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## Maternal circadian eating time and frequency are associated with blood glucose levels during pregnancy

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## Abstract

**Background**—Synchronizing eating schedules with daily circadian rhythms may improve metabolic health, but its association with gestational glycemia is unknown.

**Objective**—This study examined the association of maternal night-fasting intervals and eating episodes with blood glucose levels during pregnancy.

**Methods**—This was a cross-sectional study within a prospective cohort in Singapore. Maternal 24-hour dietary recalls, fasting glucose and 2-hour glucose concentrations were ascertained at 26–28 weeks' gestation for 1061 women (age  $30.7 \pm 5.1$  years). Night-fasting intervals were based on the longest fasting duration during the night (1900–0659h). Eating episodes were defined as events which provided  $>50$  kcal, with a time interval between eating episodes of at least 15 minutes. Multiple linear regressions with adjustment for confounders were conducted.

**Results**—Mean  $\pm$  standard deviation night-fasting intervals and eating episodes per day were  $9.9 \pm 1.6$  hours and  $4.2 \pm 1.3$  times per day, respectively; fasting and 2-hour glucose concentrations were  $4.4 \pm 0.5$  and  $6.6 \pm 1.5$  mmol/L, respectively. In adjusted models, each hourly increase in night-fasting interval was associated with a 0.03 mmol/L decrease in fasting glucose (95% CI: -0.06, -0.01 mmol/L), while each additional daily eating episode was associated with a 0.15 mmol/L increase in 2-hour glucose (95% CI: 0.03, 0.28 mmol/L). Conversely, night-fasting intervals and daily eating episodes were not associated with 2-hour and fasting glucose, respectively.

**Conclusions**—Increased maternal night-fasting intervals and reduced eating episodes per day were associated with decreased fasting glucose and 2-hour glucose, respectively, in the late-second trimester of pregnancy. This points to potential alternative strategies to improve glycemic control in pregnant women. This study was registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) as NCT01174875.

## Keywords

food timing; gestational diabetes; meal frequency; pregnancy diet; hyperglycemia

## Introduction

Gestational hyperglycemia contributes to adverse perinatal outcomes (1), neonatal adiposity (2,3) and long-term risk for obesity in offspring (4). These occur throughout the range of glycemia, even at levels below the diagnostic cut-off for gestational diabetes mellitus (GDM) (1–4). Evidence suggests that even modest glycemic improvement in pregnant women with mild glucose intolerance improved perinatal outcomes (5). Current dietary approaches towards glycemic control have mostly focused on diet quantity and quality, with relatively little information available on food timing and eating frequency (6). Eating or fasting at appropriate times and restricting eating hours may offer an innovative and feasible strategy to preventing gestational hyperglycemia (7).

Humans have developed intrinsic 24-hour cycles, called circadian clocks that are entrained by light and food to regulate daily physiological events, including glucose metabolism (8). Glucose tolerance and insulin secretion exhibit circadian oscillations, such that progressive reduction of insulin sensitivity,  $\beta$ -cell response and glucose tolerance is seen as the day

progresses, with insulin sensitivity reaching a nadir at night (9). A defined period of feeding and fasting is a dominant determinant of circadian rhythms in metabolic pathways (10). Accordingly, dietary regimens that restrict food intake to within specific windows and fasting thereafter, particularly when done in coordination with daily circadian rhythms following light/ dark phases (7), have been shown to improve glucose regulation (11). This helps to reset the body's circadian (peripheral) clocks, restore circadian rhythmicity in gene expression, and consequently enhance glucose and energy metabolism (7).

The importance of circadian timing of eating, which refers to eating schedules in relation to dark/ light and rest/ activity phases during the day, has been demonstrated in animal studies (11). Rodents given 8 to 12 hours food access during their active phase (i.e. night-time hours) were protected from the metabolic consequences of a high fat diet, compared to rodents fed under an ad libitum regimen (12). The literature on human studies which consider feeding and fasting intervals in sync with daily circadian rhythms has been very limited. A recent epidemiologic study from the United States reported that night-time fasting interval in women was inversely associated with 2-hour glucose and HbA1c (13). Reduced eating episodes through ensuring adequate fasting intervals between meals may also improve glycemic control (14,15). This finding was however inconsistent with other studies, possibly due to variability in diet composition and small sample size (16). Interestingly, frequent meals with high protein content have been shown to reduce glucose response throughout the day (14,17).

