Original Article

Glucagon, rather than Glucagon-Like Peptide 1, Mediates Higher Post-Lunch Glucose Excursions during Breakfast Skipping in Asian Indian Patients with Uncontrolled Type 2 Diabetes Mellitus

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Abstract

Introduction: The effect and mechanism of skipping breakfast on glycemic control in type 2 diabetes mellitus (T2DM) in Asian-Indians is unknown. **Methods:** Cross-over, within-group study recruiting 5 habitual breakfast eaters (BE) and 5 habitual breakfast skippers (BS) with uncontrolled T2DM (HbA1c 7-9%). Patients underwent testing after three days of following their usual breakfast habits and after seven days of crossing over to the other arm. Fasting values and incremental area under the curve ($iAUC_{0-180}$) of post-lunch levels of glucose, insulin, C-peptide, glucagon-like peptide 1 (GLP-1), and glucagon were measured. Continuous glucose monitoring (CGM) parameters assessed were area under the curve (AUC_{0-180}) of post-meal glucose values, 24-hour average blood glucose (ABG), time in range (TIR), and glycemic variability. **Results:** BS led to significantly higher fasting (133.5 ± 34.5 mg/dl vs 110 ± 31.50 mg/dl, $P = 0.009$) and peak post-lunch (214.6 ± 35.07 mg/dl vs 175.4 ± 39.26 mg/dl, $P < 0.001$) plasma glucose, and HOMA-IR (3.05 ± 3.89 vs 2.03 ± 1.76, $P = 0.007$) as compared to BE. Post-lunch $iAUC_{0-180}$ during BS was significantly higher for plasma glucose (7623 ± 2947.9 mg/dl × min vs 1922.4 ± 1902.1 mg/dl × min, $P < 0.001$), insulin (2460 ± 1597.50 mIU/ml × mins vs 865.71 ± 1735.73 mIU/ml × mins, $P = 0.028$), C-peptide (418.4 ± 173.4 ng/ml × mins vs 127.8 ± 117.1 ng/ml × mins, $P < 0.001$) and glucagon (7272.7 ± 4077 pg/ml × mins vs 4568.8 ± 2074.9 pg/ml × mins, $P = 0.044$) as compared to BE, while GLP-1 (1812.7 ± 883 pmol/l × mins during BS vs 1643 ± 910 pmol/l × mins during BE, $P = 0.255$) did not significantly differ between the two visits. CGM revealed a higher post-lunch AUC_{0-180} during BS. There was no difference in post-dinner AUC_{0-180}, ABG, TIR, or glycemic variability. **Conclusion:** Skipping breakfast led to higher post-lunch glucose excursions, possibly due to higher glucagon excursion and increased insulin resistance.

Keywords: Breakfast skipping, continuous glucose monitoring, glucagon, incretin, insulin resistance, type 2 diabetes mellitus

Introduction

Along with urbanization, cities have experienced dietary changes favoring increased caloric consumption and diverse sleep and eating patterns.[1] Habits like late-night dinners, skipping breakfast, and irregular sleeping patterns are considered unhealthy habits based on their negative consequences on glycemic control.[2-4] Data from previous studies have suggested that almost 19% of the studied population skipped breakfast, and most are nutritiously inadequate.[5-8] Different prospective and cross-sectional studies have given variable results on dysglycemia in patients with type 2 diabetes mellitus (T2DM) with breakfast skipping.[2,9-12] Some studies had confounding factors such as total calorie intake and late-night dinner eating, thus making the association between breakfast skipping and

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health outcomes inconclusive.[10,13–16] There is evidence that eating as per the circadian rhythm with a higher proportion of caloric intake during the first half of the day may improve metabolic parameters.[13,17,18]

Hence, we conducted a study to assess the effects of skipping breakfast on post-lunch plasma glucose levels in Asian Indian patients with T2DM. We additionally assessed the effect of breakfast on various laboratory parameters like insulin, C-peptide, glucagon, glucagon-like peptide 1 (GLP-1), homeostasis model of assessment of insulin resistance (HOMA-IR), inflammatory markers (high-sensitivity C-reactive protein [hs-CRP], interleukin-6 [IL-6]). We also assessed continuous glucose monitoring (CGM) system parameters such as the area under the curve (AUC_{0-180}) of post-breakfast, post-lunch, and post-dinner glucose, average blood glucose (ABG), and measures of glycemic variability (mean amplitude of glucose excursion [MAGE], standard deviation [SD], and continuous overall net glycemic action [CONGA]).

