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Original Research

# Dietary intake and glutathione s-transferase (m1 and t1) variants in type 2 diabetes mellitus at USU hospital, Medan, Indonesia

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## Abstract

**Background and Aims:** Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease caused by multifactorial, such as genetic and external factors, i.e. diet and lifestyle. This study aims to observe the differences of dietary intake T2DM patients in Universitas Sumatera Utara Hospital (USU) Hospital, Medan, Indonesia based on glutathione S-transferase M1 (GSTM1) and T1 (GSTT1) variants. **Material and Method:** This study was conducted on T2DM patients that were recruited at USU Hospital, Medan, Indonesia. Dietary intake was recorded by 24-hours food recall and analyzed with Nutrisurvey 2007. Multiplex PCR was performed to analyze GSTM1 and GSTT1 variants. **Results:** This study showed mean of lipid intake (g), Recommended Dietary Allowance (RDA) lipid (%), % lipid (%), and protein intake (g), RDA protein (%), % protein (%) in GSTM1 null were greater than GSTM1 positive ($37.02 \pm 2.77$ vs $23.68 \pm 4.3$, $p < 0.05$; $53.6 \pm 4.01$ vs $34.2 \pm 6.23$, $p < 0.05$; $26.17 \pm 1.22$ vs $16.33 \pm 2.23$, $p < 0.05$; and $50.11 \pm 3.64$ vs $49.86 \pm 11.79$, $p > 0.05$; $83.3 \pm 6.04$ vs $82.94 \pm 19.61$, $p > 0.05$; $16.8 \pm 0.69$ vs $14.6 \pm 1.37$, $p > 0.05$). In GSTT1 gene, the mean of lipid intake (g), RDA lipid (%), % lipid (%) was greater in GSTT1 null than GSTT1 positive ($33.32 \pm 3.92$ vs $32.66 \pm 2.84$, $p > 0.05$; $48.24 \pm 5.68$ vs $47.24 \pm 4.12$, $p > 0.05$; $23.69 \pm 1.78$ vs $22.81 \pm 1.77$, $p > 0.05$). **Conclusions:** Lipid intake, RDA lipid, % lipids, carbohydrate intake in GSTM1 positive and null or mean of energy intake in GSTT1 positive and null were significant differences in T2DM patients at USU Hospital, Medan, Indonesia.

**Keywords:** dietary intake, GSTM1, GSTT1, type 2 diabetes mellitus.

## Background and Aims

Diabetes mellitus (DM) is a metabolic disorder associated with nutrients metabolism [1]. In 2017, the prevalence of DM was 451 million people (8.4%) in the world and it is estimated that in 2045 it will increase to 693 million people (9.9%) [2]. In 2030, people with DM are estimated to be 21.3 million in Indonesia [3]. Type 2 diabetes mellitus (T2DM) generally occurs in adults. T2DM is caused by insulin resistance and about 87% to 91% of all DM patients are estimated to be T2DM [1, 2].

Oxidative stress underlies the pathogenesis of T2DM. Oxidative stress in T2DM can cause complications such as neurodegenerative, cardiovascular disorders, etc. [4–6]. Oxidative stress can be overcome by antioxidants. One of the antioxidant groups that play a role in oxidative stress defense is endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione S-transferase (GST). GST is an antioxidant that fights against oxidative stress by detoxification of endogenous and exogenous electrophilic

compounds, as well as metabolizing compounds formed from oxidative stress [7, 8].

The GST protein is synthesized based on genes encoded by predetermined nucleotide sequence. GST gene variations caused disturbance with the ability of GST enzymes in dealing with oxidative stress. Among the various types of GST, GSTM1 (Mu) and GSTT1 (Theta) are known to be polymorphic and widely studied in relation to susceptibility to disease [9].

Previous studies have shown an association between GSTM1 and GSTT1 gene variations with T2DM patients [10, 11]. The risk of suffering T2DM was related to external factors such as dietary intake. Previous studies showed differences of dietary intake in T2DM patients and healthy subjects. Dietary intake is an important determinant of insulin resistance as a pathogenesis of T2DM [12–14]. The association of dietary intake with T2DM has been known, and the relationship of GSTM1 and GSTT1 gene variations with T2DM has been widely studied, but the study about differences of dietary intake based on GSTM1 and GSTT1 variants in T2DM patients at USU Hospital has never been done.

