body fat has known to trigger inflammation. Platelet profile is a number of platelet-related parameters that can predict inflammation consisting of: platelet count (PLT), Platelet Larger Cell Ratio (P-LCR), Mean Platelet Volume (MPV). Differences in platelet profiles (PLT, P-LCR, MPV) in women with and without central obesity have only been investigated in a few studies.
Objective: To prove differences in platelet profiles in women with and without central obesity.
Materials and Methods: A cross-sectional observational study was conducted on 88 women with and without central obesity in RSND during July-September 2021. Data included age, abdominal circumference, hip circumference, PLT, P-LCR, and MPV. PLT, P-LCR, and MPV were measured using Sysmex XS-500i instrument. Statistical analysis was using Mann-Whitney test.
Results: Mean of women PLT with and without central obesity were 338.72±71.09x10^3/uL and 309.09±44.36x10^3/uL. Difference platelet levels in women with and without central obesity was p=0.022. Median MPV of women with and without central obesity were 10.5 (8.5-11.8)fL and 9.7 (8.5-11.6)fL. Difference MPV values in women with and without central obesity was p=0.000. Median P-LCR of women with and without central obesity were 28.2 (12.3-44.3)% and 21.5 (15.2-37.1)%, respectively. Difference P-LCR value in women with and without central obesity was p=0.002.
Conclusion: Platelet profiles (PLT, MPV, P-LCR) can be used as a marker of chronic low-grade inflammation in women with central obesity.

Keywords : *central obesity; MPV; PLT; P-LCR*

BACKGROUND

Obesity **is** still becoming one of major public health problem in the world whose number continues to increase every year. Results of Basic Health Research (Riskesdas) from the Ministry of Health of the Republic of Indonesia in 2018 show that there is an increase in prevalence of obesity. There was a 7 % increase in the prevalence of obesity in the age group over 18 years from 14.8% in 2013 to 21.8% in 2018. This result also conclude that proportion obesity is higher in women than men.[1]

Obesity defined by Body Mass Index (BMI) >25 kg/m^2. Obesity usually divided into 2 types namely Central Obesity and Perifer Obesity. Central obesity is more dangerous than the other type of obesity because central obesity indicates abnormal fat accumulation in the abdominal regions is highly associated with the risk of getting cardiometabolic diseases and their progression to end stage diseases or death.[2]

Central obesity cannot be defined by BMI only, another anthropometric examination is needed to determine whether someone has a type of central obesity. Waist to hip ratio (WHR) is one of the anthropometric measurements that can be used to determine central obesity. WHR measures the ratio of waist circumference to hip circumference, determining how much fat is stored in the waist, hips, and buttocks. Central obesity defined if the WHR is 0.9 in men and 0.85 in women, and the optimal cutoff value is 0.89 for men and 0.82 for women in Asian populations.[3]

Obesity is a condition that can trigger low-level inflammation caused by an increase in body fat. Excessive body fat cause adipocyte increased more cytokine pro-inflammatory such as TNF-Alpha, IL-6, and CRP which can be used as inflammatory parameters.[4] Previous studies have found that the platelet profile plays an important role in a number of diseases whose pathogenesis is strongly influenced by the inflammatory process [5]. Cohort study in 2021 also showed that there is a significant correlation between platelet profile and obesity. Platelet profile can be an alternative inflammatory parameters with the advantage of easier and cheaper examination compared to pro-inflammatory cytokines.[6]

There are several parameters of the platelet profile such as platelet count (PLT), mean platelet volume (MPV), and platelet large cell ratio (P-LCR). MPV can act as a negative or positive acute-phase reactant in a variety of different inflammatory conditions. An increase in MPV is associated with a severe inflammatory condition due to an increase in the number of circulating platelets, while a decrease in MPV is associated with

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a mild inflammatory condition. The P-LCR is an indicator of the number of larger platelets (>12 fL) in the circulation. This parameter can also be used to evaluate platelet activity. The P-LCR value is inversely proportional to the platelet count, and ~~is~~ directly proportional to Platelet Distribution Width (PDW) and MPV. P-LCR will decrease in patients with thrombocytosis and increase in thrombocytopenia .

Cecen S *et al* in their research explained that PLT increases with body weight, fat percentage, fat masses (FM), and fat mass index (FMI). In men, whereas Procalcitonin Test (PCT) did not change in these parameters. PLT in women decreases with age, increases with body weight, body mass index (BMI), fat percentage, FM, fat-free masses (FFM), and FMI while PCT increases with body weight, BMI, fat percentage, FM, FFM, and FMI.[5] Ali U, *et al* in their research stated that statistically there were differences in the MPV, P-LCR, PCT reference intervals based on gender, whereas in the PDW reference interval there were no significant differences.[7]

Several studies also have emphasized the relationship between obesity-related problems and platelet activation, but few studies have studied differences in platelet profiles in women with and without central obesity. This study was conducted to determine whether there are differences in platelet profiles in women with and without central obesity.

MATERIALS AND METHODS

This study used a cross-sectional observational research method, the study was conducted during July-September 2021 at the Diponegoro National Hospital (RSND) Semarang. This study was cross sectional study with simple random sampling. Total respondent in this study was 87 obese women that divided into 2 groups there is Central Obesity Group and Non Central Obesity Group. Respondent in this study was choosen based on inclusion criterias such as in 20-35 years old having BMI score > 25(kg/m^2).[8] Waist to Hip Ratio (WHR) > 0.85 Central Obesity Group and < 0.85 for Non Central Obesity Group, in healthy condition and willing to participate in this study.[9] Exclusion criterias were pregnant women, hemolysis specimens, frozen specimens, heart disease, diabetes mellitus, and blood disorders.

$$\text{Body Mass Index (BMI)} = \frac{Weight\ (kg)}{Height\ (m)^2} \qquad \text{Waist Hip Ratio (WHR)} = \frac{Waist\ Circumference\ (cm)}{Hip\ Circumference\ (cm)}$$

Respondent in this study was taken as much as 3 cc of blood in the EDTA tube, then examined at the RSND outpatient laboratory. Platelet count, PLCR and MPV were measured through a Complete Blood Count (CBC) examination using an automatic hematology analyzer Sysmex XS-500i.

The collected data were analyzed using SPSS software. Normality test of data in this research was conducted by Kolmogorov Smirnov. Data analysis of platelet levels was carried out by independent t-test, while the analysis of MPV and P-LCR values used the Mann Whitney test. The research has been approved by the Ethics Committee of the Diponegoro Medical Faculty Semarang Number 32/EC/KEPK/FK-UNDIP/III/2020.

RESULTS

Total of 88 respondents participated in the study, during data processing there was 1 outlier data, so it was excluded from the study. The final data used 87 samples consisting of 43 samples of the central obesity group and 44 samples of the group without central obesity.

Characteristics of the subjects in the two groups were presented in Table 1. Median of age was older in central obesity group than without central obesity. Median value of WHR in central obese group was 0.9 (0.85-0.98) and 0.79 (0.68-0.84) in the group without central obesity.

Table 1. Characteristics of research subjects

Variable	Central Obesity (n= 43)	Non Central Obesity (n= 44)	*p*
	Median (min-max)	Median (min-max)	
Age (years)[#]	31 (25-35)	30 (25-35)	0,439[M]
Weight (Kg)[#]	73,7 (61-138)	69,9 (54-100)	0,080[M]
Height (cm)	156 (145-167)	157,25 (145-167)	0,704[†]
BMI (Kg/m^2)[#]	30,83 (24,64-53,08)	29,15 (23,76-42,04)	0,075[M]
Waist Circumferance (cm)[#]	96 (79-138)	86 (72-112)	0,000*[M]
Hip Circumferance (cm)[#]	108 (90-145)	110 (93-135)	0,195[M]
WHR[#]	0,9 (0,85-0,98)	0,79 (0,68-0,84)	0,000*[M]

*significant (p<0.05); [M]*Mann Whitney*; [†]*independent t-test*.

