



Cite this: DOI: 10.1039/d2fo03060h

# Associations between oral processing, saliva, and bolus properties on daily glucose excursions amongst people at risk of type-2 diabetes†

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**Background:** A greater time spent with glucose above the normal range (TAR) has been associated with poorer glycaemic control amongst pre-diabetic individuals. Individual differences in oral processing behaviours and saliva amylase activity have been shown to influence glucose responses. **Objective:** The current study is a preliminary exploration of the associations of oral processing behaviours, bolus characteristics, and salivary amylase activity with the variability in daily glucose excursions within a free-living setting in populations with an elevated risk of type-2 diabetes. **Method:** Participant oral processing behaviour was derived from video recordings while they consumed a test meal. Post-meal bolus characteristics and saliva properties were measured. Participants were fitted with a continuous glucose monitor (CGM) which monitored blood glucose fluctuation over 7 consecutive free-living days. Dietary intake was recorded through a smartphone application and physical activity was monitored using a wrist worn accelerometer. **Results:** Participants varied in daily time spent with glucose above the normal range (>7.8 mmol l<sup>-1</sup>) from 0% to 15%. Greater saliva uptake in the bolus was associated with a higher time spent above the normal range for glucose ( $\beta = 0.067$  [95% CI = 0.015, 0.120];  $p < 0.05$ ), which remained significant after adjustment for dietary carbohydrate intake and BMI. Salivary amylase and saliva flow rate were not significantly associated with the time spent above the normal range. **Conclusion:** In addition to conventional dietary factors, more research is needed to understand how eating behaviours such as oro-sensory exposure, bolus surface area, and saliva uptake contribute to daily variations in postprandial glucose excursions among populations with a higher risk of developing type-2 diabetes.

 Received 11th October 2022,  
 Accepted 5th February 2023

DOI: 10.1039/d2fo03060h

rsc.li/food-function

## 1. Introduction

Individuals vary considerably in their blood glucose responses and prolonged periods spent above the normal range of glucose is associated with an increased risk of Type-2 diabetes

(T2D).<sup>1</sup> The normal glucose range in healthy populations varies between 3.9 mmol l<sup>-1</sup> and 7.8 mmol l<sup>-1</sup> throughout a day,<sup>2</sup> while consistently spending larger proportions of time outside of this glucose range has previously been linked to greater oxidative stress.<sup>3–5</sup> In recent years continuous glucose monitor (CGM) devices are growing in popularity as they allow people to measure their glucose continuously over time.<sup>6</sup> CGM devices measure changes in interstitial glucose level, which is the glucose found in the fluid between the cells and this provides an approximation of blood glucose. Over the course of a given day, users can track the proportions of glucose variability via Time In Range (TIR), Time Below Range (TBR) and Time Above Range (TAR).<sup>7</sup> These metrics give an indication of the quality of glycaemic control which can be used to personalize approaches to managing glucose excursions through changes in diet and physical activity.<sup>2</sup>

The oral phase of digestion occurs during mastication where the original food structure is broken down, lubricated and softened into a food bolus.<sup>8</sup> Saliva plays a key role during oral processing where salivary amylase initiates the breakdown of starch digestion within the food structure, which releases

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† Electronic supplementary information (ESI) available. See DOI: <https://doi.org/10.1039/d2fo03060h>

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glucose and stimulates initial insulin release.<sup>9,10</sup> Salivary amylase levels are known to differ widely between individuals, with between 3–17 copy number variants of the AMY 1 gene.<sup>11</sup> To date, the role of salivary amylase on glucose responses in healthy and pre-diabetic individuals have yielded equivocal findings.<sup>9,11</sup> Recent findings suggest that a greater salivary amylase activity but not AMY 1 gene was associated with reduced risk of diabetes<sup>12</sup> while Higuchi and colleagues showed that AMY 1 gene and high salivary amylase activity were correlated with lowered HbA1C.<sup>13</sup> This suggests that variation in salivary amylase activity may interact with oral processing behaviours during the oral phase of digestion<sup>14</sup> such as chews per bite and time a food spends in-mouth (*i.e.* orosensory exposure time), to influence postprandial glucose. This is further supported by a recent study from our group which also failed to show a significant role for salivary amylase activity in explaining the observed variance in glucose responses.<sup>10</sup>

Individual differences in oral processing behaviour are known to influence metabolic responses to the food ingested<sup>15</sup> and when combined with variations in saliva secretion during mastication can influence postprandial glucose responses after meals. Recent studies have demonstrated that longer mastication duration led to higher bolus saliva uptake, and this was associated with a higher postprandial glucose and insulin response.<sup>10,16</sup> Extending the degree to which a bolus is chewed has been shown to impact postprandial glucose release<sup>17–19</sup> and is associated with higher early insulin secretion, which influenced temporal changes in glucose but did not attenuate the blood glucose responses.<sup>10,20</sup> The sizes of formed bolus particles have also been shown to influence postprandial response, whereby smaller food bolus particles led to a higher blood glucose response among healthy individuals.<sup>21,22</sup>

