Effect of a Moderate Carbohydrate-Restricted Diet on DPP-4 Inhibitor Action among Individuals with Type 2 Diabetes Mellitus: A 6-Month Intervention Study

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Summary To decrease body weight and insulin resistance, a calorie-restricted diet—with minimal caloric intake required for daily activities—is the primary treatment strategy for patients with type 2 diabetes (T2D) in Japan. However, many patients cannot continue with this diet for long, because calorie restriction is difficult and nutritional balance is hard to understand. Carbohydrate-restricted diets are easier for patients than conventional calorie-restricted diet. In this study we aimed to elucidate the effects of a moderate carbohydrate-restricted diet on glucose metabolism and renal function in patients with T2D on dipeptidyl peptidase-4 (DPP-4) inhibitors. Nineteen outpatients with T2D continued on a moderate carbohydrate-restricted diet (targeting 50% of calories) for 6 mo. Meanwhile, 10 other outpatients with T2D on DPP-4 inhibitors had the conventional calorie-restricted diet using the food exchange table. No change in prescription drugs occurred for both groups during the study period. After the intervention, the carbohydrate content in dietary intake was lowered significantly from 56.8±8.3 to 46.8±10.1%, while the lipid concentration, primarily n-6 polyunsaturated fatty acids, was significantly increased. There was no significant change in protein intake. Hemoglobin A1c (HbA1c) fell from 7.22±0.74% to 6.95±0.72% (mean±SD). Furthermore, salt intake decreased significantly from 6.8±2.5 g prior to the intervention, to 5.7±1.9 g after the intervention. The estimated glomerular filtration rates (eGFR) decreased slightly, while serum creatinine levels did not change. These findings suggest that a moderate carbohydrate-restricted diet (50%) is effective in patients with T2D, without affecting kidney function.

Key Words type 2 diabetes, DPP-4 inhibitor, carbohydrate-restricted diet, HbA1c, renal function

Diabetes mellitus is characterized by hyperglycemia due to absolute or relative insulin deficiency. The frequency of type 2 diabetes (T2D) is increasing globally (1). Asians tend to eat greater amounts of rice and have a high risk of developing T2D (2, 3). According to the American Diabetes Association (ADA), blood sugar is mainly affected by the intake of carbohydrates among the three major nutrients (carbohydrate, lipid, and protein) (4). A reduction in carbohydrate intake is important for blood glucose management (5). However, an extreme reduction in carbohydrate intake is considered problematic (6).

An ADA statement, released in 2013 regarding the diet of adult patients with diabetes, includes a variety of accepted dietary patterns (i.e. Mediterranean, vegetarian, carbohydrate-restricted, low fat, and dietary approaches to stop hypertension [DASH] diets) (7).

In 2013, the Japan Diabetes Society did not advise adopting a carbohydrate-restricted diet due to the lack of clinical data. In addition, Japan Diabetes Society does not allow carbohydrate intake below 50% of the total energy intake (8).

The principles of any diet are that of adequate energy intake and nutritional balance by food exchange based on the food exchange table (9). However, many patients cannot accept these principles, resulting in these patients developing complications due to diabetes; approximately 40% of Japanese patients with T2D develop complications due to diabetic nephropathy (10).

Many patients with T2D are taking drugs. Dipeptidyl peptidase-4 (DPP-4) inhibitors are one of the most commonly used in patients with T2D. Many anti-diabetic medications cause hypoglycemia as a side effect. DPP-4 inhibitors are known to be less likely to cause hypoglycemia; therefore, a combination treatment involving DPP-4 inhibitor administration and a moderate carbohydrate-restricted diet is less likely to cause hypoglycemia. However, the effects of this combination treatment remain unclear. In order to reduce the complications

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Table 1. Characteristics of the study participants.

|                  | Control group | LC group | p value |
|------------------|---------------|----------|---------|
| n (men/women)    | 10 (7/3)      | 19 (3/16) | 0.011*† |
| Age (y)          | 69.7±6.9      | 64.8±9.4 | 0.128† |
| Height (cm)      | 163.5±8.4     | 154.3±8.2 | 0.012†† |
| Body weight (kg) | 63.1±10.2     | 57.0±8.1 | 0.124†† |
| Fasting blood sugar (mg/dL) | 138.1±19.5 | 135.9±25.1 | 0.401†† |
| Insulin (μU/mL)  | 5.74±3.62     | 6.59±3.22 | 0.270†† |
| HOMA-IR          | 1.97±1.35     | 2.11±0.98 | 0.383†† |