To date, no study has explored the association of circadian fasting intervals and eating episodes with blood glucose levels in pregnant women, a high risk population vulnerable to hyperglycemia (18). A better understanding of how fasting intervals and eating episodes associate with glucose levels in pregnant women may lead to improved strategies in gestational glycemic control. Singapore (located 1.3° North, 103.8° East) is a country which experiences sunrise and sunset daily at a consistent time (i.e. sunrise at ~0700h and sunset at ~1900h), with fairly constant day length of 12 hours throughout the year (19). This provides us a good opportunity to examine the association of circadian timing and episodes of eating with glycemic levels in free-living pregnant women. Using data from the Growing Up in Singapore Towards healthy Outcomes (GUSTO) study involving a multi-ethnic Asian population (20), we tested the hypotheses that longer maternal night-fasting interval (1900 to 0659h) was associated with lower plasma glucose concentrations; while more frequent eating episodes per day was associated with higher plasma glucose concentrations in women during the late-second trimester of pregnancy.

## Methods

### Study design and participants

Data were drawn from the GUSTO prospective cohort study, which was designed to investigate the effects of early life events on the risk of developing metabolic diseases in later life, as detailed elsewhere (20). This study was conducted according to the guidelines laid down in the Declaration of Helsinki. Ethical approval was obtained from the Domain Specific Review Board of Singapore National Healthcare Group (reference D/09/021) and

the Centralised Institutional Review Board of SingHealth (reference 2009/280/D). This study was registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) as NCT01174875.

Pregnant women attending antenatal care (<14 weeks' gestation) from June 2009 to September 2010 in KK Women's and Children's Hospital and National University Hospital, which are the two major public maternity units in Singapore, were recruited into the GUSTO study. These pregnant women were at least 18 years of age, citizens or permanent residents and had homogeneous parental ethnic groups (Chinese, Malay or Indian). Women receiving chemotherapy, psychotropic drugs or with type 1 diabetes mellitus were excluded. Informed written consent was obtained from all women.

### Data collection

Detailed interviews and measurements were conducted in the clinics at recruitment and at 26-28 weeks' gestation. Data on socioeconomic status, educational attainment, obstetric history, physical activity, sleep duration and bedtime were collected. Women were asked about their employment status since becoming pregnant and whether their current jobs included any night shifts. Night shifts were defined as working at least once a week from 0000 to 0600h. Education was recorded by asking women their highest level of educational attainment. Obstetric histories, including outcomes and gestation duration for each pregnancy, were recorded to determine parity. Physical activity during pregnancy was assessed using a structured interviewer-administered questionnaire based on three types of activities: light-moderate (leaves the person tired but not exhausted), moderate (leaves the person exhausted but not breathless) and vigorous intensity (makes the heart beat rapidly and leaves the person breathless) activities. Women reported the frequency (days per week) and duration (minutes) of performing these activities. Total energy expenditure on physical activity was computed from frequency and duration of these activities, which was expressed in metabolic equivalent task (MET-minutes/week) units. Women were classified as not highly active (<3000 MET-minutes/week) and highly active (≥3000 MET-minutes/week) (21,22). Sleep duration at night and bedtime were examined using the Pittsburgh Sleep Quality Index questionnaire (23). Sleep duration was assessed by asking question 'during the past month, how many hours of actual sleep did you get at night? This may be different than the number of hours you spend in bed.' Bedtime was assessed by asking question 'during the past month, what has been your usual bedtime?'

### Anthropometric measurement

Maternal height was measured to the nearest 0.1 cm using a Seca 213 Portable Stadiometer (SECA, Hamburg, Germany) at 26-28 weeks' gestation. Self-reported pre-pregnancy weight and measured weight at the first antenatal visit (14 weeks of gestation) were collected. Body mass index (BMI) was calculated as weight (kg) divided by height square (m<sup>2</sup>). Since maternal BMI at the first antenatal visit was strongly correlated with pre-pregnancy BMI ( $r=0.96$ ,  $p<0.001$ ) and had a lower percentage of missing data ( $n=71$ , 6.7%), it was used for analyses in this study.

## Dietary assessment

At 26-28 weeks' gestation, a 24-hour dietary recall was administered to women in the clinic by trained clinical staff using the 5-stage, multiple-pass interviewing technique (24). This required women to report an uninterrupted listing of all food and beverages consumed, answer a forgotten food list tailored for local population, provide details of time, occasions and descriptions of foods and amounts eaten, and ended with a final probe review. Standardized household measuring utensils and food pictures of various portion sizes were used to assist women in quantifying their food and beverage intakes. Separately, women were asked to complete a 3-day food diary at home. The clinical staff guided women on how to complete the 3-day food diaries (2 non-consecutive weekdays and one weekend day) during the following week. The food pictures used during the 24-hour dietary recalls were printed in the food diaries to assist women in quantifying portion sizes.

