

Original Research Article

Association between carbohydrate consumption with telomere length based on plasma malondialdehyde in Minangkabau male

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ABSTRACT

Background: Life expectancy was one indicator of human development index in the health sector, one of which was affected by the telomere shortening process. There were many factors that cause shortening of telomere, including an imbalance of carbohydrate consumption then oxidative stress. The study aimed to examine the association between carbohydrate and carbohydrate simplex (sucrose) consumption with telomere length based on plasma malondialdehyde in Minangkabau male.

Methods: The study was cross-sectional with 97 samples from male civil servant, Minangkabau ethnic, 40-50 years old in Padang City. Carbohydrate and sucrose consumptions were obtained through semiquantitative food frequency questionnaire, plasma malondialdehyde examination with thiobarbituric acid test and telomere length measured by quantitative polymerase chain reaction using O'Challagan and Fenech method.

Results: The result showed mean of telomere length was 550.43±188.47 bp; mean of carbohydrate consumption 1280.97±433.57 kcal or 48.80±16.52%; mean of sucrose consumption 179.40±126.61 kcal or 6.83±4.82% and mean plasma malondialdehyde 66.91±13.93 nmol/ml. The association of carbohydrate consumption with telomere length based on plasma malondialdehyde was obtained p-value = 0.714, 0.908 and 0.903. The relationship of sucrose consumption with telomere length based on malondialdehyde was obtained p-value=0,667 and 1,000. Differences in mean telomere length in various categories of carbohydrate and sucrose consumption obtained p-value = 0.547 and 0.559.

Conclusions: There was no significant association between carbohydrate and carbohydrate simplex (sucrose) consumption with telomere length based on plasma malondialdehyde of Minangkabau male; and no significant difference in telomere length in different categories of carbohydrate consumption and different sucrose consumption.

Keywords: Carbohydrate and Sucrose consumption, MDA levels, Telomere length

INTRODUCTION

An indicator of the human development index related to health is life expectancy. Life expectancy in Indonesia according to reports from the Human Health Development Index in 2010 is still around the age of 70.1 years and ranks 109th out of 158 listed countries.¹ To achieve a longer life expectancy, it is influenced by many

factors that cause the aging process, then cause illness, and eventually die.²

Many theories explain the aging process, broadly divided into two groups, the "use and damage" theory and program theory. Program theory includes the theory of limited cell replication, immune processes and hormone theory. According to the theory of limited cell replication, the more a person ages, the cell division process

continues which occurs at the end of the chromosome of deoxyribonucleic acid or deoxyribonucleic acid (DNA), specifically, the structure is called telomere. The shortening mechanism of telomere is what determines the lifespan of body cells which then determines the length of one's age.²

Telomeres are part of the non-coding DNA structure found at the ends of chromosomes, with a length of between 5-20 kilobases (kb).³ At the time of cell division, telomere also shortens around 30-200 bp which will eventually lead to aging.⁴ There are many factors that cause telomere shortening, including diet or consumption of food that is not balanced, one of which is the consumption of daily carbohydrates and oxidative stress that occurs in someone.²

Carbohydrates themselves can basically be grouped on carbohydrate simplex and complex carbohydrates, where carbohydrate simplex is limited to the most is 10% of the total calories needed in a day. One of the most commonly known and most widely consumed simplex carbohydrates is sucrose.⁵

In the event of hyperglycemia in people with high consumption of carbohydrates, free radicals that are formed exceed the body's antioxidant defenses, which together will increase oxidative stress in cells.⁶ One of the most common biomarkers for measuring oxidative stress levels is because the earliest and easiest measurement is to measure the increase in lipid peroxidation products formed, one of the most reliable being an increase in malondialdehyde (MDA) levels.⁷

In people with high carbohydrate consumption, hyperglycemia will occur which will cause high levels of oxidative stress, causing telomere DNA damage, which in turn will cause telomere shortening and premature aging.⁸ The purpose of this study was to analyze the association between carbohydrate consumption and telomere length based on the plasma MDA levels of Minangkabau male in Padang City.

METHODS

The research was an observational study with a cross sectional study design, conducted in all Subdistricts in Padang city and examination of plasma MDA levels and telomere length in the Biomedical Laboratory of the Faculty of Medicine, Andalas University. The study was conducted for twelve months in 2016 and 2017. The population in this study were all male civil servants (ASN) who worked in all Subdistricts in Padang City, were Minangkabau ethnic and were 40-50 years old at the time of the study.

Based on the calculation of the sample size for categorical data on the population, the sample size was 97 people. The sampling technique used in this study was simple random sampling.

