Effect of diurnal variations in the carbohydrate and fat composition of meals on postprandial glycemic response in healthy adults: a novel insight for the second-meal phenomenon

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ABSTRACT

Background: Meals, particularly carbohydrate intake, determine diurnal blood glucose (BG) excursions. However, the effect of meals with variable carbohydrate content on diurnal BG excursions remains poorly understood, despite routine consumption of meals that vary daily.

Objective: The aim of this study was to verify our hypothesis that glycemic response is elevated when a meal with a higher carbohydrate content follows a meal with a lower carbohydrate content.

Design: This was a secondary analysis of a study whose primary endpoint was energy metabolism (e.g., energy expenditure and substrate oxidation). This crossover study was designed to test BG responses to 3 types of meals with different macronutrient contents [regular meals (R), meals with a high-carbohydrate breakfast (CB), and meals with a high-fat breakfast (FB)] using a continuous glucose monitoring system. The R test included 3 meals/d with the same macronutrient composition; the CB test, a high-carbohydrate meal at breakfast, a high-fat meal at lunch, and a high-carbohydrate meal at dinner; and the FB test, a high-fat meal at breakfast, a high-carbohydrate meal at lunch, and a high-carbohydrate meal at dinner. Each test had similar daily macronutrient compositions, except CB and FB had larger variations in carbohydrate content than R. Fourteen healthy young men were tested in random order and underwent whole-body indirect calorimetry.

Results: Daily peak BG concentrations were higher for the CB (mean ± SD: 143.9 ± 25.3 mg/dL) and FB (140.2 ± 24.8 mg/dL) conditions than for the R condition (127.5 ± 15.7 mg/dL). Postprandial BG peaks after a high-carbohydrate meal were ∼20 mg/dL higher when a previous meal was relatively high-fat than when not high-fat ($P < 0.05$ for all). A multiple regression analysis indicated that the postprandial glycemic response was negatively associated with the preprandial respiratory quotient.

Conclusions: Our findings indicate that switching from high-fat to high-carbohydrate meals contributes to larger postprandial BG excursions, along with alterations in prioritization of carbohydrate utilization. This study was registered at the UMIN Clinical Trials Registry as UMIN000028895. *Am J Clin Nutr* 2018;108:332–342.

Keywords: A high-fat meal, Carbohydrate oxidation, Continuous glucose monitoring (CGM), Diurnal variations, Human calorimeter (chamber), Blood glucose management, Postprandial glycemic response

INTRODUCTION

Blood glucose (BG) levels are mainly altered by food intake. Notably, carbohydrate intake raises BG more substantially and directly than other macronutrients, and the complexity of the carbohydrate, or its glycemic index, can influence the rapidity and magnitude of those changes (1). Because all postprandial glycemic responses are factored into mean BG concentrations,

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Abbreviations used: baPWV, brachial-ankle pulse wave velocity; BG, blood glucose; CB, meals with a high-carbohydrate breakfast; CGMS, continuous glucose monitoring system; FB, meals with a high-fat breakfast; FFA, free fatty acids; HbA1c, glycated hemoglobin; HOMA-B, homeostasis model assessment of β-cell function; NIHN, National Institute of Health and Nutrition; OGTT, 2-h 75-mg oral glucose tolerance test; R, regular meals; RQ, respiratory quotient; VO_2 max, maximal oxygen uptake.

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over time they influence glycated hemoglobin (HbA1c) values (2, 3). Postprandial glycemic responses also receive much attention because of the independent effect of postprandial hyperglycemia on diabetic complications, although evidence with high certainty is still limited (4). To optimize the management of BG in medication-treated patients with type 1 or type 2 diabetes, it is important that postprandial glycemic responses and insulin needs are well understood. Taken together, understanding the postprandial glycemic response should contribute to mitigating the risk for type 2 diabetes and delaying its progression in diabetic patients.

Little is known about the relationship between diurnal variations in the macronutrient compositions of meals and BG, even though meals are generally eaten several times each day, and macronutrient composition changes between meals. Several studies have reported that protein intake appears to have a beneficial effect on the second-meal glycemic response (5, 6); however, there is notably insufficient evidence on the effect of carbohydrate and fat balance from a previous meal on the postprandial glycemic response of the next meal. Ercan et al. (7) showed that adding fat to a potato breakfast appeared to have no effect on the glycemic response of the next meal, whereas Frape et al. (8) showed that subjects who consumed a high-fat breakfast for 28 d had an elevated glycemic response at lunchtime. In addition, one study described the effect of a difference in carbohydrate intake between one meal and the next as it related to BG excursions (9). The study concluded that carbohydrates consumed at lunchtime appeared to have a more favorable effect on BG management than did carbohydrates consumed at the evening meal; however, the study did not comprehensively discuss the effect of the prior meal.

It is metabolically possible that a large variation in the carbohydrate and fat balance between one meal and the next can have a significant effect on the postprandial BG excursion. Increasing the proportion of dietary fat in a meal upregulates fat utilization, whereas increasing the proportion of dietary carbohydrate upregulates carbohydrate utilization. This natural metabolic tendency has been described in reports of acute (10) and relatively short-term (11–15) experiments, and has been thoroughly discussed by Flatt (16). Accordingly, it can be inferred that a high-fat/low-carbohydrate meal induces low carbohydrate utilization or temporal glucose intolerance; consequently, the glycemic response should be elevated in the event of an increase in carbohydrate consumption. Other lines of evidence also support our inference; for example, a short-term ($\geq$1 d) low-carbohydrate/high-fat diet induces temporal glucose intolerance (17–19). Furthermore, "second-meal phenomenon" studies indicate that levels of free fatty acids (FFA) in plasma positively correlate with the postprandial glycemic response (20–24). Based on existing findings, we hypothesized that a variation in the carbohydrate and fat balance from one meal to the next influences the postprandial glycemic response. In particular, when a higher carbohydrate content meal follows a lower carbohydrate content meal, the glycemic response is elevated, because the prioritization of carbohydrate utilization has essentially been lowered by the prior lower carbohydrate intake. The aim of the present study was to evaluate the effect of a variation in carbohydrate and fat content between meals on the postprandial glycemic response, especially postprandial peak BG concentration, along with a change in the body's metabolic prioritization of substrate utilization.

