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ORIGINAL PAPER

Flavonoid intake and its correlation to malondialdehyde serum among reproductive-aged women with obesity

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Abstract

Background: Obesity modulates oxidative stress and can be detected by examining malondialdehyde (MDA) serum levels. Flavonoids are natural bioactive ingredients that can be found in various types of vegetables and fruits that function as antioxidants to suppress that oxidative stress.

Objective: This study aimed to determine the correlation between flavonoid intake and MDA serum levels in obese women of reproductive age.

Methods: This cross-sectional study was conducted in Kendari, Indonesia from April to October 2021. The purposive sampling method was used to obtain 88 subjects who met the research criteria. Data were collected through interviews covering flavonoids intake using validated SQ-FFQ. Anthropometric measurements were performed to assess nutritional status, and laboratory tests were applied to determine MDA serum levels.

Results: The average intake of flavonoids in the subjects was 142,26 ± 56,53 mg per day. Meanwhile, the average MDA serum level in the subjects was 2.16 mol/L, ranging from 1.09 nmol/ml to 6.71 nmol/ml. There was no significant correlation between total flavonoid intake and MDA serum levels in obese women of reproductive age (r=0,188, p=0,079). However, there was a weak correlation between the intake of flavonoid subclasses, namely flavan-3-ols/flavanols and MDA serum levels (r=0.325, p=0.002).

Conclusion: We conclude that there was no correlation between total flavonoid intake and MDA serum levels. However, there was a correlation between flavan-3-ols/flavanols and MDA serum levels in the subjects.

Keywords: flavonoid intake, malondialdehyde, oxidative stress, obesity, women of reproductive age

Introduction

Obesity has become one of the triple burdens of malnutrition, with the number of its cases increasing three times since 1975 to 2016. In 2017 alone, it is associated with 4.7 million deaths worldwide.[1] The prevalence of obesity in women is 15% or considerably higher than men with around 11% of 650 million people in the world.[2] The increase in cases also occurred in Indonesia from 14.8% in 2013 to 21.8% in 2018.[3] In Kendari, the prevalence of obesity in the population aged over than 18 years is 22.3% in 2017 (25.71% for women, 13.09% for men), or relatively higher compared to the national average. While it is predicted that the prevalence will continue to rise since there is still no obesity prevention program in the city.[3,4]

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Obesity can cause a permanent oxidative stress due to reactive oxygen species (ROS). It also decreases endogenous antioxidants, triggering various non-communicable diseases such as coronary heart disease, diabetes mellitus, hypertension, malignancy, as well as female reproductive system disorders and the intergenerational transmission of obesity from mother to foetus.[5–7] Modulation of oxidative stress affects all reproductive phases of a woman's life. For instance, it can cause, among others, infertility, premature delivery, intrauterine growth restriction (IUGR) and fetal death.[8,9]

Oxidative stress in obese women can be detected by examining malondialdehyde (MDA) levels in plasma, serum, or urine. It can be measured accurately using various methods and is relatively inexpensive[10]. As it is stable in isolated body fluid samples, it is also not affected by diurnal variations and dietary fat content, which is a specific product of fat peroxidation.[10]

Consumption of fruits and vegetables has been shown to reduce oxidative damage but, nationally, consumption of vegetables and fruits in Indonesia is still below the recommended level.[3] Apriyanti et al.[11] found that risky food consumption behaviours, including the habit of consuming sweet, salty, fatty, preserved, caffeinated, and flavoured foods/drinks were mostly exhibited by obese women of productive age in Kendari.

