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# 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

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 study

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# 31 Abstract

# 32 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.

# 35 Methods and analysis

36 The <u>Res</u>tricted <u>Eating Time</u> (RESET) study is a randomised controlled parallel open-label trial. 100 women and 37 men with 1) overweight (BMI:  $\geq$ 25 kg/m<sup>2</sup>) and prediabetes (HbA<sub>1c</sub>: 39-47 mmol/mol); or 2) obesity (BMI:  $\geq$ 30 38 kg/m<sup>2</sup>) will be randomised to a control group (habitual living) or TRE (self-selected 10-hour eating window 39 within the period from 6 am to 8 pm) in a 1:1 ratio. Testing is scheduled at baseline and after 6 weeks (mid-40 intervention), 3 months (post-intervention), and 6 months (follow-up). The primary outcome is change in 41 body weight after 3 months of intervention. Secondary outcomes include changes in body composition; 42 measures of glucose metabolism including glycaemic variability, hormones, and metabolites; subjective and 43 metabolic markers of appetite, food preferences and reward; dietary intake; physical activity, sleep, 44 chronotype; gastric emptying, gastrointestinal transit time and motility; respiratory and glycolytic capacities; 45 the plasma proteome and metabolome; blood pressure, resting heart rate, and heart rate variability; and 46 energy expenditure and substrate oxidation. Motivation and feasibility will be examined based on interviews 47 at baseline and after 3 months. After the 3-month intervention, a 3-month follow-up period and subsequent 48 testing is scheduled to assess maintenance and longer-term effects.

# 49 Ethics and dissemination

50 The study has been approved by the Ethics Committee of the Capital Region of Denmark (H-18059188) and 51 the Danish Data Protection Agency. The study will be conducted in accordance with the Declaration of 52 Helsinki. Results from the study will address whether TRE is effective and feasible in improving health 53 outcomes in individuals at risk of lifestyle-related diseases and can potentially inform the design of feasible 54 health recommendations.

# 56 Trial registration

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

# 59 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.

# 81 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 <sup>39</sup> 101 metabolism.

<sub>42</sub> 102 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, 45 104 'early TRE' (eating window: 6-9 hours/day, between 8 am and 5 pm) improved glucose metabolism (19–21) 47 105 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 50 107 reductions in energy intake and body weight (11,23,24) and adiposity (24,25). Furthermore, in one of the 52 108 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 55 110 same study, maintenance was assessed at one-year follow-up. Importantly, upon completion of the 16 weeks 57 111 intervention, all eight participants in the same study were interested in continuing the regimen, and they 59 112 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, 113 114 challenges associated with social events including drinking and eating may exist (25). Nevertheless, an in-115 depth investigation of feasibility and sustainability of TRE is needed to understand motivation and potential 116 barriers for integration and maintenance in everyday life. In this study, the effects and feasibility of TRE in 10 11 117 individuals at high risk of type 2 diabetes will be assessed using an interdisciplinary approach including state-12 <sub>13</sub> 118 of-the-art and novel quantitative and qualitative methods.

#### **Objectives** 120

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The primary objective of the Restricted Eating Time (RESET) study is to investigate effects of 3 months of TRE 21 121 122 (10 hours/day) on change in body weight in individuals at high risk of type 2 diabetes. Secondary objectives <sup>24</sup> 123 are to describe changes in body weight and composition, metabolism, and behaviour and to assess aspects 26 124 related to motivation, feasibility, and maintenance during the 3-months intervention and after additional 3 \_, 28 125 months of follow-up.

#### **Hypotheses** 33 127

<sub>35</sub> 128 We hypothesise that 3 months of TRE will induce a clinically relevant weight loss in individuals with 129 overweight and obesity at high risk of type 2 diabetes (i.e. TRE superior to control). Furthermore, we expect 38 130 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** 44 132

#### 46 133 Study design

<sup>48</sup> 134 The study is a randomised controlled parallel group open-label trial (Figure 1). 100 individuals will be 49 randomised to 3 months of TRE or habitual living (control) in a 1:1 ratio. Randomisation is performed after 50 135 51 136 completion of screening and baseline testing (visit 1 (V1)). The primary outcome is assessed after 3 months 52 <sup>53</sup> 137 of intervention (V3). At baseline, mid-intervention (after 6 weeks; V2) and after the intervention (3 months; 54 55 138 V3) outcomes are assessed during test days and free-living measurements during the week following the test 56 57 139 days. After the 3-month intervention, a 3-month follow-up period and subsequent testing (V4) is scheduled <sup>58</sup> 140 to assess maintenance and longer-term effects. The trial will be performed at Steno Diabetes Center 59

Copenhagen and will be reported according to the Consolidated Standards of Reporting Trials (26). The study 141 142 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:  $\geq 25 \text{ kg/m}^2$ ) with concomitant prediabetes as defined by HbA<sub>1c</sub>: 39-47 mmol/mol (6) or b) obesity (BMI:  $\geq$ 30 kg/m<sup>2</sup>), 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, 21 149 23<sup>-</sup>150 because our focus is on preventing diabetes at an early stage. Individuals with overweight and prediabetes 151 and obesity with/without prediabetes are included to target individuals at high risk of type 2 diabetes. The 26 152 rationale of choosing 70 years as upper limit is that the potential for prevention is limited in older individuals. <sub>28</sub> 153 To prevent sources of circadian irregularity during the intervention, shift workers and individuals with a 154 partner engaged in shift work affecting the circadian rhythm of the participant are not eligible for 31 155 participation.

#### 156 Recruitment and screening

Participants are recruited through advertisements on different publicly available platforms (newspapers, 158 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 161 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 164 (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.

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#### 168 Outcomes

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#### 169 Primary outcome

The primary outcome is change in body weight (kg) from baseline (V1) to end of intervention (after 3 months, 170 171 V3). Change in body weight was chosen as the primary outcome for several reasons. First, weight loss is 12 172 associated with a reduction in all-cause mortality in individuals with obesity (28) and with improvement in <sub>14</sub> 173 glycaemic control in individuals with overweight and obesity (4). Second, according to the American Diabetes 174 Association, weight loss is recommended for all individuals with prediabetes (6). Third, body weight is easy 17 175 to measure with high precision and available in most clinical studies which enable determination of sample 19 176 size and comparison across studies.

#### <sup>21</sup> 177 Secondary exploratory outcomes 22

23 178 The secondary exploratory outcomes include a variety of metabolic and behavioural outcomes potentially 24 25 179 associated with the intervention. These include changes in body composition, hormones involved in glucose 26 <sub>27</sub> 180 metabolism and appetite regulation (e.g. pancreatic and gastrointestinal hormones), metabolites, glycaemic 28 181 variability; subjective appetite, food preferences and reward, and eating behaviour; gastric emptying, 29 30 182 gastrointestinal motility, and transit time; physical activity, dietary intake, and sleep; inflammatory markers; 31 32 183 respiratory and glycolytic capacities; the plasma proteome and metabolome; blood pressure, resting heart 33 184 rate, and heart rate variability; and energy expenditure and substrate oxidation (Table 2 and 34 <sup>35</sup> 185 ClinicalTrials.gov, identifier: NCT03854656). We describe changes from baseline to mid-intervention (after 6 36 37 186 weeks; V2), post intervention (after 3 months; V3) and follow-up testing (after 6 months; V4).

