**Original Article**
CLINICAL TRIALS AND INVESTIGATIONS

Obesity

# Time-Restricted Feeding Improves Glucose Tolerance in Men at Risk for Type 2 Diabetes: A Randomized Crossover Trial

*Amy T. Hutchison [1,2,3], Prashant Regmi[1,2,3], Emily N.C. Manoogian[4], Jason G. Fleischer[5], Gary A. Wittert[1,2,3], Satchidananda Panda[4], and Leonie K. Heilbronn[1,2,3]*

**Objective:** This study aimed to assess the effects of 9-hour time-restricted feeding (TRF), early (TRFe) or delayed (TRFd), on glucose tolerance in men at risk for type 2 diabetes.
**Methods:** Fifteen men (age 55 ± 3 years, BMI 33.9 ± 0.8 kg/m$^2$) wore a continuous glucose monitor for 7 days of baseline assessment and during two 7-day TRF conditions. Participants were randomized to TRFe (8 AM to 5 PM) or TRFd (12 PM to 9 PM), separated by a 2-week washout phase. Glucose, insulin, triglycerides, nonesterified fatty acids, and gastrointestinal hormone incremental areas under the curve were calculated following a standard meal on days 0 and 7 at 8 AM (TRFe) or 12 PM (TRFd).
**Results:** TRF improved glucose tolerance as assessed by a reduction in glucose incremental area under the curve ($P$ = 0.001) and fasting triglycerides ($P$ = 0.003) on day 7 versus day 0. However, there were no mealtime by TRF interactions in any of the variables examined. There was also no effect of TRF on fasting and postprandial insulin, nonesterified fatty acids, or gastrointestinal hormones. Mean fasting glucose by continuous glucose monitor was lower in TRFe ($P$ = 0.02) but not TRFd ($P$ = 0.17) versus baseline, but there was no difference between TRF conditions.
**Conclusions:** While only TRFe lowered mean fasting glucose, TRF improved glycemic responses to a test meal in men at risk for type 2 diabetes regardless of the clock time that TRF was initiated.

*Obesity* (2019) **27**, 724-732. doi:10.1002/oby.22449

## Introduction

Time-restricted feeding (TRF) is a novel dietary tool that time-limits energy intake for up to 12 h/d without necessarily altering diet quality or quantity. TRF reduces body weight, improves glucose and insulin profiles, and reduces insulin resistance in mouse models (1-3), even in the presence of a high-fat diet (3-5).

Few studies have explored the metabolic effects of TRF in humans. TRF reduced body weight by ~3% in individuals with obesity over 12 to 16 weeks (6,7). However, these studies were uncontrolled. Lean males also lost 0.4 kg when they followed a 13-hour TRF protocol (6 AM to 7 PM) for 2 weeks versus a control condition in which they gained +0.6 kg (8). When healthy men and women restricted eating to one meal per day during a 4-hour window (5 PM to 9 PM), modest improvements in body weight and fat mass were noted compared with the same diet that was given as three meals per day (9). In lean young men, TRF for 8 h/d (between 1 PM and 9 PM) combined with 8 weeks of resistance training reduced fat mass, fasting plasma glucose, and insulin versus a 13-h/d protocol (8 AM to 9 PM) (10). In contrast, self-selection of a 4-hour TRF window (between 4 PM and midnight) on 4 d/wk did not alter body composition in lean young men performing resistance training despite an overall reduction in energy intake (11).

Recently, a controlled feeding trial compared a 6-h/d eating period (dinner before 3 PM) with a 12-h/d eating period in men with prediabetes (12). This study carefully matched and supervised all energy intakes in both conditions. There was no change in body weight in either condition, but TRF improved postprandial insulin, insulin sensitivity, and β-cell function after 5 weeks (12). This study is the first to show that limiting

[1] Adelaide Medical School, University of Adelaide, Adelaide, South Australia, Australia. Correspondence: Leonie K. Heilbronn (leonie.heilbronn@adelaide.edu.au) [2] NHMRC Centre of Research Excellence in Translating Nutritional Science to Good Health, University of Adelaide, Adelaide, South Australia, Australia [3] Nutrition and Metabolism Theme, South Australian Health and Medical Research Institute, Adelaide, South Australia, Australia [4] Regulatory Biology Laboratory, Salk Institute for Biological Studies, La Jolla, California, USA [5] Integrative Biology Laboratory, Salk Institute for Biological Studies, La Jolla, California, USA.

**Funding agencies:** The research was funded by a Royal Adelaide Hospital Research grant (561110093). LKH was supported by an Australian Research Council Future Fellowship FT120100027. PR was supported by an Adelaide Scholarships International (ASI) Scholarship.

**Disclosure:** SP received author fees from Rodale Books for the upcoming book *The Circadian Code*. The other authors declared no conflict of interest.

**Author contributions:** ATH and LKH designed and conducted the study. ATH, PR, ENCM, and JGF analyzed data. All authors contributed to data interpretation and preparation of the manuscript. LKH had full access to the data and had primary responsibility for the final publication.

**Clinical trial registration:** ClinicalTrials.gov identifier NCT02633722.

Amy T. Hutchison and Prashant Regmi contributed equally to this work.

Additional Supporting Information may be found in the online version of this article.

**Received:** 9 July 2018; **Accepted:** 19 January 2019; **Published online 19 April 2019. doi:10.1002/oby.22449**

food intake prior to 3 PM is beneficial for glycemic control in humans, independently of weight change. However, the feasibility of implementing a strict TRF approach daily in the general population is unclear.

There is also a known circadian impact of meal timing, with poorer glucose tolerance at night despite identical meals and equidistant fasting lengths (13,14). Whether changes in gastric emptying, which modulates glycemia, underlie this observation is unclear. The aim of this study was to examine the effects of 9-hour TRF commenced at 8 AM or 12 PM (i.e., with a phase delay) on glucose response to a mixed-nutrient meal in men at risk for type 2 diabetes. Secondary outcomes were gastric emptying, insulin and gastrointestinal hormone release, markers of appetite, weight, and glucose profiles by continuous glucose monitoring (CGM). We hypothesized that TRF would improve glycemic control and that TRF-early (TRFe) would produce greater improvements than TRF-delayed (TRFd).

