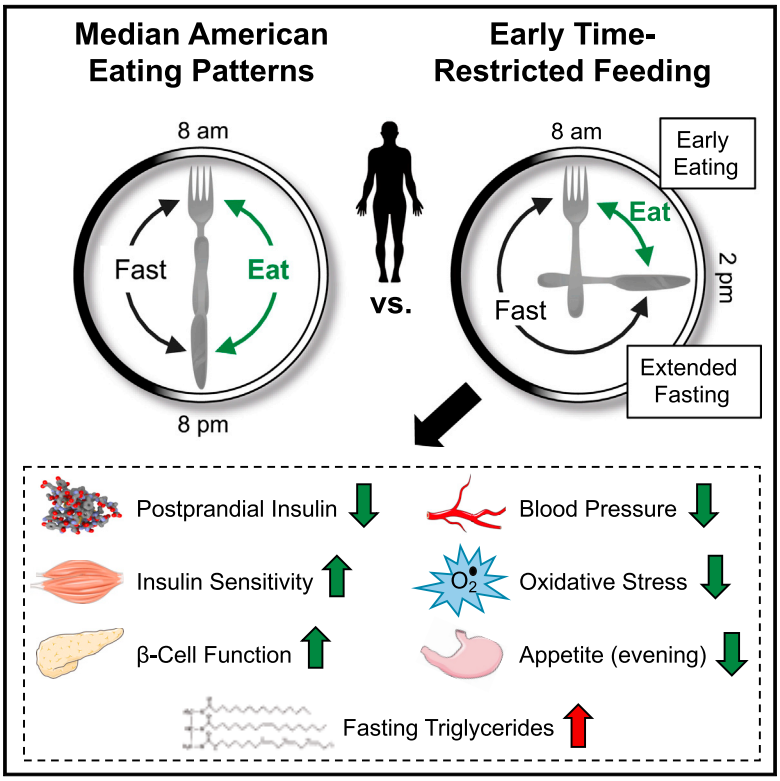
Clinical and Translational Report

Cell Metabolism

Early Time-Restricted Feeding Improves Insulin Sensitivity, Blood Pressure, and Oxidative Stress Even without Weight Loss in Men with Prediabetes

Graphical Abstract



Highlights

- Early time-restricted feeding (eTRF) increases insulin sensitivity
- eTRF also improves β cell function and lowers blood pressure and oxidative stress
- eTRF lowers the desire to eat in the evening, which may facilitate weight loss
- Intermittent fasting can improve health even in the absence of weight loss

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In Brief

Sutton et al. conduct the first supervised controlled feeding trial to test whether intermittent fasting has benefits in humans in the absence of weight loss. Prediabetic men following a form of intermittent fasting called early time-restricted feeding improved their insulin sensitivity, blood pressure, and oxidative stress levels without losing weight.

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Early Time-Restricted Feeding Improves Insulin Sensitivity, Blood Pressure, and Oxidative Stress Even without Weight Loss in Men with Prediabetes

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SUMMARY

Intermittent fasting (IF) improves cardiometabolic health; however, it is unknown whether these effects are due solely to weight loss. We conducted the first supervised controlled feeding trial to test whether IF has benefits independent of weight loss by feeding participants enough food to maintain their weight. Our proof-of-concept study also constitutes the first trial of early time-restricted feeding (eTRF), a form of IF that involves eating early in the day to be in alignment with circadian rhythms in metabolism. Men with prediabetes were randomized to eTRF (6-hr feeding period, with dinner before 3 p.m.) or a control schedule (12-hr feeding period) for 5 weeks and later crossed over to the other schedule. eTRF improved insulin sensitivity, β cell responsiveness, blood pressure, oxidative stress, and appetite. We demonstrate for the first time in humans that eTRF improves some aspects of cardiometabolic health and that IF's effects are not solely due to weight loss.