METHODS

This study was conducted at the National Institute of Health and Nutrition (NIHN) in Japan between July 2013 and July 2014. It was a secondary analysis of a study whose primary endpoint was energy metabolism (e.g., energy expenditure and substrate oxidation). The study protocol was approved by the Ethical Committee of the NIHN (approval number: 20130301–01) and was in full compliance with the provisions of the Declaration of Helsinki. Sixteen nonobese, healthy, young Japanese men were recruited and provided written informed consent for their participation in the study. Two participants withdrew from the study because of gastrointestinal effects or nonadherence to study rules (**Supplemental Figure 1**). Fourteen men [mean $\pm$ SD age: 22.2 $\pm$ 1.6 y; mean $\pm$ SD BMI (kg/m^2): 21.1 $\pm$ 1.9] were enrolled in this study. The participants were nonshift workers or university students, and had no history of chronic diseases that affect metabolism, such as diabetes, metabolic, or gastrointestinal disease. In addition, individuals regularly taking medications were excluded from the study because of possible effects on energy metabolism and BG.

Experimental design

In this randomized, crossover trial, participants' postprandial BG was assessed following 3 different types of meals: *1*) regular meals (R), *2*) meals with a high-carbohydrate breakfast (CB), and *3*) meals with a high-fat breakfast (FB). We evaluated the BG excursions using a continuous glucose monitoring system (CGMS) and a human calorimeter for evaluating energy metabolism.

An overview of the protocol is shown (**Figure 1**A). At least 1 wk before the calorimetry tests, anthropometric tests, including dual-energy X-ray absorptiometry, maximal oxygen uptake (VO_2max) test using a cycle ergometer, arterial stiffness (brachial ankle pulse wave velocity (baPWV) test, and a 2-h 75-mg oral glucose tolerance test (OGTT), were completed after an overnight fast ($\geq$12 h). Blood samples were drawn at 0, 30, 60, 90, and 120 min. BG and serum insulin were analyzed at LSI Medience Corporation and evaluated as the AUC using every time point. HbA1c was assessed at 0 min. HOMA-IR and homeostasis model assessment of β-cell function (HOMA-β) were calculated from fasting BG and fasting serum insulin levels.

During the 3 d before each calorimetry test, on days 1–3 (Figure 1B), participants consumed weight maintenance meals and kept to normal daily habits. At 1700 on day 3, all participants visited the NIHN, and had their body weight measured and the CGMS fixed. Participants entered the chamber before eating dinner (1930), and remained there until the morning of day 5. The 3 meal tests were performed randomly on day 4 of each visit. Each participant's visit for the meal tests was separated by $\geq$1 wk.

During calorimetry tests, participants were instructed to abstain from intentional vigorous physical activity such as exercise, nonscheduled sleeping and eating, chewing gum, and medications. Between tests, participants were also instructed to be consistent with regard to time spent watching television and using the computer, and to stay seated for $\geq$2 h after the start of meals, except for bathroom use. Drinking water was allowed ad libitum. Sleep was permitted between 2300 and 0700.

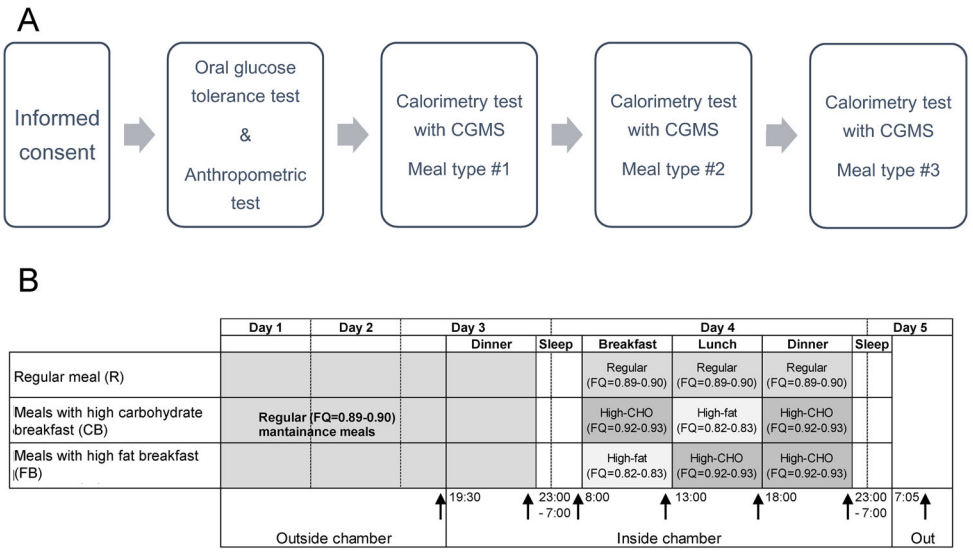

FIGURE 1 Experimental design. (A) A flow diagram of the sequence of study procedures. Participants randomly consumed 3 types of meals with different macronutrient compositions and were tested in the human calorimeter after performing an oral glucose tolerance test and an anthropometric test. (B) An overview of the calorimeter tests performed with a CGMS. Participants had free-living time and were provided with all meals during days 1–3, after which time they remained within a whole-body indirect calorimeter (chamber) and wore a CGMS from the evening of day 3 until the morning of day 5. Three types of meals were randomly received by participants, with a washout interval of ≥1 wk. Black arrows show when the CGMS calibrations were performed using self-measured blood finger-stick glucose monitoring. CGMS, continuous glucose monitoring system; CHO, carbohydrate; FQ, food quotient [calculated according to the equation: FQ = (0.81 × % protein intake ± 0.71 × % fat intake ± 1 × % carbohydrate intake)/100].

Tests and dietary treatments

During the 3 d before each calorimetry test, participants consumed the provided weight maintenance diet (15% of calories from protein, 25% from fat, and 60% from carbohydrate). Energy requirements during the 3 d before each calorimetry test were calculated for each participant based on their estimated basal metabolic rate × 1.7 or 1.9 (physical activity level of 1.7 or 1.9). The basal metabolic rate was estimated using age, gender, height, and body weight per Ganpule's equation (25). The daily total energy intake for each participant was 2000, 2500, or 3000 kcal/d, depending on their estimated energy requirements. The physical activity level was estimated using the International Physical Activity Questionnaire criteria (26). The methods used to calculate the energy requirements were identical to those described in a previous study (27).