# Methods

## Participants

Fifteen men were recruited into this randomized crossover trial (Figure 1). One participant withdrew from the study after completing the TRFe condition only because of scheduling conflicts. All participants habitually ate breakfast and did not perform shift work. Inclusion criteria were age 30 to 70 years, waist circumference ≥102 cm, weight stability (within 5% of screening weight) for >6 months prior to study entry, nonsmoker, being sedentary or lightly active (i.e., took part in <2 moderate- to high-intensity exercise sessions per week), consuming <140 g/wk of alcohol, no prior diagnosis of type 2 diabetes, not taking antihyperglycemic medication, and no personal history of cardiovascular disease, eating disorders, or major psychiatric disorders. All participants were assessed for their risk of developing diabetes by the Australian Type 2 Diabetes Risk Assessment Tool (AUSDRISK) score (15). A score of 9 to 11 indicates intermediate risk (1 in 30 will develop diabetes within 5 years, $n = 1$), 12 to 15 indicates high risk (1 in 14, $n = 2$), 16 to 19 indicates very high risk (1 in 7, $n = 6$), and ≥ 20 indicates 1 in 3 will develop diabetes within 5 years ($n = 6$). The Royal Adelaide Hospital Research Ethics Committee approved the study protocol, and all participants provided written informed consent prior to their inclusion. This study was registered as a clinical trial.

## Study design

After an initial screening visit, participants underwent a baseline assessment for 1 week (Figure 1). Whole body composition was measured by dual-energy x-ray absorptiometry (Lunar Prodigy; GE Healthcare,

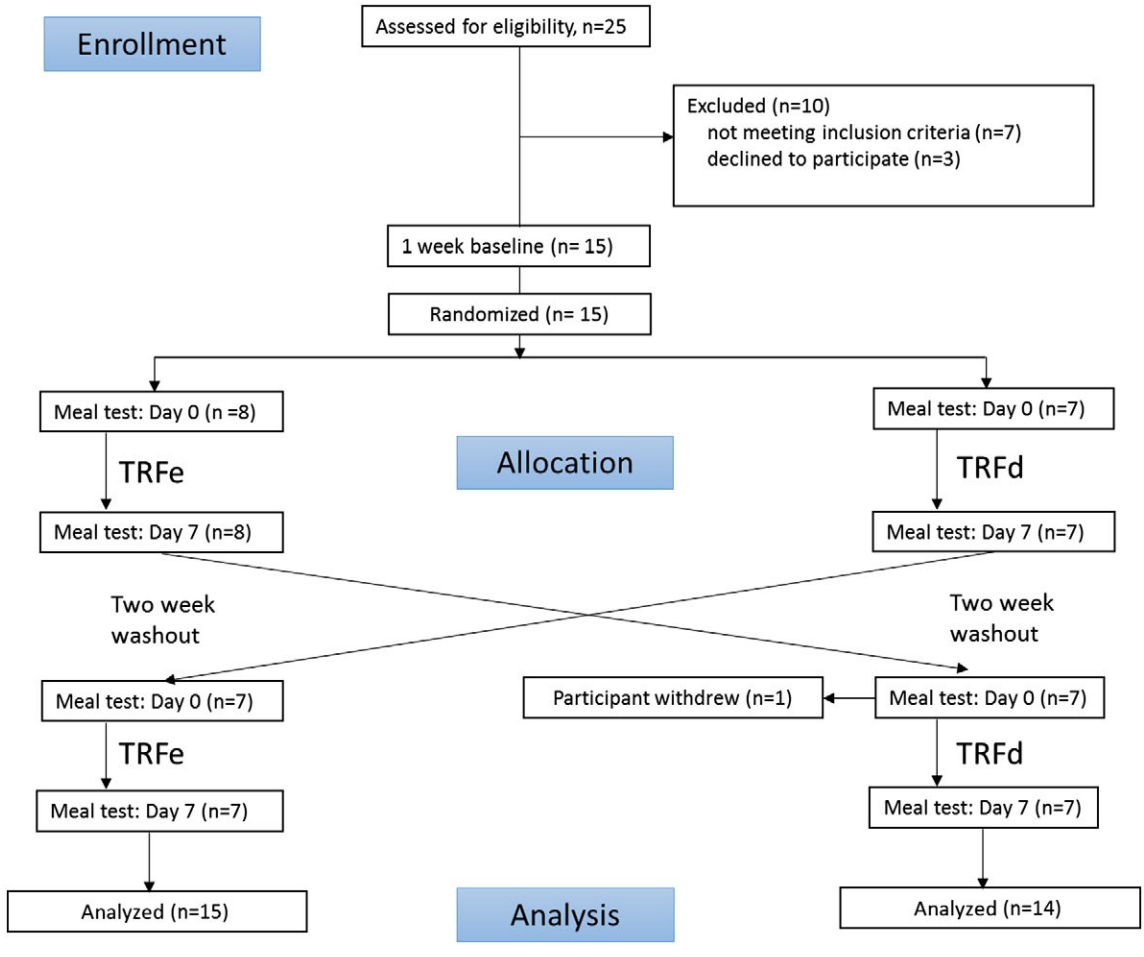


**Figure 1** Participant recruitment and study flow diagram. TRFe: time-restricted feeding between 8 AM and 5 PM; TRFd: time-restricted feeding between 12 PM and 9 PM. [Colour figure can be viewed at wileyonlinelibrary.com]

Madison, Wisconsin). Participants were fitted with a CGM device to measure blood glucose (Dexcom G4 Platinum, San Diego, California) and an accelerometer (MF-SW/DD100; BodyMedia SenseWear, Sydney, New South Wales, Australia) to measure activity and sleep patterns for the 7 days. Participants also kept a diary briefly describing the meal, snack, or beverage that was eaten and the time that this food was consumed.

Following the baseline monitoring period, participants were randomized to one of two TRF protocols for 7 days in a crossover design, separated by a 2-week washout period (Figure 1). These were TRFe (eating window between 8 AM and 5 PM) and TRFd (eating window between 12 PM and 9 PM). Participants were asked to maintain their usual lifestyles, including sleep patterns, to consume their habitual diet within the specified TRF times in each condition, and to record the times they started and finished eating each day. Outside the eating window, participants were allowed to consume water and limited amounts (one to two servings) of very low-calorie drinks and foods (e.g., herbal tea, diet drinks, mints, gum containing <4 kcal/serving) as a tool that may increase compliance.

