BMJ Open

Protocol for a randomised controlled trial on the effects of time-restricted eating on body weight, behaviour, and metabolism in individuals at high risk of type 2 diabetes - The Restricted Eating Time (RESET) study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-037166
Article Type:	Protocol
Date Submitted by the Author:	21-Jan-2020
Complete List of Authors:	Quist, Jonas; Steno Diabetes Center Copenhagen Christensen, Marie; Steno Diabetes Center Copenhagen Clemmensen, Kim; Steno Diabetes Center Copenhagen Pedersen, Hanne; Steno Diabetes Center Copenhagen; iMotions A/S Bjerre, Natasja; Steno Diabetes Center Copenhagen Størling, Joachim; Steno Diabetes Center Copenhagen Blond, Martin; Steno Diabetes Center Copenhagen Wewer Albrechtsen, Nicolai; University of Copenhagen; Rigshospitalet Holst, Jens; Faculty of Health Sciences, University of Copenhagen, The NNF Center for Basic Metabolic Research, Department of Biomedical Sciences Torekov, Signe; University of Copenhagen, Department of Biomedical Sciences Vistisen, Dorte; Steno Diabetes Center Copenhagen Jørgensen, Marit; Steno Diabetes Center Copenhagen; University of Southern Denmark Panda, Satchidananda; Salk Institute for Biological Studies Brock, Christina; Aalborg Universitet Finlayson, G; University of Leeds Færch, Kristine; Steno Diabetes Center Copenhagen
Keywords:	DIABETES & ENDOCRINOLOGY, Diabetes & endocrinology < INTERNAL MEDICINE, NUTRITION & DIETETICS

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Title: Protocol for a randomised controlled trial on the effects of time-restricted eating on body weight, behaviour, and metabolism in individuals at high risk of type 2 diabetes - The Restricted Eating Time (RESET) study

Authors: Jonas Salling Quist[1], Marie Møller Christensen[1], Kim Katrine Bjerring Clemmensen[1], Hanne Pedersen[1,2], Natasja Bjerre[1], Joachim Størling[1], Martin Bæk Blond[1], Nicolai Jacob Wewer Albrechtsen[3,4,5], Jens Juul Holst[3,6], Signe Sørensen Torekov[3,6], Dorte Vistisen[1], Marit Eika Jørgensen[1,7], Satchidananda Panda[8], Christina Brock[9], Graham Finlayson[10], Kristine Færch[1,3]

Author affiliations:

1. Steno Diabetes Center Copenhagen, Gentofte, Denmark
2. iMotions A/S, Frederiksberg, Denmark
3. Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
4. Department of Clinical Biochemistry, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark
5. Novo Nordisk Foundation Center for Protein Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
6. Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
7. National Institute of Public Health, University of Southern Denmark, Copenhagen, Denmark
8. Salk Institute of Biological Studies, La Jolla, CA, USA
9. Mech-Sense, Department of Gastroenterology and Hepatology, Aalborg University Hospital and Clinical Institute Aalborg University, Aalborg, Denmark
10. School of Psychology, Faculty of Medicine & Health, University of Leeds, Leeds, UK

Correspondance: Jonas Salling Quist, Clinical Prevention Research, Steno Diabetes Center Copenhagen, Niels Steensens Vej 6, Building NSK 2.07, DK-2810 Gentofte, Denmark, e-mail: jonas.salling.quist@regionh.dk

Word count: 5,043

Abstract

Introduction

The aim of this study is to investigate effects of TRE on change in body weight and describe changes in behaviour and metabolism in individuals at high risk of type 2 diabetes.

Methods and analysis

The Restricted Eating Time (RESET) study is a randomised controlled parallel open-label trial. 100 women and men with 1) overweight (BMI: ≥25 kg/m^2) and prediabetes (HbA_{1c}: 39-47 mmol/mol); or 2) obesity (BMI: ≥30 kg/m^2) will be randomised to a control group (habitual living) or TRE (self-selected 10-hour eating window within the period from 6 am to 8 pm) in a 1:1 ratio. Testing is scheduled at baseline and after 6 weeks (mid-intervention), 3 months (post-intervention), and 6 months (follow-up). The primary outcome is change in body weight after 3 months of intervention. Secondary outcomes include changes in body composition; measures of glucose metabolism including glycaemic variability, hormones, and metabolites; subjective and metabolic markers of appetite, food preferences and reward; dietary intake; physical activity, sleep, chronotype; gastric emptying, gastrointestinal transit time and motility; respiratory and glycolytic capacities; the plasma proteome and metabolome; blood pressure, resting heart rate, and heart rate variability; and energy expenditure and substrate oxidation. Motivation and feasibility will be examined based on interviews at baseline and after 3 months. After the 3-month intervention, a 3-month follow-up period and subsequent testing is scheduled to assess maintenance and longer-term effects.

Ethics and dissemination

The study has been approved by the Ethics Committee of the Capital Region of Denmark (H-18059188) and the Danish Data Protection Agency. The study will be conducted in accordance with the Declaration of Helsinki. Results from the study will address whether TRE is effective and feasible in improving health outcomes in individuals at risk of lifestyle-related diseases and can potentially inform the design of feasible health recommendations.

Trial registration

The trial is registered at ClinicalTrials.gov, identifier: NCT03854656

Strengths and limitations of the study

- The study includes state-of-the-art and novel technologies to assess effects of the intervention on food preferences and reward, the gastrointestinal tract, respiratory and glycolytic capacities, as well as proteomics and metabolomics.
- The interdisciplinary nature of the study and assessment of feasibility and sustainability using qualitative methods allows understanding of the participants' experiences and potential barriers and strategies for integration and maintenance of time-restricted eating (TRE) in everyday life.
- The duration of the trial does not allow for the investigation of long-term effects and hard endpoints, but the follow-up visit allows for evaluation of maintenance 3 months after the end of the intervention.
- Assessment of dietary intake in free-living conditions is challenging and adherence to the intervention is assessed based on participants' self-reported daily eating windows.
- Except of reminders regarding reporting of daily eating windows, no support to comply with the prescribed intervention is provided to the participants during the trial; adherence is therefore entirely dependent on the motivation and self-determination of the participants.
- The strict TRE regime (same eating window from day to day) and the burden of the visits may lead to the recruitment of people with specific lifestyles that allow for participation, which could reduce generalisability.

Introduction

Overweight, obesity, and prediabetes increase the risk of developing type 2 diabetes and cardiovascular disease (1–3). Weight loss is associated with improved glycaemic control and cardiometabolic health among individuals with prediabetes and type 2 diabetes (4,5); therefore, the development of effective, feasible, and sustainable weight loss strategies is essential. Current prevention and treatment of obesity and type 2 diabetes include energy restricted diets and increased levels of physical activity (6). However, adherence and maintenance to such strategies is difficult (7,8), underscoring an unmet need for more acceptable and feasible regimens.

Circadian rhythms are ~24-hour rhythms of behaviour and metabolism that are closely related to the daily light/dark cycle and sleep-wake patterns (9,10). Timing of food intake may affect the circadian rhythms of metabolic organs (9). Factors including the 24-hour availability of energy-dense foods and different eating and sleep patterns during weekdays and weekends (i.e. 'social jetlag') may lead to an irregular feeding-fasting rhythm (11,12). Observational studies suggest that irregular eating patterns and late night food consumption are associated with increased cardiometabolic risk (13). Experimental studies in rodents and humans have shown that circadian misalignment of food intake and sleep may have adverse effects on energy balance, glucose metabolism, and appetite regulation (14–17), suggesting a great therapeutic potential of aligning food intake to circadian rhythms of metabolism. Studies in rodents and flies suggest that time-restricted feeding is associated with improvements in metabolic health including improved glucose and lipid metabolism and reductions in adiposity and systemic inflammation (9,18). However, there is a lack of randomised controlled trials investigating the effects of timing of food intake on human behaviour and metabolism.

Recent cross-over intervention studies in humans have investigated short-term (4 days to 5 weeks) effects of time-restricted eating (TRE) under well-controlled conditions. Among men at high risk of type 2 diabetes, 'early TRE' (eating window: 6-9 hours/day, between 8 am and 5 pm) improved glucose metabolism (19–21) and reduced appetite (19,22). A few small pilot intervention studies (n=8-23) have investigated effects of 10-16 weeks of TRE (eating window: ~8-12 hours/day) in individuals with overweight and obesity and reported reductions in energy intake and body weight (11,23,24) and adiposity (24,25). Furthermore, in one of the studies, in which a clinically relevant weight loss (3.9%) was observed, the participants felt more energetic and reported less hunger and improved sleep quality; however, no control group was included (11). In the same study, maintenance was assessed at one-year follow-up. Importantly, upon completion of the 16 weeks intervention, all eight participants in the same study were interested in continuing the regimen, and they maintained weight loss at follow-up (3.4%) (11), suggesting that TRE may be feasible, acceptable, and

sustainable. Additionally, the long fasting period during 'early TRE' seems to be well tolerated (19); however, challenges associated with social events including drinking and eating may exist (25). Nevertheless, an in-depth investigation of feasibility and sustainability of TRE is needed to understand motivation and potential barriers for integration and maintenance in everyday life. In this study, the effects and feasibility of TRE in individuals at high risk of type 2 diabetes will be assessed using an interdisciplinary approach including state-of-the-art and novel quantitative and qualitative methods.

Objectives

The primary objective of the Restricted Eating Time (RESET) study is to investigate effects of 3 months of TRE (10 hours/day) on change in body weight in individuals at high risk of type 2 diabetes. Secondary objectives are to describe changes in body weight and composition, metabolism, and behaviour and to assess aspects related to motivation, feasibility, and maintenance during the 3-months intervention and after additional 3 months of follow-up.

Hypotheses

We hypothesise that 3 months of TRE will induce a clinically relevant weight loss in individuals with overweight and obesity at high risk of type 2 diabetes (i.e. TRE superior to control). Furthermore, we expect that weight loss is maintained in the TRE group at the 3-months follow-up visit (i.e. TRE superior to control).

Methods and analysis

Study design

The study is a randomised controlled parallel group open-label trial (Figure 1). 100 individuals will be randomised to 3 months of TRE or habitual living (control) in a 1:1 ratio. Randomisation is performed after completion of screening and baseline testing (visit 1 (V1)). The primary outcome is assessed after 3 months of intervention (V3). At baseline, mid-intervention (after 6 weeks; V2) and after the intervention (3 months; V3) outcomes are assessed during test days and free-living measurements during the week following the test days. After the 3-month intervention, a 3-month follow-up period and subsequent testing (V4) is scheduled to assess maintenance and longer-term effects. The trial will be performed at Steno Diabetes Center

Copenhagen and will be reported according to the Consolidated Standards of Reporting Trials (26). The study protocol follows the Standard Protocol Items: Recommendations for Interventional Trials statement (27).

Participants

Women and men, 30-70 years of age, with a) overweight (BMI: ≥25 kg/m^2) with concomitant prediabetes as defined by HbA_{1c}: 39-47 mmol/mol (6) or b) obesity (BMI: ≥30 kg/m^2), who are eligible according to the inclusion and exclusion criteria (Table 1) will be included.

Eligibility criteria

Inclusion and exclusion criteria are listed in Table 1. We include high-risk individuals 30-70 years of age, because our focus is on preventing diabetes at an early stage. Individuals with overweight and prediabetes and obesity with/without prediabetes are included to target individuals at high risk of type 2 diabetes. The rationale of choosing 70 years as upper limit is that the potential for prevention is limited in older individuals. To prevent sources of circadian irregularity during the intervention, shift workers and individuals with a partner engaged in shift work affecting the circadian rhythm of the participant are not eligible for participation.

Recruitment and screening

Participants are recruited through advertisements on different publicly available platforms (newspapers, webpages, pharmacies, etc.). A pre-screening is performed as a telephone interview focusing on the participant's age, BMI, and habitual eating window to reduce the number of screen failures. Participants who are eligible based on the pre-screening, receive written information about the study and are scheduled for a screening visit. At the screening visit participants provide oral and written informed consent and a health examination including medical history and assessment of inclusion and exclusion criteria is performed (Table 1). After the screening, eligible participants will be scheduled for four visits (V1-V4, Figure 1). Baseline testing (V1) takes place as soon as possible and within 6 weeks from the screening visit (V0). The first participant signed consent on February 25, 2019, and participants are recruited continuously.

Outcomes

Primary outcome

The primary outcome is change in body weight (kg) from baseline (V1) to end of intervention (after 3 months, V3). Change in body weight was chosen as the primary outcome for several reasons. First, weight loss is associated with a reduction in all-cause mortality in individuals with obesity (28) and with improvement in glycaemic control in individuals with overweight and obesity (4). Second, according to the American Diabetes Association, weight loss is recommended for all individuals with prediabetes (6). Third, body weight is easy to measure with high precision and available in most clinical studies which enable determination of sample size and comparison across studies.

Secondary exploratory outcomes

The secondary exploratory outcomes include a variety of metabolic and behavioural outcomes potentially associated with the intervention. These include changes in body composition, hormones involved in glucose metabolism and appetite regulation (e.g. pancreatic and gastrointestinal hormones), metabolites, glycaemic variability; subjective appetite, food preferences and reward, and eating behaviour; gastric emptying, gastrointestinal motility, and transit time; physical activity, dietary intake, and sleep; inflammatory markers; respiratory and glycolytic capacities; the plasma proteome and metabolome; blood pressure, resting heart rate, and heart rate variability; and energy expenditure and substrate oxidation (Table 2 and ClinicalTrials.gov, identifier: NCT03854656). We describe changes from baseline to mid-intervention (after 6 weeks; V2), post intervention (after 3 months; V3) and follow-up testing (after 6 months; V4).

Study visits and free-living assessment periods

The study includes identical test days and free-living assessment periods at baseline (before randomisation, V1) and post intervention (V3). The test day at V3 is scheduled after 12 weeks intervention; however, participants are instructed to follow their group allocation during the subsequent one-week free-living assessment period (i.e. 13th week of the intervention). Mid-intervention testing after 6 weeks (V2) includes a short test day and a subsequent free-living assessment period during which participants follow their group allocation. After the 13 weeks follow-up period (26 weeks from baseline), a short test day (V4) is scheduled to assess maintenance of potential intervention effects. If possible, all four test days are scheduled on similar week days and at the same time in the morning. An overview of the study visits is presented in Table 2.

Test days

All clinical examinations are conducted at Steno Diabetes Center Copenhagen. Participants arrive in the morning at ~8 am after a ~12-hour overnight fast. All participants are instructed to have a last meal between 7-8 pm the day prior to the test days to minimize potential acute effects of varying fasting duration on the outcomes of interest (29). Furthermore, no alcohol consumption or strenuous physical activity are allowed 48 hours prior to testing. The participants are instructed to avoid physically demanding transportation to the research facility.

Anthropometry

Height is measured using a stadiometer (SECA, Vogel&Halke, Hamburg, Germany) and body weight is measured using a digital scale (Tanita BWB-620A, Amsterdam, The Netherlands) while participants are wearing only light clothes/underwear. Waist circumference is measured at the midpoint between the lowest point of the lowest rib and the highest point of the iliac crest. Hip circumference is measured at the point of the greater femoral trochanter. An average of two repeated measurements of hip and waist circumference is used. In case of >3 cm difference between the two measurements a third measurement is conducted, and the average of the two closest measurements is used. Body composition (fat mass and fat free mass) is measured using whole-body Dual-energy X-ray Absorptiometry (Discovery, Hologic, Bedford, MA, USA). A urine sample is collected, and a pregnancy test is performed for all fertile women <60 years before the scan.

Blood pressure and resting heart rate

Blood pressure (mmHg) and resting heart rate (beats per minute) are measured three times with 2 minutes intervals using a digital blood pressure monitor (UA-852, A&D Instruments, Abingdon, UK) after a minimum of 10 min rest, and the average of the two lowest values of three consecutive measurements are used to avoid falsely high blood pressure caused by an unfamiliar and potentially stressful environment.

