High or low glycemic index (GI) meals at dinner results in greater postprandial glycemia compared with breakfast: a randomized controlled trial

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ABSTRACT

Introduction While circadian control of glucose metabolism is well known, how glycemic index (GI) of carbohydrate-rich meals interacts with time of consumption (breakfast or dinner) to influence postprandial (PP) glucose homeostasis is less well established. The objective of the study was to assess markers of PP glucose homeostasis following high or low GI test meals (TM) consumed either at breakfast or at dinner and following consumption of the subsequent standardized meals (SSM).

Research design and methods Randomized crossover trial in 34 healthy, Chinese, elderly volunteers (mean±SEM age, 56.8±0.83 years), who completed 4 separate study sessions per-protocol, consisting of a high-GI breakfast, low-GI breakfast, high-GI dinner and low-GI dinner TM, followed by a SSM at the subsequent eating occasion. Blood samples were taken for 3 hours after each TM and SSM for glucose, insulin, glucagon, free fatty acids (FFA) and triglycerides (TG) measurements.

Results Consuming TM at dinner produced greater PP glycemia than breakfast both after TM and SSM (both p<0.0001), irrespective of GI. High-GI TM also produced greater PP glycemia than low-GI TM, both after TM and SSM (both p<0.01), irrespective of time of consumption. No interaction between GI and time were found on PP glycemia, indicating parallel, but independent effects. Combined total areas under the curve of TM+SSM for PP glucose (p<0.0001), PP TG (p<0.0001) and PP FFA (p<0.0001) were all greater when TM taken during dinner compared with breakfast.

Conclusions Carbohydrate-rich meals consumed at dinner leads to significantly worse PP glucose homeostasis than when consumed at breakfast, on top of the independent GI effect of the meal. This may have implications to future type 2 diabetes risk. Moreover, future studies investigating GI/glycemic load (GL) and disease risk associations should factor in timing of GL consumption as an additional variable.

Trial registration number NCT02927600.

Significance of this study

What is already known about this subject?
► Although diurnal variation in glucose metabolism via circadian control is well known, how this interacts with glycemic index/glycemic load (GI/GL) of meals to effect postprandial metabolism following the meals and following the subsequent meals is not established.

What are the new findings?
► This study has eloquently shown that carbohydrate-rich meals, particularly those high in GI, consumed during dinner, as compared with breakfast, gave rise to significantly adverse postprandial glucose homeostasis both after the meals and after the subsequent standardized meals.

How might these results change the focus of research or clinical practice?
► Given the worse glycemic control later during the day, it is advisable to focus attention on the carbohydrate quality and quantity of the evening meals, particularly in preventing future risk of type 2 diabetes and related cardiometabolic diseases.
► Future studies investigating GI/GL and disease risk associations should factor in timing of GL consumption as an additional variable.

INTRODUCTION

Postprandial glucose (PPG) homeostasis is an important determinant for the risk of chronic diseases, in particular type 2 diabetes and cardiovascular diseases.[1–3] Since the concept of glycemic index (GI) was propagated by Jenkins *et al*,[4] low GI foods have been advocated to improve PPG and thereby manage and prevent type 2 diabetes, obesity and the risk of other cardiometabolic diseases.[5–9] However, recent reviews have shown inconsistent effects of low GI foods in the prevention and management of chronic diseases.[10–12] Part of these inconsistencies may be explained by diurnal variations in glucose homeostasis, which is often not considered in epidemiological studies. In fact, there has been a recent renaissance in research surrounding

the circadian control of metabolism.[13–15] The circadian rhythm is principally controlled centrally via the suprachiasmatic nucleus, as well as directly or indirectly through the peripheral clocks found in majority of the tissues to influence endocrine and metabolic functions including insulin secretion and insulin sensitivity.[16 17] While the central clock is mainly regulated by light-dark cycles, the peripheral clocks are much more influenced by behavioral patterns including fasting-feeding, sleep-awake cycles as well as by the dietary composition and the size of the meals.[18] Therefore, eating meals at times mismatched to the internal clock, including having a nocturnal lifestyle have been shown to increase cardiometabolic disease burden.[19] There are also suggestions of biological preferences for certain macronutrients at specific times of the day (eg, carbohydrate in the morning), as discussed in more detail elsewhere.[20]

Several studies have been undertaken which investigated how timing of meal intake interacts with nutrient profile to modulate metabolic homeostasis,[18 21 22] although majority of these evidence to date have come from controlled animal studies. More specifically, very few randomized controlled trials in humans, within the normal dietary context of consuming three mixed meals per day (at breakfast, lunch and dinner), have thoroughly explored how carbohydrate quality, particularly the GI/glycemic load (GL) of the meals interact with the timing of the meal intake to influence PPG homeostasis. Moreover, to the best of our knowledge, no study has investigated how the timing of the meal interacts with the GI of the same meal to influence the glycemic and insulinemic responses of the subsequent meal. Given that Asians have a greater predisposition to develop type 2 diabetes and prediabetes resulting from their distinctive metabolic phenotype, particularly in relation to carbohydrate metabolism, as compared with the Western Caucasian populations,[23 24] undertaking well-controlled trials in Asians to investigate interactions between meal timings and GI is imperative. We therefore investigated the effects of consuming high or low GI test meals (TM) either at breakfast or at dinner on various markers of PP glucose homeostasis immediately following TM as well as the PP period following the subsequent standardized meal (SSM).

