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Wyatt, P, Berry, SE, Finlayson, G orcid.org/0000-0002-5620-2256 et al. (12 more authors) (2021) Postprandial glycaemic dips predict appetite and energy intake in healthy individuals. *Nature Metabolism*, 3 (4). pp. 523-529. ISSN 2522-5812

<https://doi.org/10.1038/s42255-021-00383-x>

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Published in final edited form as:

Nat Metab. 2021 April 01; 3(4): 523–529. doi:10.1038/s42255-021-00383-x.

Postprandial glycaemic dips predict appetite and energy intake in healthy individuals

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Summary

Understanding how to modulate appetite in humans is key to developing successful weight loss interventions. Here, we show that postprandial glucose dips 2-3h after a meal are a better predictor of postprandial self-reported hunger and subsequent energy intake than peak glucose 0-2h and glucose iAUC 0-2h. We explore the link between postprandial glucose, appetite, and subsequent energy intake in 1070 participants from a UK discovery and US validation cohort, consuming

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Role of the sponsor: The sponsor, Zoe Global Ltd, was directly involved in study design, data collection and analysis for this manuscript.

Conflict of interest statement: TD Spector, SE Berry, AM Valdes, PW Franks, A Chan are consultants to Zoe Global Ltd ("Zoe"). J Wolf, G Hadjigeorgiou, H Al Khatib, P Wyatt and I Linenberg are or have been employees of Zoe. JE Blundell is a member of Zoe Global scientific Advisory Board. Other authors have no conflict of interest to declare.

Author contributions: Obtained funding: J.W., G.H., T.D.S.. Study design and developed concept: P.W., A.M.V., Data collection: S.E.B., D.A.D., H.A.K., L.H.N., A.T.C., R.O'D. G.F. Data analysis: P.W., A.M.V., Study coordination: S.E.B., H.A.K., D.A.D., G.H., J.W. I.B. Writing the manuscript P.W. J.B., A.M.V.. All authors reviewed and revised the final manuscript.

8,624 standardised meals followed by 71,715 ad libitum meals, using continuous glucose monitors to record postprandial glycemia. For participants eating each of the standardised meals, the average postprandial glucose dip 2-3h relative to baseline level predicts an increase in hunger 2-3h ($r=0.16$ $P<0.001$), shorter time until next meal ($r=-0.14$ $P<0.001$), greater energy intake 3-4h ($r=0.19$ $P<0.001$) and greater energy intake 24h ($r=0.27$ $P<0.001$). Results are directionally consistent in the US validation cohort. These data provide a quantitative assessment of the relevance of postprandial glycemia in appetite and energy intake modulation.

Funding—Zoe Global Ltd, Wellcome Trust, NIHR.

Introduction

Obesity represents a major global health challenge, with its prevalence in adults and children increasing worldwide(1). Effective short term interventions have, for the most part, failed to translate into successful long-term healthy behaviour(2). Multiple mechanisms make intentional weight loss difficult and facilitate weight regain ((3),(4). In addition to decreases in resting energy expenditure (5) there is also considerable evidence for increases in postprandial appetite following hypocaloric diets and caloric restriction (e.g. (6),(7),(8)). Attenuating hunger after weight loss has been proposed as a strategy for preventing weight regain(9).

Appetite control is influenced by tonic and episodic signalling systems, formerly known as long-term and short-term control. In the last decade research has shown that processes associated with fat-free mass and resting metabolic rate represent tonic determinants of energy intake (10), confirming consistent outcomes from several studies (eg(11) for review). Equally importantly, research continues to be focussed on episodic processes in the physiology of satiety with the goal of identifying key post-prandial events that influence the feeling of hunger after eating, and the occurrence and size of subsequent meals. Postprandial satiety is influenced by a series of physiological events following eating, including gastric distension, the release of gastrointestinal peptides and plasma metabolites. Prominent among these metabolites is plasma glucose, modulated by insulin, which has long been regarded as one of the most potentially interesting markers of postprandial satiety.

Animal models have shown that insulin regulates appetite by activating insulin receptors that increase the hypothalamic expression of appetite suppressing (anorexigenic) peptides, and acts on other neurons to inhibit the expression of appetite stimulating (orexigenic) peptides. In parallel, insulin and leptin act to decrease food intake through appetite suppression and changes in energy homeostasis(12). Investigation of glucose-insulin dynamics in some human studies (13,14) suggests that lower glycemic loads and lower glycemic responses can result in lower postprandial appetite and energy intake, particularly in select overweight populations(15).

The notion that blood glucose is involved in appetite expression was originally proposed by Mayer in his 'glucostatic hypothesis' (16). However, appetite research in the past decades has focused on seemingly more potent appetite signals such as leptin, GLP-1 and other peptides and their receptors. To date, the role of glucose in appetite expression is not resolved and has not been investigated in free-living conditions. In the current study we

revisited Mayer's idea and investigated the potential role of glucose in appetite control in a large-scale real-world environment. We hypothesize that glucose dynamics after a meal influence self-reported appetite, alertness and subsequent energy intake (calories). We have tested this by taking advantage of digital devices, specifically continuous glucose monitors, wearables, and mobile apps. In the largest such study to date we assessed postprandial glucose and appetite in 1,110 healthy adults from the UK and US without diabetes, following them for 2 weeks at home as they consumed both standardised breakfast and ad libitum meals. By using continuous glucose monitors and a series of standardised meals at breakfast and recording all ad libitum food intake it has been possible to estimate the effect of postprandial glycemia on postprandial appetite and energy intake.

Methods

Study Participants

The PREDICT 1 Cohort 1 study(17) (Personalised REsponses to Dietary Composition Trial) was a two-centre study conducted between 2018 and 2019. The first participant was enrolled on 4 August 2018, the last clinical visit was completed on 24 April 2019, with the primary cohort based at King's College London, UK, and a validation cohort (that underwent the same profiling as in the UK) assessed at Massachusetts General Hospital (MGH) in Boston, Massachusetts, USA. In the UK, participants (target enrolment = 1,000, total enrolled n=1010) were recruited from the TwinsUK cohort, a prospective cohort study and online advertising. In the US, participants (target enrolment = 100, total enrolled = 100) were recruited through online advertising, research participant databases and *Rally for Research* (<https://rally.partners.org/>), an online recruiting portal for research trials. Ethical approvals for the studies were obtained in the UK Ethics was granted by the London - Hampstead Research Ethics Committee (REC approval 18/LO/0663) Research Ethics Committee and Integrated Research Application System (IRAS 236407) and by the Partners Healthcare Institutional Review Board (IRB 2018P002078). The informed consent and ethical committee approvals covered all analysis reported in the current study in addition to the key primary outcomes described in Berry et al (18). The trial is registered at ClinicalTrials.gov (registration number: NCT03479866) and was run in accordance with the Declaration of Helsinki and Good Clinical Practice. Descriptive characteristics of participants included in this substudy are shown in Supplementary Table 1.

