Late-night-dinner deteriorates postprandial glucose and insulin whereas consuming dinner dividedly ameliorates them in patients with type 2 diabetes: A randomized crossover clinical trial

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ABSTRACT

Background and Objectives: The aims of this study is to explore the acute effect of consuming dinner at different timing on postprandial glucose and hormone in patients with type 2 diabetes. **Methods and Study Design:** Eight patients (age 70.8±1.9 years, HbA1c 7.6±0.6 %, BMI 23.3±3.2, mean±SD) were randomly assigned in this crossover study. Patients consumed the test meals of dinner at 18:00 on the first day, and dinner at 21:00 or divided dinner (vegetable and rice at 18:00 and vegetable and the main dish at 21:00) on the second or third day. Postprandial glucose, insulin, glucagon, free fatty acid (FFA), active glucagon-like peptide-1 (GLP-1), and active glucose-dependent insulinotropic polypeptide (GIP) concentration after dinner were evaluated. **Results:** Both incremental area under the curve (IAUC) 2h for glucose and insulin were higher in dinner at 21:00 than those in dinner at 18:00 (IAUC glucose: 449±83 vs 216±43 mmol/L×min, $p<0.01$, IAUC insulin:772±104 vs 527±107 μU/mL × min, $p<0.01$, mean ± SEM). However, in divided dinner both IAUC 4h for glucose and insulin tended to be lower than those of dinner at 21:00 (IAUC glucose: 269±76 mmol/L × min, $p=0.070$, IAUC insulin: 552±114 μU/mL × min, $p=0.070$). IAUC of active GLP-1 and active GIP demonstrated no difference among different dinner regimen. **Conclusions:** Consuming late-night-dinner (21:00) deteriorates postprandial glucose and insulin compared with those of early-evening-dinner (18:00) whereas consuming dinner dividedly ameliorates them.

Key Words: diet, postprandial glucose, insulin, dinner, meal timing, diabetes

INTRODUCTION

Many epidemiological research reports that shift workers are associated with increased risk of obesity, diabetes, and cardiovascular diseases, partly due to the circadian misalignment.[1-3] Additionally skipping breakfast or late-night meal have been demonstrated to increase the risk of weight gain.[4-7] According to the Japanese government survey, more than 30 to 35% of Japanese men aged 20 to 40 years old consumed the dinner after 21:00.[8] Consuming carbohydrate-rich meals at late night was associated with glucose intolerance and decreased insulin sensitivity in both people with and without type 2 diabetes.[9-11]

Our group has previously reported that consuming late-night-dinner deteriorates postprandial glucose concentration and glucose excursions, whereas dividing dinner, eating vegetable and carbohydrate early in the evening at 18:00 followed by vegetables and the main

dish (protein) at 21:00, significantly ameliorates them in both people with and without diabetes.[12,13]

Postprandial glucose concentration and the mean amplitude of glycemic excursions (MAGE) are influenced by meal size,[14] the amount of carbohydrates,[15] the macronutrient composition,[16] intestinal absorption,[17] gastric emptying,[18,19] meal sequence,[20,21] meal timing,[12,13,22,23] and hormones.[10,16,18,19,21] Incretion hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) play an important role for postprandial glucose concentration. GLP-1 and GIP are secreted from gut in response to consumption of nutrients and enhances insulin secretion in glucose dependent manner to lower the blood glucose concentration. GLP-1 suppresses glucagon secretion and delays gastric emptying rate, as a result ameliorates postprandial glucose elevation.[16,18,19,21] GIP facilitates fat accumulation and increases body fat mass. However, the effect of different dinner timing on hormonal secretion includes incretin hormones has not been extensively studied in patients with type 2 diabetes. In this randomized controlled crossover study, we assessed the post-dinner responses of glucose, insulin, glucagon, active GLP-1, and active GIP among three different regimens (dinner at 18:00, at 21:00, and divided dinner: eating vegetable and carbohydrate at 18:00, and vegetables and the main dish at 21:00) in individuals with type 2 diabetes.

MATERIALS AND METHODS

Participants

Study participants were recruited from the outpatient at Kajiyama Clinic, Kyoto, Japan. The study was conducted from September 2015 to November 2015. We included the patients with type 2 diabetes aged 60-75 years old with a body mass index (BMI) <30 kg/m^2, HbA1c 6.5 to 8.5%, and have not engaged work a night shift work within the previous 2 years. Participants were excluded in cases of insulin treatment, steroid use, or having severe diabetic complications. The participants habitually woke up between 06:00 and 08:00 and sleep between 22:00 and 24:00. The study protocol conformed to the 1975 Declaration of Helsinki and was approved by the Ethics Committee of Kyoto Women's University (27-9) and was registered (UMIN-CTR clinical trial registration number: UMIN000019144). The study was fully explained to the participants prior to the starting and written informed consent was obtained.

Study design

This study was a randomized crossover within-patient clinical trial. Two weeks prior to the study, the patients underwent an examination to obtain their anthropometric measurements with blood sampling for fasting plasma glucose and Hemoglobin A1c (HbA1c) after an overnight fast. The patients' characteristics such as medical history and current medication use were also examined.