Blood pressure

|                  | Control group | LC group | p value |
|------------------|---------------|----------|---------|
| Systolic (mmHg)  | 132.5±16.9    | 127.6±14.7 | 0.448†† |
| Diastolic (mmHg) | 69.0±8.6      | 73.5±10.8 | 0.230†† |
| History of diabetes (y) | 8.4±4.5 | 13.2±5.9 | 0.023*†† |

*p<0.05.
† Chi square test (Fisher exact probability).
†† Unpaired t-test.
LC, low carbohydrate; HOMA-IR, homeostatic model assessment for insulin resistance.

METHODS

Participants. Nineteen patients with T2D, who were outpatients at clinics in Shimonoseki City, Japan, were included in this study. None had severe complications such as neuropathy, nephropathy, retinopathy, or ketoacidosis; additionally no patients had other severe diseases such as renal failure, decompensated liver cirrhosis, or malignant diseases. All the patients were treated pharmacologically (i.e. with DPP-4 inhibitors alone), with no changes to their treatment during the study period. The age of the patients ranged between 50 and 80 y.

Intervention. The intervention was performed over 6 mo, between May 2015, and May 2016. During the intervention, the patients were randomized into two groups: 12 patients were treated with a conventional calorie-restricted diet (control group), and the other 20 patients were treated with a carbohydrate-restricted diet (low carbohydrate [LC] group). Two patients in the control group dropped out during intervention: one due to personal work commitments, and the other moved outside of the study area. Thus, only 10 patients in the control group completed the study. One patient in the LC group failed to continue the diet because of hospital admission due to a bone fracture. Thus, in total, 19 patients were included in the LC group.

During the intervention, all participants (in both groups) received dietary guidance once a month directed from a registered dietitian who had vast experience educating patients with T2D on calorie-restricted diets. The dietary guidance for the LC group was focused on moderate restriction of carbohydrates (targeting 50% carbohydrate) and the additional intake of fat in order to maintain the total calorie intake, whereas for the control group, calorie restriction according to a food exchange table was required. The participants were instructed to reduce salt intake in order to prevent future complications.

Biochemical examination of blood was performed using a GA08 III automatic biochemical analyzer (A&T Corporation, Tokyo, Japan). Hemoglobin A1c (HbA1c) levels were analyzed via high performance liquid chromatography using HLC-723G9 ( Tosoh Corporation, Tokyo, Japan).

Nutritional and statistical analysis. Patients recorded in a diary all their meals for 3 d, which were not consecutive, but two weekdays and one day during the weekends. All the data are expressed as mean ± standard deviation (SD). Nutritional intake was calculated from the records using Excel Eiyo-kun version 6 (Kenpaku Co. Ltd., Tokyo, Japan), and then added on to Microsoft Excel (Microsoft Corp., Redmond, WA, USA). Statistical analyses were performed on Excel and EZR (11), and included t-tests, analysis of variance (ANOVA), Friedman rank sum tests, and post-hoc Bonferroni analysis; independent group t-tests were used when comparing between two groups. The effect of strain was evaluated using ANOVA, followed by post-hoc Bonferroni tests for pairwise comparisons of significant variables. A p-value less than 0.05 was considered to be statistically significant for all experiments.

This study was conducted in compliance with the Declaration of Helsinki, and ethical clearance was granted by the Suzuka University of Medical Science Clinical Research Ethics Committee (No. 182). Informed consent was obtained from all participants.

RESULTS

There were no differences in the basic characteristics such as age, body weight, HbA1c, fasting blood glucose, insulin, homeostatic model assessment for insulin resistance (HOMA-IR), and blood pressure, between patients and controls. However, the ratio of females to males in the LC group was higher than that in the control group.
while height was lower, and the history of diabetes was significantly longer (Table 1).

The HbA1c in the LC group significantly decreased by more than 0.2% by the 4th, 5th, and 6th months, in comparison with the baseline values, although there was no change in control group HbA1c (Fig. 1B). Body weight of both the control and LC groups did not significantly change during the intervention (Fig. 1A).

