Fructooligosaccharide Supplementation Did Not Affect Plasma Glucagon-like Peptide-1 and Postprandial Glucose Levels in Type 2 Diabetes Mellitus

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ABSTRAK
Tujuan: Mengetahui pengaruh pemberian fruktooligosakarida (FOS) terhadap kadar glucagon-like peptide-1 (GLP-1) dan glukosa darah dua jam postprandial (2 JPP) pada diabetes melitus (DM) tipe 2.
Metode: Studi pre-post test design melibatkan 30 pasien DM tipe 2 yang beraobat jalan di poliklinik Metabolik Endokrin RSCM dan Klinik Dokter Keluarga FKUI dari bulan Maret hingga Juni 2010. Subyek diberi 10 gram FOS satu kali per hari selama empat minggu berturut-turut disertai konseling gizi. Pemeriksaan kadar GLP-1 menggunakan metode ELISA dan glukosa darah 2 JPP dengan metode heksokinase. Perbedaan rerata dihitung menggunakan uji Wilcoxon. Data dinyatakan bermakna bila p<0,05.
Hasil: Kadar GLP-1 (puasa, menit ke-10 dan 120 setelah makan) sebelum perlakuan adalah 3,1 (1,33–6,37), 3,3 (2,08–6,03) dan 3,0 (1,24–5,77) ng/mL, sedangkan kadar setelah perlakuan adalah 3,0 (1,68–5,36), 3,2 (2,09–7,48), dan 3,0 (1,92–6,03) ng/mL (p>0,05). Kadar 2 JPP sebelum perlakuan 198,5 (111–376) mg/dL dan setelah perlakuan 211 (108–403) mg/dL (p>0,05).

INTRODUCTION
Glucagon-like peptide-1 (GLP-1) is an incretin hormone, which has an important role in lowering prandial glucose level. In pancreatic islets, GLP-1 stimulates insulin secretion and suppresses glucagon secretion. Hormone GLP-1 is secreted in response to a meal, beginning within minutes and last for 30–60 min, and overlapping second phase that prolong secretion until 60–120 min after a meal. The effect of GLP-1 on glucose metabolism are preserved in type 2 diabetes mellitus (DM), but its secretion in response to a meal ingestion is reduced.¹ Synthesis of GLP-1 occurs in endocrine L cells, located along distal intestine (mostly in the ileum and colon).² In caeco-colon, the fermentation of fructooligosaccharide (FOS) into short chain fatty acid (SCFA) promotes proglucagon mRNA expression in mature intestinal L cells.³

In vitro study showed that FOS supplementation increased GLP-1 level, mostly in colon,⁴ Cani et al found significant increase GLP-1 secretion through promoting proglucagon mRNA.⁵ Study showed that FOS supplementation in type 2 DM patients decreased fasting plasma glucose (FPG),⁶ but other studies showed no decrease.⁷ Despite many studies examining the effect of FOS on GLP-1 level (in animal) and on blood glucose level (in human), the influence of FOS on GLP-1 secretion and its response to postprandial blood glucose levels in human has not been established. We decided to assess the influence of FOS supplementation on GLP-1 and two-hour postprandial (2-h PP) blood glucose levels.

METHODS
This subject is the one group pre and post-test design was carried out at Metabolic and Endocrine Clinic, Dr.Cipto Mangunkusumo Hospital and in Family Physician Clinic, Kayu Putih, Jakarta from March to June 2010. This study had been approved by The Committee of Medical Research Ethics of the Faculty of Medicine, University of Indonesia. Informed consent from all subjects was obtained at the time of enrollment.

Inclusion criteria: 1. Patients with type 2 DM (according to Indonesian Endocrinology Association [PERKENI] criteria) in the outpatient setting, who already taking biguanide alone (at any dose) or in combination with sulphonylurea or glinid. 2. The level of 2-hour postprandial blood glucose was more than 145 mg/dL. 3. Not using GLP-1 mimetic or dipeptidyl peptidase IV (DPP IV) inhibitor agent. 3. Provides written consent. Exclusion criteria: 1. Already treated with insulin, 2. Impaired renal (creatinine >1.2 g/dL) and liver (albumin <3 g/dL) function. 3. Pregnant or breastfeeding.

Ten grams of FOS from Orafti P95 (BENEO-Orafti, Tienen, Belgium) was included in beverages and administered daily for 28 days accompanied with nutritional counselling. Beverages were packed in 12 g aluminium foil sachets and stored in room temperature. Each subject received seven sachets of beverages weekly and were asked to drink one sachet per day, several minutes before meal. Subjects were asked to return their empty sachets to determine their compliance before receive...
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new supply. Composition of beverages are listed in table 1.