INTRODUCTION

Intermittent fasting (IF)—the practice of alternating periods of eating and fasting—has emerged as an effective therapeutic strategy for improving multiple cardiometabolic endpoints in rodent models of disease, ranging from insulin sensitivity and ectopic fat accumulation to hard endpoints such as stroke and diabetes incidence (Antoni et al., 2017; Harvie and Howell, 2017; Mattson et al., 2017; Patterson and Sears, 2017). The first clinical trials of IF in humans began about a decade ago, including trials on alternate-day fasting (Catenacci et al., 2016; Heilbronn et al., 2005a, 2005b), alternate-day modified fasting (ADMF) (Bhutani et al., 2013; Eshghinia and Mohammadzadeh, 2013; Halberg et al., 2005; Hoddy et al., 2014, 2016; Johnson et al., 2007; Klempel et al., 2013; Kroeger et al., 2018; Soeters et al., 2009; Trepanowski et al., 2017a, 2017b; Varady et al., 2009, 2013; Wegman et al., 2015), the 5:2 diet (Carter et al., 2016; Harvie et al., 2011, 2013, 2016), and the fasting-mimicking diet (Brandhorst et al., 2015; Choi et al., 2016; Wei et al., 2017; Williams et al., 1998). Data from these trials suggest that IF has similar benefits in humans: IF can reduce body weight or body fat, improve insulin sensitivity, reduce glucose and/or insulin levels, lower blood pressure, improve lipid profiles, and reduce markers of inflammation and oxidative stress (Bhutani et al., 2013; Brandhorst et al., 2015; Carter et al., 2016; Catenacci et al., 2016; Eshghinia and Mohammadzadeh, 2013; Halberg et al., 2005; Harvie et al., 2011, 2013, 2016; Heilbronn et al., 2005a, 2005b; Hoddy et al., 2014, 2016; Johnson et al., 2007; Klempel et al., 2013; Trepanowski et al., 2017b; Varady et al., 2009, 2013; Wegman et al., 2015; Wei et al., 2017; Williams et al., 1998).

However, it was unknown whether these benefits are solely due to weight loss. Many have speculated that IF improves cardiometabolic health more than conventional dieting, even when matched for weight loss. Indeed, data in rodents suggest that IF improves cardiometabolic endpoints even when food intake and/or body weight is matched to the control group (Anson et al., 2003; Belkacemi et al., 2012; Hatori et al., 2012; Olsen et al., 2017; Sherman et al., 2012; Woodie et al., 2017; Wu et al., 2011; Zarrinpar et al., 2014). However, preliminary evidence in humans suggests that the benefits of IF are due mostly or only to weight loss (Halberg et al., 2005; Harvie et al., 2011; Soeters et al., 2009; Trepanowski et al., 2017b). Initially, a single-arm, 2-week trial reported that IF improves insulin sensitivity even when participants are approximately weight stable (Halberg et al., 2005), but the study was uncontrolled. Later, two better controlled, randomized crossover trials reported that IF did not improve glucose or lipid metabolism (Carlson et al., 2007; Soeters et al., 2009; Stote et al., 2007). More recently, the longest IF study in humans reported that adults who practiced ADMF for 1 year were not any healthier than conventional dieters who lost a similar amount of weight, yet they had a higher attrition rate (Trepanowski et al., 2017b). However, none of these studies matched food intake and meal frequency or supervised participants to ensure that they were following the prescribed dietary intervention. Drawing a parallel to metabolic (bariatric) surgery—which is widely believed to be more effective than conventional caloric restriction—four studies now show that the most popular form of metabolic surgery, called Roux-en-Y gastric bypass surgery, is no better or may be even worse at improving

glycemic control than "calorie-matched" weight loss (Campos et al., 2010; Isbell et al., 2010; Jackness et al., 2013; Lingvay et al., 2013). Such findings underscore the critical need to determine whether the benefits of interventions such as IF are mediated only through weight loss or through mechanisms that are independent of weight loss.

To test whether IF can have benefits independent of weight loss, we therefore decided to perform a proof-of-concept trial using a relatively new form of IF called time-restricted feeding (TRF). TRF is a type of IF that extends the daily fasting period between dinner and breakfast the following morning, and, unlike most forms of IF, it can be practiced either with or without reducing calorie intake and losing weight. Since the median American eats over a 12-hr period (Kant and Graubard, 2014), we define TRF as a form of IF that involves limiting daily food intake to a period of 10 hr or less, followed by a daily fast of at least 14 hr. Studies in rodents using feeding windows of 3–10 hr report that TRF reduces body weight, increases energy expenditure, improves glycemic control, lowers insulin levels, reduces hepatic fat, prevents hyperlipidemia, reduces infarct volume after stroke, and improves inflammatory markers, relative to grazing on food throughout the day (Belkacemi et al., 2010, 2011, 2012; Chung et al., 2016; Duncan et al., 2016; García-Luna et al., 2017; Hatori et al., 2012; Kudo et al., 2004; Manzanero et al., 2014; Olsen et al., 2017; Park et al., 2017; Philippens et al., 1977; Sherman et al., 2011, 2012; Sundaram and Yan, 2016; Woodie et al., 2017; Wu et al., 2011; Zarrinpar et al., 2014). We chose to test TRF over other forms of IF in part because TRF consistently improves health endpoints in rodents, even when food intake and/or body weight is matched to the control group (Belkacemi et al., 2012; Hatori et al., 2012; Olsen et al., 2017; Sherman et al., 2012; Woodie et al., 2017; Wu et al., 2011; Zarrinpar et al., 2014).