Heart rate variability

Heart rate variability and cardiovascular reflex are measured by electrocardiography using a handheld device (Vagus^{TM}, Medicus Engineering, Aarhus, Denmark) during four consecutive tests: 1) resting heart rate is measured while the participant is in the supine position holding the device; 2) heart rate response to standing up from the supine position; 3) heart rate response to inhalation and exhalation is measured in the seated position; 4) heart rate response to increased intrathoracic pressure (Valsalva manoeuvre).

Resting energy expenditure and substrate oxidation

Resting energy expenditure and substrate oxidation are measured for 30 min using indirect calorimetry and a ventilated hood (Vyntus CPX, CareFusion, Hoechberg, Germany) with the participant resting in the supine position in a quiet room. Energy expenditure (30) and substrate oxidation (31) are calculated based on respiratory gas exchange i.e. carbon dioxide production and oxygen consumption.

Blood samples

Venous blood samples are collected in the fasting state at all four test days (V1-V4) and postprandially during a mixed meal test at V1 and V3 at time points 15, 30, 45, 60, 90, 120, 180, and 240 min via a catheter in an antecubital vein. Analyses include assessment of HbA_{1c}, circulating levels of glucose and lipids, inflammatory markers, and hormones involved in regulation of appetite and metabolism (e.g. insulin, glucagon, glucagon-like peptide-1, glucose-dependent insulinotropic polypeptide, peptide YY, acylated ghrelin). Furthermore, metabolomics and proteomics will be applied, and assessment of circulating proteins and metabolites that correlate with low-grade inflammation and markers of lipid metabolism will be captured using mass-spectrometry driven analyses of the plasma proteome and metabolome (32,33). Gene expression of pro- and anti-inflammatory proteins including cytokines and chemokines, and genes involved in energy metabolism of isolated peripheral blood mononuclear cells will be measured by real-time polymerase chain reaction. Cellular bioenergetic activity (mitochondrial respiration and glycolysis) of isolated peripheral blood mononuclear cells will be determined using a Seahorse XFe24 Analyzer (34). The seahorse technology measures real-time oxygen consumption rate as an indicator of mitochondrial activity and extracellular acidification rate as an indicator of glycolytic activity. Thus, these measurements will provide mechanistic knowledge of TRE-induced metabolic changes at the cellular level.

Meal test

At V1 and V3, after assessments in the fasting state, participants are asked to consume a standard breakfast meal (300 g, 498 kcal, 49% of total energy (%E) carbohydrate, 34 E% fat, 17 E% protein) consisting of bread roll, rye bread, cheese, yoghurt, muesli, butter, marmalade, and 150 ml water. For the following four hours after initiation of the meal, blood samples are collected and subjective appetite is assessed using visual analogue scales (35) at time points 15, 30, 45, 60, 90, 120, 180, and 240 min.

Subjective appetite

Hunger, satiety, fullness, thirst, estimated prospective food consumption, and desire for sweet, salt, and fat, and potential nausea are rated by the participants using electronic visual analogue scales (35) after the blood samples in the basal state at all four test days (V1-V4) and postprandially at V1 and V3.

Food preferences and food reward

At V1 and V3, components of food reward and biometric responses to standardised photographic images of foods will be assessed in the fasting state and 60 min after ingestion of the standardised breakfast meal to examine meal-induced changes in food reward and responses to food stimuli. At V2 and V4 only fasting measurements of food reward and biometric responses will be performed. In a computerised questionnaire, different food reward outcomes (food choice, implicit wanting, explicit liking, and explicit wanting) are measured using the Leeds Food Preference Questionnaire (36–38) in combination with measures of autonomic nervous system activity including arousal estimated from galvanic skin response (Biopac MP160, Biopac Systems Inc, Goleta, CA, USA), emotional response using facial expression analyses (AFFDEX algorithm, Affectiva, MA, USA), and motivated visual attention using eye tracking (Tobii X2-60, Tobiipro, Stockholm, Sweden). The Leeds Food Preference Questionnaire is integrated into a biometric software platform (iMotions) to enable simultaneous collection of data on eye tracking, galvanic skin response, and facial expressions.

Questionnaires

At V1-V4, participants fill in questionnaires regarding health and wellbeing, gastrointestinal and autonomic symptoms, eating behaviour, chronotype, sleep, and physical activity (Table 2). At V1, participants fill in a questionnaire regarding sociodemographic characteristics including age, sex, ethnicity, education, occupation, civil status, children, and personal and household income.

Interviews

Interviews will be conducted at V1, V3, and V4 to obtain insights into the participants' experiences and perceptions of the intervention. This will provide an understanding of the feasibility and integration of the intervention into the everyday life of the participants as well as maintenance of the regimen. At V1, all participants will be interviewed for approx. 25 min to examine their reasons and motivation for participation, their expectations towards the intervention, and their everyday life activities and eating practices.

At V3 and V4, all participants in the TRE group will be invited to individual semi-structured interviews of approx. 45 min to explore feasibility and maintenance of TRE in everyday life. If participants withdraw from

the study, they will be invited to individual interviews about their reasons for doing so and their experiences with the intervention. The interviews will draw on social practice theory (39) and will be recorded and transcribed verbatim. Malterud's systematic text condensation approach (40) will be used to analyse the interview data.

Free-living assessment period

A one-week free-living assessment period is scheduled after each of the test days at V1-V3, and includes the procedures described below. Participants are instructed to follow group allocation during the assessment period at V2 and V3. After the one-week free-living assessment period at V3, the participants will be instructed to live as they wish during the subsequent 3-months follow-up period.

Gastric emptying, gastrointestinal transit times and motility

At V1 and V3, participants will be instructed to ingest a wireless motility capsule (SmartPill™, Medtronic, MN, USA) immediately after ingesting the standardised breakfast meal with 150 ml water. The capsule measures pH, temperature, and pressure through the gastrointestinal tract until expulsion. From these data, regional transit time, pH profile, and motility in different parts of the gastrointestinal tract can be estimated (41–43). Participants will be instructed to wear a SmartPill™ receiver unit within 0.25 meters of their body for the following week or until expulsion of the capsule. As this is an expensive measurement, only the first ~60 participants will be offered the capsule.

Events related to the gastrointestinal tract

At V1 and V3, participants register all events related to the gastrointestinal tract (passing stool, eating, sleeping, and gastrointestinal symptoms such as nausea, vomiting, pain in the abdominal region, bloating etc.), until expulsion of the SmartPill, using an 'event button' on the SmartPill™ receiver. Additionally, the participants register the time and type of each event in a diary.

Physical activity and sleep

Physical activity and sleep will be measured using accelerometry. Participants will be equipped with one accelerometer on the thigh and one on the lower back for one week (Axivity AX3, Newcastle upon Tyne, UK). Concomitant with wearing the accelerometers, participants will be asked to fill out a physical activity and sleep diary.

Continuous glucose monitoring

A one-week continuous glucose monitoring (CGM) system (Ipro™, Medtronic Denmark A/S, Copenhagen, Denmark) will be initiated at the test days at V1-V3. The CGM will be attached to the lower part of the abdomen in the morning at the test days. Participants will be instructed to measure blood glucose levels using a glucometer (Contour XT, Ascensia Diabetes Care Denmark ApS, Copenhagen, Denmark) four times a day during the measurement period for calibration of the CGM (before breakfast, lunch, main evening meal, and bedtime).

Dietary intake

During the week following V1, V2, and V3, participants will be asked to fill in a food record diary (pen and paper) for three days (day 1, 3, and 5 after the test day; two weekdays and one weekend day). Participants will be instructed to register weight, time, and content of all meals and beverages (except water). During the same week, participants will be asked to register their eating window (see *assessment of adherence*).

Participants will be instructed to send the CGM, SmartPill™ receiver, accelerometers, and diaries to the researchers after completion of each assessment period.

Gut microbiome

Participants will be provided with a kit for stool samples including storage equipment at V0 and V2. They will be instructed to collect and immediately freeze (-20 °C) three samples from the same stool sample ≤72 hours before test days at V1 and V3. The participants will transport the samples to the laboratory in provided cooling bags and the samples will be stored at -80 °C until analysis. Bacterial DNA and RNA will be purified from the stool samples. The microbial content, composition, and function will be estimated based on sequencing of the microbiome.

Randomisation and intervention

After completing baseline testing, participants are randomly allocated to either the control group or the TRE group. Randomisation is performed in blocks varying in size, unknown to the researchers, to ensure an equal distribution of participants in the two groups in case the study, for unexpectedly reasons, must be terminated before inclusion of all participants. When participants leave the research facilities at the test day at V1, they receive a sleeve with a combination lock which contains information about group allocation. On day 7, when all baseline assessments are completed, the participants are provided with the code for the locker by an

investigator. This approach ensures that participants are blinded to the group allocation during the 7-day free-living assessment period. Over the phone, the investigator provides a detailed description and introduction to the specific group allocation. For practical reasons, randomisation is open for participants and research staff. However, the outcome assessors (data analysts) will be blinded during the statistical analyses of all experimental outcomes.

Time-restricted eating

Participants allocated to the TRE group are instructed to consume all foods and beverages (except water) within a self-selected time window of 10 hours/day between 6 am and 8 pm for the 13-week intervention. Furthermore, the participants are instructed to keep the eating window stable during the entire week and advised to select a window starting at least 2 hours after habitual wake-up time and 3 hours before habitual bedtime if possible. Participants are advised to follow the Danish dietary recommendations. No other dietary restrictions are prescribed.

Control

Participants allocated to habitual living are advised to follow the Danish dietary recommendations but are otherwise instructed to continue their habitual lifestyle during the 13 weeks intervention.

Assessment of adherence

All participants are asked to register time for initiating first and terminating last eating/drinking episode (except water) every day from the test day at V1-V4. Every week during the 26-week period (intervention and follow-up), a link to an online form will be sent by e-mail to the participants for them to register the time for eating/drinking episodes for the previous week. In case participants in the control group restrict their eating window to less than their habitual ≥12 hours/day or if the eating window of participants in the TRE group deviate from their self-selected 10-hour eating window ≥4 days during the first week, the participant will be contacted per telephone to ensure that the participant has understood the concept of their designated group allocation. During the first week of the intervention participants in the TRE group can change their eating window once, in case they are not satisfied with the originally selected window. After the first week no changes are allowed. To ensure similar contact with participants in the control group and the TRE group, the participants will not be contacted in case of non-adherence after the first week. However, if the participants fail to register their eating window, they will be contacted only to remind them to register. No other feedback is provided during the intervention. To account for variations in daily eating windows

around 10 hours, participants in the TRE group are considered adherent if their eating window is less than 11 hours/day. Adherence to the intervention is calculated as number/percentage of days during the intervention the participants' eating window is <11 hours/day. *Per protocol* is defined as ≥80% compliance. The eating window will be calculated for both groups, but no compliance criterion is applied in the control group.

Statistical methods

Sample size determination

There is strong evidence for the clinical relevance of a weight reduction of 3% in people with overweight or obesity, with or without prediabetes (44). No RCT has investigated the effects of TRE on weight loss in individuals with overweight or obesity at a high risk of type 2 diabetes. However, Gill and Panda investigated effects of a 16-week TRE intervention on changes in body weight in eight healthy overweight individuals (BMI >25 kg/m^2) with a habitual eating window >14 hours/day and observed a mean reduction of body weight of 3.9% (3.3 kg, 95% CI: 0.9-5.6 kg); however, no control group was included (11). Per inclusion criteria individuals with a BMI ≥25 kg/m^2 are included in the RESET study. For the participants with a BMI of 25 kg/m^2 (with an expected mean height of 170 cm) a change in weight of 3% will correspond to ~2 kg. Thus, in order to detect a minimal clinically relevant difference in weight change of 3% across the allowed BMI range, the trial was dimensioned to detect a difference in change of 2 kg between the TRE group and the control group. In a recent randomised controlled trial examining the effects of 13 weeks of either exercise or pharmacological therapy on cardiometabolic health in individuals with overweight or obesity and prediabetes, the SD for within-group changes in body weight in the control group was 2.6 kg (Færch et al., under review). We expect that the SD for within-group changes will be similar in the RESET trial, but to account for uncertainties we increased the SD by 20%, resulting in an SD of 3.1 kg. In order to detect a 2 kg (SD 3.1) difference in weight change with a desired statistical power of 0.8 (two-tailed test, alpha 0.05), a total of 40 participants is required in each group. To allow for a 20% drop-out in each group, we plan to include 50 participants in each of the two groups.

Statistical analysis plan

Intention-to-treat analysis including all randomised participants will be performed after the last participant has participated in the last visit. Additionally, *per protocol* analysis will be performed including participants who are compliant during the intervention. Data will be presented with the use of standard descriptive statistics. Descriptive statistics will be shown as mean (SD) for normally distributed data and as median (Q1;

Q3) for non-normally distributed data. Changes from baseline and differences in delta values between groups will be analysed using linear mixed-effects models with the outcome as a function of group, time and group x time interaction and including a participant-specific random intercept. Outcomes with a known/expected bimodal distribution due to sex differences will be adjusted for sex. Adequacy of assumptions of normality and homogeneity of variances will be assessed using graphical methods and, if necessary, data will be log-transformed for analysis and back-transformed for presentation. If model assumptions are not met by logarithmically transformation, non-parametric statistical tests will be performed. *P*-values <0.05 (two-tailed) are considered statistically significant. Results will be presented as estimated mean differences in changes with 95% confidence intervals and p-values when relevant.

Patient and Public Involvement Statement

During the study we enter into dialogue with participants about their experiences of the test days, examinations, participant information etc. with the aim to understand and improve participants' experiences in current and future studies of TRE.

Ethics and dissemination

All equipment used in the studies meet the requirement for patient safety. The total amount of blood taken at each visit is maximally 300 ml, which is less than a standard blood donation of 450 ml and considered safe. Participants will be instructed not to donate blood during the trial. There may be some discomfort associated with swallowing the SmartPill™. The risk of capsule retention in individuals without known stenosis is only 0.75% and in such a case, a pro-motility drug is often sufficient to mobilize the capsule. Alternatively, endoscopy can be performed to retrieve the capsule. Body composition is measured using Dual-energy X-ray Absorptiometry with a radiation dose less than 0.01 mSv, which corresponds to less than one day of normal background radiation. There is no expected discomfort or risks associated with ingestion of the meals, food reward measurements or biometric measurements (eye tracking, galvanic skin response, facial expression analyses). Participants are covered by the Patient Compensation Association according to the Danish Act on the Right to Complain and Receive Compensation within the Health Service. The intervention is considered safe in individuals with overweight and obesity (45).

The study has been approved by the Ethics Committee of the Capital Region of Denmark (H-18059188) and will be conducted in accordance with the Declaration of Helsinki. Approval of data and biobank has been

obtained from the Danish Data Protection Agency. The study is registered at ClinicalTrials.gov (identifier: NCT03854656).

All study-related information will be recorded, handled, and stored in a way that allows accurate reporting, interpretation and verification. Source data will be registered in the electronic data management system REDCap. For CGM measurements, source data will be registered in a web-based software (CareLink™, Medtronic) using the participant's study ID. Source data from Dual-energy X-ray Absorptiometry, biometric measurements, food preferences, Vagus™, SmartPill™, accelerometers and indirect calorimetry are registered on the device or related hardware and uploaded to a secured logged drive to which only project staff has access. Sponsor/investigator will provide direct access to source data/documents for regulatory inspection.

Positive and negative as well as inconclusive study results will be presented at conferences and published in international peer-reviewed journals in accordance with the CONSORT guidelines. All co-authors must comply with the International Committee of Medical Journal Editors guidelines.

Discussion

Implementation and maintenance of traditional strategies for weight loss and early prevention of type 2 diabetes, i.e. increased physical activity and dietary restrictions, is difficult for many (46–48), because it is time consuming and requires insights into the type and amount of foods eaten. In a public health perspective, there is a strong need for feasible lifestyle strategies to combat the current type 2 diabetes epidemic. TRE is a simple eating pattern regime which extends the daily fasting period and potentially synchronizes food intake with circadian rhythms of metabolism and may therefore represent a feasible lifestyle modification strategy. The evidence from animal studies and well-controlled human studies suggest that TRE has the potential to improve a variety of cardiometabolic risk factors in metabolically vulnerable individuals and may be a feasible and sustainable regimen (18); however, randomised controlled trials are lacking. Using an interdisciplinary approach, the present study will examine effects of TRE on weight changes and explore effects on cardiometabolic health and behaviour, as well as on participants' motivation and experiences with TRE.

