

Title : Glycemic variability is associated with quality of life and treatment satisfaction in patients with type 1 diabetes

Running title: Glycemic variability and patient-reported outcomes

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Patients with type 1 diabetes have greater glycemic variability than that in patients with type 2 diabetes. However, neither glucose variability nor hypoglycemia is detected precisely by HbA1c. This study investigated whether glycemic variability assessed by continuous glucose monitoring influences quality of life (QOL) and treatment satisfaction in patients with type 1 diabetes.

The study was conducted in Kyoto University Hospital between September 2011 and June 2012. The study protocol was approved by IRB (UMIN Clinical Trial Registry UMIN000005833). Twenty-eight patients with type 1 diabetes aged  $\geq 18$  years were included in analyses [age:  $45.9 \pm 14.5$  (mean  $\pm$  SD) years; diabetes duration:  $15.0 \pm 8.2$  years; 57% female; 21% using insulin pump; HbA1c:  $8.1 \pm 1.2\%$  ( $64.5 \pm 13.0$  mmol/mol)]. Glycemic variability in everyday life was assessed for a 72-hour period using CGMS<sup>®</sup> system Gold<sup>™</sup> (Medtronic, Northridge, CA) and mean absolute glucose (MAG) change was calculated as a glycemic variability measure. This is a summation of all absolute changes in glucose divided by the time over which the measurements were taken (1). QOL and treatment satisfaction were evaluated by the diabetes quality-of-life measure (DQOL) and the diabetes treatment satisfaction questionnaire (DTSQ) (2, 3, 4). We divided the participants into two groups by HbA1c level:  $< 8\%$  ( $64.0$  mmol/mol), the good/fair-control group (n=14);  $\geq 8\%$  ( $64.0$  mmol/mol), the poor-control group (n=14), considering HbA1c as a potential intermediate variable between glycemic variability and patient-reported outcomes (5). Potential

confounding factors were use of carbohydrate-counting with insulin adjustment, age, gender, diabetes duration, use of insulin pump and the Clarke hypoglycemic score.

Glycemic variability correlated negatively with DQOL in the good/fair-control group ( $r = -0.65, P = 0.01$ ), while there was no correlation in the poor-control group ( $r = 0.05, P = 0.87$ ) (Fig.1). Glycemic variability correlated negatively with Treatment Satisfaction across all patients ( $r = 0.40, P = 0.03$ ). No significant confounding effect was identified by stepwise selection.

Our study identifies the important association of glycemic variability with diabetes-related QOL and treatment satisfaction in patients with type 1 diabetes. Interestingly, the association between glycemic variability and diabetes-related QOL, which measures satisfaction, impact, social worries and diabetes worries, was limited to the group with good/fair-glycemic control, indicating that the contribution of glycemic variability to QOL is emphasized by better glycemic control. On the other hand, the insignificant association in the poor-glycemic control group may imply other important predictors of QOL in patients with poorer glycemic control. The strong points of this study include use of a relatively new indicator, MAG change. It differs from standard deviation, a measure of how spread-out data values are around the mean, in that it represents not only dispersion but also the rate of change of blood glucose. Although the limitations of our study include small sample size and exclusion of 12 of 40 participants whose CGM data were less than 48 hours because of

disconnection of the sensor, calibration errors, or out-of-range data, this is the first report about the important association between glycemic variability and patient-reported outcomes in type 1 diabetes.

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Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. S.A.-T. designed the study, collected and researched data, analyzed data, interpreted data and wrote the manuscript. K.I. designed the study, collected and researched data, analyzed data, interpreted data and reviewed and edited the manuscript. S.F. designed the study, analyzed data, interpreted data, reviewed and edited the manuscript.

A.H., S.H., K.T., Y.F., K.N. and D.T. collected data and contributed to discussions. N.I. reviewed and edited the manuscript and contributed to discussions. N.I. is the guarantor of this work and, as such, had full access to all the data in this study and takes responsibility for the data and accuracy of the data analysis.

Access to Questionnaires:

DTSQ: [www.healthpsychologyresearch.com](http://www.healthpsychologyresearch.com)

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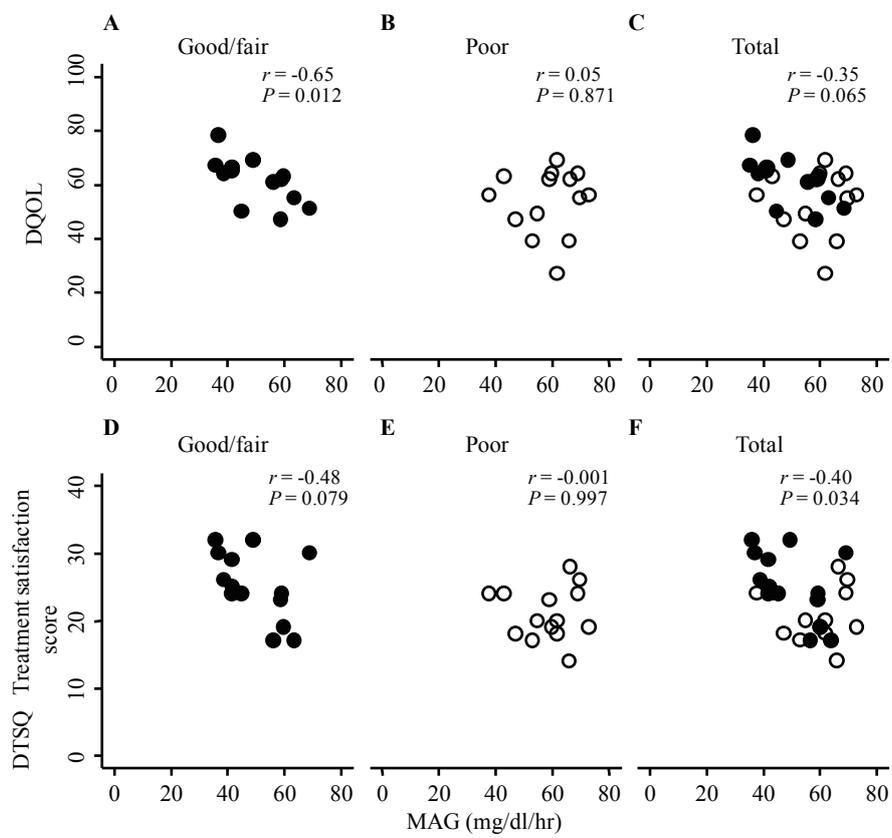
## Figure legends