In humans, four pilot trials of TRF (4–10-hr feeding periods) have been conducted to date. Surprisingly, the results of TRF in humans appear to depend on the time of day of the eating window (Carlson et al., 2007; Gill and Panda, 2015; Moro et al., 2016; Stote et al., 2007; Tinsley et al., 2017). Restricting food intake to the middle of the day ("mid-day TRF" [mTRF]) reduced body weight or body fat, fasting glucose and insulin levels, insulin resistance, hyperlipidemia, and inflammation (Gill and Panda, 2015; Moro et al., 2016). However, restricting food intake to the late afternoon or evening (after 16:00 hr.; "late TRF" [lTRF]) either produced mostly null results or worsened postprandial glucose levels, β cell responsiveness, blood pressure, and lipid levels (Carlson et al., 2007; Stote et al., 2007; Tinsley et al., 2017).

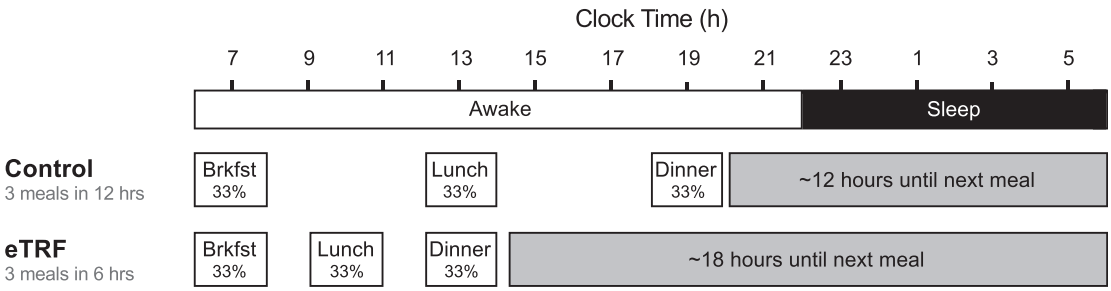
The circadian system, or internal biological clock, may explain why the effects of TRF appear to depend on the time of day. Glucose, lipid, and energy metabolism are all regulated by the circadian system, which upregulates them at some times of day and downregulates them at others (Poggiogalle et al., 2018; Scheer et al., 2009). For instance, in humans, insulin sensitivity, β cell responsiveness, and the thermic effect of food are all higher in the morning than in the afternoon or evening, suggesting that human metabolism is optimized for food intake in the morning (Morris et al., 2015a, 2015b; Poggiogalle et al., 2018; Scheer et al., 2009). Indeed, studies in humans show that eating in alignment with circadian rhythms in metabolism by increasing food intake at breakfast time and by reducing it at dinnertime improves glycemic control, weight loss, and lipid levels and also reduces hunger (Garaulet et al., 2013; Gill and Panda, 2015; Jakubowicz et al., 2013a, 2013b; Jakubowicz et al., 2015; Keim et al., 1997; Ruiz-Lozano et al., 2016). This suggests that the efficacy of IF interventions may depend not only on weight loss but also on the time of day of food intake. Moreover, these data from circadian studies suggest that combining two different meal timing strategies—IF and eating in alignment with circadian rhythms—may be a particularly beneficial form of IF. We call such a combined intervention "early time-restricted feeding" (early TRF; eTRF), and we define it as a subtype of TRF in which dinner is eaten in the mid-afternoon. To date, however, there had been no trials of eTRF in humans.

We therefore decided to test eTRF in our proof-of-concept trial. Our goals were 2-fold: (1) to determine whether eTRF can improve cardiometabolic health and (2) to determine whether IF can have benefits independent of weight loss and food intake. Our objective was not to examine the effectiveness or feasibility of eTRF but rather to determine the efficacy of eTRF when participants strictly adhere to their assigned meal times, food intake is precisely matched and monitored, and no weight loss occurs—that is, to measure the pure physiologic effects of eTRF uncontaminated by non-adherence. As such, our study is both the first clinical trial of eTRF and the most rigorously controlled trial of any form of IF in humans. We hypothesized that eTRF would improve glycemic control, improve vascular function, and reduce markers of inflammation and oxidative stress even when food intake is matched and no weight loss occurs.