Study Protocol

The full study protocol is described by Berry (17). In brief, the study consisted of two phases: a 1-day clinical baseline visit which included a breakfast and lunch challenge followed by a 13-day at-home study phase. For this study we focused only on the at home phase. During the at-home phase (days 2-14), participants consumed standardised breakfasts (including a repeat of the clinic breakfast and lunch meal, an OGTT and 6 isocaloric meals of which 5 were in duplicate) as described in the protocol (17), varying in macronutrient composition. Analyses of most primary outcomes have been described in (18). Secondary analyses on hunger and postprandial responses after 2-hours are reported here.

Participants were asked to fast for 3 hours following the standardised breakfast meals, and were then free to eat ad libitum. In later phases of the study, some participants were asked to consume meals with different fasting periods; these meals are excluded from the present analysis. Details of the meals included in the analysis are given in Supplementary Table 2. During the assessment period, participants wore continuous glucose monitors (CGM) and accelerometers to assess physical activity and sleep.

Zoe study app and dietary assessment

The Zoe study app prompted participants to report their hunger and alertness levels on visual analogue scales (from 0 to 10) truncated from Flint et al (19)], by displaying the questions “how hungry are you?” and “how alert are you?” above the scales, at 0 minutes (time of logging) and regular intervals thereafter following the logging of a standardised meal. Participants were supported throughout the study with reminders and communication from study staff through a mobile application (Zoe study app).

Any dietary intake during the study, including test meals, ad libitum meals and accompanying drinks, was recorded in the Zoe study app by participants with the exact time at consumption and ingredient quantities, so that compliance could be monitored by study staff. Only test meals that were completed according to instructions were included in analyses.

QC of the app data: Inclusion criteria for meals

Meals were included in the analysis if they met certain characteristics. Listed below are the exclusions applied to the overall PREDICT 1 Study dataset for the current analysis:

1090 participants completed 9340 standardised meals, of which; 9078 meals were scored as compliant with the study protocol; 8797 meals also had meal valid glucose readings, 8792 meals also occurred during days where the caloric intake was plausible (<6000 kcal), 8683 meals also were fasted until 175 minutes (within 10%, or +/- 100 kcal) and 8624 meals were also part of a daily calorie record that contained at least 5 dishes.

When considering the free-living meals, calorie records were adjusted to remove suspicious and incomplete values. Meal calories were substituted with the food category average calories when missing, or when the reported value was more than 2 standard deviations from the food category average. These adjustments did not affect the significance of the results. When calculating *Time until next meal*, only meals in excess of 50kcal were included in the analysis to avoid noise from tea, coffee and other non-sugar sweetened beverages.

For the energy intake in the 24 hours after a meal we used a window of time (-4 hours to +20 hours relative to the set meal time) because participants were instructed to eat the set breakfast immediately after waking, and there is some natural variation in waking times. This introduces the risk that the 24hr period following the set breakfast may include the *following day's* set breakfast. By calculating the window from -4hr to +20hr, we minimise this issue.

The distributions of all outcome traits are presented in Extended Data Figure 2 (UK cohort) and Extended Data Figure 3 (US cohort).

Data Analysis

From the overall PREDICT 1 dataset ($n=1,110$) a subset was selected for inclusion in this study (see **QC of the app data** for inclusion criteria), corresponding to the responses to the standardised breakfast meals taken at home ($n=1,070$, $m=8,624$) and the subsequent free-living meals consumed over the course of 24h ($m=71,715$). We denote the number of individuals who consumed the meal with ‘n’ and to the number of meals consumed with ‘m’.

From the overall set of meals included in the study, two subsets were analysed:

- a) Meals from participants who ate each of the types of standardised meals at least once ($n=763$, $m=5667$)
- b) Meals eaten in duplicate ($n=1053$, $m=6428$)

(see **Statistical Analysis** for power calculations for these two sets of data)

Meal Baseline calculations

The standard approach to the calculation of glycemic responses such as Glucose iAUC_{0-2h} and Glucose Rise_{0-2h} from clinic data is to consider the average glucose level in the 30 minutes before the start of the meal as the “meal baseline”. This baseline can itself therefore be a source of variability in Glucose responses, especially those immediately after waking. Between two repeats of the same meal for the same individual, there was a ($r=0.76$ $p<0.001$) association between the meal baseline values.

We took advantage of the larger dataset provided by the Continuous Glucose monitors to establish a more stable “average baseline” for each individual. This was calculated as the average of the meal baselines for each of the meals included in the study for that individual. By taking a more stable baseline, we were able to improve the repeatability of the glycemic responses that were studied, and observe stronger associations between the Glucose Dip_{2-3h} and the postprandial measures. This choice of baseline does not affect the direction or significance of the main results of this study. A comparison of the main results is presented in Supplementary Table 4

The phases of the study where measures were taken are illustrated in Figure 1 and are as follows:

Average Baseline Level: for each participant, the mean glucose concentration throughout the 30 minutes before each meal eaten in the study, measured across all meals in the study for that participant.

Phase 1: Pre-Meal: The phase up to 30 minutes before the standardised meal starts. It is characterised by;

- **pre-meal Hunger & Alertness:** the level of hunger and alertness declared by the participant, in the period +/-5mins around the start of consumption of

the meal. Readings are self-reported on a scale of 0-100. Larger values indicate increased perception of hunger and alertness. Hunger and Alertness was asked at 0, 30, 90, 120 and 150 minutes after each standardised meal

Phase 2: Standardised Meal: The phase from 0 to +2 hours is the conventional time period for measuring glucose response. It is characterised by:

- **Glucose Rise_{0-2h}:** the maximum level above the baseline within the 2h period, as a percentage of the *Average Baseline Level*;
- **Glucose iAUC_{0-2h}:** the incremental area under the glucose curve, measured relative to the *Average Baseline Level*

Phase 3: Post-Meal: The phase +2 hours to +3 hours after the meal. Participants who consumed additional food (except water) before the end of phase 3 (+3h hours, +/- 5 mins) were excluded from the analysis.