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#### 41 42 188 Study visits and free-living assessment periods

44 189 The study includes identical test days and free-living assessment periods at baseline (before randomisation, 45 46 190 V1) and post intervention (V3). The test day at V3 is scheduled after 12 weeks intervention; however, 47 191 participants are instructed to follow their group allocation during the subsequent one-week free-living 48 49 192 assessment period (i.e. 13<sup>th</sup> week of the intervention). Mid-intervention testing after 6 weeks (V2) includes a 50 <sub>51</sub> 193 short test day and a subsequent free-living assessment period during which participants follow their group 52 194 allocation. After the 13 weeks follow-up period (26 weeks from baseline), a short test day (V4) is scheduled 53 54 195 to assess maintenance of potential intervention effects. If possible, all four test days are scheduled on similar 55 56 196 week days and at the same time in the morning. An overview of the study visits is presented in Table 2.

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#### Test days 8

9 All clinical examinations are conducted at Steno Diabetes Center Copenhagen. Participants arrive in the 0 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 1 2 outcomes of interest (29). Furthermore, no alcohol consumption or strenuous physical activity are allowed 3 48 hours prior to testing. The participants are instructed to avoid physically demanding transportation to the 4 research facility.

#### 5 Anthropometry

Height is measured using a stadiometer (SECA, Vogel&Halke, Hamburg, Germany) and body weight is 6 7 measured using a digital scale (Tanita BWB-620A, Amsterdam, The Netherlands) while participants are 8 wearing only light clothes/underwear. Waist circumference is measured at the midpoint between the lowest 9 point of the lowest rib and the highest point of the iliac crest. Hip circumference is measured at the point of 0 the greater femoral trochanter. An average of two repeated measurements of hip and waist circumference 1 is used. In case of >3 cm difference between the two measurements a third measurement is conducted, and 2 the average of the two closest measurements is used. Body composition (fat mass and fat free mass) is 3 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. 4

#### 5 Blood pressure and resting heart rate

6 Blood pressure (mmHg) and resting heart rate (beats per minute) are measured three times with 2 minutes 7 intervals using a digital blood pressure monitor (UA-852, A&D Instruments, Abingdon, UK) after a minimum 8 of 10 min rest, and the average of the two lowest values of three consecutive measurements are used to 9 avoid falsely high blood pressure caused by an unfamiliar and potentially stressful environment.

#### 0 Heart rate variability

1 Heart rate variability and cardiovascular reflex are measured by electrocardiography using a handheld device 2 (Vagus<sup>™</sup>, Medicus Engineering, Aarhus, Denmark) during four consecutive tests: 1) resting heart rate is 3 measured while the participant is in the supine position holding the device; 2) heart rate response to standing 4 up from the supine position; 3) heart rate response to inhalation and exhalation is measured in the seated 5 position; 4) heart rate response to increased intrathoracic pressure (Valsalva manoeuvre).

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# 227 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.

# 232 Blood samples

233 Venous blood samples are collected in the fasting state at all four test days (V1-V4) and postprandially during <sup>18</sup> 234 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 20 235 antecubital vein. Analyses include assessment of HbA<sub>1c</sub>, circulating levels of glucose and lipids, inflammatory 236 markers, and hormones involved in regulation of appetite and metabolism (e.g. insulin, glucagon, glucagon-<sup>23</sup> 237 like peptide-1, glucose-dependent insulinotropic polypeptide, peptide YY, acylated ghrelin). Furthermore, 25 238 metabolomics and proteomics will be applied, and assessment of circulating proteins and metabolites that 27 239 correlate with low-grade inflammation and markers of lipid metabolism will be captured using mass-<sup>28</sup> 240 spectrometry driven analyses of the plasma proteome and metabolome (32,33). Gene expression of pro- and 30 241 anti-inflammatory proteins including cytokines and chemokines, and genes involved in energy metabolism <sub>32</sub> 242 of isolated peripheral blood mononuclear cells will be measured by real-time polymerase chain reaction. 243 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 <sub>37</sub> 245 measures real-time oxygen consumption rate as an indicator of mitochondrial activity and extracellular 246 acidification rate as an indicator of glycolytic activity. Thus, these measurements will provide mechanistic 40 247 knowledge of TRE-induced metabolic changes at the cellular level.

### 48 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.

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# 255 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.

# 13 259 Food preferences and food reward

At V1 and V3, components of food reward and biometric responses to standardised photographic images of 15 260 261 foods will be assessed in the fasting state and 60 min after ingestion of the standardised breakfast meal to <sup>18</sup> 262 examine meal-induced changes in food reward and responses to food stimuli. At V2 and V4 only fasting 20 263 measurements of food reward and biometric responses will be performed. In a computerised questionnaire, 264 different food reward outcomes (food choice, implicit wanting, explicit liking, and explicit wanting) are <sup>23</sup> 265 measured using the Leeds Food Preference Questionnaire (36-38) in combination with measures of 25 266 autonomic nervous system activity including arousal estimated from galvanic skin response (Biopac MP160, 27<sup>267</sup> Biopac Systems Inc, Goleta, CA, USA), emotional response using facial expression analyses (AFFDEX 268 algorithm, Affectiva, MA, USA), and motivated visual attention using eye tracking (Tobii X2-60, Tobiipro, 30 269 Stockholm, Sweden). The Leeds Food Preference Questionnaire is integrated into a biometric software <sub>32</sub> 270 platform (iMotions) to enable simultaneous collection of data on eye tracking, galvanic skin response, and 271 facial expressions.

# 272 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, cocupation, civil status, children, and personal and household income.

# 277 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.

57 283At V3 and V4, all participants in the TRE group will be invited to individual semi-structured interviews of5859 284approx. 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 285 286 with the intervention. The interviews will draw on social practice theory (39) and will be recorded and 287 transcribed verbatim. Malterud's systematic text condensation approach (40) will be used to analyse the 288 interview data.

#### 290 Free-living assessment period

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291 A one-week free-living assessment period is scheduled after each of the test days at V1-V3, and includes the 17 292 procedures described below. Participants are instructed to follow group allocation during the assessment <sub>19</sub> 293 period at V2 and V3. After the one-week free-living assessment period at V3, the participants will be 294 instructed to live as they wish during the subsequent 3-months follow-up period.

#### 23 24 295 Gastric emptying, gastrointestinal transit times and motility

<sub>26</sub> 296 At V1 and V3, participants will be instructed to ingest a wireless motility capsule (SmartPill™, Medtronic, MN, 27 297 USA) immediately after ingesting the standardised breakfast meal with 150 ml water. The capsule measures 28 29 298 pH, temperature, and pressure through the gastrointestinal tract until expulsion. From these data, regional 30 31 299 transit time, pH profile, and motility in different parts of the gastrointestinal tract can be estimated (41–43). 32 Participants will be instructed to wear a SmartPill<sup>™</sup> receiver unit within 0.25 meters of their body for the 300 33 34 301 following week or until expulsion of the capsule. As this is an expensive measurement, only the first ~60 35 36 302 participants will be offered the capsule.

39 303 Events related to the gastrointestinal tract

41 304 At V1 and V3, participants register all events related to the gastrointestinal tract (passing stool, eating, 42 305 sleeping, and gastrointestinal symptoms such as nausea, vomiting, pain in the abdominal region, bloating 43 44 306 etc.), until expulsion of the SmartPill, using an 'event button' on the SmartPill<sup>™</sup> receiver. Additionally, the 46 307 participants register the time and type of each event in a diary.

#### 49 308 Physical activity and sleep

51 309 Physical activity and sleep will be measured using accelerometry. Participants will be equipped with one 52 310 accelerometer on the thigh and one on the lower back for one week (Axivity AX3, Newcastle upon Tyne, UK). 53 54 311 Concomitant with wearing the accelerometers, participants will be asked to fill out a physical activity and 55 56 312 sleep diary.

