

Effect of consumption of vegetables and fruit on nitric oxide levels and T-glutathione levels in type 2 diabetes mellitus patients

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is closely related to cardiovascular disease and oxidative stress. It is characterized by vascular dysfunction, such as endothelial cell inability to produce nitric oxide (NO) or decreased NO availability. Glutathione (GSH), an antioxidant that contributes to preventing free radicals and reducing oxidative stress. Several studies have shown that the intake of foods such as vegetables and fruits containing both GSH and NO or their components can increase it and maintain NO homeostasis. This study aims to see how different vegetables and fruit consumption habits affect NO levels and T-GSH levels in blood plasma. This research method is analytic observational with cross sectional findings, the sample used is 85 subjects in the form of archives of biological materials. Data on habits of consuming vegetables and fruits were collected through interviews, NO levels and T-GSH levels in plasma were determined biochemically using the Elabscience® NO and GSH Assay Kit, then analyzed using the Mann Whitney test. According to the findings, the mean levels of NO and T-GSH in the blood plasma of T2DM patients, respectively, in patients who had the habit of consuming vegetables and fruits and those who did not, were for NO (108.34 ± 97.03) $\mu\text{mol/L}$ and (135.97 ± 112.87) $\mu\text{mol/L}$, for T-GSH were (13.54 ± 3.34) $\mu\text{mol/L}$ and (12.73 ± 1.44) $\mu\text{mol/L}$. There was, however, no statistically significant difference between the two parameters ($p > 0.05$). This study provides information that the habit of consuming vegetables and fruit does not provide a significant difference to the levels of NO and levels of T-GSH in blood plasma in patients with T2DM. But these habits still need to be done for health maintenance.

Keywords: diabetic, fruit, nitric oxide, T2DM, T-Glutathione, vegetables

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INTRODUCTION

Diabetes is one of the world's top ten diseases that causes death with a significant percentage increase of 70% since 2000 (Kochanek, 2019). Globally, in 2018 it was estimated that the prevalence of diabetes at various ages in the United States was 34.2 million people, or 10.5 percent of the US population (CDC, 2020). Indonesia is ranked seventh among the ten countries with the greatest number of diabetics (Kemenkes RI, 2020). Diabetes mellitus is a metabolic disease characterized by chronic hyperglycemia caused by insulin secretion, insulin action, or both (Kharroubi, 2015). The American Diabetes Association (ADA) claims diabetes is classified as type 1 diabetes, type 2 diabetes, other types, and gestational diabetes mellitus (Kharroubi, 2015) and the majority of the world's population has Type 2 Diabetes Mellitus (T2DM) (Galicia-Garcia et al., 2020).

Diabetes is a risk factor for cardiovascular disease, which is exacerbated in T2DM patients by oxidative stress, thereby causing endothelial dysfunction (Hoang et al., 2013; Strain & Paldánus, 2018). Endothelial dysfunction may also occur as a result of decreased NO bioavailability and activity of antioxidant enzymes (Hoang et al., 2013). Endothelial nitric oxide synthase (NOS) generates nitric oxide (NO) in blood vessels by converting the semi-essential cationic amino acid L-arginine into NO (González & Carlos Rivas, 2020). NOS activity has been shown to be impaired in hyperglycemia and insulin resistance (Shatanawi et al., 2020).

Glutathione is a tripeptide that is abundant in body tissues. Glutathione is essential for lowering oxidative stress, increasing detoxification of the metabolic system and immune system regulation (Minich & Brown, 2019). Studies have reported that L-cysteine (LC) supplementation decreased oxidative stress markers in both T2DM patients and healthy subjects (Jain et al., 2016). In addition, the provision of antioxidants as a therapeutic strategy is also recommended to prevent diabetes complications and restore insulin sensitivity (Rajendiran et al., 2018). Epidemiological studies reveal a strong association between dietary antioxidant intake and protection against diabetes (Rajendiran et al., 2018).

The degree of variability in an individual's ability to produce glutathione (GSH) is influenced by genetic variability in the enzymes involved in its production and/or regeneration. Some of these enzymes require nutritional cofactors (Minich & Brown, 2019). GSH level in circulating and tissue decline with age and diabetes (Jain et al., 2016).

Vegetables and fruits contain antioxidants, which can lower the risk of developing chronic diseases such as diabetes. 85% of nitrate intake is contributed by vegetables, which increase NO levels (Bahadoran et al., 2015; Jideani et al., 2021). This has been confirmed by several studies discussing the beneficial therapeutic effects of nitrates/nitrites in T2DM (Bahadoran et al., 2015). Furthermore, a number of investigations have shown that L-arginine supplementation which can be obtained from vegetables and fruits is a method to increase endothelial NO production and reverse endothelial dysfunction (Jablęcka et al., 2012; Mulwa et al., 2020). Vegetables and fruits also contain glutathione which can increase GSH levels in blood plasma (Park et al., 2014).

