Potentiation of the early-phase insulin response by a prior meal contributes to the second-meal phenomenon in type 2 diabetes

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Lee SH, Tura A, Mari A, Ko SH, Kwon HS, Song KH, Yoon KH, Lee KW, Ahn YB. Potentiation of the early-phase insulin response by a prior meal contributes to the second-meal phenomenon in type 2 diabetes. Am J Physiol Endocrinol Metab 301: E984–E990, 2011. First published August 9, 2011; doi:10.1152/ajpendo.00244.2011.—Improved glucose tolerance following a sequential meal is known as the second-meal phenomenon. We aimed to investigate its extent and underlying mechanisms in patients with type 2 diabetes. Metabolic responses after lunch in 12 diabetic patients were compared on two separate days: one with (Day BL) and another without (Day FL) breakfast. The responses of hormones were calculated by the incremental area under the curve (iAUC) values for 180 min after each meal. Indexes of early-phase insulin secretion were assessed, and β-cell function was estimated by mathematical modeling. [iAUCglucose(180–360 min)] was significantly lower on Day BL than on Day FL (181 ± 43 vs. 472 ± 29 mmol·liter−1·min, P = 0.0005). The magnitude of the The second-meal phenomenon [iAUCglucose(180–360 min) on Day BL/Day FL] was 35 ± 9%. The peak levels of insulin and C-peptide were attained 45 min earlier after the second meal than after the first meal. iAUCglucose(180–360 min) correlated negatively with iAUCinsulin(180–210 min) (r = −0.443, P = 0.0300), insulinogenic index (r = −0.769, P < 0.0001), acute C-peptide response (r = −0.596, P = 0.0021), and potentiation factor [i.e., potentiation effect on insulin secretion] ratio (180–360)/(0–20) (r = −0.559, P = 0.0045), while correlated positively with free fatty acid level before lunch (r = 0.679, P = 0.0003). The second-meal phenomenon was evident in patients with type 2 diabetes. Potentiation of the early-phase insulin response by a prior meal contributes to this phenomenon in type 2 diabetes.

free fatty acid; mathematical modeling; potentiation factor

THE EFFECT OF A PRIOR MEAL ON IMPROVING GLUCOSE TOLERANCE AFTER A SUBSEQUENT MEAL, KNOWN AS THE SECOND-MEAL PHENOMENON, OR STAUB-TRAUGOTT EFFECT, WAS FIRST OBSERVED ALMOST A CENTURY AGO (11, 28, 32). THE SECOND-MEAL PHENOMENON HAS BEEN OBSERVED CONSISTENTLY IN HEALTHY PEOPLE (2, 16, 31, 33), BUT ITS PRESENCE IS CONTROVERSIAL IN PEOPLE WITH DIABETES MELLITUS. PAST OBSERVATIONS INSISTED THAT IT IS ABSENT AFTER ORAL OR INTRAVENOUS GLUCOSE ADMINISTRATION (10, 25, 26), BUT RECENT STUDIES WITH SETTINGS RESEMBLING EVERYDAY MEAL INGESTION HAVE DOCUMENTED THE SECOND-MEAL PHENOMENON IN TYPE 2 DIABETIC PATIENTS (4, 15, 23). BECAUSE POSTPRANDIAL GLUCOSE LEVEL AND GLUCOSE FLUCTUATIONS CONTRIBUTE TO THE ACTIVATION OF OXIDATIVE STRESS, LEADING TO INCREASED RISK OF DIABETIC COMPLICATIONS INCLUDING CARDIOVASCULAR EVENTS, LOWERING GLYCEMIC EXCERSION AND GLUCOSE VARIABILITY ARE NOW CONSIDERED MAJOR TREATMENT TARGETS IN DIABETIC PATIENTS (5, 6, 27). UNDERSTANDING THE EXTENT OF AND PRECISE MECHANISMS RESPONSIBLE FOR THE SECOND-MEAL PHENOMENON MIGHT BE USEFUL FOR ACHIEVING THESE GOALS.