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## 14 *Continuous glucose monitoring*

A one-week continuous glucose monitoring (CGM) system (Ipro<sup>™</sup>, 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).

### 321 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<sup>™</sup> receiver, accelerometers, and diaries to the
 researchers after completion of each assessment period.

### 328 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

4 investigator. This approach ensures that participants are blinded to the group allocation during the 7-day 343 5 6 344 free-living assessment period. Over the phone, the investigator provides a detailed description and 345 introduction to the specific group allocation. For practical reasons, randomisation is open for participants 8 9 346 and research staff. However, the outcome assessors (data analysts) will be blinded during the statistical 10 11 347 analyses of all experimental outcomes.

#### 13 348 Time-restricted eating 14

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15 349 Participants allocated to the TRE group are instructed to consume all foods and beverages (except water) 16 17 350 within a self-selected time window of 10 hours/day between 6 am and 8 pm for the 13-week intervention. 18 <sub>19</sub> 351 Furthermore, the participants are instructed to keep the eating window stable during the entire week and 20 352 advised to select a window starting at least 2 hours after habitual wake-up time and 3 hours before habitual 21 22 353 bedtime if possible. Participants are advised to follow the Danish dietary recommendations. No other dietary 23 <sub>24</sub> 354 restrictions are prescribed.

#### 27 355 Control

29 356 Participants allocated to habitual living are advised to follow the Danish dietary recommendations but are 31 357 otherwise instructed to continue their habitual lifestyle during the 13 weeks intervention.

#### 36 359 Assessment of adherence

37 38 360 All participants are asked to register time for initiating first and terminating last eating/drinking episode <sup>39</sup> 361 (except water) every day from the test day at V1-V4. Every week during the 26-week period (intervention 40 41 362 and follow-up), a link to an online form will be sent by e-mail to the participants for them to register the time 42 43 363 for eating/drinking episodes for the previous week. In case participants in the control group restrict their 44 364 eating window to less than their habitual ≥12 hours/day or if the eating window of participants in the TRE 45 46 365 group deviate from their self-selected 10-hour eating window  $\geq$ 4 days during the first week, the participant 47 <sub>48</sub> 366 will be contacted per telephone to ensure that the participant has understood the concept of their 49 367 designated group allocation. During the first week of the intervention participants in the TRE group can 50 51 368 change their eating window once, in case they are not satisfied with the originally selected window. After the 52 <sub>53</sub> 369 first week no changes are allowed. To ensure similar contact with participants in the control group and the 54 370 TRE group, the participants will not be contacted in case of non-adherence after the first week. However, if 55 56 371 the participants fail to register their eating window, they will be contacted only to remind them to register. 57 <sub>58</sub> 372 No other feedback is provided during the intervention. To account for variations in daily eating windows 59

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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.

### 79 Statistical methods

### Sample size determination

There is strong evidence for the clinical relevance of a weight reduction of 3% in people with overweight or 81 82 obesity, with or without prediabetes (44). No RCT has investigated the effects of TRE on weight loss in 83 individuals with overweight or obesity at a high risk of type 2 diabetes. However, Gill and Panda investigated 84 effects of a 16-week TRE intervention on changes in body weight in eight healthy overweight individuals (BMI 85 >25 kg/m<sup>2</sup>) with a habitual eating window >14 hours/day and observed a mean reduction of body weight of 86 3.9% (3.3 kg, 95% CI: 0.9-5.6 kg); however, no control group was included (11). Per inclusion criteria 87 individuals with a BMI  $\geq$  25 kg/m<sup>2</sup> are included in the RESET study. For the participants with a BMI of 25 kg/m<sup>2</sup> 88 (with an expected mean height of 170 cm) a change in weight of 3% will correspond to ~2 kg. Thus, in order 89 to detect a minimal clinically relevant difference in weight change of 3% across the allowed BMI range, the 90 trial was dimensioned to detect a difference in change of 2 kg between the TRE group and the control group. 91 In a recent randomised controlled trial examining the effects of 13 weeks of either exercise or 92 pharmacological therapy on cardiometabolic health in individuals with overweight or obesity and 93 prediabetes, the SD for within-group changes in body weight in the control group was 2.6 kg (Færch et al., 94 under review). We expect that the SD for within-group changes will be similar in the RESET trial, but to 95 account for uncertainties we increased the SD by 20%, resulting in an SD of 3.1 kg. In order to detect a 2 kg 96 (SD 3.1) difference in weight change with a desired statistical power of 0.8 (two-tailed test, alpha 0.05), a 97 total of 40 participants is required in each group. To allow for a 20% drop-out in each group, we plan to 98 include 50 participants in each of the two groups.

### 399 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 404 405 will be analysed using linear mixed-effects models with the outcome as a function of group, time and group 406 x time interaction and including a participant-specific random intercept. Outcomes with a known/expected 407 bimodal distribution due to sex differences will be adjusted for sex. Adequacy of assumptions of normality 11 408 and homogeneity of variances will be assessed using graphical methods and, if necessary, data will be log-13 409 transformed for analysis and back-transformed for presentation. If model assumptions are not met by 410 logarithmically transformation, non-parametric statistical tests will be performed. P-values < 0.05 (two-tailed) 16 411 are considered statistically significant. Results will be presented as estimated mean differences in changes 18 412 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**

<sup>35</sup> 420 All equipment used in the studies meet the requirement for patient safety. The total amount of blood taken 36 37 421 at each visit is maximally 300 ml, which is less than a standard blood donation of 450 ml and considered safe. 38 <sub>39</sub> 422 Participants will be instructed not to donate blood during the trial. There may be some discomfort associated <sup>40</sup> 423 with swallowing the SmartPill<sup>™</sup>. The risk of capsule retention in individuals without known stenosis is only 41 42 424 0.75% and in such a case, a pro-motility drug is often sufficient to mobilize the capsule. Alternatively, 43 <sub>44</sub> 425 endoscopy can be performed to retrieve the capsule. Body composition is measured using Dual-energy X-ray 45 426 Absorptiometry with a radiation dose less than 0.01 mSv, which corresponds to less than one day of normal 46 47 427 background radiation. There is no expected discomfort or risks associated with ingestion of the meals, food 48 49 428 reward measurements or biometric measurements (eye tracking, galvanic skin response, facial expression 50 429 analyses). Participants are covered by the Patient Compensation Association according to the Danish Act on 51 52 430 the Right to Complain and Receive Compensation within the Health Service. The intervention is considered 53 54 431 safe in individuals with overweight and obesity (45). 55

<sup>56</sup> 432 The study has been approved by the Ethics Committee of the Capital Region of Denmark (H-18059188) and 57 58 433 will be conducted in accordance with the Declaration of Helsinki. Approval of data and biobank has been 59

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obtained from the Danish Data Protection Agency. The study is registered at ClinicalTrials.gov (identifier: 434 435 NCT03854656).

436 All study-related information will be recorded, handled, and stored in a way that allows accurate reporting, <sup>10</sup> 437 interpretation and verification. Source data will be registered in the electronic data management system 12 438 REDCap. For CGM measurements, source data will be registered in a web-based software (CareLink™, 439 Medtronic) using the participant's study ID. Source data from Dual-energy X-ray Absorptiometry, biometric <sup>15</sup> 440 measurements, food preferences, Vagus™, SmartPill™, accelerometers and indirect calorimetry are 17 441 registered on the device or related hardware and uploaded to a secured logged drive to which only project 19<sup>442</sup> staff has access. Sponsor/investigator will provide direct access to source data/documents for regulatory <sup>20</sup> 443 inspection.