RESULTS AND DISCUSSION

We performed a 5-week, randomized, crossover, isocaloric and eucaloric controlled feeding trial testing eTRF in men with prediabetes. In brief, participants adopted an eTRF schedule (6-hr daily eating period, with dinner before 15:00 hr) and a control schedule (12-hr eating period) for 5 weeks each, separated by a washout period of approximately 7 weeks. Participants chose a habitual time between 06:30 and 08:30 hr to start eating breakfast every day, and lunch and dinner were timed accordingly. For example, participants who ate breakfast at 07:00 hr then ate lunch and dinner at 10:00 hr and 13:00 hr in the eTRF arm and at 13:00 hr and 19:00 hr in the control arm (Figure 1). During the intervention phases, participants were required to eat only food provided by study staff, were fed enough food to maintain their weight, and ate all meals while being monitored by study staff. Furthermore, food intake was matched on a meal-by-meal basis across the two arms to eliminate any confounding effects from differences in food intake or meal frequency. As a result, our trial is the most rigorously controlled trial of IF in humans to date, achieving a level of rigor intermediate between metabolic ward conditions and a standard outpatient feeding trial (in which food is provided, but food intake is not measured, monitored, or enforced). The primary endpoints were glucose tolerance, postprandial insulin, and insulin sensitivity as measured using a 3-hr oral glucose tolerance test (OGTT), while the secondary endpoints were cardiovascular risk factors and markers of inflammation and oxidative stress. Metabolic hormones were added later as an exploratory outcome. Differences between meal timing schedules were assessed by comparing the two within-arm changes against each other; these treatment effects are denoted by Δ.

A Meal Timing Interventions



B Study Menus

Day 1	Day 2	Day 3	Day 4	Day 5
Breakfast	**Breakfast**	**Breakfast**	**Breakfast**	**Breakfast**
Bagel Cream Cheese Tropical Fruit Cup 1% Milk *Boiled Egg *Cheddar Cheese	Oatmeal Scrambled Egg Whites 1% Milk Butter Grape Juice Graham Crackers Peanut Butter	Waffles Maple Syrup Butter Blackberries Boiled Egg 1% Milk Pears	Cereal: Honey Nut Cheerios Scrambled Egg Whites Fruit Cocktail 1% Milk Butter Vanilla Wafers	Blueberry Muffin Boiled Egg Tropical Fruit Cup 1% Milk Granola Bar Peanut Butter
Lunch	**Lunch**	**Lunch**	**Lunch**	**Lunch**
Whole Wheat Bread Ham Lettuce Tomatoes Mayo & Mustard Pretzels Peaches Hummus Dip	Hamburger Bun Grilled Chicken Breast Lettuce Tomatoes Mayo & Mustard Fruit Salad Yogurt (Strawberry) *Cheese Crackers	Whole Wheat Bread Turkey Lettuce Tomatoes Mayo & Mustard *Swiss Cheese Baked Tortilla Chips Salsa with Black beans Sour Cream	Whole Wheat Pita Bread Chicken Salad Lettuce Tomatoes Sun Chips Grapes Chocolate Pudding	Chicken Pesto Pasta Lettuce Tomatoes Fat-free Italian Dressing Pineapple Chunks 1% Milk
Dinner	**Dinner**	**Dinner**	**Dinner**	**Dinner**
Alfredo Pasta with Chicken Capri Mixed Vegetables Dinner Roll, White Butter Pears	Catfish Almondine Rice Pilaf Green Beans Butter Dinner Roll, Wheat Peaches 1% Milk	Lemon Sage Chicken Wild Rice Blend California Blend Vegetables Butter Dinner Roll, White Pineapple Chunks Mozzerella String Cheese	Spaghetti and Meatballs Broccoli Butter *Dinner Roll, Wheat 1% Milk	Pork Chop Rosemary Garlic Potatoes Carrots Butter Dinner Roll, White *Parmesan Cheese 1% Milk

** Food was or was not served, depending on participant's calorie level.*

Figure 1. Dietary Interventions

(A) Meal timing interventions. An example schedule for a person who eats breakfast at 07:00 hr.

(B) Study menus. Food was prepared according to a 5-day sequence of menus. Each menu provided three meals/day and was composed of 35% fat, 50% carbohydrate, and 15% protein. Caloric intake was tailored to each participant's unique energy requirements, and each meal provided about 33% of daily caloric needs.

See also Figure S1 and Table S1.