## Material and Method

### Study design and patients

The cross-sectional study was carried out after obtaining approval from the Ethics Committee of Medical Faculty, Universitas Sumatera Utara (USU) EC No.116.KEPK FK USU-RSUP HAM in the year 2019. Fifty of T2DM patients recruited from endocrine polyclinic at USU Hospital according to PERKENI (Indonesian Endocrinology Association) guideline. All respondents were informed about the study protocol with written information and signed the informed consent.

### Food recall and clinical data collection

Dietary intake was recorded using a 24-hour food recall questionnaire and using food model. Dietary intake data was analyzed by Nutrisurvey 2007. Examination of fasting blood glucose (FBG) was done after the patient underwent fasting for 8–12 hours the night before. Two hours post prandial blood glucose (2hPPG) were examined two hours after meal. Fasting blood glucose, 2hPPG and HbA1c tests were performed using Cobas 6000 analyzer with the principle of hexokinase and immunoturbidimetry (Roche Diagnostics, Switzerland) at USU Hospital Laboratory.

### Genotyping GSTM1 and GSTT1 gene

Examination of GSTM1 and GSTT1 gene variants was carried out at Integrated Laboratory Medical Faculty of USU through several stages. DNA was isolated using DNA extraction kit (Promega, USA) and was extracted using buffy coat from peripheral leukocyte blood. DNA isolates measured the concentration and purity at 260/280 absorbents. DNA was stored at –80°C until further analysis.

Multiplex PCR method is performed to analyze genetic variation in GSTM1 and GSTT1. The primers were used for amplification showed in table 1. PCR reaction was 25 µL with the composition: 1 µL each primers forward and reverse, 12.5 µLTaq PCR master mix (Promega, US), 1 µL DNA sample, and nuclease free water add up to 25 µL. PCR was carried out with a pre-denaturation of 95°C for 5 minutes, denaturation of 94˚C for 30 seconds, primary anneling 63°C for 30 seconds, elongation 72˚C for 30 seconds. The amplification cycle was 35 cycles and post elongation was carried out at 72°C for 10 minutes.

The PCR products were analyzed by electrophoresis using 2% agarose (Invitrogen). The presence of DNA bands of PCR products was observed on the UltraViolet transluminator. The documentation showed that 215 bp fragment indicates GSTM1 variant and 480 bp fragment indicates GSTT1 variant with β-actin gene control at 92 bp [15].

### Statistical analysis

Dietary intake data, GSTM1 and GSTT1 gene variations were analyzed using a statistical


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Table 1: Primers of GSTM1 and GSTT1.

| Gene | Forward | Reverse |
|---|---|---|
| GSTM1 | 5′-GAACTCCCTGAAAAGCTAAAGC-3′ | 5′-GTTGGGCTCAAATATACGGTGG- 3′ |
| GSTT1 | 5′-TTCCTTACTGGT CCTCACATCTC-3′ | 5′-TCACCGGATCATGGC CAGCA-3′ |
| β-actin | 5′-AATGTGAACATGTGGGACTTTGTG-3’ | 5′-CGCCAGTTCAGGACATTAGGAC-3′ |

program based on Windows computers with a significance level of 5% ($p < 0.05$). Differences of mean dietary intake levels were analyzed by Mann Whitney test.

## Results

The characteristics of the study subjects were shown in table 2. Differences in characteristics and patterns of dietary intake based on GSTM1 and GSTT1 gene variants in T2DM patients can be seen in tables 3 and 4.

In this study population, out of fifty T2DM patients, 35 (70%) patients with GSTM1 null were found. There were differences in characteristics in each of these GSTM1 variant groups, but there were no statistically significant differences ($p > 0.05$). Dietary intake based on GSTM1 gene showed significant differences in mean lipid intake, RDA lipid, % lipid, % carbohydrate and vitamin E intake in GSTM1 positive compared to GSTM1 null ($p < 0.05$) (Table 3).