Materials and Methods

Study design

This was an open-label, interventional, within-group, cross-over study. Between August 2021 and March 2022, patients who met the inclusion and exclusion criteria were recruited from a diabetes clinic run by an Endocrinology Department in a tertiary-care hospital.

Patients

Patients with T2DM with BMI 23–35 kg/m^2, glycosylated hemoglobin (HbA1c) levels between 7% and 9% on stable lifestyle and/or oral metformin therapy were included in the study. Patients on other oral antidiabetic medications and with chronic comorbidities from diabetes, such as cardiovascular disease, cerebrovascular disease, proliferative diabetic retinopathy, or gastroparesis, night shift workers, with a history of alternate medications, glucocorticoid use, impaired renal function, impaired liver function, pregnancy/lactation, psychiatric disorders including illicit drug or alcohol abuse and patients who had undergone bariatric surgery were excluded from the study.

Study protocol

After screening, a detailed demographic, physical, and anthropometric examination was performed, and the findings were recorded in a Case Record Form. All patients were on a modified diet for at least the preceding three months, given by a registered dietician, based on their diabetes, BMI, and physical activity as per standard guidelines. The patients were stratified according to their usual breakfast habits. Patients who habitually skipped breakfast were classified to the habitual breakfast skippers (HBS) arm, whereas those who routinely ate breakfast were classified to the habitual breakfast eaters (HBE) arm.

A CGM system was inserted on day 0 and patients were instructed to follow their usual breakfast habits for three days. Both arms underwent testing on day three of the study, during which HBE had their BE visit, and HBS had their BS visit. After this, they were switched to the other arm for the next seven days during which HBS ate breakfast and HBE skipped breakfast. They were tested again on day 10 when HBE had their BS visit and HBS had a BE visit [Figure 1a]. Throughout the study, the patients ate the same modified meals as they were on, with similar macronutrients, comprising 1400 kcal during breakfast skipping visit and 1800 kcal during breakfast eating visit, divided into 15.5% proteins, 22.6% fats, and 61.8% carbohydrates [Table 1]. Patients were instructed to be consistent with their meal timings and patterns and to maintain a food diary, which the dietician subsequently analyzed. The CGM helped to check mealtime compliance.

On each test visit day, the patients arrived at the laboratory after 8–12 hours of fasting. Both breakfast and lunch were home-cooked meals brought to the center and consumed before the investigator.

During the BE visit, samples were collected at 0, 30, 60, 90, 120, and 180 minutes post breakfast. Only a fasting sample was collected during the BS visit. Post-lunch samples were collected at 0, 30, 60, 90, 120, and 180 minutes post-lunch during both visits [Figure 1b]. Blood samples were collected for plasma glucose, serum insulin, GLP-1, glucagon, and C-peptide. hs-CRP and IL-6 were analyzed only from fasting samples. The incremental area under the curve ($iAUC_{0-180}$) was calculated for post-lunch values over the baseline pre-lunch value for both days.

Laboratory parameters

Special tubes were prepared to collect samples for GLP-1 and glucagon. In a 5 ml glass tube, 750 microliters of distilled water were taken. One tablet of cOmplete™ Mini Protease Inhibitor Cocktail (Sigma Aldrich Company) was added to it. The tablet was mixed gently with distilled water by tapping till the tablet completely dissolved. A total of 75 microliters of this solution were put into ethylenediamine tetraacetate (EDTA) tubes. Each tube was stored at 20°C temperature and was used within 2 weeks of preparations. In each tube, 1 ml of blood was collected yielding 500 microliter plasma. Samples for GLP-1, glucagon, IL-6, and hs-CRP were centrifuged, separated, aliquoted, and stored at -80°C.