- **Glucose Dip_{2-3hr}:** The difference between the lowest glucose reading in hours 2-3, and the *Average Baseline Level*, as a percentage of *Average Baseline Level*. The *Glucose Dip_{2-3hr}* is expressed as a percentage in order to adjust for differences in participants' baseline levels. Positive *Glucose Dip_{2-3hr}* values indicate states of mild hypoglycemia, negative *Glucose Dip_{2-3hr}* values indicate that blood glucose levels remain elevated above baseline level.
- **Change In Hunger_{2-3hr}:** The difference between a participant's pre-meal hunger and the average hunger reading in hours 2-3.
- **Change In Alertness_{2-3hr}:** The difference between a participant's pre-meal alertness and the average alertness reading in hours 2-3.

Phase 4: Ad libitum meals: After 3 hours, participants record their food intake using a mobile app.

- **Time until next meal:** The number of minutes after consumption of the standardised meal that elapsed before the next meal of at least 50 kcal was consumed;
- **Energy intake_{3-4h}:** The initial consumption is totalled over hours 3-4 including drinks;

Energy intake_{24h}: The total daily consumption covers the full period from 4 hours before the standardised breakfast to 20 hours after/

The key measures are summarized in Table 1. The Consort Diagram for number of participants and the types of meals are shown in Extended Data Figure 1.

Statistical analyses—In order to test for associations between glucose parameters, hunger and energy intake, standard linear regression models were used. $P < .05$ was considered as nominally statistically significant, and error bars are shown throughout as 95% confidence intervals. All key measures were approximately normally distributed (γ_2 : 0.7 to 2.26), except time to next meal and energy intake 3-4h. Distributions are shown in Extended

Data Figure 2. Linear regressions were adjusted for age, sex, BMI and weight. Data from questionnaires, clinical visits and laboratory data was entered using comma delimited files, Excel spreadsheets and Microsoft Access. CGM data was imported from text files into the analysis pipeline. Analyses were carried out in R 3.4.2. Core Team, Python 3.7, using Pandas 0.25.1, Scipy 1.3.1, Pingouin 0.3.3.

The key analyses included in this study are multiple linear regressions, with Pearson's correlations coefficients and 95% CIs used to report effect sizes. Statistical power for the key analyses refer to (i) differences between individuals, for which the sample size is $n=763$ (see **Data Analysis: Set (a)**), corresponding to the results described in Figure 2), and (ii) differences between $m=6428$ meals consumed in duplicate; for which the sample size is therefore $m=3214$ pairs of meals (see **Data Analysis: Set(b)**), corresponding to the results described in Figure 3). The power for the analyses for (i) was carried out adjusting for 5 traits, namely change in hunger, change in alertness, time to next meal, energy intake in the 3 hours after the meal and energy intake in the 24 hours after the meal, and hence an alpha level of 0.01 was chosen. The necessary correlation effect size needed to achieve 80% power with $p<0.01$ for this sample size is $r=0.124$. For the correlations for (ii) to achieve 80% power at the same alpha level the Pearson's coefficient needs to be $r=0.061$ or higher.

Results

The descriptive characteristics of study participants, including their fasting levels of circulating glucose and insulin and their insulin secretion indices from the hospital day meal are presented in Supplementary Table 1. For each type of standardised meal, the mean and standard deviation of responses for the post-prandial measures are presented in Table 1 (data for the validation cohort is shown in Supplementary Table 2(b)). The macronutrient content of the standardised and free-living meals analysed is shown in Supplementary Table 3.

To assess the repeatability of self-reported measures of hunger, alertness, and energy intake, we explored the 6,428 standardised breakfasts that were eaten in duplicate on different days (see Extended Data Figure 1 for meal numbers). Each of the glycemic measures was statistically repeatable, though as expected the strength of correlations varied; Glucose Rise_{0-2h} ($r=0.73$ $P<0.001$; US: $r=0.64$ $P<0.001$), Glucose Dip_{2-3h} ($r=0.58$ $P<0.001$; US: $r=0.57$ $P<0.001$). Intraclass correlations (ICC) for these measures fell within the moderate to good range: Glucose Rise_{0-2h} (ICC(2,1)=0.81; 95% CI [0.78, 0.83]; US: ICC(2,1)=0.78; 95% CI [0.69, 0.85]), Glucose Dip_{2-3h} (ICC(2,1)=0.62; 95% CI [0.59, 0.66]; US: ICC(2,1)=0.68; 95% CI [0.56, 0.78]).

1) Glucose Dips_{2-3h} predict subsequent hunger and calorie intake

Participants were instructed to eat a range of standardised breakfasts, followed by a 3 hour fast. The post-prandial measures for those participants who consumed each of the 5 core meals (High Carb, High Fat, OGTT, UK Average, High Fibre) ($n=763$, $m=5667$; see Supplementary Table 2 for meals included) were averaged for each participant. We examined whether there was an association between glycemic responses - peak glucose rise in hours 0-2 (Glucose Rise_{0-2h}), the glucose incremental area above baseline in hours 0-2

(iAUC_{0-2h}), and the subsequent Glucose Dip_{2-3h} - and postprandial measures of self-reported hunger, alertness, time until next meal, and subsequent energy intake.

Across the postprandial measures, we found a stronger correlation with Glucose Dips 2-3h than with Glucose Rise_{0-2h}, or Glucose iAUC_{0-2h} (Table 2). The Glucose Dip_{2-3h} was statistically significantly associated with a change in Hunger_{2-3h} ($r=0.16$ $P<0.001$; US: $r=0.12$ $P=0.315$), Time until next meal ($r=-0.14$ $P<0.001$; US: $r=-0.16$ $P=0.160$), Energy intake_{3-4h} ($r=0.19$ $P<0.001$; US: $r=0.24$ $P=0.032$), Energy intake_{24h} ($r=0.27$ $P<0.001$; US: $r=0.21$ $P=0.065$). The association with change in Alertness_{2-3h} ($r=-0.04$ $P=0.313$; US: $r=0.02$ $P=0.875$) was not significant. The Glucose Dip_{2-3h} was as predictive of postprandial responses as the participant's self-reported hunger.

Figure 2 illustrates the difference by dividing the participants into quartiles of Glucose Dip_{2-3h}. The participants with the largest average Glucose Dip_{2-3h} (Q4) also had on average a +9% (95% CI 5,13) increase in reported Hunger_{2-3h}, a -2% (95% CI 1,5) decrease in Alertness level_{2-3h}, a -24 minutes (95% CI 15,33) shorter time until the next meal, a +75 kcal (95% CI 47,103) higher Energy intake_{3-4h}, and a +312 kcal (95% CI 226,398) higher overall Energy intake_{24h}, compared to the participants with the smallest Glucose Dip_{2-3h} (Q1).