Dietary intervention with natural bioactive foods such as polyphenols has become an alternative approach in overcoming obesity and metabolic diseases. It is a novel way since it can modulate physiological and molecular pathways, that performing in energy metabolism, stimulating the beta oxidation, inhibiting adipocyte differentiation and counteracting oxidative stress.[12] Flavonoid is the most polyphenol subclass consumed by humans and it has antioxidant, anti-inflammatory and anti-carcinogenic effects.[13] However, the epidemiological evidence is limited and usually inconsistent. Most of the studies on flavonoid intake and MDA were also intervention trials.[12] For the examples, a study conducted by Hirano found the effects of green tea consumption can lower MDA levels in human.[14] Meanwhile, Gonzalez et al.[15] in 2013 found an inverse relationship between flavonoid intake and MDA levels. Ultimately, Alipour et al.[16] showed that flavonoid intake was not significantly associated with MDA levels.

Such inconsistency in research on flavonoid intake and MDA serum levels as parameters of oxidative stress shows a gap to fill in this area. Moreover, similar studies, especially in women of reproductive age with obesity, have never been conducted in Indonesia. To our knowledge, this is the first study about flavonoid intake and oxidative stress in obese women in Indonesia. In addition, it can be a reference for a further study on food consumption or antioxidants sources to overcome oxidative damage and reduce the risk of various diseases. Thus, this study was conducted to determine flavonoid intake and its correlation with MDA serum levels in obese women of reproductive age in Kendari, Indonesia.

Methods

Subjects and study design

This cross-sectional study was carried out during the Covid-19 pandemic in Kendari, Southeast Sulawesi, Indonesia. Purposive sampling was used to obtain samples. The inclusion criteria for this study were women aged 19-45 years old with body mass index (BMI) ≥25 kg/m^2 who voluntarily agreed to take part in the study by signing informed consent. Those who were pregnant, smoker (active or passive), on diet and/or exercised for weight loss program, had chronic diseases (i.e. heart diseases, diabetes mellitus and hypertension) used multivitamin and/or herbal and/or on medication, consumed alcohol, and reached menopause, were exempted from this study. Sample size was determined based on correlation analysis (α=0.05; β=0.20; r=0.3), and the sum of samples was 85 subjects. However, out of 105 subjects who signed the informed consent, 17 subjects did not meet the criteria, therefore only 88 samples were further analysed.

Data collection

Data collection was conducted from April to October 2021 after it was submitted and approved by the Ethics Committee of Faculty of Medicine, University of Indonesia – Cipto Mangunkusumo Hospital (No. 593/UN2.F1/ETIK/PPM.00.02/2021, protocol number 21-06-0601). There were 3 enumerators with an education background in nutrition assisted in this study. Data obtained through the interview process, physical examination and laboratory examination.

Characteristic data

Subject's characteristic data including age, education, occupation and income were obtained through interviews. Education was categorised into high (graduated from diploma and above), moderate (graduated from junior or high school) and low (graduated from elementary school and below). Occupation was categorised into employee and not employee. Income was categorised into two groups, more than, and less than or equal to the value of minimum wage (UMK) in Kendari (Rp 2,768,592).[17] The participants of this study are those who are considered obese by the Nutritional status based on the BMI in kg/m^2 according to WHO Asia-Pacific criteria. Those who have BMI 25 - 29.9 kg/m^2 were assigned to Group of Obese 1. Meanwhile, the rest of participants (BMI $\geq$ 30 kg/m^2) were assigned to Group of Obese 2.

Flavonoid intake assessment

The assessment of flavonoid intake was done through an interview by 3 enumerators using a validated semi-quantitative food frequency questionnaire (SQ-FFQ), food model and food photo book. Subjects were asked to recall the food consumed in the last 6 months based on the list in SQ-FFQ. First, the quantity of food consumed was assessed by household size (URT) which is compared with the food photo book guide for individual food consumption surveys (SKMI 2014)[18] of the Ministry of Health of the Republic of Indonesia. Second, the data was processed using Nutrisurvey 2007. Based on the USDA Database for the Flavonoid Content of Selected Foods, we find that total flavonoid intake comprised six flavonoid subclasses, namely flavonols, flavones, flavanones, flavan-3-ol, anthocyanidins, and isoflavonoids.[12]

Using the USDA database as a reference, we then calculated participants' flavonoid levels. The total flavonoid intake was measured by the number of flavonoid subclass intakes in mg/day. The intake of the flavonoid subclass (mg/day) was obtained from the weight of the food (gr) divided by 100 multiplied the flavonoid content (mg) minus retention (0.85 for antosianidin and 0.5 for other subclasses).[12] If the raw weight was the same as the cooked weight, the flavonoid intake was calculated without retention.