SUBJECTS AND METHODS
Study population and recruitment
This study was undertaken in men and women of Chinese ethnic origin, 50–70 years of age and having a BMI between 18.5 and 24.9 kg/m^2. Exclusion criteria were: smoking, waist circumference >90 cm (males) and >85 cm (females), blood pressure ≥150/90 mm Hg, fasting glucose ≥7 mmol/L, having alcohol consumption >4 units per day, having history of anemia, suffering from any medical conditions that may affect study results, being allergic/intolerant to any of the test foods or any common foods or ingredients, taking any prescribed

medications or dietary supplements likely to interfere with study end points, being on any special or restrictive diet, for female volunteers: not reached menopause or being on hormone replacement therapy. The volunteers who took part in this study were recruited via advertisement in local newspaper, approaching potential volunteers from our center (Clinical Nutrition Research Centre (CNRC)) recruitment database, as well as through the word of mouth from August 2016 through to October 2017. Interested volunteers were invited to attend the CNRC for a screening and consent visit, following an overnight fast. Volunteers' suitability was determined using a Health and Lifestyle Questionnaire and through various anthropometric measurements. Participants' height (Seca 217, Seca GmbH, Germany), weight (Tanita BC-418, Tanita, Japan) and waist circumference were measured. Seated resting blood pressure was measured using an automated sphygmomanometer (Omron HEM907, Omron, Japan) and their fasting glucose using the HemoCue 201 (Radiometer, Denmark).

Study design and dietary intervention
This was a randomized, controlled, crossover, acute dietary intervention trial. The randomization was carried out using Medidata Balance (Medidata Solutions, USA) and the study team undertook recruitment and randomization. Each volunteer completed four separate intervention sessions for the study, in a random order, consisting of high-GI breakfast (Hi-Br), low-GI breakfast (Lo-Br), high-GI dinner (Hi-Di) and low-GI dinner (Lo-Di) as TM. All TM contained approximately 75 g available carbohydrates, made from either high GI rice (GI:92) or low GI rice (GI:55), flavored with a small amount of chicken seasoning and 20 g green leafy vegetables to ensure palatability. The GI values for the two rice were obtained by taking the measured values of the same types of rice used in two separate studies within our own research group.[25 26] Sessions were separated by a washout period of a minimum of 3 days. The meals immediately prior to each TM were standardized using standard meal (SM), meaning the same SM prior to each TM (high or low GI TM, taken either at breakfast or at dinner) was used in order to minimize any variations in baseline metabolite concentrations immediately prior to the TM, arising from any carryover effects from the volunteer's previous meal. For the two breakfast TM sessions, the volunteers attended the study center on the test day (day 1) at 7.30 am following an overnight fast lasting 13 hours, rested for approximately 1 hour following arrival and then had a cannula inserted around 8.30 am. After waiting for 15 min following cannula insertion, baseline blood sample (T0 hr) was obtained. At around 9.00 am, the volunteer consumed the TM breakfast (Hi-Br or Lo-Br), following which blood samples were obtained every 15 min for the first 1 hour and every 30 min for the subsequent 2 hours. Following this, another blood sample was drawn about 5 min before the SSM (standard lunch), which was consumed at 3.5 hours following the TM breakfast.

Blood samples were again drawn every 15 min for the first 1 hour and every 30 min for the subsequent 2 hours following the SSM consumption, following which the cannula was removed and the volunteers were allowed to go home. Similarly, during the dinner TM sessions, the volunteers attended the study center at 5.00 pm on test day (day 1), after previously eating a standardized lunch (SM, provided) at 12.30 pm on the same day. They rested for approximately 1 hour following their arrival and then had a cannula inserted around 6.00 pm on day 1. After waiting 15 min following cannula insertion, baseline blood sample (T0 hr) was obtained. At around 6.30 pm, the volunteer consumed the test dinner (Hi-Di or Lo-Di) following which blood samples were obtained every 15 min for the first 1 hour and every 30 min for the subsequent 2 hours. Following this, the cannula was removed and the volunteers were allowed to go home. They were then asked to fast overnight (no food or drink other than water for the following 10 hours, approximately) and return to the study center the following morning (day 2) at 7.30 am, rested for approximately 1 hour following arrival and then had a cannula inserted around 8.30 am on day 2. After waiting for 15 min following cannula insertion, baseline blood sample (T0 hr) was obtained. At around 9.00 am (approximately 14.5 hours following their test dinner the evening before), volunteers consumed the SSM (standard breakfast) following which blood samples were drawn every 15 min for the first 1 hour and every 30 min for the subsequent 2 hours. The sequence and types of the TM and the standardized meals before and after TM (ie, SM and SSM, respectively) were identical irrespective of the intervention session, except for the time of consumption. The constituents of the test and standardized meals as well as their calorie and macronutrient compositions are shown in online supplementary table 1. The schematic of study design is shown in online supplementary figure 1.