The associations between the Glucose Dips_{2-3h} and the postprandial measures were strongest with the OGTT set meal (which also preceded the largest dips). For each of the set meal subgroups considered independently, the associations between the glucose dip and the postprandial measures were directionally consistent with the overall results and statistically significant, with the exception of (a) Change in Alertness_{2-3h}, which was not significantly associated at the subgroup level and (b) the High Carb muffin, where the glucose dip was not significantly associated with Energy intake_{3-4h} ($r=0.04$, $p=0.349$).

Glucose Dips_{2-3h} had modest and non-significant correlations with individual characteristics, such as age, weight and BMI - except for sex, where males ($P=0.005$) had slightly larger dips. Glucose Dips_{2-3h} were associated with lower fasting levels of Insulin and C-Peptide, as measured in clinic; Plasma Glucose ($r=-0.05$ $P=0.186$; US: $r=-0.12$ $P=0.321$), Insulin ($r=-0.10$ $P=0.008$; US: $r=0.02$ $P=0.883$), C-Peptide ($r=-0.14$ $P<0.001$; US: $r=-0.11$ $P=0.357$). Glucose dips_{2-3h} were associated with the Glucose Rise_{0-2h}, but not with Glucose iAUC₀₋₂; Glucose iAUC_{0-2h} ($r=0.05$ $P=0.166$; US: $r=-0.15$ $P=0.189$), Glucose Rise_{0-2h} ($r=0.12$ $P=0.003$; US: $r=0.07$ $P=0.557$)

After adjusting for these factors, the postprandial measures (except Change in alertness_{2-3hr}) remained significantly correlated with the Glucose Dips_{2-3h}; Change in hunger_{2-3hr} ($r=0.12$ $P=0.002$; US: $r=-0.01$ $P=0.904$), Change in alertness_{2-3hr} ($r=-0.06$ $P=0.109$; US: $r=0.04$ $P=0.756$), Time until next meal ($r=-0.15$ $P<0.001$; US: $r=-0.24$ $P=0.038$), Energy intake_{3-4h} ($r=0.18$ $P<0.001$; US: $r=0.23$ $P=0.055$), Energy intake_{24h} ($r=0.25$ $P<0.001$; US: $r=0.12$ $P=0.316$)

2) Glucose Dips_{2-3h} in identical meals consumed by the same person

To understand if the association between Glucose Dips_{2-3h}, hunger, alertness and postprandial energy intake was present even when adjusting for individual characteristics, we used each person as their own control and compared their own responses to the standardised breakfasts that were repeated in duplicate, following an overnight fast, separated by 2-5 days (n=1053, m=6428; see Supplementary Table 2 for meal numbers).

The difference between the first and second repeat of each meal was calculated. We then measured the degree to which the difference in Glucose Dip_{2-3h} was associated with the difference in each of the postprandial measures. Even when holding constant both the food and the individual, Glucose Dips_{2-3h} were modestly but significantly correlated with time until next meal ($r=-0.06$ $P<0.001$), Energy intake_{3-4h} ($r=0.08$ $P<0.001$), and Energy intake_{24h} ($r=0.06$ $P<0.001$). The association with change in Hunger_{2-3hr} ($r=0.04$ $P=0.232$) and change in Alertness_{2-3hr} ($r=-0.06$ $P=0.076$) was directional, but not significant. None of the correlations in the smaller US validation cohort were significant. Figure 3 illustrates these effects, by comparing the postprandial measures across the largest (above +10%) and smallest (below -10%) differences in Glucose Dip_{2-3h}.

The correlation between difference in glucose dips and difference between the five outcomes shown in Figure 3 for each pair of meals was computed separately for each of four type of standardised meals included in the analysis, namely High Carb, High Protein, OGTT and “UK Average”. The resulting correlation coefficients were then meta-analysed using a fixed effects and a random effects estimate. The results are reported in Supplementary Table 5 and are very similar to those reported in Figure 3.

The fact that associations between Glucose Dips_{2-3h} and postprandial measures still exist between repeated meals for the same individual shows that this relationship is not fully driven by differences between individuals. It is possible that contextual factors (such as exercise, sleep and other meals in the previous day) may be responsible for differences in glucose dips_{2-3h} but we did not explore this avenue further in this study.

Discussion

The notion that blood glucose is involved in appetite was described by Mayer in the ‘glucostatic hypothesis’ (16). This concept - that a decline in blood glucose influenced appetite - was subjected to intense study (20)(21,22) but has lost support from front line researchers, partly because of a lack of evidence of any suppression of eating by high glucose availability (23). It has been argued that inadequate delivery of glucose to the brain (‘neuroglucopenia’ or ‘glucoprivation’) activates neurocircuits that drive feeding along with wide-ranging neuroendocrine and autonomic responses(24). A sustained increase in food intake follows, overriding the control exerted by the energy homeostasis system, i.e., irrespective of body fuel stores or plasma levels of leptin or insulin (24,25). However, this mechanism has been dismissed as not relevant to normal feeding and considered as an “emergency response” (e.g.(26)). More recently, an observational (n=31) study (31) of obese and healthy individuals in free-living conditions found that the glucose nadir preceding a meal had statistically significant ability to predict hunger and subsequent energy intake. In

the present study, using continuous glucose monitors in an at-home setting in >8,000 controlled meals and > 70,000 ad libitum meals we have been able to show that postprandial glucose dips are associated with appetite expression and energy intake in normal feeding in healthy individuals under what can be regarded as real-world conditions.

We demonstrate in the largest study to date the importance of glucose dynamics in the regulation of hunger and subsequent energy intake in real life scenarios. We report that the key postprandial glycemic measure linked to hunger and subsequent food intake is the Glucose Dip_{2-3h}, not the Glucose Rise_{0-2h} nor the glucose iAUC_{0-2h}. Whilst some of the correlations we report are relatively modest, they reflect the complexity of real-world eating decisions. It is notable that the Glucose Dip_{2-3h} was as good a predictor of subsequent energy intake as the participants' self-reported Hunger_{2-3h}.

We found these effects to be consistent for the same individual consuming the same meal on two occasions - the meals with larger Glucose Dips_{2-3h} have larger subjectively declared hunger, and greater energy intake afterwards. These results were directionally consistent with those in the US validation cohort, although not all results were significant in this smaller cohort.