Table 2: Patients' characteristic.

| Characteristic | Category | n (%) |
|---|---|---|
| Age (y.o) | <40 | 2 (4) |
| | 41–50 | 10 (20) |
| | 51–60 | 18 (36) |
| | 61–70 | 14 (28) |
| | >70 | 6 (12) |
| Gender | Female | 24 (48) |
| | Male | 26 (52) |
| Duration of suffering DM | <5 years | 29 (58) |
| | >5 years | 21 (42) |

From fifty T2DM patients in this study, GSTT1 positive were found in 27 subjects. Based on subject characteristics, there was an association of differences in levels of 2 h PPG between GSTT1 positive and GSTT1 null variants ($p < 0.05$), but there were none in FBG, HbA1c levels and duration of suffering DM. There were significant differences in the mean of energy, vitamin E and calcium intake between GSTT1 positive and GSTT1 null ($p < 0.05$), but not in other dietary intake (table 4).

## Discussion

T2DM is a chronic metabolic disease caused by multifactorial reasons. Genetic variations are known to be associated with occurrence of T2DM, such as variations in the glutathione S-transferase (GST), GSTM1, and GSTT1 genes. In this study, the number of T2DM patients was greater in GSTM1 null than GSTM1 positive (70% vs. 30%) whereas GSTT1 positive was greater than GSTT1 null (54% vs. 46%). Previous studies have shown similar results where the distribution of GSTM1 null was greater than GSTM1 positive (62% vs. 38%), and GSTT1 positive was greater than GSTT1 null (65% vs. 35%) in T2DM patients in Egypt [11]. Different results from the T2DM study in Chinese population showed that GSTM1 positive was greater than GSTM1 null and GSTT1 null was greater than GSTT1 positive [16]. Study on T2DM patients in Iraq showed that both GSTM1 and GSTT1 null were greater than GSTM1 and GSTT1 positive [17]. The results of previous studies on T2DM patients showed that GSTM1 and GSTT1 positive were greater than GSTM1 and GSTT1 null in Brazil [10].

Genetic variations occur due to differences in the sequence of nucleotides between

Table 3: Dietary intake and GSTM1 gene variant.

| Characteristic and dietary intake | GSTM1 | | p value |
|---|---|---|---|
| | Positive (n = 15; 30%) | Null (n = 35; 70%) | |
| Fasting Glucose (mg/dL) | 202.91 ± 32.22 | 232.66 ± 13.28 | 0.312 |
| 2hPPG (mg/dL) | 285.94 ± 30.94 | 302.13 ± 15.19 | 0.601 |
| HbA1c (%) | 8.89 ± 0.61 | 8.99 ± 0.36 | 0.881 |
| Duration of suffering DM (year) | 8.73 ± 1.71 | 6.37 ± 1.03 | 0.229 |
| Energy (kcal) | 1355.04 ± 180.65 | 1303.4 ± 65.11 | 0.738 |
| RDA Energy (%) | 61.42 ± 8.78 | 60.25 ± 3.13 | 0.876 |
| % Energy (%) | 66.56 ± 8.87 | 63.94 ± 3.19 | 0.730 |
| Lipid (g) | 23.68 ± 4.3 | 37.02 ± 2.77 | 0.015* |
| RDA Lipid (%) | 34.2 ± 6.23 | 53.6 ± 4.01 | 0.011* |
| % Lipid (%) | 16.33 ± 2.23 | 26.17 ± 1.22 | 0.001* |
| Carbohydrate (g) | 205.64 ± 25.74 | 170.2 ± 9.12 | 0.110 |
| RDA Carbohydrate (%) | 70.72 ± 8.85 | 58.56 ± 3.13 | 0.210 |
| % Carbohydrate (%) | 69.6 ± 3.4 | 57.02 ± 1.55 | 0.003* |
| Protein (g) | 49.86 ± 11.79 | 50.11 ± 3.64 | 0.979 |
| RDA Protein (%) | 82.94 ± 19.61 | 83.3 ± 6.04 | 0.982 |
| % Protein (%) | 14.6 ± 1.37 | 16.8 ± 0.69 | 0.118 |
| Vitamin C (mg) | 15.4±2.92 | 13.04 ± 3.39 | 0.672 |
| Vitamin E (mg) | 16.41 ± 1.93 | 7.63 ± 3.54 | 0.006* |
| Kalsium (Ca) (mg) | 385.52 ± 142.75 | 224.91 ± 73.22 | 0.409 |
| Magnesium (Mg) (mg) | 207.24 ± 74.17 | 187.54 ± 23.36 | 0.743 |
| Besi (Fe) (mg) | 5.67 ± 1.93 | 5.06 ± 0.56 | 0.689 |
| Zinc (Zn) (mg) | 5.12 ± 1.39 | 4.83 ± 0.34 | 0.788 |