The dependent variable in this study are carbohydrate consumption and sucrose consumption, with the intermediate variables is MDA levels, and the independent variables of the telomere length of Minangkabau male. Carbohydrate consumption is defined as the daily carbohydrate consumption of respondents based on the semiquantitative FFQ questionnaire obtained from interviews and processing through nutrisurvey. The measuring scale used is ordinal, where carbohydrate consumption is said to be low if carbohydrate consumption <40% of total energy needs, sufficient if carbohydrate consumption is 40-60% of total energy needs and high if carbohydrate consumption is >60% of total energy needs. Sucrose consumption was the daily sucrose consumption of respondents based on the semiquantitative FFQ questionnaire obtained from interviews and processing through nutrisurvey. The measuring scale is ordinal, where consumption of sucrose is said to be sufficient if sucrose consumption is $\leq 10\%$ of total energy requirements and high if sucrose consumption is >10% of total energy needs.

MDA levels are levels of lipid peroxidation products that are toxic to cells to determine a person's oxidative stress state. MDA examination and telomere length was carried out by taking the respondent's vein blood sample by trained laboratory personnel, then conducting an examination at the Biomedical Laboratory of the Medical Faculty of Andalas University. To determine the MDA level of each sample using a kit from Biovision. Colorimetric analysis was then carried out based on thiobarbituric acid (TBA) reactivity and measured by spectrophotometry to obtain MDA levels in nmol/ml. The measuring scale is ordinal, where plasma MDA levels are said to be low if MDA levels <mean and high if MDA levels \geq mean.

Telomere length is the length of DNA segment at the end of the eukaryote cell chromosome in leukocytes. Telomere length examination was carried out by taking the blood sample of the respondent and then analyzing it according to the modification of the O'Challaghan and Fenech method and measuring it using real time PCR until the telomere total length was obtained in basepair (bp). The measuring scale is ordinal, where the telomere length is said to be short if the telomere length is <mean and long if the telomere length is \geq mean.

Data analysis was carried out through two stages, namely: (A) Univariate analysis, carried out on each research variable. (B) Bivariate Analysis, which includes two tests, namely: 1) Bivariate analysis with intermediate variables, to see the relationship between independent variables and dependent variables based on intermediate variables. The statistical test used is the chi-square test with a confidence level of 95%. 2) Bivariate analysis for different tests, to find out whether there are differences in telomere length on different carbohydrate consumption using the one way ANOVA test and whether there are differences in telomere length at different sucrose

consumption using independent t-test with a confidence level of 95%.

RESULTS

Characteristics of research subjects

The subjects in this study amounted to 97 males, were Civil Servants (ASN), aged 40-50 years when the study took place, and were ethnic Minangkabau. It is known that the subject has a telomere length which varies with an average of 550.43 bp. Subjects had carbohydrate consumption that varied with an average of 48.80%, had a mean consumption of sucrose 6.83% and plasma MDA

levels which also varied with an average of 66.91 nmol/ml (Table 1).

Respondents were then categorized based on telomere length, carbohydrate consumption, sucrose consumption and plasma MDA levels (Table 2).

Based on Table 2, it can be seen that most respondents (53.60%) have shorter telomeres; 22.70% of respondents consume high amounts of carbohydrates; 19.60% of respondents consumed high amounts of carbohydrate simplex (sucrose) and most respondents (52.60%) had high plasma MDA levels.

Table 1: Average consumption of carbohydrates in Kcal and %, sucrose consumption in Kcal and %, plasma MDA levels and telomere length.

| Variable | n | Average±SD | Minimum | Maximum |
|------------------------------------|----|----------------|---------|---------|
| Telomere Length (bp ¹) | 97 | 550.43±188.47 | 292.00 | 1034.00 |
| Carbohydrate Consumption (kcal) | 97 | 1280.97±433.57 | 422.96 | 3294.80 |
| Carbohydrate Consumption (%) | 97 | 48,80±16,52 | 16.11 | 125.52 |
| Sucrose Consumption (kcal) | 97 | 179.40±126.61 | 18.80 | 863.20 |
| Sucrose Consumption (%) | 97 | 6.83±4.82 | 0.72 | 32.88 |
| Plasma MDA level (nmol/ml) | 97 | 66.91±13.93 | 33.60 | 95.20 |

¹bp = basepair

Table 2: Frequency distribution of respondents based on carbohydrate, sucrose, plasma MDA, and telomere length consumption categories.

| Variable | f | % |
|---------------------------------|----|-------|
| Telomere length | | |
| Short | 52 | 53.60 |
| Long | 45 | 46.40 |
| Carbohydrate consumption | | |
| Low | 30 | 30.90 |
| Sufficient | 45 | 46.40 |
| High | 22 | 22.70 |
| Sucrose consumption | | |
| Sufficient | 78 | 80.40 |
| High | 19 | 19.60 |
| Plasma MDA level | | |
| Low | 46 | 47.40 |
| High | 51 | 52.60 |

Association of carbohydrate consumption and telomere length based on plasma MDA levels in Minangkabau male

Table 3 shows data on carbohydrate consumption with the telomere length of Minangkabau male based on plasma MDA levels, where there are 7.21% of respondents who consume carbohydrates in the high category, have high plasma MDA levels and shorter telomere lengths.