SEVERAL HYPOTHESES HAVE BEEN SUGGESTED TO EXPLAIN THE SECOND-MEAL PHENOMENON, INCLUDING INCREASED MUSCLE GLYCOGEN STORAGE IN ASSOCIATION WITH SUPPRESSION OF PRESSURE FREE FATTY ACID (FFA) CONCENTRATION (4, 7, 15, 16, 34), DECREASED HEPATIC INSULIN CLEARANCE (33), SLOWED GASTRIC EMPTYING (9, 19), INCREASED INCRETIN HORMONE SECRETION (9, 19), AND STRONGER GLUCOSE POTENTIATION AND SUPPRESSION OF ENDOSKNE GLOWE PRODUCTION (2). MOST STUDIES DENY THE ROLE OF INCREASED INSULIN RELEASE (2, 4, 7, 9, 15, 16, 33), ALTHOUGH ONE RECENT STUDY REPORTED THE STIMULATION OF INSULIN SECRETION BY WHEY PROTEIN CONSUMED BEFORE A CARBOHYDRATE MEAL (19). THE DIFFERENT RESULTS OF VARIOUS STUDIES MIGHT REFLECT DIFFERENCES IN THE SUBJECT CHARACTERISTICS OR IN THE SIZE, COMPOSITION, OR ROUTE OF ADMINISTRATION OF THE MEAL. TO DATE, DATA ON THE ROUTINE BREAKFAST-LUNCH TEMPORAL SEQUENCE AND DETAILED ANALYSIS OF THE MECHANISM IN TYPE 2 DIABETIC PATIENTS ARE SCARCE.

IN THIS STUDY, WE AIMED TO DETERMINE WHETHER THE SECOND-MEAL PHENOMENON IS PRESENT IN ASIAN PEOPLE WITH WELL-CONTROLLED TYPE 2 DIABETES. WE HYPOTHEZIZED THAT SEVERAL FACTORS MIGHT CONTRIBUTE TO THE SECOND-MEAL PHENOMENON IN TYPE 2 DIABETIC PATIENTS AND EXAMINED THE ROLE OF THOSE POSSIBLE MECHANISMS: 1) DIFFERENCES IN THE AMOUNT AND THE PATTERN OF INSULIN SECRETION, 2) AUGMENTATION OF Β-CELL FUNCTION, 3) SUPPRESSION OF PRESSURE FFA CONCENTRATION, AND 4) INCREASED INCRETIN RESPONSE.

MATERIALS AND METHODS

Patients. Twelve patients with type 2 diabetes participated in the study. All participants had diabetes for less than 5 yr and a glycated hemoglobin (HbA1c) level less than 8% on recruitment. Subjects aged between 40 and 70 yr with a body mass index (BMI) between 18.5 and 30 kg/m² were included. All subjects were without anemia, had normal renal and hepatic function, and did not have severe diabetic complications such as cardiovascular disease, cerebrovascular disease, proliferative diabetic retinopathy, or gastroparesis. All were insulin naïve, and patients taking oral hypoglycemic agents other than sulfonylurea or metformin, or who were taking medications known to affect gastric motility, were excluded. All subjects agreed to participate after providing oral and written consent. The Institutional Review Board of the Clinical Research Coordinating Center at St. Vincent’s Hospital approved the study protocol (no. VC10EIS0007).
Study protocol. Meal tolerance tests (MTT) were performed on two separate days within 2 wk in a random order. On Day BL (breakfast + lunch), the same meals were provided at 0900 and 1200. On Day FL (fasting + lunch), only one meal was taken, at 1200. The interval between the two meals was designed to mimic the breakfast-lunch temporal sequence and to be appropriate for studying the potentiation effect of the prior meal. The mixed meal consisted of two calorie bars (Sojjoy; Otsuka Pharmaceutical, Tokyo, Japan) and a drink (New-care; Daesang Welllife, Seoul, Korea) containing 470 kcal [58 g carbohydrate, 19 g fat (7.28 g saturated fatty acid) and 17 g protein; fiber content 7.2 g], and this was consumed within 10 min. The protein content was provided by soy, dairy, fruits, and nuts. The sources of the fiber were whole soy, fruits, nuts, and maltodextrin. All antidiabetic treatment was withdrawn at least 3 days before the MTT. After an overnight fast, the subjects were tested in a seated position. A cannula was inserted into a vein on the dorsum of one hand, and a heating pad was applied to obtain arterialized blood. Blood was drawn at 0, 15, 30, 45, 60, 90, 120, and 180 min after ingestion of each meal into chilled EDTA tubes pretreated with 10 µl of dipeptidyl peptidase-IV inhibitor (Millipore, Billerica, MA) and 10 µl/ml blood of protease inhibitor (Sigma-Aldrich, St. Louis, MO). Blood samples were also collected for measurement of serum FFA concentrations. All samples were centrifuged at 4°C within 1 h and stored at −70°C until analysis.