Blood sample collection and analyses

Blood samples were collected using an intravenous cannula by a qualified research nurse. Blood samples were either collected in serum vacutainer tubes (BD, USA) for the measurements of glucose, insulin and triglycerides (TG) or collected in K_2 EDTA plasma vacutainer tubes (BD, USA) for the measurements of glucagon and free fatty acids (FFA), concentrations. The serum tubes were centrifuged within approximately 1 hour of collection after clot was formed. The plasma tubes were stored on ice immediately on collection and centrifuged within 30 min of collection. All blood tubes were centrifuged at $1500 \times g$ for 10 min at 4°C. Serum and plasma samples were then aliquoted into individual cryotubes and immediately stored at −80°C until analyses. Serum glucose, insulin and TG were measured using the standardized clinical chemistry method of the National University Hospital Referral Laboratories, Singapore, whereby glucose and TG were measured using AU 5800 clinical chemistry analyzer (Beckman Coulter, USA)

and insulin was measured using UniCel DxI 800 Access Immunoassay System (Beckman Coulter, USA). Plasma FFA was measured by an in vitro enzymatic colorimetric method (Wako, Germany), while glucagon was measured by ELISAs (Mercodia, Sweden).

Statistical analyses

Sample size calculations and statistical analyses were implemented using R V.3.2.3 (R Foundation for Statistical Computing, Austria). Our previous data showed that a low GI breakfast significantly decreased interstitial glucose after breakfast and after lunch compared with a high GI breakfast.[27] By assuming a lower effect size on glucose in an older population (75% of the observed effect size in the reference study), a sample size of 35 volunteers was required ($1-\beta$: 80%; $\alpha = 0.05$).

Incremental areas under the curve (iAUCs) were calculated for each individual for each specific meal (TM and SSM). Incremental pertained to the correction of the baseline values immediately prior to the respective meals (ie, at −5 min of the meal, respectively for TM and SSM). To do this, each respective baseline values were subtracted from the values observed after each meal at each given time point until the end of the 3-hour measurement period. The combined total AUC (tAUC) for each session was calculated by adding the total AUCs of both the TM and SSM within each session.

Linear mixed models were applied explaining the iAUCs or tAUCs combined of the meals according to the GI, the timing of intervention (using a 2×2 factorial design) as well as the interaction between the two factors: iAUC or tAUC $\sim GI_{high/low} \times time\ of\ intervention_{breakfast/dinner}$.

Assumptions were made for there being no carryover effect of one session to the next.

The above-mentioned model was used to make the following comparisons and obtain contrast estimates:
► High GI versus low GI (adjusting for time of intervention);
► Dinner versus breakfast (adjusting for GI);
► Within breakfast intervention: high GI versus low GI;
► Within dinner intervention: high GI versus low GI;
► Within high GI: dinner versus breakfast;
► Within low GI: dinner versus breakfast.

The same sets of analyses were performed on secondary outcomes such as blood insulin, TGs, FFAs and glucagon. Significance was set at p<0.05. Adjustments for multiplicity were done using the False Discovery Rate method (also known as Hochberg and Benjamini method).

RESULTS
Subjects and baseline characteristics

A total of 62 volunteers were screened, 48 volunteers enrolled, 37 completed all 4 study sessions and 34 volunteers completed the study as per-protocol. The recruitment (Consolidated Standards of Reporting Trials) flow chart including the number of volunteers at the various stages of the study is shown in figure 1.
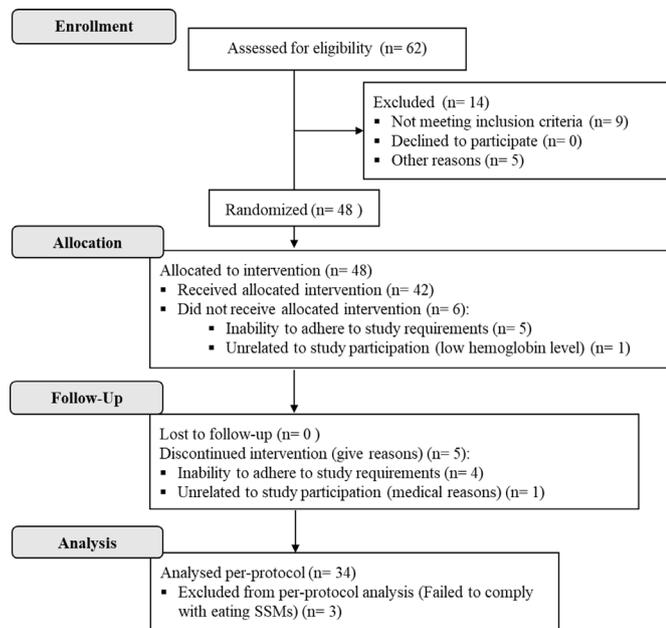
Figure 1 Consolidated Standards of Reporting Trials diagram. A total of 62 volunteers were screened, 48 volunteers enrolled, 37 completed all 4 study sessions and 34 volunteers completed the study as per-protocol. Of the total withdrawn participants, six withdrew from the study before the commencement of study intervention but after informed consent (ie, before session 1), four volunteers withdrew due to their inability to adhere to the study requirements (eg, study food consumption, not able to provide samples or undertake measurements) and one volunteer withdrew for medical reasons. SSM, subsequent standardized meal.