In order to compare the differences in the dietary intakes between the groups, we analyzed the nutritional value thereof. Total energy intake for both groups did not significantly change, whereas that of the LC group was constantly less than that of the control group (Fig. 2A). Mean carbohydrate intake as a percentage of total energy intake (E%) of the LC group was approximately 60% at baseline, which did not differ from that of the control group (Fig. 2B). During and after the intervention, the E% of carbohydrates in the LC group decreased significantly from 56.1±8.3 to 46.8±10.1, whereas that of the control group did not significantly change. We recommended that participants in the LC group increase their fat intake in order to maintain their total

Fig. 1. Body weight and HbA1c. (A) Relative body weight before, during, and after the intervention. Body weight at time zero just before the intervention was used as baseline. (B) HbA1c before, during, and after the intervention. A solid line indicates the LC group. A broken line indicates the control group. *p<0.05, **p<0.01 (t-test between the groups). *p<0.05, **p<0.01 (ANOVA with post hoc Bonferroni test). LC, low carbohydrate; HbA1c, hemoglobin A1c; ANOVA, analysis of variance.

Fig. 2. Dietary intake. (A) Total energy per standard body weight, and %energy of (B) carbohydrate, (C) fat, and (D) protein of dietary intake before, during, and after the intervention. A solid line indicates the LC group. A broken line indicates the control group. *p<0.05, **p<0.01 (t-test between groups). *p<0.05, **p<0.01 (ANOVA with post hoc Bonferroni test). LC, low carbohydrate; ANOVA, analysis of variance.
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The intake of fat was compared between the groups (Table 2). The contents of fat intake were compared during and after the intervention (Fig. 2C). The fat intake significantly decreased during and after the intervention. Indeed, the E% of fat in the LC group increased, while the E% of protein in both groups did not significantly change during or after the intervention (Fig. 2D). Salt intake decreased significantly from 2.88 ± 0.6 to 1.25 ± 0.1 g (before) to 6.8 ± 0.7 g (after).

Restriction of carbohydrate intake may result in increased protein intake, which can place a burden on renal function; however, protein intake in the LC group did not increase during the study period. Therefore, we examined the eGFR of the LC group. As a result, eGFR of the LC group significantly decreased slightly (Fig. 3A). Additionally, serum creatinine levels in the LC group did not significantly change compared to baseline.

**DISCUSSION**

In this study, we aimed to effectively control blood sugar among patients with T2D by introducing a moderate-carbohydrate-restricted diet with concurrent DPP-4 inhibitor administration. As a result, the HbA1c levels and eGFR of patients in the LC group significantly decreased after the intervention.

In a previous study, severely-restricted dietary carbohydrate intake lowered HbA1c and body mass index after a short period, however, attrition was high over the study period (12). In the present study, attrition