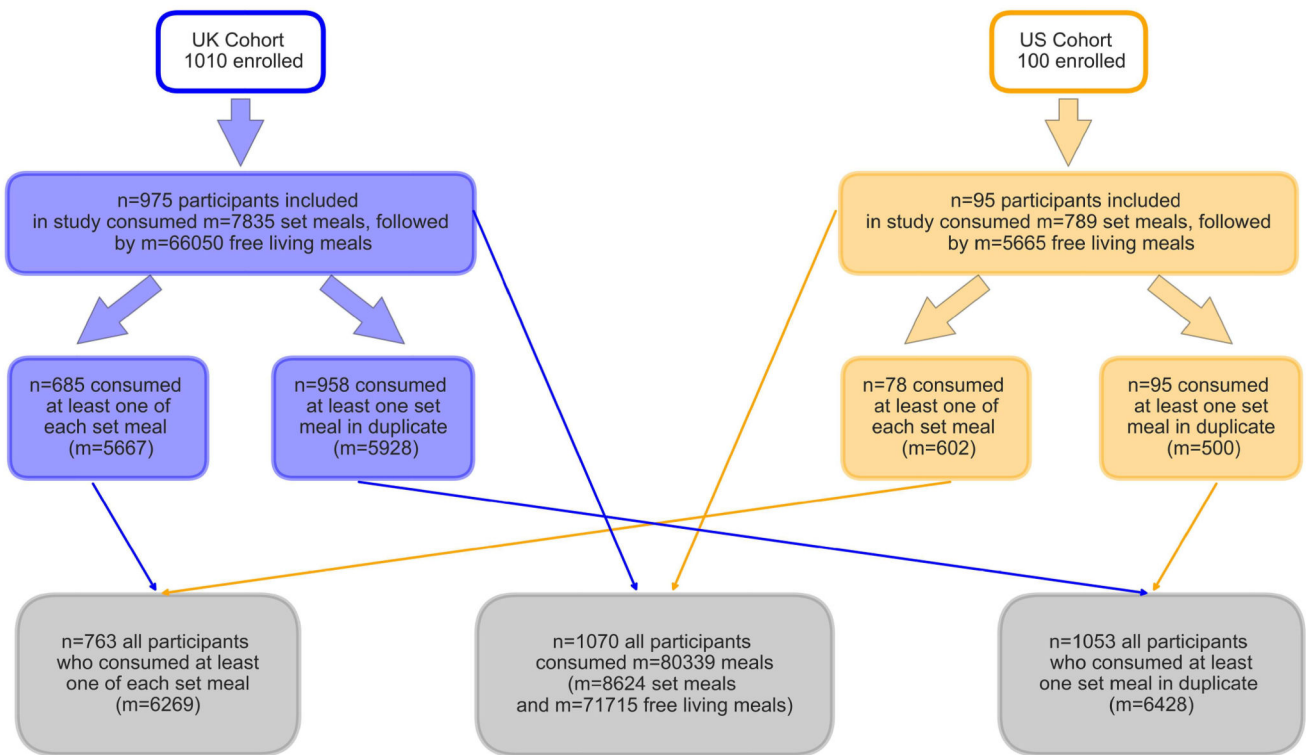
We have not fully explored all the possible causes of Glucose Dips in this study; but the findings suggest that both individual characteristics and dietary factors are likely important. Comparing individuals, Glucose Dips_{2-3h} are negatively associated with fasting C-peptide and insulin levels. Between the standardised meals, the largest glucose dips followed the meal with the largest glucose rise (OGTT). Our analysis may partly explain why observational epidemiological ((27)(28)) studies have shown strong correlations between foods with high glycemic loads (such as potatoes and sugar sweetened beverages (29)) and weight gain – as consumption of such foods could lead to glucose dips and subsequent hunger. The link between postprandial blood glucose and satiety suggests novel approaches to predicting and managing hunger using glucose data, especially if it were available in real-time.

The associations demonstrated in our studies are a realistic reflection of how appetite control operates under real world conditions, allowing variation in contextual variables such as exercise, sleep, or meal sequence. These factors may contribute to the observed in-person variability in Glucose Dips. It should be noted that the degree of association (correlation values) between glucose dips and appetite variables is relatively small, and therefore contributes only partly to postprandial satiety. In heavily controlled laboratory studies with uniform conditions and with participants' physiology and behaviour tightly constrained, much higher correlations can be observed; however, these scientifically ideal conditions are not typical of real-world human activities. The data set reported in this paper is unique - most of the meals were self-determined and consumed according to the individuals' own schedules. The decision to eat is determined by many social, psychological and physiological factors, and glucose dips are just one part of the picture. Our data confirm that in the realworld blood glucose dips are a physiological mediator of this dietary risk factor, although much of the variance remains to be accounted for.