The R test required the consumption of 3 meals per day with a macronutrient composition (15% of calories from protein, 25% from fat, and 60% from carbohydrate) reflecting that observed among Japanese adults (28). The CB test included a high-carbohydrate meal at breakfast (15% of calories from protein, 15% from fat, and 70% from carbohydrate), a high-fat meal at lunch (15% of calories from protein, 50% from fat, and 35% from carbohydrate), and a high-carbohydrate meal at dinner. The FB test included a high-fat meal at breakfast, a high-carbohydrate meal at lunch, and a high-carbohydrate meal at dinner (Figure 1B). The total daily macronutrient composition was set with equal proportions among the 3 types of test (15% of calories from protein, 25% from fat, and 60% from carbohydrate). The fatty acid profiles of meals were almost equally proportioned among the 3 tests: the ratios of saturated to monounsaturated and to polyunsaturated fatty acids were 1.4:1.4:0.8 for the R test and 1.4:1.4:1.0 for the CB and FB tests. The energy requirements during the calorimetry tests were calculated individually using each participant's estimated basal metabolic rate × 1.3 (physical activity level). The energy intake was 1900, 2100, 2300, or 2500 kcal/d. The dietary macronutrient contents for each meal test are shown in **Table 1**, and the meal menus are shown in **Supplemental Table 1**. Each food item provided in the diet was a commercial food product (fruit juice, butter, hamburger, packaged food, etc.).

Blood glucose assessments

BG concentrations were measured continuously using the CGMS (CGMS-Gold, Medtronic MiniMed). Calibrations were performed 7 times using a self-monitoring BG device (Glutest Neo Super, Sanwa Kagaku Kenkyusho Co., Ltd). Each participant performed the following calibrations: ∼20–30 min before meal ingestion, before bed, and at the end of the test (1900 and 2240 on day 3, 0740, 1240, 1740, and 2240 on day 4, and 0715 on day 5).

The mean BG excursion values were calculated, and the peak and minimum BG excursion values were extracted from the collected CGMS data. Glucose variability, including the SD and CV, were also calculated (29).

Energy metabolism assessments

Energy expenditure and substrate utilization, which included the respiratory quotient (RQ) and carbohydrate oxidation, were measured by human calorimetry using a mass spectrometer (AR-1000A, Arco System). The details of the calorimetry system and the method of calculating energy expenditure and substrate utilization were reported previously (27). Energy expenditure and carbohydrate oxidation were calculated every 30 min. RQs were calculated during every preprandial (1 h) and postprandial (5 h) period. The accuracy of the calorimeter during all tests was

TABLE 1
Macronutrient compositions and energy metabolism in each meal test[1]

Variables	Meal tests		
	R	CB	FB
Macronutrient contents			
Carbohydrate, g	297 ± 27	293 ± 23[a]	293 ± 23[a]
Fat, g	56 ± 3	58 ± 5[a]	58 ± 5[a]
Protein, g	76 ± 5	75 ± 5	75 ± 5
Carbohydrate, % of EI	59.6 ± 0.9	58.6 ± 0.2[a]	58.6 ± 0.2[a]
Fat, % of EI	25.2 ± 0.7	26.2 ± 0.1[a]	26.2 ± 0.1[a]
Protein, % of EI	15.2 ± 0.3	15.1 ± 0.2	15.1 ± 0.2
Food quotient	0.898 ± 0.002	0.895 ± 0.000[a]	0.895 ± 0.000[a]
Total energy intake, kcal	1990 ± 153	1997 ± 157	1997 ± 157
Energy intake by meal, kcal			
Breakfast	663 ± 51	677 ± 50[a]	643 ± 59[a,b]
Lunch	663 ± 51	643 ± 59[a]	677 ± 50[a,b]
Dinner	663 ± 51	677 ± 50[a]	677 ± 50[a]
Carbohydrates per meal, g			
Breakfast	99 ± 9	118 ± 9[a]	56 ± 6[a,b]
Lunch	99 ± 9	56 ± 6[a]	118 ± 9[a,b]
Dinner	99 ± 9	118 ± 9[a]	118 ± 9[a]
Energy metabolism: 24-h energy expenditure, kcal	1888 ± 166	1893 ± 157	1907 ± 165
Energy balance, kcal	102 ± 110	104 ± 100	90 ± 113
24-h carbohydrate oxidation, g	208 ± 39	218 ± 31	208 ± 34
Carbohydrate balance, g	89 ± 39	75 ± 23	85 ± 19

[1]Data are means ± SDs. Food quotient = (0.81 × % protein intake + 0.71 × % fat intake + 1 × % carbohydrate intake)/100. Energy balance = total energy intake − 24-h energy expenditure. Carbohydrate balance = total carbohydrate intake − 24-h carbohydrate oxidation. [a]$P < 0.05$ vs. the R condition. [b]$P < 0.05$ vs. the CB condition. CB, meals with a high-carbohydrate breakfast; EI, energy intake; FB, meals with a high-fat breakfast; R, regular meals.

determined by an alcohol combustion test: over 6 h, the mean ± SD accuracy of energy expenditure and the RQ were 100.1% ± 1.5% and 97.9% ± 0.3%, respectively.

The energy balance was calculated by subtracting the 24-h energy expenditure from the daily energy intake. The carbohydrate balance was calculated by subtracting the 24-h carbohydrate oxidation from the daily carbohydrate intake. Substrate utilization prioritization was assessed using the 1-h preprandial RQ and the 1-h preprandial carbohydrate oxidation rate at 0700–0800, 1200–1300, and 1700–1800.

Statistical analyses

A linear mixed effect model with Bonferroni correction was used to assess the main effect of each meal type on all BG and energy metabolism variables. Repeated-measures ANOVA with Bonferroni correction was used to assess the diurnal change in BG, such as between breakfast and lunch for each type of meal, the differences in postprandial glycemic response after each high-carbohydrate meal, and the differences in energy metabolism between the different types of meals.

Pearson correlation analysis was performed to assess the associations between variables for postprandial glycemic responses at each designated meal point (14 participants × 3 conditions × 3 meals), substrate utilization prioritization, OGTT, and physiologic functions.