The results of blood examination were platelet count, MPV value, and P-LCR. The difference in platelet profiles between two groups can be seen in Table 2. There was a significant difference in platelets count between two group (p=0.022). MPV values in central obesity group had a median of 10.5 (8.5-11.8) fL, and the median among non central obesity group was 9.7 (8.5-11.6) fL. There was a significant difference in MPV values between two groups in this study (p=0.000). P-LCR central obesity group had a median of 28.2 (12.3-44.3)%, and the other group was 21.5 (15.2-37.1)%. There was a significant difference in the P-LCR value between two groups (p=0.002).

Table 2. Differences in platelet profiles in women with and without central obesity

Variable	Normal Range [10-12]	Central Obesity (n= 43)		Non Central Obesity (n= 44)		*p*
		Mean ± SD	Median (min-max)	Mean ± SD	Median (min-max)	
PLT (x10^3/uL)	150-400	338,72 ± 71,09	329 (168-458)	309,09 ± 44,36	301 (219-424)	0,022*†
MPV (fL)	7.2-11.2	10,54 ± 0,86	10,5 (8,5-11,8)	9,82 ± 0,8	9,7 (8,5-11,6)	0,000^{*M}
P-LCR (%)	15-35	28,2 ± 7,23	28,2(12,3-44,3)	23,4 ± 6,66	21,5 (15,2-37,1)	0,002^{*M}

MPV, *mean platelet volume*; PLT, *platelet*/ trombosit; P-LCR, *platelet large cell ratio*; *p*: †*independent t-test*; ^{M}*Mann Whitney*. *significant (p<0.05).

DISCUSSION

Obesity is an excessive accumulation of fat due to an imbalance between energy intake and energy expenditure for a long time.[3] Obese individuals with high visceral adiposity have increased expression of monocyte-chemotactic protein-1 (MCP-1) and infiltration macrophages in visceral fat compared to subcutaneous fat. Visceral adipose tissue from obese subjects was also found to secrete higher levels of plasminogen activator inhibitor-1 (PAI-1), IL-6, TNF-Alpha, and leptin and lower levels of adiponectin compared to non-obese subjects.

The results of the study by Han S, *et al* said that PLT was positively related to BMI, waist circumference, WHR, and percentage of total fat mass. A similar relationship was found between PCT and body fat. However, there was no significant relationship between MPV, PDW, P-LCR with body fat.[13] In line with this study, there was a significant difference in PLT in women with and without central obesity (p = 0.022) in accordance with Cecen S, *et al* in his study that said PLT, PCT, and PDW is increased with adipose tissue, especially in obese female individuals.[5] This is because under inflammatory conditions, proinflammatory cytokine IL-6 secreted from adipose tissue increases the maturation of megakaryocyte precursors and may be the cause of increased PLT in obesity.[5]

Obesity causes chronic low-grade inflammation. Chronic low-grade inflammation in obese patients is different from inflammatory conditions in general, because there are no signs or symptoms of inflammation, but it is similar to inflammatory conditions due to activation of inflammatory mediators.[14] Aktas G, *et al* in their study compared MPV as biomarker of inflammation between Diabetes Mellitus (DM) patients with medication and Diabetes Mellitus (DM) patient without medication. This study aims to observed relationship MVP with obesity index, body mass index (BMI) and waist circumference. The result was that MPV, BMI, and waist circumference were significantly higher in patients with uncontrolled DM compared to patients with controlled DM, where waist circumference and BMI were significantly higher in patients with uncontrolled DM.[15] In this study, there were significant differences in MPV values between two groups (p=0.000). Central Obesity group having higher median MVP value than Non Central Obesity Group.

MPV is a parameter that inform about platelet size and activation. An increased MPV value indicates the presence of large, newer, denser, and more active platelets.[16] MPV is also a marker of platelet reactivity and acts as an acute-phase reactant. A high MPV is an indicator of high-grade inflammation while a low MPV is an indicator of low-grade inflammation, because obesity is known to be a low-grade inflammation, so the expected MPV value in this study from two groups still categorized in normal range.[17] LCR in women with central obesity where the P-LCR value in women with central obesity has median of 28.2 (12.3-44.3%), while the median in women without central obesity is 21.5 (15.2-37, 1) %.

Result of this study shows that there is significant differences PLT, MVP value between Central obesity group and Non Central Obesity group contradict with result from Alshehri O, *et al* study. This study shows that PLT, MPV and PDW in obese patients were not significantly different compared to non-obese patient.[17]

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CONCLUSIONS

There are significantly differences platelet profiles (PLT, MPV, P-LCR) in women with and without central obesity. Platelet profiles (PLT, MPV, P-LCR) can be used as a marker of inflammation in women with central obesity.

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Psychosocial stress, food preferences, and screen time with nutritional status of women of reproductive age in Sukamulya Village, Tangerang Regency

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ABSTRACT

Background: The increase in age and the pandemic conditions experienced cause Women of Reproductive Age (WRA) to encounter many environmental issues that disturb their psyche, resulting in psychosocial stress. A strategy for dealing with stress is called coping with stress. A higher screen time and high sugar, salt, or fat to deal with stress might change nutritional status.

Objective: This study aims to determine the relationship between psychosocial stress, food preferences, and screen time with the nutritional status of WRA in Sukamulya Village, Tangerang Regency.

Materials and Methods: This research design is cross-sectional and was conducted in March 2022 in Sukamulya Village, Tangerang Regency. The research sample amounted to 55 participants with a purposive sampling technique. The questionnaires used were Psychosocial Stress Assessment Instrument, Food Frequency Questionnaires, and recall screen time. Data analysis using the Chi-Square test.

Results: The majority of participants experienced psychosocial stress (61.8%), food preferences low in sugar, salt, and fat (63.6%), and most of them were in the high screen time category (52.7%). The results showed that there was no relationship between psychosocial stress and food preferences with nutritional status ($p > 0.05$), but there was a relationship between screen time and nutritional status ($p = 0.011$).

Conclusion: In this study, food preferences and psychosocial stress were not factors that affected the nutritional status.

***Keywords** : BMI; food preferences; psychosocial stress; screen time; women of reproductive age*

BACKGROUND

Women of Reproductive Age (WRA) is a group of productive women ranging from 15-49 years regardless of their marital status.[1] Nutritional status is one of the things that need to be considered in this group. Based on data from the World Health Organization (WHO), in 2014, those aged ≥18 years in the world experienced underweight as many as 462 million people, and 1.9 billion were overweight.[2] In 2016, globally, 9.4% of women aged >19 years were underweight.[3] According to the National Basic Health Research in 2018, among women >18 years in Indonesia, 7.8% were underweight, 15.1% were overweight, and 29.3% were obese.[4] In Banten Province, the prevalence of nutritional status based on BMI in women aged ≥18 years is 7.25% underweight, 15.54% overweight, and 30.05% obese. More specifically, Tangerang Regency has an overweight incidence rate above the overweight incidence rate in Banten Province, which is 17.03%.[5]

A preliminary survey conducted previously at Sukamulya Village, Tangerang Regency, Banten Province on 25 women aged 15-33 years found that 28% were overweight and obese. As many as 70% who are overweight are women aged >20 years and 52% of women complain of feeling depressed due to a condition or problem, especially family and economic conflicts. Their preferred food preferences are salty and savory snacks and foods. In addition, 68% have high screen time which is more than 2 hours per day. Based on these results, the research was conducted in the Sukamulya Village area.