Table 1 Composition of the beverage

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>FOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount per serving</td>
<td>12</td>
</tr>
<tr>
<td>Calories</td>
<td>43.14 kcal</td>
</tr>
<tr>
<td>Total sugar</td>
<td>0.68 g</td>
</tr>
<tr>
<td>Water</td>
<td>0.25 g</td>
</tr>
<tr>
<td>Ash</td>
<td>0.24 g</td>
</tr>
</tbody>
</table>

FOS = fructooligosaccharides; g = gram; mg = miligram; kcal = kilocalories

We enrolled subjects consecutively from their physicians. Sample size was calculated using the formula for one group pre and post test design. Total sample required was calculated based on level of 2-h postprandial blood glucose. Using 10 mg/dL as the mean difference and 20.35 mg/dL as the standard deviation, with α= 0.05 and power of study equal to 80%, a minimum sample size of 33 subjects was considered adequate. Thirty subjects (90.9%) completed the study.

MEASUREMENT

Dietary assessment used 24-h food recall; food model and household utensils were used as memory aids in assessing portion size to minimize bias. Three-day non consecutive food records were assessed during intervention. All dietary intake were analysed with Nutrisurvey 2007. Body mass index was referred to Asia Pacific parameter. Height was measured using microtoise to the nearest 0.1 cm and weight to the nearest 10 gram with a digital balance (Secasensa 804, Germany).

Thirteen mililiters of blood (9 mL into EDTA-tubes, and 4 mL into sodium floride containing tubes) was collected by venipuncture after fasting period, 10 minutes (for GLP-1) and 120 minutes after breakfast (for GLP-1 and 2-h postprandial). Plasma was separated within 60 minutes and stored at -70°C until assay. GLP-1 level was measured with human GLP-1 EIA kit (Yahara Institute Inc, Japan), and 2-h postprandial blood glucose level was measured with hexokinase enzymatic method (ADVIA, Germany).

STATISTICAL ANALYSIS

Statistical analysis was done with Statistical Package for Social Science (SPSS) programme version 11.5 software. Normally distributed data was presented as mean ± standard deviation, while data not normally distributed was presented as median (minimum-maximum). Normality was assessed by Shapiro-Wilk method. Differences in mean were assessed by paired t-test for the normally distributed data and Wilcoxon test for data not normally distributed. Food record data was not normally distributed, assessed by Friedman method. All statistical significance for the test was at p <0.05 level.

RESULT

Out of 30 study subjects, 23 subjects were females. The mean age was 60.3 ± 8.26 year. Half subjects were categorized as obese, mostly grade I (43.3%).

Data from 24-h food recall showed wide range intake of all nutrients (energy, carbohydrate, protein, fat, and FOS). All nutrients intake were not different during intervention, except fiber (p<0.05), but did not affect main outcome (table 2).

During intervention, two subjects felt nausea, three subjects had diarrhea, and four subjects had flatulence. FOS supplementation reduced constipation in eight subjects. After four weeks of intervention, GLP-1 level was not significantly increased (p>0.05), and 2-h postprandial blood glucose level did not significantly decreased (p>0.05). (table 3).

DISCUSSION

The majority patients in this study were 55–64 years, similar to type-2 DM prevalence in the developing countries (45–64 years range), and was also similar compared to type 2 DM worldwide, which showed that among >60 years of age the prevalence was higher among females.

The level of GLP-1 in this study did not significantly increase (p>0.05), in terms of duration of the disease. It also seems not affected by age, sex, BMI, diet compliance, and duration of DM. This occurrence may due to: First, since this study did not use DPP IV inhibitor agent, low GLP-1 level was linked to DPP IV activity to degrade the active GLP-1 in circulation within several minutes. Second, blood specimen was taken at 10 and 120 minutes postprandial, which allow nutrient entrance in the proximal gastrointestinal (gastric and small intestine). It induces first phase GLP-1 secretion, whereas the prolonged time second phase GLP-1 secretion from colonic L-cells not yet occurred.

In vitro study showed that the beneficial effect of FOS consumption in rat diabetes model are linked to an increase of GLP-1 level and its precursor, proglucagon mRNA. Animal study by Cani and co-workers concluded that FOS increased colonic GLP-1 level and doubled colonic proglucagon level, and also proposed

Table 2 Energy and nutrients intake during study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>1st week</th>
<th>2nd week</th>
<th>3rd week</th>
<th>p†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy intake (kcal)</td>
<td>1649.9</td>
<td>1394.5</td>
<td>1172.5</td>
<td>1260.7</td>
<td>0.67*</td>
</tr>
<tr>
<td>(% Carbohydrate intake/TER)</td>
<td>74.5 (45–114.6)</td>
<td>44.6 (29.8–93.4)</td>
<td>45.9 (24.3–97.8)</td>
<td>45.1 (28.8–102)</td>
<td>0.98*</td>
</tr>
<tr>
<td>(% Protein intake/TER)</td>
<td>19.5 (7–40.9)</td>
<td>13.9 (48–28.7)</td>
<td>13.2 (4.2–38)</td>
<td>12.2 (4.9–34.6)</td>
<td>0.55*</td>
</tr>
<tr>
<td>(% Fat intake/TER)</td>
<td>13.9 (17.6–89.2)</td>
<td>33.1 (12.3–60.7)</td>
<td>28.7 (3.2–76.1)</td>
<td>29.5 (11.4–81.2)</td>
<td>0.62*</td>
</tr>
<tr>
<td>Fiber intake (g/1000 kcal)</td>
<td>12.8 (2.4–29.9)</td>
<td>10.9 (5.8–27.6)</td>
<td>12.3 (4.9–22.1)</td>
<td>9.3 (4.1–20.6)</td>
<td>0.03</td>
</tr>
<tr>
<td>FOS intake (g)</td>
<td>0.1 (0–2.5)</td>
<td>0.3 (0–4.2)</td>
<td>0.3 (0–2.4)</td>
<td>0.6 (0–3.2)</td>
<td>0.65*</td>
</tr>
</tbody>
</table>

