Reduction of Dipeptidyl Peptidase 4 Activity in Patients with Type 2 Diabetes Mellitus Who Consumed Fruits Before Meals

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ABSTRACT

Objectives Some patients with type 2 diabetes mellitus (T2DM) have high expression of dipeptidyl peptidase 4 (DPP4) enzymes that breaks down glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP1) hormones. In the process of eating carbohydrates, K and L cells in the human small intestine produce GIP and GLP1 respectively that stimulate pancreatic β cells to release insulin. Recent evidence has revealed that fruit consumption before meals reduces fasting blood glucose level in patients with T2DM. Therefore, this study aimed to investigate the effect of fruit consumption before meals on DPP4 activity among patients with T2DM.

Methods A randomized control trial study with pre-posttest control group design was performed in 18 T2DM patients. They were randomly divided into 2 groups: 10 in the control (C) group eating a standardized diet and the other in the treatment (T) group eating fruit before consumption of standardized diet. Fasting and postprandial serum DPP4 activities in 30 min were measured using an H-Gly-Pro-pNA substrate in the 1st and 7th day’s intervention. Paired Sample t-Tests and Wilcoxon were used to analyze mean DPP4 activity in both groups with p value < 0.05.

Results In 1st day intervention, mean fasting DPP4 activity in the C group (189.42±116.27 nmol/ min/ mg protein) was higher than mean fasting DPP4 activity in the T group (121.75±74.86 nmol/ min/ mg protein). Reduced postprandial DPP4 activity was found in the T group (116.86+61.44) and increased in the C group (287,15+146.72). After 7 days intervention, reduction of fasting and postprandial DPP4 activities was observed in the C and T groups respectively. Meanwhile, mean fasting DPP4 activity in the T group remained unchanged but followed by increasing postprandial DPP4 activity.

Conclusions Fruit consumption before meals reduces fasting and increases postprandial DPP4 activities in patients with T2DM.

Keywords: Type 2 diabetes mellitus, Fruit consumption, DPP4 activity

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INTRODUCTION

The prevalence of Diabetes Mellitus (DM) patients in Indonesia remains to increase and occupy the seventh top rank around the world (1). Prevalence of diabetes in the WHO South-East Asia Region shows the amount of DM patients in Indonesia reach 8,426,000 in 2000 and will rise by 2.5% in 2030 (2). According to RisetKesehatan Dasar/ RIKESDAS report in 2018, the prevalence of diabetes mellitus patients ≥ 15 years old increased 5% from 2013. Baseline study in some of Health
Primary Care in Central Java showed most of the DM patients have regularly taken their medicine but have low physical activity and difficulty arrange their daily intake.

Up to this moment, diet therapy was the first part of Diabetes Mellitus (DM) Management besides physical exercise, oral antidiabetic agents, and nutrition education. AsosiasiPersatuanEndokrinologi Indonesia/ Perkeni has given the simple dietary management principle that is 3 J (time eating regularly, appropriate consume variety and amount of food) for DM patients. Some recent studies show other modification on diet therapy with change the order of type of meal (vegetables or protein-carbohydrate) to improve glycaemic excursions of DM patients (3) (4).

In traditionally meal patterns, the fruit has been known to consume as a dessert. Some health practitioner restricts even prohibit DM patients to consume fruit because its fructose contained that can increase blood glucose. Even though, fruit rich in vitamins and minerals also fiber have most of the health benefits for the human gut and decrease T2DM and metabolic disease incidents (5,6). Recent evidence has revealed that fruit consumption before meals reduces fasting blood glucose level in patients with T2DM.

The incretin hormones glucagon-like-peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are released from K and L cells in the human small intestine during oral ingestion of nutrients. Incretins stimulate pancreatic β cells to release insulin while GLP-1 that affect blood glucose surge through inhibits glucagon release and gastric emptying (7,8). Some patients with type 2 diabetes mellitus (T2DM) have higher expression of plasma DPP4 activity that breaks down incretin hormones (9–11). Therefore, this study aimed to investigate the effect of fruit consumption before meals on DPP4 activity among patients with T2DM.

**METHODS**

This study used a randomized control trial with a pre-post-test control group design to assess the effects of eating fruit before meals in people with type 2 diabetes (T2DM). A selective sample method was used to determine our subject. A total of 18 elderly subjects (16 female and 2 male) who members of two Primary Health Cares in Tasikmadu and Kartasura Districts followed this study. Eligible subjects criteria: subjects had to be between the ages of 40-65 years old, being treated with add-on hypoglycemic agents (metformin and sulfonylurea, not DPP-4 inhibitors), without insulin, not on diet therapy, did not smoke, had poor glycemic control and did low physical exercise (< 45 minutes/day, <3 times/week). During the period of study all subjects took their medications at the usual dose, frequency, and time.

