Blood glucose responses to 3-days of early or late time-restricted feeding in obese adults

Background information

“Diabesity” - the pandemic we mustn’t forget

Worldwide obesity has nearly tripled since 1975 (WHO, 2020) which increases an individual's risk of several chronic diseases, including type 2 diabetes (T2D) and cardiovascular disease. The “diabesity” epidemic is a global health challenge. The International Diabetes Federation (IDF) predicts the prevalence of T2D to continue rising by ~10% in the next decade, thus 552 million people with T2D by 2030 (Whitling et al. 2011). Dietary intervention is effective in preventing, treating and even reversing T2D (Lean et al. 2019). However, a recognised short coming is long-term adherence, maintenance of weight loss and concomitant improvements in cardiometabolic profile.

Time restricted eating - a novel, acceptable approach

Today’s society allows 24/7 access to food leading to around the clock eating patterns that can have an impact on glucose metabolism (see next paragraph). An emerging dietary approach for improving cardiometabolic health in obese, at risk T2D, is time-restricted eating (TRE) (Gabel & Varady, 2020). This approach is to consume all daily calories within a prescribed daily time window, followed by a prolonged period of fasting over a 24 hour period. Recent studies have demonstrated that an 8 hour window to consume food, followed by 16 hours of fasting, improves glucose regulation and lipid profile in obese adults (Chow et al. 2020; Kesztyus et al. 2019). Noteworthy, Kesztyus et al. (2019) showed that adherence to 8 hour TRE was 85% over 12 weeks. Previous more extreme approaches, such as intermittent fasting (i.e. 4 hours feeding followed by 20 hours fasting) or alternate day fasting (abstaining from calories over 24 hours for several days per week) have also shown benefit; however these more extreme approaches are less tolerable in the longer term.

Circadian regulation of metabolism

Chrononutrition (i.e. the timing of nutrient intake) is recognised to have additional health benefits (Parr et al. 2020b). Circadian rhythms tightly regulate metabolism. For example insulin secretion is reduced in the evening, thus eating late at night can result in impaired postprandial insulin sensitivity and subsequently lead to greater fat storage. In contrast, cortisol peaks in the morning and temporarily increases hepatic glucose output. Delaying breakfast until later could therefore reduce postprandial glycaemia as cortisol-induced liver glucose output does not add to raising postprandial glucose concentrations. There is evidence that earlier TRE (eTRE) elicits favourable metabolic effects. For example, 5 weeks of TRE improved (independent of weight loss) insulin sensitivity compared to a 12 hour eating window in pre-diabetics (Sutton et al. 2018). However, it remains unclear whether findings are a result of reduced calorie intake by restricting the eating window in general or physiological adaptations in energy metabolism.

Early versus delayed time restricted eating

To date, one study compared glycaemic control outcomes in a crossover design 7 days of early (eTRE) with delayed (dTRE) eating (Hutchison et al. 2019) in men with prediabetes. Despite eTRE reducing fasting glucose compared to unrestricted feeding, no differences were detected between eTRE and dTRE in other glycaemic outcomes. However, no data was presented on food intake (total energy intake or macronutrient composition) during the unrestricted or time-restricted periods, which makes data interpretation difficult, potential differences in habitual diet during the separate testing periods might have interfered with the data.

Furthermore, it is unknown whether currently shown improvements in glycaemic control are the result of a cumulative or adaptive effect of multiple days of TRE or already observed acutely. For example, Parr et al. 2020a showed that after 5 days of TRE, blood glucose concentrations over 24 hours were lower compared to extended feeding, which

was mainly attributed to lowered nocturnal blood glucose. However, it was unclear whether such an effect is a direct, acute result of the feeding strategy or a result of adaptation. The beneficial free-living effect of eTRE on glucose control has been demonstrated in young males (Jones et al. 2020), however, it remains unknown in obese, prediabetic adults.

Aims and objectives

Aim 1: Investigate the differences in i) early TRE and ii) late TRE on free-living blood glucose concentrations.