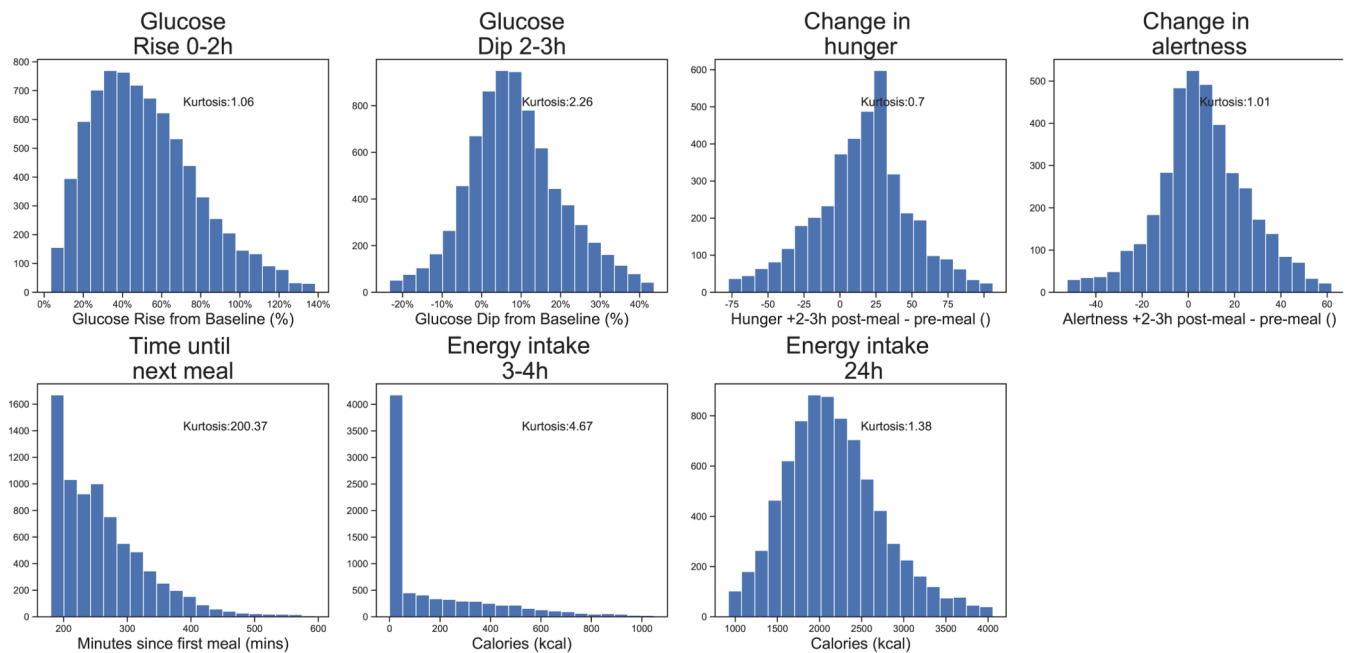
We note several study limitations. Firstly, given the scale, it was not practical to measure appetite hormones for any of the participants nor measure insulin sensitivity directly with a euglycemic clamp, nor to record insulin dynamics for the at home meals. Secondly, for the ad libitum meals the meal content is self-reported. Great care was taken to ensure the quality and accuracy of these records, as outlined in the methods, and we believe the scale and real-life context of this study more than compensates for this limitation. Thirdly, we relied on the self-reported compliance of the participants with the fasting protocols - 109 meals were excluded from participants that ate before the end of the specified fasting period. (See *QC of the app data: Inclusion criteria for meals*) - these participants may also have been responding to blood glucose dips. Fourthly, whilst we were able to study energy intake immediately after a meal and total energy intake over 24 hours, we were not able to monitor if participants compensated for short term changes in energy intake over a period of weeks or months. In addition, there was limited ethnic diversity in our study population (97% white). In addition, we have not included some potential confounders in the models, including sleep and physical activity. Finally, we note that some of the outcomes studied, specifically the visual analogue scale for scoring hunger was not perfectly normally distributed. However, we have analysed a very large number of meals (8624 standardised meals and 71,715 ad libitum meals) and linear models are known to be robust to the violation of the assumption of normality when the sample size is large enough for the central limit theorem to be at work (30).

In conclusion, our data show for the first time in a large-scale controlled study of healthy individuals representative of the general population that postprandial glucose dips are common and lead to increased hunger and energy consumption in real world conditions.

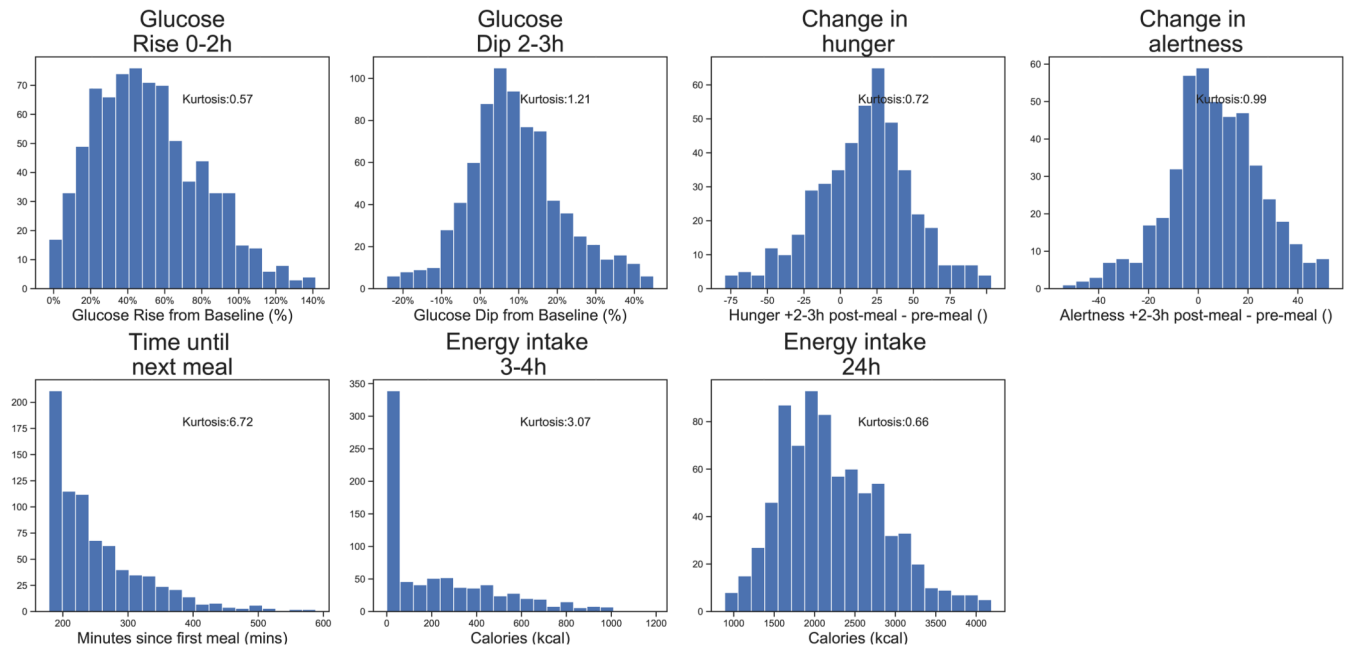
Extended Data



Extended Data Figure 1.



Extended Data Figure 2.



Extended Data Figure 3.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We express our sincere thanks to the participants of the PREDICT 1 study. We thank the staff of Zoe Global, the Department of Twin Research and Massachusetts General Hospital for their tireless work in contributing to the running of the study and data collection. We thank Abbott for their support with using their CGMs. This work was supported by Zoe Global Ltd also received support from grants from the Wellcome Trust (212904/Z/18/Z) and the Medical Research Council (MRC)/British Heart Foundation Ancestry and Biological Informative Markers for Stratification of Hypertension (AIMHY; MR/M016560/1). PWF was supported in part by grants from the European Research Council (CoG-2015_681742_NASCENT), Swedish Research Council, Novo Nordisk Foundation and the Swedish Foundation for Strategic Research (IRC award). AMV was supported by the National Institute for Health Research Nottingham Biomedical Research Centre. TwinsUK is funded by the Wellcome Trust, Medical Research Council, European Union, Chronic Disease Research Foundation (CDRF), Zoe Global Ltd and the National Institute for Health Research (NIHR)-funded BioResource, Clinical Research Facility and Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust in partnership with King's College London.