Our study began with baseline assessments for estimating energy and macronutrient need of the subjects. Baseline assessment included measurement of anthropometric (height and body weight), biochemical (fasting and 2h postprandial blood glucose), physical clinical (systolic and diastolic blood pressure), dietary intake in 24 hours, personal history (long suffering of DMT2, physical exercise habit). The body weight (BW) of subjects was measured using a SMIC digital personal scale (China) and the body height used a GEA Medical SH-2A (China). Blood glucose levels were measured using Semi-Automatic Chemistry Analyser BM-sp7000S (China) and blood pressure used GEA Mercury Sphygmomanometer (China).
The intervention period was done in 14 days which gave the nutrition counseling section in the first week and the next week after that all subjects applied the new pattern meal (eating fruit first every meal) and consumed a standardized diet. All subjects were divided into 2 groups: 10 subjects in control (C) and 8 subjects in the treatment (T) group. The first and seventh days of treatment were done in the Primary Health Cares. While the second and sixth days of treatment, subjects in each group did the procedure treatment at home and were monitored by enumerators.

Subjects had to arrange their daily meal plans based on their daily energy requirements calculated using Perkeni equations. In the first and seventh days of treatment, subjects consumed a standardized diet that consisted of carbohydrates (a mixture of brown and white rice), protein (tofu, egg or meat), 100 g mixed vegetables, and 1 piece of banana. The total energy and nutrient values of the standardized diet were displayed in Table 1. The daily meal plans for daily energy needs can range from 1100 until 2100 kcals. The total energy was distributed into 3 main meals and 2 snacks per day that allocated as follows one-fifth of total energy/ days was given in breakfast and dinner while one-third for lunch. The proportion of daily macronutrient needs consisting of 55% carbohydrate, 25% fat, and 20% protein. In particularly, fiber needs follow Institute of Medicine/ IOM (2005) (12) and American Dietetic Association/ ADA (2016) (13) recommendation that adults must consume14g fiber/1000 kcal equivalent to 25g/day for women and 38 g/day for men. Subjects recorded their food and time of meal consumption in daily intake book.

<table>
<thead>
<tr>
<th>DI (Day)</th>
<th>E (kcal)</th>
<th>P (g)</th>
<th>L (g)</th>
<th>CH (g)</th>
<th>F (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>521.72 ±64.25</td>
<td>17.66 ±3.42</td>
<td>17.93±9.15</td>
<td>76.74±9.15</td>
<td>7.20±0.55</td>
</tr>
<tr>
<td>7th</td>
<td>562.63 ±69.02</td>
<td>18.16 ±4.03</td>
<td>22.01±9.08</td>
<td>76.59±9.08</td>
<td>7.70±0.55</td>
</tr>
</tbody>
</table>

DI: Days of Intervention; E: Energy; P: Protein; L: Fat; CH: Carbohydrate; F: Fiber; g: gram

Blood samples were collected in the first and seventh days of intervention. Fasting blood samples were taken before subjects eating and postprandial blood samples were taken 30 minutes after subjects eat. The blood sample was diluted with PBS pH 7.4 (40 µL) and added 50 µL of 2 mM H-Gly-Pro-pNA substrate (Sigma- Aldrich, USA) (50 µL). Once samples were mixed completely with the substrate, the DPP-4 activity was measured spectrophotometrically at λ=405 nm at room temperature, every 10 min for 60 min. Finally, the DPP-4 enzyme activity was calculated using the Beer-Lambert formula which we performed previously (14).

The data were analyzed using SPSS (version 21.0). Descriptive data are presented as means ± standard deviations. Baseline data were analyzed with Independent t-Test for normally distributed data and Mann Whitney U for vice versa. DPP-4 enzyme activity, energy, and macronutrient from daily intake were analyzed with parametric (Paired samples t-Test) and non-parametric (Wilcoxon) tests. The p-value <0.05 was considered significant.
RESULTS

Our baseline study showed characteristics participants in both of group (Table 2). All participants were elderly aged with mean age >50 years old. C group had higher average of BMI, systolic blood pressure, protein and fiber intake than that of P group although it did not differ significantly. Whereas P group had higher fasting blood glucose levels, length duration of T2DM, more energy intake, fat intake and carbohydrate intake than C group but did not significantly differ. All participants in both groups had overweight, normal diastolic blood pressure and higher 2h-postprandial blood glucose levels and low physical exercise.