Many factors can cause nutritional problems, including psychosocial stress. A survey in 2020 by WHO on 130 countries found that 89% of these countries included mental health and psychosocial support plans in dealing with COVID-19. This survey shows that COVID-19 has an impact on psychosocial health.[6] Previous research in 2018 showed a relationship between psychosocial stress and adolescent nutritional status. The nature of stress influences individual capacity to adapt to stress and individual character, so psychosocial stress must be appropriately managed so as not to cause depression. Psychosocial stress was found to be one of the factors of weight gain in adolescents through the mechanism of changes in food consumption and choice.[7]

Food choices or preferences when dealing with stress tend to be high-energy foods such as high sugar, salt, and fat. Preference for sweet, salty, and fatty foods was found to have a significant relationship with

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nutritional status.[8] There is an assumption that the consumption of sweet, salty, and fatty foods is a strategy to turn off one's feelings and memories regarding unpleasant things or events.[9] Consumption of these three food types and sedentary activity will cause fat accumulation.

Furthermore, higher sedentary activity, especially during the COVID-19 pandemic, may increase screen time activity. Research on Semarang in 2016 showed that higher screen time and lower physical activity might increase the risk of being overweight and obese. Screen time may lead to increased energy intake and altered metabolic processes.[10]

According to the results of research conducted at the University of Tanjungpura that there is a relationship between stress and the body mass index of female students at the Faculty of Medicine.[11] Meanwhile, the results of Zaini's research (2020) show that there is no significant relationship between levels of psychosocial stress with the nutritional status of female health students in Jember Regency because stress does not directly affect the nutritional status of female students.[12] Research related to food preferences in 2017 stated that food preferences also have a weak relationship with the nutritional status of female students. Food preferences can not directly affect nutritional status except through the level of adequacy of energy consumed.[13] However, another study also in 2017 stated that there is a relationship between food preferences and sweet or salty taste and nutritional status.[8] Consuming high-energy foods with high screen time will increase nutritional status.[14]

To our best knowledge, no research mentioned the relationship between psychosocial stress, food preferences, and screen time on nutritional status. So, this research aimed to analyze that relationship in WRA in Sukamulya Village, Tangerang Regency.

MATERIALS AND METHODS

The research design used cross-sectional. This research was conducted in three neighbourhoods in Sukamulya Village, Tangerang Regency in March-August 2022. The ethical approval for this research was obtained from the Universitas Esa Unggul Research Ethics Commission, no. 0922-02.031/DPKE-KEP/FINAL-EA/UEU/II/2022. Furthermore, this research also received approval from the participants concerned before the study began by filling out a statement of consent to participate in the study.

The sampling technique used was purposive sampling. Where samples were taken according to the consideration of the characteristics and criteria inclusion to obtain information and data in accordance with the research objectives. The total population was 86 WRA aged 19-34 years at the research place. Due to the condition of the pandemic, it is possible to limit the research area so that research is carried out in 3 neighborhoods of one village. Sampling is also based on those who are willing to be used as research samples to achieve a minimum sample size. The minimum sample in this study is 50 participants and an additional 10% to avoid dropping out. This amount is calculated based on the two-proportion test formula. There were 55 participants in this study.

Eligible participants fulfilled the following criteria: (1) aged between 19 and 34; (2) owns and use electronic devices (smartphone, laptop/computer, and television); (3) residents and domiciled in the research place; (4) present when the research was conducted and in good health; (5) willing to be research participants. Meanwhile, participants who met the following criteria were excluded: (1) university student; (2) pregnant; (3) works in a specific profession with demands for work in front of electronic screens and also has school-age children; (4) changes residence outside of the research place; (5) did not participate in the series of research data collection to completion; and (6) resigned as participants.

Independent variables in this study include psychosocial stress, food preferences, and screen time with the dependent variable including nutritional status. Data collection used interview techniques for food preference questionnaires and screen time, while self-filling forms were used for psychosocial stress data. The characteristic data questionnaire contains age, employment status, and monthly salary. Anthropometric measurements are weight and height using digital scales and digital microtoice. Body Mass Index (BMI) is calculated as weight in kilograms divided by height in meters squared. BMI is categorized into two categories, normal and abnormal (underweight and obese), according to the classification of the nutritional status of Indonesians by the Ministry of Health of the Republic of Indonesia, where the nutritional status is underweight if the BMI <18.5 kg/m^2, 18.5-25.0 kg/m^2 is normal, and obese if the BMI value is >25.0 kg/m^2.[15]

Psychosocial Stress Assessment Instrument (IPSP) used to measure level of stress psychosocial. This questionnaire consists of 35 events experienced during the last six months and one additional blank item (number 36) if there is another event that participants can mention themselves. The sum of the scores is interpreted into seven categories. These categories are: not experiencing stress (0); experiencing low or little

stress (1-8); being mild stress (9-16); moderate stress (25-33); high stress (25-33); very high stress (34-40); and catastrophic stress (>41). Based on these seven categories, they were further categorized into two major categories: stress and non-stress. The category of stress if the score and interpretation of the IPSP are included in the low to catastrophic stress category. IPSP has been tested by previous studies to be used for further research.[16]

The sweet, salty, and fatty food preferences questionnaire used the FFQ and were categorized into two categories: food preferences high in sugar, salt, and fat and low in sugar, salt, and fat. The list of sweet, salty, and fatty foods is obtained from data on food commonly consumed and found around the research place. If the FFQ score is more excellent/equal to the average score of the entire sample, it is categorized as a food preference high in sugar, salt, and fat.[17]

The screen time recall questionnaire simplifies the Adult Sedentary Behavior Questionnaire (ASBQ) to collect average screen time data for the previous four days on weekdays and weekends. Screen time duration is included in the High Screen Time (HST) category if the average screen time in four days is higher than the median of data, which is 210 minutes per day. The median data is used as the cut off point because there are no rules that state the amount of screen time limit for adults. Numerical data from the screen time variable were tested for normal data using the Kolmogorov-Smirnov, and showed that the data were not normally distributed ($P < 0.005$). So, the cut off point used in this study is the median data.

All data were analyzed using SPSS version 25.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were used for the univariate test to determine the frequency distribution of the characteristics and all variables in this study. In addition, the Chi-Square test was used to analyze the relationship between each independent and dependent variable with a significance level of 0.05.[18]

RESULTS

The characteristics of the participants are presented in Table 1.

Table 1. Characteristics of Participants and Research Variables (n = 55)

Characteristics	n (%)
Age	
Late teens (19-25 years)	19 (34.5)
Early adults (26-34 years)	36 (65.5)
Employment status	
Unemployed	35 (63.6)
Employed	20 (36.4)
Monthly salary	
No income	35 (63.6)
Low income (< IDR 4,230,792/month)	11 (20.0)
High income (≥ IDR 4,230,792/month)	9 (16.4)
Psychosocial stress	
Stress	34 (61.8)
No stress	21 (38.2)
Food preferences	
Food preferences high in sugar, salt, and fat	20 (36.4)
Food preferences low in sugar, salt, and fat	35 (63.6)
Screen time	
HST (> 210 minutes/day)	26 (47.3)
LST (≤ 210 minutes/day)	29 (52.7)
BMI	
Abnormal	22 (40.0)
Normal	33 (60.0)

HST: High Screen Time, LST: Low Screen Time, BMI: Body Mass Index. IDR 4,230,792/month: regional minimum wage for Tangerang Regency 2022.

The age of the majority of participants is in the range of 26-34 years, an early adult group (65.5%). A total of 35 participants (63.6%) were housewives. Most employed participants had low income or < IDR 4,230,792/month (20.0%). It can also be seen in Table 1 that most of the participants experienced psychosocial stress (61.8%). Participants with high food preferences for sugar, salt, and fat, as many as 20 people (36.4%), and screen time were found mainly in the HST category (52.7%). Most participants had a BMI in the normal category (60.0%).

The results of the Chi-Square analysis in Table 2 show that psychosocial stress (p = 0.428) and food preferences (p = 0.567) were not associated with nutritional status. However, there was a significant relationship between screen time and nutritional status (p = 0.011).