During the test period, each patient consumed the identical test meals for breakfast at 08:00, lunch at 13:00 at home for 3 days and the different dinner regimens were provided at Kyoto Women's University for the 3 consecutive evenings (Figure 1). On the first evening they consumed identical dinner at 18:00. On the second evening the patients were divided into two groups: one group consumed dinner at 21:00 on the second day and the divided dinner on the third day; other group consumed divided dinner on the second day and dinner at 21:00 on the third day. Patients were randomly assigned to each group by flipping a coin. Blood samples were withdrawn at 0, 30, 60, and 120 min after the dinner by the physicians of Kyoto Prefectural University of Medicine at Kyoto Women's University (Figure 1). The incremental area under the curves (IAUC) 2h for glucose, insulin, glucagon, active GLP-1, and active GIP were calculated by the trapezoidal method above the baseline value at 18:00 for dinner at 18:00 and the baseline value at 21:00 for dinner at 21:00. In divided dinner IAUC 4h were calculated sum of the baseline value at 18:00 for the first meal (2h) and the baseline concentration at 21:00 for the second meal (2h). The levels of postprandial and IAUC for glucose, insulin, glucagon, free fatty acid (FAA), active GLP-1, and active GIP were compared within-patient among three days of the consumption of the identical dinner at different times.

Test meals

Each patient consumed an identical breakfast, lunch, and dinner for consecutive 3 days. The composition of the test meals were shown in Table 1 and the nutritional contents were analyzed by computer software (Excel Eiyo-kun, Kenpakusya, for Windows v. 7.0, Tokyo, Japan). The test meals consisted of boiled white rice, white bread, milk, tomato (100 g × 3), spinach (80 g × 2), broccoli (60 g), and frozen meal set of fried fish for lunch and gluten-meat steak for dinner (Tokatsu Foods Co., Ltd., Yokohama, Kanagawa, Japan). The test meals were adjusted to meet the caloric requirement of each patient by adjusting the amount of carbohydrate with the mean of a sample population prescribed a 1,725 kcal diet plan. The test meals had the same macronutrient content and composition within-patient. The patients

consumed the first dish of vegetables for 5 min, then the main dish for 5 min, and rice/bread for 5 min at each meal time. The test meals of breakfast and lunch were prepared by the patients according the protocol prescribed for each patient by the dietitian of the clinic except the frozen lunch box were provided by the research group and consumed at each patient's home. The test meals of dinner were prepared and served by the study group at Kyoto Women's University for 3 consecutive days at different time. The patients were asked to avoid alcohol consumption and excessive physical activity 2 days preceding the study day and during the study period. Water, green tea, tea, and coffee without sugar or milk were permitted to the patients. Each patient recorded each meal time and measured all food and consumed the identical test meals during the study period. The dietitians in the study group obtained the food record of each patient and confirmed the compliance of the study protocol.

Measurements

HbA1c were determined by high-performance liquid chromatography (HPLC). Blood samples for determining glucose, insulin, glucagon, FFA, active GLP-1, and active GIP were collected 0, 30, 60, 120 min after the dinner (Figure 1) and analyzed by LSI Medience (LSI Medience Corp., Tokyo, Japan). Plasma glucose (IATRO LQ, GLU, LSI, Medience Corp., Tokyo, Japan) and FFA (NEFA-SS Eiken Kagaku Corp., Tokyo, Japan) were measured by enzyme methods. Hormones were measured using the following assays; Serum insulin by chemiluminescence immunoassay (Architect Insulin, Abbott Corp., Tokyo, Japan), pancreatic glucagon by radioimmunoassay (RIA, SML, Sceti Medical Labo Corp., Tokyo, Japan), active GLP-1 by enzyme-linked immuno sorbent assay (ELISA, GLUCAGON-LIKE PEPTIDE-1 ACTIVE, Merch Millipore Corp., Darmstadt, Germany), active GIP by ELISA (Human GIP, Active form Assay Kit, IBL Corp., Gumma, Japan).

Statistical analyses

Data are reported as mean ± SEM unless otherwise stated. The sample size was calculated based on the incremental glucose peak of our previous study.[12] According to that study, a sample size of 7 patients was calculated to have at least 80% power (G*Power 3.1, Heinrich-Heine-Universität Düsseldorf, Germany), therefore, 8 patients were recruited to participate the study. The primary outcomes were postprandial blood glucose and insulin and the second outcomes are the IAUC for glucose, insulin, active GLP-1, and active GIP. Since a normal distribution and homogeneity of all parameters could not be confirmed by Shapiro-Wilk and Levene test, we performed a paired comparison by Wilcoxon matched-pairs signed rank test

followed by post hoc Bonferroni's inequality (p<0.017) when Friedman's test revealed significant effects for parameters (p<0.05). All analyses were performed with SPSS Statistics version 22 software (IBM Corp., Armonk, NY, USA).

RESULTS

Patients' Characteristics

Eight patients with type 2 diabetes agreed to participate the study and their characteristics were shown in Table 2. Three patients were controlled by diet, whereas five others taking oral hypoglycemic agents [sulphonylureas (n=5), dipeptidyl peptidase (DPP)-4 inhibitors (n=2), α-glucosidase inhibitors (n=2), metformin (n=2), and thiazolidinedione (n=1)]. The patients were asked to maintain all medications at their pre-study doses during the study period except the patients stopped taking DPP-4 inhibitors 1 day before and during the study period. Because half-lives of DPP-4 inhibitors used for the participants in this study were 6 to 10 hours, we decided to ask the participants to stop taking DPP-4 inhibitors one day before the study for the ethical benefit of the participants.