Data analysis

Data were analyzed using SPSS version 20.0. Normality of the data distribution was determined by Kolmogorov Smirnof test. Data distribution was considered normal when p value $\geq$ 0,05. Data are presented in the form of mean ± standard deviation (SD) if normally distributed ($p \geq 0.05$), and in the form of median (minimum-maximum) if not normally distributed ($p < 0.05$). Categorical data were presented in the form of frequency distribution (n,%). Continuous data were presented in the form of median (minimum-maximum). The correlation between two variables was analyzed using the Pearson correlation test if the data distribution was normal, or the Spearman Rank correlation test if the data distribution was not normal. The possible range of values for the correlation coefficient (r) is -1 to 1. A correlation of -1 indicates a perfect negative correlation, and a correlation of 1 indicates a perfect positive correlation. Value 0,8 to 1 indicate very strong correlation, 0,6 to 0,8 indicate strong correlation, 0,4 to 0,6 indicate moderate correlation, 0,2 to 0,4 indicate weak correlation, and < 0,2 indicate very weak correlation. If the correlation coefficient is greater than zero, it is a positive relationship. Conversely, if the value is less than zero, it is a negative relationship. The correlations were considered significant if the p value < 0,05. Nutrisurvey 2007 was used to perform analysis of zinc intake.

Anthropometric measurement

Anthropometric measurements were performed to obtain data of heights and weights. Heights were measured using Microtoise Staturmeter 200 cm (with 0.1 cm accuracy). Weight measurement was done by using GEA SMIC ZT120. Both anthropometric measurements were performed twice, and then were used for body mass index (BMI) calculations. Nutritional status assessments were based on the BMI in kg/m^2 according to WHO Asia-Pacific criteria.

Laboratory examination

The blood tests were conducted as a screening process to determine the levels of fasting blood glucose, high density lipoprotein-cholesterol (HDL-C) and triglyceride. Those tests were also to determine the level of malondialdehyde (MDA) serum as an oxidative stress marker. The blood tests were performed in collaboration with Maxima Kendari Laboratory, while the examination of oxidative stress level was carried out at the Biochemistry Laboratory, Faculty of Medicine, University of Indonesia in Jakarta.

Five ml of blood samples were taken from the cubital vein, which was then centrifuged at 3000 rpm for 10 minutes to obtain the serum. The samples were examined using the Automated Clinical Analyser TMS 1024i to assess fasting blood glucose, HDL-C and triglyceride levels. Blood samples that passed the screening were afterward sent to the Biochemistry Laboratory of the Faculty of Medicine, University of Indonesia in Jakarta, for MDA serum level examination. The MDA level analysis was done using the TBA method. A total of 2 ml of serum was mixed with 1 ml of 20% TCA, followed by adding 2 ml of 0.67% TBA. The mixture was then allowed to stand for 10 minutes in a boiling water bath. It is then cooled using an ice-cold water bath. It was subsequently centrifuged at 6000 rpm for 30 seconds and its absorbance was read at 530 nm using a spectrophotometer.

Statistical Analysis

Data were analysed by using IBM SPSS version 26.0. Normality test was done by using Kolmogorov Smirnov. The data distribution was considered normal when the p value > 0.05. Continuous data were presented as mean ± SD or median (minimum-maximum). Categorical data were presented as a frequency distribution (n, %). Descriptive statistics of total flavonoid intake were expressed as mean ± SD and flavonoid subclasses as median (min-max). Additional analysis Independent T-Test and Mann-Whitney Test were done to determine the flavonoid intake difference and the MDA serum levels between the group of obese 1 and obese 2. MDA serum levels were expressed as mean ± SD or median (min-max). Spearman's correlation was used to determine whether there was a relationship between flavonoid intake and MDA serum levels. The level of significance was set at $p<0.05$.