During baseline study period, we found almost of T2DM patients never got nutrition education, have uncontroled blood glucose levels, low daily intake, suffer DM for < 10 year with no complication disease, and can not fill recommended intensity physical exercise 150 min/ week. In nutrition education section, basic information came from T2DM patients, they told about restriction of rice and sweetened foods consumption would reached good blood glucose levels. They changed rice intake with other carbohydrate source like potato, cassava, sweet cassava. Moreover they reduce their time meal become 2 times/ day and added their snack time.

The alteration of DPP-4 enzyme activity in C and T group showed in figure 1. After nutrition education section, eating standardized diet and eating fruit first every meal, decrease mean DPP-4 enzyme activity in 0 min was found at C and T group. In the 1st days intervention, the higher average of DPP-4 enzyme activity in 0 min appeared in C group compare with T group respectively (189.42±116.27; 132.59±72.09 nmol/ min/ mg protein). After 1 week intervention period, the mean DPP-4 enzyme activity in 0 min decreased in both group. The average DPP-4 activity in T group was significantly decreased compare with C group(p=0.017).

Table 2 Participants Characteristics (n=18)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (C)</th>
<th>Treatment (T)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>53.02 ± 7.28</td>
<td>55.88 ± 4.19</td>
<td>0.425</td>
</tr>
<tr>
<td>Body Mass Index, kg/m2</td>
<td>26.79 ± 1.73</td>
<td>25.16 ± 5.64</td>
<td>0.473</td>
</tr>
<tr>
<td>Systolic Blood Pressure, mmHg</td>
<td>126 ± 16.74</td>
<td>126.25 ± 15.98</td>
<td>0.890</td>
</tr>
<tr>
<td>Diastolic Blood Pressure, mmHg</td>
<td>85 ± 6.90</td>
<td>85 ± 7.96</td>
<td>0.873</td>
</tr>
<tr>
<td>Fasting Blood Glucose, mg/dL</td>
<td>221.50 ± 105.84</td>
<td>220.90 ± 87.52</td>
<td>0.982</td>
</tr>
<tr>
<td>2h-Postprandial Blood Glucose, mg/dL</td>
<td>316.50 ± 154.60</td>
<td>316.50 ± 125.05</td>
<td>0.997</td>
</tr>
<tr>
<td>Duration of T2DM, years</td>
<td>4.65 ± 5.48</td>
<td>7.31 ± 5.43</td>
<td>0.099</td>
</tr>
<tr>
<td>Exercise Intensity, min/week</td>
<td>105.50 ± 22.54</td>
<td>89.38 ± 6.78</td>
<td>0.107</td>
</tr>
<tr>
<td>Recall</td>
<td>0.69 ± 375.68</td>
<td>0.69 ± 459.12</td>
<td>0.572</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>34.11 ± 19.96</td>
<td>32.48 ± 19.42</td>
<td>0.862</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>31.94 ± 19.67</td>
<td>37.39 ± 10.05</td>
<td>0.564</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>129.31 ± 43.37</td>
<td>140.20 ± 54.42</td>
<td>0.642</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>6.72 ± 1.96</td>
<td>5.97 ± 3.35</td>
<td>0.550</td>
</tr>
</tbody>
</table>

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The differences of DPP-4 enzyme activity in 1st and 7th at both groups also observed in 30 min after eat standardized diet (Figure 2). In the 1st days intervention, higher mean DPP-4 enzyme activity was found in C group (287.15+146.72 nmol/min/mg protein). After intervention period significantly decreased of DPP-4 enzyme activity appeared in C (p=0.013). Whereas significantly increase mean DPP-4 enzyme activity was showed in T group (p=0.027). Furthermore, C group showed more decreased mean DPP-4 enzyme activity in 30 min (136 nmol/min/mg protein) and T group have a little increase (4 nmol/min/mg protein).
After 1 week intervention, the addition of carbohydrate and fiber consumption occurred in both groups (Figure 3A and 3B). Carbohydrate intake in C group increased 4% while T group increased 21% from the first days' intervention. Fiber intake in T group could reach 1.5 times compared with C group only 1 time from the first days' intervention. After nutrition education and intervention, carbohydrate and fiber intake in T group significantly increased (p=0.033; p=0.012).