## Testing days

Metabolic testing was conducted prior to (day 0) and on day 7 of each intervention. Participants arrived at our research facility at either 7:30 AM (TRFe) or 11:30 AM (TRFd) after fasting from 5 PM or 9 PM, respectively, to standardize the length of the fast. Body weight and waist and hip circumference were measured in a gown after voiding, and blood pressure was measured after a 10-minute seated rest. A 20-gauge cannula was inserted into an antecubital vein of the nondominant arm, and a fasting blood sample (20 mL) was collected, followed by a baseline breath sample. Participants were given a mixed-nutrient liquid test meal (474 mL, 700 kcal; 57% carbohydrate, 28% fat, 15% protein) (Ensure Plus; Abbott Laboratories, Abbott Park, Illinois) labeled with 100 mg of $^{13}C$ sodium acetate (Cambridge Isotopes Inc., Tewksbury, Massachusetts) and asked to consume the entire drink within 2 minutes. Test drinks were given at 8 AM (TRFe) or at 12 PM (TRFd), according to study protocol. Breath samples were collected every 5 minutes for the first 30 minutes of the meal test (0, 5, 10, 15, 20, 25, and 30 minutes) and at 45, 60, 75, 90, 120, 150, and 180 minutes. Venous blood was sampled immediately prior to the test meal (0 minutes) and at 15, 30, 60, 90, 120, and 180 minutes. Blood was collected in ice-chilled EDTA vacuum tubes, and 40 µL of 200mM 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride and 20 µL of dipeptidyl peptidase-IV inhibitor were added to 2 mL of whole blood for the measurement of gastrointestinal hormones (ghrelin, peptide YY [PYY], glucagon-like peptide-1 [GLP-1], glucose-dependent insulinotropic peptide [GIP], and amylin). Plasma was separated immediately by centrifugation at 3,000 rpm for 15 minutes at 4°C and immediately snap frozen to −80°C for later analysis.

## Gastric emptying and appetite responses

$^{13}CO_2$ concentrations in exhaled air from end-expiratory breath samples were analyzed with the use of infrared spectroscopy (FANci2 breath test analyzer; Fischer Analysen Instrumente GmbH, Leipzig, Germany). The half-emptying time was calculated after curve fitting of the $^{13}C$ exhalation to a modified power exponential function (16) in GraphPad Prism 7.02 (GraphPad Software, La Jolla, California). Measures of appetite (hunger, fullness, and desire to eat during the meal test) were quantified using visual analog scales administered at 0, 30, 60, 120, and 180 minutes.

## Continuous glucose monitoring

All participants were fitted with a CGM during baseline and both intervention periods. A glucose-oxidase-based electrochemical sensor was inserted under the skin on the abdomen and remained *in situ* for each 7-day period. The electrode transmitted interstitial glucose measurements every 5 minutes wirelessly to a receiver where they were stored until the participant returned to the department. This produced 12 readings per hour. Participants calibrated their CGM twice daily by finger prick (OneTouch Verio IQ; Lifescan, Inc., Milpitas, California).

## Activity

Energy expenditure, number of steps, and time spent sleeping were measured by the SenseWear armband, a multisensor monitor worn on the back of the right upper arm (over the triceps muscle) per manufacturer instructions. This device continuously records physiological data during daily activities, and the information is integrated and processed by software using algorithms that utilize an individual's physical characteristics (sex, age, height, and weight). Energy expenditure estimated by the armband correlated strongly with estimates from doubly labeled water (17).

## Biochemical measures

Blood glucose was measured using a commercially available enzymatic kit on an AU480 clinical analyzer (Beckman Coulter, Inc., Brea, California). Nonesterified fatty acid (NEFA) concentrations were measured using an enzymatic spectrophotometric assay (NEFA-HR; Wako Diagnostics, Mountain View, California). Insulin, ghrelin, GLP-1, GIP, amylin, and PYY were measured from the same sample in duplicate using a MILLIPLEX magnetic bead-based quantitative immunoassay (MilliporeSigma, Burlington, Massachusetts), with MAGPIX instrumentation. Samples from each participant were analyzed within the same run to minimize interassay variation.

## Statistical analysis

For all biochemical outcomes, incremental area under the curve (iAUC) was calculated using the trapezoidal rule. Statistical analysis was performed using linear mixed modeling, with effects of mealtime (meal commenced at 8 AM or 12 PM), TRF (day 0 vs. day 7), and sequence (TRFe followed by TRFd, or vice versa) as fixed variables. A differential effect of mealtime in each condition was tested via the interaction between mealtime and TRF. Student *t* tests for carryover effects were conducted on the day 0 to day 7 differences, and no significant effects were found for any of the outcomes. For CGM data, accelerometry, and weight, a separate model was constructed with measures from the 7-day baseline monitoring period included and treatment (baseline, TRFe, or TRFd) as a fixed factor.

For the CGM analysis, the fed and fasting windows were determined from meal log data. The "fasting" window lasted from 4 hours after the latest occasion a participant ate in a given condition until the earliest occasion a participant ate in that condition. Mean fasting glucose for a participant in a given condition (baseline, TRFe, and TRFd) was calculated over this time window and across all days the participant spent in that condition. The "fed" window was calculated from the earliest eating occasion until the latest meal occasion, plus 4 hours to allow for postprandial fluctuations in blood glucose. We also calculated 3-hour fasting glucose concentrations as the mean of glucose measurements obtained from CGM for the 3 hours preceding the first meal of each day in all three conditions. Mean amplitude of glycemic excursions (MAGE) (18), mean of daily differences (MODD), and continuous

overall net glycemic action (CONGA) (19) were calculated from CGM data as described by others. Because of technical issues, CGM data were lost from one participant during baseline ($n = 14$) and from one during TRFd ($n = 13$). Body weight change during baseline was calculated as weight on day 0 (phase 1) minus weight at start of baseline.

Sample size requirements were calculated on statistical power functions using within-subject contrasts for the primary end point Δglucose iAUC. The study was powered to detect a 1.4-mmol/L/min difference in glucose iAUC between groups, assuming an SD of 1.25 mmol/L/min (unpublished data), with $\alpha < 0.05$ and statistical power of 80%. Statistical analysis was performed in SPSS Statistics (version 24; IBM Corp., Armonk, New York). All data are presented as mean ± SEM, with $P < 0.05$ considered significant.

**TABLE 1** Baseline characteristics of participants

| | |
|---|---|
| Age (y) | 55 ± 3 |
| Body weight (kg) | 105.7 ± 2.6 |
| BMI (kg/m$^2$) | 33.9 ± 0.8 |
| Waist circumference (cm) | 115 ± 2 |
| Waist-hip ratio | 0.99 ± 0.01 |
| SBP (mmHg) | 141 ± 3 |
| DBP (mmHg) | 87 ± 2 |
| Fasting glucose (mmol/L) | 5.8 ± 0.1 |
| Total fat mass (kg) | 32.5 ± 1.7 |
| Total lean mass (kg) | 62.5 ± 2.3 |
| Fat mass (%) | 35.1 ± 1.2 |
| Diabetes risk score (AUSDRISK) | 19.8 ± 0.8 |

All data are mean ± SEM; $n = 15$.
AUSDRISK, Australian Type 2 Diabetes Risk Assessment Tool; DBP, diastolic blood pressure; SBP, systolic blood pressure.