Multiple regression analyses with a stepwise method were used to evaluate 5-h mean BG, peak glycemic response, and 1- or 2-h postprandial BG as dependent variables, to identify predictive variables that may explain the postprandial glycemic responses at each designated meal (14 participants × 3 conditions × 3 meals). Model 1 was constructed as a full model to explore the physiologic predictors of postprandial glycemic response. Model 2 was constructed to identify the influence of a prior meal's carbohydrate and fat balance on postprandial glycemic response. Models 3 and 4 were constructed to consider the clinical availability of the variables. Model 3 was constructed for individuals lacking a measurement for every preprandial BG concentration in real life. Model 4 was constructed for individuals lacking a measurement for carbohydrate content for every meal in real life. Accordingly, in Model 1, fat-free mass, fat mass, $\dot{V}O_2$ max adjusted for weight, mean baPWV, HbA1c, HOMA-IR, HOMA-B, fasting BG, fasting serum insulin, BG AUC during OGTT (BG AUC), serum insulin AUC during OGTT (insulin AUC), 1-h preprandial mean BG, 1-h preprandial RQ, and the carbohydrate content (in grams) were included as independent variables to identify predictive variables that could explain the postprandial glycemic responses. In Model 2, fat-free mass, fat mass, $\dot{V}O_2$ max adjusted for weight, mean baPWV, HbA1c, HOMA-IR, HOMA-B, fasting BG, fasting serum insulin, BG AUC, insulin AUC, 1-h preprandial mean BG, prior meal (binomial variables: 0 = the macronutrient composition of the prior meal contained the same macronutrient content or more carbohydrates than the current meal, 1 = more fat was contained in the prior meal than in the current meal), and the carbohydrate content were included as independent variables to identify predictive variables explaining the postprandial BG responses. In Model 3, the 1-h preprandial mean BG was excluded from Model 2. In Model 4, the carbohydrate content was excluded from Model 3. The values for BG AUC and insulin AUC were divided

TABLE 2
Results of BG variables and comparisons between individuals[1]

Variable	Types of test meals				
	R ($n = 13$)	CB ($n = 13$)	FB ($n = 13$)	CB ($n = 14$)	FB ($n = 14$)
Mean BG, mg/dL					
Awake	94.9 ± 9.4	98.1 ± 7.8	98.1 ± 8.9	97.9 ± 7.6	97.4 ± 9.0
Nocturnal	78.3 ± 9.6	77.6 ± 9.1	77.1 ± 9.7	77.7 ± 8.8	76.7 ± 9.5
24 h	89.0 ± 9.0	90.9 ± 7.0	90.7 ± 7.8	90.7 ± 6.7	90.1 ± 7.9
Median BG, mg/dL					
Awake	94.1 ± 8.2	93.8 ± 7.0	94.3 ± 7.7	93.7 ± 6.8	93.7 ± 7.7
Nocturnal	79.2 ± 10.4	79.7 ± 8.6	78.4 ± 10.1	79.6 ± 8.3	78.0 ± 9.8
24 h	86.2 ± 8.8	88.4 ± 6.1	87.3 ± 7.7	88.3 ± 5.9	86.8 ± 7.8
Peak BG, mg/dL	127.5 ± 15.7	145.4 ± 25.7$^{\#}$	141.5 ± 25.3*	143.9 ± 25.3*	140.2 ± 24.8
Minimum BG, mg/dL	60.2 ± 13.6	56.1 ± 11.9	57.8 ± 12.8	56.4 ± 11.5	57.6 ± 12.3
BG, SD, mg/dL					
Awake	12.5 ± 3.5	16.5 ± 7.1	15.4 ± 6.0	16.2 ± 6.9$^{\#}$	15.2 ± 5.9
Nocturnal	5.7 ± 2.9	7.6 ± 3.9	7.0 ± 3.2	7.4 ± 3.9	6.9 ± 3.1
24 h	13.3 ± 3.1	17.3 ± 6.2*	16.5 ± 6.1	17.0 ± 6.1$^{\#}$	16.2 ± 6.0
BG, CV					
Awake	0.133 ± 0.040	0.166 ± 0.066	0.155 ± 0.053	0.164 ± 0.064	0.154 ± 0.051
Nocturnal	0.076 ± 0.045	0.102 ± 0.056	0.093 ± 0.047	0.098 ± 0.056	0.092 ± 0.046
24 h	0.152 ± 0.040	0.190 ± 0.064$^{\#}$	0.182 ± 0.062	0.186 ± 0.063	0.180 ± 0.060

[1]Data are means ± SDs. A single participant's CGMS data during the R test were excluded because an error occurred when entering calibration values. Evaluation of differences in variables between conditions was therefore performed by repeated-measures ANOVA with Bonferroni correction in 13 participants, and a mixed effect model with Bonferroni correction in 14 participants. $^{\#}P < 0.1$, $^{*}P < 0.05$ vs the R condition. BG, blood glucose; CB, meals with a high-carbohydrate breakfast; CGMS, continuous glucose monitoring system; FB, meals with a high-fat breakfast; R, regular meals.

by 100 when entered into the stepwise analysis as independent variables. When the variance inflation factor of the independent variable was >2 as a result of multiple regression analysis, the variable was rejected from the model and the analysis was then repeated.

We based our sample size calculation on results from a previous Japanese study that examined the short-term effect of a high-fat diet on glycemic response during an OGTT (18). We used an expected difference in peak glycemic response of 36 ± 27 mg/dL between the R, CB, and FB conditions, with the power set at 80% and an α level (2-tailed) of 1.66%, considering the effects of multiple comparison. This gave a sample size of 12 subjects. Statistical analyses were performed by using SPSS 23 (IBM SPSS). Data are means ± SDs unless otherwise specified.

RESULTS

Missing data

One participant's OGTT data could not be obtained owing to illness during a blood collection. Another participant's CGMS data during the R test were excluded because an error occurred when entering the calibration values. Mean values of the major BG variables in both 13 and 14 participants are presented in **Table 2**.

OGTTs and anthropometric tests

Table 3 shows results for mean blood biochemistry and physiologic functioning variables. No participants were identified as having diabetes, nor were there significant differences in body weight between the 3 types of meals tested before entering the calorimeter: R, 64.0 ± 7.6 kg; CB, 63.7 ± 7.4 kg; and FB, 63.8 ± 7.4 kg.

Total energy expenditure and energy balance during calorimetry tests

As shown in Table 1, there were no significant differences in 24-h total energy expenditure or energy balance among the types of meals tested. The 24-h total carbohydrate oxidation and carbohydrate balance resulting from CB meals was >10 g higher and lower, respectively, than with the R and FB meals; however, the differences were not significant using the Bonferroni method.