The Chi-Square statistical test results showed that low carbohydrate consumption was not significantly associated with telomere length based on Minangkabau male Plasma MDA levels (p = 0.714), while carbohydrate consumption was not significantly associated with telomere length based on plasma MDA levels of Minangkabau male (p = 0.908), and high carbohydrate consumption was not significantly associated with telomere length based on Minangkabau male MDA levels (p = 0.903).

So, there is no significant relationship between carbohydrate consumption with telomere length based on plasma MDA levels of Minangkabau male.

Association of sucrose consumption with telomere length based on plasma MDA level of Minangkabau male

Table 4 shows the relationship of sucrose consumption with the telomere length of Minangkabau male based on Plasma MDA levels, where there were 8.25% of respondents with high sucrose consumption, having high plasma MDA levels and short telomere length.

Chi-Square statistical test results show that sufficient sucrose consumption is not significantly associated with telomere length based on Minangkabau male plasma MDA levels (p = 0.667) and at high sucrose consumption not significantly associated with telomere length based on

plasma MDA levels (p = 1,000). So, there is no significant relationship between sucrose consumption and

telomere length based on plasma MDA levels of Minangkabau male.

Table 3: Data on association between consumption of carbohydrates and telomere length based on plasma MDA level of Minangkabau male.

| Carbo-hydrate consumption | Plasma MDA level | Telomere length | | Total | p |
|---------------------------|------------------|-----------------|-----------|-------------|-------|
| | | Short | Long | | |
| Low | Low | 8(8.25 %) | 7(7.21 %) | 15(15.46 %) | 0.714 |
| | High | 6(6.19 %) | 9(9.28 %) | 15(15.46 %) | |
| Sufficient | Low | 15(15.46 %) | 8(8.25 %) | 23(23.71 %) | 0.908 |
| | High | 13(13.40 %) | 9(9.28 %) | 22(22.68 %) | |
| High | Low | 3(3.09 %) | 5(5.15 %) | 8(8.25%) | 0.903 |
| | High | 7(7.21 %) | 7(7.21 %) | 14(14.43 %) | |

Table 4: Data on the relationship of sucrose consumption with telomere length based on plasma MDA levels of Minangkabau male.

| Sucrose consumption | Plasma MDA Level | Telomere Length | | Total | p |
|---------------------|------------------|-----------------|-------------|-------------|-------|
| | | Short | Long | | |
| Sufficient | Low | 23(23.71 %) | 18(18.56 %) | 41(42.27 %) | 0.667 |
| | High | 18(18.56 %) | 19(19.59 %) | 37(38.14 %) | |
| High | Low | 3(3.09 %) | 2(2.06 %) | 5(5.15 %) | 1.000 |
| | High | 8 (8.25 %) | 6(6.19 %) | 14(14.43 %) | |

Mean difference of telomere length in various categories of carbohydrate consumption and sucrose consumption

Based on Table 5, it can be seen that there are differences in telomere mean length in various categories of carbohydrate and sucrose consumption. However, the ANOVA statistical test results showed a value of p = 0.547, which means that there was no significant difference in mean telomere length based on differences in carbohydrate consumption categories.

The results of the independent t-test statistical test also showed a value of p = 0.559, which means that there were no significant differences in mean telomere length based on differences in sucrose consumption categories.

Table 5: Average distribution of telomere length in various categories of carbohydrate consumption and sucrose consumption.

| Variable | Telomere Length Average±SD (bp) | p |
|---------------------------------|---------------------------------|-------|
| Carbohydrate Consumption | | |
| Low | 575.13±217.84 | 0.547 |
| Sufficient | 528.24±172.43 | |
| High | 562.14±180.23 | |
| Sucrose Consumption | | |
| Sufficient | 555.99±193.53 | 0.559 |
| High | 527.63±168.95 | |

DISCUSSION

Nationally, the male population consumes more energy from carbohydrates (62.5%) than the female population (59.5%).⁹ In this study, it was found that carbohydrate consumption of Minangkabau male was at an average of 48.80% and respondents were more in the category of adequate carbohydrate consumption (40-60% of total energy needs), which was 46.40%. The results of this study are almost the same as the results of the study of Kark JD et al (2012) in 609 Israeli adults, where the average carbohydrate consumption was 48.60%.¹⁰