Participants

Controlled feeding trials are very demanding because they require participants to eat all meals under supervision for weeks. This, in turn, makes recruitment challenging since most people cannot take time off work daily to eat their meals while being monitored. As shown in Figure S1, 934 individuals expressed interest in trying eTRF and applied to participate in the trial. Of these, most were excluded for being unable to eat all meals under supervision. Ultimately, 130 men were screened in the clinic, and, of those, 18 had both elevated HbA1c levels and impaired glucose tolerance indicative of prediabetes, and 15 met all eligibility requirements. Twelve men were enrolled in order to have the requisite eight completers. Of the four who did not complete the intervention, two withdrew for unrelated medical reasons (severe neck pain necessitating surgery, abnormally low potassium levels at baseline that did not improve over time), and another two withdrew because of unexpected changes to their work schedule. The eight overweight men with prediabetes who completed the trial (aged 56 ± 9 years; 6 Caucasian, 1 African-American, 1 South Asian) had a mean BMI of 32.2 ± 4.4 kg/m^2, fasting glucose of 102 ± 9 mg/dL, fasting insulin of 25.1 ± 14.5 mU/L, and 2-hr glucose tolerance of 154 ± 17 mg/dL (Table S1). At screening, their mean blood pressure was at the lower end of the prehypertensive range (systolic: 123 ± 8 mm Hg; diastolic: 82 ± 7 mm Hg), while their mean lipid levels were in the normal ranges.

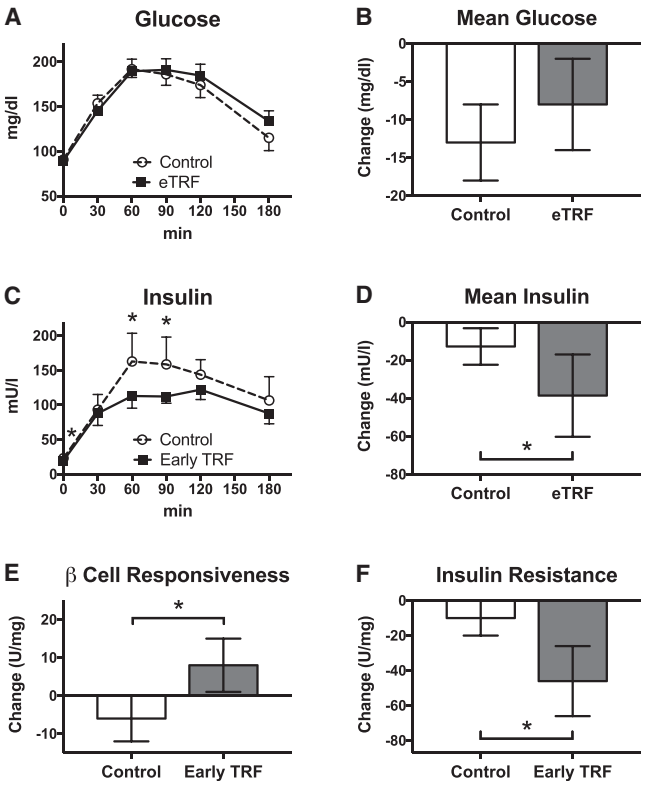


Figure 2. Glycemic Control

eTRF did not affect individual (A) or mean values (B) for glucose during a 3-hr OGTT. However, eTRF did lower insulin levels at multiple time points (C) and mean insulin levels (D). Overall, eTRF improved β cell responsiveness (E) and insulin resistance (F), as measured by the insulinogenic index and the incremental AUC ratio, respectively. (Post-intervention values shown above for glucose in A and insulin in C were adjusted for differences at baseline.) All data are paired, with n = 8 completers in each arm. Data are presented as least-squares mean ± SEM, with the exceptions of (A) and (C), which display the data as raw mean ± SEM. *$p \leq 0.05$.

See also Figure S2 and Table S2.

Compliance

Compliance was outstanding: participants who completed the trial were 100.0% ± 0.0% and 98.9% ± 1.8% compliant to eating the provided meals when following the eTRF and control schedules, respectively. Every exception to eating the provided food (aside from a single meal by one participant and 3 sick days by another participant) was approved ahead of time by study staff, and food intake was then re-calculated and matched in the second arm of the trial. Furthermore, participants were 98.2% ± 2.9% and 99.0% ± 1.9% compliant to adhering to the required meal times when following the eTRF and control schedules, respectively. In addition, body weight was approximately stable, and changes in body weight were similar between arms (−1.4 ± 1.3 kg versus −1.0 ± 1.1 kg; Δ = −0.5 ± 0.3 kg; p = 0.12). Importantly, since food intake was matched across arms, the lack of a treatment effect for body weight suggests that TRF does not impact energy expenditure in humans. We suspect that the non-significant difference in the within-arm change in body weight was due to a reduction in glycogen levels and the accompanying loss of water weight, which arises from the longer fasting duration on the eTRF schedule.