Analytic methods. The plasma concentrations of insulin, C-peptide, intact glucagon-like peptide-1 (iGLP-1), and total glucose-dependent insulinotropic polypeptide (tGIP) were measured by multiplexed bio-marker immunoassays using Luminex xMAP technology (cat. no. HMH-34K, Millipore). Because the insulinotropic effect of GIP is greatly reduced in diabetic patients, we measured the total form without discriminating between the active and inactive forms. However, we measured the active form of GLP-1, which can reflect the true extent of incretin action. The intra-assay and interassay coefficients of variation were respectively 3 and 10% for insulin, 4 and 8% for C-peptide, 1 and 15% for iGLP-1, and 4 and 6% for iGIP. Plasma glucose levels were measured by the hexokinase method using Pureauto S GLU reagent (Daiichi Pure Chemicals, Tokyo, Japan), and serum FFA concentrations were measured using enzymatic colorimetric test with SICDIA NEFAZYME (Shinyang Chemical, Seoul, Korea).

Calculations and modeling analysis. The extent of the second-meal phenomenon was assessed as the ratio of the iAUC values for glucose during 180–360 min on Day BL relative to those on Day FL. Because some previous reports used another method, which compared the rise of glucose after the second-meal and the prior meal on the same day, we also adopted this analysis. The insulinogenic index (∆ insulin(180–210 min) / ∆ glucose(180–210 min)) and acute C-peptide response [ACR, ∆ C-peptide(180–210 min) / ∆ glucose(180–210 min)] were calculated as markers of early-phase insulin secretion after lunch. Insulin sensitivity was estimated by calculating the oral glucose insulin sensitivity (OGIS) index (21) and the Stumvoll index (30) after the lunch. The parameters of β-cell function were analyzed by mathematical modeling using glucose and C-peptide data (20, 22). Briefly, the insulin secretion rate (ISR) was determined by C-peptide deconvolution and was calculated as the sum of two components. The first component originates from the dose-response relationship between the ISR and glucose concentrations. The slope of the dose-response curve is denoted as glucose sensitivity, which quantifies the ability of β-cells to respond to changes in glucose concentration. This component is modulated by a time-dependent potentiation factor (PF), which expresses the potentiation effect on insulin secretion. Some potentiating mechanisms include prolonged exposure to hyperglycemia, nonglucose substrates, incretin hormones, or neural effects. The PF was constrained to have a mean value of 1 during the 360-min duration of the study. The ratio of the PF between two time intervals was denoted the PF ratio (PFR).

The second ISR component describes the anticipated response when glucose concentration increases during the initial part of the MTT and is determined by a parameter denoted as rate sensitivity, which is a surrogate of first-phase insulin secretion.

Statistical analysis. All statistical analyses were performed using the SAS 9.1 package (SAS Institute, Cary, NC). The data are expressed as means ± SE except where noted. The AUC was calculated according to the trapezoidal rule and is shown as total AUC (tAUC) or iAUC values. The baseline values used for the iAUC for lunch were the values at 180 min. Differences between study days were compared using the Wilcoxon signed rank test. Spearman correlation analysis was used to examine the relationships between the AUC values of glucose concentration and other parameters. A P value < 0.05 was considered significant.

RESULTS

Baseline characteristics. The age, duration of diabetes, and Hb A1c levels of the 12 participants (4 men and 8 women) were 54.0 ± 7.3 yr, 20.6 ± 15.3 mo, and 6.7 ± 0.6%, respectively (mean ± SD). Their BMI and waist-to-hip ratio were 23.4 ± 2.6 kg/m² and 0.91 ± 0.05, respectively. One patient was being treated with sulfonylurea, three with metformin, five with sulfonylurea plus metformin, and three were treatment naïve.