**Table 2. Intake of fat.**

|                      | Control                  | LC                        | Student t-test |
|----------------------|--------------------------|----------------------------|----------------|
|                      | Friedman                 | Bonferroni                | Friedman       | Bonferroni    |                      |
|                      | rank sum test            |                           | rank sum test  |                           |                      |
| Total fatty acid (g) | Before 38.15 ± 12.49     | 0.003**                   | 31.14 ± 10.88  | 0.018*                   | 0.153               |
|                      | During 30.40 ± 7.70      | 0.006**                   | 40.22 ± 14.29  | 0.014*                   | 0.023               |
|                      | After 37.84 ± 12.28      | 0.672                     | 40.89 ± 15.13  | 0.019*                   | 0.564               |
| Saturated fatty acid (g) | Before 12.10 ± 3.89     | 0.061                     | 11.02 ± 4.52   | 0.532                    | 0.509               |
|                      | During 9.86 ± 3.42       | 0.010                     | 12.18 ± 4.37   | 0.460                    | 0.130               |
|                      | After 12.18 ± 3.88       | 0.513                     | 12.14 ± 5.17   | 0.460                    | 0.984               |
| Monounsaturated fatty acid (g) | Before 16.31 ± 5.92    | 0.082                     | 12.73 ± 5.86   | <0.001**                 | 0.138               |
|                      | During 12.92 ± 4.02      | 0.010                     | 17.81 ± 6.67   | 0.004**                  | 0.021               |
|                      | After 16.50 ± 6.60       | 0.866                     | 17.96 ± 7.50   | 0.007**                  | 0.595               |
| Polysaturated fatty acid (g) | Before 9.82 ± 3.72      | 0.670                     | 8.03 ± 2.49    | 0.001**                 | 0.194               |
|                      | During 8.07 ± 2.06       | 0.254                     | 10.18 ± 4.01   | 0.057                    | 0.073               |
|                      | After 9.12 ± 2.88        | 0.866                     | 11.24 ± 3.35   | <0.001**                 | 0.190               |
| n-3 Polysaturated fatty acid (g) | Before 1.82 ± 1.25      | 0.150                     | 1.46 ± 0.60    | 0.076                    | 0.402               |
|                      | During 1.60 ± 0.57       | 1.914                     | 1.76 ± 0.84    | 0.074                    | 0.565               |
|                      | After 1.90 ± 0.79        | 0.673                     | 1.90 ± 0.57    | 0.013                    | 0.975               |
| n-6 Polysaturated fatty acid (g) | Before 7.89 ± 2.68      | 0.407                     | 6.50 ± 2.16    | <0.001                   | 0.178               |
|                      | During 6.43 ± 1.77       | 0.254                     | 8.34 ± 3.31    | 0.074                    | 0.053               |
|                      | After 7.18 ± 2.22        | 0.514                     | 9.28 ± 2.97    | <0.001**                 | 0.042               |
| Cholesterol (mg)     | Before 366 ± 166         | 0.273                     | 281 ± 141      | 0.036*                   | 0.190               |
|                      | During 284 ± 107         | 0.089                     | 289 ± 137      | 2.714                    | 0.912               |
|                      | After 313 ± 113          | 0.673                     | 345 ± 98       | 0.164                    | 0.451               |

*p<0.05, **p<0.01,
Bonferroni, vs base-line value before the intervention.
LC, low carbohydrate.
was low. Additionally, it has been reported that diets promoting severe carbohydrate restriction lead to ketoacidosis (13, 14). Johnston et al. reported that it was difficult to regain weight following a moderate carbohydrate-restricted diet (15). Snorgaard et al. reported that low-to-moderate carbohydrate-restricted diets reduced HbA1c levels in patients with T2D (16). These reports indicate that a moderate carbohydrate-restricted diet is effective for control of T2D. We also examined the combined effect of this diet and DPP-4 inhibitor administration, and determined that this combination was indeed useful for patients with T2D. Among patients on a moderate carbohydrate-restricted diet, n-6 polyunsaturated fatty acid dietary intake significantly increased. This is likely explained through the decrease in carbohydrate intake. There were no changes in blood cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides levels (data not shown). Furthermore, salt intake also decreased significantly. This is likely due to decreased intake of side dishes along with the decrease in carbohydrate intake. Since DPP-4 inhibitors are known to be less likely to cause hypoglycemia, a carbohydrate restricted diet, combined with DPP-4 inhibitor administration, is less likely to cause hypoglycemia. Fifty percent of carbohydrates were coached, although this actually came down to 46.8%, which might have resulted from participants being overly conscious of the need for carbohydrate restriction.

Some limitations of this study should be noted. First, it is feared that an increase in protein intake accompanying carbohydrate-restricted diets adversely affects renal function (17). As protein intake and E% of protein did not significantly change after the intervention, the decrease in eGFR could be caused by reasons other than changes in protein load. Second, the study period was 6 mo, and no further results have been explored. It is therefore necessary to follow-up with patients for a longer period of time.

In conclusion, our study indicates that moderate restriction of carbohydrates decreases HbA1c levels among Japanese patients with T2D, treated with DPP-4 inhibitors. Moderate restriction of carbohydrate intake could be an alternative diet option for Japanese patients with T2D who experience difficulty with the conventional calorie-restricted diet using the food exchange table. However, further investigation is required to confirm the safety of the moderate carbohydrate-restricted diet. In particular, patient renal function should be examined on a regular basis, even if protein intake does not increase.

Disclosure of state of COI
We have no conflicts of interest to declare.

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