Adverse Events

There were no serious adverse events. There were about one dozen adverse events identified as possibly related to the study intervention. These included vomiting (one participant in the eTRF arm), frequent urination and drowsiness (one participant in the control arm), and headaches, increased thirst, and diarrhea (each of which afflicted two participants in the eTRF arm and one participant in the control arm). Unrelated to the study intervention, one participant reported a worsening of neck pain requiring surgery during the washout period, which precipitated his withdrawal from the study.

eTRF Reduces Insulin Levels and Improves Insulin Sensitivity and β Cell Responsiveness

Participants underwent 3-hr OGTTs in the morning at baseline and post-intervention for each study arm. As shown in Figures 2 and S2, 5 weeks of eTRF did not affect fasting glucose (Δ = −2 ± 2 mg/dL; p = 0.49) or glucose levels at any time point during the 3-hr OGTT ($p \geq 0.13$). Consequently, mean glucose levels were unchanged (Δ = 5 ± 5 mg/dL; p = 0.40). However, eTRF did affect insulin levels. eTRF decreased fasting insulin by 3.4 ± 1.6 mU/L (p = 0.05) and decreased insulin levels at t = 60 min and 90 min post-load ($p \leq 0.01$). In aggregate, eTRF reduced mean and peak insulin values by 26 ± 9 mU/L (p = 0.01) and 35 ± 13 mU/L (p = 0.01), respectively. We also investigated the impact of eTRF on OGTT-derived indices of β cell responsiveness and insulin resistance. eTRF increased the insulinogenic index, a marker of β cell responsiveness, by 14 ± 7 U/mg (p = 0.05) and decreased insulin resistance, as measured by the 3-hr incremental AUC ratio, by 36 ± 10 U/mg (p = 0.005).

Although 5 weeks of eTRF did not improve glucose levels, it dramatically lowered insulin levels and improved insulin sensitivity and β cell responsiveness. This is consistent with several other trials in humans that suggest that IF may be more effective at reducing insulin levels and improving insulin sensitivity than at lowering glucose levels (Bhutani et al., 2013; Harvie et al., 2011, 2013; Heilbronn et al., 2005a, 2005b; Trepanowski et al., 2017b; Wegman et al., 2015; Williams et al., 1998).

In our trial, the reductions in insulin levels were largest in participants with worse hyperinsulinemia at baseline, and these improvements were driven more (but not exclusively) by differences at baseline (see Figure S2). Much to our surprise, even after the 7-week washout period, all but one participant who first completed the eTRF intervention entered the second arm of the trial with substantially lower (≥25%) mean postprandial insulin levels. (The only exception was one participant who traveled multiple time zones away during his washout period.) Although these within-subject differences at baseline were not statistically significant for the four men who followed eTRF first (−46 ± 14 mU/L; p = 0.55) or for all eight completers (−20 ± 14 mU/L; p = 0.13), our linear mixed models did uncover statistically significant sequence and period effects. (By comparison, the insulin sensitivity endpoint was not affected by either sequence or period effects, while β cell responsiveness was affected only by period effects.) This suggests that eTRF may have longer-lasting benefits even after being discontinued. As a result, the