Aim 2: Investigate the difference in the acute (1 day) and accumulative (3 day) response of free-living blood glucose during the two conditions.

Objective 1: To achieve aims one and two, the two conditions will be isocaloric and eucaloric. A randomised cross-over design will be employed, where 17 obese adults are provided with their food consumption for 3 days. Continuous glucose monitors (CGM) will determine free living blood glucose levels, and dietary compliance will be monitored throughout.

Aim 3: Investigate the differences in i) early TRE and ii) late TRE fasting blood biochemistry.

Objective 2: To achieve aim three, pre- and post- fasting blood samples will be taken for each of the two conditions to investigate any changes in metabolic profile. Full phenotypic data will be collected at baseline (i.e. components of Metabolic Syndrome and HOMA-IR).

Programme of work

Participants

Adult men and women (aged 45 - 60 years) who are classed obese and overweight (BMI ≥25 kg/m^2) and have central adiposity (defined by IDF ethnicity specific threshold for waist circumference) will be recruited from local weight management clinics where well-established relationships with Manchester Metropolitan University already exist. Only those who typically eat/drink for greater than 12 hours per day and are not currently

meeting ACSM activity guidelines of at least 150 min of moderate intensity exercise per week will be recruited.

Exclusion criteria will include smokers, type 1 or 2 diabetes, those who are pregnant, alcohol consumption >14 units weekly, those with disturbed eating patterns or diagnosed eating disorders or intolerance/ allergies to certain foods supplied in the diet. Also, participants will be excluded if they had recent major body weight change (+/- 3kg in the last month), are shift workers, or diagnosed and medicated diseases of the following: ischemic heart disease, heart disease, respiratory disease, chronic kidney disease and endocrine conditions. Special communication needs and/or unable to understand verbal and written information in English. Full ethical procedures (e.g. informed consent, right to withdraw) will be adhered to.