Table 2. Relationship Between Psychosocial Stress, Food Preference, and Screen Time with Nutritional Status

Variable	BMI				Total		p
	Abnormal		Normal				
	n	%	n	%	n	%	
Psychosocial stress							
Stress	15	44.1	19	55.9	34	100.0	0.428
No stress	7	33.3	14	66.7	21	100.0	
Food preferences							
Food preferences high in sugar, salt, and fat	7	35.0	13	65.0	20	100.0	0.567
Food preferences low in sugar, salt, and fat	15	42.9	20	57.1	35	100.0	
Screen time							
HST	15	48.3	11	27.6	26	100.0	0.011*
LST	7	24,1	22	26.9	29	100.0	

*significant p<0.05, HST: High Screen Time, LST: Low Screen Time, BMI: Body Mass Index

DISCUSSION

The majority of participants in this study were in the early adult age range, which is 26-35 years.[19] In addition, most of the participants in this study are also unemployed. However, some of them work as factory employees. The married participants mostly are housewives, so to meet their needs, especially food, only comes from their husbands. Work is one factor that affects the nutritional status of WRA by describing the level of activity and economic welfare through the amount of income. Their low financial status makes it difficult to fulfil their nutritional and food needs. Higher-income households can enable them to consume more diverse and nutritious foods, thus affecting their nutritional status.[20]

The majority of working participants have low incomes. Low income has a positive correlation with the quality of food spending. Significantly, low food consumption among low-income people has lower nutritional quality because they purchase less healthful foods, fewer fruits and vegetables, and more sugary beverages.[20] Low income and financial status also trigger stress for WRA.

The most common stress triggers found in the participants in this study were stress due to the economy and stress due to the environment and workload. Based on the results of the bivariate test, it was found that there was no relationship between psychosocial stress and nutritional status. Participants with psychosocial stress were found to have the most normal nutritional status. This finding is probably because the participant's age has entered the level of emotional and psychological maturity so that they can manage stress well without involving changes in eating patterns. Late teens to adults >18 already have more mature emotions than middle teens.[20] In addition, the coping stress they do also do not lead to changes in appetite to more or less, so the mechanism of stress on nutritional status through the presence of eating disorders in this study was not found to be associated.

Psychosocial stress is an individual's body response related to their interaction with social threat situations, including social exclusion and evaluation.[21] Psychosocial stressors come from various phenomena in their environment, both the living, work, or community environment, that can interfere mentally. This study showed that as many as 61.8% experienced psychosocial stress. The highest cause of stress is due to economic problems.[22] Research on students in China in 2017 also showed that 19.6% reported high levels of uncertainty stress. Also, in this study, 8.6% of students reported high levels of life stress associated with low family income.[22] Allegedly, due to the impact of the COVID-19 pandemic in recent years, some have lost their job and have no additional income, which has led to economic problems that cause psychosocial stress. Individuals with low socioeconomic status are two to three times more likely to stress.[23]

When stress occurs, the perceived threat will activate the hypothalamic-pituitary-adrenal (HPA) neuroendocrine axis, stimulating cortisol secretion.[24] Insulin and cortisol can act synergistically to regulate lipogenesis. Furthermore, increased cortisol stimulates gluconeogenesis which results in insulin resistance. Increased cortisol under psychosocial stress can increase brain activation to stress and reward motivation pathways, thereby increasing the desire for high-calorie foods such as those high in sugar, salt, and fat.[25]

Individual coping stress varies and is not always related to dietary changes. As in this study, based on interviews, most participants stated that they did not vent or express an unpleasant condition that they felt.

Usually, going out, praying, or playing with smartphones, and especially playing with children for those who are married, makes them calmer, and things that trigger psychosocial stress do not become a heavy burden to think about and disturb. These results are similar to research conducted on health students in the Jember Regency, that there is no relationship between psychosocial stress and nutritional status of students, with the majority aged 19-21 years. Psychosocial stress does not directly correlate with nutritional status but through behavioral patterns of nutritional fulfilment. From a stress perspective, not everyone exposed to psychosocial stressors will experience stress.[26]

Food preferences were also not found to be associated with nutritional status. Most participants have low sugar, salt, and fat food preferences. Financial limitations experience does not allow them to choose foods or snacks that are high in sugar, salt, fat and food sources of animal side dishes such as red meat. The high-energy foods they usually consume are often the only ones available at nearby stalls such as instant noodles and crackers so consumption of sugar, salt and fat is not too varied and high. The majority of them have a normal BMI. Similar to the research conducted on 350 nursing students at the Medan Health Polytechnic, there was no relationship between food preference and nutritional status and a weak relationship.[12] In contrast to the research conducted in Teresina on 1,036 school adolescents, there is a relationship between food preferences and nutritional status in adolescents.[8]

There is an assumption that consuming sweet, salty, and fatty foods is a strategy to improve mood.[9] However, in this study, most participants did not use these types of food to cope with stress. Based on the results of the study, 63.6% of participants had food preferences that were low in sugar, salt and fat. According to these data, it shows that most of the participants are neutral and not excessive towards the consumption of foods high in sugar, salt and fat. Chances are when they are stressed, there are other activities they do as stress coping.

In addition, the economic factor also allows them to choose food depending on the availability of the family's economy so that they rarely consume various foods or snacks high in sugar, salt, and fat or high-fat animal side dishes such as meat. The high-energy foods that are most often consumed on average are mostly only available at stalls, such as crackers and instant noodles, so the consumption pattern of sugar, salt, and fatty foods is not too high and varied. An earlier study based on the survey in Inner Mongolia noted that high socioeconomic groups consume relatively more high-fat foods such as red meat, high calories, and sugar than low socioeconomic groups.[13]

Furthermore, factors suspected to be the trigger for the increase in nutritional status of participants apart from food preferences high in sugar, salt, and fat are due to the effect of using contraceptive injections/pills considering that most of them are married. According to the participants' statements, their weight significantly increased after marriage and regular use of contraception. Married participants in this study were 78.2% and almost all of them used contraception, both pills and injections. Based on previous research in Surabaya, it was stated that the effect of using injectable contraceptives for three months or more on weight gain.[27] So, in this case, food preferences for sugar, salt, and fat are not the main factors of changes in the nutritional status of participants.

Most participants with high sugar, salt, and fat preferences were found in participants with normal nutritional status, and some were underweight. Previous research stated that individuals with underweight and normal nutritional status prefer sweet foods.[8] This is thought to affect these participants' preference scores for sugar, salt, and fat foods. The sweetness intensity does not predict the number of calories of sweet food or drink.(28) Also, sweet foods and drinks tend to be high in sugar or simple carbohydrates that are very easily absorbed by the body. Metabolism in underweight and normal nutritional status persons tends to be faster. So, high consumption of sugar, salt, and fat does not show significant changes to their nutritional status. Similar to a review conducted in 2021, which stated that sweet or salty food preferences did not differ according to individual BMI. The incidence of obesity also could not be proven by the high consumption of sweets as expected.[28]

The incidence of obesity is also caused by excessive caloric intake accompanied by a lack of physical activity and switching to screen time behavior. Screen time is the time an individual spends in front of a digital media screen. The majority of participants in this study were in the HST category. The bivariate test results showed a relationship between screen time and nutritional status. Participants with HST were mainly obese, and participants with the LST category had a more normal nutritional status. It was found that participants of young age (<25 years) and unmarried tend to use smartphones more, so physical activity becomes very rare. According to previous research on students at the University of Hong Kong that an increase in smartphone addiction was accompanied by a decrease in physical activity.[29]

Screen time reduces physical activity because it tends to be done by sitting and staring at the screen for a long time and indirectly affects the condition of weight gain.[30] Participants who are married and have families, along with watching television, eat their children's food that has not been eaten. Hence, if done continuously, it can increase the participant's energy intake when this situation is accompanied by decreased physical activity, which might be causes nutritional problems.