Diurnal changes in BG concentrations, RQ, and carbohydrate oxidation within meal types

Dynamic diurnal changes in BG concentrations were evident with each type of meal (**Figure 2**A); however, there were no significant differences in the 24-h mean BG concentrations between the different types of meals: R, 89.0 ± 9.0 mg/dL, $n = 13$; CB, 90.7 ± 6.7 mg/dL, $n = 14$; FB, 90.1 ± 7.9 mg/dL, $n = 14$. Peak BG concentrations with CB (143.9 ± 25.3 mg/dL; $P = 0.03$; $n = 14$) and FB (140.2 ± 24.8 mg/dL; $P = 0.11$; $n = 14$) were higher than with R (127.5 ± 15.7 mg/dL; $n = 13$).

There was no significant difference in the SD or CV of BG between the types of meals when compared using a mixed effect model with Bonferroni correction (Table 2). However, with CB, the SD of BG during wakefulness ($P = 0.084$) and for a 24-h period ($P = 0.053$) tended to be higher than for R.

Dynamic diurnal changes occurred in BG concentrations and carbohydrate oxidation rates with each type of meal (Figure 2B). For R, there were no differences in peak or mean BG concentrations between meals in a day, whereas for CB and FB, there were significant differences in peak and mean BG concentrations between other meals within the day (**Supplemental Figure 2**). There appears to be an inverse relationship in the

TABLE 3
Mean parameter values and correlation analyses between predictive variables and postprandial glycemic responses in each meal[1]

			5-h postprandial period		Time (postprandial BG)	
	Mean ± SD		Mean BG	Peak BG	1 h	2 h
Carbohydrate intake, g	—	*r*	0.481*	0.496*	0.469*	0.382*
		P	<0.001	<0.001	<0.001	<0.001
Carbohydrate proportion, %	—	*r*	0.424*	0.463*	0.443*	0.376*
		P	<0.001	<0.001	<0.001	<0.001
1-h preprandial RQ	—	*r*	−0.364*	−0.382*	−0.423*	−0.417*
		P	<0.001	<0.001	<0.001	<0.001
1-h preprandial mean carbohydrate oxidation, g/min	—	*r*	−0.286*	−0.282*	−0.327*	−0.356*
		P	0.001	0.002	<0.001	<0.001
1-h preprandial mean BG, mg/dL	—	*r*	0.448*	0.273*	0.217*	0.255*
		P	<0.001	0.002	0.016	0.004
Fat-free mass, kg	53.2 ± 6.8	*r*	0.190*	0.114	0.083	0.033
		P	0.035	>0.1	>0.1	>0.1
Fat mass, kg	8.5 ± 2.1	*r*	−0.026	0.034	0.063	0.056
		P	>0.1	>0.1	>0.1	>0.1
VO_2 max, mL · kg^{-1} · min^{-1}	43.5 ± 4.8	*r*	0.266*	0.126	0.174	0.216*
		P	0.003	>0.1	0.055	0.016
baPWV, cm/s	1075.1 ± 45.1	*r*	−0.172	−0.064	−0.071	−0.121
		P	0.058	>0.1	>0.1	>0.1
Fasting BG, mg/dL	82.3 ± 5.9	*r*	0.342*	0.037	0.042	0.133
		P	<0.001	>0.1	>0.1	>0.1
Fasting serum insulin, μU/mL	2.6 ± 0.9	*r*	−0.008	−0.003	0.034	0.018
		P	>0.1	>0.1	>0.1	>0.1
HbA1c, NGSP, %	5.1 ± 0.3	*r*	−0.027	−0.001	0.087	0.032
		P	>0.1	>0.1	>0.1	>0.1
HOMA-IR	0.54 ± 0.19	*r*	0.032	0.010	0.037	0.042
		P	>0.1	>0.1	>0.1	>0.1
HOMA-β, %	54.4 ± 31.0	*r*	−0.198*	−0.051	−0.036	−0.129
		P	0.028	>0.1	>0.1	>0.1
OGTT BG at 120 min, mg/dL	93.4 ± 15.0	*r*	0.223*	0.206*	0.149	0.266*
		P	0.017	0.028	>0.1	0.004
BG AUC during OGTT, mg/dL per 120 min	13,017.7 ± 2236.9	*r*	0.271*	0.300*	0.246*	0.262*
		P	0.003	0.001	0.008	0.005
Serum insulin AUC during OGTT, μU/dL per 120 min	2592.3 ± 832.1	*r*	−0.165	0.007	−0.013	−0.032
		P	0.079	>0.1	>0.1	>0.1
Δ serum insulin AUC/Δ BG AUC, μU/mg	0.206 ± 0.09	*r*	−0.137	−0.139	−0.099	−0.103
		P	>0.1	>0.1	>0.1	>0.1

*Statistically significant correlations. baPWV, brachial-ankle pulse wave velocity; BG, blood glucose; HbA1c, glycated hemoglobin; HOMA-β, homeostasis model assessment of β-cell function; NGSP, National Glycohemoglobin Standardization Program; OGTT, oral glucose tolerance test; RQ, respiratory quotient; VO_2 max, maximal oxygen uptake.

transition between preprandial substrate utilization and postprandial glycemic response (Supplemental Figure 2).

Glycemic responses when identical high-carbohydrate meals are consumed

The BG peaks for CB and FB were observed after dinner and lunch, respectively. The peak BG concentrations after eating an identical high-carbohydrate meal were ∼20 mg/dL higher when a previous meal was high-fat compared with the other conditions ($P < 0.05$ for all, **Figure 3**). The 5-h mean BG concentrations were also significantly higher when a previous meal was high-fat compared with the other conditions ($P \leq 0.001$ for all). Focusing on the postprandial glycemic response after dinner for the CB and FB conditions, the peak and 5-h mean BG concentrations for the CB condition were significantly higher than for the FB condition ($P = 0.001$ and $P < 0.001$, respectively), although the participants consumed identical dinner meals during both conditions.

Prediction of the postprandial glycemic response based on substrate utilization prioritization and the other variables

The results of a correlation analysis between predictive variables and postprandial glycemic response are shown in **Table 3** and **Supplemental Figure 3**. Carbohydrate intake had the strongest association with all variables of postprandial glycemic response ($r = 0.382$–0.496). The 1-h preprandial RQ was significantly negatively associated with all variables for the postprandial glycemic response ($r = -0.364$ to -0.423).