## Results

Fifteen men (mean age 55 [SEM 3] years, BMI 33.9 [SEM 0.8] kg/m$^2$) were recruited into the study. Baseline characteristics are presented in Table 1. Body weight was lower on day 7 compared with day 0 ($P < 0.001$) for each treatment, but there were no significant differences between treatments (all $P > 0.66$; baseline: −0.8 ± 0.3 kg; TRFe: −1.3 ± 0.2 kg; TRFd: −0.8 ± 0.2 kg). Participants' mean self-reported eating windows are shown in Supporting Information Figure S1. The mean first eating occasion (SEM) occurred at 7:45 AM (16 minutes) during baseline, 8:22 AM (10 minutes) in TRFe, and at 12:15 PM (14 minutes) in TRFd. The mean last eating occasions were 7:18 PM (37 minutes), 4:37 PM (21 minutes), and 7:56 PM (16 minutes) in baseline, TRFe, and TRFd, respectively.

All effect sizes and $P$ values for each variable are given in Supporting Information Table S1. There were no significant mealtime by TRF interactions for any of the variables reported.

### Insulin and glucose responses to meal test

There was a significant effect of TRF ($P = 0.001$) and mealtime ($P = 0.002$) on glucose iAUC. TRF reduced glucose iAUC by ~36% (−1.6 ± 0.4 mmol/L/h), whereas consuming a meal at 12 PM resulted in a ~21% (0.87 ± 0.5 mmol/L/h) higher glucose iAUC (Figure 2A). There was no effect of mealtime or TRF on fasting glucose or fasting insulin, although a trend toward a reduction in insulin iAUC was observed as a result of TRF ($P = 0.09$; Figure 2B).

### Gastrointestinal hormone, triglyceride, and NEFA responses to meal test

There was an effect of mealtime, but not TRF, on fasting ghrelin and ghrelin iAUC. Fasting ghrelin was lower at 8 AM versus 12 PM ($P = 0.01$), while ghrelin iAUC was lower at 12 PM versus 8 AM ($P = 0.038$; Figure 3A). TRF, but not mealtime, reduced fasting GLP-1 ($P = 0.002$; Figure 3B), while there was no effect of TRF or mealtime on postprandial GLP-1. There was no effect of mealtime or TRF on fasting or postprandial GIP (Figure 3C), amylin (Figure 3D), or PYY (Figure 3E).

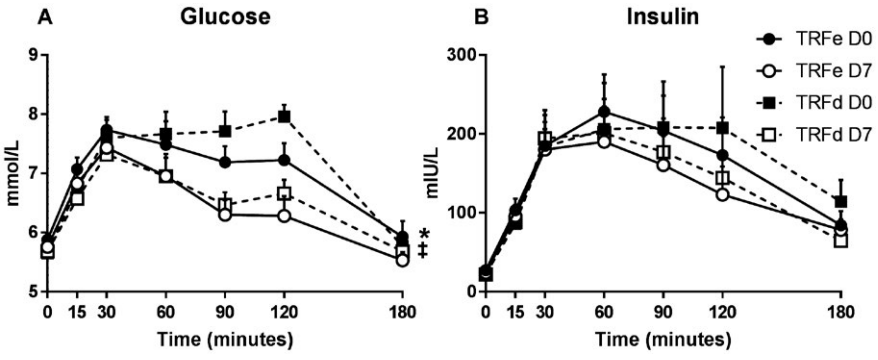


**Figure 2** (**A**) Glucose and (**B**) insulin iAUC responses to 3-hour, standardized, mixed-nutrient meal test at baseline (D0) and after 7 days (D7) of time-restricted feeding commenced early (TRFe, 8 AM-5 PM) or with a phase delay (TRFd, 12 PM-9 PM). Data are presented as mean ± SEM. Statistical analysis was performed using linear mixed modeling, with effects of meal time (meal commenced at 8 AM or 12 PM), TRF (day 0 vs. day 7), and sequence as fixed variables. *Effect of TRF (day 0 vs. day 7; $P < 0.05$) on iAUC. ‡Effect of time of day (8 AM vs. 12 PM; $P < 0.05$) on iAUC. iAUC, incremental area under curve.