Less is known about whether oral processing behaviours, bolus and saliva properties are associated with habitual daily glucose excursions among individuals at a higher risk of T2D. The impact of oral processing on glucose release over time has been studied acutely in healthy and diabetic populations using a CGM following an acute fixed carbohydrate test meal.<sup>23,24</sup> Saito and colleagues monitored blood-glucose variations across 3 meal sessions in healthy participants and showed that eating at a faster pace led to higher glucose release throughout the day.<sup>23</sup> The impact of macronutrients on glucose excursion has shown that addition of protein or fat to carbohydrate meal<sup>25</sup> and consumption of vegetables before a carbohydrate meal can delay glucose spikes in individuals with diabetes when monitored over CGM.<sup>26</sup> It remains unclear how oral processing behaviour, and saliva secretion interact to influence food bolus properties and subsequent glucose changes among individuals who are at risk of T2D.

The current study presents a preliminary exploration of the associations between differences in individual oral processing behaviours, bolus characteristics at swallow, and salivary amylase activity to the variability in daily glucose excursion above normal range in a free-living population with a higher risk of type-2 diabetes over a 7-day period.

## 2. Materials and methods

### 2.1. Subjects

Participants ( $N = 33$ ) were enrolled from the Singapore Multi-Ethnic Cohort.<sup>27</sup> All participants were aged between 21–60 years and were classified as being at risk for T2D based on either having elevated HbA1c levels (42–47 mmol mol<sup>-1</sup>; pre-diabetes), a BMI in the overweight range (25–30 kg m<sup>-2</sup>) or an immediate family history of diabetes. Additional recruitment criteria included individuals with healthy dentition and normal mastication abilities. Exclusion criteria included having a known sensitivity to medical-grade adhesives, allergies to ingredients of the study meal, pregnancy or lactation, long-term medication use, and self-reported medical conditions (bleeding disorders, diabetes mellitus of any type, hypertension, thyroid disease, myocardial infarction, cancer, psychosocial impairment). All participants provided informed consent and the study was approved by the National Healthcare Group Domain Specific Review Board (NHG DSRB, Ref: 2018/01220), Singapore. This trial is registered at clinical-trial.gov (NCT04522063).

### 2.2. Study procedure

A detailed description of participants and measures are described elsewhere.<sup>10</sup> In brief, participants were invited to attend two laboratory visits separated by 10 days. On the first visit (day 1), anthropometric measurements, blood pressure, and blood were collected. This was followed by a mixed meal tolerance test where participants consumed a fixed carbohydrate meal following a standardized protocol.<sup>28</sup> Each participant's meal consumption was video recorded to facilitate the analysis of oral processing behaviours. Separately, participants chewed a fixed portion of the carbohydrate meal to the point of swallow and expectorated for bolus analysis. CGM device was fitted to each participant's upper arm to measure glucose levels intermittently and a smartphone application was also provided for participants to record food and beverage consumption for the subsequent 10-days. During a second visit (day 11 onwards) participants had their CGM removed and completed a series of oral processing, bolus, and saliva measures.<sup>10</sup> Full details of the methods used for saliva and bolus analysis are reported elsewhere.<sup>10</sup>

### 2.3. Test meal and bolus collection

The fixed carbohydrate meal (Egg Rice, Tesco brand, NTUC FairPrice, Singapore) consisting of 50 g available carbohydrates, 6.1 g total fat and 6.8 g protein was served to the participants. The amount of test meal served was 169.5 g (1.73 kcal g<sup>-1</sup>) and drinking water (250 ml) was given to the participants after meal completion.

For bolus collection, an additional test meal sample (5 g) was provided to participants to 'chew and spit' along with a fixed amount of water (25 g) to ensure complete recovery of the sample. The measurement was done in duplicate. Each participant's bolus sample was measured Fiji image analysis<sup>29</sup> for number of particles, average particle Feret size (mm<sup>2</sup>) and



total bolus surface area (mm<sup>2</sup>) at swallow. Total bolus surface area represents the sum of the surface area of all bolus particles at expectoration (mm<sup>2</sup>). Bolus saliva uptake was estimated at the point of swallow.

$$\text{Bolus saliva uptake (\%)} = \frac{\text{weight of wet bolus(g)} - \text{weight of food(g)}}{\text{weight of food(g)}} \times 100$$

#### 2.4. Saliva collection

Using passive drool method, participants were asked to mimic gum chewing with a piece of parafilm (0.29 g, Parafilm M PM996) for 5-minutes. The measurement was conducted in duplicate with a 2-minute rest time in between. Saliva flow rate (g min<sup>-1</sup>) was calculated from average saliva volume collected. Stimulated salivary amylase activity (U ml<sup>-1</sup>) were obtained through colorimetric assay (Salimetrics Assay #1-1902, Salimetrics, LLC.) and cell imaging reader (Cytation 5, Winooski, VT, USA).