The results of a similar study conducted on adolescents in suburban Philadelphia, there is a relationship between screen time and BMI.[31] Gadget addiction had a negative impact on health, stress management, spiritual health, nutrition, and physical activity.[32] Physical inactivity due to screen time causes fat accumulation and causes obesity.

The strength of this research is to discuss the relationship between psychosocial stress, food preferences, and screen time on nutritional status in a more specific age group (WRA). This study also shows the results that high screen time along with other factors in the form of contraceptive use can improve nutritional status in WRA.

There are limitations in this study where it is difficult for the participants to remember the amount of screen time when using the screen time recall technique. Second, because this research was conducted house-to-house, so it can not control the environmental conditions when in participant's house. Sometimes for some participants, the interview process be in a hurry.

For further research, similar topics should also conduct with a different design, such as a case-control study, to find the causal relationship. Next, research can investigate the correlation between contraceptive pills and injections on nutritional status. Participants can fill in data daily for data collection, especially during screen time, to reduce bias.

CONCLUSIONS

The present study suggested a significant relationship between screen time and the nutritional status of WRA. There is no relationship between psychosocial stress, food preferences, and nutritional status. Provision of education through social activities related to nutrition and physical activity by Health Service officers, especially nutritionists to increase participants' awareness of the importance of exercising and maintaining optimal body weight as well as consumption of blood-boosting supplements for young women to fulfill iron before pregnancy. The goal is for them to further reduce screen time which should only be a maximum of 2-3 hours per day and engage in physical activities such as sports to achieve optimal nutritional status.

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Liprotide-encapsulated vitamin D_3 modulates circulated PTH levels and improved bone microstructure

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ABSTRACT

Background: vitamin D (25(OH)D) is a fat-soluble vitamin that is unstable in the gastrointestinal environment and has low bioavailability. A protein-lipid complex (liprotide) can be used as a shell to increase vitamin D stability and bioavailability. Liprotide can also serve as a delivery system for transporting vitamin D to its intended site. Little attention has been paid to utilizing liprotide as a delivery system for vitamin D and evaluating its functional activity.

Objective: to investigate the effect of liprotide-encapsulated vitamin D_3 on PTH levels and bone microstructure in vitamin D and calcium (VD-Ca) deficient rats.

Materials and Methods: an overall of 24 Wistar rats had been divided into four groups, a normal control group (K), a VD-Ca group without treatment (K-), a VD-Ca group with 180 IU/200 gBW/day free vitamin D_3 (FVD3), and a VD-Ca group with 180 IU/200 gBW/day liprotide-encapsulated vitamin D_3 (LVD3). Before and after 28 days of vitamin D intervention, blood samples were taken and analysed for serum PTH levels. The microstructure of the bone was analyzed using the Scanning Electron Microscope (SEM).

Results: the VD-Ca rats supplemented with vitamin D_3 (FVD3 and LVD3) had a significant decrease in serum PTH levels ($p<0.001$) and improved bone microstructure ($p<0.05$) compared to the (K-) group. The reduction of PTH in the LVD3 group was higher compared to the FVD3 group. The bone microstructure between the FVD3 and LVD3 groups is significantly different, as seen in the Ct.Wi parameter, with the LVD3 group having a higher Ct.Wi than the FVD3 group.

Conclusion: liprotide-encapsulated vitamin D_3 improves the serum PTH level and bone microstructure in a rat model of vitamin D and calcium deficiency.

***Keywords** : bone microstructure; encapsulation; liprotide; PTH; vitamin D_3*

BACKGROUND

Vitamin D is a crucial fat-soluble vitamin for maintaining calcium homeostasis and bone health.[1,2] Several diseases, such as diabetes, hypertension, autoimmune diseases, metabolic syndrome, some cancers, and infectious diseases are all linked to vitamin D.[1] Vitamin D could be obtained externally from the plant in the form of vitamin D_2 (*ergocalciferol*) and from the animal in the form of vitamin D_3 (*cholecalciferol*).[3] The latter is relatively easier to metabolize and therefore is more effective in maintaining vitamin D levels.[4] Despite the sources, it is important to keep vitamin D levels in the body adequate.

Many people are vitamin D deficient (25(OH)D serum levels <20 ng/mL).[5] As many as 60% of adults and 30% of children worldwide have inadequate vitamin D levels.[6] Among the reasons for Vitamin D deficiencies are excessive use of sunblock, covered clothing, minimum outside activities, season, latitude, age, skin pigmentation, and poor intake of foods containing vitamin D and calcium. Vitamin D deficiency raises the possibility of secondary hyperparathyroidism, progressive bone loss, and increased bone turnover.[7,8] Bone is one of the main organ targets for vitamin D. Active vitamin D (1,25(OH)2D) is involved in maintaining bone formation, calcium homeostasis, and neuromuscular function.[9] Calcium homeostasis is maintained by regulating calcium mobilization from bone, renal calcium reabsorption, and intestinal calcium absorption. When serum calcium is low, it triggers the production and secretion of PTH, which induces 1,25(OH)2D synthesis in the kidneys and bone calcium resorption.[10]

Food fortification and vitamin D supplementation have been used to tackle vitamin D deficiency. Unfortunately, oral supplementation is challenged by vitamin D instability in the gastrointestinal tract, as seen by its low solubility and bioavailability and slow absorption into the tissue.[4,11] Several biosystems have been developed to address limitations in vitamin D supplementation, including the lipid-based biosystem.[12] A lipid-based system, such as liposomes, has amazing biocompatibility; however, this system suffers from instability,

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partially controlled particle size, and low encapsulation efficiency.[4] Recently bioconjugation of protein to the lipid-based system has been developed to improve stability, bioavailability and even delivery. Liprotide is a complex consisting of fatty acids surrounded by partially denatured protein.[13,14] This system is promising as an oral delivery system due to its stability and ability to deliver fat-soluble active ingredients to the target.[15] Also, the fatty acid in the system ensures optimal absorption of fat-soluble nutrients in the intestine.[16]

Several studies have documented the optimization strategy of liprotide as a delivery system of various substrates. However, little attention has been paid to utilizing liprotide as a delivery system for vitamin D and evaluating its functional activity, and it has not been widely used in adults who are susceptible to vitamin D deficiency. Therefore, this study's objective was to investigate the effects of liprotide-encapsulated vitamin D_3 on bone microstructure and PTH levels of the model of the vitamin D-deficient rats.

MATERIALS AND METHODS

Research Design

This study used a true experimental pre-post control group design. The experiments in this study were compiled with the bioethical research established by The Medical/Health Research Bioethics Commission, Sultan Agung Islamic University (Approval Number: 29/I/2022/Bioethics Commission).

Sample Vitamin D preparation

The materials used were obtained from Sigma-Aldrich: vitamin D_3/*cholecalciferol* (5.00936.0010), β-lactoglobulin (BLG, L0130), and oleic acid (OA, Y0001479). Other materials used to support this research include potassium hydroxide (KOH, 1310-58-3), PBS OmniPur® liquid concentrate (6506-1LCN), absolute ethanol (1.00983.2500, 99%), and MilliQ water.

Preparation of liprotide begins with 38 mg/mL oleic acid dissolved in ethanol. 6 mg/mL β-lactoglobulin (β-Lg) was dissolved in 1.5 mg/mL oleic acid with 10 mM KOH solution (pH 10.5). The solution was incubated at 45°C for 30 minutes and then terminated. After that, the pH value of β-lactoglobulin and oleic acid (β-Lg-AO) solution was adjusted to pH 7.4 using PBS solution. The next step is vitamin D_3 encapsulated with liprotide, in which 30 mg/mL of vitamin D_3 (*cholecalciferol*) is dissolved in ethanol and diluted with MilliQ water. Afterwards, vitamin D_3 was mixed with the liprotide. The sample was homogenized using a vortex and kept at room temperature.[13] Encapsulation efficiency and vitamin D concentration were seen using HPLC (Shimadzu corp LC20AD® L20105130725 series, Japan) and SEM (JEOL® JSM-6510LA series, Japan). This sample preparation was carried out in the Diponegoro University Integrated Laboratory, Semarang.