HbA1c was processed using high-performance liquid chromatography (Bio-Rad D10 analyzer; inter-assay coefficient of variation (CV) 1%, intra-assay CV 0.8%). Plasma glucose was estimated using the Glucose Oxidase Peroxidase (GOD-POD) method (ERBA CHEM-7 [TRANSASIA], inter-assay CV 1-2.5%, intra-assay CV 1.2-2.3%). Serum insulin was estimated by chemiluminescence immunoassay (Beckman-Coulter Access-2; inter-assay CV 3.5%, intra-assay CV 2.0). HOMA-IR was calculated using a standard formula ([fasting plasma glucose (mg/dl) × fasting serum insulin levels (mIU/ml)]/405)].[19] Hs-CRP was analyzed by immunoturbidimetry (Abbott Architect; inter-assay CV 0.5-2.4%, intra-assay CV 0.7–4%). IL-6 was estimated using an electrochemiluminescence immunoassay (Roche COBAS

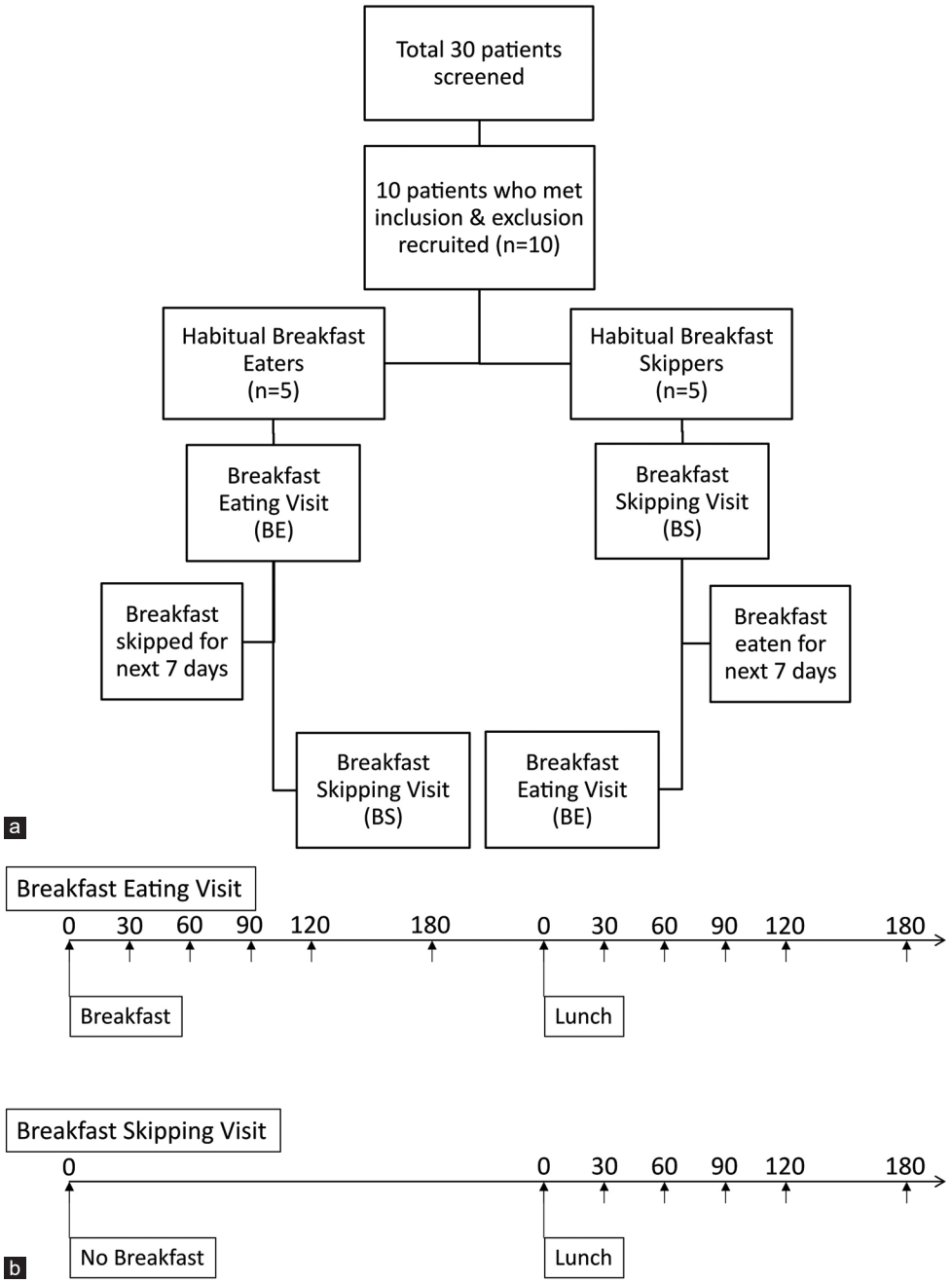

Figure 1: Study protocol. (a) Flow diagram depicting recruitment and cross-over of study participants. (b) Schematic illustration of timings for blood collection on breakfast eating and breakfast skipping visits

Table 1: Calorie content distribution of meals consumed during breakfast eating and skipping days

Day	Breakfast 08:00-09:00 h			Lunch 12:30–13:30 h			Evening Snacks 16:30–17:30 h			Dinner 20:00–21:00 h		
	P	F	C	P	F	C	P	F	C	P	F	C
Breakfast Eating	15.6%	24%	60%	15.1%	22.9%	62.5%	16.1%	19.6%	67.9%	15.2%	24%	56.8%
		400 kcal			600 kcal			200 kcal			600 kcal	
Breakfast Skipping		No breakfast		15.1%	22.9%	62.5%	16.1%	19.6%	67.9%	15.2%	24%	56.8%
					600kcal			200kcal			600kcal	

P: Proteins %; F: Fats %; and C: Carbohydrates %

e411; inter-assay CV 8.5%, intra-assay CV 6%). GLP-1 levels were measured using the enzyme-linked immunosorbent assay (ELISA) method (Merck Millipore, catalog number EZGLP1T-36K; inter-assay CV 1-2%, intra-assay CV <10%).

Glucagon levels were measured using the ELISA method (R and D Systems, Quantikine, catalog number DGCG0; inter-assay CV 1-2%, intra-assay CV <10%).

Continuous glucose monitoring system