Figure 1. Correlation between glycemic variability and DQOL, and between glycemic variability and DTSQ treatment satisfaction. A, D: Good/fair-control group (closed circles). B, E: Poor-control group (open circles). C, F: Superimposed data for the good/fair-control group (closed circles) and the poor-control group (open circles).

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Figure 1



Carbohydrate intake is associated with time spent in the euglycemic range in patients  
with type 1 diabetes

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## ABSTRACT AND KEYWORDS

### Abstract

#### **Aims/Introduction**

Greater glycemic variability and lack of predictability are important issues for patients with type 1 diabetes. Dietary factors are one of the contributors to this variability, but how closely diet is linked to glycemic fluctuation on a daily basis has not been investigated. We examined the association between carbohydrate intake and glycemic excursion in outpatients.

#### **Materials and Methods**

Thirty-three patients with type 1 diabetes were included in the analyses [age:  $44.5 \pm 11.7$  (mean  $\pm$  SD) years; diabetes duration:  $15.1 \pm 8.3$  years; 64% female; 30% using insulin pump; HbA1c:  $8.1 \pm 1.3\%$ ]. Time spent in euglycemia (70-180mg/dl), hyperglycemia ( $>180$ mg/dl) and hypoglycemia ( $< 70$ mg/dl) of consecutive 48-h periods of continuous glucose monitoring data were collected together with simultaneous records of dietary intake, insulin dose and physical activity. Correlation analyses and multiple regression analyses were used to evaluate the contribution of carbohydrate intake to time spent in the target glycemic range.

#### **Results**

In multiple regression analyses, carbohydrate intake ( $\beta=0.53$ ,  $P=0.001$ ), basal insulin dose per kilogram per day ( $\beta = -0.31$ ,  $P = 0.034$ ) and diabetes duration ( $\beta=0.30$ ,  $P=0.042$ ) were independent predictors of time spent in euglycemia. Carbohydrate intake ( $\beta=-0.51$ ,  $P=0.001$ ) and insulin pump use ( $\beta=-0.34$ ,  $P=0.024$ ) were independent predictors of time spent in hyperglycemia. Insulin pump use ( $\beta=0.52$ ,  $P<0.001$ ) and bolus insulin dose per kilogram per day ( $\beta=0.46$ ,  $P=0.001$ ) were independent predictors of time spent in hypoglycemia.

### **Conclusion**

Carbohydrate intake is associated with time spent in euglycemia in patients with type 1 diabetes.

Keywords:

Diabetes Mellitus, Type 1

Dietary carbohydrates

Glycemic control

## INTRODUCTION

Glucose variability is greater in type 1 diabetes patients than it is in type 2 diabetes patients<sup>1-3</sup> and the lack of predictability is an important issue for type 1 diabetes patients and medical staff in daily practice. Hypoglycemia is a complication of diabetes treatment and the frequency of severe hypoglycemia increases when glucose is lowered. Larger glucose variability is an additional risk factor for severe hypoglycemia<sup>4</sup>. Minimizing glucose variability is therefore a plausible method for offsetting the increased risk of hypoglycemia associated with tight glycaemic control. On the other hand, type 1 diabetes patients with larger glycaemic variability retain higher HbA1c, partly because of the difficulty in raising the insulin dosage or physical activity due to fear of hypoglycemia<sup>4,5</sup>. Larger glucose variability is associated with lower quality of life and lower satisfaction of treatment<sup>6</sup>.

Clinical factors contributing to hypoglycemia and glycaemic variability have been investigated and can be summarized as dietary factors, physical activity, insulin regimen, monitoring and pathophysiological conditions<sup>7-11</sup>. Dietary factors have been studied little, however. Maahs et al. reported a positive association between carbohydrate intake and glucose excursion during 1-4h after the first meal of the day<sup>9</sup>. On the other hand, lower carbohydrate intake was associated with higher HbA1c in the Diabetes Control and Complications Trial<sup>12</sup>. Further research is required to learn the

details of the association of carbohydrate intake with glycemic control and glycemic variability on a daily basis.