Daily energy and macronutrient intakes were assessed using a nutrient analysis software (Dietplan Version 7, Forestfield Software) based on food composition database of locally available foods (25). For mixed dishes not found in the local database, nutrient analyses of recipes were conducted using the nutrient software. For other food items not found in the database, nutrient information was obtained from food labels or the United States Department of Agriculture (USDA) national nutrient database (26). Women with reported implausible energy intakes of less than 500 kcal/day or greater than 3500 kcal/day were excluded from this study, as done in other epidemiologic studies (27,28).

Night-fasting interval was determined based on the longest fasting interval between calorie-containing food or beverage consumption from 1900 to 0659h. The 24-hour dietary pattern was assumed to follow a similar trend the next day, allowing us to obtain a complete night period which was defined according to local timing from sunset to sunrise (1900 to 0659h), as employed in our previous studies (28,29). Percentage of energy intake during night-time was calculated as the amount of energy intake from 1900 to 0659h (kcal) / total energy intake per day (kcal) x 100%. Eating episodes were defined as events which provided at least 210 kJ (~50 kcal), with a time interval between eating episodes of at least 15 minutes (30). Recent studies have revealed that eating episodes as defined using this criteria could best predict variance in total energy intake (31) and correlate strongly with total energy intake (32).

## Plasma glucose analysis

At 26-28 weeks' gestation, women underwent a 75-g Oral Glucose Tolerance Test (OGTT) to diagnose GDM. Women had an overnight fast of 8 to 10 hours prior to blood collection. Plasma glucose concentrations at 0 (fasting glucose, FG) and 120 minutes (2-hour glucose) following the oral glucose load were measured by colorimetry [Advia 2400 Chemistry system (Siemens Medical Solutions Diagnostics) and Beckman LX20 Pro analyzer (Beckman Coulter)]. GDM was diagnosed according to the 1999 World Health Organization (WHO) criteria: 7.0 mmol/L for FG and/or 7.8 mmol/L for 2-hour glucose (33).

## Statistical analysis

Categorical data were presented as frequencies and percentages, while continuous data were presented as means and standard deviations. The night-fasting intervals and eating episodes were graded into tertiles to assess associations with maternal characteristics using one-way ANOVA for continuous variables and Fisher's exact test for categorical variables. The trends of dietary intakes and glucose levels across tertiles of night-fasting intervals and eating episodes were examined using non-parametric test for trend. Multiple linear regressions were performed to examine the associations of maternal night-fasting intervals and eating episodes with FG or 2-hour glucose. Night-fasting intervals and eating episodes in continuous form were entered simultaneously into the same model and adjusted for potential confounders. These confounders were selected *a priori* based on literature review (8,13,28,34), including maternal age, ethnicity, education, employment status, night shift status, parity, BMI, physical activity, sleep duration, bedtime, total energy intake and percentage of energy intake during night-time. We repeated the analysis by using the night-fasting intervals and eating episodes in categorical form (tertiles) to show their graded associations with glucose levels. Tertile 2 was used as the reference group based on its closest value with the respective mean night-fasting intervals and eating episodes.

Taking into account the possible influence of diet composition on the associations of night-fasting intervals and eating episodes with glucose levels, substitution models were additionally performed, so as to examine the relative change of one dietary macronutrient to another under isocaloric conditions (i.e. keeping total energy constant) (35). For example, a higher protein, lower carbohydrate diet was examined in a multivariate model where percentages of energy from protein and fat, and total daily energy intake were simultaneously included. This is because, when fat and total energy intakes were kept constant, the only macronutrient that could decrease as protein increased was carbohydrate (substitution of protein for carbohydrate). Similarly, a higher fat, lower protein diet was examined when carbohydrate and total energy intakes were kept constant (substitution of fat for protein). In other words, by keeping the total energy intake constant, inclusion of any two macronutrients in the model represented the change of one macronutrient to another.

Sensitivity analyses were conducted using the 3-day food diary data from a subset of women for whom complete data were available (n=186). Owing to the small sample size, night-fasting intervals and eating episodes were analyzed in continuous form but not categorical form (tertile). The same confounders were adjusted in the multiple linear regression analysis. Results were presented as beta coefficient ( $\beta$ ) and 95% confidence intervals (CI). All statistical analyses were performed using Stata 13.1 (USA). A 2-tailed *P* value of <0.05 was considered to be statistically significant.

## Results

### Characteristics of the participants

Of 1237 enrolled women with singleton pregnancies, 1158 had completed the 24-hour dietary recalls. Of these, 13 women were excluded as they reported implausible energy intakes of less than 500 kcal/day (n = 3) or greater than 3500 kcal/day (n = 10). We further



excluded 84 women with missing OGTT results. A final sample of 1061 (85.8%) women was included in this study. Compared to the excluded women ( $n = 176$ , 14.2%), those included were older ( $P = 0.046$ ) and majority were Chinese ( $P = 0.038$ ). No significant differences in maternal characteristics were observed for BMI, education, employment status, night shift status, parity, physical activity, sleep duration and bedtime between included and excluded women (all  $P \geq 0.05$ ).