In this study, the sucrose consumption of Minangkabau male was obtained at an average of 6.83% and consumed more sucrose in the sufficient category (<10% of total energy) which was as much as 80.40%. The results of this study are almost the same as the results of a study by Leung et al (2014) which found that consumption of sucrose-rich sweet drinks in American adults aged 35-65 years ranged from 8.9 to 9.9% (236-260 kcal) or in the sufficient category.¹¹

Malondialdehyde (MDA) is one of the lipid peroxidation products that are toxic to cells. The high level of MDA in plasma shows an increase in free radicals and a decrease in antioxidants in the body.¹² In this study, the average MDA level of the Minangkabau ethnic male plasma was 66.91 nmol/ml and more had plasma MDA levels in the high category of 52.60%. This result is higher than the results of Palmieri et al (2014) in Italy which produced

plasma MDA levels in adult males is 2.5 nmol/ml.¹³ This difference in results may be due to racial (ethnic) differences, other than that due to differences in reagents or kits used. In Palmieri et al's study, plasma MDA measurements using thiobarbituric acid reactive substances (TBARS) tests with reagent assay kits (Abnova, Taiwan), while this study with Bio Vision's Lipid Peroxidation Assay Kit.¹⁴

Telomeres function to maintain chromosomal stability and integrity and play an important role in determining the amount of cell division that can be done normally.¹⁵ Telomere length is influenced by many factors including age, genetics, epigenetic and environmental improvements, economic and social conditions, physical exercise, weight and smoking.¹⁶ The results showed that the absolute telomere length of Minangkabau male aged 40-50 years ranged from 292 bp-1034 bp with an average telomere length of 550.43 bp. This result is lower than the standard telomere absolute examination results by O'Callaghan and Fenech (2011) without distinguishing sex in old age i.e. 380.43 bp-1891 bp with a mean of 941.30 bp.¹⁷ This difference occurs because the telomere length of humans is influenced by many factors including telomere length at birth, gender, age, race differences, environment, smoking, physical exercise, eating habits, disease and obesity.⁸

Based on the results of the study, it was also found that there was no relationship between carbohydrate consumption and telomere length based on plasma MDA levels of Minangkabau male. The results of this study are in line with the results of the cross sectional study of 840 adults in the United States aged 45-84 years conducted by Nettleton and friends (2008) who obtained the same results that food consumption from the carbohydrate group was not significantly associated with telomere length the person.¹⁸

In this study, it was also found that there was no relationship between sucrose consumption and telomere length based on plasma MDA levels of Minangkabau male. The results of this study are in line with the results of a cross-sectional study conducted by Calzon and friends (2015) in 287 male and female Spaniards, in a review made by Freitas-Simoes (2016) mentioning that sweet drinks (rich in sucrose) are not significantly associated with telomere length.¹⁹ Likewise, with the results of research conducted by Lee et al (2015) in 1958 middle-aged and older Korean adults found that there was no significant relationship between carbonated sweet drinks (high in sucrose) and telomere length.²⁰

Research shows that short-term limited caloric intake increases mitochondrial numbers and improves respiratory chain function, reduces ROS production and ultimately prevents shortening of telomere length.²¹

In this study, it was found that telomere results were longer in respondents who consumed lower amounts of

carbohydrates, although there was no significant difference in telomere length with different carbohydrate consumption among respondents after statistical tests. In this study also obtained longer telomeres in respondents who consumed sufficient amounts of sucrose and shorter telomeres in respondents who consumed high amounts of sucrose, although there was no significant difference in telomere length with respondent's sucrose consumption after statistical tests. The results of this study are almost the same as the results of a study by Zhou et al (2016) in China, which shows that at different telomere lengths (short, medium and long), there is no significant difference in carbohydrate consumption.²¹

CONCLUSION

Minangkabau male consume enough carbohydrates, consume sufficient amounts of sucrose, have high plasma MDA levels and have short telomere lengths. There is no relationship between carbohydrate consumption or sucrose consumption with telomere length based on plasma MDA levels of Minangkabau male or it can be said that carbohydrate consumption or high consumption of sucrose will not directly cause high levels of plasma MDA which then affects the telomere length of Minangkabau male, because of the many factors that affect telomere length. There were no significant differences in mean telomere length based on carbohydrate consumption and different consumption of sucrose.

Further research is suggested to examine other factors that influence the telomere shortening process, especially in Indonesia or especially for the Minangkabau ethnic group, because of the limited research on this subject, examining the role of telomerase enzymes, other nutrients and endogenous antioxidants in telomere shortening and sample separation between the sick and the healthy.

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