Recently, continuous blood glucose monitoring (CGM) has made it possible to monitor in detail glucose fluctuations in daily life, and several indicators of intraday and interday glycemic variability are now available<sup>4</sup>. Time spent in glycemic target range is a simple and absolute assessment of glycemic control, reflecting both mean glucose level and glucose excursions, and is sensitive to interventions<sup>13</sup>.

To investigate the overall influence of carbohydrate intake on glycemic levels including both excursion and mean, we evaluated the association between carbohydrate intake and time spent in target glycemic range by use of simultaneous dietary records and CGM in outpatient settings.

## **MATERIALS AND METHODS**

### **Participants**

This cross-sectional study was conducted in Kyoto University Hospital between September 2011 and June 2012. Patients aged eighteen years or older who were diagnosed with type 1 diabetes and were treated with Basal-Bolus therapy (multiple daily injection (MDI) or continuous subcutaneous insulin infusion (CSII)) were eligible for enrollment. Patients were excluded if they had renal insufficiency ( $Cr_{e} \geq 1.5$  mg/dl),

liver failure, acute infection, psychological comorbidities or dementia, or were pregnant, taking steroid medication, or had received pancreas or islet transplantation. The study protocol was approved by the Kyoto University Graduate School and Faculty of Medicine, Ethics Committee and registered on University hospital Medical Information Network in Japan (UMIN000005833). Written informed consent was obtained from all participants.

### **Continuous Glucose Monitoring**

All subjects underwent a 72-h period of monitoring using CGMS<sup>®</sup> system Gold<sup>™</sup> (Medtronic, Northridge, CA). The CGMS<sup>®</sup> consists of a glucose oxidase-based sensor inserted subcutaneously in an abdominal site and attached via cable to a monitor. The monitor takes a reading every 10 seconds and accumulates an average every 5 minutes for a total of 288 readings per day. The participants were instructed to enter at least four, daily metered blood glucose measurements for calibration using conventional glucose meters. They also kept records of insulin dose and whether or not they conducted bolus adjustment by carbohydrate-counting while wearing CGMS<sup>®</sup>. The CGM data could be read only after CGMS<sup>®</sup> was removed. The data from CGMS<sup>®</sup> were downloaded using Minimed solutions CGMS sensor 3.0C software. Further data management was performed using Microsoft (Seattle, WA) Excel. According to

Medtronic's recommendation, the criteria that a minimal correlation of 0.79 between the sensor glucose and meter blood glucose values with a mean absolute error <28% were used to determine the accuracy of the CGM data. Patients whose CGM data deviated from these criteria were rescheduled for wearing CGMS<sup>®</sup> with dietary record and physical activity monitoring, and this second set of CGM data was used for the analyses so long as the subjects met the criteria. Glucose values outside the range of 40-400mg/dl were reported as  $\leq 40$  or  $\geq 400$  mg/dl. The first complete, consecutive 48-hour period of valid CGM data beginning from midnight was analyzed for each subject.

### **Glycemic indices**

Mean, standard deviation (SD), and time spent in euglycemia (70-180mg/dl), in hyperglycemia (>180mg/dl) and in hypoglycemia (< 70mg/dl) were calculated from CGM data. Time spent in target range, expressed as percentage in a total 48-hour monitoring period, has been adopted in several studies for assessing CGM data<sup>13, 14</sup>. Time spent in euglycemia is a direct index of appropriate glycemic control and can reflect both mean glucose and glucose excursions<sup>13</sup>.

### **Dietary data**

Patients recorded everything they ate for the 72-hour period using recording

sheets and photographs of foods. We used two, complete, consecutive whole-day records from 0:00 to 24:00. The photographs were taken before and after consumption with a ruler placed beside the foods as a scale for estimation of the size of the portions. The validity of assessing diet using photographs as food intake records has been reported<sup>15</sup>. Registered dietitians estimated the amount of foods from the recording sheets and photographs and calculated the weight of the ingredients, carbohydrate, fat, protein, fiber and ethanol, using computer software program Excel Eiyokun version 4.5 (Kenpaku Co. Ltd, Tokyo, Japan). Excel Eiyokun is a program designed to calculate amounts of ingredients based on standard tables of food composition in Japan<sup>16, 17</sup>.

The composition of fat (% of energy) and protein (% of energy) were calculated as follows: fat (g)  $\times 9$ /total energy (kcal) and protein  $\times 4$ /total energy (kcal), respectively (g). The composition of carbohydrate (% of energy) was calculated as 100-(fat (% of energy) +protein (% of energy)). These calculations were used to determine fat intake, protein intake and carbohydrate intake in the following analyses.

We defined snacking after dinner as a small intake of food and/or beverage with energy after dinner excepting those for treating hypoglycemia, and “Snacking after dinner” was coded 1 for any episode of snacking after dinner during the two days, and 0 otherwise. “Late dinner” was coded 1 for any dinner after 21:00 during the two days, and 0 otherwise.