444 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.

#### <sup>31</sup> 448 Discussion

33 449 Implementation and maintenance of traditional strategies for weight loss and early prevention of type 2 35 450 diabetes, i.e. increased physical activity and dietary restrictions, is difficult for many (46-48), because it is 451 time consuming and requires insights into the type and amount of foods eaten. In a public health perspective, 38 452 there is a strong need for feasible lifestyle strategies to combat the current type 2 diabetes epidemic. TRE is 40 453 a simple eating pattern regime which extends the daily fasting period and potentially synchronizes food 42 454 intake with circadian rhythms of metabolism and may therefore represent a feasible lifestyle modification 43 455 strategy. The evidence from animal studies and well-controlled human studies suggest that TRE has the 44 45 456 potential to improve a variety of cardiometabolic risk factors in metabolically vulnerable individuals and may 457 be a feasible and sustainable regimen (18); however, randomised controlled trials are lacking. Using an <sup>48</sup> 458 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 50 459 . 52 460 TRE.

54 461 Implicit in the trial design is that the intervention is only relevant and feasible if the participants can uphold 55 <sub>56</sub> 462 the TRE regime without frequent contact with the research staff; otherwise we find it unlikely that this 57 58 463 intervention will have great relevance in the public at large (who are likely less motivated than those opting 59 464 to partake in an RCT). Eating is integrated in the rhythms and social relations in everyday life. Since TRE affects 60

eating practices, it is important to get insight into participants' experiences with TRE and to identify potential 465 466 barriers and strategies for integration and maintenance of this regimen. Thus, the findings from the present 467 study will address whether TRE is an acceptable intervention which can improve health outcomes in 468 individuals at risk of lifestyle-related diseases. As such, it will potentially inform the design of future large-11 469 scale studies and feasible health recommendations.

#### <sup>16</sup> 471 Authors' contributions

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19 472 JSQ and KF conceived the idea and initiated the study. KF is principal investigator and JSQ, HP, MMC, KKBC, 473 and NB are co-investigators. All authors contributed to the design of the study. JSQ drafted the manuscript. 22 474 All authors have critically reviewed the manuscript and approved the final version.

### <sup>27</sup> 476 Funding and economical compensation

<sup>29</sup> 477 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 31 478 scholarship from Aalborg University and an industrial PhD scholarship from Innovation Fund Denmark. We 480 cover documented, reasonable travel expenses if the participant lives more than 12 km from Steno Diabetes 36 481 Center Copenhagen. Participants will not receive any other financial compensation for participating in the 38 482 study.

#### **Competing interests** 42 484

Steno Diabetes Center Copenhagen is a hospital providing health services for the public health care system. 44 485 45 486 Steno Diabetes Center Copenhagen is partly funded by the Novo Nordisk Foundation through unrestricted 46 <sup>47</sup> 487 grants. The Novo Nordisk Foundation has no economic interests in the study. The Novo Nordisk Foundation 48 49 488 will not have influence on the study design, data collection, analysis, interpretation of data, the writing of the 50 489 study report or any publication and the decision to submit the paper for publication. The investigators 51 <sup>52</sup> 490 employed at Steno Diabetes Center Copenhagen will not benefit economically from conducting the study. 53 54 491 HP is co-investigator on the project which is part of her Industrial PhD project in collaboration with iMotions 55 56 492 A/S, where HP is employed. iMotions A/S is a collaborator on the project and gives advice for the use and <sup>57</sup> 493 analysis of biometric methods in the study design phase. SP has published a book, The Circadian Code, 58 59 494 focusing on the concept of TRE.

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$     \begin{array}{r}       39 \\       40 \\       41 \\       42 \\       613 \\       42     \end{array} $	Figure	e legend
44 614 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60	Figure	e 1: Study design.

# Table 1. Inclusion and exclusion criteria

# Inclusion criteria

- Age: ≥30 to ≤70 years
- Body mass index ≥30 kg/m<sup>2</sup> or body mass index ≥25 kg/m<sup>2</sup> in combination with pre-diabetes (HbA1c ≥39 to <48 mmol/mol)</li>
- 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<sub>1c</sub>  $\geq$ 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<sup>™</sup>

- 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

	<b>VU</b>	V1	V2	
I me, weeks from start of intervention	-/1	-1	6	1
Participant Information	v			
Informed consent	X			
Medical history (individual and family)	X			
In- and exclusion criteria	Х	.,	.,	
Pregnancy test (fertile women only)		X	X	>
Efficacy outcomes				
HbA <sub>1C</sub>	Х	Х	Х	>
Body weight	Х	Х	Х	>
Waist and hip circumference		Х	Х	>
Body composition (DXA scanning)		Х	Х	>
Blood pressure and resting heart rate	Х	Х	Х	>
Stool sample		Х		X
Fasting blood samples		Х	Х	>
Postprandial blood samples		Х		>
Indirect calorimetry		Х		>
Heart rate variability (Vagus™)		Х		>
Mixed meal test with SmartPill™		Х		)
Event registration related to SmartPill™		Х		>
Physical activity and sleep measurement		Х	Х	>
Food records		Х	Х	>
Continuous glucose monitoring		Х	Х	>
Fasting food reward and biometric measurements		Х	Х	Х
Postprandial food reward and biometric measurements		Х		>
Questionnaires				
Sociodemographic characteristics		Х		
Health and wellbeing		Х	Х	)
Physical activity		Х	Х	>
Fasting appetite sensations		Х	Х	)
Postprandial appetite sensations		Х		)
Gastrointestinal symptoms		Х	Х	>
Autonomic symptoms		Х		
Pain		X		
Sleep quality and sleepiness		X	Х	>
Chronotype		Х	Х	>
Night eating		Х	Х	>
Eating behaviour and control over eating		Х	Х	>
Interviews				
Interview (all participants) <sup>2</sup>		Х		
Interview (all participants in the TRF group) <sup>3</sup>				X

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.

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# **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

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Secondary Subject Heading:	Diabetes and endocrinology, Nutrition and metabolism, Research methods
Keywords:	DIABETES & ENDOCRINOLOGY, Diabetes & endocrinology < INTERNAL MEDICINE, NUTRITION & DIETETICS





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6	2	time-restricted eating on body weight, behaviour, and metabolism in individuals at high risk of type 2
7 8	3	diabetes - The <u>Res</u> tricted <u>E</u> ating <u>T</u> ime (RESET) study
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55 56	28	
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58 59	29	Word count: 5,350
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#### 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 <u>Res</u>tricted <u>Eating Time</u> (RESET) study is a randomised controlled parallel-group open-label trial. 100 women and men with 1) overweight (BMI:  $\geq$ 25 kg/m<sup>2</sup>) and prediabetes (HbA<sub>1c</sub>: 39-47 mmol/mol); or 2) obesity (BMI: ≥30 kg/m<sup>2</sup>) 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

# 59 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.

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# 79 Introduction

80 Overweight and prediabetes increase the risk of developing type 2 diabetes and cardiovascular disease (1– 81 3). Weight loss is associated with improved glycaemic control and cardiometabolic health among individuals 82 with prediabetes and type 2 diabetes (4,5); therefore, the development of effective, feasible, and sustainable 83 weight loss strategies is essential. Current prevention and treatment of obesity and type 2 diabetes include 84 energy restricted diets and increased levels of physical activity (6). However, adherence and maintenance to 85 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, 

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challenges associated with social events including drinking and eating may exist (25). Nevertheless, an indepth 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 stateof-the-art and novel quantitative and qualitative methods.