Implicit in the trial design is that the intervention is only relevant and feasible if the participants can uphold the TRE regime without frequent contact with the research staff; otherwise we find it unlikely that this intervention will have great relevance in the public at large (who are likely less motivated than those opting to partake in an RCT). Eating is integrated in the rhythms and social relations in everyday life. Since TRE affects

eating practices, it is important to get insight into participants' experiences with TRE and to identify potential barriers and strategies for integration and maintenance of this regimen. Thus, the findings from the present study will address whether TRE is an acceptable intervention which can improve health outcomes in individuals at risk of lifestyle-related diseases. As such, it will potentially inform the design of future large-scale studies and feasible health recommendations.

Authors' contributions

JSQ and KF conceived the idea and initiated the study. KF is principal investigator and JSQ, HP, MMC, KKBC, and NB are co-investigators. All authors contributed to the design of the study. JSQ drafted the manuscript. All authors have critically reviewed the manuscript and approved the final version.

Funding and economical compensation

KF is sponsor and principal investigator. The sponsor and investigators have no economic interest in the results of the study. The study is funded by an unrestricted grant from the Novo Nordisk Foundation, a PhD scholarship from Aalborg University and an industrial PhD scholarship from Innovation Fund Denmark. We cover documented, reasonable travel expenses if the participant lives more than 12 km from Steno Diabetes Center Copenhagen. Participants will not receive any other financial compensation for participating in the study.

Competing interests

Steno Diabetes Center Copenhagen is a hospital providing health services for the public health care system. Steno Diabetes Center Copenhagen is partly funded by the Novo Nordisk Foundation through unrestricted grants. The Novo Nordisk Foundation has no economic interests in the study. The Novo Nordisk Foundation will not have influence on the study design, data collection, analysis, interpretation of data, the writing of the study report or any publication and the decision to submit the paper for publication. The investigators employed at Steno Diabetes Center Copenhagen will not benefit economically from conducting the study. HP is co-investigator on the project which is part of her Industrial PhD project in collaboration with iMotions A/S, where HP is employed. iMotions A/S is a collaborator on the project and gives advice for the use and analysis of biometric methods in the study design phase. SP has published a book, The Circadian Code, focusing on the concept of TRE.

References

1. Færch K, Vistisen D, Johansen NB, Jørgensen ME. Cardiovascular risk stratification and management in pre-diabetes. Curr Diab Rep. 2014;14(6).
2. Perreault L, Færch K. Approaching pre-Diabetes. J Diabetes Complications. 2014;28(2):226–33.
3. Valdez R, Yoon PW, Liu T, Khoury MJ. Family History and Prevalence of Diabetes in the U.S. Population: The 6-year results from the National Health and Nutrition Examination Survey (1999 2004). Diabetes Care. 2007;30(10):2517–22.
4. Gummesson A, Nyman E, Knutsson M, Karpefors M. Effect of weight reduction on glycated haemoglobin in weight loss trials in patients with type 2 diabetes. Diabetes, Obes Metab. 2017;19(9):1295–305.
5. le Roux CW, Astrup A, Fujioka K, Greenway F, Lau DCW, Van Gaal L, et al. 3 years of liraglutide versus placebo for type 2 diabetes risk reduction and weight management in individuals with prediabetes: a randomised, double-blind trial. Lancet. 2017;389(10077):1399–409.
6. American Diabetes Association. 3. Prevention or delay of type 2 diabetes: Standards of medical care in diabetes-2019. Diabetes Care. 2019;42(January):S29–33.
7. Anderson JW, Konz EC, Frederich RC, Wood CL. Long-term weight-loss maintenance: A meta-analysis of US studies. Am J Clin Nutr. 2001;74(5):579–84.
8. Lemstra M, Bird Y, Nwankwo C, Rogers M, Moraros J. Weight loss intervention adherence and factors promoting adherence: a meta-analysis. Patient Prefer Adherence. 2016;10:1547–59.
9. Melkani GC, Panda S. Time-restricted feeding for prevention and treatment of cardiometabolic disorders. Vol. 595, Journal of Physiology. 2017. p. 3691–700.
10. Longo VD, Panda S. Fasting, Circadian Rhythms, and Time-Restricted Feeding in Healthy Lifespan. Cell Metab. 2016;23(6):1048–59.
11. Gill S, Panda S. A Smartphone App Reveals Erratic Diurnal Eating Patterns in Humans that Can Be Modulated for Health Benefits. Cell Metab. 2015;22(5):789–98.
12. Wittmann M, Dinich J, Merrow M, Roenneberg T. Social jetlag: misalignment of biological and social time. Chronobiol Int. 2006;23(1–2):497–509.
13. St-Onge M-P, Ard J, Baskin ML, Chiuve SE, Johnson HM, Kris-Etherton P, et al. Meal Timing and Frequency: Implications for Cardiovascular Disease Prevention: A Scientific Statement From the American Heart Association. Circulation. 2017;135:00–00.
14. Arble DM, Bass J, Behn CD, Butler MP, Challet E, Czeisler C. Impact of Sleep and Circadian Disruption on Energy Balance and Diabetes : A Summary of Workshop Discussions. Sleep. 2015;38(12):1849–60.
15. Garaulet M, Gómez-Abellán P. Timing of food intake and obesity: a novel association. Physiol Behav. 2014;134(2014):44–50.
16. Scheer FAJL, Hilton MF, Mantzoros CS, Shea SA. Adverse metabolic and cardiovascular consequences of circadian misalignment. Proc Natl Acad Sci U S A. 2009;106(11):4453–8.
17. Gonnissen HKJ, Hulshof T, Westerterp-Plantenga MS. Chronobiology, endocrinology, and energy- and food-reward homeostasis. Obes Rev. 2013;14(5):405–16.
18. Chaix A, Manoogian ENC, Melkani GC, Panda S. Time-Restricted Eating to Prevent and Manage Chronic Metabolic Diseases. Annu Rev Nutr. 2019;39(1):12.1-12.5.
19. Sutton EF, Beyl R, Early KS, Cefalu WT, Ravussin E, Peterson CM. Early Time-Restricted Feeding Improves Insulin Sensitivity, Blood Pressure, and Oxidative Stress Even without Weight Loss in Men with Prediabetes. Cell Metab. 2018;27(6):1212–1221.e3.
20. Jamshed H, Beyl RA, Manna DL Della, Yang ES, Ravussin E, Peterson CM. Early Time-Restricted Feeding Improves 24-Hour Glucose Levels and Affects Markers of the Circadian Clock, Aging, and

Autophagy in Humans. Nutrients. 2019;11(1234):1–16.

21. Hutchison AT, Regmi P, Manoogian ENC, Fleischer JG, Wittert GA, Panda S, et al. Time-Restricted Feeding Improves Glucose Tolerance in Men at Risk for Type 2 Diabetes: A Randomized Crossover Trial. Obesity. 2019;27(5):724–32.

22. Ravussin E, Beyl RA, Poggiogalle E, Hsia DS, Peterson CM. Early Time-Restricted Feeding Reduces Appetite and Increases Fat Oxidation But Does Not Affect Energy Expenditure in Humans. Obesity. 2019;27(8):1244–54.

23. Gabel K, Hoddy KK, Haggerty N, Song J, Kroeger CM, Trepanowski JF, et al. Effects of 8-hour time restricted feeding on body weight and metabolic disease risk factors in obese adults: A pilot study. Nutr Heal Aging. 2018;4(4):345–53.

24. Wilkinson MJ, Manoogian ENC, Zadourian A, Lo H, Fakhouri S, Shoghi A, et al. Ten-Hour Time-Restricted Eating Reduces Weight, Blood Pressure, and Atherogenic Lipids in Patients with Metabolic Syndrome. Cell Metab. 2019;31(1):92–104.e5.

25. Antoni R, Robertson TM, Robertson MD, Johnston JD. A pilot feasibility study exploring the effects of a moderate time-restricted feeding intervention on energy intake, adiposity and metabolic physiology in free-living human subjects. J Nutr Sci. 2018;7.

26. Schulz KF, Altman DG, Moher D, Group C. CONSORT 2010 Statement : Updated Guidelines for Reporting Parallel Group Randomized Trials OF TO. BMJ. 2013;340(c332).

27. Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin JA, et al. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. BMJ. 2013;346(e7586).

28. Ma C, Avenell A, Bolland M, Hudson J, Stewart F, Robertson C, et al. Effects of weight loss interventions for adults who are obese on mortality, cardiovascular disease, and cancer: systematic review and meta-analysis. BMJ. 2017;359(j4849).

29. Hulmán A, Færch K, Vistisen D, Karsai J, Nyári TA, Tabák AG, et al. Effect of time of day and fasting duration on measures of glycaemia: Analysis from the Whitehall II Study. Diabetologia. 2013;56(2):294–7.

30. Weir JBD V. New methods for calculating metabolic rate with special reference to protein metabolism. J Physiol. 1949;109(1–2):1–9.

31. Frayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. J Appl Physiol. 1983;55(2):628–34.

32. Geyer PE, Kulak NA, Pichler G, Holdt LM, Teupser D, Mann M. Plasma Proteome Profiling to Assess Human Health and Disease. Cell Syst. 2016;2(3):185–95.

33. Galsgaard KD, Winther-Sørensen M, Ørskov C, Kissow H, Poulsen SS, Vilstrup H, et al. Disruption of glucagon receptor signaling causes hyperaminoacidemia exposing a possible liver-alpha-cell axis. Am J Physiol Endocrinol Metab. 2018;314(1):E93–103.

34. Jones N, Piasecka J, Bryant AH, Jones RH, Skibinski DOF, Francis NJ, et al. Bioenergetic analysis of human peripheral blood mononuclear cells. Clin Exp Immunol. 2015;182(1):69–80.

35. Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. Int J Obes. 2000;24(1):38–48.

36. Finlayson G, King N, Blundell JE. Is it possible to dissociate “liking” and “wanting” for foods in humans? A novel experimental procedure. Physiol Behav. 2007;90(1):36–42.

37. Dalton M, Finlayson G. Psychobiological examination of liking and wanting for fat and sweet taste in trait binge eating females. Physiol Behav. 2014;136:128–34.

38. Finlayson G, King N, Blundell J. The role of implicit wanting in relation to explicit liking and wanting for food: Implications for appetite control. Appetite. 2008;50(1):120–7.

39. Warde A. Consumption and theories of practice. J Consum Cult. 2005;5(2):131–53.
40. Malterud K. Systematic text condensation: A strategy for qualitative analysis. Scand J Public Health. 2012;40(8):795–805.
41. Maqbool S, Parkman HP, Friedenberg FK. Wireless capsule motility: Comparison of the smartPill® GI monitoring system with scintigraphy for measuring whole gut transit. Dig Dis Sci. 2009;54(10):2167–74.
42. Farmer AD, Wegeberg AML, Brock B, Hobson AR, Mohammed SD, Scott SM, et al. Regional gastrointestinal contractility parameters using the wireless motility capsule: inter-observer reproducibility and influence of age, gender and study country. Aliment Pharmacol Ther. 2018;47(3):391–400.
43. Zarate N, Mohammed SD, O'Shaughnessy E, Newell M, Yazaki E, Williams NS, et al. Accurate localization of a fall in pH within the ileocecal region: Validation using a dual-scintigraphic technique. Am J Physiol - Gastrointest Liver Physiol. 2010;299(6):1276–86.
44. Jensen MD, Ryan DH, Apovian CM, Ard JD, Comuzzie AG, Donato KA, et al. 2013 AHA/ACC/TOS Guideline for the Management of Overweight and Obesity in Adults. Circulation. 2014;129(25 suppl 2):S102–38.
45. Gabel K, Hoddy KK, Varady KA. Safety of 8-h time restricted feeding in adults with obesity. Appl Physiol Nutr Metab. 2018;44(1):107–9.
46. Wing RR, Phelan S. Long-term weight loss maintenance. Am J Clin Nutr. 2005;82(1 Suppl):222S–225S.
47. Wadden TA, Neiberg RH, Wing RR, Clark JM, Delahanty LM, Hill JO, et al. Four-year weight losses in the look AHEAD study: Factors associated with long-term success. Obesity. 2011;19(10):1987–98.
48. Ulen CG, Huizinga MM, Beech B, Elasy TA. Weight regain prevention. Clin Diabetes. 2008;26(3):100–13.

Figure legend

Figure 1: Study design.

Table 1. Inclusion and exclusion criteria

Inclusion criteria • Age: ≥30 to ≤70 years • Body mass index ≥30 kg/m^2 or body mass index ≥25 kg/m^2 in combination with pre-diabetes (HbA1c ≥39 to <48 mmol/mol) • Habitual eating/drinking window ≥12 hours (including foods/snacks and energy containing beverages e.g. soft drinks (except of water)) and an eating/drinking window of ≥14 hours minimum one day per week **Exclusion criteria** • Daily smoking • For women: pregnancy, planned pregnancy (within the study period) or lactating • Frequent travels over time zones (more than one return trip/travel over times zones (>one-hour time difference) during the 13-week intervention). • Shift work or partner engaged in shift work (if it affects the person's sleep and eating pattern) • Unable to understand the informed consent and the study procedures • Self-reported history of an eating disorder during the past three years • Self-reported weight change (>5 kg) within three months prior to inclusion • Known diabetes or diabetes detected at screening (HbA_{1c} ≥48 mmol/mol) • Uncontrolled medical issues including but not limited to cardiovascular, pulmonary, rheumatologic, hematologic, oncologic, infectious, gastrointestinal or psychiatric disease; endocrine disease; immunosuppression • Current treatment with medication or medical devices which significantly affect glucose metabolism, appetite, or energy balance • Current treatment with antidepressants • Bariatric surgery • Implanted or portable electro-mechanical medical device such as a cardiac pacemaker, defibrillator or infusion pump • Celiac disease, Crohn's disease, ulcerative colitis or proctitis • Alcohol/drug abuse or in treatment with disulfiram at time of inclusion • Concomitant participation in other intervention studies • Not able to eat ≥85% of the test meal because of e.g. allergy
Specific exclusion criteria for participants receiving SmartPill™
• Gastrointestinal symptoms or diseases such as regular (weekly) abdominal pain, dysphagia, gastric bezoars, strictures, fistulas, bowel obstructions or diverticulitis • Current treatment with medication or medical devices which significantly affect gastrointestinal motility or transit time (prokinetics, antidiarrheals, laxatives, or opioids) • Gastrointestinal surgery within 3 months before inclusion
Other criteria for withdrawal and exclusion after inclusion
• Participant's withdrawal of the informed consent • Pregnancy or other safety concerns – judged by the investigator

615

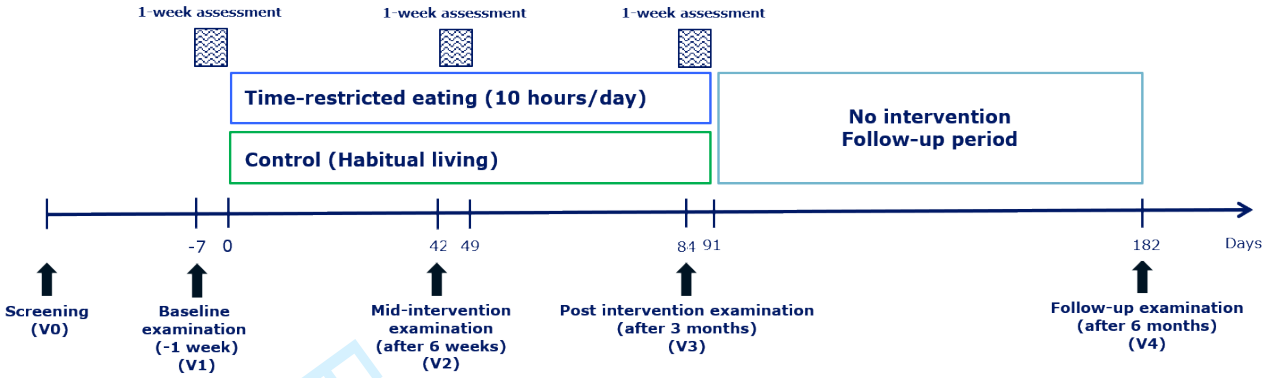
Table 2. Overview of study visits

Visit	V0	V1	V2	V3	V4
Time, weeks from start of intervention	-7[1]	-1	6	12	26
Participant information					
Informed consent	X				
Medical history (individual and family)	X				
In- and exclusion criteria	X				
Pregnancy test (fertile women only)		X	X	X	X
Efficacy outcomes					
HbA_{1C}	X	X	X	X	X
Body weight	X	X	X	X	X
Waist and hip circumference		X	X	X	X
Body composition (DXA scanning)		X	X	X	X
Blood pressure and resting heart rate	X	X	X	X	X
Stool sample		X		X	
Fasting blood samples		X	X	X	X
Postprandial blood samples		X		X	
Indirect calorimetry		X		X	
Heart rate variability (Vagus™)		X		X	
Mixed meal test with SmartPill™		X		X	
Event registration related to SmartPill™		X		X	
Physical activity and sleep measurement		X	X	X	
Food records		X	X	X	
Continuous glucose monitoring		X	X	X	
Fasting food reward and biometric measurements		X	X	X	X
Postprandial food reward and biometric measurements		X		X	
Questionnaires					
Sociodemographic characteristics		X			
Health and wellbeing		X	X	X	X
Physical activity		X	X	X	X
Fasting appetite sensations		X	X	X	X
Postprandial appetite sensations		X		X	
Gastrointestinal symptoms		X	X	X	X
Autonomic symptoms		X			
Pain		X			
Sleep quality and sleepiness		X	X	X	X
Chronotype		X	X	X	X
Night eating		X	X	X	X
Eating behaviour and control over eating		X	X	X	X
Interviews					
Interview (all participants)[2]		X			
Interview (all participants in the TRE group)[3]				X	X

1. Max 6 weeks before baseline testing (V1); 2. Interview regarding motivation for participation; 3. Interview regarding feasibility and maintenance. V0: screening; V1: baseline testing; V2: mid-intervention testing (after 6 weeks); V3: post intervention testing (after 3 months); V4: follow-up testing (after 6 months). Abbreviations: DXA: Dual-energy X-ray Absorptiometry; TRE: Time-restricted eating.