### **Physical activity level**

We measured the physical activity using Lifecoder PLUS<sup>®</sup> (Suzuken, Nagoya, Japan) during CGM. This method involves uniaxial accelerometry and has been validated for assessment of physical activity-related energy expenditure<sup>18</sup>. The patients attach it onto a belt when they awake and take it off before they go to sleep. The device samples acceleration at 32Hz and assesses values ranging from 0.06 to 1.94g (1.00g is equal to the acceleration of free fall). A maximum pulse over 4 seconds is taken for the acceleration value and activity is categorized into eleven levels. The activity levels are subsequently converted to calculate energy expenditure due to various activities (kcal). Total energy expenditure (TEE) is calculated from the sum of BMR, thermic effect of food ( $=1/10(\text{TEE})$ ) and energy expenditure due to activity. BMR is calculated from body weight, height, sex and age using a standard Japanese formula<sup>19</sup>. The physical activity level is calculated as follows:  $\text{TEE (kcal)} / \text{BMR (kcal)}$ . The average of two consecutive days that coincided with the two days of CGM data was used.

### **Coefficient of variation of R-R intervals (CVR-R)**

We measured CVR-R for assessment of autonomic neuropathy<sup>20, 21</sup>. The R-R

interval was measured by electrocardiography for 1 minute in the supine position after at least 3 minutes rest using CardioStar FCP-7301 (Fukuda Denshi, Tokyo, Japan). The CVR-R was calculated by dividing the standard deviations (SD) by the mean (M): CV (%) = (SD/M) × 100

### **Laboratory measurements**

Glycemic control was assessed by HbA1c and glycated albumin (GA). In all patients, HbA1c and GA levels were evaluated within 2 weeks after CGM was initiated. HbA1c was measured via reversed-phase cation exchange chromatography, using ADAMS™ A1c HA-8180 (Arkray, Kyoto, Japan). The CV of within-run reproducibility and between-run reproducibility were reported to be within 1%. The value for HbA1c (%) was estimated as a National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) calculated by the formula  $\text{HbA1c (\%)} = 1.02 \times \text{HbA1c JDS (\%)} + 0.25\%$ , measured by the previous Japanese standard substance and measurement methods and HbA1c (NGSP) <sup>22</sup>. GA was measured via enzymatic method, using JCA-BM8000 series (JOEL, Tokyo, Japan). The CV of within-run reproducibility is 0.8-1.0% and that of between-run reproducibility is 1.2%. Serum C-peptide was measured by Chemiluminescent Enzyme Immunoassay, LUMIPULSE® Presto C-peptide (Fuji Rebio, Tokyo, Japan). The CV of within-run reproducibility is 1.96-2.97% and that of

between-run reproducibility is 1.06-2.60%. The measurement range of serum C-peptide is 0.02-30 ng/ml. For C-peptide measurement, blood samples were centrifuged immediately at 3,000 rpm for 5 min. Serum was stored at -80°C and measured within 1 month after collection.

### **Glucagon stimulation test**

A glucagon stimulation test was done after overnight 8h fast. Tests were rescheduled if the subject had a capillary glucose value <70mg/dl. Serum C-peptide was measured before and 6 minutes after the intravenous injection of 1mg glucagon<sup>23</sup>.

### **Hypoglycemia**

The participants were asked about their experience of hypoglycemia over the past year on the day of starting CGM and a Clarke score (0 = no hypoglycemia;  $\geq 4$  = hypoglycemia unawareness) was calculated<sup>24</sup>.

### **Statistical analyses**

Analysis was performed using STATA<sup>®</sup> 11.2 (StataCorp LP, TX). Pearson's product-moment correlation coefficient was used to evaluate the relationship between glycemic indices and clinical variables including dietary intake. We then performed

multiple regression analyses for association between carbohydrate intake and glycemic indices adjusting for other plausible determinants of glycemic indices. We started from an empty model and added variables by significance level less than 0.10 from the followings candidate variables: age, diabetes duration, BMI, blood pressure, CSII use, carbohydrate-counting, fiber intake, snacking after dinner episode, late dinner, insulin dose per kilogram per day, physical activity, CVR-R, lipid profiles, C-peptide and Clarke score in the order of correlation coefficients with glycemic indices. Two-sided  $P < 0.05$  was considered statistically significant.

## RESULTS

A total of 40 patients were recruited. Diet therapy for these patients was based on advice from nutritionists. Of the 40 patients, one was found to be in a non-insulin-dependent-state by serum C-peptide level<sup>25</sup>. In three patients, available CGM data covered less than 48 hours because of disconnection of the sensor or calibration errors. One patient failed to measure CVR-R because of atrial fibrillation. In another patient, there were missing data due to problems with Lifecoder<sup>®</sup> PLUS. Another patient reported that he drank a large amount of alcohol (equivalent to more than 60g of pure ethanol per day) during monitoring because of a celebration party. These seven patients were excluded from the analysis. None of the 33 remaining patients had clinical thyroid

disease or were diagnosed with clinical ketosis.

The clinical characteristics and the measurements of the 33 patients analyzed are shown in Table 1. The mean age was 44.5 years and most of them were not obese. Their C-peptide levels were very low and most were undetectable by highly sensitive measurement. The distribution of intake of the three macronutrients is shown in Figure 1. Carbohydrate intake correlated negatively with fat intake ( $r = -0.93$ ,  $P < 0.001$ ), although there was no significant correlation between carbohydrate intake and protein intake ( $r = -0.33$ ,  $P = 0.061$ ) or between protein intake and fat intake ( $r = -0.04$ ,  $P = 0.819$ ).