The detailed demographic characteristics of study participants is shown in table 1. There were no differences in demographic characteristics between those enrolled into the study as compared with the per-protocol population, except for a larger proportion of male participants in the final per-protocol population. All female participants were postmenopausal.

The postprandial substrate and hormone concentrations following TM and SSM for up to 3-hour measurement periods after each meal during various intervention sessions are shown in figure 2. Baseline substrate and hormone concentrations before TM did not differ between high and low GI interventions, within breakfast or within dinner interventions (Hi-Br vs Lo-Br and Hi-Di vs Lo-Di). However, baseline glucose, glucagon and TG concentrations immediately prior to TM were significantly greater in Hi-Di than Hi-Br TM sessions and TG concentration was greater in Lo-Di than Lo-Br TM sessions. Immediately prior to the SSM, baseline glucose and glucagon concentrations were greater following Lo-Br than Hi-Br TM sessions. Baseline TG concentration was greater while FFA was lower in Lo-Di than Hi-Di TM sessions. Baseline glucose and insulin concentrations were greater following breakfast TM sessions compared with dinner TM sessions, while that of glucagon, TG and FFA concentrations were lower following breakfast TM

Table 1 Subjects' baseline characteristics

| Variable	Level	All subjects enrolled	Per-protocol population
N		48	34
BMI (kg/m^2)		22.1±0.24	22.3±0.29
Height (cm)		164.1±1.11	165.7±1.35
Weight (kg)		59.7±1.24	61.6±1.57
Waist circumference (cm)		76.2±1.11	77.8±1.39
Hip circumference (cm)		94.0±0.77	94.7±0.79
SBP (mm Hg)		124.5±1.76	122.6±2.09
DBP (mm Hg)		78.3±1.11	78.7±1.40
Fasting glucose (mmol/L)		4.6±0.07	4.6±0.10
Heart rate (bpm)		61.7±1.47	62.2±2.00
Age (years)		56.9±0.71	56.8±0.83
Gender	Female	22	13
	Male	26	21
Smoker	No	48	34
Alcohol consumption	No	40	28
	Yes	8	6

Data are expressed as mean±SEM; gender, smoker and alcohol consumption are expressed as number of subjects.
BMI, body mass index; DBP, diastolic blood pressure; N, number of subjects; SBP, systolic blood pressure.

sessions as compared with dinner sessions. The baseline substrate and hormone concentrations and the respective comparisons are shown in online supplementary table 2.

Postprandial metabolic responses (iAUCs) following TM and SSM

The iAUCs (which adjusts for baseline) after TM and SSM following various TM sessions are shown in table 2. It should be noted that for Br TM sessions, the SSM were the subsequent lunch and for the Di TM sessions the SSM were the subsequent breakfast.

As expected, iAUCs of PPG (p<0.0001) and PPI (p<0.0001) following high GI TM was significantly greater than low GI TM, irrespective of meal time. More pertinently, iAUCs of PPG (p<0.0001) and PPI (p<0.05) following dinner TM was also greater than breakfast, irrespective of the GI content of the meals. While the interactions of GI:meal time were not significant for both PPG and PPI immediately after the TM (online supplementary table 3), the relative differences in iAUCs of PPG between dinner versus breakfast, irrespective of GI was greater than that between high versus low GI, irrespective of meal time. Similarly, iAUCs of PPG following SSM after high GI TM was significantly greater than the low GI TM sessions (p<0.01), irrespective of meal time, although the iAUCs of PPI following SSM were not different between the high and low GI TM sessions. The iAUCs of both PPG (p<0.0001) and PPI (p<0.0001) following SSM after
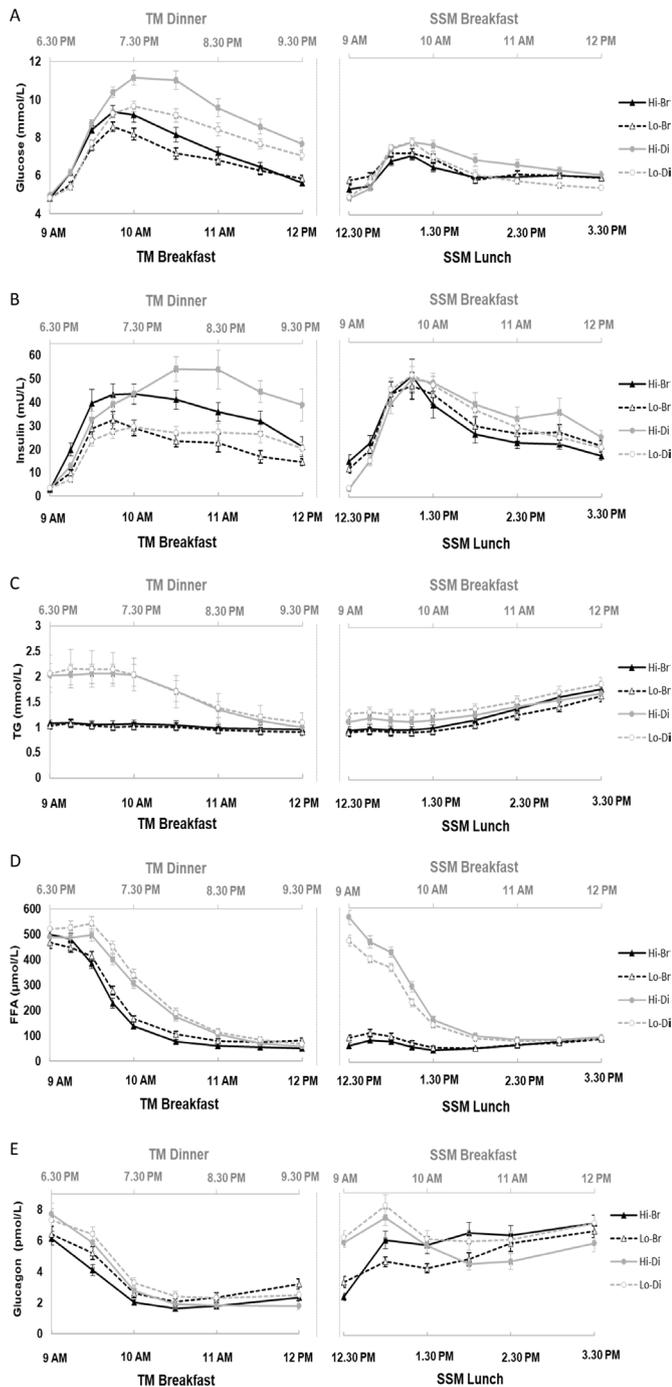
Figure 2 Postprandial substrate and hormone concentrations following test meals (TM) and subsequent standard meals (SSM) for up to 3-hour measurement periods after each meal. Mean±SEM concentrations of serum glucose (A), serum insulin (B), serum triglycerides (TG) (C), plasma free fatty acids (FFA) (D) and plasma glucagon (E) after high-glycemic index breakfast (Hi-Br), low-glycemic index breakfast (Lo-Br), high-glycemic index dinner (Hi-Di), low-glycemic index dinner (Lo-Di) TM and SSM. For all variables, n=34 subjects, except for FFA, n=29.