# 17 **Objectives**

The primary objective of the <u>Res</u>tricted <u>Eating Time</u> (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.

# 124 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).

# 129 Methods and analysis

# 130 Study design

1 The study is a single-centre, parallel-group, randomised, controlled, superiority, open-label trial (Figure 1). 2 100 individuals will be randomised to 3 months of TRE or habitual living (control) in a 1:1 ratio. Habitual living 3 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 4 5 outcome is assessed after 3 months of intervention (V3). At baseline, mid-intervention (after 6 weeks; V2) 6 and after the intervention (3 months; V3) outcomes are assessed during test days and free-living 7 measurements during the week following the test days. After the 3-month intervention, a 3-month follow-8 up period and subsequent testing (V4) is scheduled to assess maintenance and longer-term effects. The trial 59

will be performed at Steno Diabetes Center Copenhagen and will be reported according to the Consolidated 139 140 Standards of Reporting Trials (26). The study protocol follows the Standard Protocol Items: 141 Recommendations for Interventional Trials statement (27). The trial is registered at ClinicalTrials.gov 142 (identifier: NCT03854656) and a copy of the World Health Organization Trial Registration Data Set is supplied 10 11 143 in Supplementary Table 1. 12

#### 16 145 **Participants**

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<sup>18</sup> 146 Women and men, 30-70 years of age, with a) overweight (BMI: ≥25 kg/m<sup>2</sup>) with concomitant prediabetes as 19 defined by HbA<sub>1c</sub>: 39-47 mmol/mol (6) or b) obesity (BMI:  $\geq$ 30 kg/m<sup>2</sup>), who are eligible according to the 20 147 21 22 <sup>148</sup> inclusion and exclusion criteria (Table 1) will be included.

#### 24 149 Eligibility criteria 25

<sup>26</sup> 150 Inclusion and exclusion criteria are listed in Table 1. We include high-risk individuals 30-70 years of age, 27 28 151 because our focus is on preventing diabetes at an early stage. Individuals with overweight and prediabetes 29 <sub>30</sub> 152 and obesity with/without prediabetes are included to target individuals at high risk of type 2 diabetes. The 31 153 rationale of choosing 70 years as upper limit is that the potential for prevention is limited in older individuals. 32 33 154 To prevent sources of circadian irregularity during the intervention, shift workers and individuals with a 34 <sub>35</sub> 155 partner engaged in shift work affecting the circadian rhythm of the participant are not eligible for 36 156 participation. 37

#### Recruitment and screening 39 157

41 158 Participants are recruited through advertisements on different publicly available platforms (newspapers, 42 43 159 webpages, pharmacies, etc.). A pre-screening is performed as a telephone interview focusing on the 44 160 participant's age, BMI, and habitual eating window to reduce the number of screen failures. Participants who 45 46 161 are eligible based on the pre-screening, receive written information about the study and are scheduled for a 47 48 162 screening visit. At the screening visit participants provide oral and written informed consent to medical staff, 49 163 and a health examination including medical history and assessment of inclusion and exclusion criteria is 50 51 164 performed (Table 1). After the screening, eligible participants will be scheduled for four visits (V1-V4, Figure 52 53 165 1). Baseline testing (V1) takes place as soon as possible and within 6 weeks from the screening visit (V0). The 54 166 first participant signed consent on February 25, 2019, and participants are recruited continuously. 55

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Outcomes

Primary outcome

sample size and comparison across studies.

Study visits and free-living assessment periods

Secondary exploratory outcomes

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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

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

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. 13<sup>th</sup> 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.

weeks; V2), post intervention (after 3 months; V3) and follow-up testing (after 6 months; V4).

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# 199 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.

# 06 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.

# 216 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.

# 221 Heart rate variability

Heart rate variability and cardiovascular reflex are measured by electrocardiography using a handheld device
(Vagus<sup>™</sup>, 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).

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#### 228 Resting energy expenditure and substrate oxidation

229 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 230 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.

#### 233 Blood samples

234 Venous blood samples are collected in the fasting state at all four test days (V1-V4) and postprandially during <sup>18</sup> 235 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 20 236 antecubital vein. Analyses include assessment of HbA<sub>1c</sub>, circulating levels of glucose and lipids, inflammatory 237 markers, and hormones involved in regulation of appetite and metabolism (e.g. insulin, glucagon, glucagon-<sup>23</sup> 238 like peptide-1, glucose-dependent insulinotropic polypeptide, peptide YY, acylated ghrelin). Furthermore, 25 239 metabolomics and proteomics will be applied, and assessment of circulating proteins and metabolites that 20 27 240 correlate with low-grade inflammation and markers of lipid metabolism will be captured using mass-<sup>28</sup> 241 spectrometry driven analyses of the plasma proteome and metabolome (32,33). Gene expression of pro- and 30 242 anti-inflammatory proteins including cytokines and chemokines, and genes involved in energy metabolism <sub>32</sub> 243 of isolated peripheral blood mononuclear cells will be measured by real-time polymerase chain reaction. 244 Cellular bioenergetic activity (mitochondrial respiration and glycolysis) of isolated peripheral blood 35 245 mononuclear cells will be determined using a Seahorse XFe24 Analyzer (34). The seahorse technology <sub>37</sub> 246 measures real-time oxygen consumption rate as an indicator of mitochondrial activity and extracellular <sup>38</sup> 247 acidification rate as an indicator of glycolytic activity. Thus, these measurements will provide mechanistic 40 248 knowledge of TRE-induced metabolic changes at the cellular level. Serum and plasma will be stored in a 42 249 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 253 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.

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#### Subjective appetite 257

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258 Hunger, satiety, fullness, thirst, estimated prospective food consumption, and desire for sweet, salt, and fat, 259 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. 10 260

#### 13 261 Food preferences and food reward 14

15 262 At V1 and V3, components of food reward and biometric responses to standardised photographic images of 16 263 foods will be assessed in the fasting state and 60 min after ingestion of the standardised breakfast meal to 17 <sup>18</sup> 264 examine meal-induced changes in food reward and responses to food stimuli. At V2 and V4 only fasting 19 measurements of food reward and biometric responses will be performed. In a computerised questionnaire, 20 265 21 266 different food reward outcomes (food choice, implicit wanting, explicit liking, and explicit wanting) are 22 <sup>23</sup> 267 measured using the Leeds Food Preference Questionnaire (36-38) in combination with measures of 24 25 268 autonomic nervous system activity including arousal estimated from galvanic skin response (Biopac MP160, 26 27<sup>269</sup> Biopac Systems Inc, Goleta, CA, USA), emotional response using facial expression analyses (AFFDEX 28 270 algorithm, Affectiva, MA, USA), and motivated visual attention using eye tracking (Tobii X2-60, Tobiipro, 29 30 271 Stockholm, Sweden). The Leeds Food Preference Questionnaire is integrated into a biometric software 31 <sub>32</sub> 272 platform (iMotions) to enable simultaneous collection of data on eye tracking, galvanic skin response, and 33 273 facial expressions. 34

#### 274 Questionnaires

<sup>38</sup> 275 At V1-V4, participants fill in questionnaires regarding health and wellbeing, gastrointestinal and autonomic 39 40 276 symptoms, eating behaviour, chronotype, sleep, and physical activity (Table 2). At V1, participants fill in a 42 277 questionnaire regarding sociodemographic characteristics including age, sex, ethnicity, education, 43 278 occupation, civil status, children, and personal and household income. 44

#### 279 Interviews

48 Interviews will be conducted at V1, V3, and V4 to obtain insights into the participants' experiences and 280 49 50 281 perceptions of the intervention. This will provide an understanding of the feasibility and integration of the 51 52 282 intervention into the everyday life of the participants as well as maintenance of the regimen. At V1, all 53 <sub>54</sub> 283 participants will be interviewed for approx. 25 min to examine their reasons and motivation for participation, 55 284 their expectations towards the intervention, and their everyday life activities and eating practices. 56

57 285 At V3 and V4, all participants in the TRE group will be invited to individual semi-structured interviews of 58 <sub>59</sub> 286 approx. 45 min to explore feasibility and maintenance of TRE in everyday life. If participants withdraw from

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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<sup>™</sup> 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<sup>™</sup> 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.