The CGM used for the present study was the FreeStyle Libre Pro Flash Glucose Monitoring System, which was blinded to the patients and required the reader to read the data. CGM data were exported to Microsoft Excel using FreeStyle Libre Pro software on the manufacturer's website. As per the manufacturer, the first 12 hours of the CGM are unreliable; hence, data recordings of the first 12 hours were not considered. $AUC_{0\text{-}180\ min}$ was calculated for CGM glucose values of 3 hours post-breakfast, post-lunch, and post-dinner on both days. 24-hour ABG was calculated for 08:00 hours on test day to 08:00 hours the next day on both test days. Other CGM parameters assessed were time above range (TAR), time in range (TIR), and time below range (TBR). MAGE, SD, and CONGA were used as measures of glycemic variability (GV).[20]

Statistical analysis

The sample size was calculated using the software GPower 3.1. According to a study conducted by Jakubowicz *et al*.,[12] post-lunch glucose at $AUC_{0\text{-}180}$ was 30,566.3 ± 185.1 mg/dL × min while eating breakfast and 41,804.7 ± 310.9 mg/dL × min while skipping breakfast. Assuming 95% confidence intervals and 80% power, we found the minimum sample size of two patients, one in each group. We decided to study 10 patients.

All analyses were performed using Microsoft Excel 2019 and SPSS Statistics v28.0. $AUC_{0\text{-}180\ min}$ and $iAUC_{0\text{-}180\ min}$ were calculated using an algorithm on MS Excel and GraphPad Prism 9.0 using the trapezoidal method. ABG was calculated as the average of all glucose readings in the given time. MAGE and CV were determined using EasyGV software version 9.0.R2 (EasyGV, University of Oxford).

The normality of data was checked using the Shapiro-Wilk test. If a normal distribution was found, paired T-tests were applied between the two means. If the data was not normally distributed, the Wilcoxon matched-pairs signed rank test was applied between two medians. A P value of <0.05 was considered significant.

Ethical aspects

This study was approved by the institutional ethics committee at Topiwala National Medical College and BYL Nair Charitable Hospital (ECARP/2020/137 dated 18/02/2021). The procedures in the study were in accordance with the guidelines laid down in the Declaration of Helsinki, 1964, and revised thereafter. Written informed consent was obtained for participation in the study and use of the patient data for research and educational purposes.