Incremental concentration of glucose and insulin

In Figure 2 A and C we demonstrated incremental concentration of blood glucose and blood insulin after consuming each dinner to show the significant difference of incremental increase of glucose and insulin concentration among 3 different dinner time. Incremental glucose concentration at 60 min and 120 min (60 min; 4.45±0.93 vs 2.03±0.51mmol/L × min, p=0.008. 120 min; 6.75±1.22 vs 3.35±0.84 mmol/L × min, p=0.008, mean±SEM) and IAUC 2h for glucose were all significantly higher in dinner at 21:00 than those of dinner at 18:00 (449±83 vs 216±43 mmol/L × min, p=0.008, Figure 2 A and B), although there was no significant difference at 0 min of plasma glucose concentrations among three different dinner timing (dinner at 18:00; 8.35±0.58, dinner at 21:00; 7.02±0.30, divided dinner ; 8.91±0.74 mmol/L). IAUC 2h for insulin was also significantly higher in dinner at 21:00 than in dinner at 18:00, (772±104 vs 527±107 μU/mL × min, p=0.008, Figure 2 D), while pre-dinner insulin concentration was significantly lower in dinner at 21:00 than dinner at 18:00 and divided dinner (5.51±1.55 vs 8.91±1.43, vs 10.21±2.35 μU/mL, respectively, both p=0.008).

On the other hand, when the patients consumed the first meal with tomato and rice at 18:00 in divided dinner incremental glucose at 30 min, 60 min, and 120 min were all significantly lower than those in dinner at 21:00 (30 min; 0.42±0.45 vs 1.40±0.46 mmol/L, p=0.008. 60 min; 2.58±0.97 mmol/L, p=0.016. 120 min; 3.06±1.37 mmol/L, p=0.008, Figure 2A).

Incremental glucose at 120 min in the second meal with vegetable and the main dish at 21:00 of divided dinner was significantly lower than those of dinner at 21:00 and dinner at 18:00 (-0.34±0.93 mmol/L vs. dinner at 21:00, vs. dinner at 18:00, both p=0.008, Figure 2 A). Incremental insulin at 60 min and 120 min in the first meal of divided dinner were significantly lower than those in dinner at 21:00 (60 min; 1.80±1.50 vs 7.58±1.54 μU/mL, p=0.016. 120 min; 2.36±2.24 vs 11.3±1.97 μU/mL, p=0.008, Figure 2C) and incremental insulin at 120 min in the second meal of divided dinner was also significantly lower than that in dinner at 21:00 (1.49±2.66 μU/mL, p=0.016, Figure 2 C). Both IAUCs for glucose and insulin in divided dinner, which were the sum of IAUC 2h of the first meal (18:00 to 20:00) and IAUC 2h of the second meal (21:00 to 23:00), were tended to be lower than those in dinner at 21:00 (IAUC for glucose; 269±76 mmol/L × min, p=0.070, Figure 2B. IAUC for insulin; 552±114 μU/mL × min, p=0.070, Figure 2 D), but there were no difference between dinner at 18:00 and divided dinner.

Postprandial concentration of glucagon and FFA

The glucose and insulin concentrations increase after eating, while FFA and glucagon concentrations decrease after eating, therefore in Figure 3 A and B we demonstrated postprandial concentrations of glucagon and FFA. Postprandial glucagon at 60 min and 120 min in the first meal of divided dinner were significantly lower than those of dinner at 18:00 (60 min; 35.6±3.1 vs 40.4±3.3 pmol/L, p=0.008. 120 min; 31.7±3.0 vs 36.7±3.4 pmol/L, p=0.008, Figure 3 A). Postprandial glucagon at 30 min in the second meal of divided dinner was significantly lower than those of dinner at 21:00 and dinner at 18:00 (33.0±4.1 vs 40.5±3.6, vs 39.5±3.3 pmol/L, both p=0.008). Postprandial glucagon at 60 min in the second meal of divided dinner was also lower than that of dinner at 21:00 (36.2±3.9 vs 39.1±4.9 pmol/L, p=0.008) and tended to be lower than that of dinner at 18:00 (40.4±3.3 pmol/L, p=0.070). The FFA at 0 min and 30 min in dinner at 21:00 were tended to be higher than those of dinner at 18:00, but not statistically significant (0 min; 0.693±0.095 vs 0.328±0.063 mEq/L, p = 0.070. 30 min; 0.541±0.057 vs. 0.294±0.034 mEq/L, p=0.070, Figure 3B).

Postprandial concentration of active GLP-1 and active GIP

The effects of consuming dinner at different timing on incretin secretion were shown in Figure 4. There were no significant difference in concentration of postprandial active GLP-1 and IAUC for active GLP-1 among 3 different meal timing (Figure 4 A and B). However, incremental active GIP at 60 min in the first meal of divided dinner was significantly lower

than that of dinner at 21:00 (10.7±8.0 vs. 25.9±9.8 pmol/L, p=0.008), and incremental active GIP at 120 min in the first meal of divided dinner was also significantly lower than that of dinner at 18:00 (2.21±7.5 vs. 26.0±13.9 pmol/L, p=0.008) and tended to be lower than that of dinner at 21:00 (40.6±13.3 pmol/L, p=0.070, Figure 4 C), although IAUC for active GIP showed no significant difference among three days (Figure 4 D).