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#### 316 Continuous glucose monitoring

A one-week continuous glucose monitoring (CGM) system (Ipro<sup>™</sup>, Medtronic Denmark A/S, Copenhagen, 317 318 Denmark) will be initiated at the test days at V1-V3. The CGM will be attached to the lower part of the 10 319 abdomen in the morning at the test days. Participants will be instructed to measure blood glucose levels 320 using a glucometer (Contour XT, Ascensia Diabetes Care Denmark ApS, Copenhagen, Denmark) four times a 12 13 321 day during the measurement period for calibration of the CGM (before breakfast, lunch, main evening meal, 14 15 322 and bedtime).

#### 18 323 Dietary intake

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324 During the week following V1, V2, and V3, participants will be asked to fill in a food record diary (pen and 22 325 paper) for three days (day 1, 3, and 5 after the test day; two weekdays and one weekend day). Participants 24 326 will be instructed to register weight, time, and content of all meals and beverages (except water). During the 327 same week, participants will be asked to register their eating window (see assessment of adherence).

328 Participants will be instructed to send the CGM, SmartPill<sup>™</sup> receiver, accelerometers, and diaries to the 30 329 researchers after completion of each assessment period.

#### 330 *Gut microbiome*

35 331 Participants will be provided with a kit for stool samples including storage equipment at VO and V2. They will 37 332 be instructed to collect and immediately freeze (-20 °C) three samples from the same stool sample ≤72 hours 333 before test days at V1 and V3. The participants will transport the samples to the laboratory in provided 40 334 cooling bags and the samples will be stored at -80 °C until analysis. Bacterial DNA and RNA will be purified 42 335 from the stool samples. The microbial content, composition, and function will be estimated based on 336 sequencing of the microbiome.

#### 47 <sub>48</sub> 338 **Randomisation and intervention**

After completing baseline testing, participants are randomly allocated to either the control group or the TRE 50 339 51 340 group. Randomisation is performed in blocks varying in size, unknown to the researchers, to ensure an equal 52 <sup>53</sup> 341 distribution of participants in the two groups in case the study, for unexpectedly reasons, must be terminated 54 55 342 before inclusion of all participants. The randomisation list was generated by an external statistician and 56 57 343 uploaded to the electronic data management system REDCap (8.10.18, Vanderbilt University, TN, USA). <sup>58</sup> 344 When participants leave the research facilities at the test day at V1, they receive a sleeve with a combination 59

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59 60 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.

### 351 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.

### 8 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.

### 362 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  $\geq$ 12 hours/day or if the eating window of participants in the TRE group deviate from their self-selected 10-hour eating window  $\geq$ 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 375 376 around 10 hours, participants in the TRE group are considered adherent if their eating window is less than 377 11 hours/day. Adherence to the intervention is calculated as number/percentage of days during the 378 intervention the participants' eating window is <11 hours/day. Per protocol is defined as  $\geq$ 80% compliance. 11 379 The eating window will be calculated for both groups, but no compliance criterion is applied in the control 13 380 group. Regardless to the degree of adherence all participants allocated to both groups will be invited for test 381 days with emphasis on participating in V3 and if participants are not willing to attend a full test day they will 16 382 be asked to come in for a measurement of the primary outcome.

21 384 Statistical methods

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#### 24 <sup>385</sup> Sample size determination

There is strong evidence for the clinical relevance of a weight reduction of 3% in people with overweight or 26 386 27 387 obesity, with or without prediabetes (45). No RCT has investigated the effects of TRE on weight loss in 28 29 388 individuals with overweight or obesity at a high risk of type 2 diabetes. However, Gill and Panda investigated 30 31 389 effects of a 16-week TRE intervention on changes in body weight in eight healthy overweight individuals (BMI 32 390 >25 kg/m<sup>2</sup>) with a habitual eating window >14 hours/day and observed a mean reduction of body weight of 33 34 391 3.9% (3.3 kg, 95% CI: 0.9-5.6 kg); however, no control group was included (11). Per inclusion criteria 35 individuals with a BMI  $\geq$  25 kg/m<sup>2</sup> are included in the RESET study. For the participants with a BMI of 25 kg/m<sup>2</sup> 36 392 37 393 (with an expected mean height of 170 cm) a change in weight of 3% will correspond to ~2 kg. Thus, in order 38 <sup>39</sup> 394 to detect a minimal clinically relevant difference in weight change of 3% across the allowed BMI range, the 40 41 395 trial was dimensioned to detect a difference in change of 2 kg between the TRE group and the control group. 42 396 In a recent randomised controlled trial examining the effects of 13 weeks of either exercise or 43 44 397 pharmacological therapy on cardiometabolic health in individuals with overweight or obesity and 45 46 398 prediabetes, the SD for within-group changes in body weight in the control group was 2.6 kg (Færch et al., 47 48 399 under review). We expect that the SD for within-group changes will be similar in the RESET trial, but to 49 400 account for uncertainties we increased the SD by 20%, resulting in an SD of 3.1 kg. In order to detect a 2 kg 50 51 401 (SD 3.1) difference in weight change with a desired statistical power of 0.8 (two-tailed test, alpha 0.05), a 52 <sub>53</sub> 402 total of 40 participants is required in each group. To allow for a 20% drop-out in each group, we plan to <sup>54</sup> 403 include 50 participants in each of the two groups. 55

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### 406 Statistical analysis plan

407 Intention-to-treat analysis including all randomised participants will be performed after the last participant 408 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 12 <sup>410</sup> statistics. Descriptive statistics will be shown as mean (SD) for normally distributed data and as median (Q1; <sup>13</sup> 411 Q3) for non-normally distributed data. Changes from baseline and differences in delta values between groups 15 412 will be analysed using linear mixed-effects models with the outcome as a function of group, time and group 17 413 x time interaction and including a participant-specific random intercept. Outcomes with a known/expected <sup>18</sup> 414 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-22 416 transformed for analysis and back-transformed for presentation. If model assumptions are not met by <sup>23</sup> 417 logarithmically transformation, non-parametric statistical tests will be performed. P-values < 0.05 (two-tailed) 25 418 are considered statistically significant. The potential impact of missing data on the primary outcome will be 27 419 evaluated in a sensitivity analysis based on multiple imputation in which participants with missing data at <sup>28</sup> 420 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.