The multiple regression analysis performed to evaluate the peak postprandial glycemic response is shown in **Table 4**. For Model 1, the 1-h preprandial RQ was identified as an independent predictor of the peak glycemic response. Besides the amount of carbohydrate in a meal, the BG AUC, 1-h preprandial mean BG, and fasting glucose were selected as predictors of the peak glycemic response. For Models 2–4, the macronutrient composition of a prior meal was also identified as an independent predictor of the peak BG response. The associations were similar

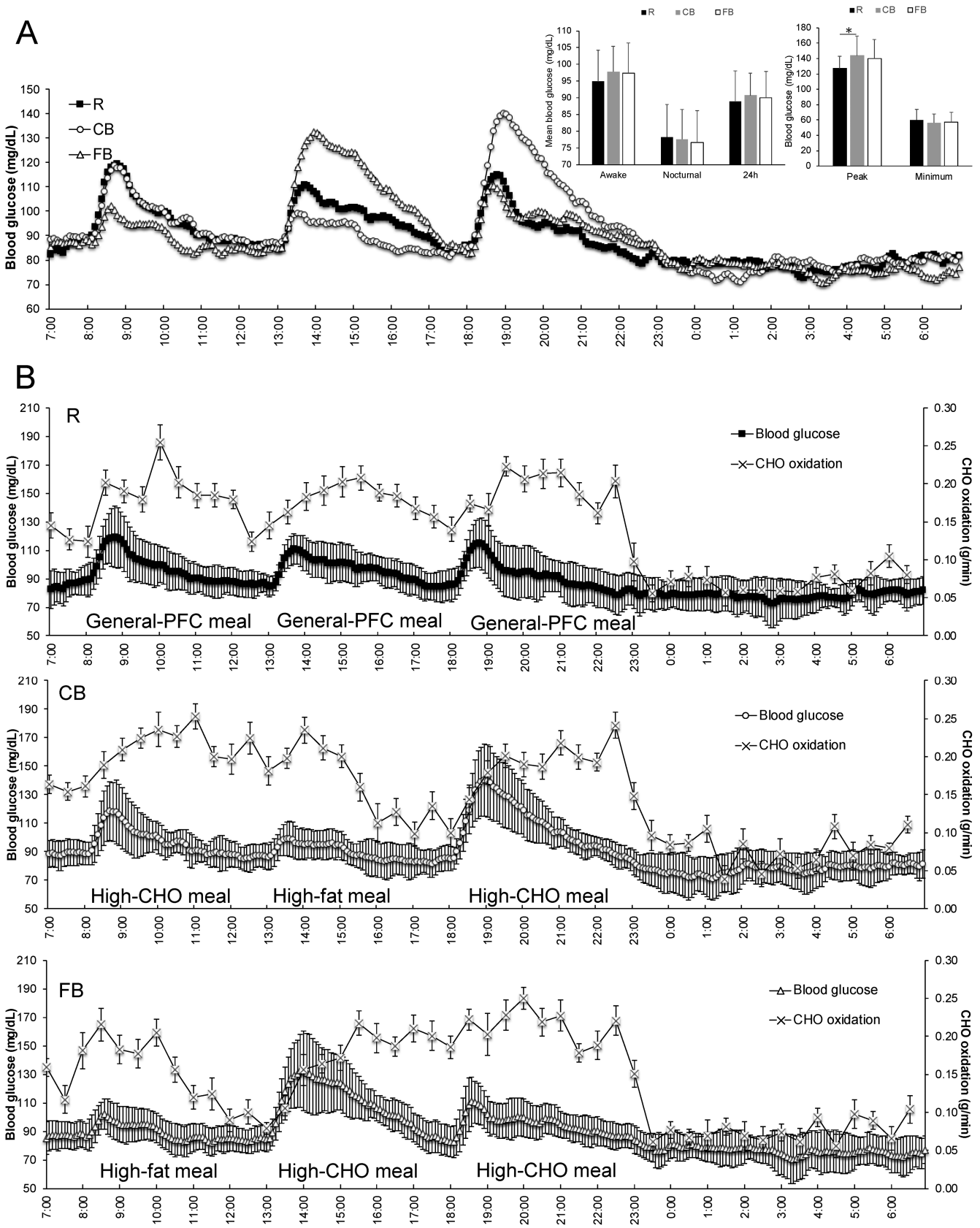

FIGURE 2 The results of diurnal variations in BG. (A) Line plots of the diurnal variation in BG for meals with various macronutrient compositions and bar graphs (mean ± SD) of the mean, peak, and minimum blood glucose concentrations during different periods within a day. (B) Line plots of the diurnal changes in BG (mean ± SD) and carbohydrate oxidation (mean ± SE) by type of meal. R, $n = 13$; CB and FB, $n = 14$; $^{*}P < 0.05$. BG, blood glucose; CB, meals with a high-carbohydrate breakfast; CHO, carbohydrate; FB, meals with a high-fat breakfast; PFC, protein, fat, and carbohydrate; R, regular meals.