Table 1 shows the characteristics of the participants. Women with longer night-fasting intervals were older ( $P = 0.007$ ), had higher BMI ( $P = 0.004$ ), more likely to be Chinese ( $P = 0.002$ ) and had earlier bedtimes ( $P < 0.001$ ). Women with more eating episodes were also found to be older ( $P = 0.004$ ), but had lower BMI ( $P < 0.001$ ), more likely to be Indian ( $P < 0.001$ ), attained higher education ( $P < 0.001$ ), employed ( $P = 0.028$ ) and not highly active ( $P = 0.002$ ). No significant differences in night shift status, parity and sleep duration were noted across tertiles of night-fasting intervals and eating episodes per day ( $P \geq 0.05$ ).

Table 2 shows the dietary intakes and glucose levels of the participants. Women with longer night-fasting intervals reported lower total energy intakes ( $P < 0.001$ ), higher percentages of total protein intakes ( $P = 0.003$ ), lower percentages of energy intakes during night-time ( $P < 0.001$ ) and fewer eating episodes per day ( $P < 0.001$ ). These women were also found to have lower FG ( $P = 0.004$ ) than those with shorter night-fasting intervals. Women with more frequent eating episodes reported greater total energy intakes ( $P < 0.001$ ), lower percentages of total protein intakes ( $P = 0.020$ ) and shorter night-fasting intervals ( $P < 0.001$ ). These women showed no significant differences in glucose levels across tertiles of eating episodes.

### Association of night-fasting intervals and eating episodes with glucose levels

Table 3 shows the association of maternal night-fasting intervals and eating episodes with glucose levels during pregnancy. Each hourly increase in maternal night-fasting intervals was significantly associated with a 0.03 mmol/L decrease in FG after adjustment for confounders (95% CI: -0.06, -0.01;  $P = 0.014$ ), implying that 3-4 hours longer night-fasting intervals could reduce FG by a small but clinically meaningful  $\sim 0.10$  mmol/L. When night-fasting intervals were graded into tertiles, women from tertile 1 (lowest tertile) showed no difference in FG ( $\beta$ : -0.05 mmol/L; 95% CI: -0.14, 0.05;  $P = 0.33$ ), but those from tertile 3 (highest tertile) were found to have a 0.15 mmol/L decrease in FG (95% CI: -0.25, -0.06;  $P = 0.002$ ) compared to women from tertile 2, suggesting fasting for 11-12 hours at night had greatest association with FG. No significant association was detected between maternal eating episodes per day and FG ( $\beta$ : -0.02; 95% CI: -0.06, 0.02;  $P = 0.24$ ).

Using 2-hour glucose as the outcome variable, no significant association was found in relation to maternal night-fasting intervals ( $\beta$ : 0.04; 95% CI: -0.05, 0.12;  $P = 0.36$ ). On the other hand, each additional daily eating episode was significantly associated with a 0.15 mmol/L increase in 2-hour glucose after adjustment for confounders (95% CI: 0.03, 0.28;  $P = 0.018$ ). Compared to women from tertile 2 of eating episodes, those from tertile 3 (highest tertile) were significantly associated with higher 2-hour glucose ( $\beta$ : 0.31; 95% CI: 0.01, 0.61;  $P = 0.045$ ). Interaction tests between fasting intervals or eating episodes and BMI in relation to glucose levels were performed; however, no significant interactions were found ( $P$ -interaction  $\geq 0.05$ ) (data not shown).

In sensitivity analyses based on 3-day food diaries, each hourly increase in maternal night-fasting intervals was associated with a 0.07 mmol/L decrease in FG (95% CI: -0.14, -0.01;  $P = 0.043$ ); while each additional eating episode was associated with a 0.28 mmol/L increase in 2-hour glucose after adjustment for confounders (95% CI: 0.01, 0.55;  $P = 0.044$ ) among the subsample of women.

## Discussion

In an Asian cohort, pregnant women were found to have an average of 9.9 hours of night-fasting intervals and 4.2 eating episodes each day. This study provides new evidence showing that longer maternal night-fasting intervals were associated with lower FG; whereas more frequent daily episodes of eating were associated with higher 2-hour glucose in women during the late-second trimester of pregnancy, after accounting for various demographic and lifestyle factors, as well as diet composition. These findings suggest that both circadian timing of food intake and number of eating episodes should be considered in managing gestational glycemia. Maternal FG could be controlled through modifying fasting intervals at night, which allows synchronization with the body's circadian resting rhythms; while maternal 2-hour glucose could be controlled by adjusting the number of eating episodes per day.