Results

Baseline patient characteristics

The study enrolled 5 HBE and 5 HBS [Figure 1a]. The period of recruitment and follow-up was between August 2021 and March 2022. All ten patients (5 men and 5 women) completed the study. Patients were 45.2 ± 9.9 years old with 2.25 ± 1.1 years of T2DM duration and had HbA1c of 7.38 ± 0.40%. The patients were overweight or obese, with an average BMI of 28.3 ± 4.4 kg/m^2. The mean fasting plasma glucose was 131.9 ± 7.7mg/dl, and post-prandial plasma glucose was 180.4 ± 17.7mg/dl.

Plasma glucose levels

Fasting plasma glucose was significantly higher during the BS visit than during the BE visit (110 ± 31.50 mg/dl during BE vs 133.5 ± 34.50 mg/dl during BS, $P = 0.009$). However, the pre-lunch plasma glucose did not significantly differ when the patients ate breakfast (133.50 ± 88.25 mg/dl during BE vs 114.00 ± 27.00 mg/dl during BS, $P = 0.169$). The peak post-lunch plasma glucose was significantly higher on the BS visit than on the BE visit (175.4 ± 39.26 mg/dl during BE vs 214.6 ± 32.05 mg/dl during BS, $P < 0.001$). The $iAUC_{0\text{-}180\ min}$ post-lunch plasma glucose values were also significantly higher during the BS visit than the BE visit (1922.4 ± 1902.1 mg/dl × mins during BE vs 7623 ± 2947.9 mg/dl × mins during BS, $P < 0.001$) [Table 2] [Figure 2a].

Serum Insulin, C-peptide, GLP-1 and Glucagon levels

Fasting serum insulin levels were higher during the BS visit but did not reach statistical significance (7.45 ± 5.10 mIU/ml during BE vs 9.15 ± 12.55 mIU/ml during BS, $P = 0.059$) [Figure 2b]. Fasting values of C-peptide, GLP-1, and glucagon were not significantly different between the two visits. The $iAUC_{0\text{-}180\ min}$ of post-lunch insulin, C-Peptide, and glucagon was significantly higher on BS than BE. Post-lunch $iAUC_{0\text{-}180\ min}$ of GLP-1 did not differ significantly between the two visits. [Table 2 and Figure 2c-e].

Continuous glucose monitoring profile

As calculated by CGM readings, the $AUC_{0\text{-}180\ min}$ of post-breakfast was higher during BE and post-lunch values were higher during BS. The $AUC_{0\text{-}180\ min}$ of the post-dinner glucose on CGM was similar between the two visits (29211 ± 4498 mg/dl × mins during BE vs 30298 ± 8880 mg/dl × mins during BS; $P = 0.58$) [Table 3]. The 24-hour ABG was also not different on the two days (138.3 ± 27.5 mg/dl during BE vs 133.4 ± 39.9 mg/dl during BS vs, $P = 0.364$) [Figure 3]. The TAR, TIR, and TBR were similar in the two conditions. There was no difference in measures of glycemic variability [Table 3].

HOMA-IR, IL-6 and hs-CRP levels

HOMA-IR calculated on the BS visit was higher than on the BE visit (2.03 ± 1.76 during BE vs 3.05 ± 3.89 during BS, $P = 0.007$), implying higher insulin resistance on the skipping day. The IL-6 and hs-CRP levels did not differ between the two meal visits [Table 2].

Discussion

As suggested by epidemiological studies, there is a probable link between breakfast skipping and body weight gain,