Carbohydrate intake correlated positively with time spent in euglycemia ( $r = 0.48$ ,  $P = 0.005$ ) and negatively with time spent in hyperglycemia ( $r = -0.50$ ,  $P = 0.003$ ) (Figure 2). Conversely, fat intake showed a negative correlation with time spent in euglycemia ( $r = -0.44$ ,  $P = 0.011$ ) and a positive correlation with time spent in hyperglycemia ( $r = 0.45$ ,  $P = 0.009$ ). Neither carbohydrate intake nor fat intake correlated with time spent in hypoglycemia ( $r = 0.20$ ,  $P = 0.274$  and  $r = -0.15$ ,  $P = 0.400$ , respectively). In addition, there was no significant relationship between protein intake and time spent in euglycemia ( $r = -0.17$ ,  $P = 0.331$ ), hyperglycemia ( $r = 0.23$ ,  $P = 0.206$ ) or hypoglycemia ( $r = -0.14$ ,  $P = 0.425$ ).

The relationship between insulin dose and time spent in euglycemia,

hyperglycemia and hypoglycemia are shown in Figure 3. Total insulin dose per kilogram per day was positively correlated with time spent in hypoglycemia ( $r = 0.47$ ,  $P = 0.006$ ) but not with time spent in euglycemia ( $r = -0.15$ ,  $P = 0.409$ ) or hyperglycemia ( $r = -0.15$ ,  $P = 0.400$ ). Basal insulin dose per kilogram per day was not correlated with time spent in euglycemia ( $r = -0.27$ ,  $P = 0.135$ ), hyperglycemia ( $r = 0.11$ ,  $P = 0.543$ ) or hypoglycemia ( $r = 0.18$ ,  $P = 0.303$ ). Bolus insulin dose per kilogram per day showed a positive correlation with time spent in hypoglycemia ( $r = 0.48$ ,  $P = 0.005$ ) but not with time spent in euglycemia ( $r = -0.03$ ,  $P = 0.879$ ) or hyperglycemia ( $r = -0.25$ ,  $P = 0.152$ )

We performed multiple regression analyses for time spent in euglycemia, hyperglycemia and hypoglycemia by forward selection from possible determinants considering simple correlation coefficients. Independent variables for time spent in euglycemia were selected from carbohydrate intake, basal insulin dose per kilogram per day, diabetes duration, carbohydrate-counting, fiber, triglyceride, snacking after dinner, systolic blood pressure, physical activity, CVR-R, BMI, CSII use, Clarke score, late dinner, age and glucagon-stimulated C-peptide in this order. Independent variables for time spent in hyperglycemia were selected from carbohydrate intake, CSII use, CVR-R, bolus insulin dose per kilogram per day, age, carbohydrate-counting, triglyceride, late dinner, diabetes duration, snacking after dinner, glucagon-stimulated C-peptide, BMI, fiber, systolic blood pressure, Clarke score and physical activity, in this order.

Independent variables for time spent in hypoglycemia were selected from CSII use, bolus insulin dose per kilogram per day, fiber, age, CVR-R, late dinner, carbohydrate intake, BMI, physical activity, systolic blood pressure, glucagon-stimulated C-peptide, snacking after dinner, diabetes duration, triglyceride, carbohydrate-counting and Clarke score, in this order. We found that time spent in euglycemia was significantly predicted by carbohydrate intake ( $\beta = 0.53$ ,  $P = 0.001$ ), basal insulin dose per kilogram per day ( $\beta = -0.31$ ,  $P = 0.034$ ) and diabetes duration ( $\beta = 0.30$ ,  $P = 0.042$ ) and that time spent in hyperglycemia was predicted by carbohydrate intake ( $\beta = -0.51$ ,  $P = 0.001$ ) and CSII use ( $\beta = -0.34$ ,  $P = 0.024$ ) (Table 2). Time spent in hypoglycemia was predicted by CSII use ( $\beta = 0.52$ ,  $P < 0.001$ ) and bolus insulin dose per kilogram per day ( $\beta = 0.46$ ,  $P = 0.001$ ).

We also analyzed the relationship between carbohydrate intake and HbA1c or GA as indicators of average glycemia over several months or weeks, respectively, and the relationship between carbohydrate intake and glycemic indices calculated from CGM data (Figure 4). There was no significant correlation between carbohydrate intake and HbA1c ( $r = -0.14$ ,  $P = 0.438$ ) or GA ( $r = -0.10$ ,  $P = 0.590$ ). On the other hand, carbohydrate intake was significantly correlated with mean glucose calculated from CGM data ( $r = -0.48$ ,  $P = 0.005$ ), SD ( $r = -0.37$ ,  $P = 0.033$ ) calculated from CGM data and also time spent in euglycemia ( $r = 0.48$ ,  $P = 0.005$ ) calculated from CGM data. The

strongest correlation was observed between HbA1c and GA ( $r = 0.85$ ,  $P < 0.001$ ) and a relatively strong correlation was observed between HbA1c and mean, between GA and mean, and between mean and time spent in euglycemia ( $r = 0.70$ ,  $r = 0.65$  and  $r = -0.69$ , respectively, all  $P < 0.001$ ). Time spent in euglycemia was moderately correlated with HbA1c ( $r = -0.45$ ,  $P = 0.008$ ), but not with GA ( $r = -0.31$ ,  $P = 0.083$ ).