Animal Study

This study used a true experimental with pre-post control group design. A total of 24 male Wistar rats, aged eight weeks, weighed 150-200 gr, were housed in individual cages under a typical temperature of 22±2°C, light-controlled (12h/12h alternating light and dark), and humidity 60-70%. They were fed a standard diet AIN-93M[17] and had ad libitum access to water. Experimental animal treatments were carried out at the Experimental Animal Laboratory, Center for Food and Nutrition Studies, Gadjah Mada University, Yogyakarta.

After a one-week acclimatization period, the rats were randomly divided into four groups (six animals per group): a normal control group (K), a VD-Ca group without treatment (K-), a VD-Ca group with 180 IU/200 gBW/day free vitamin D_3 (FVD3), and a VD-Ca group with 180 IU/200 gr BW/day liprotide-encapsulated vitamin D_3 (LVD3). The dosage is based on the Endocrine Society Clinical Practice Guidelines, and it has been proven in previous studies that a daily dose of 10,000 IU of vitamin D can effectively treat vitamin D deficiency in humans without posing any risk of harm.[5,18] This dose was converted into a dose for rats by a conversion factor (0.018) to obtain a dose of 180 IU/200 g BW/day. The normal control group rats were fed a standard diet. The rats in groups K-, FVD3, and LVD3 were fed a VD-Ca-deficient diet. The VD-Ca deficient diets were prepared by modification of AIN-93M; vitamin D_3 and Ca were excluded from the formulation. After two weeks on a diet, VD-Ca deficiency rats were confirmed by measuring serum 25(OH)D levels and serum calcium levels (respectively, control = 82.67±4.15 ng/mL; deficient = 16.02±0.61 ng/mL and control = 12.06±0.18 mg/dL; deficient = 5.80±0.39 mg/dL). Blood samples were taken via retro-orbital plexus. FVD3 was diluted in virgin coconut oil, and LVD3 was diluted in water. Vitamin D supplementation was carried out daily for 28 days. One mL/kg body weight of vitamin D (final dose of 180 IU/200 gr BW) was used in FVD3 and LVD3 groups. Weekly body weight and daily food intake measurements were made. The rats in all groups had free access to water. Rats were fasted for 12 hours, given ketamine anesthesia (100 mg/kg

body weight), and then cervical dislocation was used to finish the experiment. This animal study can be seen in Figure 1.

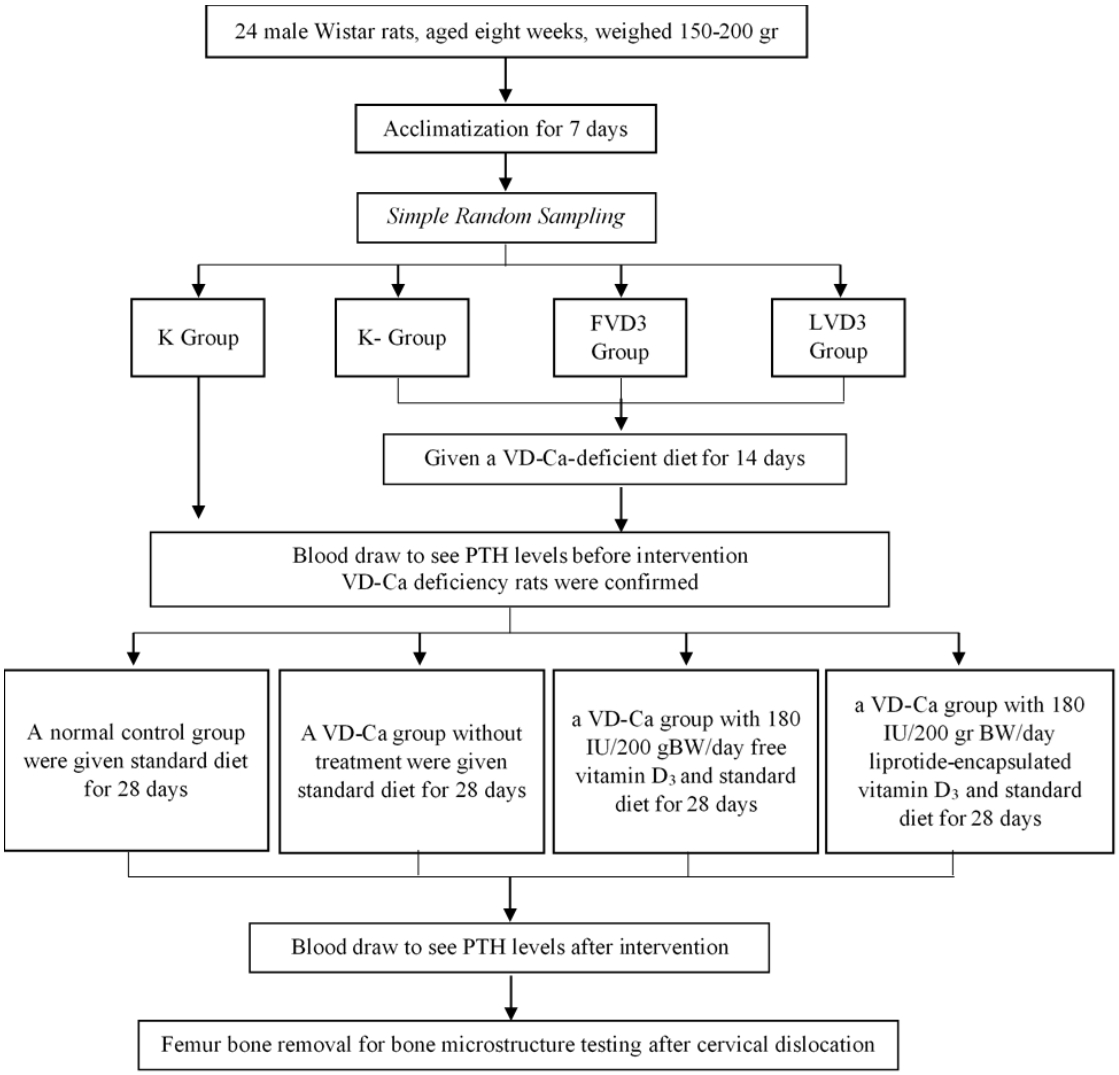

Figure 1. Research Workflow

Analysis of Serum PTH

Blood samples were taken before and after vitamin D_3 treatment. Enzyme-Linked Immunosorbent Assay (ELISA) was used to measure the levels of serum PTH.[19] The assay was performed according to the protocols provided by the ELISA kit (Fine Test).

Analysis of Bone Microstructure Parameters

Left femurs were each sliced longitudinally using a diamond disc saw from the intercondylar line to the diaphysis after being dried to a constant weight at 60°C.[20] Then, the bone samples were coated with a conductive material (gold) using a sputtering machine. Bone measurements were performed on the metaphysis area containing trabecular and cortical bones. The microstructure of the rat's femur (trabecular thickness [Tb.Th], trabecular separation [Tb.Sp], and cortical width [Ct.Wi]) was observed using Scanning Electron Microscope (SEM) with two magnifications (15x and 85x). Tb.Th parameter measurement was carried out by measuring the trabecular diameter in mm; Tb.Sp measures the distance between the segmented trabecular margins in mm; and Ct.Wi measures cortical thickness in mm.[21]

Statistical Analysis

All quantitative data are expressed as mean ± SD (standard deviation). Data analysis was carried out using statistical data processing program (IBM SPSS® Statistics version 25). The Shapiro-Wilk test was used to examine the data's normality. One-Way ANOVA was used to analyze the variations in PTH levels and bone

microstructure parameters between groups, and then a Post-Hoc statistical test was performed. Data were considered statistically significant if the $p<0.05$.