DISCUSSION

This is the first study to assess the acute effect of different dinner timing on postprandial glucose and hormone responses in patients with type 2 diabetes. The main finding in this study is that the consumption of the late-night-dinner (21:00) is associated with postprandial higher concentration of glucose and insulin compared with those in early-evening-dinner (18:00) in patients with type 2 diabetes. Incremental glucose at 120 min and IAUC 2h for glucose in consuming the late-night-dinner (21:00) were both 2-fold higher than those of early-evening-dinner (18:00). Incremental insulin level at 120 min and IAUC 2h for insulin in dinner at 21:00 were both about 1.5-fold higher than those in dinner at 18:00. It was remarkable that only 3 hours delay from 18:00 to 21:00 of consuming identical meal caused significant higher postprandial glucose and insulin concentration (Figure 2). Meanwhile, consumption of dinner dividedly (consuming tomato and carbohydrate at 18:00, and vegetable and the main dish at 21:00) resulted to divide the postprandial concentration of glucose, insulin, glucagon, active GLP-1, and active GIP as shown in Figure 2 to 4. Moreover, both IAUC 4h for glucose and insulin in divided dinner (the sum of IAUC of the first meal and the second meal) were tended to be lower than those in dinner at 21:00, but almost unchanged between those in dinner at 18:00 and divided dinner. On the other hand, IAUC for active GLP-1 and active GIP demonstrated no difference in consuming identical dinner of three different time. Thus, we found that the changing dinner time and consuming dinner dividedly affected not only the postprandial glucose concentration but also the postprandial insulin concentration.

One of the reason for the higher postprandial glucose and insulin concentration observed in consuming dinner at 21:00 compared with those in dinner at 18:00 may be related to the circadian rhythm because the diurnal variations of insulin resistance is higher at night than in the daytime.[24-26] Several reports have suggested that meal timing influenced on the clock systems. Jakubowicz D et al reported that skipping breakfast adversely affected the clock and clock controlled gene expression. Skipping breakfast was correlated with increased postprandial glycemic response whereas breakfast consumption affected clock and clock-

controlled gene expression leading to normal glucose excursions in both people with and without diabetes.[27, 28] Also, the diet-induced thermogenesis (DIT) is 40 to 50% lower in the evening than in the morning in healthy individuals.[29] Though, the reports of the circadian rhythm of incretin hormones are still controversial. Lindgren O et al reported that the secretion of GLP-1 and GIP were more pronounced in the morning than in the afternoon 25 while Takahashi M et al. reported that the GIP secretion was higher in the evening than in the morning.[28] Thus, disorder of the circadian rhythm may lead to increased insulin resistance and elevated postprandial glucose concentration. However, consuming identical meals at 21:00 resulted in significantly higher concentration of postprandial glucose and insulin than those of dinner at 18:00 was not consistent with the theory of circadian rhythms because in this study all dinner were consumed during the evening from 18:00 to 21:00. The mechanism of meal timing on circadian rhythms and hormone secretion is still unclear, since we did not investigate the influences of clock controlled gene expression nor DIT in this study.

Therefore, another possible reason for the different postprandial glucose and insulin concentration observed in this study may be explained by the pre-dinner FFA and insulin concentration. The long fasting time (8 hours) in dinner at 21:00 may have led the elevated pre-dinner FFA and concomitant with decreased plasma insulin concentrations. High plasma FFA and low plasma insulin concentrations augmented postprandial glucose and insulin concentration, because the acute elevation of FFA increases insulin resistance and possibly increases hepatic glucose production.[30-32] Thus, increased plasma FFA and decreased plasma insulin at pre-dinner would be one of the reasons for increasing postprandial glucose and insulin concentrations in consuming dinner at 21:00.

Furthermore, the amelioration of glucose and insulin responses in divided dinner can be explained by the small amount of nutrients, particularly the amount of carbohydrate in each meal. The amount of carbohydrate in the second meal of divided dinner at 21:00 was 25.3 g which was much smaller than that of dinner at 21:00 and dinner at 18:00 (carbohydrate 104.2 g). Therefore, the reduction of the postprandial glucose and insulin responses observed in divided dinner was influenced by the amount of carbohydrate. However, the reverse regimen in divided dinner which was consuming vegetable and the main dish at 18:00 following tomato and carbohydrate at 21:00 raised the postprandial glucose concentration in our study (data now shown). Thus, large carbohydrate meals should be avoided at late evening.

Alsalim W et al reported that the increasing meal size elicited a caloric-dependent insulin response due to increased β-cell secretion achieved by increased GIP and GLP-1 secretion.[14,33] Moreover, it has been reported that amino acids and peptides directly stimulate

L cell activity to secrete GLP-1 to potentiate insulin secretion from β-cell and associated with regulation of food intake whereas the fat intake stimulate K cell activity to secrete GIP.[34] Therefore, because the amount of calorie, protein, and fat in each meal of divided dinner were smaller than those of dinner at 18:00 and dinner at 21:00, the concentration of postprandial glucose, insulin, glucagon, active GLP-1, and active GIP might be divided by two separate consumption.