Table 2: Laboratory parameters

	Breakfast Eating visit	Breakfast Skipping visit	P
Fasting values			
Fasting Plasma Glucose (mg/dl)*	110±31.50	133.5±34.50	0.009
Insulin (mIU/ml)*	7.45±5.10	9.15±12.55	0.059
HOMA-IR*	2.03±1.76	3.05±3.89	0.007
C-peptide (ng/ml)	2.3±0.71	2.46±0.94	0.362
GLP-1 (pmol/l)	26.38±11.04	27.33±11.33	0.732
Glucagon (pg/ml)*	88.15±125.53	88.55±66.18	0.185
Post-lunch values			
Pre-lunch plasma glucose (mg/dl)*	133.50±88.25	114.00±27.00	0.169
Plasma glucose $iAUC_{0-180}$ (mg/dl×mins)	1922.47±1902.14	7623±2947.94	<0.001
Peak post-lunch plasma glucose (mg/dl)	175.4±39.26	214.6±35.07	<0.001
Insulin $iAUC_{0-180}$ (mIU/ml×mins)*	865.71±1735.73	2460±1597.50	0.028
C-Peptide $iAUC_{0-180}$ (ng/ml×mins)	127.8±117.1	418.4±173.4	<0.001
GLP-1 $iAUC_{0-180}$ (pmol/l×mins)	1643±910	1812.7±883	0.255
Glucagon $iAUC_{0-180}$ (pg/ml×mins)	4568.8±2074.9	7272.7±4077	0.044
Inflammatory markers			
IL-6 (pg/ml)	5.8±2.32	5.18±2.2	0.447
hs-CRP (mg/l)*	1.80±6.93	2.45±7.38	0.672

Fasting and post-lunch values of glucose, insulin, GLP-1, glucagon, C-peptide, and inflammatory markers during breakfast eating and breakfast skipping visits. *Values expressed in Median±IQR. All other values are expressed in Mean±SD. HOMA-IR: homeostasis model of assessment of insulin resistance; GLP-1: glucagon-like peptide 1; iAUC: incremental area under the curve; hs-CRP: high-sensitivity C-reactive; and IL-6: interleukin-6

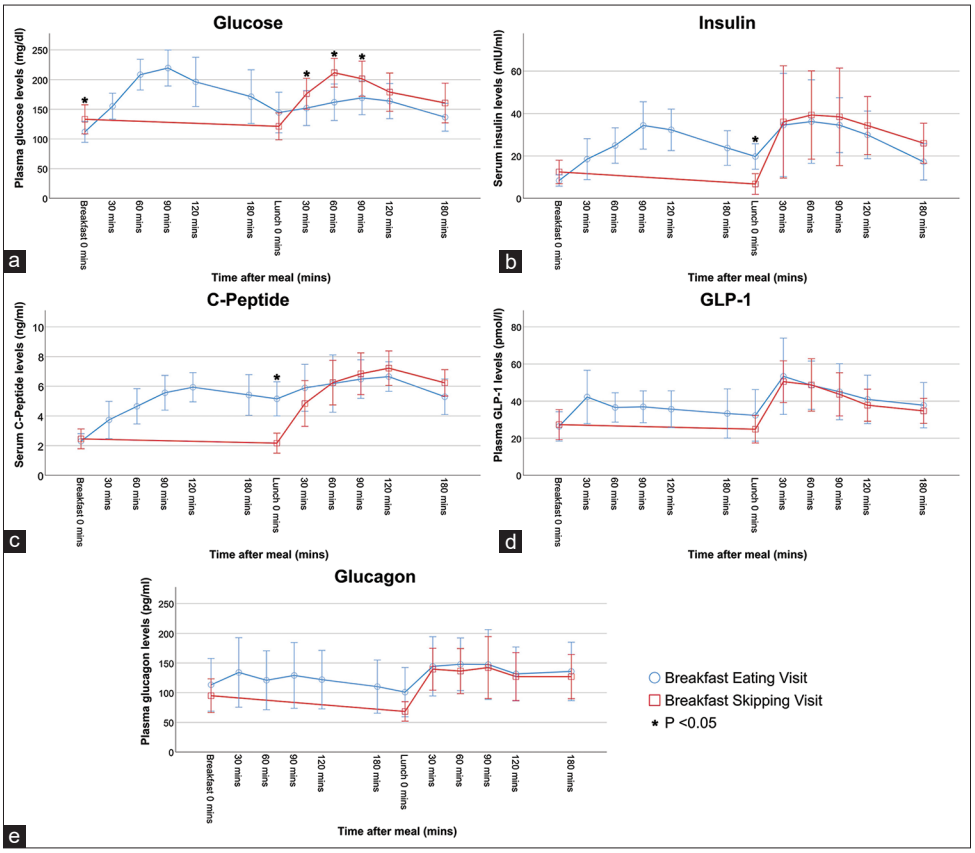

Figure 2: Laboratory parameters. Levels of glucose (a), insulin (b), C-peptide (c), GLP-1 (d), and glucagon (e) for 180 mins post-breakfast and post-lunch during breakfast eating and breakfast skipping visits. * *P* value < 0.05