RESULTS

PTH Levels

Table 1. shows the PTH levels of VD-Ca deficient rats before and after two weeks of vitamin D supplementation treatment. VD-Ca deficient rats without treatment (K-) and healthy rats on a standard diet (K) were used as the control experiment. Two weeks after the initiation of the induction of VD-Ca deficiency, the average PTH levels in K-, FVD3, and LVD3 groups (pre-treatment) were above the normal limit (10-65 pg/mL). Post-treatment, PTH levels increased significantly in the normal control group and the VD-Ca deficient without treatment group. The One-Way ANOVA test showed a significant difference in PTH levels after intervention in all groups ($p<0.001$). VD-Ca deficient rats that received FVD3 or LVD3 showed a significant reduction in PTH levels, while the K- group remained high. Notably, the reduction in PTH levels in the LVD3 group was higher than in the FVD3 group. According to similar test results that have been confirmed in earlier studies, VD-Ca deficient rats had vitamin D levels of 15.76 ± 0.36 ng/ml; however, after receiving LVD3, their levels rose to 69.45 ± 3.40 ng/ml.

Table 1. PTH Levels (pg/mL)

Group	Average PTH Levels ± SD			p^r
	Pre-treatment	Post-treatment	Delta pre-post	
K (Normal control)	34.37 ± 3.06	37.17 ± 3.27	2.80 ± 0.63	0.000*
K- (VD-Ca without treatment)	187.38 ± 4.18	189.87 ± 3.27	2.50 ± 0.94	0.001*
FVD3 (VD-Ca with free vitamin D_3)	190.28 ± 3.11	60.83 ± 3.00	-129.45 ± 5.04[ab]	0.000*
LVD3 (VD-Ca with liprotide-encapsulated vitamin D_3)	186.21 ± 1.78	42.78 ± 2.66	-143.43 ± 2.80[abc]	0.000*
p^q			0.000*	

[q]One-Way ANOVA (n=6), followed by Bonferroni Post-Hoc test ([a]Indicates $p<0.05$ versus normal control group; [b]Indicates $p<0.05$ versus VD-Ca group without treatment; [c]Indicates $p<0.05$ versus VD-Ca group with free vitamin D_3); [r]Paired t-test;*significant ($p<0.05$).

Bone Microstructure Parameters

The statistical analysis microstructure parameter of rat's femurs are shown in Figure 2. The lowest Tb.Th and Ct.Wi values were found in the K- group. The Tb.Sp in the K- group is bigger than the K, FVD3, and LVD3 groups. While the Tb.Th and Tb.Sp parameters did not significantly differ between the FVD3 and LVD3 groups, the Ct.Wi parameter did, with the LVD3 group having a higher Ct.Wi than the FVD3 group. The One-Way ANOVA test showed a significant difference in bone microstructure parameters after intervention in all groups ($p<0.05$).

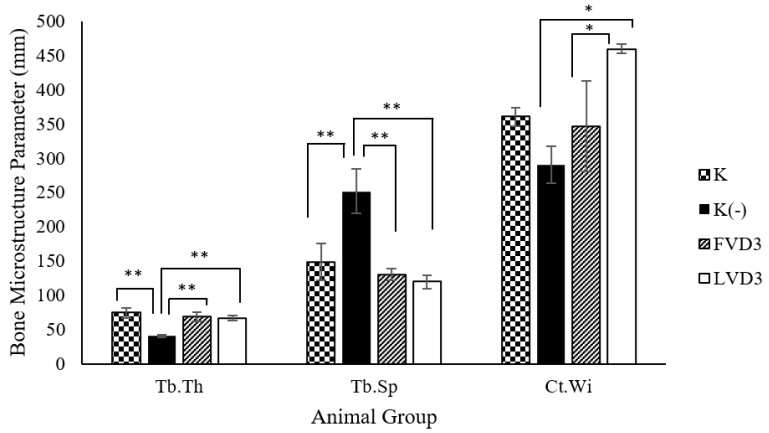

Figure 2. Bone Microstructure Parameters

The bone microstructure parameters of all groups, including Trabecular thickness (Tb.Th), Trabecular separation (Tb.Sp), and Cortical width (Ct.Wi)). Data shown are means; error bars show SD. Data were analyzed by one-way ANOVA ($p<0.05$, n=6), followed by Tamhane's Post-Hoc test. *Indicates $p<0.05$; **Indicates $p<0.001$). K, normal control group; K-, VD-Ca group without treatment; FVD3, VD-Ca group with 180 IU/Kg/day free vitamin D_3; LVD3, VD-Ca group with 180 IU/Kg/day liprotide-encapsulated vitamin D_3.

For comparison purposes, Figure 3. represents the micrograph SEM analysis of the inner part of the left femur. The femoral metaphysis was used to analyze the microstructure of bone. The area that was used to measure Ct.Wi is denoted by white arrows. The squares correspond to Figures 3E–H and show the ranges used to calculate Tb.Th and Tb.Sp. Double-edged arrows and double-edged diamond arrows are used to denote Tb.Th and Tb.Sp. According to these micrographs, the femoral cortical bone of the K- group was thinner than the rest of the groups (Figure 3A-D, see arrows). Figure 3E-H shows femoral metaphysis in detail. The K- group trabecular bone had the highest porosity, indicated by the thinness of Tb.Th and the dimensions of Tb.Sp, compared to the rest of the groups. Although it is not very noticeable, there is a porosity difference between the FVD3 and LVD3 groups. The LVD3 group has lower porosity than the FVD3 group.

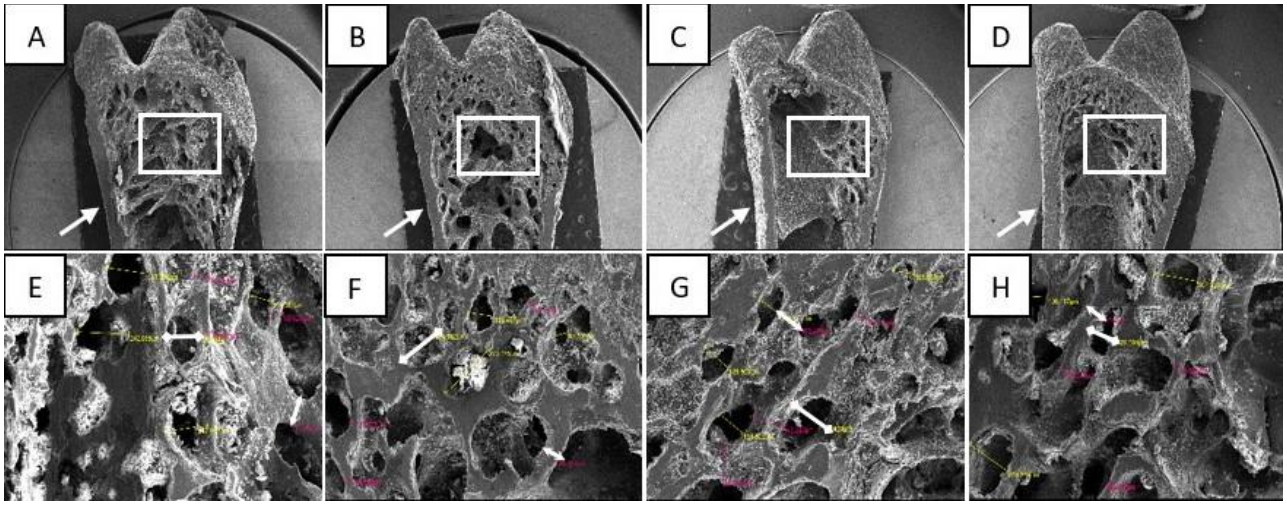

Figure 3. SEM Images of The Inside Left Femur

Left to right: K, normal control group; K-, VD-Ca group without treatment; FVD3, VD-Ca group with 180 IU/Kg/day free vitamin D_3; LVD3, VD-Ca group with 180 IU/Kg/day liprotide-encapsulated vitamin D_3 (**A-D**) shows the interior of the rat's femur that was cut longitudinally from the intercondylar line to the diaphysis (15x), (**E-H**) shows the femoral metaphysis (trabecular area) (85x).