Across time, human activity and feeding have generally occurred during the day, while rest and fasting typically occurred at night. We speculate that shortened night-fasting intervals might lead to misalignment with the day-night rhythms of glucose homeostasis. This could disrupt functions of metabolic regulator pathways implicated in glucose homeostasis (12,36). In mice, synchronization of the feeding-fasting cycle with dark-light phases of the day improves oscillations of circadian clock components in the liver and hepatic glucose metabolism (12), maintains intestinal barrier integrity and minimizes bacterial translocation which can promote systemic inflammation (37), impairing hepatic glucose regulation. In addition, reduced eating episodes with a longer fasting interval between meals may help to generate sharp feeding-fasting cycles, which consolidate circadian patterns in gene expression and circadian activation of various metabolic pathways (7).

In the current study, we showed that longer maternal night-fasting intervals were associated with lower FG. This finding took into account macronutrient intakes, suggesting that besides diet composition and quality, it is important to also consider the circadian timing of food intake when managing gestational glycemia. This finding is also supported by animal studies where rodents with 12-hour restricted feeding during the active phase (dark phase) had reduced FG (38), independent of the diet composition (39). On the other hand, we found no association between maternal night-fasting intervals and 2-hour glucose. This stands at odds with a recent epidemiologic study involving 1066 women from the United States National Health and Nutrition Examination Survey (NHANES) (13). The authors found that increased night-fasting interval was associated with lower 2-hour glucose; however FG was not examined in the study (13). The NHANES study defined night-fasting as the time between the last and first meal intake of the next day, which could have included extended fasting resulting from events such as breakfast skipping. Our study defined night-fasting using a narrower period by restricting it within a specified time from 1900 to 0659h, consistent with



the dark phase of the day. Extended fasting may result in different glycemic response and was not examined in this study.

Our finding of frequent eating and higher 2-hour glucose is consistent with several previous randomized crossover trials (14,15), but not others (17,40). Trials involving healthy adults showed that consumption of frequent meals at 6 or 14 times resulted in higher glucose levels across the day compared to those who consumed 3 meals (14,15), suggesting that frequent eating episodes results in poorer glycemic control. However, this observation contrasts with findings from Solomon et al. (40) and Kanaley et al. (17) who found no difference in glucose response over a period of 8 to 12-hour when comparing a high frequency (6 or 12 meals) and a low frequency (2 or 3 meals) eating pattern in lean and obese adults. Similarly, no association was observed between eating episodes and 2-hour glucose in the United States NHANES study (13). Discrepancies in findings may be partly due to differences in the diet composition and definition of eating episodes used across studies.

Maternal glycemia has been shown to significantly impact offspring metabolic health. Evidence from the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study involving 23,316 pregnant women reported strong, continuous associations of maternal FG and 2-hour glucose levels below those diagnostic of diabetes with increased birth weight, cord blood serum C-peptide values (an index of fetal  $\beta$ -cell function) (1) and neonatal adiposity (2). Similarly, data from our GUSTO cohort also demonstrated continuous associations between maternal glucose levels and excessive neonatal adiposity which extended across the range of glycemia (3). In particular, raised FG was associated with increased offspring adiposity in the first 36 months of life among non-obese women (4). This indicates that even moderate changes in glucose levels may influence fetal growth. Although it raises the possibility that shortened night-fasting intervals and increased eating episodes during pregnancy can adversely impact offspring metabolic health through increased maternal FG and 2-hour glucose respectively, this hypothesis needs to be addressed in further studies or randomized trials.

The strengths of this study included a large sample of pregnant women and the ability to factor for a wide array of socio-demographic, health and lifestyle variables in our analyses. However, we recognized and considered the following limitations. First, the use of single day self-reported dietary data to assess women's night-fasting intervals and eating episodes might be subjected to response bias and inter-day variations. Information on intakes of complex vs simple carbohydrates, and plant vs animal protein was not available for these analyses. However, our findings were supported by results based on sensitivity analyses using the 3-day food diaries. Second, no glucose measurement was done in between FG and 2-hour glucose, and thus we were not able to determine the post-OGTT response using the trapezoid method (41) which serves as a better measure for glucose tolerance. Our findings were also limited by the lack of data on serum insulin, C-peptide and insulin growth factors, which would have allowed for the assessment on insulin sensitivity and  $\beta$ -cell response to mechanistically explain our findings. In addition, blood samples and dietary assessment were only collected at one-time point in the late-second trimester which restricted our ability to evaluate the time course effects of eating behavior on glycemic control. Third, we did not aim to examine the impact of snacking on glucose levels, therefore eating episodes were not

differentiated between main meals and snacks. Forth, differences in age and ethnicity were noted between included and excluded women. This could introduce a potential selection bias, but were controlled for in the analyses. Fifth, no objective measure was used to capture physical activity and sleep data. Sixth, the effect estimates might be affected by unadjusted or residual confounding factors that remained such as artificial light exposure at night. Finally, our study recruited Asian participants which may limit the generalizability of our findings to populations of other ethnic groups.