#### 2.5. Behavioural coding of meal oral processing

A webcam (Logitech HD c310) was used to record oral processing behaviours during the test meal on day 1, and *post-hoc* behavioural coding analysis was used to derive objective quantitative measures of oral processing behaviours, using a standardized behavioural coding approach described previously.<sup>30</sup> The frequencies of 'bite', 'chew', 'swallow' and duration of 'time where food is spent in mouth' were coded by a trained coder using an annotation software (ELAN 5.8, Max Planck Institute for Psycholinguistics, The Language Archive, Nijmegen, Netherlands). The coded behavioural data was used to derive a series of oral processing measures including average bite size (g per bite), average eating rate (g min<sup>-1</sup>), time where food spends in the mouth (seconds). A second trained coder validated the coding data by re-coding a random sample of 10% of the recordings, where coding was deemed acceptable when ≥80% agreement was achieved between coders.

#### 2.6. Continuous glucose monitoring

Participant glucose level was repeatedly measured at 15-minute intervals (*i.e.*, 96 measurements per day per person) using a CGM device (Freestyle Libre; Abbott Diabetes Care, Witney, Oxon, UK) between the two laboratory visits. Although the CGM sensor can store up to 8 hours of glucose measurements, participants were instructed to scan the CGM sensor every 6 hours to transfer the data using a paired reader device to avoid data loss. To avoid behavioural modification from reading the CGM values, the reader device was masked with an opaque sticker. As the glucose measurement from the CGM device is most accurate after the first 3 days of wear,<sup>31</sup> it was determined *a priori* only free-living CGM measurements from day 4 to day 10 (*i.e.*, 7 days) would be used for analysis. In addition, only days with CGM measurements for at least 16 hours were considered as valid and analysed. Glucose values between 3.0 mmol l<sup>-1</sup> and 7.8 mmol l<sup>-1</sup> were considered as in the normal range. For each participant on each

day, their daily percentage of time above range (TAR, >7.8 mmol L<sup>-1</sup>) and daily average glucose level were calculated using the CGM measurements from the corresponding day.

#### 2.7. Dietary and physical activity assessments

An event-contingent Ecological Momentary Assessment (EMA) was used for dietary recording *via* a smartphone application ("the app") developed for this study (Jana Care Inc., Boston, MA, USA). Participants were guided to log all foods and beverages consumed during day 4 to day 10. A text reminder was sent on day 3. Participants logged meal types (*i.e.*, breakfast, lunch, dinner, or snack), food and beverage names, and portion sizes corresponding to what they had eaten.<sup>32,33</sup> The food composition database from Health Promotion Board Singapore and a comprehensive database of US foods was used to derive participants' energy and nutrient intake on each day.<sup>32,33</sup> Mean daily energy and carbohydrate intakes were calculated for each participant. Days with reported energy intakes below 500 kcal or greater than 5000 kcal were excluded. Carbohydrate was expressed as a percentage of total energy intake.

Each participant wore an accelerometer (Actigraph - Model wGT3x + BT) on the non-dominant wrist between the two laboratory visits. An open-source R-package 'GGIR' (version 1.11-0)<sup>34</sup> was used to derive daily time spent in moderate-to-vigorous physical activity (MVPA). Days with less than 16 hours of wear time were excluded in the analysis. Full details of the analyses for dietary and physical activity assessments are reported elsewhere.<sup>35</sup>

#### 2.8. Statistical analysis

Participants' baseline characteristics are presented with descriptive statistics (mean ± SD). Participant's 24-hour glucose measurements were summarized and plotted based on participants' average daily TAR levels (0, 0 to <5%, or 5 to <15%). The selected glucose target range is determined *a priori* before conducting the analysis, which is consistent with other analysis of this study.<sup>35</sup> Generalised additive model was applied to smooth the time series curves. The Generalized Estimating Equation (GEE) models were used to examine the association of participants' glucose TAR on each day (the dependent variable) with the oral processing, saliva, and bolus characteristics (fixed factors). TAR levels are taken as continuous variable. An independent within-cluster correlation structure was used, where participants served as clusters (random factor). To avoid compromising the statistical reliability due to the modest sample size, the main analysis examined one fixed factor (univariate) in each GEE models, instead of multiple fixed factors simultaneously. Sensitivity analyses were performed, which included one additional fixed factor as a covariate at a time (*i.e.*, BMI, daily carbohydrate and sugar intakes (% energy), and total daily and daily 10 minutes moderate-to-vigorous physical activity). Covariates such as age, gender, and other demographic variables were not significantly associated with daily glucose percentage above range, hence no further adjustments were made to these variables. To compare the relative association effect sizes by different factors, regression coefficient was also standardized using a published approach<sup>36</sup>



by adjusting to the standard deviation of the independent and dependent variables. Statistical significance was set at 5% ( $p < 0.05$ ). Analysis was performed using SPSS (IBM SPSS statistics, version 26) and R statistical software (version 3.6.1). R package 'geepack' (version 1.3-2) was used for GEE models.

### 3. Results

#### 3.1. Participants' characteristics

A total of 33 individuals, but seven were excluded from the analyses: one did not fulfil the inclusion criteria, three withdrew because of sensor discomfort, and one had surgery before study commencement and two others were removed due to test meal video playback error. The remaining 26 participants provided valid data on CGM (165 days), food intake, oral processing, saliva, and bolus properties, and were thus included in the final analysis.