616

Figure 1. Study design



BMJ Open

Protocol for a single-centre, parallel-group, randomised, controlled, superiority trial on the effects of time-restricted eating on body weight, behaviour, and metabolism in individuals at high risk of type 2 diabetes - The Restricted Eating Time (RESET) study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-037166.R1
Article Type:	Protocol
Date Submitted by the Author:	07-May-2020
Complete List of Authors:	Quist, Jonas; Steno Diabetes Center Copenhagen Christensen, Marie; Steno Diabetes Center Copenhagen Clemmensen, Kim; Steno Diabetes Center Copenhagen Pedersen, Hanne; Steno Diabetes Center Copenhagen; iMotions A/S Bjerre, Natasja; Steno Diabetes Center Copenhagen Størling, Joachim; Steno Diabetes Center Copenhagen Blond, Martin; Steno Diabetes Center Copenhagen Wewer Albrechtsen, Nicolai; University of Copenhagen; Rigshospitalet Holst, Jens; Faculty of Health Sciences, University of Copenhagen, The NNF Center for Basic Metabolic Research, Department of Biomedical Sciences Torekov, Signe; University of Copenhagen, Department of Biomedical Sciences Vistisen, Dorte; Steno Diabetes Center Copenhagen Jørgensen, Marit; Steno Diabetes Center Copenhagen; University of Southern Denmark Panda, Satchidananda; Salk Institute for Biological Studies Brock, Christina; Aalborg Universitet Finlayson, G; University of Leeds Færch, Kristine; Steno Diabetes Center Copenhagen
Primary Subject Heading:	Diabetes and endocrinology
Secondary Subject Heading:	Diabetes and endocrinology, Nutrition and metabolism, Research methods
Keywords:	DIABETES & ENDOCRINOLOGY, Diabetes & endocrinology < INTERNAL MEDICINE, NUTRITION & DIETETICS

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Title: Protocol for a single-centre, parallel-group, randomised, controlled, superiority trial on the effects of time-restricted eating on body weight, behaviour, and metabolism in individuals at high risk of type 2 diabetes - The Restricted Eating Time (RESET) study

Authors: Jonas Salling Quist[1], Marie Møller Christensen[1], Kim Katrine Bjerring Clemmensen[1], Hanne Pedersen[1,2], Natasja Bjerre[1], Joachim Størling[1], Martin Bæk Blond[1], Nicolai Jacob Wewer Albrechtsen[3,4,5], Jens Juul Holst[3,6], Signe Sørensen Torekov[3,6], Dorte Vistisen[1], Marit Eika Jørgensen[1,7], Satchidananda Panda[8], Christina Brock[9], Graham Finlayson[10], Kristine Færch[1,3]

Author affiliations:

1. Steno Diabetes Center Copenhagen, Gentofte, Denmark
2. iMotions A/S, Frederiksberg, Denmark
3. Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
4. Department of Clinical Biochemistry, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark
5. Novo Nordisk Foundation Center for Protein Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
6. Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
7. National Institute of Public Health, University of Southern Denmark, Copenhagen, Denmark
8. Salk Institute of Biological Studies, La Jolla, CA, USA
9. Mech-Sense, Department of Gastroenterology and Hepatology, Aalborg University Hospital and Clinical Institute Aalborg University, Aalborg, Denmark
10. School of Psychology, Faculty of Medicine & Health, University of Leeds, Leeds, UK

Correspondance: Jonas Salling Quist, Clinical Prevention Research, Steno Diabetes Center Copenhagen, Niels Steensens Vej 6, Building NSK 2.07, DK-2820 Gentofte, Denmark, e-mail: jonas.salling.quist@regionh.dk

Word count: 5,350

Abstract

Introduction

The aim of this study is to investigate effects of time-restricted eating (TRE) on change in body weight and describe changes in behaviour and metabolism in individuals at high risk of type 2 diabetes.

Methods and analysis

The Restricted Eating Time (RESET) study is a randomised controlled parallel-group open-label trial. 100 women and men with 1) overweight (BMI: ≥25 kg/m^2) and prediabetes (HbA_{1c}: 39-47 mmol/mol); or 2) obesity (BMI: ≥30 kg/m^2) will be randomised to a control group (habitual living) or TRE (self-selected 10-hour eating window within the period from 6 am to 8 pm) in a 1:1 ratio. Testing is scheduled at baseline and after 6 weeks (mid-intervention), 3 months (post-intervention), and 6 months (follow-up). The primary outcome is change in body weight after 3 months of intervention. Secondary outcomes include changes in body composition; measures of glucose metabolism including glycaemic variability, hormones, and metabolites; subjective and metabolic markers of appetite, food preferences and reward; dietary intake; physical activity, sleep, chronotype; gastric emptying, gastrointestinal transit time and motility; respiratory and glycolytic capacities; the plasma proteome and metabolome; blood pressure, resting heart rate, and heart rate variability; and resting energy expenditure and substrate oxidation. Motivation and feasibility will be examined based on interviews at baseline and after 3 months. After the 3-month intervention, a 3-month follow-up period and subsequent testing is scheduled to assess maintenance and longer-term effects.

Ethics and dissemination

The study has been approved by the Ethics Committee of the Capital Region of Denmark (H-18059188) and the Danish Data Protection Agency. The study will be conducted in accordance with the Declaration of Helsinki. Results from the study will address whether TRE is effective and feasible in improving health outcomes in individuals at risk of lifestyle-related diseases and can potentially inform the design of feasible health recommendations.

Trial registration

The trial is registered at ClinicalTrials.gov, identifier: NCT03854656

Strengths and limitations of this study

- The study includes state-of-the-art and novel technologies to assess effects of the intervention on food preferences and reward, the gastrointestinal tract, respiratory and glycolytic capacities, as well as proteomics and metabolomics.
- The interdisciplinary nature of the study and assessment of feasibility and sustainability using qualitative methods allows understanding of the participants' experiences and potential barriers and strategies for integration and maintenance of time-restricted eating (TRE) in everyday life.
- The duration of the trial does not allow for the investigation of long-term effects and hard endpoints, but the follow-up visit allows for evaluation of maintenance 3 months after the end of the intervention.
- Except of reminders regarding reporting of daily eating windows, no support to comply with the prescribed intervention is provided to the participants during the trial; adherence is therefore entirely dependent on the motivation and self-determination of the participants.
- While the broadness of the inclusion criteria allows for the recruitment of a study population more alike to the general population at risk for type 2 diabetes and cardiovascular disease it at the same time increases the risk for heterogeneity in the effects of some of the secondary outcomes.

Introduction

Overweight and prediabetes increase the risk of developing type 2 diabetes and cardiovascular disease (1–3). Weight loss is associated with improved glycaemic control and cardiometabolic health among individuals with prediabetes and type 2 diabetes (4,5); therefore, the development of effective, feasible, and sustainable weight loss strategies is essential. Current prevention and treatment of obesity and type 2 diabetes include energy restricted diets and increased levels of physical activity (6). However, adherence and maintenance to such strategies is difficult (7,8), underscoring an unmet need for more acceptable and feasible regimens.

Circadian rhythms are ~24-hour rhythms of behaviour and metabolism that are closely related to the daily light/dark cycle and sleep-wake patterns (9,10). Timing of food intake may affect the circadian rhythms of metabolic organs (9). Factors including the 24-hour availability of energy-dense foods and different eating and sleep patterns during weekdays and weekends (i.e. 'social jetlag') may lead to an irregular feeding-fasting rhythm (11,12). Observational studies suggest that irregular eating patterns and late night food consumption are associated with increased cardiometabolic risk (13). Experimental studies in rodents and humans have shown that circadian misalignment of food intake and sleep may have adverse effects on energy balance, glucose metabolism, and appetite regulation (14–17), suggesting a great therapeutic potential of aligning food intake to circadian rhythms of metabolism. Studies in rodents and flies suggest that time-restricted feeding is associated with improvements in metabolic health including improved glucose and lipid metabolism and reductions in adiposity and systemic inflammation (9,18). However, there is a lack of randomised controlled trials investigating the effects of timing of food intake on human behaviour and metabolism.

Recent cross-over intervention studies in humans have investigated short-term (4 days to 5 weeks) effects of time-restricted eating (TRE) under well-controlled conditions. Among men at high risk of type 2 diabetes, 'early TRE' (eating window: 6-9 hours/day, between 8 am and 5 pm) improved glucose metabolism (19–21) and reduced appetite (19,22). A few small pilot intervention studies (n=8-23) have investigated effects of 10-16 weeks of TRE (eating window: ~8-12 hours/day) in individuals with overweight and obesity and reported reductions in energy intake and body weight (11,23,24) and adiposity (24,25). Furthermore, in one of the studies, in which a clinically relevant weight loss (3.9%) was observed, the participants felt more energetic and reported less hunger and improved sleep quality; however, no control group was included (11). In the same study, maintenance was assessed at one-year follow-up. Importantly, upon completion of the 16 weeks intervention, all eight participants in the same study were interested in continuing the regimen, and they maintained weight loss at follow-up (3.4%) (11), suggesting that TRE may be feasible, acceptable, and sustainable. Additionally, the long fasting period during 'early TRE' seems to be well tolerated (19); however,

challenges associated with social events including drinking and eating may exist (25). Nevertheless, an in-depth investigation of feasibility and sustainability of TRE is needed to understand motivation and potential barriers for integration and maintenance in everyday life. In this study, the effects and feasibility of TRE in individuals at high risk of type 2 diabetes will be assessed using an interdisciplinary approach including state-of-the-art and novel quantitative and qualitative methods.

Objectives

The primary objective of the Restricted Eating Time (RESET) study is to investigate effects of 3 months of TRE (10 hours/day) on change in body weight in individuals at high risk of type 2 diabetes. Secondary objectives are to describe changes in body weight and composition, metabolism, and behaviour and to assess aspects related to motivation, feasibility, and maintenance during the 3-months intervention and after additional 3 months of follow-up.

Hypotheses

We hypothesise that 3 months of TRE will induce a clinically relevant weight loss in individuals with overweight and obesity at high risk of type 2 diabetes (i.e. TRE superior to control). Furthermore, we expect that weight loss is maintained in the TRE group at the 3-months follow-up visit (i.e. TRE superior to control).

Methods and analysis

Study design

The study is a single-centre, parallel-group, randomised, controlled, superiority, open-label trial (Figure 1). 100 individuals will be randomised to 3 months of TRE or habitual living (control) in a 1:1 ratio. Habitual living was chosen as the comparator to evaluate the effects of TRE when included in everyday routines. Randomisation is performed after completion of screening and baseline testing (visit 1 (V1)). The primary outcome is assessed after 3 months of intervention (V3). At baseline, mid-intervention (after 6 weeks; V2) and after the intervention (3 months; V3) outcomes are assessed during test days and free-living measurements during the week following the test days. After the 3-month intervention, a 3-month follow-up period and subsequent testing (V4) is scheduled to assess maintenance and longer-term effects. The trial

will be performed at Steno Diabetes Center Copenhagen and will be reported according to the Consolidated Standards of Reporting Trials (26). The study protocol follows the Standard Protocol Items: Recommendations for Interventional Trials statement (27). The trial is registered at ClinicalTrials.gov (identifier: NCT03854656) and a copy of the World Health Organization Trial Registration Data Set is supplied in Supplementary Table 1.

Participants

Women and men, 30-70 years of age, with a) overweight (BMI: ≥25 kg/m^2) with concomitant prediabetes as defined by HbA_{1c}: 39-47 mmol/mol (6) or b) obesity (BMI: ≥30 kg/m^2), who are eligible according to the inclusion and exclusion criteria (Table 1) will be included.

Eligibility criteria

Inclusion and exclusion criteria are listed in Table 1. We include high-risk individuals 30-70 years of age, because our focus is on preventing diabetes at an early stage. Individuals with overweight and prediabetes and obesity with/without prediabetes are included to target individuals at high risk of type 2 diabetes. The rationale of choosing 70 years as upper limit is that the potential for prevention is limited in older individuals. To prevent sources of circadian irregularity during the intervention, shift workers and individuals with a partner engaged in shift work affecting the circadian rhythm of the participant are not eligible for participation.

Recruitment and screening

Participants are recruited through advertisements on different publicly available platforms (newspapers, webpages, pharmacies, etc.). A pre-screening is performed as a telephone interview focusing on the participant's age, BMI, and habitual eating window to reduce the number of screen failures. Participants who are eligible based on the pre-screening, receive written information about the study and are scheduled for a screening visit. At the screening visit participants provide oral and written informed consent to medical staff, and a health examination including medical history and assessment of inclusion and exclusion criteria is performed (Table 1). After the screening, eligible participants will be scheduled for four visits (V1-V4, Figure 1). Baseline testing (V1) takes place as soon as possible and within 6 weeks from the screening visit (V0). The first participant signed consent on February 25, 2019, and participants are recruited continuously.

Outcomes

Primary outcome

The primary outcome is mean change in body weight (kg) from baseline (V1) to end of intervention (after 3 months, V3). Change in body weight was chosen as the primary outcome for several reasons. First, weight loss is associated with a reduction in all-cause mortality in individuals with obesity (28) and with improvement in glycaemic control in individuals with overweight and obesity (4). Second, according to the American Diabetes Association, weight loss is recommended for all individuals with prediabetes (6). Third, body weight is easy to measure with high precision and available in most clinical studies which enable determination of sample size and comparison across studies.

Secondary exploratory outcomes

The secondary exploratory outcomes include a variety of metabolic and behavioural outcomes potentially associated with the intervention. These include changes in body composition, hormones involved in glucose metabolism and appetite regulation (e.g. pancreatic and gastrointestinal hormones), metabolites, glycaemic variability; subjective appetite, food preferences and reward, and eating behaviour; gastric emptying, gastrointestinal motility, and transit time; physical activity, dietary intake, and sleep; inflammatory markers; respiratory and glycolytic capacities; the plasma proteome and metabolome; blood pressure, resting heart rate, and heart rate variability; and energy expenditure and substrate oxidation (Table 2 and ClinicalTrials.gov, identifier: NCT03854656). We describe changes from baseline to mid-intervention (after 6 weeks; V2), post intervention (after 3 months; V3) and follow-up testing (after 6 months; V4).