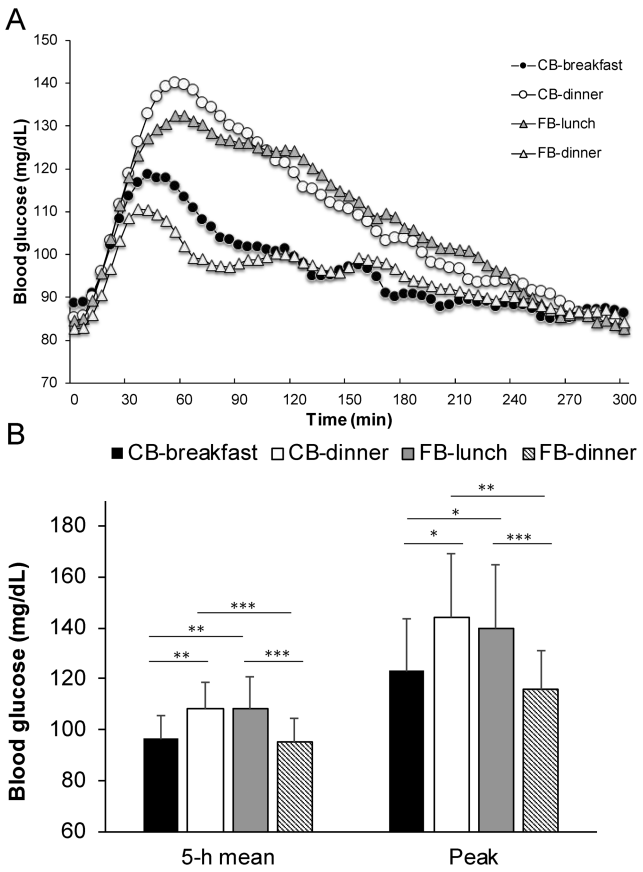

FIGURE 3 Postprandial glycemic response after each high-carbohydrate meal tested when it followed a prior meal that was high in carbohydrate or fat content. (A) Line plots of postprandial BG values by meal type. (B) Bar graphs showing postprandial BG values. The postprandial BG values after dinner when a high-fat meal was consumed at lunch (CB), and after lunch when a high-fat meal was consumed at breakfast (FB), were significantly higher than they were after breakfast (CB) and after dinner when a high-carbohydrate meal was consumed at lunch (FB). Data are means ± SDs. $^{*}P < 0.05$, $^{**}P = 0.001$, $^{***}P < 0.001$. BG, blood glucose; CB, meals with a high-carbohydrate breakfast; FB, meals with a high-fat breakfast; R, regular meals.

for 5-h mean BG, 1-h postprandial BG, and 2-h postprandial BG (**Supplemental Tables 2–4**).

DISCUSSION

Most previous studies have focused on the influence of the carbohydrate content of a single meal or a habitual diet (i.e., dietary content averaged over ≥1 d) on BG behavior. In reality, however, the carbohydrate content and/or the macronutrient composition changes from meal to meal. Therefore, we focused on the influence of variations in carbohydrate content between meals. Our results show that there was no significant difference in mean BG concentrations between the hours one is awake and the hours one is asleep, or over 24-h related to meal types; there were, however, large changes in postprandial glycemic responses when the carbohydrate-to-fat ratio changed from one meal to the next. Glycemic response increases were particularly evident when participants switched from a high-fat meal to a high-carbohydrate meal, as opposed to the reverse, or in the absence of switching the macronutrient content. Consequently, the peak BG concentrations of the CB and FB tests were higher than for the R test, although the FB test was not significant after Bonferroni correction, and the postprandial glycemic response after dinner with CB was higher than with FB, even though the participants consumed identical dinner meals (Figure 3). Our results further suggest that circadian rhythm does not contribute to the increased glycemic response to a prior high-fat meal, because there were no differences in either the peak or the mean postprandial glucose concentrations between dinner for the CB condition and lunch for the FB condition. These results support our hypothesis and provide useful information regarding clinical and experimental uses of BG-lowering agents. A remarkable finding of the present study is that prioritization of preprandial substrate utilization predicts postprandial glycemic response; the multiple regression analysis revealed that this factor is an independent predictor of glycemic response. In addition, we presented evidence (Figure 2 and Supplemental Figure 2) indicating that carbohydrate oxidation changed in response to prior carbohydrate intake, consistent with our hypothesis. According to previous epidemiologic studies, no definitive evidence exists to support the effect of carbohydrate quantity and macronutrient balance on the risk for type 2 diabetes (30); consequently, the effect of these factors on diabetes risk warrants further investigation. Our results may offer a potential explanation for the results reported in a previous study (30), and emphasize the importance of dietary content at each meal and each subsequent meal.

Previous studies have reported that fermentable dietary fiber consumption (23, 31–33), low glycemic–index meal consumption (34–41), high-protein meal consumption (5, 6), or meal skipping (20–22, 42, 43) can influence the postprandial glycemic response at the next meal, an occurrence commonly referred to as the "second-meal phenomenon." To understand the mechanism of this phenomenon, these studies investigated several biochemical or physiologic factors: FFA (5, 21–24, 31, 33, 39, 43), insulin responsiveness (22, 43), gastric emptying (6, 33), and absorption (35–38). Several studies have found a large difference in FFA levels during the preprandial period before the second meal, and that there is an association between preprandial FFA concentration and postprandial glycemic response, which could be because of insulin resistance associated with the elevation of FFA (44). However, a previous study found that FFA concentration was similarly suppressed following consumption of a high-fat meal compared with consumption of a high-carbohydrate meal, and that differences in FFA concentration at 5 h after intake were minimal (45). This result suggests that the FFA concentration might not have affected the glycemic response in the present study, although FFA data are needed to confirm this. However, in the same study, triglyceride levels increased after a high-fat meal, and the separation at 5 h after intake was relatively large (45); consequently, triglycerides may be mainly oxidized for energy production from fat during the postprandial period. The common point is that increases in FFA or triglycerides indicate increased fat utilization via β-oxidation. These results suggest that the monitoring of metabolic prioritization of substrate utilization may improve glucose management. Insulin responsiveness may also explain our results, as suggested by Jakubowicz et al. (43). For example, a previous study showed that a second glucose stimulus is associated with a larger insulin release than the first (46). This physiologic reaction is considered to be a mechanism for raising the carbohydrate utilization capacity. In contrast, the effects of

TABLE 4
Multiple regression analysis using independent variables for peak postprandial BG[1]

	B	SE	β	P	ΔR^2	R^2	R^2 adjusted
Model 1							
Constant	188.745	41.171		<0.001		0.504	0.481
Carbohydrate intake, g	0.339	0.061	0.396	<0.001	0.260		
BG AUC during OGTT/100, mg/dL per 120 min	0.319	0.075	0.310	<0.001	0.091		
1-h preprandial RQ	−173.340	37.415	−0.334	<0.001	0.064		
1-h preprandial mean BG, mg/dL	0.771	0.182	0.315	<0.001	0.059		
Fasting BG, mg/dL	−0.761	0.292	−0.204	0.011	0.031		
Model 2							
Constant	34.200	21.790		0.119		0.557	0.537
Prior meal	23.191	3.811	0.440	<0.002	0.260		
1-h preprandial BG, mg/dL	0.819	0.172	0.334	<0.001	0.127		
Carbohydrate intake, g	0.253	0.061	0.295	<0.000	0.077		
BG AUC during OGTT/100, mg/dL per 120 min	0.331	0.071	0.322	<0.001	0.067		
Fasting BG, mg/dL	−0.692	0.273	−0.185	0.013	0.026		
Model 3							
Constant	45.751	11.359		<0.001		0.460	0.445
Prior meal	19.198	4.072	0.364	<0.001	0.260		
Carbohydrate intake, g	0.306	0.066	0.357	<0.001	0.106		
BG AUC during OGTT/100, mg/dL per 120 min	0.315	0.072	0.306	<0.001	0.094		
Model 4							
Constant	73.780	10.447		<0.001		0.355	0.343
Prior meal	27.112	4.019	0.514	<0.001	0.260		
BG AUC during OGTT/100, mg/dL per 120 min	0.316	0.078	0.307	<0.001	0.095		