Glucose, insulin, and C-peptide responses after MTT. Peak plasma glucose levels after lunch were lower on Day BL than on Day FL (9.38 ± 0.52 vs. 10.06 ± 0.46 mmol/l), tAUC glucose(180–360 min) was significantly lower on Day BL than on Day FL (181 ± 43 vs. 472 ± 29 mmol-litre−1-min, P = 0.0005). The magnitude of the second-meal phenomenon was 35 ± 9%. The glucose responses to the MTT were significantly lower after lunch than after breakfast on Day BL; the tAUC values were 1,457 ± 81 and 1,540 ± 74 mmol-litre−1-min, respectively (P = 0.0269), and the iAUC values were 181 ± 43 and 353 ± 43 mmol-litre−1-min, respectively (P = 0.0024). tAUC and iAUC ratios averaged 94 ± 2% and 47 ± 14%, respectively (Fig. 1A and Table 1). The insulin response to the MTT was more rapid after the second meal than after the first meal. The peak insulin level was attained earlier after the second meal (at 45 min) than after the first meal (at 90 min). The tAUC insulin(180–360 min) was significantly higher on Day FL than on Day BL. The tAUC insulin(180–360 min) was significantly higher on Day BL because of the carryover effect of the first meal. By contrast, iAUC insulin(180–210 min), which reflects early-phase insulin secretion, was significantly higher on Day BL than on Day FL (2,007 ± 390 vs. 1,011 ± 226 pmol-litre−1-min, P = 0.0010). The responses of C-peptide and its AUC values mimicked that of insulin (Fig. 1, B and C, and Table 1). The insulinogenic index (2,313 ± 426 vs. 726 ± 116, P = 0.0005) and ACR (3,832 ± 591 vs. 2,167 ± 296, P = 0.0024) were also significantly higher on Day BL than on Day FL.

Incretin hormone responses after the MTT. The iGLP-1 and tGIP responses to meals showed a similar pattern and reached a peak level 30–45 min after both the first and second meals. tAUC iGLP-1(180–360 min) was higher on Day BL than on Day FL (2,986 ± 494 vs. 2,514 ± 341 pmol-litre−1-min, P = 0.0425), but tAUC GIP(180–360 min) did not differ significantly between days. The iAUC iGLP-1(180–210 min) and tAUC GIP(180–210 min) values were nearly identical on the two days (Fig. 1, D and E, and Table 1).

FFA response after MTT. Fasting FFA levels were similar on Day BL (0.69 ± 0.07 mmol/l) and Day FL (0.67 ± 0.04 mmol/l). On Day BL, the FFA level decreased rapidly, reached
are means (H11006)/220)/(60 –180), which quantifies the increase in PF from the 360)/(0 –20), which quantifies the mean PF level during the shift in the dose-response curve and thus the ISR at 6.5 mmol/l glucose was significantly higher on Day BL (Fig. 1B and Table 1). After correction for the PF, the difference in the ISR at the reference glucose level disappeared. The PFR(180 – 360)/(0–20), which quantifies the mean PF level during the second meal compared with baseline, and the PFR(200–220)/(60–180), which quantifies the increase in PF from the end of the first meal to the early phase of the second meal, were also significantly higher on Day BL. Basal ISR, β-cell glucose sensitivity, and rate sensitivity were similar on the two days (Table 2).

Predictors of glucose excursion after lunch. In the linear correlation analysis, iAUCglucose(180–360 min) correlated negatively with iAUCinsulin(180–210 min) (r = −0.443, P = 0.0300), insulinogenic index (r = −0.769, P < 0.0001), ACR (r = −0.596, P = 0.0021), PFR(180–360)/(0–20) (r = −0.559, P = 0.0045), and PFR(200–220)/(160–180) (r = −0.471, P = 0.0201). iAUCglucose(180–360 min) correlated positively with FFA180 (r = 0.679, P = 0.0003; Fig. 3). However, no significant associations were found between iAUCglucose(180–360 min) and β-cell glucose sensitivity (r = −0.139, P = 0.5167), β-cell rate sensitivity (r = −0.199, P = 0.3509), OGIS (r = 0.366, P = 0.0785), or Stumvoll index (r = −0.155, P = 0.4702). FFA180 correlated negatively with the insulinogenic index (r = −0.545, P = 0.0059), ACR (r = −0.412, P = 0.0454), PFR(180–360)/(0–20) (r = −0.708, P = 0.0001), and PFR(200–220)/(160–180) (r = −0.618, P = 0.0013).