dinner TM sessions were significantly greater than breakfast TM sessions, irrespective of the GI of previous TM. Indeed, there was a significant GI:meal time interaction (p<0.05; online supplementary table 3) for the iAUC of

PPI following SSM, whereby for breakfast TM sessions, the high GI TM gave rise to a lower iAUCs of PPI after SSM than low GI TM session (p<0.05), although this association was not existent during dinner TM sessions.

For postprandial TG, while dinner had greater iAUCs than breakfast after TM, irrespective of GI (p<0.001), no differences were found between high versus low GI, irrespective of meal time. However, for PP TG after SSM, while high GI TM sessions gave rise to greater iAUCs than low GI (p<0.05), the iAUC of TG after SSM during dinner TM sessions were lower than the breakfast sessions (p<0.0001). These observations though were most likely related to the differences in baseline concentrations of TG immediately prior to the SSM between various intervention sessions, possibly due to the differences between the dinner and breakfast sessions in the intermeal durations between TM and SSM. The postprandial suppression of FFA (inverse iAUC) following TM was significantly greater during breakfast than during dinner, irrespective of GI (p<0.05), although there was no difference between high and low GI per se. Finally, postprandial glucagon suppression did not differ between treatments following TM. As for postprandial glucagon after SSM, there was significant interaction between GI:meal time (p<0.0001; online supplementary table 3) and the detailed iAUC values and comparisons are shown in table 2. In general, the PP glucagon response following SSM (lunch) after breakfast interventions were significantly greater as compared with the SSM (breakfast) after dinner interventions, irrespective of the GI of the meals.

Combined postprandial metabolic responses (tAUCs) of TM and SSM

The combined tAUCs of TM and SSM and the respective comparisons are shown in table 3. The combined tAUC of PP glycemia for high GI TM intervention session was significantly greater than the low GI. Similarly, the combined tAUC of PP glycemia for dinner was also greater than breakfast. There being a significant GI:meal time interaction (p=0.001; online supplementary table 4), while the high versus low GI difference was significant only during dinner interventions, the same was not true for the breakfast interventions. However, the dinner versus breakfast comparisons were significant, irrespective of the GI of the meals. The combined tAUC of PP insulin were also significantly greater for high versus low GI (p<0.0001), as well as for dinner vs breakfast (p<0.01) interventions.

The combined tAUC of PP TG was significantly greater during dinner intervention as compared with breakfast (p<0.0001), although not between high versus low GI interventions. Similarly, irrespective of GI, there was a significant increase in combined tAUC of FFA during dinner TM as compared with breakfast TM session (p<0.0001), although such effects were not found during high versus low GI comparisons.