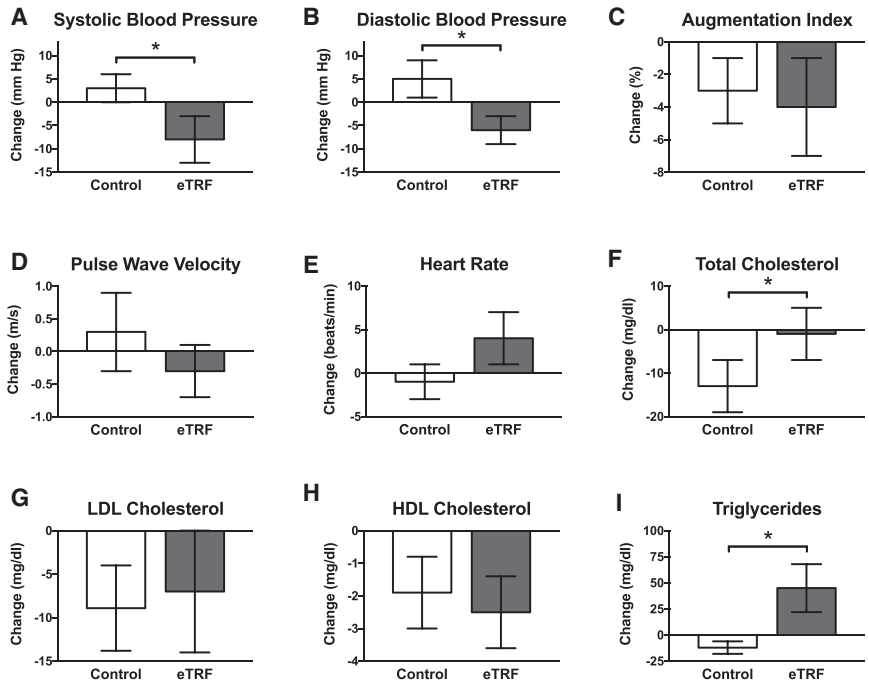


Figure 3. Cardiovascular Disease Risk Factors

eTRF dramatically lowered systolic blood pressure (A) and diastolic blood pressure (B) in the morning. However, it increased or tended to increase morning values for resting heart rate (E), triglycerides (I), and, in turn, total cholesterol (F). The augmentation index (C), pulse wave velocity (D), LDL cholesterol (G), and HDL cholesterol (H) were unaffected.

All data are paired, with n = 8 completers in each arm. Data are presented as least-squares mean ± SEM. *p $\leq$ 0.05.

See also Table S2.

true effect size for improvements in mean postprandial insulin could be smaller or larger than the 26 ± 9 mU/L decrease that we observed, and our study therefore merits replication to confirm the effect size in men with prediabetes. Nonetheless, all but one of our participants experienced improvements of 5 mU/L or greater in mean postprandial insulin levels on eTRF relative to the control schedule, suggesting that such effects are real. Interestingly, the one participant whose insulin levels worsened on eTRF had reported a long history of overnight shift work prior to enrolling in the trial. Given that circadian rhythms are altered in adults who perform overnight shift work, it will be important to determine whether some subpopulations have altered circadian rhythms and would benefit more from alternative meal timing interventions.

An important consideration in interpreting our results is that our data may underestimate the glycemic benefits of eTRF for two reasons. First, we did not match the fasting duration prior to testing: participants fasted for about 18 hr prior to testing in the eTRF arm but for only 12 hr in the control arm. Acute fasting induces insulin resistance and worsens β cell responsiveness even after only 24 hr, and this is mediated—at least partially—through elevation of triglycerides and/or free fatty acids from lipolysis (Antoni et al., 2016; Browning et al., 2012; Halberg et al., 2005; Salgin et al., 2009). In one trial, 24 hr of fasting decreased insulin sensitivity the following morning by 54% and the acute response of insulin, a marker of β cell responsiveness, by 22% (Salgin et al., 2009). In retrospect, given that the eTRF arm involved fasting for 18 hr prior to testing and that we observed a large 57 ± 13 mg/dL increase in triglyceride levels at the start of the OGTT (described below), it is quite remarkable that we found an improvement in both β cell responsiveness and insulin sensitivity. This limitation can be resolved in future studies by matching the fasting duration in both arms on the day prior to testing.

The second reason why our data may underestimate the glycemic benefits of eTRF is that we measured glucose levels only in the morning. Although we observed no difference at habitual breakfast time, eTRF may still lower mean 24-hr glucose levels simply by shifting the timing of lunch and dinner to earlier in the day, when the circadian system promotes better glucose tolerance (Poggiogalle et al., 2018). In future studies, it will be important to measure glucose metabolism over a 24-hr period to determine whether improvements in insulin levels and insulin sensitivity—without an accompanying reduction in glucose levels—is indeed a hallmark of IF or is an artifact of not measuring glucose levels over the 24-hr day.

In sum, our results show that eTRF can be used to treat insulin resistance and to improve pancreatic β cell function; however, its effects on 24-hr glucose levels remain to be determined.

eTRF Lowers Blood Pressure but Does Not Affect Arterial Stiffness, LDL Cholesterol, or HDL Cholesterol

As shown in Figure 3, 5 weeks of eTRF lowered morning levels of systolic and diastolic blood pressure by 11 ± 4 mm Hg (p = 0.03) and 10 ± 4 mm Hg (p = 0.03), respectively, relative to the control schedule. This is a surprisingly and dramatically large improvement for a dietary intervention of only 5 weeks that did not induce weight loss; it is on par with the effectiveness of anti-hypertensive medications such as angiotensin-converting enzyme (ACE) inhibitors (Heran et al., 2008). Although other IF trials have observed improvements in blood pressure (Bhutani et al., 2013; Eshghinia and Mohammadzadeh, 2013; Varady et al., 2009; Wei et al., 2017), none have reported effects this large. Given some evidence that elevated insulin levels may directly increase blood pressure (Bhanot and McNeill, 1996; Biston et al., 1996; Persson, 2007), one possibility is that the improvements in blood pressure were driven by the reduction in insulin levels. Another possibility is that eTRF promotes natriuresis by shifting salt intake to earlier in the daytime when sodium excretion is upregulated by the circadian system (Johnston et al., 2016).