Study visits and free-living assessment periods

The study includes identical test days and free-living assessment periods at baseline (before randomisation, V1) and post intervention (V3). The test day at V3 is scheduled after 12 weeks intervention; however, participants are instructed to follow their group allocation during the subsequent one-week free-living assessment period (i.e. 13th week of the intervention). Mid-intervention testing after 6 weeks (V2) includes a short test day and a subsequent free-living assessment period during which participants follow their group allocation. After the 13 weeks follow-up period (26 weeks from baseline), a short test day (V4) is scheduled to assess maintenance of potential intervention effects. If possible, all four test days are scheduled on similar week days and at the same time in the morning. An overview of the study visits is presented in Table 2.

Test days

All clinical examinations are conducted at Steno Diabetes Center Copenhagen. Participants arrive in the morning at ~8 am after a ~12-hour overnight fast. All participants are instructed to have a last meal between 7-8 pm the day prior to the test days to minimize potential acute effects of varying fasting duration on the outcomes of interest (29). Furthermore, no alcohol consumption or strenuous physical activity are allowed 48 hours prior to testing. The participants are instructed to avoid physically demanding transportation to the research facility.

Anthropometry

Height is measured using a stadiometer (SECA, Vogel&Halke, Hamburg, Germany) and body weight is measured using a digital scale (Tanita BWB-620A, Amsterdam, The Netherlands) while participants are wearing only light clothes/underwear. Waist circumference is measured at the midpoint between the lowest point of the lowest rib and the highest point of the iliac crest. Hip circumference is measured at the point of the greater femoral trochanter. An average of two repeated measurements of hip and waist circumference is used. In case of >3 cm difference between the two measurements a third measurement is conducted, and the average of the two closest measurements is used. Body composition (fat mass and fat free mass) is measured using whole-body Dual-energy X-ray Absorptiometry (Discovery, Hologic, Bedford, MA, USA). A urine sample is collected, and a pregnancy test is performed for all fertile women <60 years before the scan.

Blood pressure and resting heart rate

Blood pressure (mmHg) and resting heart rate (beats per minute) are measured three times with 2 minutes intervals using a digital blood pressure monitor (UA-852, A&D Instruments, Abingdon, UK) after a minimum of 10 min rest, and the average of the two lowest values of three consecutive measurements are used to avoid falsely high blood pressure caused by an unfamiliar and potentially stressful environment.

Heart rate variability

Heart rate variability and cardiovascular reflex are measured by electrocardiography using a handheld device (Vagus^{TM}, Medicus Engineering, Aarhus, Denmark) during four consecutive tests: 1) resting heart rate is measured while the participant is in the supine position holding the device; 2) heart rate response to standing up from the supine position; 3) heart rate response to inhalation and exhalation is measured in the seated position; 4) heart rate response to increased intrathoracic pressure (Valsalva manoeuvre).

Resting energy expenditure and substrate oxidation

Resting energy expenditure and substrate oxidation are measured for 30 min using indirect calorimetry and a ventilated hood (Vyntus CPX, CareFusion, Hoechberg, Germany) with the participant resting in the supine position in a quiet room. Energy expenditure (30) and substrate oxidation (31) are calculated based on respiratory gas exchange i.e. carbon dioxide production and oxygen consumption.

Blood samples

Venous blood samples are collected in the fasting state at all four test days (V1-V4) and postprandially during a mixed meal test at V1 and V3 at time points 15, 30, 45, 60, 90, 120, 180, and 240 min via a catheter in an antecubital vein. Analyses include assessment of HbA_{1c}, circulating levels of glucose and lipids, inflammatory markers, and hormones involved in regulation of appetite and metabolism (e.g. insulin, glucagon, glucagon-like peptide-1, glucose-dependent insulinotropic polypeptide, peptide YY, acylated ghrelin). Furthermore, metabolomics and proteomics will be applied, and assessment of circulating proteins and metabolites that correlate with low-grade inflammation and markers of lipid metabolism will be captured using mass-spectrometry driven analyses of the plasma proteome and metabolome (32,33). Gene expression of pro- and anti-inflammatory proteins including cytokines and chemokines, and genes involved in energy metabolism of isolated peripheral blood mononuclear cells will be measured by real-time polymerase chain reaction. Cellular bioenergetic activity (mitochondrial respiration and glycolysis) of isolated peripheral blood mononuclear cells will be determined using a Seahorse XFe24 Analyzer (34). The seahorse technology measures real-time oxygen consumption rate as an indicator of mitochondrial activity and extracellular acidification rate as an indicator of glycolytic activity. Thus, these measurements will provide mechanistic knowledge of TRE-induced metabolic changes at the cellular level. Serum and plasma will be stored in a biobank for future analyses.

Meal test

At V1 and V3, after assessments in the fasting state, participants are asked to consume a standard breakfast meal (300 g, 498 kcal, 49% of total energy (%E) carbohydrate, 34 E% fat, 17 E% protein) consisting of bread roll, rye bread, cheese, yoghurt, muesli, butter, marmalade, and 150 ml water. For the following four hours after initiation of the meal, blood samples are collected and subjective appetite is assessed using visual analogue scales (35) at time points 15, 30, 45, 60, 90, 120, 180, and 240 min.

Subjective appetite

Hunger, satiety, fullness, thirst, estimated prospective food consumption, and desire for sweet, salt, and fat, and potential nausea are rated by the participants using electronic visual analogue scales (35) after the blood samples in the basal state at all four test days (V1-V4) and postprandially at V1 and V3.

Food preferences and food reward

At V1 and V3, components of food reward and biometric responses to standardised photographic images of foods will be assessed in the fasting state and 60 min after ingestion of the standardised breakfast meal to examine meal-induced changes in food reward and responses to food stimuli. At V2 and V4 only fasting measurements of food reward and biometric responses will be performed. In a computerised questionnaire, different food reward outcomes (food choice, implicit wanting, explicit liking, and explicit wanting) are measured using the Leeds Food Preference Questionnaire (36–38) in combination with measures of autonomic nervous system activity including arousal estimated from galvanic skin response (Biopac MP160, Biopac Systems Inc, Goleta, CA, USA), emotional response using facial expression analyses (AFFDEX algorithm, Affectiva, MA, USA), and motivated visual attention using eye tracking (Tobii X2-60, Tobiipro, Stockholm, Sweden). The Leeds Food Preference Questionnaire is integrated into a biometric software platform (iMotions) to enable simultaneous collection of data on eye tracking, galvanic skin response, and facial expressions.

Questionnaires

At V1-V4, participants fill in questionnaires regarding health and wellbeing, gastrointestinal and autonomic symptoms, eating behaviour, chronotype, sleep, and physical activity (Table 2). At V1, participants fill in a questionnaire regarding sociodemographic characteristics including age, sex, ethnicity, education, occupation, civil status, children, and personal and household income.

Interviews

Interviews will be conducted at V1, V3, and V4 to obtain insights into the participants' experiences and perceptions of the intervention. This will provide an understanding of the feasibility and integration of the intervention into the everyday life of the participants as well as maintenance of the regimen. At V1, all participants will be interviewed for approx. 25 min to examine their reasons and motivation for participation, their expectations towards the intervention, and their everyday life activities and eating practices.

At V3 and V4, all participants in the TRE group will be invited to individual semi-structured interviews of approx. 45 min to explore feasibility and maintenance of TRE in everyday life. If participants withdraw from

the study, they will be invited to individual interviews about their reasons for doing so and their experiences with the intervention. The interviews will draw on social practice theory (39) and will be recorded and transcribed verbatim. Malterud's systematic text condensation approach (40) will be used to analyse the interview data.

Free-living assessment period

A one-week free-living assessment period is scheduled after each of the test days at V1-V3, and includes the procedures described below. Participants are instructed to follow group allocation during the assessment period at V2 and V3. After the one-week free-living assessment period at V3, the participants will be instructed to live as they wish during the subsequent 3-months follow-up period.

Gastric emptying, gastrointestinal transit times and motility

At V1 and V3, participants will be instructed to ingest a wireless motility capsule (SmartPill™, Medtronic, MN, USA) immediately after ingesting the standardised breakfast meal with 150 ml water. The capsule measures pH, temperature, and pressure through the gastrointestinal tract until expulsion. From these data, regional transit time, pH profile, and motility in different parts of the gastrointestinal tract can be estimated (41–43). Participants will be instructed to wear a SmartPill™ receiver unit within 0.25 meters of their body for the following week or until expulsion of the capsule. As this is an expensive measurement, only the first ~60 participants will be offered the capsule.

Events related to the gastrointestinal tract

At V1 and V3, participants register all events related to the gastrointestinal tract (passing stool, eating, sleeping, and gastrointestinal symptoms such as nausea, vomiting, pain in the abdominal region, bloating etc.), until expulsion of the SmartPill, using an 'event button' on the SmartPill™ receiver. Additionally, the participants register the time and type of each event in a diary.

Physical activity and sleep

Physical activity and sleep will be measured using accelerometry. Participants will be equipped with one accelerometer on the thigh and one on the lower back for one week (Axivity AX3, Newcastle upon Tyne, UK). Concomitant with wearing the accelerometers, participants will be asked to fill out a physical activity and sleep diary.

Continuous glucose monitoring

A one-week continuous glucose monitoring (CGM) system (Ipro™, Medtronic Denmark A/S, Copenhagen, Denmark) will be initiated at the test days at V1-V3. The CGM will be attached to the lower part of the abdomen in the morning at the test days. Participants will be instructed to measure blood glucose levels using a glucometer (Contour XT, Ascensia Diabetes Care Denmark ApS, Copenhagen, Denmark) four times a day during the measurement period for calibration of the CGM (before breakfast, lunch, main evening meal, and bedtime).

Dietary intake

During the week following V1, V2, and V3, participants will be asked to fill in a food record diary (pen and paper) for three days (day 1, 3, and 5 after the test day; two weekdays and one weekend day). Participants will be instructed to register weight, time, and content of all meals and beverages (except water). During the same week, participants will be asked to register their eating window (see *assessment of adherence*).

Participants will be instructed to send the CGM, SmartPill™ receiver, accelerometers, and diaries to the researchers after completion of each assessment period.

Gut microbiome

Participants will be provided with a kit for stool samples including storage equipment at V0 and V2. They will be instructed to collect and immediately freeze (-20 °C) three samples from the same stool sample ≤72 hours before test days at V1 and V3. The participants will transport the samples to the laboratory in provided cooling bags and the samples will be stored at -80 °C until analysis. Bacterial DNA and RNA will be purified from the stool samples. The microbial content, composition, and function will be estimated based on sequencing of the microbiome.

Randomisation and intervention

After completing baseline testing, participants are randomly allocated to either the control group or the TRE group. Randomisation is performed in blocks varying in size, unknown to the researchers, to ensure an equal distribution of participants in the two groups in case the study, for unexpectedly reasons, must be terminated before inclusion of all participants. The randomisation list was generated by an external statistician and uploaded to the electronic data management system REDCap (8.10.18, Vanderbilt University, TN, USA). When participants leave the research facilities at the test day at V1, they receive a sleeve with a combination

lock which contains information about group allocation. On day 7, when all baseline assessments are completed, the participants are provided with the code for the lock by an investigator. This approach ensures that participants are blinded to the group allocation during the 7-day free-living assessment period. Over the phone, the investigator provides a detailed description and introduction to the specific group allocation. For practical reasons, randomisation is open for participants and research staff. However, the outcome assessors (data analysts) will be blinded during the statistical analyses of all experimental outcomes.

Time-restricted eating

Participants allocated to the TRE group are instructed to consume all foods and beverages (except water) within a self-selected time window of 10 hours/day between 6 am and 8 pm for the 13-week intervention. Furthermore, the participants are instructed to keep the eating window stable during the entire week and advised to select a window starting at least 2 hours after habitual wake-up time and 3 hours before habitual bedtime if possible. Participants are advised to follow the Danish dietary recommendations (44). No other dietary restrictions are prescribed.

Control

Participants allocated to habitual living are advised to follow the Danish dietary recommendations (44) but are otherwise instructed to continue their habitual lifestyle during the 13 weeks intervention.

Assessment of adherence

All participants are asked to register time for initiating first and terminating last eating/drinking episode (except water) every day from the test day at V1-V4. Every week during the 26-week period (intervention and follow-up), a link to an online form will be sent by e-mail to the participants for them to register the time for eating/drinking episodes for the previous week. In case participants in the control group restrict their eating window to less than their habitual ≥12 hours/day or if the eating window of participants in the TRE group deviate from their self-selected 10-hour eating window ≥4 days during the first week, the participant will be contacted per telephone to ensure that the participant has understood the concept of their designated group allocation. During the first week of the intervention participants in the TRE group can change their eating window once, in case they are not satisfied with the originally selected window. After the first week no changes are allowed. To ensure similar contact with participants in the control group and the TRE group, the participants will not be contacted in case of non-adherence after the first week. However, if the participants fail to register their eating window, they will be contacted only to remind them to register.

No other feedback is provided during the intervention. To account for variations in daily eating windows around 10 hours, participants in the TRE group are considered adherent if their eating window is less than 11 hours/day. Adherence to the intervention is calculated as number/percentage of days during the intervention the participants' eating window is <11 hours/day. *Per protocol* is defined as ≥80% compliance. The eating window will be calculated for both groups, but no compliance criterion is applied in the control group. Regardless to the degree of adherence all participants allocated to both groups will be invited for test days with emphasis on participating in V3 and if participants are not willing to attend a full test day they will be asked to come in for a measurement of the primary outcome.

Statistical methods

Sample size determination

There is strong evidence for the clinical relevance of a weight reduction of 3% in people with overweight or obesity, with or without prediabetes (45). No RCT has investigated the effects of TRE on weight loss in individuals with overweight or obesity at a high risk of type 2 diabetes. However, Gill and Panda investigated effects of a 16-week TRE intervention on changes in body weight in eight healthy overweight individuals (BMI >25 kg/m^2) with a habitual eating window >14 hours/day and observed a mean reduction of body weight of 3.9% (3.3 kg, 95% CI: 0.9-5.6 kg); however, no control group was included (11). Per inclusion criteria individuals with a BMI ≥25 kg/m^2 are included in the RESET study. For the participants with a BMI of 25 kg/m^2 (with an expected mean height of 170 cm) a change in weight of 3% will correspond to ~2 kg. Thus, in order to detect a minimal clinically relevant difference in weight change of 3% across the allowed BMI range, the trial was dimensioned to detect a difference in change of 2 kg between the TRE group and the control group. In a recent randomised controlled trial examining the effects of 13 weeks of either exercise or pharmacological therapy on cardiometabolic health in individuals with overweight or obesity and prediabetes, the SD for within-group changes in body weight in the control group was 2.6 kg (Færch et al., under review). We expect that the SD for within-group changes will be similar in the RESET trial, but to account for uncertainties we increased the SD by 20%, resulting in an SD of 3.1 kg. In order to detect a 2 kg (SD 3.1) difference in weight change with a desired statistical power of 0.8 (two-tailed test, alpha 0.05), a total of 40 participants is required in each group. To allow for a 20% drop-out in each group, we plan to include 50 participants in each of the two groups.