insulin resistance (IR), and T2DM.[2,5,15] The timing of the meal influences the post-prandial increase in energy expenditure and blood glucose levels, and skipping breakfast may reduce 24-hour energy expenditure while increasing blood glucose levels.[2,4,12] The extent of post-prandial rise in plasma glucose depends on the quantity and nature of

Table 3: Continuous glucose monitoring (CGM) parameters

	Breakfast Eating visit	Breakfast Skipping visit	*P*
Post-meal CGM profile			
Pre-lunch AUC_{0-180} (mg/dl×mins)*	30135±9851.25	19395±5298.75	0.005
Post-lunch AUC_{0-180} (mg/dl×mins)	27013.5±6544.4	32031±7626.8	0.019
Post-dinner AUC_{0-180} (mg/dl×mins)	29211±4498	30298±8880	0.58
Entire day CGM profile			
TIR (%)*	90.1±23.6	84.9±38.7	0.213
TAR (%)*	8.85±25.2	9.38±27	0.205
TBR (%)*	0±1.8	0±2.08	0.581
ABG (mg/dl)	138.3±27.5	133.4±39.9	0.364
Glycemic variability			
SD (mg/dl)	33.6±7.9	35.08±12.2	0.665
MAGE (mg/dl)*	86.5±35.1	103.1±46.7	0.333
CONGA (mg/dl)*	108.8±36.7	108.4±37.1	0.203

Post-meal and 24-hour CGM profile during breakfast eating and breakfast skipping visits. *Values expressed in Median±IQR. All other values are expressed in Mean±SD. AUC: area under the curve; TIR: time in range; TAR: time above range; TBR: time below range; ABG: average blood glucose; SD: standard deviation; MAGE: mean average glycemic excursion; CONGA: continuous overall net glycemic action

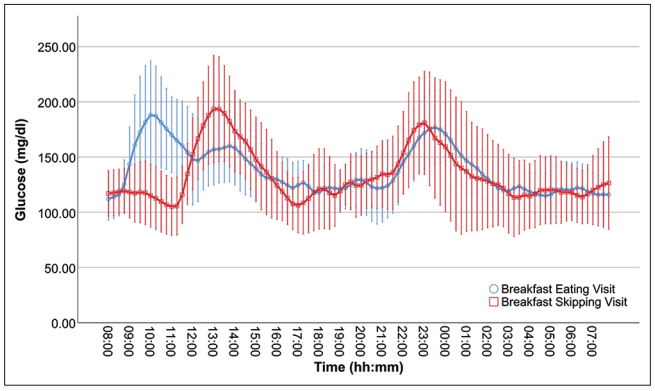

Figure 3: 24-hour Continuous glucose monitoring profile. Continuous glucose monitoring system readings through 24 hours during breakfast eating and breakfast skipping days

food ingested and the metabolic state immediately before eating. The previous meal modulates the post-prandial glycemic responses to a subsequent meal, termed the second-meal phenomenon.[11] In the present study, we tried to understand the influence of breakfast skipping on blood glucose dynamics and incretin hormone responses in patients with T2DM.

The omission of breakfast resulted in a significantly higher glycemic response after lunch than when breakfast was consumed. Breakfast omission increased post-lunch glucose excursion as evidenced by the higher $iAUC_{0-180}$ and post-lunch peak glucose despite lower pre-lunch glucose. The post-lunch insulin $iAUC_{0-180}$ was higher on the BS day without any difference in the GLP-1 $iAUC_{0-180}$. We got a significantly higher and rapid post-lunch glucagon excursion during BS, which may be responsible for the marked rise in post-lunch glucose excursion. Glucagon metabolism in T2DM is altered, being suppressed on prolonged fasting (as may have been pre-lunch during the BS visit) and increases post-prandially.[21,22] This increase in glucagon is attributed to non-pancreatic sources and is responsible for the increase in hepatic gluconeogenesis leading to a higher post-meal glucose excursion despite higher insulin levels during BS.[21,23]