Result

Among 105 subjects who were willing to take part in the study, there were 17 subjects who did not meet the criteria, so that the remaining 88 subjects were further analysed **(Figure 1).** The median age of 88 subjects was 28 (19-45) years old. The majority of subjects were employees with low income (below the UMK) and most of them were assigned to Group of Obese 1. Therefore, the BMI median value of all the subjects was 27.58 (25.02-39.20) kg/m^2. Subject's characteristics data is shown as **Table 1.**

The average total flavonoid intake in the subjects was 142,26 ± 56,53 mg per day. Total flavonoid intake in the Group of Obese 2 was higher than the Group of Obese 1. After performing the Independent T-Test analysis ($p > 0.05$), we found that there was no significant difference in total flavonoid between both groups. The result can be seen in **Table 2**. In addition, we found that the median value of MDA serum levels was 2.16 (1.09-6.71) nmol/ml. Furthermore, after taking the Mann-Whitney Test ($p > 0.05$) based on the subjects' obesity status, there was no significant difference in MDA serum levels in both groups, as seen in **Table 3.**

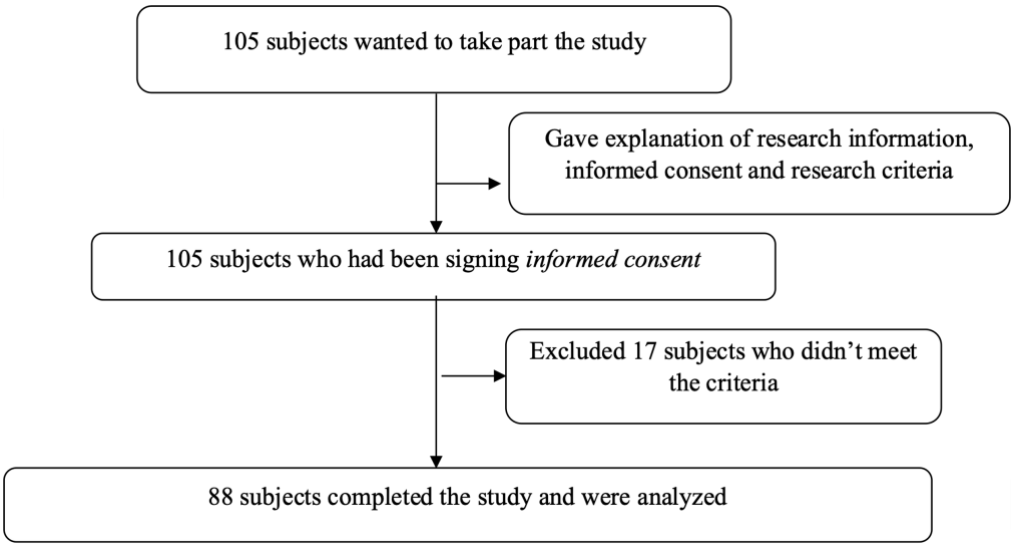

Figure 1. Subjects selection procedure

Table 1. Subjects' characteristics

Variabel	**Hasil**
Age (years)	28 (19-45)**
Education level, n (%)	
Low	-
Moderate	44 (50)
High	44 (50)
Occupation, n (%)	
Employee	49 (55.7)
Unemployee	39 (44.3)
Income, n (%)	
≤ UMK	75 (85.2)
> UMK	13 (14.8)
Antropometry	
Weight, (kg)	64.4 (55.00-99.25)**
Height, (cm)	153 (145-170)**
Body Mass Index, (kg/m^2)	27.58 (25.02-39.20)**
Nutritional status, n (%)	
Obese 1	70 (79.5)
Obese 2	18 (20.5)
Fasting blood glucose (mg/dl)	81,5 (44-144)**
High Density Lipoprotein (mg/dl)	50.16 ±10.08*
Triglyseride (mg/dl)	93 (34-473)**