Baseline characteristics of participants are shown in Table 1. These participants ( $N = 26$ , 9 females) had a mean ( $\pm$ SD) age of 45.3 (9.8) years, a BMI of 27.4 (1.8) kg m<sup>-2</sup>, baseline fasting glucose of 4.8 (0.3) mmol L<sup>-1</sup> and an HbA1c level of 5.4 (0.4) %. The average measurement hours per day during the valid days were 23.8 (0.7) hours for the accelerometer and 21.8 (1.8) hours for the glucose monitoring. During the 7-day study period, there were 179 participant-days with valid CGM data, of which 145 participant-days had valid accelerometer data and 142 participant-days had valid dietary data. 118 participant-days had all three types of valid data available simul-

taneously. On average, the participants provided 5.7 (0.9) days of valid accelerometer data, 6.4 (1.3) days of valid glucose monitoring data, and 6.1 (1.3) days of valid dietary data per person during the 7-day study period for the analysis. The average stimulated saliva flow rate 1.3 (1.0) g min<sup>-1</sup> and stimulated  $\alpha$ -amylase activity 143.2 (79.3) U mL<sup>-1</sup> were within the normal range of previously reported values.<sup>37</sup> Participants oral processing behaviours for the test meal were similar to those reported in previously published literature<sup>10</sup> for a similar meal and participants for (mean  $\pm$  SEM) bite size 5.6 (1.3) g per bite, eating rate 35.0 (13.9) kcal min<sup>-1</sup>, bolus saliva uptake 43.0 (27.1) g, bolus saliva incorporation rate 5.5 (3.6) g min<sup>-1</sup>, bolus surface area 2105.8 (429.6) mm<sup>2</sup>, and bolus particle size 1.3 (0.6) mm<sup>2</sup>.

Based on the inclusion criteria, 25 participants recruited had a BMI of >25 (TAR 0%:  $n = 6$ ; TAR 0 to <5%:  $n = 15$ ; TAR 5 to 15%:  $n = 4$ ). Amongst the 4 participants with elevated BMI of >25, 1 had HbA1c of >6% (or 42 mmol mol<sup>-1</sup>). Significant differences between the 3 TAR groups were observed for mean glucose ( $p < 0.01$ ), TAR/day ( $p < 0.01$ ) and TIR day ( $p = 0.043$ ). Participants who spent 5 to 15% in TAR had highest mean glucose level of 5.6 (0.2) mmol L<sup>-1</sup>, lowest TBR of 0.8 (1.1)% and TIR 89.4 (4.3)%.

#### 3.2. Continuous glucose monitoring (CGM) profiles

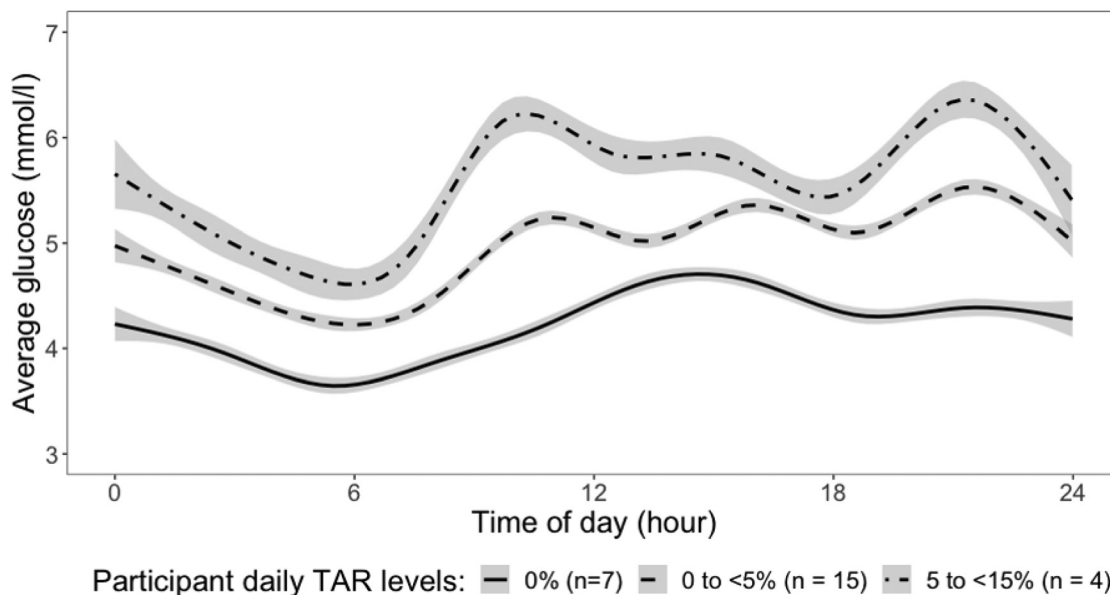
Fig. 1 summarizes the 24-hour mean glucose level of participants from 7 days CGM data into 3 groups based on the time spent above the normal range (3.0–7.8 mmol l<sup>-1</sup>) for blood glucose. Most participants ( $n = 15$ ) spent between 0 and 5% of time per day with glucose above the normal range, while 7 par-