DISCUSSION

This study's main goal was to determine whether LVD3 might lower serum PTH levels and enhance bone microstructure in a rat model of vitamin D and calcium deficiency. We demonstrated that administration of LVD3 for four weeks recovered PTH levels and improved bone microstructure.

Vitamin D and PTH are inversely related.[22] The K- group has high PTH levels, which indicates that vitamin D deficiency increases PTH levels. Similar to a previous study, vitamin D deficiency is frequently associated with high-normal or elevated PTH levels.[23] This was also shown in our previous study, which found that rat with vitamin D deficiency had low vitamin D_3 levels of 15.58 ng/mL and high PTH levels of 187,38 pg/mL. PTH levels increased in the K and K-groups following treatment. It could be because each group has gained weight. Changes in PTH levels can be influenced by environmental factors, such as lifestyle (smoking, alcohol consumption, BMI, diet), pollutants, and genetic factors.[24] Vitamin D_3 supplementation (in the form of FVD3 or LVD3) in VD-Ca deficient rats can significantly reduce PTH levels. Our findings are consistent with a prior investigation that found vitamin D treatment might considerably lower PTH levels and raise serum calcium.[25] The average decrease in PTH levels in the LVD3 group was higher than in the FVD3 group. This trend is most likely due to improved vitamin D_3 stability as LVD3. The previous study showed that encapsulation shields bioactive substances from pH and enzyme breakdown and allows controlled release.[26] Therefore, it is assumed that liprotide serve as a method for delivering vitamin D_3 to its intended recipient in a controlled manner. It also can protect vitamin D_3 from damage, increase absorption efficiency, and increase bioavailability.[27,28] Improvement in stability and bioavailability may strengthen the effect of vitamin D supplementation on PTH levels. As explained in a previous study, the transport and delivery of nutraceuticals in encapsulation is a complex process that involves biological processes, from digestion to their implementation in targeted cells or tissues.[29]

Vitamin D deficiency can affect bone structure. In this study, we observed changes in the microstructure of the femur bone. The femur is the longest and sturdiest bone in the body and has the highest

calcium content.[20,30] The four components of bone structure are the cortical bone, trabecular bone, periosteum, and endosteum.[31] The cortical bone forms a dense outer shell that surrounds the bone, found mainly in the diaphysis and less in the metaphyses and epiphyses.[32] Trabecular bone is found inside the bone, forming a porous network at the epiphyses and being found at the metaphyses separated by growth plates.[33,34] Trabecular bone is a metabolically active tissue associated with calcium homeostasis, unlike cortical bone, which is thick, dense, and metabolically inactive.[20] Bone remodelling can increase porosity and decrease bone mass. Therefore, alterations in the fibers of the cortical and trabecular tissues are correlated with the strength and mechanical characteristics of bone. The K- group had lower Tb.Th and Ct.Wi than the FVD3 and LVD3 group. In contrast, the K- group had a higher Tb.Sp than the FVD3 and LVD3 group. Vitamin D deficiency causes a lower Tb.Th, lower Ct.Wi, and higher Tb.Sp. Similar to the previous study, low vitamin D levels indicate increased bone turnover and weakened bone structure in trabecular bone, where the amount of trabecular bone is low and Tb.Sp is high.[35] From this, it is also known that vitamin D_3 is effective in improving bone structure. The LVD3 group had a higher Ct.Wi than the FVD3 group. The LVD3 group also had the lowest average Tb.Sp value among all groups. Liprotide as a vitamin D_3 delivery system can protect, increase the stability and solubility of vitamin D_3 in water, increasing bioavailability, and deliver vitamins to their targets in a controlled manner.[4,36] LVD3 has the potential to protects and delivers vitamin D_3, and the effect is visible in improving bone structure.

In this study, FVD3 and LVD3 were administered orally. Based on the results of a previous study, oral administration of vitamin D_3 can rapidly improve vitamin D status in patients with vitamin D deficiency conditions.[37] After ingestion, FVD3 and LVD3 pass through the stomach at a pH of 4.5 to 6.0. According to previous research, the β-Lg complex and vitamin D_3 are resistant to digestion by the stomach enzyme pepsin.[38] The hydrophobic nature of the denatured amino acid in β-Lg may inhibit enzymatic hydrolysis of the pepsin β-Lg complex, and vitamin D_3 is susceptible to the entire GI tract, including the presence of proteolytic enzymes.[28] It is reasonable to assume that the liprotide structure that encapsulates vitamin D_3 remains intact and stable during acid digestion in the stomach before degrading in the GI tract implying that liprotide could be an effective vehicle for increasing vitamin D_3 bioavailability. Meanwhile, FVD3 is thought to be almost completely degraded at the end of digestion. An in vitro study demonstrated that 90% of the unprotected vitamin D_3 was lost during six hours in simulated intestinal fluid (SIF).[11]

Vitamin D_3 is absorbed into the small intestine by enterocytes with the help of bile salts, monoglycerides, and free fatty acids.[39,40] Most of the ingested vitamin D is taken up by the chylomicrons into the lymphatic system and stored in adipose tissue and muscle before entering the bloodstream.[41] The remaining portion is moved to the liver by the vitamin D binding protein (VDBP), where it is hydroxylated by the enzyme 25-hydroxylase (CYP27A1) to biologically inactive 25(OH.)D or calcidiol.[42,43] With the aid of the enzyme 1-hydroxylase, 25(OH.)D is rehydroxylated in the kidney to the biologically active 1,25(OH)2D or calcitriol.[42,43] 1,25(OH)2D enters the bloodstream with VDBP and is transported to target tissues like the kidney, bone, and gut.[44] Calcium and phosphorus mobilization, absorption, and reabsorption are all regulated by active vitamin D. In carrying out this biological action, 1,25(OH)2D bind to the VDR found in osteoblasts to release biochemical signals that lead to the formation of mature osteoclasts.[9,45]

PTH is the main stimulator for producing active vitamin D by increasing the activity of CYP27B1.[41,46] 1,25(OH)2D and PTH influence bone homeostasis.[47] When vitamin D and calcium levels are low, the parathyroid glands will release PTH to induce CYP27B1 synthesis, increasing from 1,25(OH)2D.[43] In addition, PTH can also increase the absorption of calcium in the kidneys and intestines, increasing the body's calcium levels. Calcium levels are permanently monitored by calcium-sensing receptors (CaSR) in the parathyroid glands.[40,48] PTH can also increase the release of fibroblast growth factor-23 (FGF-23), produced by mature osteoblasts and bone cells.[47] Prolonged high PTH levels can cause a decrease in trabecular bone and cortical bone microstructure.[49–51] Thus, vitamin D and calcium deficiencies are frequently linked to increased bone turnover, fracture risk, and decreased bone mass.[52]

The age of the Wistar rats used in this study might be comparable to that of an adult human.[53] When comparing the ages of humans and animals, there is no easy way to determine the age difference because the results depend on the factors that are examined.[54] It can be cautiously applied to humans in future research.

CONCLUSIONS

In summary, this study shows that liprotide derived from β-lactoglobulin-oleic acid can protect and deliver vitamin D_3 to the target, as evidenced by a decrease in serum PTH levels and an improvement in bone

microstructure. More research is required to determine how it works in humans and how liprotide-encapsulated vitamin D_3 is absorbed in vitro.

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