### 425 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.

# 430 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<sup>™</sup>. 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 436 437 Absorptiometry with a radiation dose less than 0.01 mSv, which corresponds to less than one day of normal 438 background radiation. There is no expected discomfort or risks associated with ingestion of the meals, food 439 reward measurements or biometric measurements (eye tracking, galvanic skin response, facial expression 10 11 440 analyses). Participants are covered by the Patient Compensation Association according to the Danish Act on 12 13 441 the Right to Complain and Receive Compensation within the Health Service. The intervention is considered 14 442 safe in individuals with overweight and obesity (46). 15

17 443 The study has been approved by the Ethics Committee of the Capital Region of Denmark (H-18059188) and 18 19<sup>444</sup> will be conducted in accordance with the Declaration of Helsinki. Approval of data and biobank has been <sup>20</sup> 445 obtained from the Danish Data Protection Agency. Consent from the Ethics Committee of the Capital Region 21 22 446 of Denmark to all previous and future amendments to the protocol have and will be obtained before these 23 24 447 are instated (Supplementary Table 2).

<sup>25</sup> 448 The study is registered at ClinicalTrials.gov (identifier: NCT03854656). No Data monitoring committee has 26 27 449 been appointed for the trial due to the perceived very low risk of harms. 28

29 29 30 450 All study-related information will be recorded, handled, and stored safely in a way that allows accurate <sup>31</sup> 451 reporting, interpretation and verification. Source data will be registered in the electronic data management 32 33 452 system REDCap (8.10.18, Vanderbilt University, TN, USA). For CGM measurements, source data will be 34 35<sup>453</sup> registered in a web-based software (CareLink™, Medtronic) using the participant's study ID. Source data from <sup>36</sup> 454 Dual-energy X-ray Absorptiometry, biometric measurements, food preferences, Vagus™, SmartPill™, 37 38 455 accelerometers and indirect calorimetry are registered on the device or related hardware and uploaded to a 39 40 456 secured logged drive to which only project staff has access. Investigators at Steno Diabetes Center <sup>41</sup> 457 Copenhagen will have access to the full data set. Sponsor/investigator will provide direct access to source 42 43 458 data/documents for regulatory inspection. Access to the full protocol and data can be obtained from the 44 45 <sup>459</sup> principal investigator. Since we expect no harms associated with the intervention, information on <sup>46</sup> 460 harms/side-effects will not be systematically recorded. 47

48 461 Positive and negative as well as inconclusive study results will be presented at conferences and published in 49 50 462 international peer-reviewed journals in accordance with the CONSORT guidelines, no publication restrictions 51 52 463 have been imposed. All co-authors must comply with the International Committee of Medical Journal Editors 53 54 464 guidelines and no professional writers will be engaged in the writing process.

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#### Discussion 467

Implementation and maintenance of traditional strategies for weight loss and early prevention of type 2 468 469 diabetes, i.e. increased physical activity and dietary restrictions, is difficult for many (47-49), because it is 10 470 time consuming and requires insights into the type and amount of foods eaten. In a public health perspective, 11 471 there is a strong need for feasible lifestyle strategies to combat the current type 2 diabetes epidemic. TRE is 12 <sup>13</sup> 472 a simple eating pattern regime which extends the daily fasting period and potentially synchronizes food 14 15 473 intake with circadian rhythms of metabolism and may therefore represent a feasible lifestyle modification 16 17 474 strategy. The evidence from animal studies and well-controlled human studies suggest that TRE has the <sup>18</sup> 475 potential to improve a variety of cardiometabolic risk factors in metabolically vulnerable individuals and may 19 20 476 be a feasible and sustainable regimen (18); however, randomised controlled trials are lacking. Using an 21 22 477 interdisciplinary approach, the present study will examine effects of TRE on weight changes and explore <sup>23</sup> 478 effects on cardiometabolic health and behaviour, as well as on participants' motivation and experiences with 24 25 479 TRE.

27 28 480 Implicit in the trial design is that the intervention is only relevant and feasible if the participants can uphold 29 481 the TRE regime without frequent contact with the research staff; otherwise we find it unlikely that this 30 31 482 intervention will have great relevance in the public at large (who are likely less motivated than those opting 32 483 to partake in an RCT). Eating is integrated in the rhythms and social relations in everyday life. Since TRE affects 33 <sup>34</sup> 484 eating practices, it is important to get insight into participants' experiences with TRE and to identify potential 36 485 barriers and strategies for integration and maintenance of this regimen. Thus, the findings from the present 37 486 study will address whether TRE is an acceptable intervention which can improve health outcomes in 38 <sup>39</sup> 487 individuals at risk of lifestyle-related diseases. As such, it will potentially inform the design of future large-40 41 488 scale studies and feasible health recommendations.

#### 46 490 Authors' contributions

48 491 JSQ and KF conceived the idea and initiated the study. KF is principal investigator and JSQ, HP, MMC, KKBC, 49 <sup>50</sup> 492 and NB are co-investigators. JSQ, MMC, KKBC, HP, NB, JS, MBB, NJWA, JJH, SST, DV, MEJ, SP, CB, GF, KF 51 52 493 contributed to the design of the study. JSQ drafted the manuscript. MMC, KKBC, HP, NB, JS, MBB, NJWA, JJH, 53 54 494 SST, DV, MEJ, SP, CB, GF, KF critically reviewed the manuscript. JSQ, MMC, KKBC, HP, NB, JS, MBB, NJWA, JJH, <sup>55</sup> 495 SST, DV, MEJ, SP, CB, GF, KF approved the final version. 56

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# 497 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.

# 509 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.

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# Table 1. Inclusion and exclusion criteria

# Inclusion criteria

- Age: ≥30 to ≤70 years
- Body mass index ≥30 kg/m<sup>2</sup> or body mass index ≥25 kg/m<sup>2</sup> in combination with pre-diabetes (HbA<sub>1c</sub> ≥39 to <48 mmol/mol)</li>
- 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<sub>1c</sub> ≥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<sup>™</sup>

- 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

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Time, weeks from start of intervention	-1				
	-/-	-1	6	12	26
Participant information					
nformed consent	Х				
Medical history (individual and family)	Х				
n- and exclusion criteria	Х				
Pregnancy test (fertile women only)		Х	Х	Х	Х
Efficacy outcomes					
HbA <sub>1C</sub>	Х	Х	Х	Х	Х
Body weight	Х	Х	Х	Х	Х
Waist and hip circumference		Х	Х	Х	Х
Body composition (DXA scanning)		Х	Х	Х	Х
Blood pressure and resting heart rate	Х	Х	Х	Х	Х
Stool sample		Х		Х	
Fasting blood samples		Х	Х	Х	Х
Postprandial blood samples 🔍		Х		Х	
ndirect calorimetry		Х		Х	
Heart rate variability (Vagus™)		Х		Х	
Mixed meal test with SmartPill™		Х		Х	
Event registration related to SmartPill™		Х		Х	
Physical activity and sleep measurement		Х	Х	Х	
Food records		Х	Х	Х	
Continuous glucose monitoring		Х	Х	Х	
Fasting food reward and biometric measurements		Х	Х	Х	Х
Postprandial food reward and biometric measurements		Х		Х	
Questionnaires					
Sociodemographic characteristics		Х			
Health and wellbeing		Х	Х	Х	Х
Physical activity		Х	Х	Х	Х
Fasting appetite sensations		Х	Х	Х	Х
Postprandial appetite sensations		Х		Х	
Gastrointestinal symptoms		Х	Х	Х	Х
Autonomic symptoms		Х			
Pain		Х			
Sleep quality and sleepiness		X	Х	Х	Х
Chronotype		Х	Х	Х	Х
Night eating		Х	Х	Х	Х
Eating behaviour and control over eating		Х	Х	Х	Х
nterviews					
nterview (all participants) <sup>2</sup>		Х			
nterview (all participants in the TRE group) <sup>3</sup>				Х	Х

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.