## DISCUSSION

This study demonstrates a clear association of carbohydrate intake and favorable glycemic control during a consecutive two day period in a daily life setting of type 1 diabetes patients. Notably, carbohydrate intake showed no association with HbA1c or GA in this study, indicating that the association of carbohydrate intake with euglycemia does not represent long-term interaction but rather short-term interaction, on a daily basis.

The existing evidence of association of carbohydrate intake and glycemic control was estimated by food frequency questionnaires, which assesses long-term nutritional exposure, and HbA1c<sup>12, 26</sup>, and there has been no association shown between carbohydrate intake estimated by dietary records, which assesses short-term nutritional exposure, and HbA1c<sup>27, 28</sup>. In the present study, carbohydrate intake was calculated by dietary record during continuous monitoring of glucose levels. Mean glucose level

during monitoring is generally the best index for correlating CGM with HbA1c, and showed an association with carbohydrate intake, while HbA1c did not. One reason may be that carbohydrate intake estimated by dietary record is not a good measure of chronic exposure to carbohydrate.

There was no trend found between carbohydrate intake and time spent in hypoglycemia, but a significant inverse association between carbohydrate intake and time spent in hyperglycemia was observed. Carbohydrate intake in the participants of this study had a strong inverse correlation with fat intake, and the contributions of carbohydrate intake and fat intake to time in hyperglycemia and time in euglycemia were in the opposite direction. The contributions of carbohydrate intake were larger than those of fat intake. On the other hand, protein intake showed no association with time in any range. Our results indicate that there is a relationship between increased carbohydrate intake or reduced fat intake and better short-term glucose control, which is less time in hyperglycemia and more time in euglycemia.

Insulin dose is also an important factor influencing glycemic control in type 1 diabetes and is reported to correlate with carbohydrate intake<sup>28-30</sup>. We included insulin dose in the multiple regression models and observed an independent negative association between basal insulin dose and time spent in euglycemia and an independent positive association between bolus insulin dose and time spent in hypoglycemia but no

association between insulin dose and time spent in hyperglycemia. This may suggest that higher insulin dose per body weight is a risk for hypoglycemia. CSII use was another factor associated with time spent in hypoglycemia and also a factor reducing the time spent in hyperglycemia. CSII, therefore, may contribute to reduce hyperglycemia but may tend to increase hypoglycemia, possibly more so under tight glycaemic control.

Diabetes duration independently predicted euglycemia; patients with longer duration spent a longer time in euglycemia. Given that the CGM data in this study were not shown to the patients in real time, they were assumed to manage their glycaemic level based on SMBG and their experience. Patients may become skilled in managing their glycaemic fluctuation through their long experience. Carbohydrate-counting was used by seven out of the 33 patients on the days that glucose was monitored.

Carbohydrate-counting seemed to show a weak contribution to increased time spent in euglycemia, but the contribution did not reach statistical significance. Among the other plausible predictors that we included in the multiple regression models, fiber intake showed a tendency to reduce hypoglycemia, but the contribution was not significant when it was included in the model together with CSII use and insulin dose. The C-peptide level was undetectable by highly sensitive measurement in most patients in this study, even after glucagon stimulation, and a dummy variable for C-peptide did not show a significant effect on time in target range or other indicators of glycaemic

variability. Hypoglycemia-associated autonomic failure can also increase hypoglycemia<sup>10</sup>. However, in our data CVR-R was not an independent predictor of time spent in hypoglycemia. One reason for this may be that the CVR-R used in this study was not under deep breathing but only resting condition. Another limitation of our study is the relatively small sample size.

A strength of our study is that our carbohydrate intake measurement does not reflect dietary history but only the amount of carbohydrate actually taken during the period that the glucose level was continuously monitored. Time spent in a target glycemic range that includes both mean glucose level and glucose excursions on a daily basis is a suitable index for facilitation of glycemic control. We performed additional analyses using other indexes of glycemic variability, including MAGE, CV and mean absolute glucose change, but there were no significant findings.

Our data showed that carbohydrate intake ranged from 40% to 60% of total energy intake, while the guideline recommends that carbohydrate account for 50 - 60%<sup>31</sup> of intake. Patients who consumed carbohydrate accounting for less than 50% of total energy spent a shorter time in euglycemia and a longer time in hyperglycemia. This partly may be due to the fact that the insulin regimen was based mainly on carbohydrate intake, which is not appropriate for a diet consisting of relatively high fat. Although fat content has been considered more important in controlling body weight

than in controlling glycemic level<sup>11</sup>, fat intake delays the postprandial rise in blood glucose and makes peak glucose occur later<sup>32</sup>. It may therefore contribute to the mismatch of the insulin regimen and the glycemic level and be a cause of glycemic variability and unpredictability. Further research is required to confirm these findings in experimental settings focusing on postprandial glucose or in other groups of patients whose intake is of a different composition of the three major nutrients from that of the present study.

In conclusion, sufficient carbohydrate intake is clearly associated with favorable glycemic control in patients with type 1 diabetes using MDI or CSII.

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## Figure legends

### Figure 1

Distribution of intake of three macronutrients (% of energy) shown by two-way scatter plot. (a) Fat intake and carbohydrate intake. (b) Carbohydrate intake and protein intake. (c) Protein intake and fat intake.

### Figure 2

(a) Correlations between carbohydrate intake and time spent in euglycemia, hyperglycemia and hypoglycemia. (b) Correlations between fat intake and time spent in euglycemia, hyperglycemia and hypoglycemia.