Table 1. Comparisons of iAUC and iAUC values after lunch on the two days

<table>
<thead>
<tr>
<th></th>
<th>iAUC(180–360 min)</th>
<th>iAUC(180–360 min)</th>
<th>iAUC(180–210 min)</th>
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<tbody>
<tr>
<td></td>
<td>Day BL</td>
<td>Day FL</td>
<td>Day BL</td>
</tr>
<tr>
<td>Glucose, mmol/l·min</td>
<td>1,457 ± 81</td>
<td>1,486 ± 64</td>
<td>181 ± 43</td>
</tr>
<tr>
<td>Insulin, pmol/l·min</td>
<td>46,230 ± 4,815</td>
<td>32,810 ± 3,073*</td>
<td>17,183 ± 2,541</td>
</tr>
<tr>
<td>C-peptide, pmol/l·min</td>
<td>20,538 ± 15,844</td>
<td>155,992 ± 13,578*</td>
<td>40,816 ± 5,977</td>
</tr>
<tr>
<td>iGLP-1, pmol/l·min</td>
<td>2,986 ± 494</td>
<td>2,514 ± 341*</td>
<td>1,173 ± 292</td>
</tr>
<tr>
<td>tGIP, pmol/l·min</td>
<td>7,968 ± 1,374</td>
<td>7,277 ± 1,149</td>
<td>3,373 ± 877</td>
</tr>
<tr>
<td>FFA, mmol/l·min</td>
<td>67,6 ± 4,3</td>
<td>68,6 ± 4,7</td>
<td>16,5 ± 3,3</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. tAUC, total area under the curve; iAUC, incremental area under the curve; BL, breakfast + lunch; FL, fasting + lunch; iGLP-1, intact glucagon-like peptide-1; tGIP, total glucose-dependent insulinotropic polypeptide. *P < 0.05 vs. Day BL.
The present study demonstrates that the second-meal phenomenon is evident in patients with type 2 diabetes. We have also shown that the different insulin response to the second vs. the first meal is an important part of this phenomenon. The first meal potentiated the insulin response during the second meal and particularly in the early phase. The increase in the PF during the second meal is a key mechanism, and the suppression of FFA concentration and the role of incretin hormones also seem to be associated with the second-meal phenomenon.

Whether the second-meal phenomenon is present to a meaningful extent in type 2 diabetic patients is controversial. A small amount of glucose (5 g) given 30 min before breakfast failed to ameliorate postprandial hyperglycemia (10). Experiments including three-hourly intravenous glucose tolerance tests showed that the glucose disappearance rate changed very little between loads and that the restoration of the second-meal phenomenon after sulfonylurea treatment was inconsistent (25, 26). However, the settings included in previous studies were not those of everyday meal ingestion. Sequential ingestion of mixed meals including carbohydrate and protein at 3- to 4-h intervals shows that the second-meal phenomenon is preserved in type 2 diabetic patients (4, 15, 23). Following the second meal, the blood glucose response may be reduced by up to 95% compared with the response after the first meal. In our study, the glucose excursion after lunch on Day BL was 35% of that on Day FL. To exclude the possibilities of diurnal variation in the glucose, insulin, or gut hormone responses (18), we designed the experiment to administer the MTT on two separate days instead of comparing the responses after breakfast and after lunch on the same day. Comparing the glucose response after lunch and after breakfast on the same day, as in previous studies (4, 23), confirmed the presence of the second-meal phenomenon.

Investigating the mechanisms responsible for the second-meal phenomenon is important for extending our understanding of the physiology of glucose metabolism and for achieving target glucose levels more effectively. First, we observed differences in the insulin response to the second vs. the first meal. The peak levels of insulin and C-peptide were attained 90 min after the first meal (breakfast on Day BL), whereas peak insulin levels were attained 90 min after the first meal (breakfast on Day BL and lunch on Day FL). Most previous studies have denied the enhancement of insulin release after the second meal phenomenon after sulfonylurea treatment was inconsistent (25, 26). However, the settings included in previous studies were not those of everyday meal ingestion. Sequential ingestion of mixed meals including carbohydrate and protein at 3- to 4-h intervals shows that the second-meal phenomenon is preserved in type 2 diabetic patients (4, 15, 23). Following the second meal, the blood glucose response may be reduced by up to 95% compared with the response after the first meal. In our study, the glucose excursion after lunch on Day BL was 35% of that on Day FL.