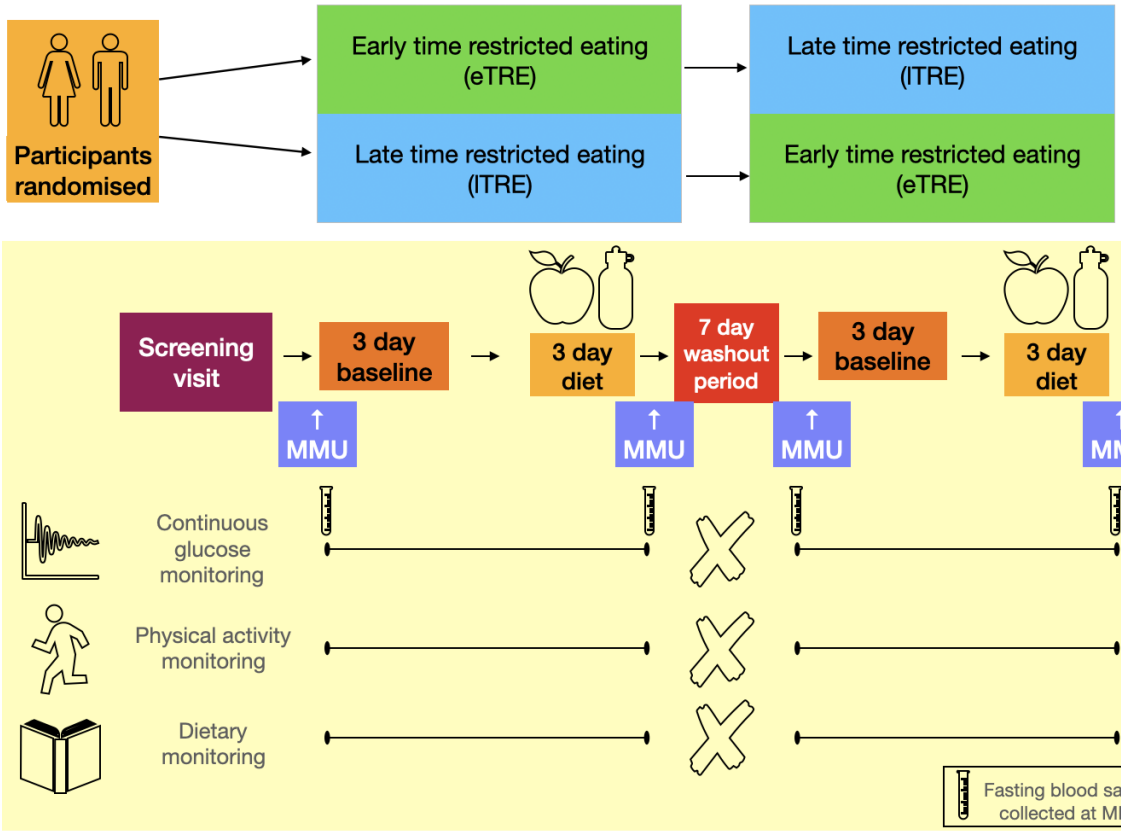
Screening

Initial telephone screening will ask for self-reported height, weight (to determine BMI), waist circumference if known, and the International Physical Activity Questionnaire (IPAQ) to determine activity status. Laboratory screening will include anthropometrics, body composition, blood pressure, capture of medical records and family history as well as 3-day assessment of physical activity (accelerometer) and written dietary records to reflect habitual diet. Females will be asked for menstrual and contraceptive information.

Study design

In a randomized crossover trial, participants will follow two days of time restricted feeding. During this trial, both early time-restricted eating (early TRE: eating window between 8 AM and 4 PM) or late time-restricted eating (late TRE: eating window between 12 AM and 8 PM) will be utilised. All meals will be the same on the trial days but will be consumed at different prescribed times. The diet will contain three main meals and two snacks to be consumed in free-living conditions. Before and after each condition, a fasting blood sample will be collected. During the condition and 3-days before (i.e. 6 days for each condition), participants will be wearing a continuous glucose monitoring (CGM) device to measure

blood glucose and an accelerometer to measure activity and sleep pattern. The diet for the days will be provided and instructions to the participants when to consume the meals will be explained. Participants will be asked to report their consumption plus any non-caloric fluids (coffee, tea, water) and standardize these in all conditions. The morning after a final trial day, participants will be interviewed about their attitudes towards the diet patterns. The wash out period between experimental conditions will be at least 7 days before the other condition is analysed.



	Breakfast	Lunch	Dinner	Snacks
Early time restricted eating (eTRE)	8am	12pm	4pm	10am & 2pm
Late time restricted eating (lTRE)	12pm (noon)	4pm	8pm	1pm & 6pm

Figure 1: Schematic of study protocol, timing of measurements and timing of meals.

Dietary intervention

The diet in each of the conditions will be isocaloric and eucaloric and matched with regards to total energy intake and macronutrient composition per meal. Relative energy intake distribution will be in line with recommendations from the NHS (breakfast 20%, lunch 30%, dinner 30%, snacks 2 x 10%). Diet composition details and timings of each meal or snack are presented in supporting document 2. Total daily energy intake on trial was based on REE obtained from Mifflin St-Jeor equations multiplied by factor 1.4 for physical activity levels. Macronutrient composition was CHO: 50% of EI, Fat: 30% of EI and PRO: 20% of EI. It is recognised that the energy intake and macronutrient composition during the study conditions may be different to that of the participants usual diet. Their habitual dietary intake will be collected and analysed, however this is notoriously under reported in obese adults (Rennie et al. 2007). Our approach in this cross-over design (where each participant is acting as its own control) is to standardise based on the NHS recommendations ensuring parity between participants.

Experimental measures

3 day screening of physical activity and dietary status:

- Objectively monitored physical activity using a wrist worn accelerometer (GENEActiv); >90% wear time will be required.
- Habitual daily food intake using a paper, or electronic (participants preference) food diary. On receipt, any further information required will be obtained verbally by the researcher and noted, the diaries will be analysed using Nutritics software.