**Table 1** Participants' characteristics

|  | $N = 26$ (male = 17) | TAR 0%<br>( $n = 7$ , male = 5) | TAR 0 to <5%<br>( $n = 15$ , male = 10) | TAR 5 to <15%<br>( $n = 4$ , male = 2) | $p$ -Value |
|--|----------------------|---------------------------------|---|--|------------|
| Age (years)  | 45.3 (9.8)           | 46.1 (11.8)                     | 44.1 (9.4)                              | 48.0 (9.4)                             | 0.727      |
| BMI (kg m <sup>-2</sup> )                          | 27.4 (1.8)           | 26.5 (1.5)                      | 27.8 (2.0)                              | 27.5 (0.6)                             | 0.304      |
| HbA1c (%)  | 5.4 (0.4)            | 5.4 (0.2)                       | 5.3 (0.4)                               | 5.8 (0.5)                              | 0.216      |
| Mean glucose/day (mmol L <sup>-1</sup> )           | 4.8 (0.6)            | 4.2 (0.5)                       | 4.9 (0.3)                               | 5.6 (0.2)                              | <0.001*    |
| TAR/day (%)  | 2.4 (3.6)            | 0.0 (0.0)                       | 1.5 (1.1)                               | 9.8 (3.7)                              | <0.001*    |
| TBR/day (%)  | 2.5 (6.7)            | 6.5 (11.8)                      | 1.2 (3.0)                               | 0.8 (1.1)                              | 0.183      |
| TIR/day (%)  | 95.1 (7.2)           | 93.5 (11.8)                     | 97.3 (3.7)                              | 89.4 (4.3)                             | 0.043*     |
| <b>Saliva properties<sup>a</sup></b>               |                      |                                 |   |  |            |
| Stimulated $\alpha$ -amylase (U mL <sup>-1</sup> ) | 143.2 (79.3)         | 107.1 (75.6)                    | 149.1 (71.6)                            | 175.2 (112.6)                          | 0.511      |
| Saliva flow rate (g min <sup>-1</sup> )            | 1.3 (1.0)            | 1.1 (0.7)                       | 1.3 (1.1)                               | 1.7 (0.6)                              | 0.338      |
| <b>Bolus characteristics<sup>b</sup></b>           |                      |                                 |   |  |            |
| Average particle size (mm <sup>2</sup> )           | 1.3 (0.6)            | 1.3 (0.4)                       | 1.4 (0.7)                               | 1.1 (0.1)                              | 0.849      |
| Total surface area (mm <sup>2</sup> )              | 2105.8 (429.6)       | 2292.6 (431.3)                  | 2020.7 (371.9)                          | 2098.2 (634.9)                         | 0.426      |
| Saliva incorporation rate (g min <sup>-1</sup> )   | 5.5 (3.6)            | 5.0 (3.3)                       | 5.0 (4.0)                               | 7.8 (1.7)                              | 0.117      |
| <b>Oral processing behaviours</b>                  |                      |                                 |   |  |            |
| Bite size (g per bite)                             | 5.6 (1.3)            | 5.3 (1.1)                       | 5.6 (1.4)                               | 6.1 (1.2)                              | 0.653      |
| Time in mouth (seconds)                            | 591.0 (266.4)        | 717.7 (367.6)                   | 497.5 (175.5)                           | 719.8 (272.7)                          | 0.074      |
| Eating rate (kcal min <sup>-1</sup> )              | 35.0 (13.9)          | 29.8 (13.5)                     | 39.7 (14.1)                             | 26.6 (7.7)                             | 0.074      |
| <b>Combined bolus and saliva</b>                   |                      |                                 |   |  |            |
| Saliva uptake (g) <sup>b</sup>                     | 43.0 (27.1)          | 35.8 (20.2)                     | 37.6 (27.6)                             | 71.6 (18.6)                            | 0.076      |

<sup>a</sup> Missing data: 1 participant did not complete saliva measures ( $n = 25$ ). <sup>b</sup> Missing data: due to incomplete recovery of bolus samples in 2 participants ( $n = 24$ ).





**Fig. 1** Average blood glucose levels over 7 days of 24-hour CGM for participants ( $n = 26$ ;  $n$  participant-days = 165) grouped into three levels according to % time per day spent with glucose level above  $7.8 \text{ mmol l}^{-1}$ : 0%; 0 to <5%; 5 to <15%. TAR: Time-Above-Range (%). The shaded area around each line represents the mean ( $\pm$ SE).

participants had all glucose levels within the normal range. Four participants experienced more frequent fluctuations with glucose level above the normal range for 5 to <15% of time per day.