*Mean ± SD
**Median (minimum-maximum)

Table 2. Subject's flavonoid intake

Variables	All n=88 (Mean±SD/ Median(min-maks))	Obese 1 n=70 (Mean±SD/ Median(min-maks))	Obese 2 n=18 (Mean±SD/ Median(min-maks))	*p*
Flavonoid	142,26 ± 56,53	137,06 ± 51,9	162,49 ± 69,82	0,089*
Flavonol	20,22 (1,65-71,79)	19,61 (1,65-60,34)	28,73 ± 17,12	0,118**
Flavon	1,46 (0,13-22,77)	1,46 (0,13-22,7)	1,26 (0,43-22,77)	0,84**
Flavanon	5,68 (0,00-53,92)	5,68 (0-53,92)	5,95 (0,01-49,59)	0,918**
Flavan-3-Ol	27,66 (0,02-94,79)	29,63 ± 22,71	33,75 ± 24,01	0,5*
Antosianidin	0,63 (0,00-13,51)	0,38 (0-13,51)	1,57 (0-4,59)	0,228**
Isoflavonoid	55,53 (2,67-213,43)	55,52 (2,67-213,43)	79,48 ± 50,85	0,066**

**Independent T-Test*
***Mann-Whitney Test*

Table 3. Subject's malondialdehyde serum levels

Variable	All n=88 (Mean±SD/ Median(min-maks))	Obese 1 n=70 (Mean±SD/ Median(min-maks))	Obese 2 n=18 (Mean±SD/ Median(min-maks))	*p*
MDA serum levels (nmol/ml)	2,16 (1,09-6,71)	2,17 (1,2-6,7)	2,28 ± 0,77	0,466*

**Mann Whitney Test*

Table 4. Correlation between flavonoid intake and malondialdehyde serum level

Variables	Malondialdehyde serum levels					
	All n=88		Obese 1 n=70		Obese 2 n=18	
	r	P[b]	r	P[b]	r	p
Total flavonoid intake	0,188	0,079	0,172	0,155	0,390	0,110[a]
Flavonol	-0,074	0,492	-0,046	0,706	-0,139	0,582[a]
Flavon	-0,097	0,370	-0,136	0,262	0,056	0,826[b]
Flavanon	-0,095	0,377	-0,099	0,413	-0,065	0,797[b]
Flavan-3-Ol	0,325	0,002†	0,305	0,010†	0,585	0,011[a]†
Antosianidin	-0,029	0,789	-0,046	0,705	0,053	0,834[b]
Isoflavonoid	0,094	0,383	0,063	0,604	0,422	0,081[a]

[a]*Pearson*
[b]*Spearman*
†: statistically significant

This study found no correlation between flavonoid intake and MDA serum levels in all subjects (r=0.188, p=0.079), both in the Group of Obese 1 (r=0.172, p=0.155) and Group of Obese 2 (r=0.39, p=0.11). However, there was a positive correlation between flavan-3-ols intake and MDA serum levels in all subjects (r=0.325, p=0.002), both in the Group of Obese 1 (r=0.305, p=0.01) and Group of Obese 2 (r=0.585, p=0.011). Data are summarised in **Table 4.**

Discussion

This study found no correlation between flavonoid intake and MDA serum levels in obese women of reproductive age in Kendari (p>0.05). The therapeutic effect of flavonoids is 250-400 mg per day.[19] Meanwhile, the intake of flavonoids in the subject is only 142.26 ± 56.53 mg per day. The result is in line with the study by Alipour et al.[16] which showed no association between flavonoid intake and MDA levels (p > 0.05) in 170 healthy women aged 20-48 years in Iran. In contrast, a study on 159 elderly people in Spain using the SQ-FFQ to estimate flavonoid intake and its association with plasma MDA conducted by Gonzalez et al.[15] showed a negative relationship between flavonoid intake and MDA through multiple regression analysis.