The present study explored the influence of dinner timing on glucose and hormone responses in patients with type 2 diabetes, although some limitations should be mentioned. First, the study population consisted of small number of Japanese individuals who had different duration, medication, and severity of type 2 diabetes, therefore it is unclear whether the present results could be appropriately applied to patients with type 1 diabetes or individuals without diabetes, or to other racial groups. Second, as this study investigated the acute effects of dinner timing on glucose and hormones responses, long-term dietary interventions should be conducted to determine whether the effect of consumption of dinner dividedly would be effective for glycemic control and hormone responses. Third, the oral hypoglycemic medications may have influenced the postprandial glucose and hormone responses in the patients who were taking such medications. The patients maintained all medications at their pre-study doses during the study period except the patients who were taking DPP-4 inhibitors stopped taking them one day before the study and during the study period. Moreover, since this study was a cross-over within-patient trial, we suggest that the effect of oral hypoglycemic medications on glycemic responses would be limited. Additionally, the gastric emptying rate is critical in early and overall postprandial glucose excursions because enhanced GLP-1 secretion delays gastric emptying in both people with and without diabetes.[18,19,21] Although, gastric emptying rate was not measured in this study the contribution to the effect of meal timing on the postprandial glucose and hormone concentration remain unclear.

Our findings demonstrate that not only the amount of total energy, carbohydrate, and macronutrients but also consumption of meal timing is the important factors for postprandial glucose and hormone responses. The consumption of dinner in the early evening such as at 18:00 or 19:00 should be endorsed to people even though some people are not able to avoid eating late at night. Then, people who eat dinner late at night are recommended to consume dinner dividedly. For example eating sandwich or rice balls at workplaces in the early evening and eating vegetable and the main dish in later at home may contribute to improve the glycemic control and lower the risk of obesity [1,3,6,9-11] and cardiovascular diseases.[35,36]

However, our study is restricted to small numbers of Japanese patients with type 2 diabetes, and the exact mechanism of this effect remain uncertain. Further investigation are required to determine overall mechanisms of daily rhythms on different timing of consuming dinner in individuals with and without type 2 diabetes.

Conclusions

This study demonstrated that consuming late-night-dinner (21:00) deteriorates postprandial glucose and insulin concentration compared with those of early-evening-dinner (18:00) whereas consuming dinner dividedly, consuming carbohydrate in the early evening followed by vegetable and the main dish at later evening, ameliorates them in patients with type 2 diabetes. Eating dinner dividedly might improve the glycemic control for patients with type 2 diabetes who cannot avoid eating dinner at late night and it may provide a crucial contribution to the prevention of diabetic complications.

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CONFLICT OF INTEREST AND FUNDING DISCLOSURE

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Table 1. The composition and macro-nutritional contents of the test meals

	Energy (kcal)	Protein (g)	Fat (g)	Carbohydrate (g)	Fiber (g)	Detail content
Breakfast	410	18.3	12.0	59.5	5.7	White bread 90 g, tomato 100 g, broccoli 60 g, milk 200 g, strawberry jam (sugar free) 13 g
Lunch	606	23.9	11.3	100.3	7.8	Boiled white rice 200 g, frozen lunch box of fried fish with vegetable, tomato 100 g, spinach 80 g
Dinner	709	24.2	20.4	104.2	7.8	
Divided dinner						
at 1800 h	355	5.7	0.7	78.9	1.7	Boiled white rice 200 g, seasoned seaweed, tomato 100 g
at 2100 h	354	18.5	19.7	25.3	6.1	Frozen lunch box of gluten-meat steak with vegetable, spinach 80 g with fried tofu 15 g
Total	1,725	66.4	43.7	264.0	21.3	

The nutritional content of the test meals was calculated by computer software (Microsoft Excel Eiyokun for Window Ver.7.0, Kenpakusya, Tokyo).

Table 2. Characteristics of the patients with type 2 diabetes

	n=8
Male/female	5/3
Age (years)	70.8±1.9
Duration of diabetes (years)	8.5±3.1
Body weight (kg)	68.6±13.4
BMI (kg/m^2)	23.3±3.2
HbA1c (%)	7.6±0.6
Fasting plasma glucose (mmol/L)	7.2±0.8
Systolic blood pressure (mmHg)	131.8±11.0
Diastolic blood pressure (mmHg)	67.0±11.0
Diet therapy only	3
Oral glucose lowering medication	5
Sulfonylurea	5
Dipeptidyl peptidase-IV (DPP4) inhibitor	2
α-glucosidase inhibitor	2
Metformin	2
Thiazolidinedione	1
Antihypertensive medication	3
Lipid-lowering medication	6

BMI: body mass index.
Data are mean±SD or n.