In conclusion, this to our knowledge is the first study to demonstrate that fasting for a longer interval during the night and eating less frequently throughout the day were independently associated with reduced FG and 2-hour glucose respectively in women at the late-second trimester of pregnancy. Although we found modest reductions in glucose levels which may appear trivial, these improvements over time may have a cumulative effect which can help to prevent the consequences of gestational hyperglycemia. We recommend that the current nutritional management should include the practice of preserving a natural rhythm of day-time feeding and night-time sleeping patterns rather than to eat at night (8), as well as to maintain a moderate eating frequency. This will hopefully provide a novel, simple, feasible and sustainable dietary strategy for pregnant women to improve glycemic control. However, this observational study does not imply causality; long-term and large-scale randomized trials are warranted to confirm our findings and evaluate the clinical practice applicability in order to support the public health recommendations. In countries which experience seasonal variations, study design needs to be season specific and different cut-offs for feeding-fasting timing are required. Future studies should also evaluate the diurnal variations of blood glucose and, most importantly, the underlying biological and/ or social mechanisms.

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KMG, KK, SMS and Y-SC designed the GUSTO cohort study. SLL and FY designed the present study. MTC contributed to dietary data collection and analyses. SLL, PHW, NP and FM performed data management and analysis. YBC advised on the statistical analysis. SLL, JKYC, PHW, YBC, NL, MF-FC and FY interpreted the findings and revised drafts of the paper. SLL wrote the paper and had primary responsibility for final content. All authors read and approved the final manuscript.

## Abbreviations used

<b>BMI</b>	body mass index
<b>CI</b>	confidence intervals
<b>FG</b>	fasting glucose

<b>GDM</b>	gestational diabetes mellitus
<b>GUSTO</b>	Growing Up in Singapore Towards healthy Outcomes
<b>MET</b>	metabolic equivalent task
<b>NHANES</b>	National Health and Nutrition Examination Survey
<b>OGTT</b>	Oral Glucose Tolerance Test

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Table 1

Characteristics of GUSTO pregnant women according to night-fasting intervals and eating episodes per day<sup>1/</sup>

Variables	All n=1061 <sup>2</sup>	Night-fasting intervals			p <sup>4</sup>	Eating episodes per day			p <sup>4</sup>
		Tertile 1 (4.0 – 9.0h) n=397	Tertile 2 (9.1 – 10.9h) n=291	Tertile 3 (11.0 – 12.0h) n=373		Tertile 1 (1 – 3x) n=361	Tertile 2 (4x) n=331	Tertile 3 (5 – 10x) n=369	
Age, years	30.7 ± 5.1	30.1 ± 5.0	31.1 ± 5.2	31.0 ± 5.1	0.007	30.0 ± 5.5	30.1 ± 5.0	31.1 ± 4.7	0.004
BMI, kgm <sup>-2</sup>	23.6 ± 4.7	23.1 ± 4.4	24.3 ± 5.1	23.8 ± 4.7	0.004	24.4 ± 5.4	23.8 ± 4.7	22.7 ± 3.9	<0.001
Ethnicity, n (%)					0.002				<0.001
Chinese	608 (57.3)	230 (37.8)	147 (24.2)	231 (38.0)		179 (29.4)	197 (32.4)	232 (38.2)	
Malay	270 (25.4)	100 (37.0)	74 (27.4)	96 (35.6)		132 (48.9)	79 (29.3)	59 (21.9)	
Indian	183 (17.2)	67 (36.6)	70 (38.3)	46 (25.1)		50 (27.3)	55 (30.1)	78 (42.6)	
Education, n (%)					0.88				<0.001
None/ primary/ secondary	690 (65.8)	253 (36.7)	192 (27.8)	245 (35.5)		283 (41.0)	213 (30.9)	194 (28.1)	
University	358 (34.2)	137 (38.3)	97 (27.1)	124 (34.6)		74 (20.7)	114 (31.8)	170 (47.5)	
Employment status, n (%)					0.404				0.028
Unemployed	330 (31.1)	122 (37.0)	99 (30.0)	109 (33.0)		125 (37.9)	109 (33.0)	96 (29.1)	
Employed	731 (68.9)	275 (37.6)	192 (26.3)	264 (36.1)		236 (32.3)	222 (30.4)	273 (37.3)	
Night shift status, n (%)					0.34				0.43
No	1013 (95.5)	374 (36.9)	280 (27.6)	359 (35.4)		342 (33.8)	320 (31.6)	351 (34.6)	
Yes	48 (4.5)	23 (47.9)	11 (22.9)	14 (29.2)		19 (39.6)	11 (22.9)	18 (37.5)	
Parity, n (%)					0.44				0.45
Nulliparous	476 (45.3)	189 (39.7)	128 (26.9)	159 (33.4)		154 (32.4)	147 (30.9)	175 (36.8)	
Multiparous	575 (54.7)	207 (36.0)	159 (27.7)	209 (36.6)		202 (35.1)	182 (31.7)	191 (33.2)	
Physical activity, n (%)					0.41				0.002
Not highly active (<3000 MET-min/week)	860 (82.1)	326 (37.9)	242 (28.1)	292 (34.0)		278 (32.3)	263 (30.6)	319 (37.1)	
Highly active (≥ 3000 MET-min/week)	187 (17.9)	67 (35.8)	47 (25.1)	73 (39.0)		80 (42.8)	61 (32.6)	46 (24.6)	
Sleep duration, hours <sup>3</sup>	7.2 ± 1.5	7.1 ± 1.5	7.3 ± 1.4	7.3 ± 1.5	0.20	7.2 ± 1.7	7.3 ± 1.4	7.1 ± 1.3	0.53
Bedtime, 24-hour <sup>3</sup>	2307 ± 0152	2335 ± 0146	2300 ± 0124	2242 ± 0208	<0.001	2306 ± 0229	2309 ± 0110	2306 ± 0136	0.93

<sup>1/</sup>Values are means ± SDs, unless otherwise stated. BMI, body mass index at 14 weeks' gestation; GUSTO, Growing Up in Singapore Towards healthy Outcomes; MET, metabolic equivalent task.



- <sup>2</sup> Sample size ranged from 990 to 1061 due to the missing values.
- <sup>3</sup> Sample size for the data on sleep duration and bedtime were available to 688 and 690 women, respectively.
- <sup>4</sup> Based on one-way ANOVA or Fisher's exact test as appropriate;  $P < 0.05$  was considered as statistically significant.

**Table 2**  
Dietary intakes and glucose levels of GUSTO pregnant women according to night-fasting intervals and eating episodes per day<sup>1</sup>

Variables	Night-fasting intervals			Eating episodes per day			p <sup>3</sup>		
	Tertile 1 (4.0 – 9.0h) n=397	Tertile 2 (9.1 – 10.9h) n=291	Tertile 3 (11.0 – 12.0h) n=373	Tertile 1 (1 – 3x) n=361	Tertile 2 (4x) n=331	Tertile 3 (5 – 10x) n=369			
Total energy intake, kcal/d	1855 ± 555	1978 ± 570	1816 ± 548	1756 ± 520	<0.001	1548 ± 477	1847 ± 496	2163 ± 506	<0.001
Total carbohydrate intake, % energy	51.6 ± 8.8	51.7 ± 8.4	52.2 ± 9.1	51.0 ± 9.0	0.33	51.5 ± 9.7	50.9 ± 8.8	52.3 ± 7.7	0.23
Total protein intake, % energy	15.7 ± 3.9	15.3 ± 3.7	15.6 ± 4.2	16.2 ± 3.8	0.003	16.0 ± 4.1	15.9 ± 3.9	15.2 ± 3.6	0.020
Total fat intake, % energy	32.6 ± 7.6	32.9 ± 7.3	32.1 ± 7.7	32.6 ± 8.0	0.58	32.5 ± 8.6	33.0 ± 7.6	32.2 ± 6.7	0.65
Night-time energy intake, % energy <sup>2</sup>	33.1 ± 18.3	36.8 ± 17.9	34.1 ± 16.9	28.2 ± 18.7	<0.001	33.5 ± 22.2	33.5 ± 17.9	32.3 ± 13.9	0.43
Eating episodes, n/d	4.2 ± 1.3	4.6 ± 1.3	4.2 ± 1.2	3.6 ± 1.0	<0.001	2.8 ± 0.4	4.0 ± 0	5.6 ± 0.8	<0.001
Night-fasting, hours	9.9 ± 1.6	8.2 ± 0.8	10.0 ± 0.4	11.6 ± 0.4	<0.001	10.6 ± 1.4	9.8 ± 1.5	9.2 ± 1.4	<0.001
Plasma fasting glucose, mmol/L	4.4 ± 0.5	4.4 ± 0.5	4.4 ± 0.5	4.3 ± 0.4	0.004	4.4 ± 0.4	4.4 ± 0.5	4.3 ± 0.5	0.06
Plasma 2-hour glucose, mmol/L	6.6 ± 1.5	6.4 ± 1.4	6.6 ± 1.7	6.6 ± 1.4	0.08	6.5 ± 1.5	6.5 ± 1.4	6.7 ± 1.5	0.07
Gestational diabetes mellitus, n (%)					0.21 <sup>4</sup>				0.33 <sup>4</sup>
No	863 (81.3)	332 (38.5)	228 (26.4)	303 (35.1)		302 (35.0)	268 (31.1)	293 (34.0)	
Yes	198 (18.7)	65 (32.8)	63 (31.8)	70 (35.4)		59 (29.8)	63 (31.8)	76 (38.4)	

<sup>1</sup> Values are means ± SDs, unless otherwise stated, n = 1061. GUSTO, Growing Up in Singapore Towards healthy Outcomes.

<sup>2</sup> Computed from night-time energy intake (kcal)/ total energy intake per day (kcal) \*100% .

<sup>3</sup> Based on non-parametric test for trend;  $P < 0.05$  was considered as statistically significant.

<sup>4</sup> Based on fisher's exact test;  $P < 0.05$  was considered as statistically significant.

Table 3

Associations of maternal night-fasting intervals and eating episodes with glucose levels during pregnancy in the GUSTO study<sup>1</sup>

	Crude			Adjusted <sup>2</sup>			Adjusted <sup>3</sup>		
	$\beta$ (95% CI)	P	P	$\beta$ (95% CI)	P	P	$\beta$ (95% CI)	P	P
<b>Fasting glucose (mmol/L)</b>									
Night-fasting (hours)	-0.03 (-0.05, -0.01)	0.007	0.015	-0.03 (-0.06, -0.01)	0.015	-0.03 (-0.06, -0.01)	0.014	0.014	0.014
Eating episodes (n/d)	-0.02 (-0.04, 0.01)	0.14	0.20	-0.03 (-0.06, 0.01)	0.20	-0.02 (-0.06, 0.02)	0.24	0.24	0.24
Night-fasting									
Tertile 1	-0.01 (-0.08, 0.07)	0.92	0.33	-0.05 (-0.14, 0.05)	0.33	-0.05 (-0.14, 0.05)	0.33	0.33	0.33
Tertile 2	Reference		Reference	Reference		Reference		Reference	
Tertile 3	-0.09 (-0.17, -0.02)	0.010	0.002	-0.15 (-0.24, -0.06)	0.002	-0.15 (-0.25, -0.06)	0.002	0.002	0.002
Eating episodes									
Tertile 1	0.03 (-0.04, 0.10)	0.42	0.24	0.06 (-0.04, 0.15)	0.24	0.06 (-0.04, 0.15)	0.26	0.26	0.26
Tertile 2	Reference		Reference	Reference		Reference		Reference	
Tertile 3	-0.01 (-0.08, 0.07)	0.87	0.98	-0.01 (-0.09, 0.09)	0.98	0.01 (-0.09, 0.10)	0.97	0.97	0.97
<b>2-hour glucose (mmol/L)</b>									
Night-fasting (hours)	0.05 (-0.01, 0.11)	0.08	0.30	0.05 (-0.04, 0.13)	0.30	0.04 (-0.05, 0.12)	0.36	0.36	0.36
Eating episodes (n/d)	0.08 (0.01, 0.15)	0.033	0.032	0.13 (0.01, 0.25)	0.032	0.15 (0.03, 0.28)	0.018	0.018	0.018
Night-fasting									
Tertile 1	-0.23 (-0.45, 0.01)	0.05	0.38	-0.14 (-0.44, 0.17)	0.38	-0.14 (-0.44, 0.16)	0.37	0.37	0.37
Tertile 2	Reference		Reference	Reference		Reference		Reference	
Tertile 3	-0.05 (-0.28, 0.18)	0.66	0.94	-0.01 (-0.32, 0.29)	0.94	-0.03 (-0.33, 0.27)	0.85	0.85	0.85
Eating episodes									
Tertile 1	-0.04 (-0.26, 0.19)	0.76	0.32	-0.16 (-0.46, 0.15)	0.32	-0.17 (-0.48, 0.13)	0.27	0.27	0.27
Tertile 2	Reference		Reference	Reference		Reference		Reference	
Tertile 3	0.19 (-0.04, 0.41)	0.10	0.08	0.27 (-0.03, 0.57)	0.08	0.31 (0.01, 0.61)	0.045	0.045	0.045

<sup>1</sup>Data were analyzed using multiple linear regressions;  $P < 0.05$  was considered as statistically significant. GUSTO, Growing Up in Singapore Towards healthy Outcomes.<sup>2</sup>Model 1: adjusted for maternal age, ethnicity, education, employment status, night shift status, parity, body mass index, physical activity, sleep duration, bedtime, total energy intake and percentage of energy during night-time.

Model 2: adjusted for Model 1 + total percentage of energy from protein, total percentage of energy from fat.