The anti-obesity potential associated with flavonoids is quite relevant and their regulatory effects have been observed in reducing food intake and nutrient absorption, modulating adipogenesis and adipocyte life cycle, triggering thermogenesis and energy consumptions, and regulating the gut microbiota.[20] A study by Kim et al.[21] showed that the high intake of flavonoids was associated with a decrease in obesity prevalence among women. It is similar to a study conducted by Jennings, which showed a relationship between the high intake of flavonoids and the low fat mass.[22]

Flavonoids have shown promising health-enhancing effects in human cell culture, experimental animals, and clinical studies in humans. The majority of studies linking flavonoid intake to MDA levels as a biomarker of oxidative stress resulted from intervention trials.[19] Hirano[14] for instance, has looked at the effect of tea consumption which has been shown to lower MDA. Gonzalez et al.[15] found an inverse relationship between flavonoid intake and MDA. Contrarily, Alipour et al.[16] showed a different result in which flavonoid intake was not significantly associated with MDA.

The variation in the body's response to flavonoids such as differences in the composition of the gut microbiota and the food matrix can affect the metabolism and effectiveness of flavonoids. The mechanism of the antioxidant effect of flavonoids is still not clearly understood. One possible explanation for this effect is through direct reaction with reactive oxidants or the influence of the non-enzymatic antioxidant capacity on plasma. Another mechanism of flavonoids is as an antioxidant. The flavonoids' compound has the ability to donate a hydrogen atom (as a reducing agent) to free radicals. After giving away the hydrogen atom, flavonoids is then transformed into a stabilized radical phenolic compound, so that it is not easy to participate in other radical reactions.[16]

Interestingly, this study found a significant positive correlation between the intake of one of the flavonoid subclasses, namely flavan-3-ol/flavanol (r=0,325, p=0,002) and MDA serum levels. This flavonoid subclass is mostly found in tea, apples, fruit juices, chocolate, etc.[21] The result shows that an increase in MDA levels is correlated with the increasing intake of flavan-3-ol/flavanol. However, the correlation in the Group of Obese 2 (r=0.585, p=0.011) was higher than the Group of Obese 1 (r=0.305, p=0.010). The subjects' most consumed sources of flavan-3-ol were tea, bananas and apples.

An experimental study conducted by Monreal et al.[22] on the green tea consumption of obese subjects' (rich of 3 ol flavans) demonstrates a decrease in MDA levels after drinking 4 glasses of green tea per day or supplementing with 2 green tea capsules per day for 8 weeks. Another study conducted by Majo et al.[23] reveals that low concentrated flavan-3-ols have a potential to be strong antioxidants that can perform as free radical scavengers. Nevertheless, the study also shows that flavan-3-ols can go through the auto-oxidation at higher concentrations, thereby reducing their potential as antioxidants.

Several popular antioxidants have been reported to have prooxidant behaviors. There are at least three factors that can affect the function of antioxidants, converting them to prooxidants. These factors include the presence of metal ions, the concentration of antioxidants in the matrix environment and their redox potentials.[24] The prooxidant effect may also be beneficial, in which mild levels of oxidative stress generate the increase of antioxidant defense levels and biotransformation enzymes, leading to overall cytoprotection. The prooxidant activity is considered to be directly proportional to the total number of hydroxyl groups in the flavonoid molecule. The extent to which flavonoids are able to act as anti-prooxidants in vivo is still poorly understood and requires further study.[25]