Statistical analysis plan

Intention-to-treat analysis including all randomised participants will be performed after the last participant has participated in the last visit. Additionally, *per protocol* analysis will be performed including participants who are compliant during the intervention. Data will be presented with the use of standard descriptive statistics. Descriptive statistics will be shown as mean (SD) for normally distributed data and as median (Q1; Q3) for non-normally distributed data. Changes from baseline and differences in delta values between groups will be analysed using linear mixed-effects models with the outcome as a function of group, time and group x time interaction and including a participant-specific random intercept. Outcomes with a known/expected bimodal distribution due to sex differences will be adjusted for sex. Adequacy of assumptions of normality and homogeneity of variances will be assessed using graphical methods and, if necessary, data will be log-transformed for analysis and back-transformed for presentation. If model assumptions are not met by logarithmically transformation, non-parametric statistical tests will be performed. *P*-values <0.05 (two-tailed) are considered statistically significant. The potential impact of missing data on the primary outcome will be evaluated in a sensitivity analysis based on multiple imputation in which participants with missing data at follow-up will be pooled with the participants in the control group during the imputation process.
Results will be presented as estimated mean differences in changes with 95% confidence intervals and p-values when relevant. A full statistical analysis plan will be uploaded to ClinicalTrials.gov before the inclusion of participants is finalized.

Patient and Public Involvement Statement

During the study we enter into dialogue with participants about their experiences of the test days, examinations, participant information etc. with the aim to understand and improve participants' experiences in current and future studies of TRE.

Ethics and dissemination

All equipment used in the studies meet the requirement for patient safety. The total amount of blood taken at each visit is maximally 300 ml, which is less than a standard blood donation of 450 ml and considered safe. Participants will be instructed not to donate blood during the trial. There may be some discomfort associated with swallowing the SmartPill™. The risk of capsule retention in individuals without known stenosis is only 0.75% and in such a case, a pro-motility drug is often sufficient to mobilize the capsule. Alternatively,

endoscopy can be performed to retrieve the capsule. Body composition is measured using Dual-energy X-ray Absorptiometry with a radiation dose less than 0.01 mSv, which corresponds to less than one day of normal background radiation. There is no expected discomfort or risks associated with ingestion of the meals, food reward measurements or biometric measurements (eye tracking, galvanic skin response, facial expression analyses). Participants are covered by the Patient Compensation Association according to the Danish Act on the Right to Complain and Receive Compensation within the Health Service. The intervention is considered safe in individuals with overweight and obesity (46).

The study has been approved by the Ethics Committee of the Capital Region of Denmark (H-18059188) and will be conducted in accordance with the Declaration of Helsinki. Approval of data and biobank has been obtained from the Danish Data Protection Agency. Consent from the Ethics Committee of the Capital Region of Denmark to all previous and future amendments to the protocol have and will be obtained before these are instated (Supplementary Table 2).

The study is registered at ClinicalTrials.gov (identifier: NCT03854656). No Data monitoring committee has been appointed for the trial due to the perceived very low risk of harms.

All study-related information will be recorded, handled, and stored safely in a way that allows accurate reporting, interpretation and verification. Source data will be registered in the electronic data management system REDCap (8.10.18, Vanderbilt University, TN, USA). For CGM measurements, source data will be registered in a web-based software (CareLink™, Medtronic) using the participant's study ID. Source data from Dual-energy X-ray Absorptiometry, biometric measurements, food preferences, Vagus™, SmartPill™, accelerometers and indirect calorimetry are registered on the device or related hardware and uploaded to a secured logged drive to which only project staff has access. Investigators at Steno Diabetes Center Copenhagen will have access to the full data set. Sponsor/investigator will provide direct access to source data/documents for regulatory inspection. Access to the full protocol and data can be obtained from the principal investigator. Since we expect no harms associated with the intervention, information on harms/side-effects will not be systematically recorded.

Positive and negative as well as inconclusive study results will be presented at conferences and published in international peer-reviewed journals in accordance with the CONSORT guidelines, no publication restrictions have been imposed. All co-authors must comply with the International Committee of Medical Journal Editors guidelines and no professional writers will be engaged in the writing process.

Discussion

Implementation and maintenance of traditional strategies for weight loss and early prevention of type 2 diabetes, i.e. increased physical activity and dietary restrictions, is difficult for many (47–49), because it is time consuming and requires insights into the type and amount of foods eaten. In a public health perspective, there is a strong need for feasible lifestyle strategies to combat the current type 2 diabetes epidemic. TRE is a simple eating pattern regime which extends the daily fasting period and potentially synchronizes food intake with circadian rhythms of metabolism and may therefore represent a feasible lifestyle modification strategy. The evidence from animal studies and well-controlled human studies suggest that TRE has the potential to improve a variety of cardiometabolic risk factors in metabolically vulnerable individuals and may be a feasible and sustainable regimen (18); however, randomised controlled trials are lacking. Using an interdisciplinary approach, the present study will examine effects of TRE on weight changes and explore effects on cardiometabolic health and behaviour, as well as on participants' motivation and experiences with TRE.

Implicit in the trial design is that the intervention is only relevant and feasible if the participants can uphold the TRE regime without frequent contact with the research staff; otherwise we find it unlikely that this intervention will have great relevance in the public at large (who are likely less motivated than those opting to partake in an RCT). Eating is integrated in the rhythms and social relations in everyday life. Since TRE affects eating practices, it is important to get insight into participants' experiences with TRE and to identify potential barriers and strategies for integration and maintenance of this regimen. Thus, the findings from the present study will address whether TRE is an acceptable intervention which can improve health outcomes in individuals at risk of lifestyle-related diseases. As such, it will potentially inform the design of future large-scale studies and feasible health recommendations.

Authors' contributions

JSQ and KF conceived the idea and initiated the study. KF is principal investigator and JSQ, HP, MMC, KKBC, and NB are co-investigators. JSQ, MMC, KKBC, HP, NB, JS, MBB, NJWA, JJH, SST, DV, MEJ, SP, CB, GF, KF contributed to the design of the study. JSQ drafted the manuscript. MMC, KKBC, HP, NB, JS, MBB, NJWA, JJH, SST, DV, MEJ, SP, CB, GF, KF critically reviewed the manuscript. JSQ, MMC, KKBC, HP, NB, JS, MBB, NJWA, JJH, SST, DV, MEJ, SP, CB, GF, KF approved the final version.

Funding and economical compensation

Kristine Færch is sponsor and principal investigator (e-mail: kristine.faerch@regionh.dk, Phone: + 4530913061, Clinical Prevention Research, Steno Diabetes Center Copenhagen, Niels Steensens Vej 6, DK-2820, Gentofte, Denmark). The sponsor and investigators have no economic interest in the results of the study. The study is funded by an unrestricted grant from the Novo Nordisk Foundation (NNF17OC0027822), a PhD scholarship from Aalborg University and an industrial PhD scholarship from the Innovation Fund Denmark. The funders do not take part in or have any influence on: study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication. We cover documented, reasonable travel expenses if the participant lives more than 12 km from Steno Diabetes Center Copenhagen. Participants will not receive any other financial compensation for participating in the study.

Competing interests

Steno Diabetes Center Copenhagen is a hospital providing health services for the public health care system. Steno Diabetes Center Copenhagen is partly funded by the Novo Nordisk Foundation through unrestricted grants. The Novo Nordisk Foundation has no economic interests in the study. The Novo Nordisk Foundation will not have influence on the study design, data collection, analysis, interpretation of data, the writing of the study report or any publication and the decision to submit the paper for publication. The investigators employed at Steno Diabetes Center Copenhagen will not benefit economically from conducting the study. HP is co-investigator on the project which is part of her Industrial PhD project in collaboration with iMotions A/S, where HP is employed. iMotions A/S is a collaborator on the project and gives advice for the use and analysis of biometric methods in the study design phase. SP has published a book, The Circadian Code, focusing on the concept of TRE.

References

1. Lee CMY, Colagiuri S, Woodward M, Gregg EW, Adams R, Azizi F, et al. Comparing different definitions of prediabetes with subsequent risk of diabetes: An individual participant data meta-analysis involving 76 513 individuals and 8208 cases of incident diabetes. BMJ Open Diabetes Res Care. 2019;7(1):1–10.
2. Schmidt MI, Bracco PA, Yudkin JS, Bensenor IM, Griep RH, Barreto SM, et al. Intermediate hyperglycaemia to predict progression to type 2 diabetes (ELSA-Brasil): an occupational cohort study in Brazil. Lancet Diabetes Endocrinol. 2019 Apr;7(4):267–77.
3. Vistisen D, Witte DR, Brunner EJ, Kivimaki M, Tabak A, Jorgensen ME, et al. Risk of cardiovascular disease and death in individuals with prediabetes defined by different criteria: The whitehall II study. Diabetes Care. 2018;41(4):899–906.
4. Gummesson A, Nyman E, Knutsson M, Karpefors M. Effect of weight reduction on glycated haemoglobin in weight loss trials in patients with type 2 diabetes. Diabetes, Obes Metab. 2017;19(9):1295–305.
5. le Roux CW, Astrup A, Fujioka K, Greenway F, Lau DCW, Van Gaal L, et al. 3 years of liraglutide versus placebo for type 2 diabetes risk reduction and weight management in individuals with prediabetes: a randomised, double-blind trial. Lancet. 2017;389(10077):1399–409.
6. American Diabetes Association. 3. Prevention or delay of type 2 diabetes: Standards of medical care in diabetes-2019. Diabetes Care. 2019;42(January):S29–33.
7. Anderson JW, Konz EC, Frederich RC, Wood CL. Long-term weight-loss maintenance: A meta-analysis of US studies. Am J Clin Nutr. 2001;74(5):579–84.
8. Lemstra M, Bird Y, Nwankwo C, Rogers M, Moraros J. Weight loss intervention adherence and factors promoting adherence: a meta-analysis. Patient Prefer Adherence. 2016;10:1547–59.
9. Melkani GC, Panda S. Time-restricted feeding for prevention and treatment of cardiometabolic disorders. J Physiol. 2017;595(12):3691–700.
10. Longo VD, Panda S. Fasting, Circadian Rhythms, and Time-Restricted Feeding in Healthy Lifespan. Vol. 23, Cell Metabolism. 2016. p. 1048–59.
11. Gill S, Panda S. A Smartphone App Reveals Erratic Diurnal Eating Patterns in Humans that Can Be Modulated for Health Benefits. Cell Metab. 2015;22(5):789–98.
12. Wittmann M, Dinich J, Merrow M, Roenneberg T. Social jetlag: misalignment of biological and social time. Chronobiol Int. 2006;23(1–2):497–509.
13. St-Onge M-P, Ard J, Baskin ML, Chiuve SE, Johnson HM, Kris-Etherton P, et al. Meal Timing and Frequency: Implications for Cardiovascular Disease Prevention: A Scientific Statement From the American Heart Association. Circulation. 2017;135:00–00.
14. Arble DM, Bass J, Behn CD, Butler MP, Challet E, Czeisler C. Impact of Sleep and Circadian Disruption on Energy Balance and Diabetes : A Summary of Workshop Discussions. Sleep. 2015;38(12):1849–60.
15. Garaulet M, Gómez-Abellán P. Timing of food intake and obesity: a novel association. Physiol Behav. 2014;134(2014):44–50.
16. Scheer FAJL, Hilton MF, Mantzoros CS, Shea SA. Adverse metabolic and cardiovascular consequences of circadian misalignment. Proc Natl Acad Sci U S A. 2009;106(11):4453–8.
17. Gonnissen HKJ, Hulshof T, Westerterp-Plantenga MS. Chronobiology, endocrinology, and energy- and food-reward homeostasis. Obes Rev. 2013;14(5):405–16.
18. Chaix A, Manoogian ENC, Melkani GC, Panda S. Time-Restricted Eating to Prevent and Manage Chronic Metabolic Diseases. Annu Rev Nutr. 2019;39(1):12.1-12.5.
19. Sutton EF, Beyl R, Early KS, Cefalu WT, Ravussin E, Peterson CM. Early Time-Restricted Feeding

Improves Insulin Sensitivity, Blood Pressure, and Oxidative Stress Even without Weight Loss in Men with Prediabetes. Cell Metab. 2018;27(6):1212–1221.e3.

20. Jamshed H, Beyl RA, Manna DL Della, Yang ES, Ravussin E, Peterson CM. Early Time-Restricted Feeding Improves 24-Hour Glucose Levels and Affects Markers of the Circadian Clock, Aging, and Autophagy in Humans. Nutrients. 2019;11(1234):1–16.
21. Hutchison AT, Regmi P, Manoogian ENC, Fleischer JG, Wittert GA, Panda S, et al. Time-Restricted Feeding Improves Glucose Tolerance in Men at Risk for Type 2 Diabetes: A Randomized Crossover Trial. Obesity. 2019;27(5):724–32.
22. Ravussin E, Beyl RA, Poggiogalle E, Hsia DS, Peterson CM. Early Time-Restricted Feeding Reduces Appetite and Increases Fat Oxidation But Does Not Affect Energy Expenditure in Humans. Obesity. 2019;27(8):1244–54.
23. Gabel K, Hoddy KK, Haggerty N, Song J, Kroeger CM, Trepanowski JF, et al. Effects of 8-hour time restricted feeding on body weight and metabolic disease risk factors in obese adults: A pilot study. Nutr Heal Aging. 2018;4(4):345–53.
24. Wilkinson MJ, Manoogian ENC, Zadourian A, Lo H, Fakhouri S, Shoghi A, et al. Ten-Hour Time-Restricted Eating Reduces Weight, Blood Pressure, and Atherogenic Lipids in Patients with Metabolic Syndrome. Cell Metab. 2019;31(1):92–104.e5.
25. Antoni R, Robertson TM, Robertson MD, Johnston JD. A pilot feasibility study exploring the effects of a moderate time-restricted feeding intervention on energy intake, adiposity and metabolic physiology in free-living human subjects. J Nutr Sci. 2018;7.
26. Schulz KF, Altman DG, Moher D, Group C. CONSORT 2010 Statement : Updated Guidelines for Reporting Parallel Group Randomized Trials OF TO. BMJ. 2013;340(c332).
27. Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin JA, et al. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. BMJ. 2013;346(e7586).
28. Ma C, Avenell A, Bolland M, Hudson J, Stewart F, Robertson C, et al. Effects of weight loss interventions for adults who are obese on mortality, cardiovascular disease, and cancer: systematic review and meta-analysis. BMJ. 2017;359(j4849).
29. Hulmán A, Færch K, Vistisen D, Karsai J, Nyári TA, Tabák AG, et al. Effect of time of day and fasting duration on measures of glycaemia: Analysis from the Whitehall II Study. Diabetologia. 2013;56(2):294–7.
30. Weir JBD V. New methods for calculating metabolic rate with special reference to protein metabolism. J Physiol. 1949;109(1–2):1–9.
31. Frayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. J Appl Physiol. 1983;55(2):628–34.
32. Geyer PE, Kulak NA, Pichler G, Holdt LM, Teupser D, Mann M. Plasma Proteome Profiling to Assess Human Health and Disease. Cell Syst. 2016;2(3):185–95.
33. Galsgaard KD, Winther-Sørensen M, Ørskov C, Kissow H, Poulsen SS, Vilstrup H, et al. Disruption of glucagon receptor signaling causes hyperaminoacidemia exposing a possible liver-alpha-cell axis. Am J Physiol Endocrinol Metab. 2018;314(1):E93–103.
34. Jones N, Piasecka J, Bryant AH, Jones RH, Skibinski DOF, Francis NJ, et al. Bioenergetic analysis of human peripheral blood mononuclear cells. Clin Exp Immunol. 2015;182(1):69–80.
35. Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. Int J Obes. 2000;24(1):38–48.
36. Finlayson G, King N, Blundell JE. Is it possible to dissociate “liking” and “wanting” for foods in humans? A novel experimental procedure. Physiol Behav. 2007;90(1):36–42.
37. Dalton M, Finlayson G. Psychobiological examination of liking and wanting for fat and sweet taste in

trait binge eating females. Physiol Behav. 2014;136:128–34.

38. Finlayson G, King N, Blundell J. The role of implicit wanting in relation to explicit liking and wanting for food: Implications for appetite control. Appetite. 2008;50(1):120–7.

39. Warde A. Consumption and theories of practice. J Consum Cult. 2005;5(2):131–53.

40. Malterud K. Systematic text condensation: A strategy for qualitative analysis. Scand J Public Health. 2012;40(8):795–805.

41. Maqbool S, Parkman HP, Friedenberg FK. Wireless capsule motility: Comparison of the smartPill® GI monitoring system with scintigraphy for measuring whole gut transit. Dig Dis Sci. 2009;54(10):2167–74.