Continuous Glucose Monitor:

- 24-36 hours prior to the first experimental visit, participants will be fitted with a continuous glucose monitor, implanted into the subcutaneous tissue of their body.
- On completion of condition, this data will be downloaded and analysed for outcomes outlined below.

Dietary compliance, adherence and appetite:

- Participants will be asked to record their dietary intake throughout, indicating the time of meal ingestion. They will also record any adverse or unusual events such as being unable to finish a meal. If the participant suffers from an illness during the window where the modified diet is consumed, they will be instructed to consume whatever food they desire and their data will not be analysed.

Fasting blood biochemistry:

- A fasting blood sample will be taken from the antecubital vein/ capillary blood at 8am on the morning beginning a 3-day condition and 8am on the day after (i.e. 4th day).
- Analysis will be performed to determine any changes in:
  - Glucose, insulin, triglycerides, HDL, LDL, Non-Esterified Fatty Acid (NEFA), adiponectin, apolipoprotein.
  - Glucose, insulin, and NEFA will be used to determine any changes in insulin resistance (i.e. HOMA-IR and adipose IR).
  - Albumin and alkaline phosphatase to assess any changes in liver function.
  - Appetite hormones (leptin and ghrelin) to assess any changes related to hunger.

Outcomes

Primary: average AUC for blood glucose over 3-day period between three conditions.

Secondary: Compare the effects of early TRE vs late TRE on:

- To compare the time spent above 7.8 mmol.L-1 (CGM data) during the free-living period - i.e. does time restricted feeding (early TRE vs late TRE) reduce time spent in hyperglycaemia?
- Free-living glycaemic variability using data collected from the CGM (Mean Amplitude Glycaemic Excursions [MAGE] scores for example; mean postprandial glycaemia (breakfast, lunch and dinner))

- Differences in above outcomes between day 1 and day 3
- Dietary compliance (any difference in energy intake)
- Differences in metabolic profile determined by fasting blood biochemistry

Sample size calculation

Sample size calculations were run with commercially available software (G*Power 3.1.9.4) using ANOVA - repeated measures within factors. Based on previous experiments by Parr et al. 2020a, comparing TRE with extended feeding for 5 days, with an effect size f of 0.36 on nocturnal [glucose AUC] (effect size d = 0.72; f = 1/2d) and an $\alpha = 0.05$ and a power of 0.8, a sample size of 14 participants was required. To account for drop out, sample size was increased 17.

Data analysis

Tools/software: Excel, SPSS, Nutritics (diet analysis), LibreView (CGM analysis), ActivLife (physical activity).

Anonymised data will be downloaded from these tools to relevant software before data is combined in Excel. SPSS will be used to determine descriptive (e.g. mean, standard deviation) and perform inferential statistics (e.g. Dunnett's test).

Timeline

Given the existing infrastructure of this project, for example recruitment links and priority access to the human and biochemical physiology laboratories, this project is deliverable in 18 months. Full risk assessment and Covid-19 mitigations are in place to conduct the research during these unprecedented times. Ethical submission is in progress to commence the study in July 2021. Each participant has a total of 5 (one screening, four assessment) 30-45 minute study visits, separated over a period of ~4 weeks; based on the sample calculation of 17 participants required, the work is feasible. Furthermore, postgraduate students and other members of staff are also available to offer support in the logistical running of the project where appropriate.

Lay description

Obesity is a global problem with over a third of the UK population being either overweight or obese. Obesity increases the risk of several chronic diseases including type 2 diabetes (T2D), a disease characterised by poor blood sugar control which often leads to further health complications such as cardiovascular disease and ultimately premature death. Strategies to improve blood sugar control have previously looked at what to eat, for example, low -, high carbohydrate, fat or protein diets. However, evidence is conflicting and reports show that people do not maintain diets in the longer term. Thus, the recent question has shifted from 'what' to eat to 'when' to eat.