### Figure 3

(a) Correlations between total insulin dose and time spent in euglycemia, hyperglycemia and hypoglycemia. (b) Correlations between basal insulin dose and time spent in euglycemia, hyperglycemia and hypoglycemia. (c) Correlations between bolus insulin dose and time spent in euglycemia, hyperglycemia and hypoglycemia.

### Figure 4

Relationship between carbohydrate intake and glycemic indices including HbA1c,

glycated albumin, mean calculated from CGM data, standard deviation calculated from CGM data and time spent in euglycemia calculated from CGM data.

Table 1 Clinical characteristics of participants

Age (years)	44.5±14.7
Sex	
Male	12 (36)
Female	21 (64)
BMI (kg/m <sup>2</sup> )	22.6±3.6
Diabetes duration (years)	15.1±8.3
Glucagon stimulated C-peptide	
Detectable (≥ 0.02ng/ml)	5 (15)
Undetectable	28 (85)
Fasting blood glucose (mg/dl)	188±66
Insulin administration	
MDI	23 (70)
CSII	10 (30)
Carbohydrate-counting	7 (21)
HbA1c (%)	8.1±1.3
Glycated albumin (%)	24.2±4.8
Lipid profile (n=32)	
LDL cholesterol (mg/dl)	97±24
HDL cholesterol (mg/dl)	74±18
Triglyceride (mg/dl)	86±56
Clarke score	1.6±1.3
CVR-R (%)	3.3±1.8
Physical activity	1.4±0.1
Blood pressure (mmHg)	
Systolic	119±17
Diastolic	69±10
Total insulin dose/BW (U/kg)	0.69±0.21
Basal insulin dose/BW (U/kg)	0.29±0.10
Bolus insulin dose/BW (U/kg)	0.40±0.16
Glycemic indices measured by CGM	
Mean glucose (mg/dl)	160±48
SD (mg/dl)	64±16
Time spent in euglycemia (70-180 mg/dl) during 48hours (min)	1520±566
(%)	52.8±19.6
Time spent in hyperglycemia (> 180 mg/dl) during 48hours (min)	1018±692
(%)	35.3±24.0
Time spent in hypoglycemia (< 70 mg/dl) during 48hours (min)	342±403
(%)	11.9±14.0
Dietary data (average of consecutive 2 days)	

Energy (including alcohols) (kcal)	1922±400
Energy (including alcohols) /BW (kcal/kg)	33.0±5.3
Energy (kcal)	1888±370
Energy/BW (kcal/kg)	32.4±5.0
Carbohydrate intake (% of energy)	52.2±5.5
Fat intake (% of energy)	32.7±5.2
Protein intake (% of energy)	15.1±2.0
Fiber (g)	12.6±3.6
Ethanol (g)	3.6±7.4
Late dinner (%)	7 (21)
Snacking after dinner (%)	15 (45)

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Data are mean ±SD or n (%). BMI, body mass index; MDI, multiple daily injection; CSII, continuous subcutaneous insulin infusion; Total insulin dose/BW, total insulin dose per body weight; Basal insulin dose/BW, basal insulin dose per body weight; Bolus insulin dose/BW, bolus insulin dose per body weight; CGM, continuous glucose monitoring; Energy (including alcohols), total energy intake including alcohols; Energy (including alcohols)/ BW: total energy intake including alcohols per body weight, Energy, total energy intake excluding alcohols; Energy/ BW: total energy intake excluding alcohols per body weight.

Table 2 Multivariate analyses for the determinants of time spent in target range

Covariates	Time spent in euglycemia (70-180 mg/dl)		Time spent in hyperglycemia (> 180 mg/dl)		Time spent in hypoglycemia (< 70 mg/dl)	
	$\beta$	<i>P</i>	$\beta$	<i>P</i>	$\beta$	<i>P</i>
Carbohydrate intake	0.53	0.001	-0.51	0.001	---	
Basal insulin dose/BW	-0.31	0.034	---		---	
Diabetes duration	0.30	0.042	---		---	
Carbohydrate-counting	0.26	0.078	---		---	
CSII	---		-0.34	0.024	0.52	<0.001
Bolus insulin dose/BW	---		---		0.46	0.001
Late dinner	---		---		0.24	0.065
Adjusted $R^2$	0.41		0.33		0.50	
<i>P</i>	<0.001		<0.001		<0.001	

Variables were selected by forward selection method, according to the significance level for addition of variables less than 0.10, from the following candidate variables: carbohydrate intake, insulin dose per kilogram per day, diabetes duration, carbohydrate-counting, CSII use, late dinner, fiber intake, lipid profiles, snacking after dinner episode, blood pressure, physical activity, CVR-R, Clarke score, BMI, age and C-peptide. Basal insulin dose/BW, basal insulin dose per body weight; Bolus insulin dose/BW, bolus insulin dose per body weight; CVR-R, coefficient of variation of R-R intervals; CSII, continuous subcutaneous insulin infusion.