Although several studies have shown the ability of flavonoids to overcome lipid peroxidation, it turns out that flavonoids have an oxidation potential that can bring the antioxidant or pro-oxidant properties under different conditions. For instance, in high doses or when the metal ions are present, flavonoids are able to reduce Cu(II) to Cu(I) and thus initiate the formation of free radicals. In another condition, the high concentrations of phenolic compounds can turn into pro-oxidants.[24,25]

This study has not been able to prove a correlation between flavonoid intake and MDA levels in obese women of reproductive age in Kendari. Several things that could cause insignificant results, among others, are the sample size was not sufficient, especially to compare the two groups of obesity. In addition, the analysis of flavonoids intake in food used food databases from other countries, where the levels of flavonoids can be different with this study due to differences in soil type, temperature, pH, season, etc.

The database of food sources of flavonoids in Indonesia is also still very limited. One of those that is quite comprehensive is provided by a study by Sefrina et al.[12] which is used in this study. However, that research still also used information from the USDA food database. Due to the diverse variations and types of food, in determining the flavonoid level in food sources, it is necessary to carry out a more specific examination, not only by relying on existing data from other countries.

This study also relies heavily on the honesty of respondents in reporting the intake and providing information related to the exclusion criteria. Screening examinations are very limited, considering the time and funds. Therefore, no other examinations, such as endogenous antioxidant levels affecting MDA levels, were administered in this study.

Furthermore, the absence of a correlation between total flavonoids and MDA levels in this study could be due to the fact that the intake of the subjects was not sufficient to provide the desired effects. It is known that the therapeutic effect of flavonoids is 250-400 mg per day.[19] This value has not been achieved by the subjects in this study.

The limitations of this study is that it uses SQ-FFQ to assess the flavonoid intake which is very dependent on the subject's memory, so that recall bias can occur, and it is difficult to get accurate intake information. This method can also lead to underestimation or overestimation from the participants. There are also several elements causing variation of participants' food intakes such as type of food consumed in different climates and various regional zones, certain seasons that affect the phytochemical content, food processing methods, temperature and processing time. It is acknowledged that respondents did not correctly inform the amount of food they consumed because of the flat slope syndrome, and hence, it is more or less than the actual amount. This limitation was prevented by providing an explanation of the importance of food intake reports to assess the health condition of research subjects in the informed consent. To minimize this recall bias and make it easier for the subject to recall the food they consumed, food models and photos were also utilized.

This study also did not examine other factors that can affect MDA serum levels such as endogenous and exogenous antioxidant levels. The examination was not carried out due to time and funding constraints. Since this study used a cross-sectional design, we could not monitor changes in flavonoid intake and MDA levels in subjects and were unable to observe the association and causal relationship between those two variables. This study also did not distinguish between physical activity and stress

factors from the subjects affecting food selection and total energy intake, hence it is prone to bias.

However, this research is the first study to assess flavonoid intake in Kendari. Further research needs to be undertaken using a larger sample size, especially to compare the two obese groups. Other research using other designs such as case control or cohorts can also be considered. This study leaves a room to explore, especially in the field of flavonoid intake. For instance, by examining other factors which were not carried out in this study such as endogenous antioxidants, oxidative stress markers and MDA comparison. Another potential approach is conducting a validation test of sq ffq intake of flavonoids, utilizing a variety of foods that are more representative of the population. It is also necessary to conduct further studies on the foods containing flavonoids database in Indonesia, since by far, there is still limited information and similar studies to date. The future studies can therefore produce a more valid database.

Conclusion

This study found no correlation between total flavonoid intake and MDA serum levels but there was a positive correlation between one of the flavonoid subclasses, namely flavan-3-ol/flavanol. This result may be due to low intake of flavonoids that reduce the effectiveness of flavonoids as antioxidants. The findings of this study are expected to be used as a basic data for further research on the benefits and impacts of flavonoids, and factors that affect flavonoids as antioxidants, especially in reproductive age women with obesity.

Conflict of Interest

Authors declared no conflict of interest regarding this article.

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