Data availability

The data used for analyzing this study are held by the department of Twin Research at King's College London. The data can be released to bona fide researchers using our normal procedures overseen by the Wellcome Trust and its guidelines as part of our core funding. We receive around 100 requests per year for our datasets and have a meeting three times a month with independent members to assess proposals. Application is via <https://twinsuk.ac.uk/resources-for-researchers/access-our-data/>. This means that the data needs to be anonymized and conform to GDPR standards. Specifically for this paper, all the variables used in the models can be requested as well as the summary outcome measures for each person

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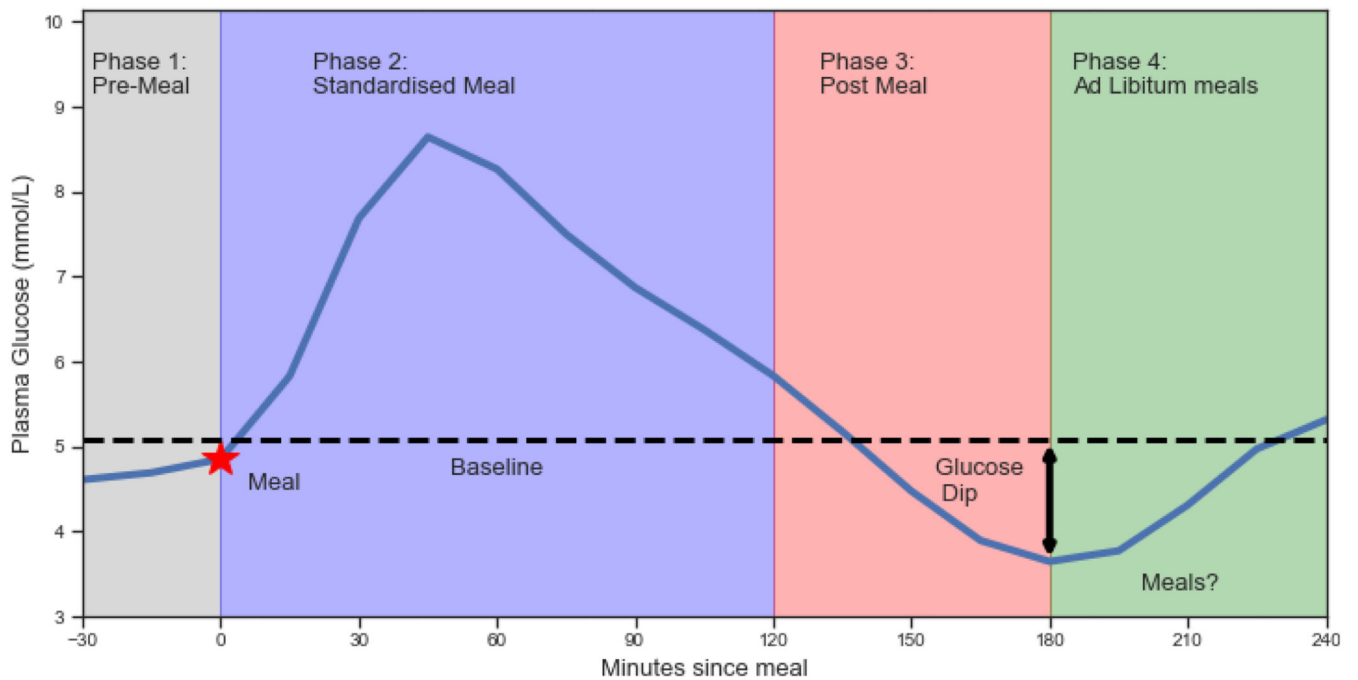


Figure 1. Average glycemic responses to standardised breakfasts, illustrating key measures used in study

(n=1,070, m=8,624). Four phases were studied (see methods) namely: **Phase 1:** Pre-Meal: (up to 30 minutes before the standardised meal starts. **Phase 2:** Standardised Meal: (from 0 to +2 hours is the conventional time period for measuring glucose response) **Phase 3:** Post-Meal: The phase +2 hours to +3 hours after the meal. All participants were asked to fast up until 3 hours post-meal so no energy intake has taken place. **Phase 4:** *Ad libitum* meals: when participants were allowed to eat again. See methods for details

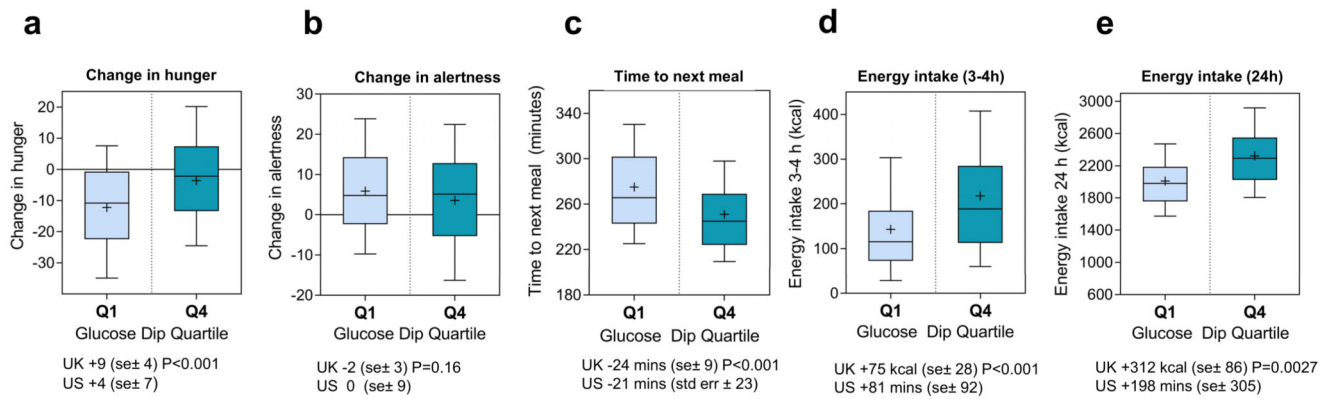


Figure 2. Postprandial measures by top and bottom quartiles of 2-3h Glucose Dip (n=763, m=5667 (UK n=685, m=5667, US n=78, m=602). Participants were divided into quartiles of their average Glucose Dip2-3h, following consumption of OGTT, High Carb, UK average, High Fat, and High Fibre standardised breakfasts (n=5667). The participants with the largest dips (Q4) are compared to those with the smallest dips (Q1), according to their Change in Hunger2-3h, Change in Alertness2-3h, Time until the next meal, ad libitum Energy Intake3-4h immediately after the end of the fasting period, and ad libitum Energy Intake24h. Boxplots showing median, means (indicated by a + sign) interquartile ranges and 90% confidence intervals are shown. P-values from two sided t-tests are reported. Sample sizes for each panel (a) change in hunger and (b) change in alertness Q1(n=161) Q4(n=162) (c) time in minutes until next meal (d) energy intake between 3 and 4 hours after the meal (e) energy intake in the 24 hours after the meal Q1(n=172) Q4(n=171). Error bars indicate 90% confidence intervals.