42. Farmer AD, Wegeberg AML, Brock B, Hobson AR, Mohammed SD, Scott SM, et al. Regional gastrointestinal contractility parameters using the wireless motility capsule: inter-observer reproducibility and influence of age, gender and study country. Aliment Pharmacol Ther. 2018;47(3):391–400.

43. Zarate N, Mohammed SD, O'Shaughnessy E, Newell M, Yazaki E, Williams NS, et al. Accurate localization of a fall in pH within the ileocecal region: Validation using a dual-scintigraphic technique. Am J Physiol - Gastrointest Liver Physiol. 2010;299(6):1276–86.

44. The Danish Veterinary and Food Administration, Ministry of Environment and Food, Danish Dietary Recommendations [Internet]. Available from: https://altomkost.dk/english/#c41067

45. Jensen MD, Ryan DH, Apovian CM, Ard JD, Comuzzie AG, Donato KA, et al. 2013 AHA/ACC/TOS Guideline for the Management of Overweight and Obesity in Adults. Circulation. 2014;129(25 suppl 2):S102–38.

46. Gabel K, Hoddy KK, Varady KA. Safety of 8-h time restricted feeding in adults with obesity. Appl Physiol Nutr Metab. 2018;44(1):107–9.

47. Wing RR, Phelan S. Long-term weight loss maintenance. Am J Clin Nutr. 2005;82(1 Suppl):222S–225S.

48. Wadden TA, Neiberg RH, Wing RR, Clark JM, Delahanty LM, Hill JO, et al. Four-year weight losses in the look AHEAD study: Factors associated with long-term success. Obesity. 2011;19(10):1987–98.

49. Ulen CG, Huizinga MM, Beech B, Elasy TA. Weight regain prevention. Clin Diabetes. 2008;26(3):100–13.

Figure legend

Figure 1: Study design.

Table 1. Inclusion and exclusion criteria

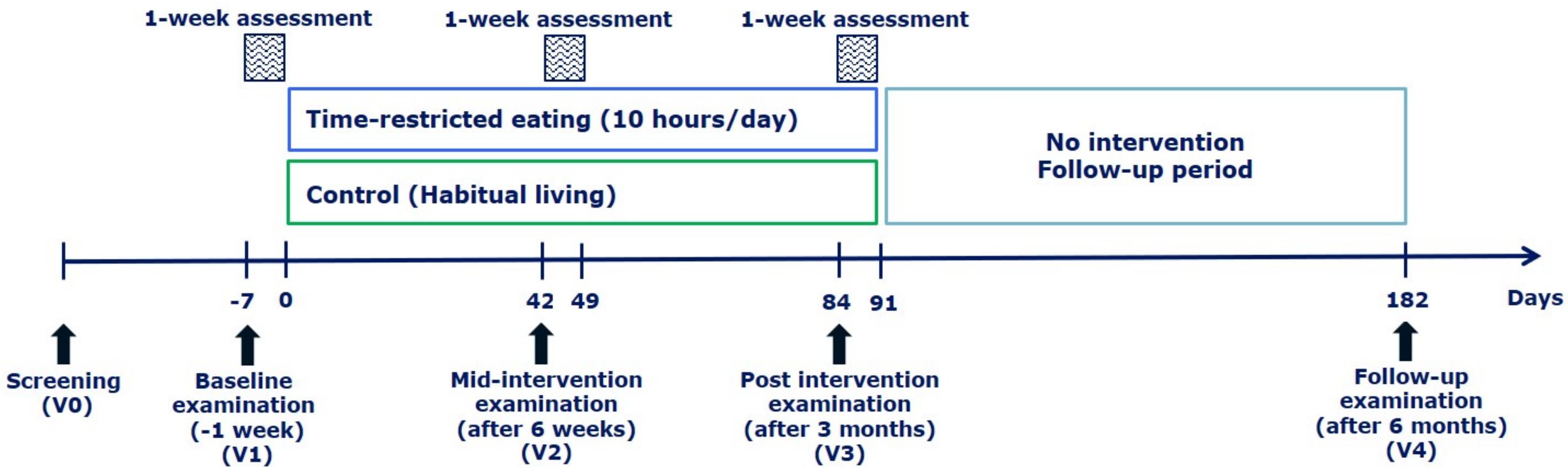
Inclusion criteria • Age: ≥30 to ≤70 years • Body mass index ≥30 kg/m^2 or body mass index ≥25 kg/m^2 in combination with pre-diabetes (HbA_{1c} ≥39 to <48 mmol/mol) • Habitual eating/drinking window ≥12 hours (including foods/snacks and energy containing beverages e.g. soft drinks (except of water)) and an eating/drinking window of ≥14 hours minimum one day per week **Exclusion criteria** • Daily smoking • For women: pregnancy, planned pregnancy (within the study period) or lactating • Frequent travels over time zones (more than one return trip/travel over times zones (>one-hour time difference) during the 13-week intervention). • Shift work or partner engaged in shift work (if it affects the person's sleep and eating pattern) • Unable to understand the informed consent and the study procedures • Self-reported history of an eating disorder during the past three years • Self-reported weight change (>5 kg) within three months prior to inclusion • Known diabetes or diabetes detected at screening (HbA_{1c} ≥48 mmol/mol) • Uncontrolled medical issues including but not limited to cardiovascular, pulmonary, rheumatologic, hematologic, oncologic, infectious, gastrointestinal or psychiatric disease; endocrine disease; immunosuppression • Current treatment with medication or medical devices which significantly affect glucose metabolism, appetite, or energy balance • Current treatment with antidepressants • Bariatric surgery • Implanted or portable electro-mechanical medical device such as a cardiac pacemaker, defibrillator or infusion pump • Celiac disease, Crohn's disease, ulcerative colitis or proctitis • Alcohol/drug abuse or in treatment with disulfiram at time of inclusion • Concomitant participation in other intervention studies • Not able to eat ≥85% of the test meal because of e.g. allergy
Specific exclusion criteria for participants receiving SmartPill™
• Gastrointestinal symptoms or diseases such as regular (weekly) abdominal pain, dysphagia, gastric bezoars, strictures, fistulas, bowel obstructions or diverticulitis • Current treatment with medication or medical devices which significantly affect gastrointestinal motility or transit time (prokinetics, antidiarrheals, laxatives, or opioids) • Gastrointestinal surgery within 3 months before inclusion
Other criteria for withdrawal and exclusion after inclusion
• Participant's withdrawal of the informed consent • Pregnancy or other safety concerns – judged by the investigator

Table 2. Overview of study visits

Visit	V0	V1	V2	V3	V4
Time, weeks from start of intervention	-7[1]	-1	6	12	26
Participant information					
Informed consent	X				
Medical history (individual and family)	X				
In- and exclusion criteria	X				
Pregnancy test (fertile women only)		X	X	X	X
Efficacy outcomes					
HbA_{1C}	X	X	X	X	X
Body weight	X	X	X	X	X
Waist and hip circumference		X	X	X	X
Body composition (DXA scanning)		X	X	X	X
Blood pressure and resting heart rate	X	X	X	X	X
Stool sample		X		X	
Fasting blood samples		X	X	X	X
Postprandial blood samples		X		X	
Indirect calorimetry		X		X	
Heart rate variability (Vagus™)		X		X	
Mixed meal test with SmartPill™		X		X	
Event registration related to SmartPill™		X		X	
Physical activity and sleep measurement		X	X	X	
Food records		X	X	X	
Continuous glucose monitoring		X	X	X	
Fasting food reward and biometric measurements		X	X	X	X
Postprandial food reward and biometric measurements		X		X	
Questionnaires					
Sociodemographic characteristics		X			
Health and wellbeing		X	X	X	X
Physical activity		X	X	X	X
Fasting appetite sensations		X	X	X	X
Postprandial appetite sensations		X		X	
Gastrointestinal symptoms		X	X	X	X
Autonomic symptoms		X			
Pain		X			
Sleep quality and sleepiness		X	X	X	X
Chronotype		X	X	X	X
Night eating		X	X	X	X
Eating behaviour and control over eating		X	X	X	X
Interviews					
Interview (all participants)[2]		X			
Interview (all participants in the TRE group)[3]				X	X

1. Max 6 weeks before baseline testing (V1); 2. Interview regarding motivation for participation; 3. Interview regarding feasibility and maintenance. V0: screening; V1: baseline testing; V2: mid-intervention testing (after 6 weeks); V3: post intervention testing (after 3 months); V4: follow-up testing (after 6 months). Abbreviations: DXA: Dual-energy X-ray Absorptiometry; TRE: Time-restricted eating.

649


1-week assessment
1-week assessment
1-week assessment
Time-restricted eating (10 hours/day)
Control (Habitual living)
No intervention
Follow-up period
-7
0
42
49
84
91
182
Days
Screening
(V0)
Baseline
examination
(-1 week)
(V1)
Mid-intervention
examination
(after 6 weeks)
(V2)
Post intervention
examination
(after 3 months)
(V3)
Follow-up
examination
(after 6 months)
(V4)

WHO International Clinical Trials Registry information for the RESET trial

Adapted from: https://apps.who.int/trialsearch/Trial2.aspx?TrialID=NCT03854656

Main

Note: This record shows only 22 elements of the WHO Trial Registration Data Set. To view changes that have been made to the source record, or for additional information about this trial, click on the URL below to go to the source record in the primary register.

Register:	ClinicalTrials.gov
Last refreshed on:	9 December 2019
Main ID:	NCT03854656
Date of registration:	18/02/2019
Prospective Registration:	Yes
Primary sponsor:	Kristine Færch
Public title:	Effect of Time-restricted Eating on Behaviour and Metabolism in Overweight Individuals at High Risk of Type 2 Diabetes RESET
Scientific title:	Effect of Time-restricted Eating on Behaviour and Metabolism in Overweight Individuals at High Risk of Type 2 Diabetes - the RESET Study
Date of first enrolment:	February 25, 2019
Target sample size:	100
Recruitment status:	Recruiting
URL:	https://clinicaltrials.gov/show/NCT03854656
Study type:	Interventional

Study design:	Allocation: Randomized. Intervention model: Parallel Assignment. Primary purpose: Prevention. Masking: None (Open Label).
Phase:	N/A

Countries of recruitment

Denmark

Contacts

Name:	Kristine Færch, PhD	**Name:**	Kristine Færch, PhD	**Name:**	Kristine Færch, PhD
Address:		**Address:**		**Address:**	
Telephone:	+45 30913061	**Telephone:**	+45 30913061	**Telephone:**	
Email:	kristine.faerch@regionh.dk	**Email:**	kristine.faerch@regionh.dk	**Email:**	
Affiliation:		**Affiliation:**		**Affiliation:**	Steno Diabetes Center Copenhagen

Key inclusion & exclusion criteria

Inclusion Criteria:

- BMI =30 kg/m2 or BMI =25 kg/m2 in combination with pre-diabetes (HbA1c =39-<48 mmol/mol)

- Habitual eating/drinking window =12 hours (including foods/snacks and energy containing beverages e.g. soft drinks (except of water)) and an eating/drinking window of =14 hours minimum one day per week

Exclusion criteria

- Daily smoking

- For women: pregnancy, planned pregnancy (within the study period) or lactating

- Frequent travels over time zones (max one return trip/travel over times zones (?one hour time difference) during the 13 weeks intervention).

- Shift work or partner engaged in shift work (if it affects the person's sleep and eating pattern)

- Unable to understand the informed consent and the study procedures

- Self-reported history of an eating disorder during the past three years

- Self-reported weight change (>5 kg) within three months prior to inclusion

- Diabetes

- HbA1c =48 mmol/mol

- Uncontrolled medical issues including but not limited to cardiovascular pulmonary, rheumatologic, hematologic, oncologic, infectious, gastrointestinal or psychiatric disease; diabetes or other endocrine disease; immunosuppression

- Current treatment with medication or medical devices which significantly affect glucose metabolism, appetite, or energy balance

- Current treatment with antidepressants

- Bariatric surgery

- Implanted or portable electro-mechanical medical device such as a cardiac pacemaker, defibrillator or infusion pump

- Celiac disease, Crohn's disease, ulcerative colitis or proctitis

- Alcohol/drug abuse or in treatment with disulfiram at time of inclusion

- Concomitant participation in other intervention studies

- Not able to eat =85% of the test meal because of e.g. allergy

Specific exclusion criteria for participants receiving SmartPillTM (n=60)

- Gastrointestinal symptoms or diseases such as regular (weekly) abdominal pain, dysphagia, gastric bezoars, strictures, fistulas, bowel obstructions or diverticulitis

- Current treatment with medication or medical devices which significantly affect gastrointestinal motility or transit time (prokinetics, antidiarrheals, laxatives, or opioids)

- Gastrointestinal surgery within 3 months before inclusion

Age minimum: 30 Years
Age maximum: 70 Years
Gender: All

Health Condition(s) or Problem(s) studied

Overweight and Obesity

PreDiabetes

Intervention(s)

Other: Time-restricted eating

Primary Outcome(s)

Change in body weight (kg) [Time Frame: Change from baseline to the end of the intervention (after 12 weeks)]

Secondary Outcome(s)

Arousal measured using galvanic skin response [Time Frame: Changes from baseline. Fasted state at all four visits (Baseline and after 6, 12, and 26 weeks) and during a mixed meal test at baseline and end of the intervention (after 12 weeks)]

Attention measured using eye tracking [Time Frame: Changes from baseline. Fasted state at all four visits (Baseline and after 6, 12, and 26 weeks) and during a mixed meal test at baseline and end of the intervention (after 12 weeks)]

Body mass index (kg/m^2) [Time Frame: Changes from baseline. Fasted state at all four visits (Baseline and after 6, 12, and 26 weeks)]

Body weight (kg) [Time Frame: Changes from baseline. Fasted state at all four visits (Baseline and after 6, 12, and 26 weeks)]

Circulating proteins that associate with low-grade inflammation and lipid metabolism [Time Frame: Changes from baseline. Fasted state at all four visits (Baseline and after 6, 12, and 26 weeks)]

Continuous overall net glycaemic action (CONGA) [Time Frame: Changes from baseline. Measured 7 days after the test days at baseline and after 6 and 12 weeks]

Daily eating/drinking window (hh:min) [Time Frame: Registrered every day (13 weeks intervention and 13 weeks follow-up period)]

Daily time spent above different glucose concentrations (e.g. >6.1 mmol/L, >7.0 mmol/L, >7.8 mmol/L, and >11.1 mmol/L) [Time Frame: Changes from baseline. Measured 7 days after the test days at baseline and after 6 and 12 weeks]

Diastolic blood pressure (mmHg) [Time Frame: Changes from baseline. Measured at all four visits (Baseline and after 6, 12, and 26 weeks)]

Emotions measured using facial expression analyses [Time Frame: Changes from baseline. Fasted state at all four visits (baseline and after 6, 12, and 26 weeks) and during a mixed meal test at baseline and end of the intervention (after 12 weeks)]

Energy intake (kcal/day) [Time Frame: Changes from baseline. Registered 3 days after the test days at baseline and after 6 and 12 weeks]

Explicit liking [Time Frame: Changes from baseline. Fasted state at all four visits (Baseline and after 6, 12, and 26 weeks) and during a mixed meal test at baseline and end of the intervention (after 12 weeks)]

Explicit wanting [Time Frame: Changes from baseline. Fasted state at all four visits (Baseline and after 6, 12, and 26 weeks) and during a mixed meal test at baseline and end of the intervention (after 12 weeks)]

Fat free mass (kg) [Time Frame: Changes from baseline. Fasted state at all four visits (Baseline and after 6, 12, and 26 weeks)]

Fat mass (kg) [Time Frame: Changes from baseline. Fasted state at all four visits (Baseline and after 6, 12, and 26 weeks)]

Fat percentage (%) [Time Frame: Changes from baseline. Fasted state at all four visits (Baseline and after 6, 12, and 26 weeks)]

Feasibility of the intervention (qualitative methods) [Time Frame: Visits at baseline and after 12 and 26 weeks. Potential drop-outs will be interviewed at the specific time point.]