TER = total energy requirement; FOS = fructooligosaccharide; g = gram; kcal = kilocalories; * = not significant; † = Friedman test

Table 3 Body mass index, GLP-1, and 2-h PP levels before and after study

<table>
<thead>
<tr>
<th>Variables</th>
<th>Before</th>
<th>After</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.3 ± 3.3</td>
<td>25.3 ± 3.3</td>
<td>0.57 t</td>
</tr>
<tr>
<td>GLP-1 (ng/mL)</td>
<td>3.1 (1.3–6.4)</td>
<td>3.0 (1.7–5.4)</td>
<td>0.13 w</td>
</tr>
<tr>
<td>Fasting</td>
<td>3.3 (2.6–6.0)</td>
<td>3.2 (2.1–5.7)</td>
<td>0.99 w</td>
</tr>
<tr>
<td>10 minutes postprandial</td>
<td>3.0 (1.2–5.8)</td>
<td>3.0 (1.9–6.1)</td>
<td>0.91 w</td>
</tr>
<tr>
<td>120 minutes postprandial</td>
<td>198.5 (111–376)</td>
<td>211 (108–403)</td>
<td>0.96 w</td>
</tr>
</tbody>
</table>

GLP-1 = glucagon-like peptide-1; t = based on Asia pacific criterion
* = not significant; t = paired t-test; w = Wilcoxon test
that, FOS through its fermentation in colon promotes the expression and secretion of colonic GLP-1.1,12 Several studies reported an increasing GLP-1 level in various animal models, but not shown in this study. This study found 2-h PP blood glucose level did not significantly decrease (p>0.05). It showed that after 28 days supplementation, blood glucose level was higher than those before intervention. It appertains contrary to previous study which showed that FOS consumption did not affect on plasma glucose.13 The absence of any effect of FOS might be related to: 1) a progressive decline of pancreatic beta-cell function (start at beginning of disease and steady decline thereafter);14,15 median duration of DM in this study was 84 (1–300) months. 2) duration of intervention might be too short to affect blood glucose level. This was in agreement with the result of two studies by Alles and Luo.16,17 Longer period of FOS supplementation is required to decrease 2-h postprandial blood glucose level. 3) noncompliance of the recommended diet. Carbohydrate intake was categorized as low (<45% of total energy requirement/TER) in half of subjects, fat intake was categorized as high (>25% of TER) in more than half of subjects and fiber intake was low (<25 g/1000 kcal) in most subjects (96.7%).12 Low carbohydrate intake will increase glucagon to insulin ratio, which may induce glycosgenolysis and gluconeogenesis.12 High fat intake may cause insulin resistance and impaired intracellular glucose metabolism; decreases the number of insulin receptors in several tissues, glucose transport into muscle and adipose tissue, which lead to increased blood glucose level and worsened glycemic control among type 2 DM.12

Low fiber intake in this study is because most subjects considered fruits were more expensive than carbohydrate or fat food source. This was similar to data from Riset Kesehatan Dasar 2007, that 93.6% Indonesian population had low intake of fruits and vegetables.12 American Diabetes Association (ADA) proposed intake of 14 gram dietary fiber per 1000 kcal daily.12 This study had some limitations. First, one group pre and post test design couldn’t determine whether the result was caused by intervention.12 Second, this study didn’t fulfill a minimum sample requirement. To avoid bias in laboratory assessment, subjects were asked to fast for 10–12 hours and ate standard meal within 10 min. We used same blood taking procedure and GLP-1 were assayed with a standard procedure.

The administration of 10 grams FOS for 28 days with nutritional counselling did not increase GLP-1 level or decrease 2-h PP blood glucose in type 2 DM patients. This lack of effect could be explained by insufficient minimum sample size, diet non compliance, short study duration or pancreatic beta-cell function loss. Thus, our finding do not suggest that FOS is an effective means to favorably affect endogenous GLP-1 or blood glucose in short duration. It remains possible that longer study may show effect on endogenous GLP-1 or blood glucose in newly diagnosed type 2 DM patients who take DPP IV inhibitor agent.

ACKNOWLEDGMENTS
The authors would like to thank Mashudi for making FOS formulation and analysing in the Laboratory of Food Chemistry and Analysis, Departement of Community Nutrition, Faculty of Human Ecology, Bogor Agricultural Institute.

REFERENCES