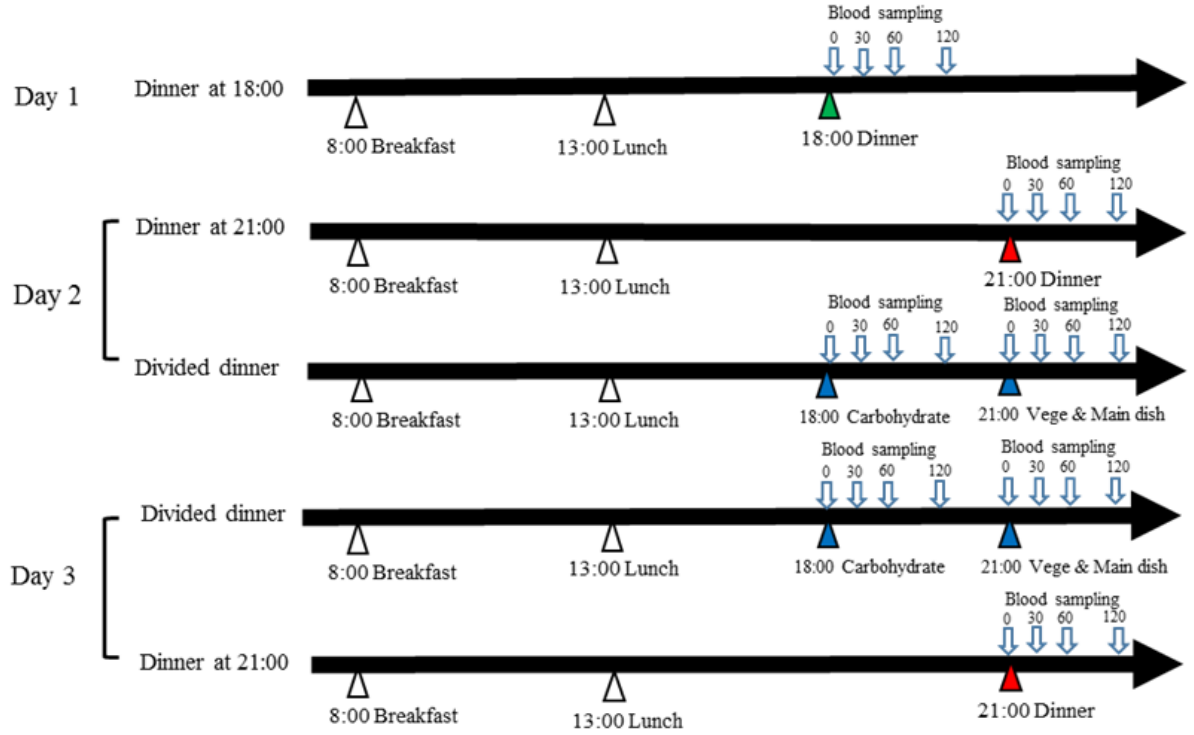

Figure 1. Schematic illustration of the study. Each patient consumed the identical test meals of breakfast at 08:00, lunch at 13:00 at home for 3 days. The patients visited Kyoto Women's University 3 consecutive evenings and consumed dinner at 18:00 on the first day, and divided dinner (tomato and rice at 18:00 followed the spinach and the main dish at 21:00) or dinner at 21:00 on the second day or the third day. Blood samples were withdrawn at 0, 30, 60, and 120 min after the dinner for 3 days.