Data are presented as mean ± standard deviation. p values: Mann-Whitney test

individuals that are related to race and ethnicity [18]. Genetic variations in GSTM1 and GSTT1 were known as factors of T2DM susceptibility. Previous studies have shown that GSTM1 and GSTT1 null decreased GST activity, thereby reducing the ability of this enzyme dealing with oxidative stress that arises in T2DM. Oxidative stress increases insulin resistance and worsens the disease [19, 20]. Genetic variation is not the only pathogenesis that caused people to suffer from T2DM. External factors also play an important role in the pathogenesis of T2DM. Genetic factors and their interactions with external factors such as dietary intake contribute to the development of T2DM. Heritability for T2DM is estimated around 26%. The interaction between genetic factors and dietary intake tends to be important, too [21–23].

In this study, significant differences were found in the mean energy intake in GSTT1 positive compared to GSTT1 null ($p < 0.05$), but there were not found in lipid, carbohydrate, and protein intakes. Energy is obtained from macronutrients such as carbohydrates, fats, and proteins. In order to be converted into energy, these nutrients undergo a metabolic process. Carbohydrate becomes glucose, fat becomes fatty acids, and protein becomes amino acids. Consumption of high carbohydrates and lipid food, in addition to low physical activity, will change the energy balance by storing energy as fat that is rarely used. This excessive macronutrients intake will


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Table 4: Dietary intake and GSTT1 gene variant.

| Characteristic and dietary intake | GSTT1 | | p value |
|---|---|---|---|
| | Positive (n = 27; 54%) | Null (n = 23; 46%) | |
| Fasting Glucose (mg/dL) | 197.78 ± 14.29 | 223.65 ± 21.53 | 0.337 |
| 2hPPG (mg/dL) | 273.65 ± 16.91 | 305.4 ± 21.38 | 0.015* |
| HbA1c (%) | 8.93 ± 0.43 | 8.98 ± 0.44 | 0.938 |
| Duration of suffering DM (year) | 7.37 ± 1.26 | 6.73 ± 1.27 | 0.729 |
| Energy (kcal) | 1363.05 ± 116.24 | 1267.05 ± 67.79 | 0.046* |
| RDA Energy (%) | 62.21 ± 5.71 | 58.72 ± 3.09 | 0.612 |
| % Energy (%) | 66.87 ± 5.71 | 62.2 ± 3.32 | 0.503 |
| Lipid (g) | 32.66 ± 2.84 | 33.32 ± 3.92 | 0.897 |
| RDA Lipid (%) | 47.24 ± 4.12 | 48.24 ± 5.68 | 0.892 |
| % Lipid (%) | 22.81 ± 1.77 | 23.69 ± 1.78 | 0.730 |
| Carbohydrate (g) | 196.73 ± 16.84 | 173.9 ± 9.96 | 0.534 |
| RDA Carbohydrate (%) | 66.22 ± 5.79 | 59.84 ± 3.42 | 0.537 |
| % Carbohydrate (%) | 68.77 ± 2.46 | 59.65 ± 2.28 | 0.535 |
| Protein (g) | 51.74 ± 7.46 | 48.03 ± 3.35 | 0.671 |
| RDA Protein (%) | 86.05 ± 12.41 | 79.85 ± 5.55 | 0.669 |
| % Protein ((%) | 16.65 ± 0.89 | 15.7 ± 0.92 | 0.468 |
| Vitamin C (mg) | 14.7 ± 2.58 | 14.67 ± 4.62 | 0.995 |
| Vitamin E (mg) | 15.4 ± 3.47 | 11.86 ± 3.95 | 0.047* |
| Kalsium (Ca) (mg) | 405.62 ± 128.89 | 117.52 ± 23.14 | 0.048* |
| Magnesium (Mg) (mg) | 216.6 ± 45.64 | 166.27 ± 24.94 | 0.361 |
| Besi (Fe) (mg) | 5.89 ± 1.13 | 4.48 ± 0.67 | 0.312 |
| Zinc (Zn) (mg) | 5.36 ± 0.83 | 4.4 ± 0.31 | 0.315 |