[1]Adjustments were as follows: Model 1: carbohydrate intake, 1-h preprandial RQ, 1-h preprandial mean BG, HbA1c, HOMA-IR, HOMA-B, fasting BG, fasting insulin, BG AUC, insulin AUC, baPWV, $\dot{V}O_2$ max adjusted for weight, fat mass, and fat-free mass; Model 2: carbohydrate intake, prior meal (binomial variables: 0 = the macronutrient composition of the prior meal contained the same macronutrient content or more carbohydrates than the current meal, 1 = more fat was contained in the prior meal than the current meal), 1 h preprandial BG, HbA1c, HOMA-IR, HOMA-B, fasting BG, fasting insulin, BG AUC, insulin AUC, baPWV, $\dot{V}O_2$ max adjusted for weight, fat mass, and fat-free mass; Model 3: Model 2 minus 1-h preprandial BG; Model 4: Model 3 minus carbohydrate intake. baPWV, brachial-ankle pulse wave velocity; HbA1c, glycated hemoglobin; HOMA-B, homeostasis model assessment of β-cell function; OGTT, oral glucose tolerance test; RQ; respiratory quotient.

gastric emptying or absorption on the glycemic response following the second meal may have been limited in the present study. A high-fat meal will delay gastric emptying and slow absorption. Therefore, if gastric emptying had contributed to the postprandial glycemic response at the second meal following the high-fat meal, the glycemic response should have decreased, but our study showed an elevated postprandial glycemic response after consumption of a high-fat meal. In addition, the concentration of residual insulin in the blood from a previous meal would contribute to the glycemic response at the next meal. However, the possibility of an effect of residual insulin was probably low in our study, as Raben et al. (45) showed that there was no difference in the 5-h insulin concentration after ingestion of a high-fat meal compared with a high-carbohydrate meal. On the other hand, if the interval between meals is shorter, the residual insulin corresponding to the difference in prior macronutrient intake will strongly influence the glycemic response at the next meal.

Our study also revealed other physiologically meaningful results. Although significant differences were not observed between the meals in the same day with the R test, the postprandial glycemic reactions appeared to decrease after each meal. This result is consistent with a previous study (9). The phenomenon may be explained by the Staub-Traugott effect (47) and/or insulin responsiveness (46). By contrast, a number of previous studies indicated that the glycemic response was higher in the afternoon and/or evening than in the morning (48–56). Although the reason for the differences is still unclear, several of these studies indicated that elevated FFA levels in the evening may affect glucose tolerance (49, 51, 54); these results may have a common mechanism with the second-meal phenomenon. Because elevated FFA concentrations generally occur after a longer period of time between meals (acute negative energy balance), there may have been differences in energy balance, and/or the interval between meals, between previous studies and the current one. Indeed, in 5 of 9 studies (49–51, 53, 55), the fasting period before dinner was 8–12 h to standardize the fasting duration between breakfast and dinner; in addition, subjects in these studies may have had negative energy balance for an entire day. However, in our protocol, we aimed to evaluate "usual" postprandial glycemic response under a general time period between meals and energy-balanced conditions. The monitoring of 24-h energy expenditure and energy balance is a strength in our study because, to our knowledge, no studies have evaluated 24-h energy balance before, even though it might influence BG levels.

There were some limitations to this study. First, we did not include participants with impaired glucose tolerance or diabetes in this study. Although more investigations may be needed to assess the effect of variations in carbohydrate-to-fat ratios between meals for such patients, our hypothesis might be applicable to diabetes patients because several studies of diabetes patients have indicated the effect of FFA on the "second-meal phenomenon" (5, 22, 39, 43). Second, we did not strictly control the carbohydrate complexity (i.e., the glycemic index and fiber content). In general, the fiber content in a high-fat meal is likely to be lower than in a high-carbohydrate meal. This may complicate the interpretation of whether it is the high fat or the low fiber intake

that caused the results in this study. We do know, however, fiber intake is associated with lowering the BG. With the CB test, the postprandial glycemic response at dinner was significantly higher than at breakfast. Even if the high-fat lunch contained less fiber, that could not explain this result. Third, participants consumed different foods between the R and the CB and FB conditions, which may have complicated the comparison of the results between R and the other tests. However, according to our current results, the glycemic index and/or glycemic load seems to be controlled to an acceptable extent because the postprandial glycemic responses after breakfast were comparable. Furthermore, the participants in this study were tested for each type of meal while sedentary (an approximate physical activity level of 1.3), so that the effect of diurnal variations in the macronutrient content of meals could be determined. Additional studies may therefore be needed to clarify the interactions between different types of meals and physical activity related to glucose excursions.

In conclusion, a large variation in carbohydrate and fat balance from one meal to the next throughout the day induces BG excursions of a greater magnitude in healthy individuals. In particular, switching the primary macronutrient from fat to carbohydrate contributes to an increased postprandial glycemic response. This response might be explained by the alteration in the prioritization of carbohydrate utilization owing to the influence of the carbohydrate-to-fat ratio of the prior meal. Conversely, our results suggest that maintaining a similar carbohydrate and fat balance over two consecutive meals appears to favor lower BG excursions over a day.

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The authors' responsibilities were as follows—TA and EY: designed the research and conducted the preliminary experiments; NN: supervised the experimental design as an attending medical doctor; TA, SN, CU, EY, and ST: conducted the experiments; NN and HT: conducted the experiments as a medical doctor; TA: analyzed the data and wrote the manuscript; SN, CU, EY, NN, HT, and ST: edited the manuscript; TA and ST: had primary responsibility for final content; and all authors: read and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

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