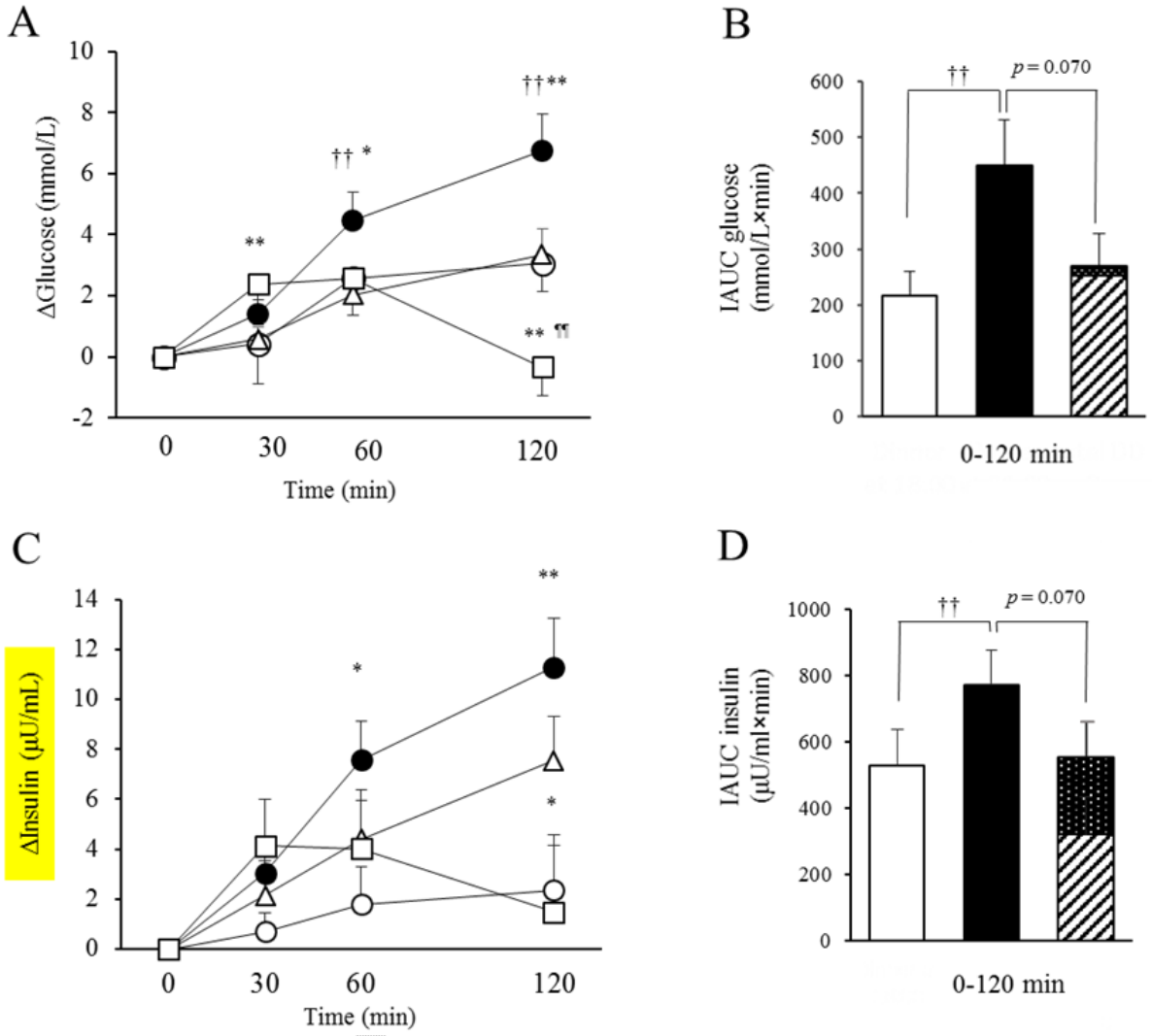

Figure 2. Time course curves are indicated for incremental glucose (A) and insulin (C) in patients with type 2 diabetes during three different dinner timing: dinner at 18:00, white triangle; dinner at 21:00, black circle; the first meal at 18:00 of divided dinner, white circle; the second meal at 21:00 of divided dinner, white square. IAUC 2h for glucose (B) and for insulin (D) are indicated as follows: dinner at 18:00, white bars; dinner at 21:00, black bars; the first meal at 18:00 of divided dinner, hatched bars; the second meal at 21:00 of divided dinner, black bars with white dots on hatched bars. IAUCs for glucose and insulin in divided dinner, which were the sum of IAUC 2h of the first meal (18:00 to 20:00) and IAUC 2h of the second meal (21:00 to 23:00). Data are means ± SEM. Data were analyzed by Wilcoxon signed-ranks test; †† $p<0.01$ for dinner at 18:00 vs. dinner at 21:00, * $p<0.05$ ** $p<0.01$ for divided dinner vs. dinner at 21:00, ¶ $p<0.01$ for divided dinner vs. dinner at 18:00.

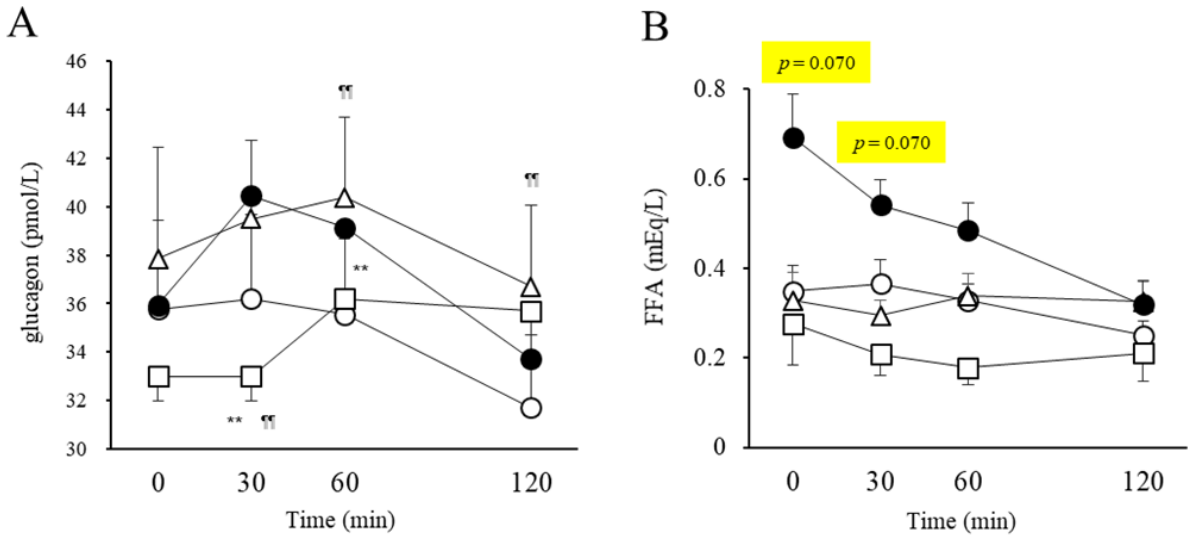

Figure 3. Time course curves are indicated for postprandial glucagon (A) and postprandial FFA (B) in patients with type 2 diabetes during three different dinner timing: dinner at 18:00, white triangle; dinner at 21:00, black circle; the first meal at 18:00 of divided dinner, white circle; the second meal at 21:00 of divided dinner, white square. Data are means ± SEM. Data were analyzed by Wilcoxon signed-ranks test; ** $p<0.01$ for divided dinner vs. dinner at 21:00, ¶ $p<0.01$ for divided dinner vs dinner at 18:00, $p=0.070$ for dinner at 21:00 vs dinner at 18:00.