This starkly contrasts Jakubowicz *et al*.,[12] who found that insulin and GLP-1 were majorly responsible for the second meal effect. Despite having a comparable study design, there are several reasons for the differences between that study and ours. Our cohort had a shorter duration of diabetes. Second, Asian Indians are a GLP-1-resistant population, with a preserved GLP-1 response even in T2DM, and have similar incretin responses despite varied glucose excursions.[22,24] A study from India demonstrated similar GLP-1 responses in patients with normal glucose tolerance, impaired fasting glucose, and impaired glucose tolerance, indicating GLP-1 resistance.[22] These could be the reasons for a similar post-lunch GLP-1 response despite higher post-lunch glucose during BS compared to BE. We have also considered iAUC, rather than AUC, for these parameters, which would better reflect the acute changes in pre-lunch conditions affecting the post-lunch excursions.[25,26] Our patients also ate lunch after 04:00 hours of breakfast (compared to 05:30 hours in the other study), making iAUC a more reliable calculation since the drop in pre-lunch values would be different.

Asian Indians also have a smaller beta-cell mass than Caucasians, probably leading to blunted second-meal insulin responses.[27] In addition, an important aspect of the second-meal effect is free fatty acid (FFA) suppression rather than an acute change in insulin secretion.[11] Although we did not assess FFAs, FFA suppression was an important finding in previous studies.[12,28]

We also found higher fasting plasma glucose and fasting serum insulin levels on BS visit, indicating higher IR. This finding of a reduction in fasting plasma glucose and insulin has shown inconsistent results in previous controlled trials.[9,12,29,30] This improvement in IR by eating breakfast could be another reason

for our patients' improvement in post-lunch glucose. Thus, the higher post-lunch glucose excursion on BS could result from a higher glucagon response and, possibly, non-suppression of FFA and increased IR. This altered dynamics of glucagon in Asian Indians with T2DM would require further study.

We did not find the persistence of this second-meal effect till dinner, which was another difference from the Jakubowicz study.[12] This could be because we evaluated the $AUC_{0\text{-}180}$ for post-dinner glucose by CGM readings, and although the patients followed the prescribed diet, the evening meal administered was not supervised. Despite the difference in the total energy intake (28.57% higher total calories on BE visit day as compared to BS visit day), the 24-hour average blood glucose was similar between the two meal conditions (138.3mg/dl during BE vs 133.4 mg/dl during BS; $P = 0.364$). In previous studies, when equal calories were given throughout the day, breakfast skipping led to higher 24-hour glucose.[4,29] When the same group, in a different study, provided higher calories during BE, they did not find a difference in 24-hour glucose. They, however, found a higher MAGE and CONGA during BS, indicating higher glycemic variability.[28] This was contrary to our study, probably due to normal non-diabetics studied in their population.

This is the first study in Asian Indian T2DM patients to understand the glycemic profile using CGM after skipping breakfast with total calorie deficit with incretin responses. Another strength of the study is the robust design of the test, where patients were habituated for at least 7 days to the breakfast condition before undergoing the test, rather than an acute change, making it possible to assess fasting values. The study's main limitation was that it had a small sample size, and long-term effects on BMI and glucose parameters were not studied. In addition, we did not study GIP and FFA responses, which could have given us better insights into mechanisms.

Conclusion

Breakfast skipping in T2DM leads to a higher post-lunch glucose excursion, which in Asian Indians could be due to a glucagon increase and IR rather than incretin-mediated insulin secretion after breakfast eating.

Acknowledgement

None.

Author contributions

PS, JVG, PKV, and NMB conceptualized and designed the study. YVC, MDH, PS, SR, NV, and AVP did data acquisition and statistical analysis. JVG, SM, PKV, and NMB supervised the study and analyzed and interpreted the data. All authors contributed to the study execution, manuscript preparation, and critical revision of the article. All authors approved of the final version.

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Conflicts of interest

There are no conflicts of interest.

Disclosure on the use of artificial intelligence

The authors declare that no artificial intelligence was used for the preparation of this manuscript.

Data availability

Data can be made available after request and due approval of institutional authority.

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