The 24-h daylight cycle has an impact on the way our bodies work, in particular our blood sugar control as well as where and how much fat we store. Additionally, societal changes have brought about around the clock eating behaviours which can also negatively impact health. Many people now eat late into the evening when our biological rhythms are designed to be in a state of fasting. Limiting the time-window to eat might be an alternative and more tolerable way to improve blood sugar levels than paying such precise and burdensome attention to counting calories or the diet composition.

Restricting daily eating time to ~8hours has been shown to improve blood sugar control in obese and T2D adults. The current study now aims to investigate if this can be optimised by eating earlier in the day. Seventeen obese, at risk T2D participants will be studied, under two separate 3-day conditions. Overall food intake will be matched but the timing of eating will be altered between early or late conditions. A range of clinically valid and gold standard research assessments will be used to understand if altering the timing of an obese adults daily eating can have an impact on blood sugar control and the other risk markers such as the amount of fat within their blood. The results will provide the first dataset of its kind and will aid our understanding of dietary approaches that are potentially more beneficial in-patient groups with impaired blood sugar control. The project is a platform for mechanistic data that has societal importance in the longer term, for example preventing progression to, and management of T2D.

References

Chow, L. S., Manoogian, E. N. C., Alvear, A., Fleischer, J. G., Thor, H., Dietsche, K., Wang, Q., Hodges, J. S., Esch, N., Malaeb, S., et al. 2020. Time-Restricted Eating Effects on Body Composition and Metabolic Measures in Humans who are Overweight: A Feasibility Study. *Obesity (Silver Spring),* 28**,** 860-869.

Gabel, K. & Varady, K. A. 2020. Current research: effect of time restricted eating on weight and cardiometabolic health. *J Physiol*.

Hutchison, A. T., Regmi, P., Manoogian, E. N. C., Fleischer, J. G., Wittert, G. A., Panda, S. & Heilbronn, L. K. 2019. Time-Restricted Feeding Improves Glucose Tolerance in Men at Risk for Type 2 Diabetes: A Randomized Crossover Trial. *Obesity (Silver Spring),* 27**,** 724-732.

Kesztyus, D., Cermak, P., Gulich, M. & Kesztyus, T. 2019. Adherence to Time-Restricted Feeding and Impact on Abdominal Obesity in Primary Care Patients: Results of a Pilot Study in a Pre-Post Design. *Nutrients,* 11.
Lean, M., McCombie, L. & McSorely, J. 2019. Trends in type 2 diabetes. *BMJ,* 366**,** l5407.

Organisation, W. H. *Obesity and overweight* [Online]. Available: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight [Accessed 22-02-2021 2021].

Parr, E. B., Devlin, B. L., Radford, B. E. & Hawley, J. A. 2020a. A Delayed Morning and Earlier Evening Time-Restricted Feeding Protocol for Improving Glycemic Control and Dietary Adherence in Men with Overweight/Obesity: A Randomized Controlled Trial. *Nutrients,* 12.

Parr, E. B., Heilbronn, L. K. & Hawley, J. A. 2020b. A Time to Eat and a Time to Exercise. *Exerc Sport Sci Rev,* 48**,** 4-10.

Rennie, K. L., Coward, A. & Jebb, S. A. 2007. Estimating under-reporting of energy intake in dietary surveys using an individualised method. *Br J Nutr,* 97**,** 1169-76.

Sutton, E. F., Beyl, R., Early, K. S., Cefalu, W. T., Ravussin, E. & Peterson, C. M. 2018. Early Time-Restricted Feeding Improves Insulin Sensitivity, Blood Pressure, and Oxidative Stress Even without Weight Loss in Men with Prediabetes. *Cell Metab,* 27**,** 1212-1221 e3.

Whiting, D. R., Guariguata, L., Weil, C. & Shaw, J. 2011. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes Res Clin Pract,* 94**,** 311-21.