### 3.3. Contributors to time spent above normal range for daily glucose excursion

Table 2 and Fig. 2 show the univariate associations between saliva, bolus, and oral processing characteristics and TAR, the daily percentage of time spent above normal glucose range of  $7.8 \text{ mmol l}^{-1}$ . The analysis included daily carbohydrate and sugar intakes as covariate as these have direct impact on glucose excursion. Neither movement (physical activity) nor dietary intakes were associated with mean glucose levels. No association was found between stimulated salivary amylase and saliva flow rate with TAR. There was a significant relationship between bolus saliva uptake and TAR, where greater

saliva uptake in the bolus is associated with a higher TAR ( $\beta = 0.067$  [0.015, 0.120];  $p = 0.012$ ). No associations were found between saliva, bolus, and oral processing characteristics and TBR (data not shown). TIR was associated with lower salivary amylase stimulation ( $\beta = -0.025$  [-0.046, -0.004];  $p = 0.017$ ) and smaller bite size ( $\beta = -1.982$  [-3.640, -0.323];  $p = 0.019$ ) (data not shown). Bolus saliva uptake remained significantly associated with TAR with consistent effect sizes after controlling for BMI, carbohydrate intake, sugar intake or physical activity (see ESI Tables 1 and 2† for model performance).

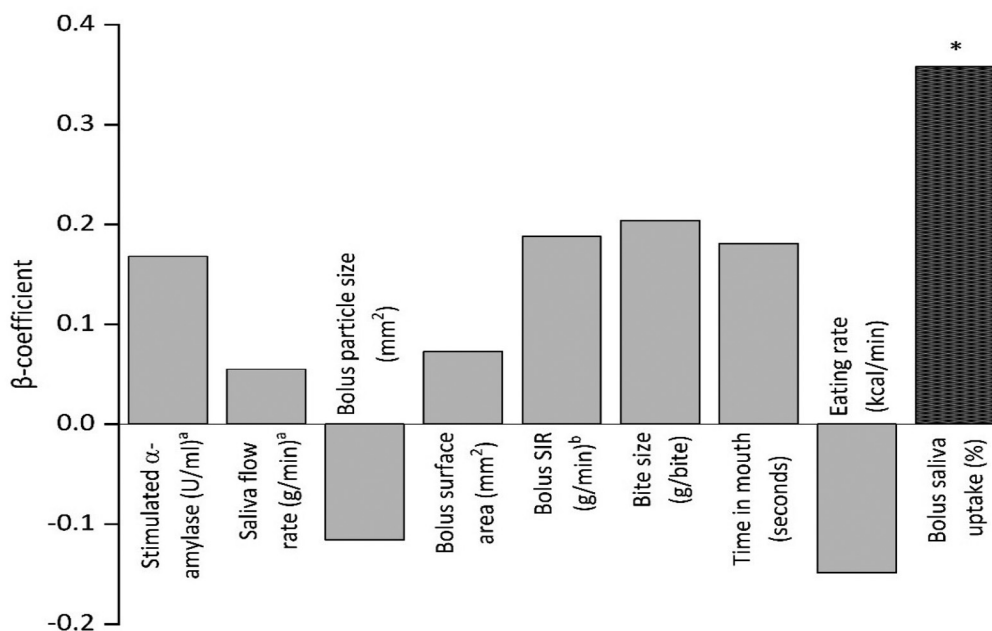
Results from sensitivity analysis showed that the adjusted associations for bolus saliva uptake with TAR remained significant ( $\beta$ , effect size) when controlled for various covariates: BMI, 0.069 (95% CI: 0.016–0.122,  $p = 0.011$ ); daily carbohydrate intake, 0.073 (95% CI: 0.017–0.128,  $p = 0.011$ ); sugar intake, 0.073 (95% CI: 0.020–0.126,  $p = 0.007$ ); daily 10-minute bout of

**Table 2** Associations between saliva, bolus, and oral processing characteristics with percentage daily Time-Above-Range (TAR) for glucose over a 7-day period

| $N = 26$                           | Variables   | Unstandardized $\beta$ (95% CI) | $p$ -Value    |
|------------------------------------|---|---------------------------------|---------------|
| Saliva properties <sup>a</sup>     | Stimulated salivary amylase activity ( $\text{U ml}^{-1}$ ) | 0.010 (-0.004, 0.025)           | 0.158         |
|                                    | Saliva flow rate ( $\text{g min}^{-1}$ )                    | 0.271 (-0.604, 1.145)           | 0.544         |
| Bolus characteristics <sup>b</sup> | Average particle size ( $\text{mm}^2$ )                     | -0.975 (-2.662, 0.712)          | 0.257         |
|                                    | Total surface area ( $\text{mm}^2$ )                        | 0.001 (-0.004, 0.006)           | 0.770         |
|                                    | Saliva incorporation rate SIR ( $\text{g min}^{-1}$ )       | 0.280 (-0.067, 0.627)           | 0.114         |
| Oral processing behaviours (OPB)   | Bite size (g per bite)                                      | 0.761 (-0.373, 1.895)           | 0.189         |
|                                    | Time in mouth (seconds)                                     | 0.003 (-0.005, 0.012)           | 0.473         |
|                                    | Eating rate ( $\text{kcal min}^{-1}$ )                      | -0.050 (-0.171, 0.072)          | 0.426         |
| Combined bolus and saliva          | Bolus saliva uptake (%) <sup>b</sup>                        | 0.067 (0.015, 0.120)            | <b>0.012*</b> |

Unstandardized  $\beta$  refers to regression coefficient from the GEE model. \* $p$  value <0.05. Positive  $\beta$ -coefficient values represent predictors associated with an increase in TAR. <sup>a</sup>Missing data: 1 participant did not complete saliva measures ( $n = 25$ ). <sup>b</sup>Missing data: due to incomplete recovery of bolus samples in 2 participants ( $n = 24$ ).