Table 2 Test meal (TM) and subsequent standard meal (SSM) incremental areas under the curve (iAUCs) of postprandial responses for various substrates and hormones

Variable	MT	GI	N	TM					SSM				
				Mean	SD	Comparison	Contrast	P value†	Mean	SD	Comparison	Contrast	P value†
Glucose	BR	Hi	34	481.99	192.50	Hi vs Lo (overall)	142.01	**<0.0001**	140.70	155.36	Hi vs Lo (overall)	70.96	**<0.01**
		Lo	34	378.64	158.75	Di vs Br (overall)	245.75	**<0.0001**	93.83	156.48	Di vs Br (overall)	155.78	**<0.0001**
	DI	Hi	34	766.40	320.61	Br: Hi vs Lo	103.35	**<0.05**	320.57	151.72	Br: Hi vs Lo	46.87	0.1836
		Lo	34	585.74	215.50	Di: Hi vs Lo	180.66	**<0.0001**	225.52	108.36	Di: Hi vs Lo	95.05	**<0. 01**
						Hi: Di vs Br	284.40	**<0.0001**			Hi: Di vs Br	179.88	**<0.0001**
						Lo: Di vs Br	207.10	**<0.0001**			Lo: Di vs Br	131.69	**<0.001**
Insulin	BR	Hi	34	5643.62	3093.41	Hi vs Lo (overall)	2729.25	**<0.0001**	2447.71	1651.66	Hi vs Lo (overall)	−232.46	0.4477
		Lo	34	3269.08	2073.61	Di vs Br (overall)	865.96	**<0.05**	3454.72	2338.81	Di vs Br (overall)	2462.41	**<0.0001**
	DI	Hi	34	6864.29	3714.81	Br: Hi vs Lo	2374.54	**<0.0001**	5684.67	3420.26	*Br: Hi vs Lo*	*−1007.01*	***<0.05***
		Lo	34	3692.37	2039.35	Di: Hi vs Lo	3083.97	**<0.0001**	5141.35	2288.56	*Di: Hi vs Lo*	*542.09*	*0.2398*
						Hi: Di vs Br	1220.67	**<0.05**			*Hi: Di vs Br*	*3236.96*	***<0.0001***
						Lo: Di vs Br	511.24	0.3481			*Lo: Di vs Br*	*1687.85*	***<0.001***
Triglycerides	BR	Hi	34	3.98	7.93	Hi vs Lo (overall)	0.77	0.7772	53.62	27.28	Hi vs Lo (overall)	7.21	**<0.05**
		Lo	34	2.58	3.60	Di vs Br (overall)	9.66	**<0.001**	42.93	21.43	Di vs Br (overall)	−13.42	**<0.001**
	DI	Hi	34	13.02	18.47	Br: Hi vs Lo	1.40	0.7465	36.72	36.57	Br: Hi vs Lo	10.69	**<0.05**
		Lo	34	12.87	18.88	Di: Hi vs Lo	0.15	0.9645	32.99	30.08	Di: Hi vs Lo	3.73	0.4473
						Hi: Di vs Br	9.04	**<0.05**			Hi: Di vs Br	−16.90	**<0.01**
						Lo: Di vs Br	10.29	**<0.01**			Lo: Di vs Br	−9.94	**<0.05**
Free fatty acids	BR	Hi	29	−59504.89	26273.50	Hi vs Lo (overall)	−3703.86	0.3504	496.98	7870.64	Hi vs Lo (overall)	−3967.37	0.1552
		Lo	30	−50405.09	18057.99	Di vs Br (overall)	8766.81	**<0.05**	−3446.39	7360.76	Di vs Br (overall)	−56096.50	**<0.0001**
	DI	Hi	29	−45272.73	22182.40	Br: Hi vs Lo	−8909.32	0.1022	−63524.99	24943.72	*Br: Hi vs Lo*	*4231.63*	*0.2678*
		Lo	29	−46774.32	26604.49	Di: Hi vs Lo	1501.59	0.7772	−51358.61	17701.28	*Di: Hi vs Lo*	*−12166.38*	***<0.01***
						Hi: Di vs Br	13972.27	**<0.05**			*Hi: Di vs Br*	*−64295.50*	***<0.0001***
						Lo: Di vs Br	3561.36	0.5193			*Lo: Di vs Br*	*−47897.49*	***<0.0001***
Glucagon	BR	Hi	34	−637.76	354.44	Hi vs Lo (overall)	−118.79	0.1233	647.97	374.81	Hi vs Lo (overall)	111.58	**<0.05**
		Lo	34	−564.90	333.75	Di vs Br (overall)	−126.52	0.1022	317.80	293.40	Di vs Br (overall)	−435.34	**<0.0001**
	DI	Hi	34	−820.98	502.17	Br: Hi vs Lo	−93.00	0.3889	−48.14	395.24	*Br: Hi vs Lo*	*347.53*	***<0.0001***
		Lo	34	−671.83	589.98	Di: Hi vs Lo	−144.58	0.1878	77.36	447.54	*Di: Hi vs Lo*	*−124.36*	*0.1195*
						Hi: Di vs Br	−152.31	0.1653			*Hi: Di vs Br*	*−671.28*	***<0.0001***
						Lo: Di vs Br	−100.73	0.3619			*Lo: Di vs Br*	*−199.39*	***<0.05***

Linear mixed models were used to compare the iAUCs following TM according to the GI and MT in a 2×2 analyses. Significance was set at p<0.05.
'Hi vs Lo overall'—when dinner and breakfast combined. 'Di vs Br overall'—when high and low GI combined.
Bold values are statistically significant.
*Adjustments for multiplicity was done using the False Discovery Rate method.
†Only when GI:MT interaction significant, subgroup analyses are shown in italics.
BR, breakfast; DI, dinner; GI, glycemic index; Hi, high GI; Lo, low GI; MT, meal time; N, number.