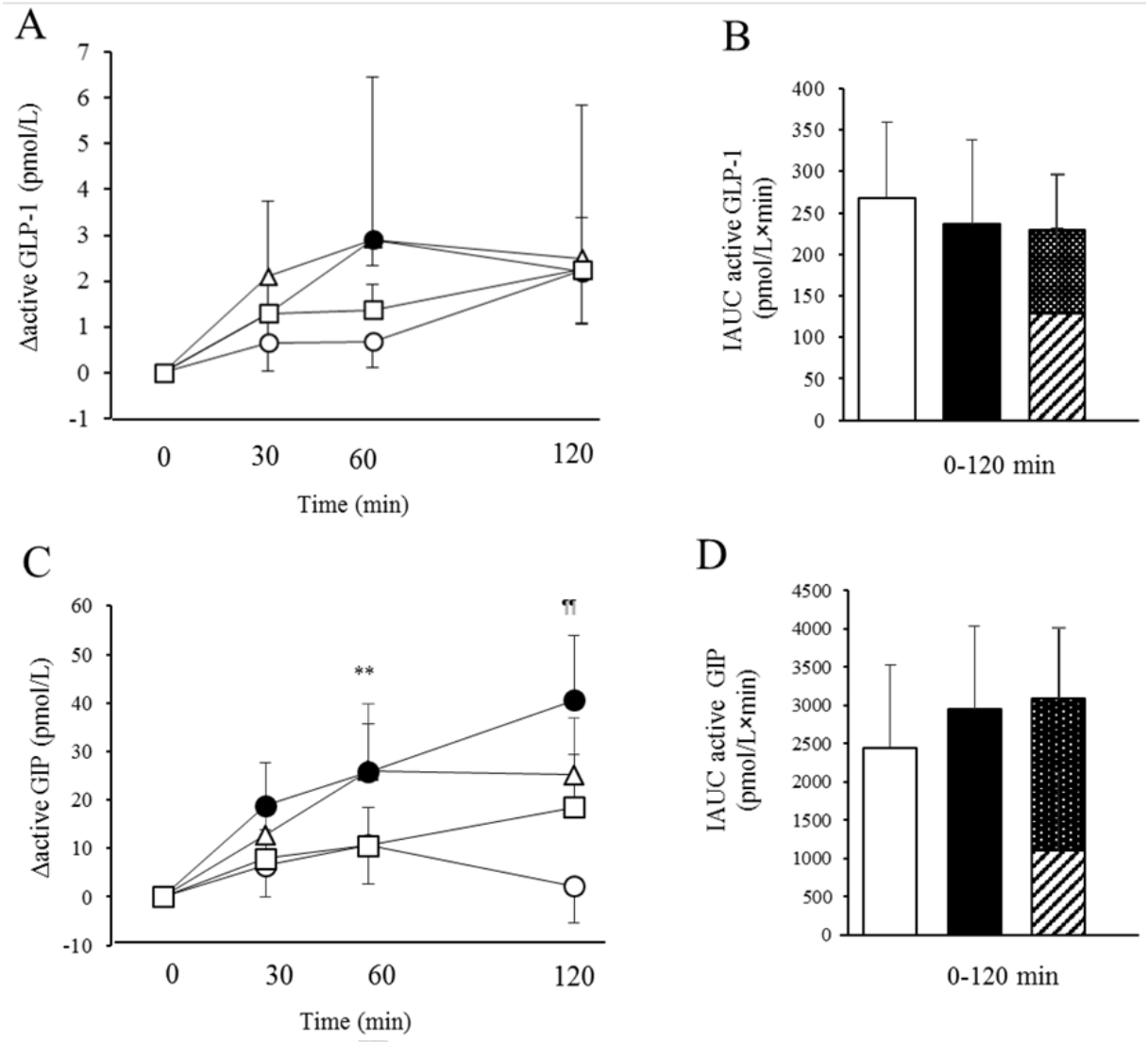

Figure 4. Time course curves are indicated for incremental active GLP-1 (A) and incremental active GIP (C) in patients with type 2 diabetes during three different dinner timing: dinner at 18:00, white triangle; dinner at 21:00, black circle; the first meal at 18:00 of divided dinner, white circle; the second meal at 21:00 of divided dinner, white square. IAUC 2h for active GLP-1 (B) and active GIP (D) are indicated as follows: dinner at 18:00, white bars; dinner at 21:00, black bars; the first meal at 18:00 of divided dinner, hatched bars; the second meal at 21:00 of divided dinner, black bars with white dots on hatched bars. IAUCs for GLP-1 and GIP in divided dinner, which were the sum of IAUC 2h of the first meal (18:00 to 20:00) and IAUC 2h of the second meal (21:00 to 23:00). Data are means ± SEM. Data were analyzed by Wilcoxon signed-ranks test; ** $p<0.01$ for divided dinner vs dinner at 21:00, ¶ $p<0.01$ for divided dinner vs. dinner at 18:00.