Table 2. Comparisons of the data from modeling analysis of β-cell function

<table>
<thead>
<tr>
<th></th>
<th>Day BL</th>
<th>Day FL</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISR at glucose level of 6.5 mmol/l, pmol·min⁻¹·m⁻²</td>
<td>100.4 ± 11.5</td>
<td>72.7 ± 7.5</td>
<td>0.0010</td>
</tr>
<tr>
<td>ISR at glucose level of 6.5 mmol/l corrected for PF, pmol·min⁻¹·m⁻²</td>
<td>63.7 ± 5.5</td>
<td>69.9 ± 9.6</td>
<td>0.4238</td>
</tr>
<tr>
<td>Basal ISR, pmol·min⁻¹·m⁻²</td>
<td>62.9 ± 5.8</td>
<td>59.4 ± 6.3</td>
<td>0.2661</td>
</tr>
<tr>
<td>Total ISR, nmol/m²</td>
<td>53.0 ± 4.2</td>
<td>33.6 ± 2.9</td>
<td>0.0005</td>
</tr>
<tr>
<td>tAUCISR(180–360 min), nmol/m²</td>
<td>27.5 ± 2.0</td>
<td>24.2 ± 2.2</td>
<td>0.0010</td>
</tr>
<tr>
<td>iAUCISR(180–360 min), nmol/m²</td>
<td>22.4 ± 1.6</td>
<td>22.0 ± 2.1</td>
<td>0.7334</td>
</tr>
<tr>
<td>tAUCISR(180–360 min), nmol/m²</td>
<td>5.1 ± 0.5</td>
<td>2.6 ± 0.3</td>
<td>0.0005</td>
</tr>
<tr>
<td>iAUCISR(180–360 min), nmol/m²</td>
<td>1.3 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>0.0771</td>
</tr>
<tr>
<td>β-Cell glucose sensitivity, pmol·min⁻¹·m⁻²·mM⁻¹</td>
<td>27.8 ± 4.2</td>
<td>28.7 ± 3.7</td>
<td>0.6772</td>
</tr>
<tr>
<td>Rate sensitivity, pmol·m⁻²·mM⁻¹</td>
<td>365.3 ± 104.3</td>
<td>257.9 ± 58.9</td>
<td>0.4238</td>
</tr>
<tr>
<td>PFR (180–360)/(0–20)</td>
<td>1.59 ± 0.11</td>
<td>1.12 ± 0.07</td>
<td>0.0010</td>
</tr>
<tr>
<td>PFR (200–220)/(160–180)</td>
<td>1.23 ± 0.07</td>
<td>0.97 ± 0.02</td>
<td>0.0068</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. ISR, insulin secretion rate; PF, potentiation factor; PFR, potentiation factor ratio.
meal (2, 4, 7, 9, 15, 16, 33). We also found that $iAUC_{\text{insulin}}(180–360 \text{ min})$ was rather higher on Day FL than on Day BL. However, the $iAUC_{\text{insulin}}(180–210 \text{ min})$, insulinogenic index, and ACR were significantly higher on Day BL than on Day FL, indicating the role of the early-phase insulin response in the mechanism responsible for the second-meal phenomenon. In support of this notion, $iAUC_{\text{glucose}}(180–360 \text{ min})$ correlated negatively with the $iAUC_{\text{insulin}}(180–210 \text{ min})$, insulinogenic index, and ACR.

Mathematical modeling of $\beta$-cell function gave additional evidence to support this hypothesis. The ISR was higher on Day BL than on Day FL throughout the first hour after lunch despite the lower glucose level. The main finding using this model was the PF pattern observed in Fig. 2B. The increased PF during the second meal on Day BL suggests that the $\beta$-cell response is generally stronger, possibly because of a priming effect. This would lead to significantly higher PFR$(180–360)/(0–20)$ and PFR$(200–220)/(160–180)$ values on Day BL, which also correlated negatively with $iAUC_{\text{glucose}}(180–360 \text{ min})$.

The model offers a unifying explanation by suggesting that the first meal potentiates the insulin-secretory response during the entire second meal and particularly in the early phase. It is interesting that a similar phenomenon has also been observed in nondiabetic healthy subjects (2). Because our patients had well-controlled diabetes and had had diabetes for a relatively short time, it remains unclear whether patients with greater impairment of $\beta$-cell function would show similar responses. A study reporting that the second-meal phenomenon was observed in diabetic patients whose diabetes was controlled with tolbutamide, but not in insulin-dependent patients (29), again raises this question.