Data are presented as mean ± standard deviation. p values: Mann-Whitney test

increase insulin resistance. Dietary habits or diet, especially carbohydrate and lipid types, are important determinants of insulin resistance. Several previous studies have shown that carbohydrate and fat intake was associated with the incidence of T2DM. High-calorie and high-fat diet will increase insulin resistance even in low-risk populations [14, 24, 25]. The high lipid intake in the GSTM1 null compared to GSTM1 positive was found to have a significant relationship between this gene variant. We suggest further research whether these two risk factors are interr-elated risk factors of T2DM.

The mean of vitamins C and E intake were lower in GSTM1 null than GSTM1 positive. Lower intake of vitamins C and E in the null group compared to the positive group was also found in the GSTT1 gene. Significant differences were found in the mean of vitamin E intake in GSTM1 and GSTT1 positive compared to GSTM1 and GSTT1 null ($p < 0.05$), but not in vitamin C. Vitamin C and vitamin E are claimed to be antioxidants that reduce oxidative stress and ROS. As an antioxidant, vitamin C is known to reduce insulin resistance by improving endothelial function thereby preventing the development of T2DM. Insulin resistance and lack of insulin production are caused by oxidative damage to β cells due to excessive ROS in the body and triggering fat peroxidation. Vitamin C works by donating its electrons to fat radicals to end the chain reaction in the fat peroxidation process. Vitamin E is the

main antioxidant in cell membranes. Previous studies have shown a protective effect of vitamin E intake against the incidence of type 2 diabetes. The action of vitamin E influencing glucose tolerance, insulin sensitivity, and insulin secretion is not yet understandable [26, 27].

In addition to macronutrient and vitamin intake, this study also assessed differences in the mean mineral intake of calcium, magnesium, iron, and zinc in the GSTM1 and GSTT1 variants. Insulin secretion is a calcium dependent process while calcium is one of the activator components of Calcium receptors (CaR). CaR can mediate cell-to-cell communication, including cells in the pancreas that allow changes in extracellular calcium (Ca2+) concentrations. Cell communication is mediated by CaR and allows β cell response to secrete insulin. Magnesium (Mg) is a mineral that makes it easy for glucose to enter cells and cofactor of various enzymes for glucose oxidation. Previous study showed low magnesium intake leading to impaired insulin secretion whereas magnesium supplementation decreased the incidence of T2DM [28, 29, 30].

Zinc (Zn) is known to improve the structure, synthesis, storage, and secretion of insulin so that they can synthesize glucose transporters that were translocated from intracellular to the plasma membrane. Glucose transporters help glucose molecules cross the cell membrane and reduce glucose buildup outside the cell. As a hemoglobin forming material, iron (Fe) is also considered important in the pathogenesis of T2DM. Macronutrient metabolic processes (carbohydrates, fats and proteins) require oxygen as a basic compound. Transport of oxygen is carried out by hemoglobin. Lack of Fe in the body causes lower amount of hemoglobin and reduced amount of oxygen being transported. Disruption of the oxygen transport process causes impairment of carbohydrate metabolism so that blood glucose levels are uncontrolled which causes an insulin resistance [31, 32].

Interestingly, this study found the mean levels of calcium, magnesium, zinc, and iron in the GSTM1 and GSTT1 null were lower than those in GSTM1 and GSTT1 positive. Some previous studies showed that GSTM1 and GSTT1 null act as a risk factor of T2DM. Further research needs to be done by including healthy subjects to analyze dietary intake and its role in GSTM1, GSTT1 gene variants as risk factors of T2DM.

## Conclusions

This study concluded that there were significant differences in mean lipid intake, RDA lipid %, lipid % carbohydrate, and vitamin E intake in GSTM1 positive compared to GSTM1 null. There were also significant differences in the mean of energy, vitamin E, and calcium intake in GSTT1 positive compared to GSTT1 null in T2DM patients at USU Hospital, Medan, Indonesia.

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## Conflict of Interest

The authors declare no conflict of interest.

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