DISUSSION

The study showed that the time of consumption (in this case breakfast vs dinner) of a carbohydrate-rich meal had significant effects on various markers of PP glucose homeostasis in the PP period immediately after the meal itself and after the SSM, on top of the independent effects of GI. While the differences between high and low GI meals on PP glycemia and insulinemia were expected, the differences in PPG and PPI between dinner and breakfast, irrespective of their GI content are likely to be physiologically relevant, with potentially significant public health implications. This observation is particularly important since majority of the epidemiological studies investigating associations between GI/GL of the diet and disease risk generally ignore considerations on assessing the timing of the consumption in addition to calculating the mean daily GI/GL contents of the foods. For example, an individual habitually consuming proportionately greater GL (amount of available carbohydrate×GI) during dinner may have an overall worse glycemic status than someone else who habitually consumes greater GL for breakfast, even though their mean daily GL may be similar. This observation may partly explain some of the heterogeneity and inconsistencies in the GI-disease associations between various studies.[10–12]

Table 3 Combined total areas under the curves (tAUCs) of test meals (TM) and subsequent standard meal (SSM) postprandial responses for various substrates and hormones

Variable	Meal time	GI	N	Mean	SD	Comparison	Contrast	P value†
Glucose	BR	Hi	34	2493.32	334.44	Hi vs Lo (overall)	181.11	**<0.0001**
		Lo	34	2423.70	328.43	Di vs Br (overall)	296.81	**<0.0001**
	DI	Hi	34	2901.62	442.64	*Br: Hi vs Lo**	*69.63*	*0.1906*
		Lo	34	2609.02	306.35	*Di: Hi vs Lo**	*292.60*	***<0.0001***
						*Hi: Di vs Br**	*408.29*	***<0.0001***
						*Lo: Di vs Br**	*185.32*	***<0.001***
Insulin	BR	Hi	34	11425.73	6261.91	Hi vs Lo (overall)	2686.65	**<0.0001**
		Lo	34	9516.74	4963.53	Di vs Br (overall)	1572.55	**<0.01**
	DI	Hi	34	13775.94	6962.72	Br: Hi vs Lo	1908.99	**<0.01**
		Lo	34	10126.83	4127.53	Di: Hi vs Lo	3464.31	**<0.0001**
						Hi: Di vs Br	2350.21	**<0.01**
						Lo: Di vs Br	794.89	0.3125
Triglycerides	BR	Hi	34	421.39	156.72	Hi vs Lo (overall)	−3.30	0.9097
		Lo	34	395.60	155.64	Di vs Br (overall)	158.25	**<0.0001**
	DI	Hi	34	550.54	278.76	Br: Hi vs Lo	25.79	0.5690
		Lo	34	582.93	404.90	Di: Hi vs Lo	−32.39	0.4981
						Hi: Di vs Br	129.16	**<0.01**
						Lo: Di vs Br	187.33	**<0.0001**
Free fatty acids	BR	Hi	29	44669.53	14708.28	Hi vs Lo (overall)	−2131.99	0.4862
		Lo	30	50423.81	17067.00	Di vs Br (overall)	36217.11	**<0.0001**
	DI	Hi	29	84738.51	17216.24	Br: Hi vs Lo	−5761.22	0.1680
		Lo	29	83241.27	18907.22	Di: Hi vs Lo	1497.24	0.7035
						Hi: Di vs Br	39846.33	**<0.0001**
						Lo: Di vs Br	32587.88	**<0.0001**
Glucagon	BR	Hi	34	1601.86	560.85	Hi vs Lo (overall)	−81.57	0.1906
		Lo	34	1563.72	570.10	Di vs Br (overall)	207.56	**<0.001**
	DI	Hi	34	1638.99	659.15	*Br: Hi vs Lo**	*55.67*	*0.5241*
		Lo	34	1885.22	766.10	*Di: Hi vs Lo**	*−218.82*	***<0.001***
						*Hi: Di vs Br**	*70.31*	*0.4604*
						*Lo: Di vs Br**	*344.80*	***<0.0001***

Linear mixed models were used to compare the combined tAUCs following TM+SSM according to the GI and MT in a 2×2 analyses. Significance was set at p<0.05.

'Hi vs Lo overall'—when dinner and breakfast combined. 'Di vs Br overall'—when high and low GI combined.

Bold values are statistically significant.

*Only when GI:MT interaction significant, subgroup analyses are shown in italics.

†Adjustments for multiplicity was done using the False Discovery Rate method.

BR, breakfast; DI, dinner; GI, glycemic index; Hi, high GI; Lo, low GI; MT, meal time; N, number.