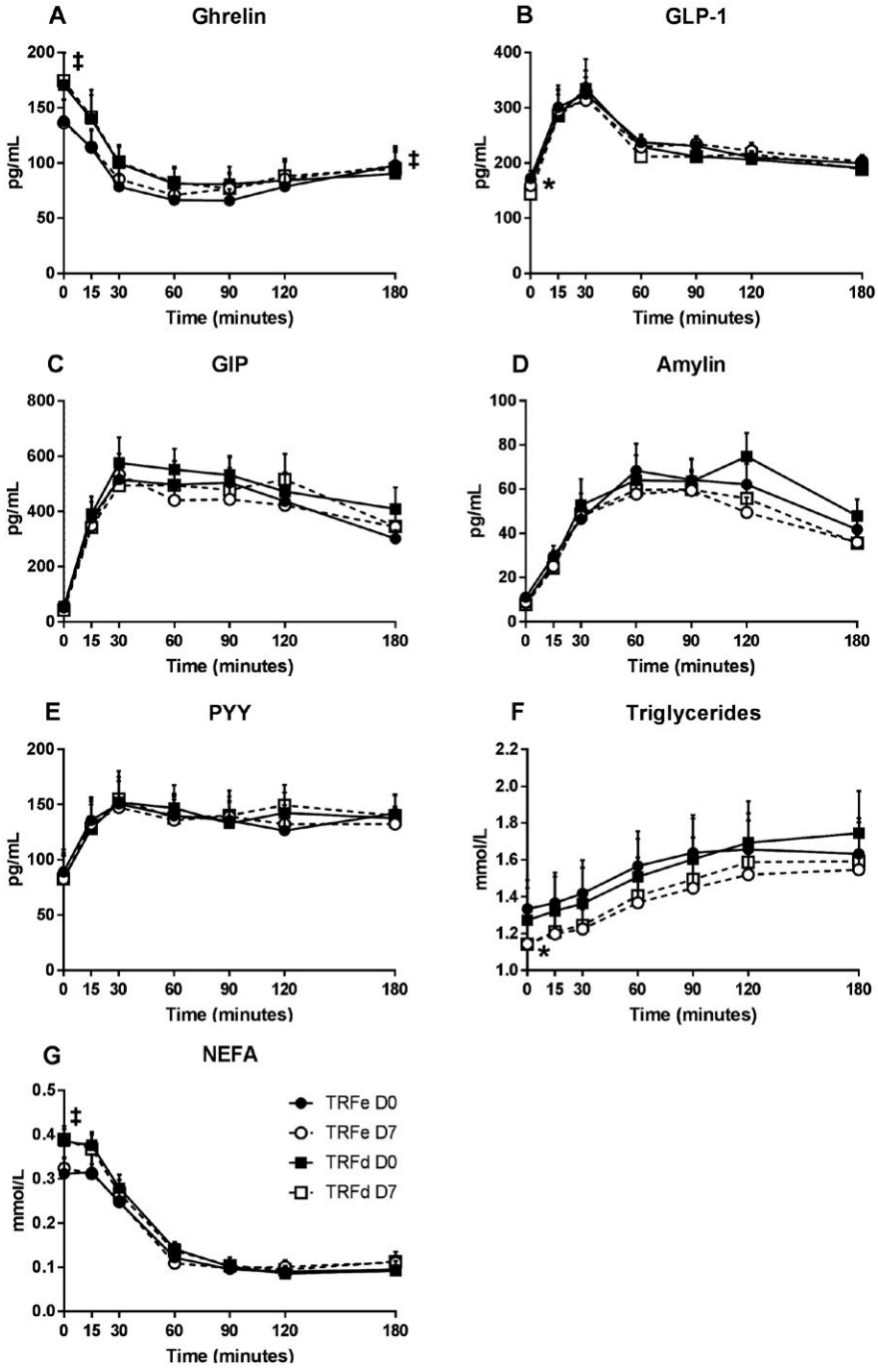


**Figure 3** (**A**) Ghrelin, (**B**) GLP-1, (**C**) GIP, (**D**) amylin, (**E**) PYY, (**F**) triglyceride, and (**G**) NEFA responses to a 3-hour, standardized, mixed-nutrient meal test at baseline (D0) and after 7 days (D7) of time-restricted feeding commenced early (TRFe, 8 AM-5 PM) or with a phase delay (TRFd, 12 PM-9 PM). Data are presented as mean ± SEM. Statistical analysis was performed using linear mixed modeling, with effects of mealtime (meal commenced at 8 AM or 12 PM) TRF (day 0 vs. day 7) and sequence as fixed variables. *Effect of TRF (day 0 vs. day 7; $P < 0.05$). ‡Effect of mealtime (8 AM vs. 12 PM; $P < 0.05$). GIP, gastric inhibitory peptide; GLP-1, glucagon like peptide 1; NEFA, nonesterified fatty acids; PYY, peptide YY.

TRF reduced fasting triglycerides ($P = 0.003$) but did not alter triglyceride iAUC ($P = 0.57$; Figure 3F). There was no effect of mealtime on fasting or postprandial triglycerides. There was an effect of mealtime, but not TRF, on fasting and NEFA iAUC (Figure 3G). Fasting NEFA was higher at 12 PM versus 8 AM ($P < 0.001$), while NEFA iAUC was lower (i.e., meal-induced suppression was greater) at 12 PM ($P = 0.001$).