Diabetes is associated with significantly lower NO and glutathione levels (Kalkan & Suher, 2013). Therefore, it is important to know how to maintain or increase levels of NO and GSH in the body, one of which is by consuming vegetables or fruits containing NO and glutathione or their components and the aim of this study to determine difference effect between vegetable and fruit consumption habits on NO levels and GSH levels.

MATERIALS AND METHOD

Materials

The equipment used in this study included syringe (3ml), Ethylene diamine tetra acetic acid (EDTA) vascular blood tube (3ml), test tube, centrifuge, vortex, micropipette, micro-plate reader (450 nm and 550 nm), 37°C water bath. The materials used in this study included NO Assay Kit E-BC-K036 and Total Glutathione/Oxidized E-BC-K097.

Methods

This is an analytic observational study with a cross-sectional research design and ethical approval number 2332/C.1/KEPK-FKUMS/VIII/2019. The sample used in the form of blood plasma from DMT2 patients as many as 85 samples at the Jetis 1 Health Center, Bantul, Yogyakarta which was obtained in the study of (Akrom, 2018). Furthermore, measurements of NO levels and T-GSH levels in the blood plasma were carried out.

All patients diagnosed with T2DM by doctors, with or without comorbidities, were included in this study. Furthermore, eligible patients were aged 18 years or older, male or female (had signed and filled out the informed consent) who were diagnosed or had a history of T2DM, had uncontrolled and controlled blood glucose levels, had HbA1C values above normal (4% -5.7%), had moderate to high adherence rates to medications as measured by MARS, compliance questionnaire, had regular treatment at the Jetis 1 Public Health Center during the study period, was willing to participate in the research. Exclusion criteria were patients with mental disorders, pregnant and resigned as respondents during the study. Patients are said to consume vegetables/fruits if they consume vegetables and fruit once a day. This data was obtained through interviews with a question form.

Measurement of NO levels and T-GSH levels in plasma

Both NO levels and T-GSH levels in plasma samples were measured by colorimetric technique using a microplate reader at 550 nm for NO and 405 nm for T-GSH, expressed in $\mu\text{mol/L}$. Measurements were made following the manufacturer's instructions from the NO Assay Kit E-BC-K036 and Total Glutathione/Oxidized Glutathione Assay Kit E-BC-K097.

Measurement of NO levels in plasma

Dilute the standard sodium nitrite solution with deionized water to serial concentrations. Then take 200 μl of serial solution and sample respectively, and add 200 μl of reagent 1 and 100 μl of reagent 2 respectively, Stir well and let stand for 15 minutes at room temperature, centrifugation for 10 min at 3,100 g, take 160 μl of supernatant for the following procedure. Then react the supernatant and chromogenic reagent while stirring for 2 minutes, let stand for 15 minutes at room temperature, immediately measure the OD of each well with a microplate reader at 550 nm. Then calculate the result by plotting on the standard curve.

Measurement of T-GSH levels in plasma

Total glutathione in tissues and body fluids was measured by the DTNB cyclic reaction. Add 400 μl of reagent 4 to 100 μl of sample (plasma) and stirred using a vortex for 30 seconds, then allowed to stand for 5 minutes at 4°C and followed by centrifugation for ten minutes at 3500 rpm. Take the supernatant, store at 4°C for measurement or -20°C overnight.

Take a total of 10 μl of the sample and also standard solution (50 $\mu\text{mol/L}$ standard GSH), add 100 μl of reagent 1 and 10 μl of reagent 2, respectively. Stir thoroughly, let stand for 2 minutes at room temperature (25°C), then 50 μl of reagent 3 is added and the time was recorded, mix fully. At 405 nm, take absorbance readings with microplate reader at 30 seconds (A1) and 630 seconds (A2), respectively.

Data Analysis

The Statistical Package for the Social Sciences was used to analyze the data collected. Demographic data (age and sex) and consumption habits of vegetables and fruit in T2DM patients were analyzed using a descriptive test presented in the form of frequency. Then the homogeneity and normality tests were carried out using the explore of descriptive tests and Kolmogorov-Smirnov test.