We investigated the possible role of FFA concentration, because suppression of FFA after the first meal has been suggested as an important mechanism for the second-meal phenomenon. The FFA level just before lunch correlated positively with the postlunch glucose increment, a finding that is consistent with previous reports showing similar results in both diabetic and nondiabetic people (4, 7, 15, 16, 34). Taylor et al. (16) showed a negative relationship between the increment in muscle glycogen signal and prelunch FFA level and hypothesized that suppressed FFA level after the first meal improves insulin action, which would facilitate postprandial muscle glycogen storage. The acute role of FFA on insulin action also has been demonstrated in a study where the different compositions of the first meals resulted in different patterns of FFA responses prior to the second meal (34). The lesser rebound of plasma FFA resulting in lower concentrations before lunch were closely related to the improved glycemic responses after lunch in nondiabetic subjects. In our data, the link between insulin resistance and the second-meal phenomenon is not certain, because the surrogate markers of insulin sensitivity did not correlate with $iAUC_{\text{glucose}}(180–360 \text{ min})$ or with FFA$^{180}$. On the other hand, FFA$^{180}$ correlated negatively with some parameters of insulin secretion and with the PFR. Prolonged elevation of plasma FFA level causes $\beta$-cell lipotoxicity and impairs insulin secretion, whereas reduction of FFA level with acipimox improves insulin secretion (8, 24). However, acute changes in blood FFA level on insulin secretion need to be further confirmed.

In addition to the effects of the composition and the glycemic index of the meal, several studies suggest that the meal frequency is an important factor in influencing the second-meal phenomenon. Improved glucose tolerance has been reported with increased meal frequency in both healthy (13, 14) and type 2 diabetic people (1, 12), despite receiving isocaloric diets. By spreading the nutrient load which prolongs the rate of glucose absorption, serum insulin levels were decreased with FFA levels being equal or suppressed. The role of tissue insulinization at a relatively low insulin concentration might be
beneficial for the hyperglycemia and hyperinsulinemia related complications by ameliorating these conditions. Taken together, these studies suggest that the meal frequency, as well as composition, is an important determinant of the second-meal phenomenon.

Some evidence has led us to hypothesize the possible roles of incretin hormones. The absence of the second-meal phenomenon after a repeated intravenous glucose tolerance test (25, 26) but preservation by MTT (4, 15, 23), and the higher tAUC of GLP-1 and glucose potentiation after the second meal (2) may provide clues about the role of incretin hormones. Other relevant findings were the stimulation of incretin secretion by a preload of whey, oil, or artificial sweetener (3, 9, 19). In our study, the incretin responses to lunch were similar regardless of whether the breakfast meal had been ingested. Nevertheless, tAUC of GLP-1 on Day BL was higher on Day BL, indicating a longer exposure to higher levels of GLP-1. It would be interesting to define the contribution of incretin hormones to the second-meal phenomenon.

The ultimate goal of investigating this phenomenon is the clinical application. In the diagnostic area, a nonfasting 50-g OGTT used as a screening tool for gestational diabetes mellitus should be interpreted with caution because prior feeding can alter the response. In one study, even lower levels of glucose were attained when an OGTT was performed after mixed-meal ingestion (17). In the treatment area, this phenomenon might provide an option for attenuating postmeal glucose excursions and reducing glucose variability, although further confirmation is needed. The optimal amounts, components, and timing of the prior meal to induce the second-meal phenomenon should be determined. On the basis of our observation, we can speculate that the extent of the second-meal phenomenon might change across the spectrum of nondiabetic to insulin-dependent subjects according to the degree of insulin-secretory capacity and insulin resistance. This might be another important factor in determining the range of therapeutic application.

One limitation of our study is that a control group was not included. We showed that potentiation of insulin secretion is present in diabetic people, but we cannot say whether this effect is normal in these people. In addition, the roles of insulin sensitivity, suppression of endogenous glucose production, and gastric emptying remain undefined. Notably, the link between insulin sensitivity and the second-meal phenomenon needs to be studied with a gold standard method such as the hyperinsulinemic-euglycemic clamp.

In conclusion, potentiation of the early-phase insulin response by a prior meal contributes to the second-meal phenomenon in type 2 diabetes. Potentiating mechanisms such as the levels of FFA or incretin hormone seem to play a role in increasing early-phase insulin secretion. These findings suggest the possibility of applying the second-meal phenomenon as a way of reducing postprandial glucose excursion.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES