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Maternal night-eating pattern and glucose tolerance during pregnancy: study protocol for a longitudinal study

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Manuscripts

1 **Maternal night-eating pattern and glucose tolerance during pregnancy: study protocol for** 2 **a longitudinal study**

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41 42 **Word count: 2969**

43
44 **Keywords:** Eating time; Glucose tolerance; Lifestyle factor; Night-eating; Pregnancy

47 **Abstract**

48 **Introduction:** Coordinating eating schedules with day-night cycles has been shown to improve
49 glucose regulation in adults, but its association with gestational glycaemia is unclear. A better
50 understanding on how eating time can influence glucose levels in pregnancy may improve
51 strategies for gestational glycaemic control. This study aims to examine the association of
52 maternal night-eating pattern with glucose tolerance at mid-pregnancy, and to investigate how
53 lifestyle factors may be related to night-eating pattern.

54 **Methods and analysis:** This is a longitudinal study to be conducted at KK Women's and
55 Children's Hospital in Singapore, where 400 pregnant women at 18-21 weeks' gestation will be
56 recruited from antenatal clinics. Information on socio-demographic, lifestyle habits and obstetric
57 outcomes will be collected. Dietary intake will be recorded using the 4-day food diary and food
58 frequency questionnaire; while 24-hour physical activity, sedentary behaviour, sleep and light
59 exposure will be captured using the Actigraph accelerometer at 18-21 weeks' gestation.
60 Continuous glucose monitoring at 18-21 weeks' gestation, oral glucose tolerance test and insulin
61 test at 24-28 weeks' gestation will be performed to assess glycaemic outcomes. Multivariable
62 generalized linear models will be used to analyse the association of maternal night-eating pattern
63 (consumption of meal and snack during 1900-0659h) with glycaemic measures, and the
64 associated factors of night-eating pattern, controlling for potential confounders.

65 **Ethics and dissemination:** Ethical approval has been granted by the Centralised Institutional
66 Review Board of SingHealth, Singapore (reference 2018/2529). The results will be presented at
67 conferences and disseminated in journal articles.

68 **Trial registration:** NCT 03803345.

69

70 Article Summary

71 Strengths and limitations of this study

- 72 • This study will provide information on maternal night-eating pattern during pregnancy
73 and its association with glycaemic outcomes, which will be useful to healthcare
74 professionals and the pregnant population in the effort of glycaemic control.
- 75 • This study will comprehensively assess the night-eating pattern, glycaemic profile and
76 lifestyle factors of pregnant women.
- 77 • Given the participants will be recruited from a single hospital, the sample may not be
78 considered representative of all pregnant women in Singapore.

80 INTRODUCTION

81 Over time, living things from fungi to humans have evolved to keep time with the earth's
82 repeated light-dark cycles. These day-night rhythms orchestrate critical aspects of human
83 physiology, from cell signalling to cellular metabolism; as well as influence habitual aspects of
84 human behaviour, including activity, sleep and energy consumption.[1] The alignment of eating
85 time with the body's circadian rhythms, known also as circadian eating, has been shown to
86 improve glucose tolerance,[2] suggesting that circadian dietary strategies may be a useful way to
87 maintain metabolic health. Pregnant women belong to a high-risk population vulnerable to
88 hyperglycaemia and its consequences. In Singapore, 20% women develop gestational diabetes
89 mellitus (GDM).[3] Even at glucose concentrations below the diagnostic cut-off for GDM, risks
90 of adverse perinatal outcomes can occur, and these risks increase continuously in association
91 with rising glucose levels during pregnancy.[4] Effective interventions to improve glycaemic
92 control in pregnancy are urgently needed.

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3 93 Although it is known that food quantity and quality influence GDM development,[5] the
4
5 94 effect of circadian eating pattern, particularly excess food consumed during the late evening or
6
7
8 95 night on glucose regulation in pregnancy, remains an important gap of knowledge. In the general
9
10 96 population, late-eating or night-eating has been associated with less healthy eating and more
11
12 97 snack intake,[6] which may be related to metabolic disorders.[7] It was found that women with
13
14 98 GDM were more likely to snack at night compared with those of normal glucose tolerance.[8]
15
16
17 99 Based on the latest nutritional guidelines from the Academy of Nutrition and Dietetics, a new
18
19 100 recommendation on meal and snack distribution has been included where women with GDM are
20
21 101 encouraged to have 3 meals and 2 or more snacks per day.[9] However, this recommendation
22
23 102 does not consider the effect of day-night or circadian cycles, and it was formed based on a
24
25
26 103 consensus approach rather than with supportive evidence.

27
28 104 Therefore, our motivation is to develop an understanding of the role of circadian timing
29
30 105 of meal and snack intakes on blood glucose levels during pregnancy, which is potentially a
31
32 106 modifiable behaviour for glycaemic control. The primary aims of this study are (i) to examine
33
34 107 the association of maternal night-eating pattern with glucose tolerance at mid-pregnancy, and (ii)
35
36 108 to investigate how lifestyle factors, specifically daily physical activity, sedentary behaviour,
37
38 109 sleep, diet quality and light exposure may be related to night-eating pattern. These lifestyle
39
40 110 factors may influence the association between night-eating pattern and glycaemic measures; yet
41
42 111 have not been evaluated previously [10]. The central hypothesis is that small evening meals and
43
44
45 112 less frequent snacking at night will be associated with better glucose tolerance at 24-28 weeks'
46
47
48 113 gestation – a period when the screening for GDM is usually done, compared to those with larger
49
50 114 evening meals and more frequent snacking at night.

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54 115

116 **METHODS AND ANALYSIS**

117 **Study design**

118 This is a prospective longitudinal study, where pregnant women at 18-21 weeks' gestation will
119 be recruited and followed until delivery. An overview of the study procedures is illustrated in
120 figure 1.

121

122 **Participants and recruitment**

123 This study will be conducted at KK Women's and Children's Hospital (KKH) in Singapore.
124 KKH houses the largest Obstetrics and Gynaecology department in Singapore, with over 10,000
125 ($\approx 30\%$) live births recorded annually. A non-probability sampling method will be used to recruit
126 pregnant women who attend scheduled antenatal clinic appointments at KKH. Those who meet
127 the selection criteria will be invited to participate in this study. Recruitment will be carried out
128 for 20 months, expected from May 2019 until Jan 2021.

129 The sample will comprise pregnant women between 18-21 weeks' gestation at recruitment,
130 age between 18-45 years, who are Singapore citizens or Singapore Permanent Residents, plan to
131 continue antenatal care at KKH, intend to deliver at KKH and able to provide written informed
132 consent. Excluded will be pregnant women with diabetes in pregnancy at recruitment as
133 confirmed by the Oral Glucose Tolerance Test (OGTT), have pre-existing type-1 or type-2
134 diabetes, chronic kidney disease, preeclampsia, multiple pregnancy, on routine night-shift work
135 for at least 3x/week currently or in the last month, use of anticonvulsant medications or oral
136 steroids currently or in the last month, and with known or suspected allergy to medical grade
137 adhesives. Participants who develop a miscarriage or undergo a termination event, unable to

1
2
3 138 comply with the study protocol or wish to discontinue participation will be withdrawn from the
4
5 139 study.

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7
8 140 Recruitment brochures that contain general information of the study will be placed in the
9
10 141 antenatal clinics. During the recruitment process, trained research staff will inform potential
11
12 142 women of the study both verbally and with written information. Women who are agreeable to
13
14 143 participate will be required to provide written informed consent. Those who decline to participate
15
16 144 will continue to receive their hospital antenatal care as usual, and care provided to each pregnant
17
18 145 woman will not be affected nor influenced by the woman's decision to either participate or not
19
20 146 participate in the study.

21 22 147 23 24 25 26 148 **Study procedures**

27
28 149 After providing written informed consent at the recruitment visit (18-21 weeks' gestation),
29
30 150 enrolled participants will be asked for information on socio-demographic and lifestyle habits. At
31
32 151 the same visit, participants will be given a food diary to record 4-day dietary intake. A
33
34 152 photographic food diary will be obtained from a subsample (50%) of participants, who will be
35
36 153 required to download a food record app to capture food images before and after eating events
37
38 154 over 4 days. This is to assess the feasibility and validity of using food record mobile app to
39
40 155 digitally capture dietary intake. All participants will also be asked to wear a continuous glucose
41
42 156 monitoring system (CGMS) sensor on the back of their upper arm to measure 24-hour glucose
43
44 157 levels over 10 consecutive days and an Actigraph accelerometer on the wrist to capture their 24-
45
46 158 hour physical activity pattern, sedentary behaviour, sleep and light exposure over 10 consecutive
47
48 159 days.

160 At 24-28 weeks' gestation, a 75-g OGTT (0, 60 and 120 min) along with fasting insulin
 161 test will be conducted. Participants will be asked to fill up an online food frequency
 162 questionnaire (FFQ) to assess food intake in the past one month. After delivery, research staff
 163 will retrieve medical notes to document obstetric outcomes.

165 **Sample size**

166 The sample size is based on estimate of correlation coefficient between maternal circadian eating
 167 time and plasma glucose at mid-pregnancy from a previous study.[10] Based on 2-sided
 168 significance level set at 5% and with 80% power, 200 pregnant women are required to detect a
 169 minimum correlation coefficient of 0.20 between night-time caloric intake and plasma glucose.
 170 Assuming that variance inflation factor arising from covariate adjustment is 1.7 and with a
 171 dropout rate of 15%, the total sample size required for primary aim is 400 pregnant women.

173 **Study measurements**

174 Baseline socio-demographic information and potential confounding variables will be collected
 175 through questionnaires at visit 1. These include age, ethnicity, education, occupation, smoking
 176 status, alcohol consumption, meal regularity, electronic media use before bedtime and mood.
 177 Health and obstetric histories will be obtained from the electronic medical notes. Table 1 shows
 178 the details of the types of data that will be collected in this study.

179 **Table 1** Data that will be collected in the study

180 Data	Visit 1 (18-21 weeks gestation)	Visit 2 (24-28 weeks gestation)	After delivery
181 Informed consent	•		
182 Eligibility criteria	•		
183 Baseline characteristics			
184 Educational attainment	•		

1			
2			
3	Occupation	•	
4	Ethnicity	•	
5	Pre-pregnancy body mass index	•	
6	Smoking status	•	
7	Alcohol intake	•	
8	Questionnaires		
9	Physical activity	•	
10	Sedentary behavior	•	
11	Sleep habit	•	
12	Light exposure	•	
13	Electronic media use before bedtime	•	
14	Mood	•	
15	Actigraphy monitoring	•	
16	Diet		
17	Meal regularity	•	
18	Food diary (paper/ mobile app)	•	
19	Food frequency questionnaire		•
20	Glycemic measures		
21	Continuous glucose monitoring	•	
22	Oral glucose tolerance test		•
23	Fasting insulin test		•
24	Obstetric information		
25	Gestational weight gain		•
26	Obstetric history		•
27	Delivery outcomes		•
28	Pregnancy complications		•
29	Birth outcomes		•
30			
31			
32			
33			
34			
35	181		
36	182	Exposure measures	
37			
38	183	Night-eating pattern	
39			
40	184	At visit 1, the research staff will guide participants to fill up the 4-day food diary (3 weekdays	
41			
42	185	and 1 weekend day). Participants will be asked to record the time, type, description and amount	
43			
44	186	of food and beverages consumed throughout the day. Pictures of household measuring utensils	
45			
46	187	and various food portion sizes are printed in the food diary to assist participants in quantifying	
47			
48	188	their food intake.	
49			
50			
51	189	Food intake will also be recorded through mobile phone food record app with image	
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54	190	capture function (Meallogger, Wellness Foundry, USA). A subsample of participants (50%) with	
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3 191 smart phone devices will be required to download this app which is free and compatible with
4
5 192 iPhone and Android platforms. The images of food/ beverages and a common known size object
6
7 193 (e.g. spoon, chopstick, pen) will be captured together before and after every eating event for
8
9 194 better portion size estimation. An information sheet describing how to capture the food images
10
11 195 correctly will be provided.
12
13

14 196 Nutrient analysis of dietary records will be performed using the Dietplan (Forestfield
15
16 197 Software, UK), which contains a local food composition database. The daytime and night-time
17
18 198 periods will be determined by the local time of sunrise and sunset, occurring at ~0700h and
19
20 199 ~1900h, respectively, throughout the year given Singapore's equatorial position.[10] Night-
21
22 200 eating pattern will be assessed based on the amount and frequency of meal and snack
23
24 201 consumption during 1900-0659h.
25
26
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28
29

30 31 203 **Diet quality**

32
33 204 Diet quality will be derived from a 125 food items electronic graphic FFQ at visit 2, where the
34
35 205 Healthy Eating Index will be calculated. This FFQ is adapted from the paper-based FFQ used by
36
37 206 the National Nutrition Survey 2010.[11] Participants will be required to indicate frequency of
38
39 207 foods consumed in the past one month, by selecting one out of six frequency options ranging
40
41 208 from '1-3 times per month' to '2-3 times per day'. Individual portion size will be asked for each
42
43 209 food, and pictures of the various portion sizes will be provided for more accurate quantification.
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47 210 48 49 211 **Physical activity, sedentary behaviour, sleep and light exposure**

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51 212 The Actigraph wGT3X-BT (Actigraph LLC, Pensacola, FL, USA) will be used to objectively
52
53 213 monitor 24-hour physical activity, sedentary behaviour, sleep and light exposure.[12,13] The
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2
3 214 wGT3X-BT is a triaxial accelerometer designed to record continuous high resolution physical
4
5 215 activity and sleep/wake information. It includes an integrated ambient light sensor that delivers
6
7 216 lux values alongside activity information. Lux is a measure of light intensity. At visit 1,
8
9
10 217 participants will wear the device on their wrist for 10 consecutive days. The device does not have
11
12 218 to be removed during aquatic activities or showering. An information sheet describing how to
13
14 219 wear the device correctly will be provided. The Actigraphy data will be downloaded using the
15
16 220 ActiLife software and processed using the R package GGIR.[14]

17
18
19 221 Questionnaires on physical activity, sedentary behaviour, sleep and light exposure will
20
21 222 also be administered at the same visit. Participants will be interviewed using the modified
22
23 223 International Physical Activity Questionnaire-Short Form (IPAQ-SF) to self-report their physical
24
25 224 activity in the past 7 days.[15] The modified questionnaire evaluates the vigorous physical
26
27 225 activity, the moderate physical activity and the walking time. We removed question asking about
28
29 226 the sitting time from the original IPAQ-SF and included it in the questionnaire used to assess
30
31 227 sedentary behaviour. The data will be computed in metabolic equivalents (MET-min/week)
32
33 228 scores. Questionnaire on sedentary behaviour which is modified from the Adult Sedentary
34
35 229 Behaviour Questionnaire (ASBQ) will be performed.[16] The questionnaire evaluates time spent
36
37 230 sedentary in the past 7 days, including sitting time at work, sitting/lying down time to watch
38
39 231 television, to use electronic devices, at mealtimes, while driving or reading. Participants will also
40
41 232 self-administer the Pittsburgh Sleep Quality Index (PSQI) questionnaire to assess their sleep
42
43 233 habits in the past month,[17] and the Harvard Light Exposure Assessment (H-LEA)
44
45 234 questionnaire to assess their main light sources exposure in hourly basis on a typical weekday
46
47 235 and weekend day.[18]

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237 **Outcomes measures**

238 The main outcome of this study will be plasma glucose levels as assessed by OGTT at mid-
239 pregnancy. The secondary outcomes include glycaemic variability based on continuous glucose
240 monitoring profile, insulin level, GDM development, GWG, delivery and birth outcomes.

242 **OGTT and insulin test**

243 Participants will undergo a 75-gram OGTT after an overnight fast of 8-10 hours at visit 2. This is
244 a routine universal test for all pregnancies at KKH. Venous fasting plasma glucose and insulin,
245 1-hour and 2-hour post-load plasma glucose levels will be measured in the KKH lab. No
246 additional blood sample will be stored and the samples will be destroyed at completion of
247 analysis. The participants will be informed of their OGTT results by the attending doctors during
248 their subsequent antenatal visits. Any abnormal findings will be treated as per clinical practice.
249 GDM will be defined according to the World Health Organization 2013 criteria.[19]

251 **Continuous glucose monitoring**

252 A 10-day continuous glucose monitoring for assessment of glycaemic variability will be initiated
253 at visit 1. The FreeStyle Libre Pro Flash Glucose Monitoring System (Abbott, Germany) will be
254 used. The CGMS sensor will be applied on the back of upper arm. No calibration for the sensor
255 is required throughout the 10 days period. Readings from the CGMS are unavailable to
256 participants in real time to avoid bias that may arise from unmasked, real time glucose readings.

258 **Gestational weight gain**

259 Maternal weight at every antenatal visit will be retrieved from the medical notes after delivery.

260 Total and rate of gestational weight gain (GWG) will be computed. Classification of GWG will
261 be performed according to the Institute of Medicine's guideline.[20]

262

263 **Delivery and birth outcomes**

264 Delivery and birth outcomes will be retrieved from the medical notes after delivery.

265

266 **Statistical analysis**

267 Statistical analyses will be performed using the SPSS statistical package (SPSS Inc., Chicago,
268 Illinois, USA) or Stata Statistical Software (Stata, College Station, TX, USA). Multivariable
269 generalized linear models will be used to examine the associations of maternal night-eating
270 pattern (amount and frequency of meal and snack intake at night) with glycaemic measures,
271 GWG and obstetric outcomes, adjusting for potential covariates. Selection of covariates will be
272 determined from literature review, directed acyclic graph and observed statistical significance
273 associations with exposures and outcomes. Multivariable generalized linear models will also be
274 used to examine associations of physical activity, sedentary behaviour, sleep, diet quality and
275 light exposure with night-eating pattern, controlling for potential covariates.

276

277 **Quality control**

278 The research staff will receive trainings on how to perform study procedures, including
279 administration of questionnaires, food diary and FFQ, handling of CGMS device and Actigraph
280 accelerometer. The research staff will be required to complete the competency assessments to
281 ensure data quality before conducting the procedures in this study. Monthly meetings will be

282 held with the principal investigator to review study procedures and data collected. An annual
283 report on study progress will be prepared.

284

285 **Data monitoring and management**

286 Participants will be anonymized and assigned with a specific ID at study entry. Data will be
287 managed using the Research Electronic Data Capture (REDCap) electronic data capture tool. To
288 ensure accuracy and completeness of data entry, data will be checked by identifying if there is
289 any outlier or missing value. The data checking process will be performed in the first 3 months
290 of the study and so on, such that the experience gained can be used to train the research staff for
291 improvement. Paper documents will be kept in a locked cabinet and electronic data will be stored
292 on password-protected computers or hard-disk drives which can only be accessed by research
293 team members. All records will be kept for at least 6 years after completing the study.

294

295 **Patient and public involvement statement**

296 The research questions, exposure and outcome measures were determined based on the
297 evaluation of knowledge gap as identified from literature review, and through discussions with
298 clinicians, researchers and health care staff who have been involved in maternal child care.

299 Although participants did not directly contribute to the development of research questions and
300 the study design, their needs and preferences were considered throughout the process.

301 Participants will be informed for their blood test results. Findings of the study will be
302 disseminated to participants at their request.

303

304 **ETHICS AND DISSEMINATION**

1
2
3 305 Participants will sign a written informed consent and be provided with written information about
4
5 306 the study. This study will be conducted according to the Helsinki Declaration. Ethical approval
6
7
8 307 has been granted by the Centralised Institutional Review Board of SingHealth (reference
9
10 308 2018/2529). This study has been registered at ClinicalTrials.gov (NCT 03803345). Findings of
11
12 309 the study will be presented at conferences and disseminated in peer-reviewed journals. Media
13
14
15 310 releases will be considered to maximize visibility of the findings to the general public.
16
17 311

19 312 **DISCUSSION**

21 313 This protocol outlines the rationale and design of a longitudinal study that aims to examine the
22
23 314 associations of night-eating pattern with glycaemic measures and obstetric outcomes among
24
25 315 pregnant women in Singapore. Lifestyle factors associated with night-eating pattern will also be
26
27
28 316 evaluated. Data from this study will contribute to narrow the gap in knowledge related to
29
30 317 maternal night-eating pattern during pregnancy, which has received relatively less attention in
31
32 318 the literature compared with general adult population.

35 319 The strengths of the study include comprehensive assessment of maternal diet using 4-
36
37 320 day food diary and FFQ, providing a representative estimate of habitual dietary intake. This
38
39 321 study will also provide evidence whether the use of mobile-based food record method with
40
41
42 322 image capture function can assist in dietary data collection and analysis. The use of Actigraph
43
44 323 allows detailed investigations and objective measures for physical activity, sedentary behaviour,
45
46 324 sleep and light exposure, to enhance data accuracy. Other than using OGTT and insulin response
47
48 325 as the glycaemic outcomes, this study will also describe maternal glycaemic variability based on
49
50 326 continuous glucose monitoring profile, giving us the opportunity to understand the gestational
51
52 327 glucose patterns which may independently contribute to GDM-related complications.[21]
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2
3 328 This study may be limited by its external validity as it will only include participants from
4
5 329 one hospital in Singapore. The use of non-probability sampling method to recruit participants
6
7
8 330 may introduce selection bias, however, this is restricted by the practical and feasible recruitment
9
10 331 mechanism at the study site. Therefore, caution will be required to extrapolate the findings to
11
12 332 general pregnant population. Nevertheless, KKH houses the largest public maternity unit in
13
14 333 Singapore, and manages approximately 30% of all live births in Singapore, across a wide socio-
15
16 334 demographic spectrum. To check for generalisability of findings, we will explore for differences
17
18
19 335 by comparing basic demographic data obtained from this study with data available from other
20
21 336 studies involving larger population of pregnant women in Singapore.[22]

22
23
24 337 This study aims to serve as a baseline reference for planning interventional clinical trial
25
26 338 to examine the effect of aligning eating time with day-night cycles on glucose regulation and
27
28 339 GDM risk in pregnancy. This may help to develop evidence-based recommendations on maternal
29
30 340 nutrition related to meal and snack distribution, in order to improve gestational glycaemic
31
32 341 control, reduce the risk of GDM, and thus improving pregnancy and childhood outcomes. Also,
33
34 342 this study may have public health implications as night-eating has become a common practice
35
36 343 and habit among urban communities.
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40 344

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43
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45
46 347 research administrator, Jinjie Lin, to the planning of this study.
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50 51 349 **Author contributions**

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2
3 350 SLL is the principal investigator of the study, along with FY, YBC, MFFC, MRF, NL, YSL,
4
5 351 KHT and BSUC as co-investigators who have contributed to the conception and design of the
6
7 352 study. SLL, FY and JKYC assisted in the development and implementation of the study. SLL
8
9 353 drafted the manuscript. SLL, YBC, MFFC, MRF, NL, YSL, KHT and FY commented, edited
10
11 354 and revised the manuscript. All authors read and approved the final manuscript.
12
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15 355

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20
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22
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24 359

25 26 360 **Competing interests**

27
28 361 None declared.
29
30
31 362

32 33 363 **Ethics approval**

34
35 364 The Centralised Institutional Review Board of SingHealth (reference 2018/2529).
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39 40 366 **Data sharing statement**

41
42 367 The majority of data collected will be published. Any unpublished, de-identified data will be
43
44 368 made available to interested persons on request.
45
46
47 369

48 49 370 **REFERENCES**

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23 424 healthy Outcomes (GUSTO) birth cohort study. *Int J Epidemiol* 2014;43:1401–9.
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29 426 **Figure 1** Flow diagram of the study design
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Figure 1 Flow diagram of the study design

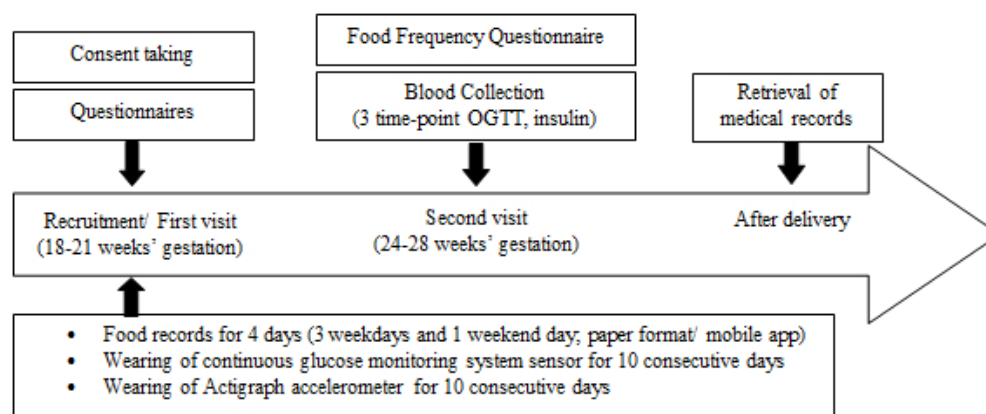


Figure 1 Flow diagram of the study design

48x20mm (300 x 300 DPI)

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Maternal night-eating pattern and glucose tolerance during pregnancy: study protocol for a longitudinal study

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Manuscripts

1 **Maternal night-eating pattern and glucose tolerance during pregnancy: study protocol for** 2 **a longitudinal study**

3
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41 42 **Word count: 2969**

43
44 **Keywords:** Eating time; Glucose tolerance; Lifestyle factor; Night-eating; Pregnancy

47 **Abstract**

48 **Introduction:** Coordinating eating schedules with day-night cycles has been shown to improve
49 glucose regulation in adults, but its association with gestational glycaemia is less clear. A better
50 understanding on how eating time can influence glucose levels in pregnancy may improve
51 strategies for gestational glycaemic control. This study aims to examine the association of
52 maternal night-eating pattern with glucose tolerance in the second trimester of pregnancy, and to
53 investigate how lifestyle factors may be related to night-eating pattern.

54 **Methods and analysis:** This is an observational longitudinal study that targets to recruit 400
55 pregnant women at 18-24 weeks' gestation from the KK Women's and Children's Hospital in
56 Singapore. Data collection includes socio-demographics, lifestyle habits and obstetric
57 information. Maternal dietary intake is collected using the 4-day food diary and food frequency
58 questionnaire; while 24-hour physical activity, sedentary behaviour, sleep and light exposure are
59 captured using the accelerometer at 18-24 weeks' gestation. Continuous glucose monitoring at
60 18-24 weeks' gestation, oral glucose tolerance test and insulin test at 24-28 weeks' gestation are
61 performed to assess glycaemic outcomes. Multivariable generalized linear models will be used to
62 analyse the association of maternal night-eating pattern (consumption of meal and snack during
63 1900-0659h) with glycaemic measures, and the associated factors of night-eating pattern,
64 controlling for potential confounders. Recruitment began in March 2019 and is estimated to end
65 in November 2020.

66 **Ethics and dissemination:** Ethical approval has been granted by the Centralised Institutional
67 Review Board of SingHealth, Singapore (reference 2018/2529). The results will be presented at
68 conferences and disseminated in journal articles.

69 **Trial registration:** NCT 03803345.

70

71 Article Summary

72 Strengths and limitations of this study

- 73 • This study will provide information on maternal night-eating pattern during pregnancy
74 and its association with glycaemic outcomes, which will be useful to healthcare
75 professionals and the pregnant population in the effort of glycaemic control.
- 76 • This study comprehensively assesses the night-eating pattern, glycaemic profile and
77 lifestyle factors of pregnant women.
- 78 • Given the participants are recruited from a single hospital, the sample may not be
79 considered representative of all pregnant women in Singapore.

81 INTRODUCTION

82 Over time, humans have evolved to keep time with the earth's repeated light-dark cycles. These
83 day-night rhythms orchestrate critical aspects of human physiology, from cell signalling to
84 cellular metabolism; as well as influence habitual aspects of human behaviour, including activity,
85 sleep and energy consumption.[1] The alignment of eating time with the body's circadian
86 rhythms, known also as circadian eating, has been shown to improve glucose tolerance,[2]
87 suggesting that circadian dietary strategies may be a useful way to maintain metabolic health.

88 Pregnant women belong to a high-risk population vulnerable to hyperglycaemia and its
89 consequences. In Singapore, 20% women develop gestational diabetes mellitus (GDM).[3] Even
90 at glucose concentrations below the diagnostic cut-off for GDM, risks of adverse perinatal
91 outcomes can occur, and these risks increase continuously in association with rising glucose

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3 92 levels during pregnancy.[4] Effective interventions to improve glycaemic control in pregnancy
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5 93 are urgently needed.
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8 94 Although it is known that food quantity and quality influence GDM development,[5] the
9
10 95 effect of circadian eating pattern,[6] specifically evening meal intake and nocturnal snacking
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12 96 behaviour on glucose regulation in pregnancy, remains an important gap of knowledge. In the
13
14 97 general population, late-eating or night-eating has been associated with less healthy eating and
15
16 98 more snack intake,[7] which may be related to metabolic disorders.[8] It was found that women
17
18 99 with GDM were more likely to snack at night compared with those of normal glucose
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20
21 100 tolerance.[9] Based on the latest nutritional guidelines from the Academy of Nutrition and
22
23 101 Dietetics, a new recommendation on meal and snack distribution has been included where
24
25 102 women with GDM are encouraged to have 3 meals and 2 or more snacks per day.[10] However,
26
27 103 this recommendation did not consider the effect of day-night or circadian cycles, and it was
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29 104 formed based on a consensus approach rather than with supportive evidence.
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33 105 Therefore, our motivation is to develop an understanding of the role of circadian timing
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35 106 for meals and snacks on blood glucose levels during pregnancy, which is potentially a modifiable
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37 107 behaviour for glycaemic control. The aims of this study are (i) to examine the association of
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39 108 maternal night-eating pattern from the aspect of amount and frequency of meals and snacks with
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41 109 glucose tolerance in the second trimester of pregnancy, and (ii) to investigate how lifestyle
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43 110 factors, specifically daily physical activity, sedentary behaviour, sleep, diet quality and light
44
45 111 exposure may be related to night-eating pattern. These lifestyle factors may influence the
46
47 112 association between night-eating pattern and glycaemic measures; yet have not been evaluated
48
49 113 previously. The central hypothesis is that small evening meals and less frequent snacking at night
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51 114 are associated with better glucose tolerance at 24-28 weeks' gestation – a period when the
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115 screening for GDM is usually done, compared to those with larger evening meals and more
116 frequent snacking at night.

117

118 **METHODS AND ANALYSIS**

119 **Study design**

120 This is an observational longitudinal study, where pregnant women at 18-24 weeks' gestation are
121 recruited and followed until delivery. An overview of the study procedures is illustrated in figure
122 1.

124 **Participants and recruitment**

125 This study is conducted at KK Women's and Children's Hospital (KKH), Singapore. KKH
126 houses the largest Obstetrics and Gynaecology department in Singapore, with over 10,000
127 ($\approx 30\%$) live births recorded annually. A non-probability sampling method is used to recruit
128 pregnant women who attend scheduled antenatal clinic appointments at KKH. Those who meet
129 the selection criteria are invited to participate in this study. Recruitment began in March 2019
130 and is estimated to end in November 2020.

131 The sample comprises pregnant women between 18-24 weeks' gestation at recruitment, age
132 ≥ 18 years, who are Singapore citizens or Singapore Permanent Residents, plan to continue
133 antenatal care at KKH, intend to deliver at KKH and able to provide written informed consent.
134 Excluded women are those with diabetes in pregnancy at recruitment as confirmed by the Oral
135 Glucose Tolerance Test (OGTT), have pre-existing type-1 or type-2 diabetes, on routine night-
136 shift work for at least 3x/week currently or in the last month, use of anticonvulsant medications/
137 oral steroids currently or in the last month, and with known or suspected allergy to medical grade

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3 138 adhesives. We also exclude pregnant women with chronic kidney disease, preeclampsia and
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5 139 multiple pregnancy due to lack of evidence to support accuracy of using continuous glucose
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7 140 monitoring system (Freestyle Libre Pro, Abbott, Germany) among these patients. Participants
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9
10 141 who develop a miscarriage or undergo a termination event, unable to comply with the study
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12 142 protocol or wish to discontinue participation are withdrawn from the study.
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14
15 143 Recruitment brochures that contain general information of the study are placed in the
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17 144 antenatal clinics. During the recruitment process, trained research staff inform potential women
18
19 145 of the study both verbally and with written information. Women who are agreeable to participate
20
21 146 provide written informed consent. Those who decline to participate continue to receive their
22
23 147 hospital antenatal care as usual, and care provided to each pregnant woman is not affected nor
24
25 148 influenced by the woman's decision to either participate or not participate in the study.
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30 31 150 **Study procedures**

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33 151 Study visits (recruitment and follow-up visits) of this study are determined based on maternal
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35 152 antenatal appointment schedules. After providing written informed consent at the recruitment
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37 153 visit (18-24 weeks' gestation), enrolled participants are interviewed for information on socio-
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39 154 demographics and lifestyle habits. At the same visit, participants are provided with a food diary
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41 155 to record 4-day dietary intake. All participants are also asked to wear a continuous glucose
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43 156 monitoring system (CGMS) sensor on the back of their upper arm to measure 24-hour glucose
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45 157 levels over 10 consecutive days, and an accelerometer on the wrist to capture their 24-hour
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47 158 physical activity pattern, sedentary behaviour, sleep and light exposure over 10 consecutive days.
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51 159 At 24-28 weeks' gestation, participants undergo a 75-g OGTT (0, 60 and 120 min) along
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53 160 with fasting insulin test. During the same period, research staff conduct an interviewer-
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3 161 administered online food frequency questionnaire (FFQ) in the antenatal clinic to assess maternal
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5 162 food intake over the past one month. After delivery, research staff retrieve medical notes to
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8 163 document obstetric outcomes.
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11 12 165 **Sample size**

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14 166 The sample size was calculated based on estimate of correlation coefficient between maternal
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16
17 167 circadian eating time and plasma glucose at 26-28 weeks of gestation from a previous study.[6]
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19 168 Based on 2-sided significance level set at 5% and with 80% power, 200 pregnant women are
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21 169 required to detect a minimum correlation coefficient of 0.20 between night-time caloric intake
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23
24 170 and plasma glucose. Assuming that variance inflation factor arising from covariate adjustment is
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26 171 1.7 and with a dropout rate of 15%, the total sample size required for primary aim is 400
27
28 172 pregnant women.
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31 32 33 174 **Study measurements**

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35 175 Baseline socio-demographic information and potential confounding variables are collected
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38 176 through questionnaires at visit 1. These include age, ethnicity (Chinese, Malay, Indian, others),
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40 177 education (none, primary, secondary, tertiary), occupation (unemployed, employed), smoking
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42 178 status (never, past smoker, active smoker, passive smoker), alcohol consumption (never,
43
44 179 monthly, weekly, daily), nausea/ vomiting (no, moderate, severe, very severe), meal regularity,
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46
47 180 electronic media use before bedtime and mood. Health and obstetric histories are obtained from
48
49 181 the electronic medical notes. Table 1 shows the details of the types of data that are collected in
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51 182 this study.
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55 184 **Table 1** Data collection in the study
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Data	Visit 1 (18-24 weeks gestation)	Visit 2 (24-28 weeks gestation)	After delivery
Informed consent	✓		
Eligibility criteria	✓		
Baseline characteristics			
Educational attainment	✓		
Occupation	✓		
Ethnicity	✓		
Pre-pregnancy body mass index	✓		
Smoking status	✓		
Alcohol intake	✓		
Nausea/ vomiting	✓		
Questionnaires			
Physical activity	✓		
Sedentary behavior	✓		
Sleep habit	✓		
Light exposure	✓		
Electronic media use before bedtime	✓		
Mood	✓		
Actigraphy monitoring	✓		
Diet			
Meal regularity	✓		
Food diary	✓		
Food frequency questionnaire		✓	
Glycemic measures			
Continuous glucose monitoring	✓		
Oral glucose tolerance test		✓	
Fasting insulin test		✓	
Obstetric information			
Gestational weight gain			✓
Obstetric history			✓
Delivery outcomes			✓
Pregnancy complications			✓
Birth outcomes			✓

185

186 **Exposure measures**187 **Night-eating pattern**

188 At visit 1, the research staff guide the participants to fill up the 4-day food diary (3 weekdays and
 189 1 weekend day). Participants are required to record the time, type, description and amount of
 190 food and beverages consumed throughout the day. Pictures of household measuring utensils and
 191 various food portion sizes are printed in the food diary to assist participants in quantifying their

192 food intake. In the case that food diary is not able to be filled up by the participant, research staff
193 conduct 24-hour recall for dietary data collection through phone interview.

194 Nutrient analysis of dietary records will be performed using the Dietplan (Forestfield
195 Software, UK), which contains a local food composition database. Based on the evidence
196 showing that sunlight is a strong environmental signal for the human circadian clock,[11] we
197 determine daytime and night-time periods according to the local time of sunrise (~0700h) and
198 sunset (~1900h),[6] which are relatively consistent throughout the year given Singapore's
199 equatorial position (1.3°N, 103.8°E).[12] With that, night-eating pattern will be assessed based
200 on the amount and frequency of meals and snacks during 1900-0659h.

202 **Diet quality**

203 Diet quality will be derived from a 125 food items electronic graphic FFQ at visit 2, where the
204 Healthy Eating Index will be calculated. This FFQ is adapted from the paper-based FFQ used by
205 the National Nutrition Survey 2010.[13] Participants are required to indicate frequency of foods
206 consumed in the past one month, by selecting one out of six frequency options ranging from '1-3
207 times per month' to '2-3 times per day'. Individual portion size is asked for each food, and
208 pictures of the various portion sizes are provided for more accurate quantification.

210 **Physical activity, sedentary behaviour, sleep and light exposure**

211 The Actigraph wGT3X-BT (Actigraph LLC, Pensacola, FL, USA) is used to objectively monitor
212 24-hour physical activity, sedentary behaviour, sleep and light exposure.[14,15] The wGT3X-BT
213 is a triaxial accelerometer designed to record continuous high resolution physical activity and
214 sleep/wake information. It includes an integrated ambient light sensor that delivers lux values

215 alongside activity information. Lux is a measure of light intensity. At visit 1, participants wear
216 the device on their wrist for 10 consecutive days. The device does not have to be removed during
217 aquatic activities or showering. An information sheet describing how to wear the device correctly
218 is provided. The Actigraphy data will be downloaded using the ActiLife software and processed
219 using the R package GGIR.[16] Variables such as energy expenditure (MET-min/day), sleep/
220 wake parameters (total sleep time, total wake time and number of awakenings) and amount of
221 light exposure (Lux) will be derived from the actigraphy data.

222 Questionnaires on physical activity, sedentary behaviour, sleep and light exposure are
223 also administered at the same visit. Participants are interviewed using the modified International
224 Physical Activity Questionnaire-Short Form (IPAQ-SF) to self-report their physical activity in
225 the past 7 days.[17] The modified questionnaire evaluates the vigorous physical activity, the
226 moderate physical activity and the walking time. We removed question asking about the sitting
227 time from the original IPAQ-SF and included it in the questionnaire used to assess sedentary
228 behaviour. The data will be computed in metabolic equivalents (MET-min/week) scores.
229 Questionnaire on sedentary behaviour which is modified from the Adult Sedentary Behaviour
230 Questionnaire (ASBQ) is performed.[18] The questionnaire evaluates time spent sedentary in the
231 past 7 days, including sitting time at work, sitting/lying down time to watch television, to use
232 electronic devices at mealtimes, while driving or reading. Participants also self-administer the
233 Pittsburgh Sleep Quality Index (PSQI) questionnaire to assess their sleep habits in the past
234 month,[19] and the Harvard Light Exposure Assessment (H-LEA) questionnaire to assess their
235 main light sources exposure in hourly basis on a typical weekday and weekend day.[20]

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237 **Outcomes measures**

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3 238 The main outcome of this study is plasma glucose levels as assessed by OGTT after visit 1,
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5 239 routinely between 24-28 weeks' gestation. The secondary outcomes include glycaemic
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8 240 variability based on continuous glucose monitoring profile, insulin level, GDM development,
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10 241 GWG, delivery and birth outcomes.

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13 14 243 **OGTT and insulin test**

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17 244 Participants undergo a 75-gram OGTT after an overnight fast of 8-10 hours at visit 2. This is a
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19 245 routine universal test for all pregnancies at KKH. The procedures of fasting and OGTT are
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21 246 explained by the research staff and nurses in the antenatal clinic before visit 2. Venous fasting
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23 247 plasma glucose and insulin, 1-hour and 2-hour post-load plasma glucose levels are measured in
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25 248 the KKH lab. The participants are informed of their OGTT results by the attending doctors
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27 249 during their subsequent antenatal visits. Any abnormal findings are treated as per clinical
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29 250 practice. GDM is defined according to the World Health Organization 2013 criteria.[21]
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33 34 252 **Continuous glucose monitoring**

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37 253 A 10-day continuous glucose monitoring for assessment of glycaemic variability is initiated at
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39 254 visit 1 by using the FreeStyle Libre Pro Flash Glucose Monitoring System (Abbott, Germany).
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41 255 The CGMS sensor is applied on the back of upper arm. No calibration for the sensor is required
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43 256 throughout the 10 days period. Readings from the CGMS are unavailable to participants in real
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45 257 time to avoid bias that may arise from unmasked, real time glucose readings. We do not perform
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47 258 this procedure at visit 2 as if the participant is diagnosed with GDM, they will receive dietary
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49 259 counselling and/ or insulin treatment which can alter the CGMS readings.
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261 **Gestational weight gain**

262 Research staff retrieve maternal weight at every antenatal visit from the medical notes after
263 delivery. Total and rate of gestational weight gain (GWG per week) will be computed.
264 Classification of GWG will be performed according to the Institute of Medicine's guidelines.[22]

266 **Delivery and birth outcomes**

267 Research staff retrieve information on delivery and birth outcomes from the medical notes after
268 delivery.

270 **Statistical analysis**

271 Statistical analyses will be performed using the SPSS statistical package (SPSS Inc., Chicago,
272 Illinois, USA) or Stata Statistical Software (Stata, College Station, TX, USA). Multivariable
273 generalized linear models will be used to examine the associations of maternal night-eating
274 pattern (amount and frequency of meal and snacks) with glycaemic measures, GWG and
275 obstetric outcomes, adjusting for potential covariates. Selection of covariates will be determined
276 from literature review, directed acyclic graph and/ or observed statistical significance
277 associations with exposures and outcomes. Interaction tests between night-eating and covariates
278 (e.g. age, ethnicity, pre-pregnancy body mass index) on glycaemic measures will be performed
279 to determine if subsequent stratification analyses are required. Multivariable generalized linear
280 models will also be used to examine associations of physical activity, sedentary behaviour, sleep,
281 diet quality and light exposure with night-eating pattern, controlling for potential covariates.

283 **Quality control**

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3 284 The research staff received trainings on how to perform study procedures, including
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5 285 administration of questionnaires, food diary and FFQ, handling of CGMS device and
6
7 286 accelerometer. The research staff were required to complete the competency assessments to
8
9 287 ensure data quality before conducting the procedures in this study. Monthly meetings are held
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11 288 with the principal investigator to review study procedures and data collected. An annual report
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13 289 on study progress will be prepared.
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19 291 **Data monitoring and management**

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21 292 Participants are anonymized and assigned with a specific ID at study entry. Data are managed
22
23 293 using the Research Electronic Data Capture (REDCap) electronic data capture tool. To ensure
24
25 294 accuracy and completeness of data entry, data are checked by identifying if there is any outlier or
26
27 295 missing value. The data checking process is performed in the first 3 months of the study and so
28
29 296 on, such that the experience gained can be used to train the research staff for improvement. Paper
30
31 297 documents are kept in a locked cabinet and electronic data are stored on password-protected
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33 298 computers or hard-disk drives which can only be accessed by research team members. All
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35 299 records will be kept for at least 6 years after completing the study.
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42 301 **Patient and public involvement statement**

43
44 302 The research questions, exposure and outcome measures were determined based on the
45
46 303 evaluation of knowledge gap as identified from literature review, and through discussions with
47
48 304 clinicians, researchers and health care staff who have been involved in maternal child care.
49
50 305 Although participants did not directly contribute to the development of research questions and
51
52 306 the study design, their needs and preferences were considered throughout the process.
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3 307 Participants will be informed for their blood test results. Findings of the study will be
4
5 308 disseminated to participants at their request.
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10 310 **ETHICS AND DISSEMINATION**

11 311 Participants sign a written informed consent and are provided with written information about the
12
13 312 study. This study is conducted according to the Helsinki Declaration. Ethical approval has been
14
15 313 granted by the Centralised Institutional Review Board of SingHealth (reference 2018/2529). This
16
17 314 study has been registered at ClinicalTrials.gov (NCT 03803345). Findings of the study will be
18
19 315 presented at conferences and disseminated in peer-reviewed journals. Media releases will be
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21 316 considered to maximize visibility of the findings to the general public.
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26 317

28 318 **DISCUSSION**

29 319 This protocol outlines the rationale and design of an observational longitudinal study that aims to
30
31 320 examine the associations of night-eating pattern with glycaemic measures and obstetric outcomes
32
33 321 among pregnant women in Singapore. Lifestyle factors associated with night-eating pattern are
34
35 322 evaluated. Data from this study will contribute to narrow the gap in knowledge related to
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37 323 maternal night-eating pattern during pregnancy, which has received relatively less attention in
38
39 324 the literature compared with general adult population.
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44 325 The strengths of the study include comprehensive assessment of maternal diet using 4-
45
46 326 day food diary and FFQ, providing a representative estimate of habitual dietary intake. The use
47
48 327 of accelerometer allows detailed investigations and objective measures for physical activity,
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50 328 sedentary behaviour, sleep and light exposure, to enhance data accuracy. Other than using OGTT
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52 329 and insulin response as the glycaemic outcomes, this study also describes maternal glycaemic
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3 330 variability based on continuous glucose monitoring profile, giving us the opportunity to
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5 331 understand the gestational glucose patterns which may independently contribute to GDM-related
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8 332 complications.[23]
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10 333 This study may be limited by its external validity as it only includes participants from one
11
12 334 hospital in Singapore. The use of non-probability sampling method to recruit participants may
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14 335 introduce selection bias, however, this is restricted by the practical and feasible recruitment
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16 336 mechanism at the study site. Therefore, caution is required to extrapolate the findings to general
17
18 337 pregnant population. Nevertheless, KKH houses the largest public maternity unit in Singapore,
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20 338 and manages approximately 30% of all live births in Singapore, across a wide socio-
21
22 339 demographic spectrum. To check for generalisability of findings, we will explore for differences
23
24 340 by comparing basic demographic data obtained from this study with data available from other
25
26 341 studies involving larger population of pregnant women in Singapore.[24]
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30 342 This study aims to serve as a baseline reference for planning interventional clinical trial
31
32 343 to examine the effect of aligning eating time with day-night cycles on glucose regulation and
33
34 344 GDM risk in pregnancy. This may help to develop evidence-based recommendations on maternal
35
36 345 nutrition related to meal and snack distribution, in order to improve gestational glycaemic
37
38 346 control, reduce the risk of GDM, and thus improving pregnancy and childhood outcomes. Also,
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40 347 this study may have public health implications as night-eating has become a common practice
41
42 348 and habit among urban communities.
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47 349

49 350 **Acknowledgements**

50
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52
53 352 research administrator, Jinjie Lin, to the planning of this study.
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3 353
45 354 **Author contributions**

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7
8 355 SLL is the principal investigator of the study, along with FY, YBC, MFFC, MRF, NL, YSL,
9
10 356 KHT and BSUC as co-investigators who have contributed to the conception and design of the
11
12 357 study. SLL, FY and JKYC assisted in the development and implementation of the study. SLL
13
14 358 drafted the manuscript. SLL, YBC, MFFC, MRF, NL, YSL, KHT and FY commented, edited
15
16 359 and revised the manuscript. All authors read and approved the final manuscript.
17
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19 360

20
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22
23
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25
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27
28 364

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30
31 365 **Competing interests**

32
33 366 None declared.
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37
38 368 **Ethics approval**

39
40 369 The Centralised Institutional Review Board of SingHealth (reference 2018/2529).
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45 371 **Data sharing statement**

46
47 372 The majority of data collected will be published. Any unpublished, de-identified data will be
48
49 373 made available to interested persons on request.
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54 375 **REFERENCES**
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25 432 Gestational-Age Infants. *Diabetes Care* 2015;38:1319-25.
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30 434 healthy Outcomes (GUSTO) birth cohort study. *Int J Epidemiol* 2014;43:1401–9.

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35 436 **Figure 1** Flow diagram of the study design

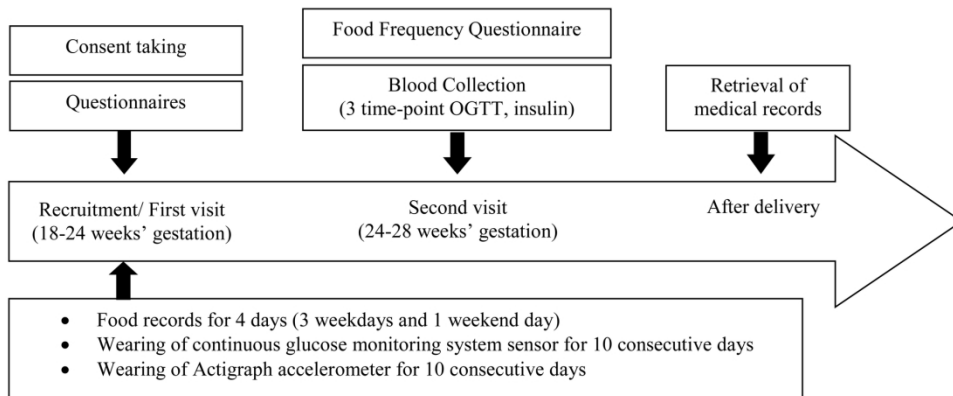


Figure 1 Flow diagram of the study design

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BMJ Open

Maternal night-eating pattern and glucose tolerance during pregnancy: study protocol for a longitudinal study

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SCHOLARONE™
Manuscripts

1 **Maternal night-eating pattern and glucose tolerance during pregnancy: study protocol for** 2 **a longitudinal study**

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41
42 **Word count: 3356**

43
44 **Keywords:** Eating time; Glucose tolerance; Lifestyle factor; Night-eating; Pregnancy

47 **Abstract**

48 **Introduction:** Coordinating eating schedules with day-night cycles has been shown to improve
49 glucose regulation in adults, but its association with gestational glycaemia is less clear. A better
50 understanding on how eating time can influence glucose levels in pregnancy may improve
51 strategies for gestational glycaemic control. This study aims to examine the association of
52 maternal night-eating pattern with glucose tolerance in the second trimester of pregnancy, and to
53 investigate how lifestyle factors may be related to night-eating pattern.

54 **Methods and analysis:** This is an observational longitudinal study that targets to recruit 200
55 pregnant women at 18-24 weeks' gestation from the KK Women's and Children's Hospital in
56 Singapore. Data collection includes socio-demographics, lifestyle habits and obstetric
57 information. Maternal dietary intake is collected using the 4-day food diary and food frequency
58 questionnaire; while 24-hour physical activity, sedentary behaviour, sleep and light exposure are
59 captured using the accelerometer at 18-24 weeks' gestation. Continuous glucose monitoring at
60 18-24 weeks' gestation, oral glucose tolerance test and insulin test at 24-28 weeks' gestation are
61 performed to assess glycaemic outcomes. Multivariable generalized linear models will be used to
62 analyse the association of maternal night-eating pattern (consumption of meal and snack during
63 1900-0659h) with glycaemic measures, and the associated factors of night-eating pattern,
64 controlling for potential confounders. Recruitment began in March 2019 and is estimated to end
65 in November 2020.

66 **Ethics and dissemination:** Ethical approval has been granted by the Centralised Institutional
67 Review Board of SingHealth, Singapore (reference 2018/2529). The results will be presented at
68 conferences and disseminated in journal articles.

69 **Trial registration:** NCT 03803345.

70

71 Article Summary

72 Strengths and limitations of this study

- 73 • This study will provide information on maternal night-eating pattern during pregnancy
74 and its association with glycaemic outcomes, which will be useful to healthcare
75 professionals and the pregnant population in the effort of glycaemic control.
- 76 • This study comprehensively assesses the night-eating pattern, glycaemic profile and
77 lifestyle factors of pregnant women.
- 78 • Given the participants are recruited from a single hospital, the sample may not be
79 considered representative of all pregnant women in Singapore.

81 INTRODUCTION

82 Over time, humans have evolved to keep time with the earth's repeated light-dark cycles. These
83 day-night rhythms orchestrate critical aspects of human physiology, from cell signalling to
84 cellular metabolism; as well as influence habitual aspects of human behaviour, including activity,
85 sleep and energy consumption.[1] The alignment of eating time with the body's circadian
86 rhythms, known also as circadian eating, has been shown to improve glucose tolerance,[2]
87 suggesting that circadian dietary strategies may be a useful way to maintain metabolic health.

88 Pregnant women belong to a high-risk population vulnerable to hyperglycaemia and its
89 consequences. In Singapore, 20% women develop gestational diabetes mellitus (GDM).[3] Even
90 at glucose concentrations below the diagnostic cut-off for GDM, risks of adverse perinatal
91 outcomes can occur, and these risks increase continuously in association with rising glucose

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3 92 levels during pregnancy.[4] Effective interventions to improve glycaemic control in pregnancy
4
5 93 are urgently needed.
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8 94 Although it is known that food quantity and quality influence GDM development,[5] the
9
10 95 effect of circadian eating pattern,[6] specifically evening meal intake and nocturnal snacking
11
12 96 behaviour on glucose regulation in pregnancy, remains an important gap of knowledge. In the
13
14 97 general population, late-eating or night-eating has been associated with less healthy eating and
15
16 98 more snack intake,[7] which may be related to metabolic disorders.[8] It was found that women
17
18 99 with GDM were more likely to snack at night compared with those of normal glucose
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21 100 tolerance.[9] Based on the latest nutritional guidelines from the Academy of Nutrition and
22
23 101 Dietetics, a new recommendation on meal and snack distribution has been included where
24
25 102 women with GDM are encouraged to have 3 meals and 2 or more snacks per day.[10] However,
26
27 103 this recommendation did not consider the effect of day-night or circadian cycles, and it was
28
29 104 formed based on a consensus approach rather than with supportive evidence.
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33 105 Therefore, our motivation is to develop an understanding of the role of circadian timing
34
35 106 for meals and snacks on blood glucose levels during pregnancy, which is potentially a modifiable
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37 107 behaviour for glycaemic control. The aims of this study are (i) to examine the association of
38
39 108 maternal night-eating pattern from the aspect of amount and frequency of meals and snacks with
40
41 109 glucose tolerance in the second trimester of pregnancy, and (ii) to investigate how lifestyle
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43 110 factors, specifically daily physical activity, sedentary behaviour, sleep, diet quality and light
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45 111 exposure may be related to night-eating pattern. These lifestyle factors may influence the
46
47 112 association between night-eating pattern and glycaemic measures; yet have not been evaluated
48
49 113 previously. The central hypothesis is that small evening meals and less frequent snacking at night
50
51 114 are associated with better glucose tolerance at 24-28 weeks' gestation – a period when the
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115 screening for GDM is usually done, compared to those with larger evening meals and more
116 frequent snacking at night.

117

118 **METHODS AND ANALYSIS**

119 **Study design**

120 This is an observational longitudinal study, where pregnant women at 18-24 weeks' gestation are
121 recruited and followed until delivery. An overview of the study procedures is illustrated in figure
122 1.

124 **Participants and recruitment**

125 This study is conducted at KK Women's and Children's Hospital (KKH), Singapore. KKH
126 houses the largest Obstetrics and Gynaecology department in Singapore, with over 10,000
127 ($\approx 30\%$) live births recorded annually. A non-probability (convenience) sampling method is used
128 to recruit pregnant women who attend scheduled antenatal clinic appointments at KKH. Instead
129 of all antenatal clinics, we only target at specific clinics with a greater number of potential
130 participants to perform the recruitment due to restricted manpower. Those who meet the
131 selection criteria are invited to participate in this study. Recruitment began in March 2019 and is
132 estimated to end in November 2020.

133 The sample comprises pregnant women between 18-24 weeks' gestation at recruitment, age
134 ≥ 18 years, who are Singapore citizens or Singapore Permanent Residents, plan to continue
135 antenatal care at KKH, intend to deliver at KKH and able to provide written informed consent.
136 Excluded women are those with diabetes in pregnancy at recruitment as confirmed by the Oral
137 Glucose Tolerance Test (OGTT), have pre-existing type-1 or type-2 diabetes, on routine night-

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3 138 shift work for at least 3x/week currently or in the last month, use of anticonvulsant medications/
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5 139 oral steroids currently or in the last month, and with known or suspected allergy to medical grade
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8 140 adhesives. We also exclude pregnant women with chronic kidney disease, preeclampsia and
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10 141 multiple pregnancy due to lack of evidence to support accuracy of using continuous glucose
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12 142 monitoring system (Freestyle Libre Pro, Abbott, Germany) among these patients. Participants
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14
15 143 who develop a miscarriage or undergo a termination event, unable to comply with the study
16
17 144 protocol or wish to discontinue participation are withdrawn from the study.

18
19 145 Recruitment brochures that contain general information of the study are placed in the
20
21 146 antenatal clinics. During the recruitment process, trained research staff inform potential women
22
23 147 of the study both verbally and with written information. Women who are agreeable to participate
24
25 148 provide written informed consent. Those who decline to participate continue to receive their
26
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28 149 hospital antenatal care as usual, and care provided to each pregnant woman is not affected nor
29
30 150 influenced by the woman's decision to either participate or not participate in the study.

31
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34 35 152 **Study procedures**

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37 153 Study visits (recruitment and follow-up visits) of this study are determined based on maternal
38
39 154 antenatal appointment schedules. After providing written informed consent at the recruitment
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41 155 visit (18-24 weeks' gestation), enrolled participants are interviewed for information on socio-
42
43 156 demographics and lifestyle habits. At the same visit, participants are provided with a food diary
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45 157 to record 4-day dietary intake. All participants are also asked to wear a continuous glucose
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47 158 monitoring system (CGMS) sensor on the back of their upper arm to measure 24-hour glucose
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49 159 levels over 10 consecutive days, and an accelerometer on the wrist to capture their 24-hour
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51 160 physical activity pattern, sedentary behaviour, sleep and light exposure over 10 consecutive days.
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3 161 At 24-28 weeks' gestation, participants undergo a 75-g OGTT (0, 60 and 120 min) along
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5 162 with fasting insulin test. During the same period, research staff conduct an interviewer-
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7 163 administered online food frequency questionnaire (FFQ) in the antenatal clinic to assess maternal
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9 164 food intake over the past one month. After delivery, research staff retrieve medical notes to
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11 165 document obstetric outcomes.
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167 **Sample size**

168 The sample size was calculated based on estimate of correlation coefficient between maternal
169 circadian eating time and plasma glucose at mid/ late pregnancy from previous studies.[6,11]
170 Based on 2-sided significance level set at 5% and with 80% power, 123 pregnant women are
171 required to detect a minimum correlation coefficient of 0.25 between night-time caloric intake
172 and plasma glucose. Assuming that variance inflation factor arising from covariate adjustment is
173 1.5 and with a dropout rate of 10%, the total sample size required for primary aim is 200
174 pregnant women.
175

176 **Study measurements**

177 Baseline socio-demographic information and potential confounding variables are collected
178 through questionnaires at visit 1. These include age, ethnicity (Chinese, Malay, Indian, others),
179 education (none, primary, secondary, tertiary), occupation (unemployed, employed), smoking
180 status (never, past smoker, active smoker, passive smoker), alcohol consumption (never,
181 monthly, weekly, daily), nausea/ vomiting (no, moderate, severe, very severe), meal regularity,
182 electronic media use before bedtime and mood. Health and obstetric histories are obtained from

183 the electronic medical notes. Table 1 shows the details of the types of data that are collected in
 184 this study.

185
 186 **Table 1** Data collection in the study

Data	Visit 1 (18-24 weeks gestation)	Visit 2 (24-28 weeks gestation)	After delivery
Informed consent	√		
Eligibility criteria	√		
Baseline characteristics			
Educational attainment	√		
Occupation	√		
Ethnicity	√		
Pre-pregnancy body mass index	√		
Smoking status	√		
Alcohol intake	√		
Nausea/ vomiting	√		
Questionnaires			
Physical activity	√		
Sedentary behavior	√		
Sleep habit	√		
Light exposure	√		
Electronic media use before bedtime	√		
Mood	√		
Actigraphy monitoring	√		
Diet			
Meal regularity	√		
Food diary	√		
Food frequency questionnaire		√	
Glycemic measures			
Continuous glucose monitoring	√		
Oral glucose tolerance test		√	
Fasting insulin test		√	
Obstetric information			
Gestational weight gain			√
Obstetric history			√
Delivery outcomes			√
Pregnancy complications			√
Birth outcomes			√

187
 188 **Exposure measures**

189 **Night-eating pattern**

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3 190 At visit 1, the research staff guide the participants to fill out the 4-day food diary (3 weekdays
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5 191 and 1 weekend day). Participants are required to record the time, type, description and amount of
6
7 192 food and beverages consumed throughout the day. Pictures of household measuring utensils and
8
9
10 193 various food portion sizes are printed in the food diary to assist participants in quantifying their
11
12 194 food intake. In the case that food diary is not able to be filled up by the participant, research staff
13
14
15 195 conduct 24-hour recall for dietary data collection through phone interview.

16
17 196 Nutrient analysis of dietary records will be performed using the Dietplan (Forestfield
18
19 197 Software, UK), which contains a local food composition database. Based on the evidence
20
21 198 showing that sunlight is a strong environmental signal for the human circadian clock,[12] we
22
23 199 determine daytime and night-time periods according to the local time of sunrise (~0700h) and
24
25 200 sunset (~1900h),[6] which are relatively consistent throughout the year given Singapore's
26
27 201 equatorial position (1.3°N, 103.8°E).[13] With that, night-eating pattern will be assessed based
28
29 202 on the amount and frequency of meals and snacks during 1900-0659h.
30
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35 204 **Diet quality**

36
37 205 Diet quality will be derived from a 125 food items electronic graphic FFQ at visit 2, where the
38
39 206 Healthy Eating Index will be calculated. This FFQ is adapted from the paper-based FFQ used by
40
41 207 the National Nutrition Survey 2010.[14] Participants are required to indicate frequency of foods
42
43 208 consumed in the past one month, by selecting one out of six frequency options ranging from '1-3
44
45 209 times per month' to '2-3 times per day'. Individual portion size is asked for each food, and
46
47 210 pictures of the various portion sizes are provided for more accurate quantification.
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54 212 **Physical activity, sedentary behaviour, sleep and light exposure**

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3 213 The Actigraph wGT3X-BT (Actigraph LLC, Pensacola, FL, USA) is used to objectively monitor
4
5 214 24-hour physical activity, sedentary behaviour, sleep and light exposure.[15,16] The wGT3X-BT
6
7
8 215 is a triaxial accelerometer designed to record continuous high resolution physical activity and
9
10 216 sleep/wake information. It includes an integrated ambient light sensor that delivers lux values
11
12 217 alongside activity information. Lux is a measure of light intensity. At visit 1, participants wear
13
14 218 the device on their non-dominant wrist for 10 consecutive days. The device does not have to be
15
16 219 removed during aquatic activities or showering. An information sheet describing how to wear the
17
18 220 device correctly is provided. The Actigraphy data will be downloaded using the ActiLife
19
20 221 software and processed using the R package GGIR.[17] Variables such as energy expenditure
21
22 222 (MET-min/day), sleep/ wake parameters (total sleep time, total wake time and number of
23
24 223 awakenings) and amount of light exposure (Lux) will be derived from the actigraphy data.
25
26
27

28 224 Questionnaires on physical activity, sedentary behaviour, sleep and light exposure are
29
30 225 also administered at the same visit. Participants are interviewed using the modified International
31
32 226 Physical Activity Questionnaire-Short Form (IPAQ-SF) to self-report their physical activity in
33
34 227 the past 7 days.[18] The modified questionnaire evaluates the vigorous physical activity, the
35
36 228 moderate physical activity and the walking time. We removed question asking about the sitting
37
38 229 time from the original IPAQ-SF and included it in the questionnaire used to assess sedentary
39
40 230 behaviour. The data will be computed in metabolic equivalents (MET-min/week) scores.
41
42

43 231 Questionnaire on sedentary behaviour which is modified from the Adult Sedentary Behaviour
44
45 232 Questionnaire (ASBQ) is performed.[19] The questionnaire evaluates time spent sedentary in the
46
47 233 past 7 days, including sitting time at work, sitting/lying down time to watch television, to use
48
49 234 electronic devices at mealtimes, while driving or reading. Participants also self-administer the
50
51 235 Pittsburgh Sleep Quality Index (PSQI) questionnaire to assess their sleep habits in the past
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236 month,[20] and the Harvard Light Exposure Assessment (H-LEA) questionnaire to assess their
237 main light sources exposure in hourly basis on a typical weekday and weekend day.[21]

238

239 **Outcomes measures**

240 The main outcome of this study is plasma glucose levels as assessed by OGTT after visit 1,
241 routinely between 24-28 weeks' gestation. The secondary outcomes include glycaemic
242 variability based on continuous glucose monitoring profile, insulin level, GDM development,
243 GWG, delivery and birth outcomes.

244

245 **OGTT and insulin test**

246 Participants undergo a 75-gram OGTT after an overnight fast of 8-10 hours at visit 2. This is a
247 routine universal test for all pregnancies at KKH. The procedures of fasting and OGTT are
248 explained by the research staff and nurses in the antenatal clinic before visit 2. Venous fasting
249 plasma glucose and insulin, 1-hour and 2-hour post-load plasma glucose levels are measured in
250 the KKH lab. The participants are informed of their OGTT results by the attending doctors
251 during their subsequent antenatal visits. Any abnormal findings are treated as per clinical
252 practice. GDM is defined according to the World Health Organization 2013 criteria.[22]

253

254 **Continuous glucose monitoring**

255 A 10-day continuous glucose monitoring for assessment of glycaemic variability is initiated at
256 visit 1 by using the FreeStyle Libre Pro Flash Glucose Monitoring System (Abbott, Germany).
257 The CGMS sensor is applied on the back of upper arm. No calibration for the sensor is required
258 throughout the 10 days period. Readings from the CGMS are unavailable to participants in real

1
2
3 259 time to avoid bias that may arise from unmasked, real time glucose readings. We do not perform
4
5 260 this procedure at visit 2 as if the participant is diagnosed with GDM, they will receive dietary
6
7 261 counselling and/ or insulin treatment which can alter the CGMS readings.
8
9

10 262

11 263 **Gestational weight gain**

12
13
14 264 Research staff retrieve maternal weight at every antenatal visit from the medical notes after
15
16 265 delivery. Total and rate of gestational weight gain (GWG per week) will be computed.
17
18

19 266 Classification of GWG will be performed according to the Institute of Medicine's guidelines.[23]
20
21

22 267

23 268 **Delivery and birth outcomes**

24
25
26 269 Research staff retrieve information on delivery and birth outcomes from the medical notes after
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28 270 delivery.
29

30 271

31 272 **Statistical analysis**

32
33 273 We will perform statistical analyses using the SPSS statistical package (SPSS Inc., Chicago,
34
35 274 Illinois, USA) or Stata Statistical Software (Stata, College Station, TX, USA). Multivariable
36
37 275 generalized linear models will be used to examine the associations of maternal night-eating
38
39 276 pattern (e.g. amount of last meal consumption in kcal, number of nightly snacking episodes) with
40
41 277 glycaemic measures, GWG and obstetric outcomes, adjusting for potential covariates. We will
42
43 278 define night-time based on the period between 1900-0659h (from sunset to sunrise) as described
44
45 279 above. We will also perform additional analysis to further define night-time based on different
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47 280 criteria (e.g. after 8pm or 9pm) to explore result differences.
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3 281 Selection of covariates will be determined from literature review, directed acyclic graph
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5 282 and/ or observed statistical significance associations with exposures and outcomes. In view of the
6
7
8 283 relation between night-fasting and plasma glucose as reported previously, [6] effect of night-
9
10 284 fasting duration will therefore be considered and adjusted in the model. Multivariable
11
12 285 generalized linear models will also be used to examine associations of physical activity,
13
14 286 sedentary behaviour, sleep, diet quality and light exposure with night-eating pattern, controlling
15
16
17 287 for potential covariates.
18

19 288 We will conduct stratified analyses to assess potential effect modification by maternal
20
21 289 age and pre-pregnancy body mass index. We will evaluate the significance of effect modification
22
23
24 290 on the multiplicative scale by including an interaction term (night-eating pattern x age or night-
25
26 291 eating pattern x pre-pregnancy body mass index) in the model.
27

28 292 We will impute missing data using multiple imputation analyses by chained equations.
29
30
31 293 [24] The number of imputations will be determined based on percentage of missing values [25]
32
33 294 and results of total imputations will be pooled using Rubin's rule.[26] To evaluate whether the
34
35 295 imputation of missing data may have affected the results, we will perform sensitivity analyses on
36
37
38 296 participants with complete data.
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40 297

41 42 298 **Quality control**

43
44 299 The research staff received training on how to perform study procedures, including
45
46
47 300 administration of questionnaires, food diary and FFQ, handling of CGMS device and
48
49 301 accelerometer. The research staff were required to complete the competency assessments to
50
51 302 ensure data quality before conducting the procedures in this study. Monthly meetings are held
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3 303 with the principal investigator to review study procedures and data collected. An annual report
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5 304 on study progress will be prepared.
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9 10 306 **Data monitoring and management**

11
12 307 Participants are anonymized and assigned with a specific ID at study entry. Data are managed
13
14 308 using the Research Electronic Data Capture (REDCap) electronic data capture tool. To ensure
15
16 309 accuracy and completeness of data entry, data are checked by identifying if there is any outlier or
17
18 310 missing value. The data checking process is performed in the first 3 months of the study and so
19
20 311 on, such that the experience gained can be used to train the research staff for improvement. Paper
21
22 312 documents are kept in a locked cabinet and electronic data are stored on password-protected
23
24 313 computers or hard-disk drives which can only be accessed by research team members. All
25
26 314 records will be kept for at least 6 years after completing the study.
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32 33 316 **Patient and public involvement statement**

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35 317 The research questions, exposure and outcome measures were determined based on the
36
37 318 evaluation of knowledge gap as identified from literature review, and through discussions with
38
39 319 clinicians, researchers and health care staff who have been involved in maternal child care.

40 320 Although participants did not directly contribute to the development of research questions and
41
42 321 the study design, their needs and preferences were considered throughout the process.
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45 322 Participants will be informed for their blood test results. Findings of the study will be
46
47 323 disseminated to participants at their request.
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52 53 325 **ETHICS AND DISSEMINATION**

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2
3 326 Participants sign a written informed consent and are provided with written information about the
4
5 327 study. This study is conducted according to the Helsinki Declaration. Ethical approval has been
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7
8 328 granted by the Centralised Institutional Review Board of SingHealth (reference 2018/2529).

9
10 329 When there are any changes in the study protocol or instruments used during the study period,
11
12 330 further ethical approval is sought, follow by re-consenting the participants whenever necessary.

13
14 331 This study has been registered at ClinicalTrials.gov (NCT 03803345). Findings of the study will
15
16 332 be presented at conferences and disseminated in peer-reviewed journals. Media releases will be
17
18 333 considered to maximize visibility of the findings to the general public.
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23 24 335 **DISCUSSION**

25
26 336 This protocol outlines the rationale and design of an observational longitudinal study that aims to
27
28 337 examine the associations of night-eating pattern with glycaemic measures and obstetric outcomes
29
30 338 among pregnant women in Singapore. Lifestyle factors associated with night-eating pattern are
31
32 339 evaluated. Data from this study will contribute to narrow the gap in knowledge related to
33
34 340 maternal night-eating pattern during pregnancy, which has received relatively less attention in
35
36 341 the literature compared with general adult population.
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39
40 342 The strengths of the study include comprehensive assessment of maternal diet using 4-
41
42 343 day food diary and FFQ, providing a representative estimate of habitual dietary intake. The use
43
44 344 of accelerometer allows detailed investigations and objective measures for physical activity,
45
46 345 sedentary behaviour, sleep and light exposure, to enhance data accuracy. Other than using OGTT
47
48 346 and insulin response as the glycaemic outcomes, this study also describes maternal glycaemic
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50 347 variability based on continuous glucose monitoring profile, giving us the opportunity to
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3 348 understand the gestational glucose patterns which may independently contribute to GDM-related
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5 349 complications.[27]
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8 350 This study may be limited by its external validity as it only includes participants from one
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10 351 hospital in Singapore. The use of non-probability sampling method to recruit participants may
11
12 352 introduce selection bias, however, this is restricted by the practical and feasible recruitment
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14 353 mechanism at the study site. Therefore, caution is required to extrapolate the findings to general
15
16 354 pregnant population. Nevertheless, KKH houses the largest public maternity unit in Singapore,
17
18 355 and manages approximately 30% of all live births in Singapore, across a wide socio-
19
20 356 demographic spectrum. To check for generalisability of findings, we will explore for differences
21
22 357 by comparing basic demographic data obtained from this study with data available from other
23
24 358 studies involving larger population of pregnant women in Singapore.[28]
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27

28 359 This study aims to serve as a baseline reference for planning interventional clinical trial
29
30 360 to examine the effect of aligning eating time with day-night cycles on glucose regulation and
31
32 361 GDM risk in pregnancy. This may help to develop evidence-based recommendations on maternal
33
34 362 nutrition related to meal and snack distribution, in order to improve gestational glycaemic
35
36 363 control, reduce the risk of GDM, and thus improving pregnancy and childhood outcomes. Also,
37
38 364 this study may have public health implications as night-eating has become a common practice
39
40 365 and habit among urban communities.
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48
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50
51 369 research administrator, Jinjie Lin, to the planning of this study.
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3 371 **Author contributions**
4

5 372 SLL is the principal investigator of the study, along with FY, YBC, MFFC, MRF, NL, YSL,
6
7 373 KHT and BSUC as co-investigators who have contributed to the conception and design of the
8
9 374 study. SLL, FY and JKYC assisted in the development and implementation of the study. SLL
10
11 375 drafted the manuscript. SLL, YBC, MFFC, MRF, NL, YSL, KHT and FY commented, edited
12
13 376 and revised the manuscript. All authors read and approved the final manuscript.
14
15
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17 377

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20

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22
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24
25
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27
28 382 **Competing interests**
29

30 383 None declared.
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35 385 **Ethics approval**
36

37 386 The Centralised Institutional Review Board of SingHealth (reference 2018/2529).
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42 388 **Data sharing statement**
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44 389 The majority of data collected will be published. Any unpublished, de-identified data will be
45
46 390 made available to interested persons on request.
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51 392 **REFERENCES**
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461 **Figure 1** Flow diagram of the study design

For peer review only

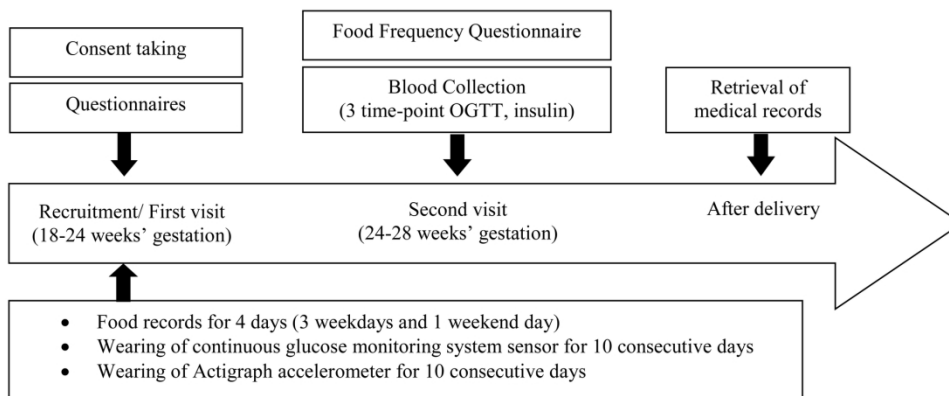


Figure 1 Flow diagram of the study design

175x101mm (300 x 300 DPI)

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Maternal night-eating pattern and glucose tolerance during pregnancy: study protocol for a longitudinal study

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Manuscripts

1 **Maternal night-eating pattern and glucose tolerance during pregnancy: study protocol for** 2 **a longitudinal study**

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41
42 **Word count: 3356**

43
44 **Keywords:** Eating time; Glucose tolerance; Lifestyle factor; Night-eating; Pregnancy

47 **Abstract**

48 **Introduction:** Coordinating eating schedules with day-night cycles has been shown to improve
49 glucose regulation in adults, but its association with gestational glycaemia is less clear. A better
50 understanding on how eating time can influence glucose levels in pregnancy may improve
51 strategies for gestational glycaemic control. This study aims to examine the association of
52 maternal night-eating pattern with glucose tolerance in the second trimester of pregnancy, and to
53 investigate how lifestyle factors may be related to night-eating pattern.

54 **Methods and analysis:** This is an observational longitudinal study that targets to recruit 200
55 pregnant women at 18-24 weeks' gestation from the KK Women's and Children's Hospital in
56 Singapore. Data collection includes socio-demographics, lifestyle habits and obstetric
57 information. Maternal dietary intake is collected using the 4-day food diary and food frequency
58 questionnaire; while 24-hour physical activity, sedentary behaviour, sleep and light exposure are
59 captured using the accelerometer at 18-24 weeks' gestation. Continuous glucose monitoring at
60 18-24 weeks' gestation, oral glucose tolerance test and insulin test at 24-28 weeks' gestation are
61 performed to assess glycaemic outcomes. Multivariable generalized linear models will be used to
62 analyse the association of maternal night-eating pattern (consumption of meal and snack during
63 1900-0659h) with glycaemic measures, and the associated factors of night-eating pattern,
64 controlling for potential confounders. Recruitment began in March 2019 and is estimated to end
65 in November 2020.

66 **Ethics and dissemination:** Ethical approval has been granted by the Centralised Institutional
67 Review Board of SingHealth, Singapore (reference 2018/2529). The results will be presented at
68 conferences and disseminated in journal articles.

69 **Trial registration:** NCT 03803345.

70

71 Article Summary

72 Strengths and limitations of this study

- 73 • This study will provide information on maternal night-eating pattern during pregnancy
74 and its association with glycaemic outcomes, which will be useful to healthcare
75 professionals and the pregnant population in the effort of glycaemic control.
- 76 • This study comprehensively assesses the night-eating pattern, glycaemic profile and
77 lifestyle factors of pregnant women.
- 78 • Given the participants are recruited from a single hospital, the sample may not be
79 considered representative of all pregnant women in Singapore.

81 INTRODUCTION

82 Over time, humans have evolved to keep time with the earth's repeated light-dark cycles. These
83 day-night rhythms orchestrate critical aspects of human physiology, from cell signalling to
84 cellular metabolism; as well as influence habitual aspects of human behaviour, including activity,
85 sleep and energy consumption.[1] The alignment of eating time with the body's circadian
86 rhythms, known also as circadian eating, has been shown to improve glucose tolerance,[2]
87 suggesting that circadian dietary strategies may be a useful way to maintain metabolic health.

88 Pregnant women belong to a high-risk population vulnerable to hyperglycaemia and its
89 consequences. In Singapore, 20% women develop gestational diabetes mellitus (GDM).[3] Even
90 at glucose concentrations below the diagnostic cut-off for GDM, risks of adverse perinatal
91 outcomes can occur, and these risks increase continuously in association with rising glucose

1
2
3 92 levels during pregnancy.[4] Effective interventions to improve glycaemic control in pregnancy
4
5 93 are urgently needed.
6
7

8 94 Although it is known that food quantity and quality influence GDM development,[5] the
9
10 95 effect of circadian eating pattern,[6] specifically evening meal intake and nocturnal snacking
11
12 96 behaviour on glucose regulation in pregnancy, remains an important gap of knowledge. In the
13
14 97 general population, late-eating or night-eating has been associated with less healthy eating and
15
16 98 more snack intake,[7] which may be related to metabolic disorders.[8] It was found that women
17
18 99 with GDM were more likely to snack at night compared with those of normal glucose
19
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21 100 tolerance.[9] Based on the latest nutritional guidelines from the Academy of Nutrition and
22
23 101 Dietetics, a new recommendation on meal and snack distribution has been included where
24
25 102 women with GDM are encouraged to have 3 meals and 2 or more snacks per day.[10] However,
26
27 103 this recommendation did not consider the effect of day-night or circadian cycles, and it was
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29 104 formed based on a consensus approach rather than with supportive evidence.
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33 105 Therefore, our motivation is to develop an understanding of the role of circadian timing
34
35 106 for meals and snacks on blood glucose levels during pregnancy, which is potentially a modifiable
36
37 107 behaviour for glycaemic control. The aims of this study are (i) to examine the association of
38
39 108 maternal night-eating pattern from the aspect of amount and frequency of meals and snacks with
40
41 109 glucose tolerance in the second trimester of pregnancy, and (ii) to investigate how lifestyle
42
43 110 factors, specifically daily physical activity, sedentary behaviour, sleep, diet quality and light
44
45 111 exposure may be related to night-eating pattern. These lifestyle factors may influence the
46
47 112 association between night-eating pattern and glycaemic measures; yet have not been evaluated
48
49 113 previously. The central hypothesis is that small evening meals and less frequent snacking at night
50
51 114 are associated with better glucose tolerance at 24-28 weeks' gestation – a period when the
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115 screening for GDM is usually done, compared to those with larger evening meals and more
116 frequent snacking at night.

117

118 **METHODS AND ANALYSIS**

119 **Study design**

120 This is an observational longitudinal study, where pregnant women at 18-24 weeks' gestation are
121 recruited and followed until delivery. An overview of the study procedures is illustrated in figure
122 1.

124 **Participants and recruitment**

125 This study is conducted at KK Women's and Children's Hospital (KKH), Singapore. KKH
126 houses the largest Obstetrics and Gynaecology department in Singapore, with over 10,000
127 ($\approx 30\%$) live births recorded annually. A non-probability (convenience) sampling method is used
128 to recruit pregnant women who attend scheduled antenatal clinic appointments at KKH. Instead
129 of all antenatal clinics, we only target at one specific clinic with a greater number of potential
130 participants to perform the recruitment due to restricted manpower. We expect the response rate
131 to be 25-35%. Those who meet the selection criteria are invited to participate in this study.
132 Recruitment began in March 2019 and is estimated to end in November 2020.

133 The sample comprises pregnant women between 18-24 weeks' gestation at recruitment, age
134 ≥ 18 years, who are Singapore citizens or Singapore Permanent Residents, plan to continue
135 antenatal care at KKH, intend to deliver at KKH and able to provide written informed consent.
136 Excluded women are those with diabetes in pregnancy at recruitment as confirmed by the Oral
137 Glucose Tolerance Test (OGTT), have pre-existing type-1 or type-2 diabetes, on routine night-

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3 138 shift work for at least 3x/week currently or in the last month, use of anticonvulsant medications/
4
5 139 oral steroids currently or in the last month, and with known or suspected allergy to medical grade
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8 140 adhesives. We also exclude pregnant women with chronic kidney disease, preeclampsia and
9
10 141 multiple pregnancy due to lack of evidence to support accuracy of using continuous glucose
11
12 142 monitoring system (Freestyle Libre Pro, Abbott, Germany) among these patients. Participants
13
14
15 143 who develop a miscarriage or undergo a termination event, unable to comply with the study
16
17 144 protocol or wish to discontinue participation are withdrawn from the study.
18

19 145 Recruitment brochures that contain general information of the study are placed in the
20
21 146 antenatal clinics. During the recruitment process, trained research staff inform potential women
22
23 147 of the study both verbally and with written information. Women who are agreeable to participate
24
25 148 provide written informed consent. Those who decline to participate continue to receive their
26
27
28 149 hospital antenatal care as usual, and care provided to each pregnant woman is not affected nor
29
30 150 influenced by the woman's decision to either participate or not participate in the study.
31
32

33 151

35 152 **Study procedures**

36
37 153 Study visits (recruitment and follow-up visits) of this study are determined based on maternal
38
39 154 antenatal appointment schedules. After providing written informed consent at the recruitment
40
41 155 visit (18-24 weeks' gestation), enrolled participants are interviewed for information on socio-
42
43 156 demographics and lifestyle habits. At the same visit, participants are provided with a food diary
44
45 157 to record 4-day dietary intake. All participants are also asked to wear a continuous glucose
46
47 158 monitoring system (CGMS) sensor on the back of their upper arm to measure 24-hour glucose
48
49 159 levels over 10 consecutive days, and an accelerometer on the wrist to capture their 24-hour
50
51
52 160 physical activity pattern, sedentary behaviour, sleep and light exposure over 10 consecutive days.
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1
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3 161 At 24-28 weeks' gestation, participants undergo a 75-g OGTT (0, 60 and 120 min) along
4
5 162 with fasting insulin test. During the same period, research staff conduct an interviewer-
6
7 163 administered online food frequency questionnaire (FFQ) in the antenatal clinic to assess maternal
8
9 164 food intake over the past one month. After delivery, research staff retrieve medical notes to
10
11 165 document obstetric outcomes.
12
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15 166

167 **Sample size**

168 The sample size was calculated based on estimate of correlation coefficient between maternal
169 circadian eating time and plasma glucose at mid/ late pregnancy from previous studies.[6,11]
170 Based on 2-sided significance level set at 5% and with 80% power, 123 pregnant women are
171 required to detect a minimum correlation coefficient of 0.25 between night-time caloric intake
172 and plasma glucose. Assuming that variance inflation factor arising from covariate adjustment is
173 1.5 and with a dropout rate of 10%, the total sample size required for primary aim is 200
174 pregnant women.
175

176 **Study measurements**

177 Baseline socio-demographic information and potential confounding variables are collected
178 through questionnaires at visit 1. These include age, ethnicity (Chinese, Malay, Indian, others),
179 education (none, primary, secondary, tertiary), occupation (unemployed, employed), smoking
180 status (never, past smoker, active smoker, passive smoker), alcohol consumption (never,
181 monthly, weekly, daily), nausea/ vomiting (no, moderate, severe, very severe), meal regularity,
182 electronic media use before bedtime and mood. Health and obstetric histories are obtained from

183 the electronic medical notes. Table 1 shows the details of the types of data that are collected in
 184 this study.

185
 186 **Table 1** Data collection in the study

Data	Visit 1 (18-24 weeks gestation)	Visit 2 (24-28 weeks gestation)	After delivery
Informed consent	√		
Eligibility criteria	√		
Baseline characteristics			
Educational attainment	√		
Occupation	√		
Ethnicity	√		
Pre-pregnancy body mass index	√		
Smoking status	√		
Alcohol intake	√		
Nausea/ vomiting	√		
Questionnaires			
Physical activity	√		
Sedentary behavior	√		
Sleep habit	√		
Light exposure	√		
Electronic media use before bedtime	√		
Mood	√		
Actigraphy monitoring	√		
Diet			
Meal regularity	√		
Food diary	√		
Food frequency questionnaire		√	
Glycemic measures			
Continuous glucose monitoring	√		
Oral glucose tolerance test		√	
Fasting insulin test		√	
Obstetric information			
Gestational weight gain			√
Obstetric history			√
Delivery outcomes			√
Pregnancy complications			√
Birth outcomes			√

187
 188 **Exposure measures**

189 **Night-eating pattern**

1
2
3 190 At visit 1, the research staff guide the participants to fill out the 4-day food diary (3 weekdays
4
5 191 and 1 weekend day). Participants are required to record the time, type, description and amount of
6
7 192 food and beverages consumed throughout the day. Pictures of household measuring utensils and
8
9
10 193 various food portion sizes are printed in the food diary to assist participants in quantifying their
11
12 194 food intake. In the case that food diary is not able to be filled up by the participant, research staff
13
14
15 195 conduct 24-hour recall for dietary data collection through phone interview.

16
17 196 Nutrient analysis of dietary records will be performed using the Dietplan (Forestfield
18
19 197 Software, UK), which contains a local food composition database. Based on the evidence
20
21 198 showing that sunlight is a strong environmental signal for the human circadian clock,[12] we
22
23 199 determine daytime and night-time periods according to the local time of sunrise (~0700h) and
24
25 200 sunset (~1900h),[6] which are relatively consistent throughout the year given Singapore's
26
27 201 equatorial position (1.3°N, 103.8°E).[13] With that, night-eating pattern will be assessed based
28
29 202 on the amount and frequency of meals and snacks during 1900-0659h.
30
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33 203

35 204 **Diet quality**

36
37 205 Diet quality will be derived from a 125 food items electronic graphic FFQ at visit 2, where the
38
39 206 Healthy Eating Index will be calculated. This FFQ is adapted from the paper-based FFQ used by
40
41 207 the National Nutrition Survey 2010.[14] Participants are required to indicate frequency of foods
42
43 208 consumed in the past one month, by selecting one out of six frequency options ranging from '1-3
44
45 209 times per month' to '2-3 times per day'. Individual portion size is asked for each food, and
46
47 210 pictures of the various portion sizes are provided for more accurate quantification.
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51 211

54 212 **Physical activity, sedentary behaviour, sleep and light exposure**

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3 213 The Actigraph wGT3X-BT (Actigraph LLC, Pensacola, FL, USA) is used to objectively monitor
4
5 214 24-hour physical activity, sedentary behaviour, sleep and light exposure.[15,16] The wGT3X-BT
6
7
8 215 is a triaxial accelerometer designed to record continuous high resolution physical activity and
9
10 216 sleep/wake information. It includes an integrated ambient light sensor that delivers lux values
11
12 217 alongside activity information. Lux is a measure of light intensity. At visit 1, participants wear
13
14 218 the device on their non-dominant wrist for 10 consecutive days. The device does not have to be
15
16 219 removed during aquatic activities or showering. An information sheet describing how to wear the
17
18 220 device correctly is provided. The Actigraphy data will be downloaded using the ActiLife
19
20 221 software and processed using the R package GGIR.[17] Variables such as energy expenditure
21
22 222 (MET-min/day), sleep/ wake parameters (total sleep time, total wake time and number of
23
24 223 awakenings) and amount of light exposure (Lux) will be derived from the actigraphy data.
25
26
27

28 224 Questionnaires on physical activity, sedentary behaviour, sleep and light exposure are
29
30 225 also administered at the same visit. Participants are interviewed using the modified International
31
32 226 Physical Activity Questionnaire-Short Form (IPAQ-SF) to self-report their physical activity in
33
34 227 the past 7 days.[18] The modified questionnaire evaluates the vigorous physical activity, the
35
36 228 moderate physical activity and the walking time. We removed question asking about the sitting
37
38 229 time from the original IPAQ-SF and included it in the questionnaire used to assess sedentary
39
40 230 behaviour. The data will be computed in metabolic equivalents (MET-min/week) scores.
41
42
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44 231 Questionnaire on sedentary behaviour which is modified from the Adult Sedentary Behaviour
45
46 232 Questionnaire (ASBQ) is performed.[19] The questionnaire evaluates time spent sedentary in the
47
48 233 past 7 days, including sitting time at work, sitting/lying down time to watch television, to use
49
50 234 electronic devices at mealtimes, while driving or reading. Participants also self-administer the
51
52 235 Pittsburgh Sleep Quality Index (PSQI) questionnaire to assess their sleep habits in the past
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236 month,[20] and the Harvard Light Exposure Assessment (H-LEA) questionnaire to assess their
237 main light sources exposure in hourly basis on a typical weekday and weekend day.[21]

238

239 **Outcomes measures**

240 The main outcome of this study is plasma glucose levels as assessed by OGTT after visit 1,
241 routinely between 24-28 weeks' gestation. The secondary outcomes include glycaemic
242 variability based on continuous glucose monitoring profile, insulin level, GDM development,
243 GWG, delivery and birth outcomes.

244

245 **OGTT and insulin test**

246 Participants undergo a 75-gram OGTT after an overnight fast of 8-10 hours at visit 2. This is a
247 routine universal test for all pregnancies at KKH. The procedures of fasting and OGTT are
248 explained by the research staff and nurses in the antenatal clinic before visit 2. Venous fasting
249 plasma glucose and insulin, 1-hour and 2-hour post-load plasma glucose levels are measured in
250 the KKH lab. The participants are informed of their OGTT results by the attending doctors
251 during their subsequent antenatal visits. Any abnormal findings are treated as per clinical
252 practice. GDM is defined according to the World Health Organization 2013 criteria.[22]

253

254 **Continuous glucose monitoring**

255 A 10-day continuous glucose monitoring for assessment of glycaemic variability is initiated at
256 visit 1 by using the FreeStyle Libre Pro Flash Glucose Monitoring System (Abbott, Germany).
257 The CGMS sensor is applied on the back of upper arm. No calibration for the sensor is required
258 throughout the 10 days period. Readings from the CGMS are unavailable to participants in real

1
2
3 259 time to avoid bias that may arise from unmasked, real time glucose readings. We do not perform
4
5 260 this procedure at visit 2 as if the participant is diagnosed with GDM, they will receive dietary
6
7 261 counselling and/ or insulin treatment which can alter the CGMS readings.
8
9

10 262

11 263 **Gestational weight gain**

12
13
14 264 Research staff retrieve maternal weight at every antenatal visit from the medical notes after
15
16 265 delivery. Total and rate of gestational weight gain (GWG per week) will be computed.
17
18

19 266 Classification of GWG will be performed according to the Institute of Medicine's guidelines.[23]
20
21

22 267

23 268 **Delivery and birth outcomes**

24
25
26 269 Research staff retrieve information on delivery and birth outcomes from the medical notes after
27
28 270 delivery.
29

30 271

31 272 **Statistical analysis**

32
33 273 We will perform statistical analyses using the SPSS statistical package (SPSS Inc., Chicago,
34
35 274 Illinois, USA) or Stata Statistical Software (Stata, College Station, TX, USA). Multivariable
36
37 275 generalized linear models will be used to examine the associations of maternal night-eating
38
39 276 pattern (e.g. amount of last meal consumption in kcal, number of nightly snacking episodes) with
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41 277 glycaemic measures, GWG and obstetric outcomes, adjusting for potential covariates. We will
42
43 278 define night-time based on the period between 1900-0659h (from sunset to sunrise) as described
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45 279 above. We will also perform additional analysis to further define night-time based on different
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47 280 criteria (e.g. after 8pm or 9pm) to explore result differences.
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3 281 Selection of covariates will be determined from literature review, directed acyclic graph
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5 282 and/ or observed statistical significance associations with exposures and outcomes. In view of the
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7
8 283 relation between night-fasting and plasma glucose as reported previously, [6] effect of night-
9
10 284 fasting duration will therefore be considered and adjusted in the model. Multivariable
11
12 285 generalized linear models will also be used to examine associations of physical activity,
13
14 286 sedentary behaviour, sleep, diet quality and light exposure with night-eating pattern, controlling
15
16
17 287 for potential covariates.
18

19 288 We will conduct stratified analyses to assess potential effect modification by maternal
20
21 289 age and pre-pregnancy body mass index. We will evaluate the significance of effect modification
22
23
24 290 on the multiplicative scale by including an interaction term (night-eating pattern x age or night-
25
26 291 eating pattern x pre-pregnancy body mass index) in the model.
27

28 292 We will impute missing data using multiple imputation analyses by chained equations.
29
30
31 293 [24] The number of imputations will be determined based on percentage of missing values [25]
32
33 294 and results of total imputations will be pooled using Rubin's rule.[26] To evaluate whether the
34
35 295 imputation of missing data may have affected the results, we will perform sensitivity analyses on
36
37
38 296 participants with complete data.
39

40 297

41 42 298 **Quality control**

43
44 299 The research staff received training on how to perform study procedures, including
45
46
47 300 administration of questionnaires, food diary and FFQ, handling of CGMS device and
48
49 301 accelerometer. The research staff were required to complete the competency assessments to
50
51 302 ensure data quality before conducting the procedures in this study. Monthly meetings are held
52
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1
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3 303 with the principal investigator to review study procedures and data collected. An annual report
4
5 304 on study progress will be prepared.
6
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8 305

9
10 306 **Data monitoring and management**

11
12 307 Participants are anonymized and assigned with a specific ID at study entry. Data are managed
13
14 308 using the Research Electronic Data Capture (REDCap) electronic data capture tool. To ensure
15
16 309 accuracy and completeness of data entry, data are checked by identifying if there is any outlier or
17
18 310 missing value. The data checking process is performed in the first 3 months of the study and so
19
20 311 on, such that the experience gained can be used to train the research staff for improvement. Paper
21
22 312 documents are kept in a locked cabinet and electronic data are stored on password-protected
23
24 313 computers or hard-disk drives which can only be accessed by research team members. All
25
26 314 records will be kept for at least 6 years after completing the study.
27
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33 316 **Patient and public involvement statement**

34
35 317 The research questions, exposure and outcome measures were determined based on the
36
37 318 evaluation of knowledge gap as identified from literature review, and through discussions with
38
39 319 clinicians, researchers and health care staff who have been involved in maternal child care.

40 320 Although participants did not directly contribute to the development of research questions and
41
42 321 the study design, their needs and preferences were considered throughout the process.
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45 322 Participants will be informed for their blood test results. Findings of the study will be
46
47 323 disseminated to participants at their request.
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53 325 **ETHICS AND DISSEMINATION**
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3 326 Participants sign a written informed consent and are provided with written information about the
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5 327 study. This study is conducted according to the Helsinki Declaration. Ethical approval has been
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7
8 328 granted by the Centralised Institutional Review Board of SingHealth (reference 2018/2529).

9
10 329 When there are any changes in the study protocol or instruments used during the study period,
11
12 330 further ethical approval is sought, follow by re-consenting the participants whenever necessary.

13
14 331 Previously collected data which are not able to be matched with the current data as collected
15
16 332 using the latest revised version will be removed and treated as missing variable, if data re-
17
18
19 333 collection is not possible. This study has been registered at ClinicalTrials.gov (NCT 03803345).

20
21 334 Findings of the study will be presented at conferences and disseminated in peer-reviewed
22
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24 335 journals. Media releases will be considered to maximize visibility of the findings to the general
25
26 336 public.

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30 31 338 **DISCUSSION**

32
33 339 This protocol outlines the rationale and design of an observational longitudinal study that aims to
34
35 340 examine the associations of night-eating pattern with glycaemic measures and obstetric outcomes
36
37
38 341 among pregnant women in Singapore. Lifestyle factors associated with night-eating pattern are
39
40 342 evaluated. Data from this study will contribute to narrow the gap in knowledge related to
41
42 343 maternal night-eating pattern during pregnancy, which has received relatively less attention in
43
44 344 the literature compared with general adult population.

45
46
47 345 The strengths of the study include comprehensive assessment of maternal diet using 4-
48
49 346 day food diary and FFQ, providing a representative estimate of habitual dietary intake. The use
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51 347 of accelerometer allows detailed investigations and objective measures for physical activity,
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53
54 348 sedentary behaviour, sleep and light exposure, to enhance data accuracy. Other than using OGTT

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3 349 and insulin response as the glycaemic outcomes, this study also describes maternal glycaemic
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5 350 variability based on continuous glucose monitoring profile, giving us the opportunity to
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8 351 understand the gestational glucose patterns which may independently contribute to GDM-related
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10 352 complications.[27]

11
12 353 This study may be limited by its external validity as it only includes participants from one
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14 354 hospital in Singapore. The use of non-probability sampling method to recruit participants may
15
16 355 introduce selection bias, however, this is restricted by the practical and feasible recruitment
17
18 356 mechanism at the study site. Therefore, caution is required to extrapolate the findings to general
19
20 357 pregnant population. Nevertheless, KKH houses the largest public maternity unit in Singapore,
21
22 358 and manages approximately 30% of all live births in Singapore, across a wide socio-
23
24 359 demographic spectrum. To check for generalisability of findings, we will explore for differences
25
26 360 by comparing basic demographic data obtained from this study with data available from other
27
28 361 studies involving larger population of pregnant women in Singapore.[28]

29
30
31 362 This study aims to serve as a baseline reference for planning interventional clinical trial
32
33 363 to examine the effect of aligning eating time with day-night cycles on glucose regulation and
34
35 364 GDM risk in pregnancy. This may help to develop evidence-based recommendations on maternal
36
37 365 nutrition related to meal and snack distribution, in order to improve gestational glycaemic
38
39 366 control, reduce the risk of GDM, and thus improving pregnancy and childhood outcomes. Also,
40
41 367 this study may have public health implications as night-eating has become a common practice
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43 368 and habit among urban communities.

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50 51 370 **Acknowledgements**

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372 research administrator, Jinjie Lin, to the planning of this study.

373

374 **Author contributions**

375 SLL is the principal investigator of the study, along with FY, YBC, MFFC, MRF, NL, YSL,
376 KHT and BSUC as co-investigators who have contributed to the conception and design of the
377 study. SLL, FY and JKYC assisted in the development and implementation of the study. SLL
378 drafted the manuscript. SLL, YBC, MFFC, MRF, NL, YSL, KHT and FY commented, edited
379 and revised the manuscript. All authors read and approved the final manuscript.

380

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384

385 **Competing interests**

386 None declared.

387

388 **Ethics approval**

389 The Centralised Institutional Review Board of SingHealth (reference 2018/2529).

390

391 **Data sharing statement**

392 The majority of data collected will be published. Any unpublished, de-identified data will be
393 made available to interested persons on request.

394

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12 420 intake with glucose tolerance among pregnant African American women. *Matern Child Nutr*
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17 464 **Figure 1** Flow diagram of the study design
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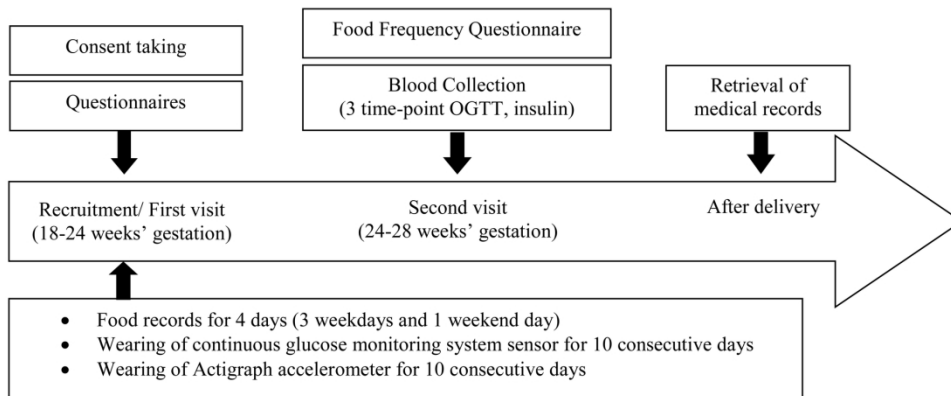


Figure 1 Flow diagram of the study design

175x101mm (300 x 300 DPI)