**Fig. 2** Standardized associations between saliva, bolus, and oral processing characteristics and daily % Time-Above-Range (glucose level  $>7.8 \text{ mmol l}^{-1}$ ) over a 7-day period ( $N = 26$ ). Positive  $\beta$ -coefficient values represent predictors associated with an increase TAR. \* $p$  value  $< 0.05$ . <sup>a</sup>Missing data: 1 participant did not complete saliva measures ( $n = 25$ ). <sup>b</sup>Missing data: 2 participants due to incomplete recovery of bolus samples ( $n = 24$ ).

moderate-to-vigorous physical activity 0.068 (95% CI: 0.008–0.126,  $p = 0.024$ ); and total daily moderate-to-vigorous physical activity, 0.067 (95% CI: 0.012–0.122,  $p = 0.017$ ).

## 4. Discussion

The current preliminary study sought to explore whether there is a relationship between oral processing parameters and time spent above the normal range for blood glucose. We showed that participant bolus saliva uptake was significantly associated with a longer time spent above the normal range for blood glucose, and that this relationship remained significant after controlling for BMI and dietary carbohydrate intake. Other factors such as saliva flow rate and  $\alpha$ -amylase activity were not significantly associated with 24-hour glucose excursions.

Bolus saliva uptake was a significant predictor of the percentage of time participants spent 'above' the target range for blood glucose. This association remained significant after adjustment for carbohydrate intake, sugar intake, physical activity and BMI. Our study suggests that it is the combined effect of both oral processing behaviours and saliva that links to the differences in glucose variation above normal range. Bolus saliva uptake is a product of a tripartite of factors involved in the oral phase of digestions that includes the rate of eating and time a foods spends in mouth, chewing efficiency to change the surface area of food particles to encourage greater saliva uptake, and the rate of saliva production and incorporation to assist bolus formation and agglomeration

before swallow.<sup>15</sup> Extended mastication increases the duration a food spends in-mouth (*i.e.* oro-sensory exposure time), allows more time for saliva uptake and action on the available starch for digestion. In addition, longer chewing duration leads to an increase in the number of food particles that are smaller in size and a larger bolus surface area, which stimulates more saliva production and greater saliva uptake by bolus particles.<sup>8,22</sup> Previous studies have reported that starch digestion increases when food particle size reduces and bolus surface area increases,<sup>10,38</sup> which stimulates greater amylase activity and early glucose release. Experiments on mixed meal tolerance test demonstrated that increasing mastication supports early glucose release and stimulates insulin secretion,<sup>10,16</sup> which suggests that greater bolus saliva uptake could change the kinetics of glucose release and may aid insulin release.<sup>8</sup> Although insulin was not included in this study, it is worth noting recent studies have found the association between glucose fluctuation above normal range and insulin resistance<sup>35,39</sup> in healthy and at risk population. These preliminary findings merit further experimentation to better understand the contribution of oral processing behaviours and bolus saliva uptake to glucose variations above normal range in pre-diabetic populations.

People vary widely in their habitual eating behaviours and individual differences in oral processing behaviour when consuming a meal can influence both the oro-exposure time and saliva uptake, which in turn could impact insulin releases. In combination, these factors could affect long-term maintenance of blood-glucose levels. It is worth noting that although our finding demonstrated slower eating rate and a higher saliva



uptake were significantly associated with higher insulin release but also a longer time spent above the normal range for blood glucose. Previous research has shown that faster eating rate and taking fewer chews per bite has been linked with higher food intake<sup>18,40</sup> and a lower satiety response.<sup>10,20</sup> The current findings highlight that food textures can directly influence an individual's eating behaviour during a meal, and previous research has shown that texture changes to a meal can influence both eating rate and energy intake.<sup>41–43</sup> Chewing for longer will slow eating rate, reduce energy intake and enhance insulin release and post-meal satiety per kcal consumed, but may stimulate a higher glucose trajectory. Further research is needed to better understand how food texture could be used as a natural approach to eating speed and influence food–saliva interactions and glucose response. In addition to food texture, it may also be possible to alter the material properties of foods to change their fracture properties and slow the available surface bolus surface area to alter saliva uptake kinetics and to better support glucose metabolism.<sup>44</sup> A recent experiment by R. Janani and colleagues demonstrated how combinations of specific food texture modifications can be used to alter micro-structural patterns of oral processing and eating speed.<sup>45</sup> A closer understanding of these changes will create new opportunities to moderate eating behaviours and metabolic response in the future.