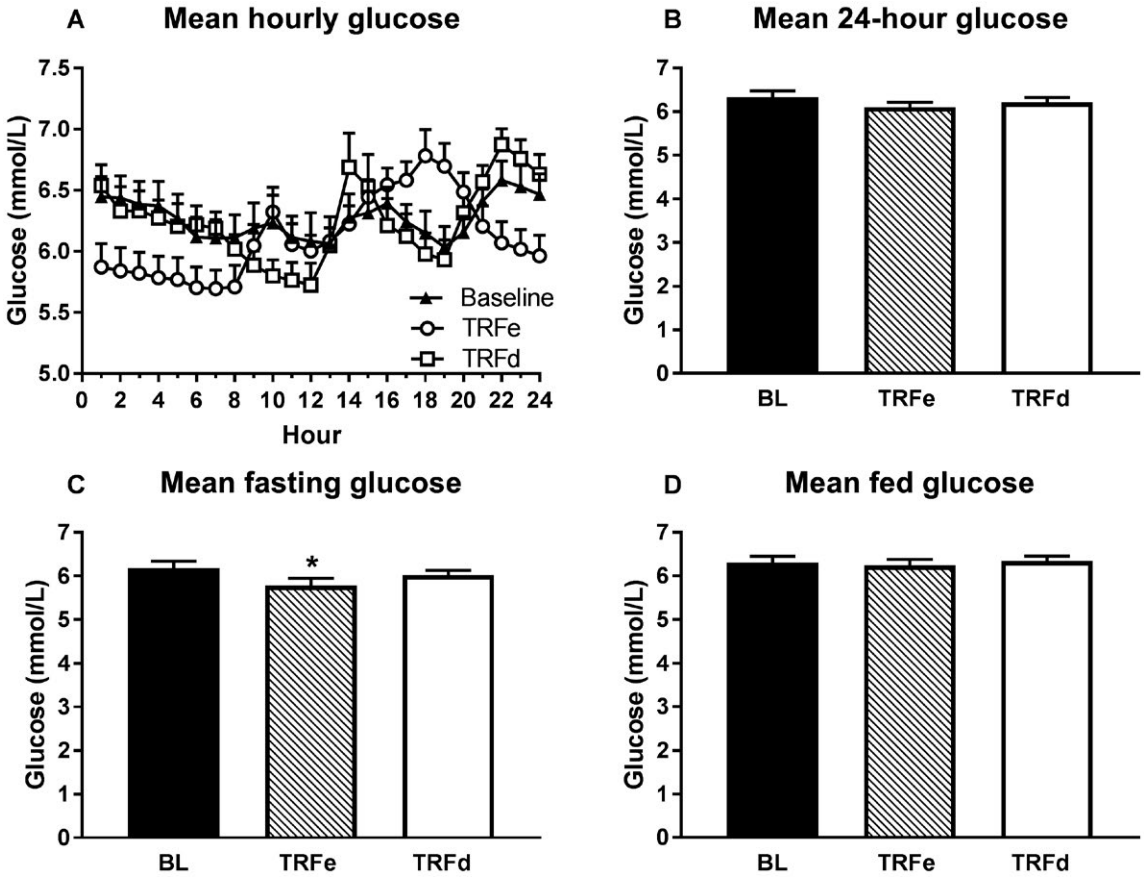


**Figure 4** (**A**) Mean hourly glucose from continuous glucose monitoring (CGM) over 7 days during baseline monitoring period (closed triangles), time-restricted feeding commenced early (TRFe, 8 AM-5 PM; open circles), or time-restricted feeding with a phase delay (TRFd, 12 PM-9 PM; open squares). Glucose readings were averaged every hour and each time period over 7 days, plotted as mean ± SEM. (**B**) Mean 24-hour blood glucose concentrations from CGM over 7 days during baseline monitoring period (black bar), TRFe (8 AM-5 PM; hatched bar), or TRFd (12 PM-9 PM; open bar). (**C**) Mean fasting blood glucose concentrations from CGM over 7 days during baseline monitoring period (black bar), TRFe (8 AM-5 PM; hatched bar), or TRFd,(12 PM-9 PM; open bar). Mean fasting glucose for a participant in a given condition (baseline, TRFe, and TRFd) was calculated between 4 hours after a participant consumed first meal until the time participant consumed last meal in that condition. (**D**) Mean fed blood glucose concentrations from CGM over 7 days during baseline monitoring period (black bar), TRFe (8 AM-5 PM; hatched bar), or TRFd (12 PM-9 PM; open bar). Mean fed glucose for a participant in a given condition (baseline, TRFe, and TRFd) was calculated from the time a participant consumed first meal until the time participant consumed last meal, plus 4 hours in a given condition. Data are mean ± SEM ($n$ = 14). Statistical analysis was performed using linear mixed modeling with effects of treatment (baseline, TRFe, and TRFd) as a fixed variable. *$P$ < 0.05 vs. baseline.

## Gastric emptying and perceived appetite responses to meal test

There was no effect of mealtime or TRF on the rate of gastric emptying. There was a significant effect of mealtime only on perceived fullness iAUC, which was increased when the meal was initiated at 8 AM versus 12 PM ($P$ = 0.038). There was no effect of mealtime or TRF on perceived hunger, fullness, or desire to eat.

## Mean glucose responses from CGM

Mean hourly glucose concentrations are shown in Figure 4A. There was no statistical difference in the mean 24-hour blood glucose concentration ($P$ = 0.37; Figure 4B), CONGA ($P$ = 0.37), MAGE ($P$ = 0.27), or MODD ($P$ = 0.22) between treatments (data not shown). There was an effect of treatment on mean fasting glucose by CGM ($P$ = 0.023; Figure 4C, Figure 5). This was significantly lower during TRFe compared with baseline ($P$ = 0.02) but not in TRFd compared with baseline ($P$ = 0.17), and there were no differences between TRF treatments ($P$ = 0.47). The mean blood glucose concentration for the 3 hours preceding the first meal of the day was reduced in TRFe versus baseline ($P$ = 0.03), with a tendency to be reduced in TRFd versus baseline ($P$ = 0.07). There was no difference between treatments ($P$ = 0.92), and there was no effect of treatment on mean fed blood glucose assessed by CGM ($P$ = 0.77; Figure 4D).

## Activity

There was no effect of treatment on total energy expenditure, number of steps, or duration of sleep assessed by accelerometry (Supporting Information Table S2).

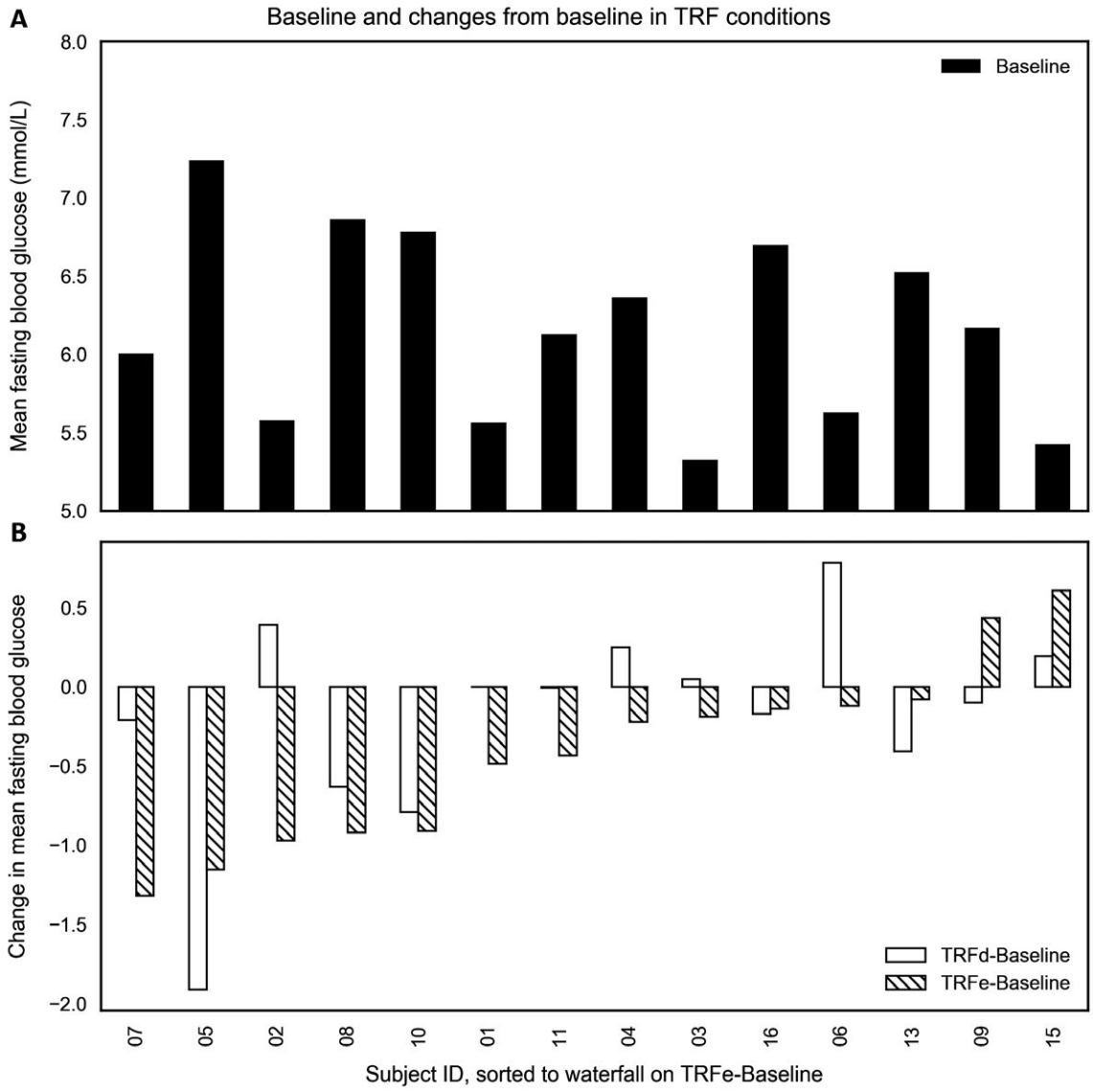


**Figure 5** Waterfall graph of mean fasting glucose measured by continuous glucose monitoring (CGM) during baseline and 7 days of time-restricted feeding. (**A**) Baseline mean fasting glucose and (**B**) change in mean fasting glucose during time-restricted feeding commenced early (TRFe, 8 AM-5 PM) or with a phase delay (TRFd, 12 PM-9 PM) from baseline (mmol/L). Mean fasting glucose was determined by CGM data between 4 hours after a participant consumed first meal until the time participant consumed last meal based on food logs. Data sorted from the largest decrease in mean glucose to increased glucose based on TRFe condition-baseline. Data from both conditions shown for each participant, stacked below participant's own baseline fasting glucose. Baseline CGM data was collected from 14 participants.