The test results showed that the data obtained were homogeneous but not normally distributed. Furthermore, the Mann Whitney test was used to determine the relationship between differences in age, sex, and consumption of vegetables and fruit with levels of NO and T-GSH in the plasma of T2DM patients. At $p < 0.05$, the results were considered statistically significant

RESULT AND DISCUSSION

Demographic Profile and Lifestyle of T2DM Patients

Table 1. Demographic profile and lifestyle of T2DM patients at Jetis 1 public health center, Bantul, Yogyakarta (statistical test: frequency distribution)

Variable		Σ (n=85)	Percentage (%)	
Demographics	1. Age	< 55	32	3.6
		\geq 55	53	62.4
	Mean \pm SD	57.6 \pm 9.1		
2. Sex	Male	33	38.8	
	Female	52	61.2	
Lifestyle	3 Consumption habits of vegetables and fruit	Yes	70	82.4
		No	15	17.6

This study involved 85 patients with a mean age of 57.6 \pm 9.1 years. The percentage of female 61.2% and male 38.8% and there were 82.4% of patients who had habit of consuming vegetables and fruit [Table 1](#).

Table 2. NO levels and T-GSH levels in blood plasma of T2DM patients at Jetis 1 public health center, Bantul, Yogyakarta

	Mean \pm SD	Minimum	Maximum
NO Levels (μ mol/L)	113.22 \pm 99.84	5.92	381.31
T-GSH Levels (μ mol/L)	13.39 \pm 3.10	6.82	22.73

Table 3. Differences in demographic and lifestyle on no levels in blood plasma of T2DM patients at Jetis 1 public health center, Bantul, Yogyakarta (Statistical Test: Mann Whitney Test, significant ($p < 0.05$))

Variable	N	NO (μ mol/L)			
		Mean \pm SD	(min-max)	<i>p</i>	
Demographic	1. Age	<55	107.73 \pm 107.04	(8.23-374.38)	0.508
		\geq 55	116.53 \pm 96.14	(5.92-381.31)	
2. Sex	Male	94.87 \pm 74.50	(5.92-252.85)	0.360	
	Female	124.86 \pm 112.16	(8.23-381.31)		
Lifestyle	3. Consumption habits of vegetables and fruit	Yes	108.34 \pm 97.03	(5.92-381.31)	0.381
		No	135.97 \pm 112.87	(8.23-374.38)	

Differences in age and sex on NO levels in blood plasma of T2DM

The results show that the average level of NO in the blood plasma of DMT2 patients is (113.22 \pm 99.84) μ mol/L ([Table 2](#)). The mean plasma NO level in T2DM patients aged less than 55 years (107.73 \pm 107.04) μ mol/L was lower than those aged more than or equal to 55 years (116.53 \pm 96.14) μ mol/L. Likewise, NO levels in male (94.87 \pm 74.50) μ mol/L are also lower than in female (124.86 \pm 112.16) μ mol/L. In T2DM patients, differences in age and sex with plasma NO levels were not significantly related ($p > 0.05$).

Differences in consumption of vegetables and fruits on NO levels in blood plasma of T2DM patients

The results (Table 3) show that the mean levels of NO in patients who consumed vegetables and fruit ($108.34 \pm 97.03 \mu\text{mol/L}$) were lower than patients who did not eat vegetables and fruit ($135.97 \pm 112.87 \mu\text{mol/L}$). There was no significant relationship between the differences in consumption habits of vegetables and fruit in patients with T2DM with NO levels ($p > 0.05$). This study is not in line with several previous studies, where the availability of NO can be increased and maintained through the consumption of vegetables and fruits that contain lots of nitrate/nitrite (Jonvik et al., 2016; Kobayashi et al., 2015).

The difference in results can be caused by various factors. How to eat such as chewing properly can also affect the bioavailability of NO. More nitrite will enter the acid-rich stomach by chewing it well first, then the nitrite will be protonated to form nitric acid which can spontaneously decompose into NO (Kobayashi, 2019). Additional diseases that may be suffered by the subject can also affect NO levels. There are some diseases that cause excessive NO production, while other conditions can cause low NO levels (Akanji et al., 2020). There are some diseases that cause excessive NO production, while other conditions can cause low NO levels. Restricting more specific inclusion and exclusion criteria may be more effective in reducing the diversity of results.