Variations in salivary amylase activity were not associated with time spent above normal glucose range, which is in line with findings from previous research.<sup>46,47</sup> Salivary amylase levels were within what can be considered a 'normal' range within our participant pool, and it is possible that salivary amylase only begins to influence postprandial glucose excursions at the extremes of the distribution, at either high or low levels of activity. Our preliminary findings highlight for the first time the impact of increased bolus saliva uptake on glucose variations, suggesting the importance of considering variations not only in saliva composition but also in bolus surface area, and oral processing behaviours such as oro-sensory exposure time.

Among participants, 15% spent between 5–15% of their time above 7.8 mmol l<sup>-1</sup> in their daily glucose excursions. Extended periods of daily glucose excursion above the normal range is indicative of a higher risk of T2D. Earlier studies have reported that fluctuations of blood glucose above the normal range can trigger reactive oxygen species and oxidative stress, leading to endothelial dysfunction and the progression of T2D.<sup>3–5,48</sup> In pre-diabetic population, early detection of glucose fluctuations above normal range may serve as a prevention strategy to the development of full-on diabetes. A personalised approach to guiding eating behaviours (*e.g.* eating pace and saliva secretion during food intake) as part of lifestyle and nutritional strategies could be applied for individuals at a higher risk of T2D, to support efforts to maintain a normal blood glucose levels within the desired range. In the present study we observed associations between natural variations in blood glucose and habitual eating behaviours in a free-living population with a higher risk of T2D. This is significant as much previous research has been conducted under lab-

oratory conditions where participants follow a prescribed oral processing regime, such as being directed to fix the number of chews per bite or eating at a specific speed. Our findings highlight that natural variations in oral processing behaviour can have an impact on glucose control.

A strength of our preliminary study was that participants were free to follow their usual diet, meal patterns, and oral processing behaviour. In addition, we focused on a pre-diabetic population and performed detailed analysis of their oral processing behaviours and bolus and saliva characteristics. The extended duration of CGM profiling at 15-minute intervals was a strength and this provided an opportunity to assess variations over a prolonged duration of 7-days. The generalizability of our study findings is limited by the loss of data and reduced sample size during the experimental duration, and the use of self-reported diet recall where we applied ecological momentary assessment (EMA) to capture dietary patterns. Self-reported food intake measures are often associated with under reporting and where participants were able to record their food intakes *via* an online diet application away from the test centre.<sup>49</sup> We did not ask participants to record their mealtimes and hence was unable to investigate the associations between circadian changes in glucose and meal timing and carbohydrate loads during meals. We collected data from participants for 10 days and discarded the first 3 days such that only 7 consecutive days were included in the final analysis. Whereas we believe this provided a representative impression of typical variations in blood glucose among a pre-diabetic populations, we recommend that future studies track eating behaviour and glucose variations for an extended period, to observe how these metabolic variations impact metabolic markers of glucose metabolism such as haemoglobin A-1C (HbA1c<sup>50</sup>).

## 5. Conclusion

We showed that bolus saliva uptake was associated with daily variations in glucose excursions above the normal range among participants at a higher risk of Type 2 diabetes, under free-living conditions. Our findings suggest the importance of considering eating behaviours in conjunction with dietary factors in the understanding of differences in individual glucose excursions. These findings require further research, and we recommend future intervention studies that evaluate the impact of manipulating oral processing behaviours on postprandial glucose excursions over time.

## Abbreviations

|     |                                 |
|-----|---------------------------------|
| BMI | Body mass index                 |
| CGM | Continuous glucose monitor      |
| EMA | Ecological momentary assessment |
| GEE | Generalized estimating equation |
| T2D | Type 2 diabetes                 |
| TAR | Time-above-range                |



## Author contributions

CW, RMVD and CGF study design. ATG and XHC data collection. JY, RMVD, ATG, CGF data analysis and interpretation. ATG and CGF writing. XHC, JY, CW, RMVD, CGF review and edit.

## Ethical statement

This study was approved by the National Healthcare Group Domain Specific Review Board (NHG DSRB, Ref: 2018/01220), Singapore.

## Clinical trial registration

This trial is registered at [clinicaltrials.gov](https://clinicaltrials.gov) (NCT04522063).

## Consent to participate

All participants gave written informed consent and were compensated for their time.

## Consent for publication

All authors have seen and approved the final version of the manuscript. The article is the authors' original work and has not received prior publication and is not under consideration for publication elsewhere.

## Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

## Code availability

Software application are available from the corresponding author on reasonable request.

## Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

The authors would like to thank Michelle Choy for her support during the data collection. The study was supported by the

Singapore Ministry of Health's National Medical Research Council under its Centre Grant Programme (NMRC/CG/M009/2017\_NUH/NUHS). A. T. G. and C. G. F. were supported by the Singapore Biomedical Research Council Food Structure Engineering for Nutrition and Health (Sub-grant Grant No. H18/01/a0/E11, Awarded to PI: Forde, C. G.).

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