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# WHO International Clinical Trials Registry information for the RESET trial

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

# 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.

<b>Register:</b>	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. Into None (Open Label).	ervention mode	el: Parallel Assignment. Prima	ary purpose: Prev	vention. Masking:
Phase:	N/A				
Countries of	of recruitment				
Denmark					
Contacts					
Name: Address:	Kristine Færch, PhD	Name: Address:	Kristine Færch, PhD	Name:	Kristine Færch, PhD
Telephone: Email: Affiliation:	+45 30913061 kristine.faerch@regionh.dk	Telephone: Email: Affiliation:	+45 30913061 kristine.faerch@regionh.dk	Address: Telephone: Email:	Steno Diabetes
Key inclusi	on & exclusion criteria			Affiliation:	Center Copenhagen

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

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- 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

 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)]

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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)]
For peer review only - http://dmjopen.dmj.com/site/adout/guidelines.xntml

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)]
For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

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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]
For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

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]

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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)]

akefulness (min) [Time Frame: Changes from baseline. Measured for 7 days after the test days at baseline and ter 6 and 12 weeks]
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Date of version	The amendments were made in the protocol and approved by the local Ethics
(date of approval from	Committee and the number and headings of the specific sections refers to the
the Ethics Committee)	protocol and not the article describing the protocol.
8 January 2019	Original
, (11 January 2019)	
5 February 2019	Amendment 01
(28 February 2019)	
	5.2 Exclusion criteria:
Inclusion of first	Diagnosis with diabetes and HbA1c ≥48 mmol/mol have been added. The trial
participant on 25	includes individuals who are at high risk of developing type 2 diabetes, but do
February 2019	not yet have the disease and these criteria were missing in the previous version.
1 Coldary 2015.	
	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 <i>per protocol</i> analysis in addition to the intention to treat analysis ir
	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	Amendment 02
October 2019)	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
	neriod after the test day
	period diter the test day.
	1 1 2 Secondary endnoints
	Respiratory and glycolytic canacities of isolated peripheral blood mononuclear
	cells (PRMCs) are only measured in facting conditions at baseline and after the
	intervention (2 months) and not as originally stated mid intervention (6 weeks)
	and at follow up (6 months) and not northrandially
	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 <i>per protocol</i> if they are compliant ≥80% of the day
	during the 3-months intervention. Previously, participants were allowed to b
	non-compliant one day per week in order to be <i>per protocol</i> but this criterion wa
	omitted due to practical challenges associated with determination of this.
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<ul> <li>eating windows. Participants are not asked to send a picture of their hand registrations as previously stated.</li> <li>c. Participants in the TRE group are allowed to change their selected eatin window once during the first week after randomisation.</li> <li><b>5.1 Inclusion criteria</b> <ul> <li>Habitual eating pattern: Previous criteria: ≥14 hours/day eating window o weekdays and eating 3 hours before bedtime' has been changed to ≥12 hours/day eating window and ≥14 hours/day 21 day/week.</li> <li>The criteria were changed due to challenges associated with recruitment only few individuals reported a habitual eating window ≥14 hours/day.</li> </ul> </li> <li><b>5.2 Exclusion criteria</b> <ul> <li>Partner engaged in shift work is only an exclusion criterion if it affects the individual's sleep or eating pattern.</li> <li>Due to financial reasons only the first 60 out of 100 participants will receiv SmartPill at the test days and specific exclusion criteria related to this met have been listed separately.</li> <li>'Not able to eat ≥85% of the test meal because of e.g. allergy' has been act ensure that the test days can be conducted.</li> <li>Concomitant participation in other research projects has been specified to 'intervention studies'.</li> <li><b>5.4 Randomisation</b></li> <li>The randomisation is not stratified by sex and age as originally stated. Stratification was discussed during the design phase but was not impleme This was a mistake and it has been omitted.</li> <li><b>6.2 Screening and 7.1.1 Questionnaires</b></li> <li>Questionnaires about sociodemographic characteristic and chronotype ar answered at the screening visit as originally stated. The was a mistake.</li> <li>The questionnaire on autonomic symptoms (COMPASS31) is only answere baseline (visit 1).</li> <li>International Physical Activity Questionnaire (IPAQ) is used in the study ar Recent Physical Activity Questionnaire (IPAQ) as originally written which mistake.</li> </ul> </li> </ul>	b. Every week participants receive an email with a link to registration of their c
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	medication in the morning before testing.

	(Destiningents will be asked to register all faced items and because in sector a
	the day before V1 and instructed to consume the same on the day before V2
	the day before V1 and instructed to consume the same on the day before V2,
	and V4' has been changed to: 'Participants will be asked to consume a last me
	between 7-8 pm the day before the test days' to standardize the fasting durat
	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™' ha
	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
	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
	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
	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	Amendment 03
(21 February 2020)	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 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents\*

Section/item Item No		Description			
Administrative in	format	ion			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym <b>Page 1</b>			
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 <b>Page 18</b>			
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			

1 2 3 4 5 6 7 8		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) <b>N/A (Page 16)</b>
9	Introduction		
10 11 12 13 14 15	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 <b>Page 4-5</b>
16 17 18		6b	Explanation for choice of comparators Page 5
20 21 22	Objectives	7	Specific objectives or hypotheses Page 5
23 24 25 26 27 28	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
29	Methods: Particip	oants, i	nterventions, and outcomes
31 32 33 34 35 26	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
37 38 39 40 41	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
42 43 44 45 46	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered <b>Page 12-13</b>
47 48 49 50 51		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
52 53 54 55 56 57 58 59 60		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 <b>N/A</b>
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 <b>Page 7-12 and Table 2</b>
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 <b>Page 6</b>
Methods: Assign	ment o	f 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 <b>Page 12</b>
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 <b>Page 12-13</b>

2 3 4 5 6	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how <b>Page 13</b>
7 8 9 10 11		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial <b>N/A</b>
13	Methods: Data co	llectio	n, management, and analysis
15 16 17 18 19 20 21 22 23	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 <b>Page 7-12</b>
24 25 26 27 28 29		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
30 31 32 33 34 35 36	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 <b>Page 16</b>
37 38 39 40 41	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 <b>Page 14-15</b>
42 43 44 45 46		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) N/A
47 48 49 50 51 52		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
53 54 55 56 57	Methods: Monitor	ing	

1 2 3 4 5 6 7 8	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 <b>Page 16</b>
9 10 11 12 13 14		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 <b>N/A</b>
15 16 17 18 19 20	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
21 22 23 24 25 26	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor <b>N/A</b>
20 27 28	Ethics and disser	ninatio	on C
29 30 31 32	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval <b>Page 16</b>
33 34 35 36 37 38 39	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
40 41 42 43 44	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) <b>Page 6</b>
45 46 47 48		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable <b>N/A</b>
49 50 51 52 53 54	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 <b>Page 16</b>
55 56 57 58 59 60	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site <b>Page 18</b>

2 3 4 5 6	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		
7 8 9 10 11	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 <b>N/A</b>		
12 13 14 15 16 17	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		
19 20 21 22		31b	Authorship eligibility guidelines and any intended use of professional writers <b>Page 16.</b>		
23 24 25 26 27		31c	Plans, if any, for granting public access to the full protocol, participant- level dataset, and statistical code Page 16		
28 29	Appendices				
30 31 32 33	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates <b>Page 6 and Supplementary material</b>		
34 35 36 37 38 39	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		
40 41					
42 43 44 45 46 47 48 49	*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 " <u>Attribution-NonCommercial-NoDerivs 3.0 Unported</u> " license.				