Table 4. Differences in demographic and lifestyle on T-GSH Levels in blood plasma of T2DM patients at Jetis 1 public health center, Bantul, Yogyakarta (statistical test: mann whitney test, significant ($p < 0.05$))

	Variabel	N	T-GSH ($\mu\text{mol/L}$)			
			Mean \pm SD	(min-max)	<i>p</i>	
Demografic	1. Age	<55	32	13.28 ± 3.47	(6.82-22.73)	0.884
		≥ 55	53	13.46 ± 2.88	(6.82-20.45)	
	2. Sex	Male	33	13.84 ± 3.08	(6.82-20.45)	0.153
		Female	52	13.11 ± 3.10	(6.82-22.73)	
Lifestyle	3. Consumption habits of vegetables and fruit	Yes	70	13.54 ± 3.34	(6.82-22.73)	0.222
		No	15	12.73 ± 1.44	(9.09-13.64)	

Differences in age and sex on T-GSH levels in blood plasma of T2DM

According to the findings, the average level of T-GSH in the blood plasma of T2DM patients was (13.39 ± 3.10) $\mu\text{mol/L}$ (Table 2). The mean plasma T-GSH level with age less than 55 years (13.28 ± 3.47) $\mu\text{mol/L}$ was slightly lower than plasma T-GSH level in patients older than or equal to 55 years (13.46 ± 2.88) $\mu\text{mol/L}$. While, the levels of T-GSH levels in male patients were (13.84 ± 3.08) $\mu\text{mol/L}$ higher than female (13.11 ± 3.10) $\mu\text{mol/L}$. In T2DM patients, it was seen that the differences in age and sex with plasma NO levels were not significant ($p > 0.05$) (Table 4).

Differences in consumption of vegetables and fruits on T-GSH levels in blood plasma of T2DM patients

The results presented in table 4 show that the the mean plasma T-GSH levels in patients who consumed vegetables and fruit ($13.54 \pm 3.34 \mu\text{mol/L}$) were lower than those who did not consume vegetables and fruit ($12.73 \pm 1.44 \mu\text{mol/L}$). No significant relationship was found between differences in vegetable and fruit consumption habits and T-GSH levels ($p > 0.05$). This may be due to the way of cooking. Peeling and chopping vegetables before cooking can remove the epidermis and have a protective effect when cooked. Cooking time, especially grilling and frying methods, can significantly affect GSH levels. In addition, although not significant, cooking at high temperatures (baking, frying, and grilling) can reduce GSH levels by up to 40% (Drinkwater et al., 2015). Any form of food processing will reduce the content of phytochemicals, including fruit plants such as Citrus plants (Zou et al., 2016).

Effect of Consumption ... (Sari et al.,)

The amount of vegetables and fruit consumed also affects the increase in glutathione peroxidase activity (Hermesdorff et al., 2012). However, it is suggested that a number of different vegetables be consumed each day for health benefits, including reducing the risk of diabetes (Dias, 2012).

The characteristics of vegetables and fruits, particularly their phytonutrient content may also have an effect. Zou et al's research stated that citrus plants contain very diverse and varied phytochemicals that can affect antioxidant capacity (Zou et al., 2016). Other supporting research shows that Se supplementation significantly increases blood glutathione (Sedighi et al., 2014), the selection of vegetables and fruits consumed with greater Se content will also have a greater chance of increasing glutathione levels.

Patients with T2DM are known to have lower plasma GSH concentrations than non-diabetic controls. Decreased synthesis as well as increased irreversible utilization of GSH through non-glycemic mechanisms may be the cause (Lutchmansingh et al., 2018). The habit of consuming vegetables and fruit regularly can improve antioxidant status. Research shows that consuming one serving of broccoli has significantly increased GST activity in plasma (Bahadoran et al., 2011; Riso et al., 2014). The same thing was seen in type 2 diabetes patients who did a low-calorie diet of 2 pieces per day, the results obtained were significantly increased glutathione reduction (Hegde et al., 2013).

Limitation

Our study has several limitations. NO and T-GSH levels in blood plasma between T2DM patients and healthy controls were not compared. The number of samples used was not too large and the number of samples between patients who consume vegetables and fruit has a large difference in numbers. Many confounding factors were not excluded in this study, including methods of preparation and cooking, sampling time, restrictions on the length of time the patient consumed vegetables and fruit. Regardless of the difficulties mentioned above, optimizing GSH levels through the intake of foods that are proven to contain GSH, either as precursors, cofactors, or whole foods, is a fairly easy, inexpensive, and safe method (Minich & Brown, 2019). Likewise, foods containing NO or its components will also increase NO levels and health status.

CONCLUSION

In conclusion, this study does not show that differences in lifestyle habits in consuming vegetables and fruit have no significant effect on NO levels and T-GSH levels in blood plasma. However, eating vegetables and fruit is still recommended as a healthy habit to prevent or inhibit various diseases. Further research is needed with a larger number of subjects and more specific inclusion criteria.

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