Figure 1

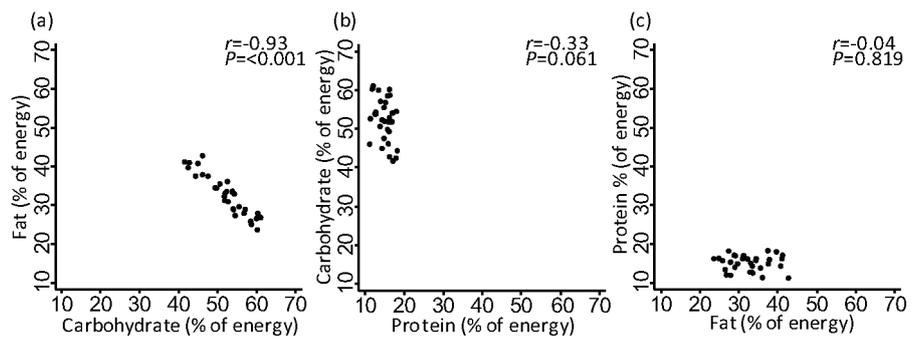


Figure 2

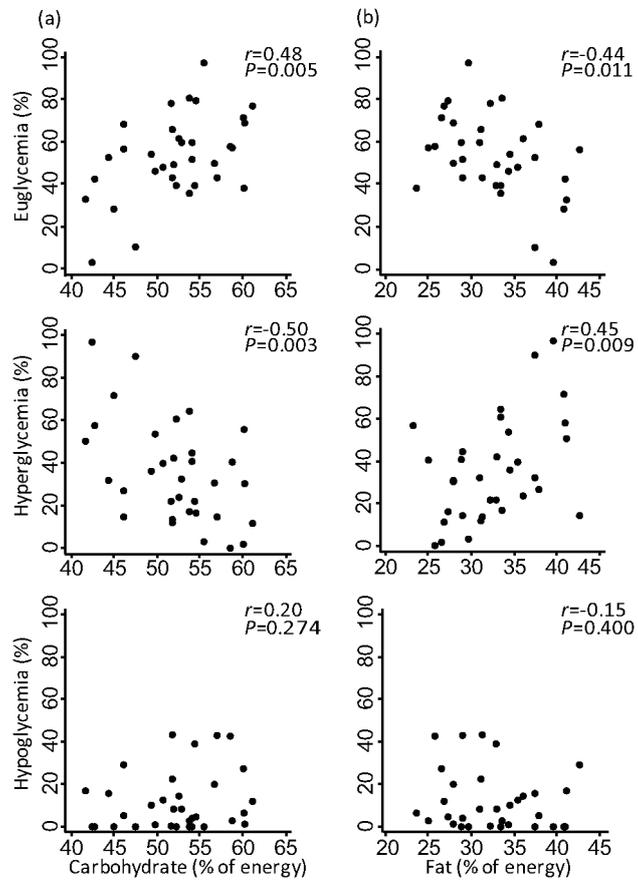


Figure 3

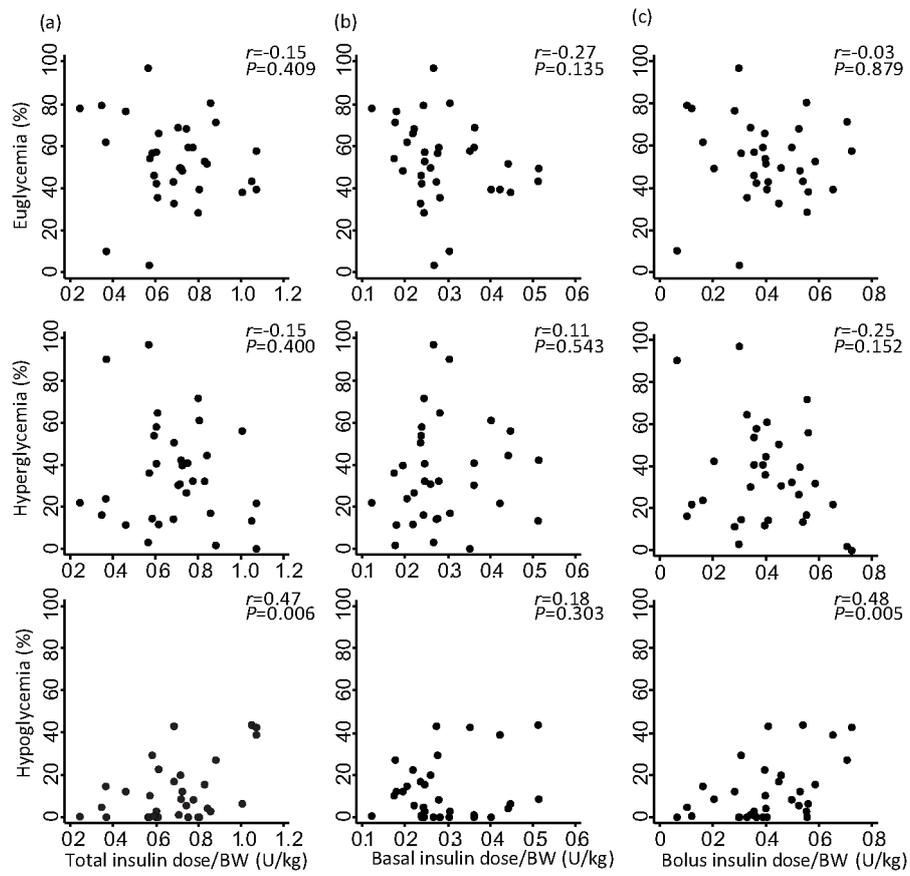


Figure 4