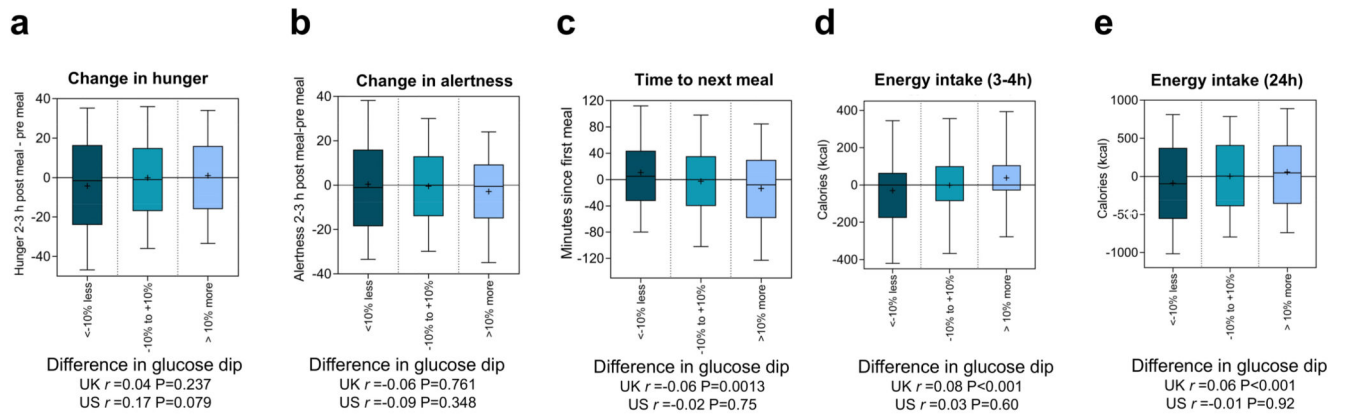


Figure 3. Differences in postprandial measures across repeated meals within individuals (n=1053, m=6428 (UK n=958; US n=95, m=500)). Standardised breakfasts that were repeated on two occasions, following an overnight fast, separated by 2-5 days. The difference between the first and second repeat of each meal was calculated. We then measured the degree to which the difference in Glucose Dip_{2-3h} was associated with the difference in each of the postprandial measures in the exploration and validation studies. For illustration, the results were grouped according to differences between the Glucose Dip_{2-3h} recorded on the first and second repeat (<-10% less, -10% to +10%, >10% more). Number of pairs of meals for each box are: for panels (a) and (b) <-10% less m= 177, -10% to +10% m=652, >10% more m=157; for panels (c) (d) and (e) <-10% less m= 501, -10% to +10% m=1960, >10% more m=503. Correlation coefficients (r) were calculated for m= 2964 and m=250 pairs of meals from the UK and US respectively. P-values from two sided tests are shown. Error bars indicate 90% confidence intervals.

Table 1
Responses to standardised meals (Exploration Cohort)

Meal	Num	Glucose Rise 0-2h (%)		Glucose Dip 2-3h (%)		Change in hunger		Change in alertness		Time until next meal (mins)		Energy intake 3-4h (kcal)		Energy intake 24h (kcal)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
High Carb	1,826	56%	24%	5%	11%	-12	28	8	20	273	87	156	263	2189	614
High Fat	381	29%	18%	8%	8%	-10	24	6	21	264	95	197	286	2267	696
High Fibre	886	50%	23%	6%	10%	-18	26	8	20	277	89	134	243	2237	647
High Protein	1,069	27%	17%	4%	9%	-16	27	7	20	272	134	163	244	2231	650
OGTT	1,808	77%	31%	19%	17%	9	28	3	25	241	85	269	283	2085	622
UK Average	1,865	45%	22%	6%	10%	-11	26	7	21	272	79	131	220	2182	632

Table 2
Associations between glucose responses and postprandial measures, between individuals

	Glucose Rise 0-2h	Glucose iAUC 0-2h	Change in hunger	Glucose Dip 2-3h
Glucose Dip 2-3h	r=0.11 P=0.003 (US: r=0.07 P=0.557)	r=0.05 P=0.166 (US: r=-0.15 P=0.189)	r=0.16 P=<0.001 (US: r=0.12 P=0.315)	
Change in hunger	r=0.02 P=0.678 (US: r=0.07 P=0.540)	r=0.02 P=0.701 (US: r=-0.05 P=0.647)		r=0.16 P=<0.001 (US: r=0.12 P=0.315)
Change in alertness	r=0.04 P=0.304 (US: r=-0.07 P=0.571)	r=0.05 P=0.169 (US: r=0.00 P=0.988)	r=-0.03 P=0.416 (US: r=0.06 P=0.625)	r=-0.04 P=0.313 (US: r=0.02 P=0.875)
Time until next meal	r=0.13 P=<0.001 (US: r=-0.09 P=0.413)	r=0.11 P=0.005 (US: r=-0.11 P=0.319)	r=-0.11 P=0.007 (US: r=-0.02 P=0.876)	r=-0.14 P=<0.001 (US: r=-0.16 P=0.160)
Energy intake 3-4h	r=-0.16 P=<0.001 (US: r=-0.03 P=0.764)	r=-0.08 P=0.036 (US: r=0.05 P=0.695)	r=0.21 P=<0.001 (US: r=0.03 P=0.810)	r=0.19 P=<0.001 (US: r=0.24 P=0.032)
Energy intake 24h	r=-0.08 P=0.036 (US: r=-0.02 P=0.835)	r=-0.05 P=0.170 (US: r=0.05 P=0.677)	r=0.18 P=<0.001 (US: r=0.10 P=0.375)	r=0.27 P=<0.001 (US: r=0.21 P=0.065)