**DISCUSSION**

Fiber has many benefits for human health. Dietary fiber was obtained from daily vegetables and fruits consumption. Fruit and vegetables may contain resistant starch and oligosaccharides that cannot digest in the stomach but go to the colon for bacterial fermentation (15). A beneficial effect from fiber digestion by colon bacteria can stimulate the growth and/or activity of one or a limited number of good bacteria and thus improves human health (16,17). In our study, we found a positive effect from eating pattern changes in form consuming fruit before meals can decrease DPP-4 enzyme activity. This finding was in line with the in vitro study of Zeng et al. (18), Bifidobacterium strain (good gut microbiota) could inhibit the DPP-IV activity. The other evidence showed that the other good gut microorganisms like Lactobacillus strains can hydrolyze proteins that present in the environment (19).

DPP-4 is a glycoprotein that acts as a transmembrane protein and released into the circulation during digestion activity (8). The reduction of mean DPP-4 activity may be related to gut microbiota activity that can breakdown the protein bond of the DPP-4 enzyme. This positive effect could reduce incretin hormone (GIP and GLP-1) damage by the DPP-4 enzyme in T2DM patients. The other finding was showed from eating behavior change based on the nutrition education section and the sample menu for T2DM (standardized diet) can reduce DPP-4 enzyme activity. While eating fruit first showed constantly reduction or small increased of DPP-4 enzyme activity. Nutrition education session, meal sample (standardized diet) and...
new habit eating fruit first can reduce DPP-4 enzyme activity. Our evidence was similar to Ouyang et al. 2015 study that indicated T2DM who followed nutrition education and implemented self-care behavior have better glycemic control.

Energy, macronutrient, and fiber intake were also evaluated to assess T2DM eating behavior after the nutrition education section and during the intervention period. All participants in the group have been consumed below recommendation energy, micronutrient and fiber personal guidelines based on Perkeni equation and recommended nutrient proportion by ADA and Perkeni. In the first days' intervention, we found the total energy, micronutrient and fiber intake in C group higher than T group, but did not differ significantly. On the contrary after nutrition education and intervention, T group showed enhancement of daily energy, macronutrient, and fiber intake. While in C group showed a decrease of total energy, protein and fat intake but not in carbohydrate and fiber intake.

Eating pattern improvement of diabetic patients appeared in our study. After nutrition education and private counseling diet therapy, all patients diabetic learned a private sample meal for them in 1st and 7th days' intervention. During intervention periods especially in 2nd until 6th days' intervention, all patients diabetic try to practice the standardized meal into their daily meal. Moreover, dietary consultants of each group did guidance and evaluation by telephone to keep their dietary adherence. In these periods all diabetic patients followed recommendation DM diet therapy independently. During our study, we found changed perception and knowledge in diabetic patients. According to their daily intake book, we found differences in variety, amount and time eating schedule. During intervention periods, all diabetic patient have regular eating time, consume another carbohydrate source (cassava, potatoes, noodle, bread) in small portion when snack time or replace rice with other that used replacement raw food guide to result in the same standard portion. The effectiveness of dietary education can improve dietary eating pattern diabetic patients thus help them control their blood glucose to reach the recommended goal. The effectiveness of dietary education can improve dietary eating pattern diabetic patients thus help them control their blood glucose to reach recommended glycemic target (20,21).

Furthermore, we finding the effectiveness of using telecommunication electronic media, record their daily intake food, and simple eating guide with sample meal menus and raw food replacement guide. Our findings in line with Krishna and Boren (2008) study that showed support care with cell phones and text messages can improve knowledge and self-efficacy in diabetic patients self-management. The active participation activity of diabetic patients helped them to regard their food habits. The active participation activity in our study was recorded their daily food intake in a book and estimated their portion meal with food scales of household size food (spoon and glass). The food recorded activity helps diabetic patients and health practitioners have known and analyzed the healthy eating pattern, based on the frequent intake of whole carbohydrates, protein and fat sources, fruits, and vegetables thus related to glycemic control in patients with diabetes (22).

In this study, we did not evaluate the association of dietary adherence to DPP-4 enzyme activity and glycemic control indicators. Further studies are therefore required for improvement of eating guide especially for T2DM in sequence, portion, and food variety to achieve controlled blood glucose levels and body weight. Besides, the length of intervention can be considered to evaluate nutrition education technic and simple and easy dietary management. Our findings from this study were appropriate to apply in prediabetes patients to improve their eating patterns and self-efficacy to disease development.
In conclusion, change the sequence of fruit consumption before meals can increase fiber intake in daily eating patterns. Furthermore, eating fruit first can reduce fasting and increase postprandial DPP4 activities in patients with T2DM.

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