## Discussion

This study examined the effects of 1 week of TRF, with and without a short phase delay, on glucoregulatory responses to a standard test meal and 24-hour glucose profiles in men at risk for type 2 diabetes. We observed that 9-hour TRF produced a ~36% reduction in the glycemic responses to a test meal, with no differences between TRF treatments. The improvement in glycemic control was not explained by changes in gastric emptying or gastrointestinal hormone release, but there was a tendency to reduce postprandial insulin. Together, these data show that TRF improves glycemic responses to a test meal in men who are at risk for type 2 diabetes, regardless of the clock time TRF was initiated.

TRF improves glucose tolerance and insulin sensitivity in mice (3,20). In humans, markers of β-cell function and insulin sensitivity were also improved by TRF (6-hour feeding period, with dinner before 3 PM) (12). In that study, the increased fasting length (18 hours versus 12 hours in the control condition) may have contributed to the result (12). To achieve equal fasting lengths in this study, meal tests were conducted at 8 AM (TRFe) or 12 PM (TRFd). As expected, we observed a reduced glucose iAUC response to the test meal given at 8 AM versus 12 PM, which reflects known variations in circadian rhythm (21,22). However, TRF improved the glucose response to the standardized test meal, regardless of whether it was initiated at breakfast or lunch. This study suggests that there may be some flexibility in the clock time that

TRF is initiated. We highlight that modest weight loss occurred during both TRF conditions, which may have contributed to the observed improvement in glycemia, rather than TRF per se. However, Sutton et al. (12) recently established that the insulin-sensitizing effects of TRF occurred independently of weight loss.

Several uncontrolled studies in humans have shown that TRF reduces body weight by 1% to 3% over a period of 2 to 16 weeks (6,8,23,24). In our study, participants lost a modest amount of weight (~1%) during TRF. However, an equivalent weight loss occurred during the baseline monitoring period, despite participants being instructed to consume their habitual diets, and was not different between conditions. This finding suggests that diet monitoring by the investigators, rather than TRF, may underpin the modest weight loss reported in past uncontrolled trials of TRF. Clearly, long-term randomized controlled trials are required to test the efficacy of TRF to reduce body weight.

Despite similar weight loss across the three treatment periods, mean fasting glucose by CGM was lower only during the TRFe treatment versus baseline. Firstly, this finding supports past evidence that TRF improves glycemic control independently of weight loss (9). Second, there was no statistical difference between TRFd and baseline. Thus, we speculate that restricting calories to an earlier time frame may be optimal for 24-hour glucose management, although there was not a statistical difference in this response between TRF conditions. However, it is well described that glucose tolerance diminishes over the course of the day under natural circadian variation (21,22,25).

While gastric emptying rate is a known modulator of the glycemic response to a meal, there was no change in gastric emptying in response to TRF. There was also no effect of TRF on gastrointestinal hormone release. However, fasting ghrelin and the postprandial suppression of ghrelin were higher at 12 PM versus 8 AM. The natural ghrelin nadir occurs in the biological morning (~8 AM) in humans, irrespective of the sleep-wake cycle (26), and correlates with circadian variations in hunger, which peak later in the day (27). This finding suggests that prescription of TRF later in the day may have greater effects on reducing appetite and may aid compliance. Studies of extended morning fasting have shown greater suppression of ghrelin when a meal is consumed around midday (i.e., breakfast skipping) compared with consuming the same meal at breakfast and lunch (28,29). Some authors have linked this to reduced insulin responses, occurring as a result of the second-meal effect, because insulin was proposed to play in role in ghrelin suppression (30). In a study by Carlson et al., consuming all calories within a single 4-hour window (4 PM to 8 PM) had no effect on morning fasting and postprandial ghrelin when compared with three meals per day after 8 weeks (31). Further exploration of how meal timing and TRF may manipulate appetite signaling is warranted.

We observed a reduction in triglycerides in response to TRF, with no difference between TRFe and TRFd. In contrast, previous observations of 8 weeks of TRF in lean males reported no change in fasting triglycerides (10) and whilst others reported increased total cholesterol and LDL and HDL cholesterol after consuming all calories as one meal per day for 6 weeks between 5 PM and 9 PM (9). It is unclear whether consumption of the meal during the morning may have altered this outcome. We also observed mealtime-related changes in fasting and postprandial NEFA, which are congruent with previous reports of diurnal rhythms of circulating NEFA and lipid metabolism (32).

This was a 1-week controlled crossover intervention that was conducted solely in overweight men. As such, our findings cannot be directly extended to women, individuals with normal weight, or those with established metabolic disturbances, such as type 2 diabetes mellitus. The study was conducted in a free-living population, and we made no attempt to standardize food intake. During the free-living periods (1-week baseline assessment prior to randomization to TRFd or TRFe and during the washout before crossing over to the alternate TRF condition), participants were instructed to continue to follow their regular dietary habits. This lack of standardization could have influenced the baseline result and may have lessened our ability to detect differences between TRFe and TRFd. The long-term feasibility of this eating pattern compared with ad libitum intake and its effectiveness in improving metabolic outcomes over longer periods require further investigation in larger populations. Finally, this study may have been underpowered to detect changes in secondary outcomes. Future studies should also consider how an individual's own chronotype may impact the magnitude of responses when TRF is commenced early or late.

In conclusion, this study has demonstrated that 1 week of TRF improves glucose responses to a meal in men at risk for type 2 diabetes, irrespective of when TRF is commenced. This trial should be repeated in larger cohorts with more tightly controlled free-living periods to confirm this result. Overall, the simplicity of TRF and the efficacy in improving glycemic outcomes indicate that large-scale, long-term randomized controlled trials are warranted.O

## Acknowledgments

Individual deidentified participant data will not be available for this clinical trial.