In support of our findings, a recent study comparing the response with a low GI dinner (taken at 8.00 pm) and breakfast (taken at 8.00 am) in a young non-diabetic population also found that the glucose iAUC at dinner was significantly greater than breakfast.[28] In their study, Leung *et al* kept the duration of the prior fasting period identical for both conditions, hence excluding the potential confounding role of the intermeal duration factor. In our study however, while the duration of the fasting periods before TM as well as before SSM were indeed variable between dinner and breakfast interventions, these meal timings and intermeal durations reflected a normal dietary pattern of 3 meals per day, that is, breakfast in the morning, lunch in the early afternoon and dinner in the evening. Similar to our findings, another study found that the timing of the day had greater effects on PPG than the difference between high and low GI meals in a young non-diabetic population.[29] One of the differences between our study and the two previously cited studies were the population recruited. Indeed, we

included an older cohort in whom glucose homeostasis was likely to be more compromised[30] and who could benefit more in terms of cardiometabolic disease prevention. The strength of our study design as compared with these earlier studies was the use of a larger sample size (34 vs 9 and 10, respectively). Moreover, we studied participants of Chinese origin, known to be at a greater risk of developing type 2 diabetes than Caucasians,[24 31] and our study had been one of the most comprehensive studies to investigate simultaneously the influences of timing and GI variations of meals on PPG responses in Asians. Overall, the results of our study and previous findings corroborate that in non-diabetic individuals, glucose metabolism is significantly worse in the evenings in comparison to the mornings, irrespective of the GI content of the meal.

While it has been long known that the composition (including GI/GL) of the previous meal affects the glycemic responses of the subsequent meal,[32–34] no previous study has investigated the effect of meal time (breakfast vs dinner) and GI variations on the subsequent meal effect concurrently. We found that both differences in the timing of previous meal (TM) intake as well as the variations in GI independently and simultaneously affected the PPG response of the SSM, clearly indicating carryover effects due to both these parameters. While differences in intermeal duration between TM dinner and SSM breakfast (14.5 hour) during dinner intervention sessions and between TM breakfast and SSM lunch (3.5 hours) during breakfast intervention sessions may confound some of the differences observed, nonetheless this was the only study design that would have reflected a normal dietary pattern.

The increased PPG response after the SSM (breakfast) following high GI TM dinner compared with the low GI TM dinner may have arisen from the significantly greater baseline concentration of FFA concentration immediately prior to SSM (breakfast) following the high GI TM dinner intervention. This is similar to a previous observation that the fasting FFA concentration was positively correlated with glucose iAUC of a standardized breakfast.[35] Furthermore, similar to our differences in fasted FFA concentration observation following an overnight fast, after high versus low GI dinner consumption the night before, an earlier study by Jenkins *et al* had also shown that consuming a bolus of 50 g glucose in comparison with continuous sipping over a 3-hour period led to greater FFA suppression over the postprandial period and significantly reduced blood glucose levels following a 5 g intravenous glucose tolerance test.[36] Of course, it is well established that there is an inverse association between plasma FFA concentration and insulin sensitivity.[37–40] Our study also showed greater suppression of FFA in the postprandial periods immediately following breakfast TM compared with the dinner TM. This, together with an increased glycemic response during the Di TM indicates reduced insulin sensitivity during dinner as compared with breakfast. Previous studies have indeed

shown reduced insulin sensitivity during the evening as compared with the morning using insulin tolerance test or frequently sampled intravenous glucose tolerance test.[41 42] Interestingly, insulin sensitivity in type 2 diabetics also changes with circadian rhythmicity, but seems to be lower in the morning than in the evening, possibly due to increased hepatic gluconeogenesis in type 2 diabetes,[43 44] which suggests that our findings may not be entirely applicable to type 2 diabetics. Furthermore, the GI values of the TM reported in this study was estimated from the GI values of the same rice types from our previous studies rather than being directly measured in this study itself. Considering potential variations in GI of rice arising from subtle differences in growth conditions or milling, the actual GI of the TM may have been slightly different. As an additional limitation, it should be noted that due to the acute design of our study, the long-term validity of our findings would need to be further confirmed. Furthermore, we investigated only one specific time for breakfast and one specific time for dinner. Thus, our findings might have been somewhat variable for other timings of these meals. Nonetheless, by simultaneously measuring several markers at the same time, this study has produced a comprehensive dataset on the chronobiological impact of food consumption of variable GI on postprandial glucose homeostasis in a non-diabetic population.

In summary, our study specifically found that carbohydrate-rich meals taken at dinner gave rise to significantly adverse profile of glucose homeostasis as compared with the same meals taken at breakfast, independent of the GI content of the meal. These findings are likely to have public health implications in Asia where carbohydrate-rich, high GI meals are often consumed late during the day (often late evenings) due to cultural practices. For example, a recent study by Gupta *et al* in healthy adults in India found that 32% of daily intake of calories were consumed between 7.00 pm and 11.00 pm, with the median dinnertime being after 10 pm.[45] With the high prevalence of prediabetes worldwide, it will be advisable to particularly focus attention on the carbohydrate quality and quantity of the evening meals. The findings of our study also has significant implications on future design of epidemiological studies investigating GI of diet and disease risk associations. We recommend that the timing of intake of carbohydrates (ie, glycemic load) should also be assessed and reported as an additional variable. The results of this study prompt further research in the area of chrononutrition, particularly to investigate long-term effects of carbohydrate consumption at different times of the day.

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