Food choice [Time Frame: Changes from baseline. Fasted state at all four visits (Baseline and after 6, 12, and 26 weeks) and during a mixed meal test at baseline and end of the intervention (after 12 weeks)]

Gastric emptying time (hours and minutes) [Time Frame: Changes from baseline. Time after consumption of the standard mixed meal at baseline and end of the intervention (after 12 weeks).]

HbA1c (mmol/mol and %) [Time Frame: Changes from baseline. All four visits (Baseline and after 6, 12, and 26 weeks)]

Heart rate (bpm) [Time Frame: Changes from baseline. Measured at all four visits (Baseline and after 6, 12, and 26 weeks)]

Heart rate response to forced exhalation during rest (valsalva maneuver) [Time Frame: Changes from baseline. Measured at visits at baseline and end of the intervention (after 12 weeks)]

Heart rate response to inhalation and exhalation [Time Frame: Changes from baseline. Measured at visits at baseline and end of the intervention (after 12 weeks)]

Heart rate response to standing up from the supine position [Time Frame: Changes from baseline. Measured at visits at baseline and end of the intervention (after 12 weeks)]

Hip circumference (cm) [Time Frame: Changes from baseline. Fasted state at all four visits (Baseline and after 6, 12, and 26 weeks)]

Hormones [Time Frame: Changes from baseline. Measured in the blood in the fasted state at all four visits (Baseline and after 6, 12, and 26 weeks) and during a mixed meal test (4 hours) at baseline and end of the intervention (after 12 weeks)]

Implicit wanting [Time Frame: Changes from baseline. Fasted state at all four visits (Baseline and after 6, 12, and 26 weeks) and during a mixed meal test at baseline and end of the intervention (after 12 weeks)]

Insulin resistance (indices) [Time Frame: At all four visits (Baseline and after 6, 12, and 26 weeks)]

Insulin sensitivity (indices) [Time Frame: At all four visits (Baseline and after 6, 12, and 26 weeks)]

Large bowel transit time (hours and minutes) [Time Frame: Changes from baseline. Time after consumption of the standard mixed meal at baseline and end of the intervention (after 12 weeks).]

Macronutrient intake (energy percentage) [Time Frame: Changes from baseline. Registered 3 days after the test days at baseline and after 6 and 12 weeks]

Mean amplitude of glycaemic excursions (MAGE) [Time Frame: Changes from baseline. Measured 7 days after the test days at baseline and after 6 and 12 weeks]

Mean glucose concentrations [Time Frame: Changes from baseline. Measured 7 days after the test days at baseline and after 6 and 12 weeks]

Metabolites [Time Frame: Changes from baseline. Measured in the blood in the fasted state at all four visits (Baseline and after 6, 12, and 26 weeks) and during a mixed meal test (4 hours) at baseline and end of the intervention (after 12 weeks)]

Microbiome content and diversity [Time Frame: Changes from baseline. Collected before or during test days at visits at baseline and after 12 weeks]

Motility index [Time Frame: Changes from baseline. Time after consumption of the standard mixed meal at baseline and end of the intervention (after 12 weeks).]

Motivation for participation (qualitative methods) [Time Frame: Visits at baseline and after 12 and 26 weeks. Potential drop-outs will be interviewed at the specific time point.]

Physical activity (counts/min) [Time Frame: Changes from baseline. Measured 7 days after the test days at baseline and after 6 and 12 weeks]

Physical activity (MET hours) [Time Frame: Changes from baseline. Measured 7 days after the test days at baseline and after 6 and 12 weeks]

Physical activity (time spent at different intensities) [Time Frame: Changes from baseline. Measured 7 days after the test days at baseline and after 6 and 12 weeks]

Physical activity energy expenditure (kcal/day) [Time Frame: Changes from baseline. Measured 7 days after the test days at baseline and after 6 and 12 weeks]

Respiratory and glycolytic capacities of isolated peripheral blood mononuclear cells (PBMCs) [Time Frame: Changes from baseline. Fasted state at all four visits (Baseline and after 6, 12, and 26 weeks) and during a mixed meal test at baseline and end of the intervention (after 12 weeks)]

Resting energy expenditure (kcal/day) [Time Frame: Changes from baseline. Measured at visits at baseline and after 12 weeks]

Satisfaction with the intervention (qualitative methods) [Time Frame: Visits at baseline and after 12 and 26 weeks. Potential drop-outs will be interviewed at the specific time point.]

Self-reported autonomic symptoms [Time Frame: Changes from baseline. Assessed at all four visits (Baseline and after 6, 12, and 26 weeks)]

Self-reported chronotype [Time Frame: Changes from baseline. Assessed at all four visits (Baseline and after 6, 12, and 26 weeks)]

Self-reported control over eating [Time Frame: Changes from baseline. Assessed at all four visits (Baseline and after 6, 12, and 26 weeks)]

Self-reported eating behavior [Time Frame: Changes from baseline. Assessed at all four visits (Baseline and after 6, 12, and 26 weeks)]

Self-reported gastrointestinal symptoms (part 1) [Time Frame: Changes from baseline. Assessed at all four visits (Baseline and after 6, 12, and 26 weeks)]

Self-reported gastrointestinal symptoms (part 2) [Time Frame: Changes from baseline. Assessed at all four visits (Baseline and after 6, 12, and 26 weeks)]

Self-reported gastrointestinal symptoms (part 3) [Time Frame: Changes from baseline. Registered 7 days after the test days at baseline and after 12 weeks]

Self-reported night eating [Time Frame: Changes from baseline. Assessed at all four visits (Baseline and after 6, 12, and 26 weeks)]

Self-reported overall health and wellbeing [Time Frame: Changes from baseline. Assessed at all four visits (Baseline and after 6, 12, and 26 weeks)]

Self-reported physical activity [Time Frame: Changes from baseline. Assessed at all four visits (Baseline and after 6, 12, and 26 weeks)]

Self-reported sleep quality [Time Frame: Changes from baseline. Assessed at all four visits (Baseline and after 6, 12, and 26 weeks)]

Self-reported sleepiness [Time Frame: Changes from baseline. Assessed at all four visits (Baseline and after 6, 12, and 26 weeks)]

Sleep duration (min) [Time Frame: Changes from baseline. Registered and measured for 7 days after the test days at baseline and after 6 and 12 weeks]

Sleep efficiency (%) [Time Frame: Changes from baseline. Measured for 7 days after the test days at baseline and after 6 and 12 weeks]

Sleep onset latency (min) [Time Frame: Changes from baseline. Registered and measured for 7 days after the test days at baseline and after 6 and 12 weeks]

Sleep timing (hh:mm) [Time Frame: Changes from baseline. Registered and measured for 7 days after the test days at baseline and after 6 and 12 weeks]

Sleep variability (min) [Time Frame: Changes from baseline. Registered and measured for 7 days after the test days at baseline and after 6 and 12 weeks]

Small bowel transit time (hours and minutes) [Time Frame: Changes from baseline. Time after consumption of the standard mixed meal at baseline and end of the intervention (after 12 weeks).]

Standard deviation of glucose concentrations [Time Frame: Changes from baseline. Measured 7 days after the test days at baseline and after 6 and 12 weeks]

Subjective appetite [Time Frame: Changes from baseline. Fasted state at all four visits (Baseline and after 6, 12, and 26 weeks) and during a mixed meal test at baseline and end of the intervention (after 12 weeks)]

Substrate oxidation (respiratory exchange ratio) [Time Frame: Changes from baseline. Measured at visits at baseline and after 12 weeks]

Systolic blood pressure (mmHg) [Time Frame: Changes from baseline. Measured at all four visits (Baseline and after 6, 12, and 26 weeks)]

Timing of dietary intake (hh:mm) [Time Frame: Changes from baseline. Registered 3 days after the test days at baseline and after 6 and 12 weeks]

Timing of physical activity (hh:mm) [Time Frame: Changes from baseline. Measured 7 days after the test days at baseline and after 6 and 12 weeks]

Total gastrointestinal transit time (hours and minutes) [Time Frame: Changes from baseline. Time after consumption of the standard mixed meal at baseline and end of the intervention (after 12 weeks).]

Variation coefficients of glucose concentrations [Time Frame: Changes from baseline. Measured 7 days after the test days at baseline and after 6 and 12 weeks]

Waist circumference (cm) [Time Frame: Changes from baseline. Fasted state at all four visits (Baseline and after 6, 12, and 26 weeks)]

Wakefulness (min) [Time Frame: Changes from baseline. Measured for 7 days after the test days at baseline and after 6 and 12 weeks]

Secondary ID(s)

NNF17OC0027822

Source(s) of Monetary Support

Please refer to primary and secondary sponsors

Secondary Sponsor(s)

Aalborg University Hospital

iMotions A/S

Salk Institute for Biological Studies

University of Copenhagen

University of Leeds

Ethics review

Results

Results available:

Date Posted:

Date Completed:

URL:

Supplementary Table 2. Amendments to the protocol

Date of version (date of approval from the Ethics Committee)	The amendments were made in the protocol and approved by the local Ethics Committee and the number and headings of the specific sections refers to the protocol and not the article describing the protocol.
8 January 2019 (11 January 2019)	Original
5 February 2019 (28 February 2019) Inclusion of first participant on 25 February 2019.	**Amendment 01** **5.2 Exclusion criteria:** Diagnosis with diabetes and HbA1c ≥48 mmol/mol have been added. The trial includes individuals who are at high risk of developing type 2 diabetes, but do not yet have the disease and these criteria were missing in the previous version. **6. Study visits.** Measurement of blood pressure at the screening visit. This information was missing in the previous version. **9.2 Statistics** Inclusion of a *per protocol* analysis in addition to the intention to treat analysis in the analysis plan. Intention to treat analysis is the primary analysis, but the *per protocol* analysis will be performed to assess the effects of the intervention among participants who were compliant during the intervention.
2 October 2019 (23 October 2019)	**Amendment 02** Specified that the primary outcome is measured after 12 weeks intervention. The intervention is 13 weeks in total including a 1-week free-living assessment period after the test day. **4.1.2 Secondary endpoints** Respiratory and glycolytic capacities of isolated peripheral blood mononuclear cells (PBMCs) are only measured in fasting conditions at baseline and after the intervention (3 months) and not as originally stated mid-intervention (6 weeks) and at follow-up (6 months) and not postprandially. **4.3.3 General information** '*During the follow-up period from week 14 to week 26, all participants will be instructed to continue their habitual lifestyle. The TRE group is allowed – but not required – to continue the TRE regime*' this has been changed to '*During the follow-up period from week 14 to week 26, all participants may live and eat as they wish*'. The original wording was not in line with the intended procedure which allows individuals from both groups to follow TRE or make other lifestyle changes during the 13 week follow-up period. **4.3.4 Compliance and contact with the participants during the study.** The procedure has been changed: a. Participants are defined as *per protocol* if they are compliant ≥80% of the days during the 3-months intervention. Previously, participants were allowed to be non-compliant one day per week in order to be *per protocol* but this criterion was omitted due to practical challenges associated with determination of this.

	b. Every week participants receive an email with a link to registration of their daily eating windows. Participants are not asked to send a picture of their hand written registrations as previously stated. c. Participants in the TRE group are allowed to change their selected eating window once during the first week after randomisation. **5.1 Inclusion criteria** Habitual eating pattern: Previous criteria: *≥14 hours/day eating window on weekdays and eating 3 hours before bedtime'* has been changed to *≥12 hours/day eating window and ≥14 hours/day ≥1 day/week.* The criteria were changed due to challenges associated with recruitment since only few individuals reported a habitual eating window ≥14 hours/day. **5.2 Exclusion criteria** Partner engaged in shift work is only an exclusion criterion if it affects the individuals' sleep or eating pattern. Due to financial reasons only the first 60 out of 100 participants will receive the SmartPill at the test days and specific exclusion criteria related to this method have been listed separately. *'Not able to eat ≥85% of the test meal because of e.g. allergy'* has been added to ensure that the test days can be conducted. Concomitant participation in other research projects has been specified to 'intervention studies'. **5.4 Randomisation** The randomisation is not stratified by sex and age as originally stated. Stratification was discussed during the design phase but was not implemented. This was a mistake and it has been omitted. **6.2 Screening and 7.1.1 Questionnaires** Questionnaires about sociodemographic characteristic and chronotype are not answered at the screening visit as originally stated. This was a mistake. The questionnaire on autonomic symptoms (COMPASS31) is only answered at baseline (visit 1). International Physical Activity Questionnaire (IPAQ) is used in the study and not Recent Physical Activity Questionnaire (RPAQ) as originally written which was a mistake. **7.0 Examinations** '*Except habitual medication'* has been added to the instructions *'No medication except habitual medication'* since participants are allowed to take habitual medication in the morning before testing.

	'*Participants will be asked to register all food items and beverages ingested on the day before V1 and instructed to consume the same on the day before V2, V3, and V4'* has been changed to: '*Participants will be asked to consume a last meal between 7-8 pm the day before the test days*' to standardize the fasting duration before testing and to minimize the burden for the participants. **7.3 Blood samples** The blood sample at 150 min has been omitted due to the total number of samples and expected limited additional information. **7.8 SmartPill™** *'Participants are asked to fast for 6 hours after ingestion of the SmartPill™'* has been added. This information was missing in the previous version. 6 hours fasting after ingestion of the pill is standard procedure to ensure that the pill has exited the stomach before the next meal is consumed. **7.12.1 Free-living physical activity and sleep** Participants are asked to register physical activity and sleep in a diary during the week after test days. Information about registration of physical activity was missing in the previous version. **7.13 Interviews** Interviews will be performed with all 100 participants at baseline (visit 1) and all participants in the TRE group after the intervention (visit 2) and not only in a subgroup as initially planned to get a broader and more thorough insight. **10.2. Source data identification and verification** 'Investigators and co-investigators' has been changed to 'project staff' since biomedical laboratory scientists have also access to source data. **12.3. Risk and symptoms for the study participants** Maximal fasting duration before test days is 14 hours and not 12 hours as previously written.
14 January 2020 (21 February 2020)	**Amendment 03** **4.1.2 Secondary endpoints** Inclusion of fasting and postprandial concentrations of plasma C-terminal telopeptide of collagen type-1 (CTX), and procollagen type 1 N-terminal propeptide (P1NP) i.e. markers of bone resorption and bone formation, respectively. These outcomes were not included in the original version.

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym **Page 1**
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry **Page 2 and 7.**
	2b	All items from the World Health Organization Trial Registration Data Set **The table is included as a Supplementary Table 1 and we refer to this in the Methods section (Page 6). The information appears throughout the manuscript, however, we refer to the table and the registration on clinicaltrials.gov for details about specific secondary outcomes.**
Protocol version	3	Date and version identifier **Supplementary Table 2**
Funding	4	Sources and types of financial, material, and other support **Page 18**
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors **Page 1 and 17**
	5b	Name and contact information for the trial sponsor **Page 18**
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities **Page 18**

	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) **N/A (Page 16)**
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention **Page 4-5**
	6b	Explanation for choice of comparators **Page 5**
Objectives	7	Specific objectives or hypotheses **Page 5**
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) **Page 5-6**
Methods: Participants, interventions, and outcomes		
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained **Page 5-6**
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) **Page 6 and Table 1**
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered **Page 12-13**
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) **N/A**
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) **Page 13-14**

	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial **N/A**
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended **Page 7-12 and Table 2**
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) **Page 5-6, Figure 1**
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations **Page 14**
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size **Page 6**

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions **Page 12**
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned **Page 12-13**
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions **Page 12-13**

Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how **Page 13**
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial **N/A**
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol **Page 7-12**
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols **Page 13-14**
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol **Page 16**
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol **Page 14-15**
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) **N/A**
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) **Page 14-15**
Methods: Monitoring		

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed **Page 16**
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial **N/A**
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct **Page 16**
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor **N/A**
Ethics and dissemination		
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval **Page 16**
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) **Page 16**
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) **Page 6**
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable **N/A**
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial **Page 16**
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site **Page 18**

Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators **Page 16**
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation **N/A**
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions **Page 16**
	31b	Authorship eligibility guidelines and any intended use of professional writers **Page 16.**
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code **Page 16**
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates **Page 6 and Supplementary material**
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable **Page 9**

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.