## References

1. Belkacemi L, Selselet-Attou G, Louchami K, Sener A, Malaisse WJ. Intermittent fasting modulation of the diabetic syndrome in sand rats. II. In vivo investigations. *Int J Mol Med* 2010;26:759-765.
2. Tsai JY, Villegas-Montoya C, Boland BB, et al. Influence of dark phase restricted high fat feeding on myocardial adaptation in mice. *J Mol Cell Cardiol* 2013;55:147-155.
3. Hatori M, Vollmers C, Zarrinpar A, et al. Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. *Cell Metab* 2012;15:848-860.
4. Chaix A, Zarrinpar A, Miu P, Panda S. Time-restricted feeding is a preventative and therapeutic intervention against diverse nutritional challenges. *Cell Metab* 2014;20:991-1005.
5. Sundaram S, Yan L. Time-restricted feeding reduces adiposity in mice fed a high-fat diet. *Nutr Res* 2016;36:603-611.
6. Gill S, Panda S. A smartphone app reveals erratic diurnal eating patterns in humans that can be modulated for health benefits. *Cell Metab* 2015;22:789-798.
7. Gabel K, Hoddy KK, Haggerty N, et al. Effects of 8-hour time restricted feeding on body weight and metabolic disease risk factors in obese adults: a pilot study. *Nutr Healthy Aging* 2018;4:345-353.
8. LeCheminant JD, Christenson E, Bailey BW, Tucker LA. Restricting night-time eating reduces daily energy intake in healthy young men: a short-term cross-over study. *Br J Nutr* 2013;110:2108-2113.
9. Stote KS, Baer DJ, Spears K, et al. A controlled trial of reduced meal frequency without caloric restriction in healthy, normal-weight, middle-aged adults. *Am J Clin Nutr* 2007;85:981-988.
10. Moro T, Tinsley G, Bianco A, et al. Effects of eight weeks of time-restricted feeding (16/8) on basal metabolism, maximal strength, body composition, inflammation, and cardiovascular risk factors in resistance-trained males. *J Transl Med* 2016;14:290.
11. Tinsley GM, Forsse JS, Butler NK, et al. Time-restricted feeding in young men performing resistance training: a randomized controlled trial. *Eur J Sport Sci* 2017;17:200-207.
12. Sutton EF, Beyl R, Early KS, Cefalu WT, Ravussin E, Peterson CM. Early time-restricted feeding improves insulin sensitivity, blood pressure, and oxidative stress even without weight loss in men with prediabetes. *Cell Metab* 2018;27:1212-1221.
13. Morgan LM, Aspostolakou F, Wright J, Gama R. Diurnal variations in peripheral insulin resistance and plasma non-esterified fatty acid concentrations: a possible link? *Ann Clin Biochem* 1999;36:447-450.
14. Gupta CC, Dorrian J, Grant CL, et al. It's not just what you eat but when: the impact of eating a meal during simulated shift work on driving performance. *Chronobiol Int* 2017;34:66-77.

15. Chen L, Magliano DJ, Balkau B, et al. AUSDRISK: an Australian Type 2 Diabetes Risk Assessment Tool based on demographic, lifestyle and simple anthropometric measures. *Med J Aust* 2010;192:197-202.
16. Braden B, Adams S, Duan LP, et al. The [13C]acetate breath test accurately reflects gastric emptying of liquids in both liquid and semisolid test meals. *Gastroenterology* 1995;108:1048-1055.
17. Johannsen DL, Calabro MA, Stewart J, Franke W, Rood JC, Welk GJ. Accuracy of armband monitors for measuring daily energy expenditure in healthy adults. *Med Sci Sports Exerc* 2010;42:2134-2140.
18. Service FJ, Molnar GD, Rosevear JW, Ackerman E, Gatewood LC, Taylor WF. Mean amplitude of glycemic excursions, a measure of diabetic instability. *Diabetes* 1970;19:644-655.
19. McDonnell CM, Donath SM, Vidmar SI, Werther GA, Cameron FJ. A novel approach to continuous glucose analysis utilizing glycemic variation. *Diabetes Technol Ther* 2005;7:253-263.
20. Belkacemi L, Selselet-Attou G, Hupkens E, et al. Intermittent fasting modulation of the diabetic syndrome in streptozotocin-injected rats. *Int J Endocrinol* 2012;2012:962012. doi:10.1155/2012/962012
21. Zimmet PZ, Wall JR, Rome R, Stimmler L, Jarrett RJ. Diurnal variation in glucose tolerance: associated changes in plasma insulin, growth hormone, and non-esterified fatty acids. *Br Med J* 1974;1:485-488.
22. Carroll KF, Nestel PJ. Diurnal variation in glucose tolerance and in insulin secretion in man. *Diabetes* 1973;22:333-348.
23. Nematy M, Alinezhad-Namaghi M, Rashed MM, et al. Effects of Ramadan fasting on cardiovascular risk factors: a prospective observational study. *Nutr J* 2012;11:69. doi:10.1186/1475-2891-11-69
24. Fakhrzadeh H, Larijani B, Sanjari M, Baradar-Jalili R, Amini MR. Effect of Ramadan fasting on clinical and biochemical parameters in healthy adults. *Ann Saudi Med* 2003;23:223-226.
25. Lee A, Ader M, Bray GA, Bergman RN. Diurnal variation in glucose tolerance. Cyclic suppression of insulin action and insulin secretion in normal-weight, but not obese, subjects. *Diabetes* 1992;41:750-759.
26. Espelund U, Hansen TK, Hojlund K, et al. Fasting unmasks a strong inverse association between ghrelin and cortisol in serum: studies in obese and normal-weight subjects. *J Clin Endocrinol Metab* 2005;90:741-746.
27. Scheer FA, Morris CJ, Shea SA. The internal circadian clock increases hunger and appetite in the evening independent of food intake and other behaviors. *Obesity (Silver Spring)* 2013;21:421-423.
28. Chowdhury EA, Richardson JD, Tsintzas K, Thompson D, Betts JA. Effect of extended morning fasting upon ad libitum lunch intake and associated metabolic and hormonal responses in obese adults. *Int J Obes (Lond)* 2016;40:305-311.
29. Chowdhury EA, Richardson JD, Tsintzas K, Thompson D, Betts JA. Carbohydrate-rich breakfast attenuates glycaemic, insulinaemic and ghrelin response to ad libitum lunch relative to morning fasting in lean adults. *Br J Nutr* 2015;114:98-107.
30. Flanagan DE, Evans ML, Monsod TP, et al. The influence of insulin on circulating ghrelin. *Am J Physiol Endocrinol Metab* 2003;284:E313-E316.
31. Carlson O, Martin B, Stote KS, et al. Impact of reduced meal frequency without caloric restriction on glucose regulation in healthy, normal-weight middle-aged men and women. *Metabolism* 2007;56:1729-1734.
32. Dallmann R, Viola AU, Tarokh L, Cajochen C, Brown SA. The human circadian metabolome. *Proc Natl Acad Sci U S A* 2012;109:2625-2629.