However, 5 weeks of eTRF did not affect the augmentation index (Δ = −1.4% ± 2.1%; p = 0.53) or pulse wave velocity (Δ = −0.5 ± 0.4 m/s; p = 0.23), which are measures of arterial stiffness. Similarly, eTRF did not affect HDL cholesterol (Δ = −0.6 ± 0.9 mg/dL; p = 0.48) or LDL cholesterol (Δ = 2 ± 6 mg/dL;

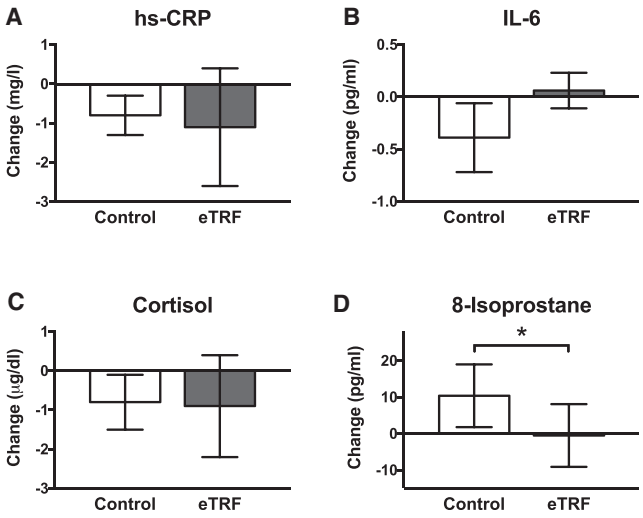


Figure 4. Inflammatory and Oxidative Stress Markers

eTRF did not affect the inflammatory markers hs-CRP (A), IL-6 (B), or cortisol (C). However, eTRF reduced levels of 8-isoprostane (D), a marker of oxidative stress to lipids. All data are paired, with n = 8 completers in each arm. Data are presented as least-squares mean ± SEM. *$p \leq 0.05$.

See also Table S2.

p = 0.75). eTRF did increase morning fasting levels of triglycerides by 57 ± 13 mg/dL (p = 0.0007), which translated into a 13 ± 5 mg/dL relative increase in morning fasting levels of total cholesterol (p = 0.02). (The relative increase in total cholesterol was driven by an improvement in the control arm rather than a change in the eTRF arm.) The elevation in circulating triglyceride levels is likely due to the longer fasting duration preceding testing (18 hr versus 12 hr in the control arm) and likely reflects triglyceride re-esterification following lipolysis and possibly also hepatic and intramuscular storage of triglyceride (Browning et al., 2012; Soeters et al., 2012). eTRF also tended to increase morning heart rate by 5 ± 3 bpm (p = 0.10) to 74 ± 7 bpm post-intervention, but the effect did not reach statistical significance. This potential increase may reflect a change in sensory nervous system activity due to the longer daily fasting duration and accompanying lipolysis (Patel et al., 2002; Pequignot et al., 1980). The increases in fasting triglycerides and potentially also heart rate merit further study—particularly in a trial that matches the fasting duration prior to testing.

eTRF Reduces Oxidative Stress but Does Not Affect Inflammatory Markers

Relative to the control arm, 5 weeks of eTRF decreased plasma levels of 8-isoprostane, a marker of oxidative stress to lipids, by 11 ± 5 pg/mL (p = 0.05) or about 14% (Figure 4). (Both sequence and period effects for 8-isoprostane were statistically significant.) The relative improvement was driven by a worsening in the control arm, suggesting that in our study, eTRF prevented 8-isoprostane levels from becoming worse when participants ate the provided study foods. However, eTRF did not affect any markers of inflammation; morning fasting levels of hs-CRP (Δ = −0.3 ± 1.0 mg/L; p = 0.77), cortisol (Δ = −0.1 ± 1.3 μg/dL; p = 0.95), and IL-6 (Δ = 0.45 ± 0.27 pg/mL; p = 0.12) were all unchanged.

Only one prior TRF trial has measured inflammatory markers, and it reported a reduction in IL-1β, but not in IL-6 or TNF-α (Moro et al., 2016). In general, most clinical trials report that IF does not affect hs-CRP, TNF-α, or IL-6 (Bhutani et al., 2013; Halberg et al., 2005; Harvie et al., 2011, 2013; Moro et al., 2016; Trepanowski et al., 2017b; Wei et al., 2017), indicating that IF does not affect most inflammatory markers in humans. By contrast, our finding of an improvement in oxidative stress relative to the control arm is in agreement with an 8-week IF trial that reported dramatic reductions in 8-isoprostane, nitrotyrosine, protein carbonyls, and 4-hydroxynonenal adducts (Johnson et al., 2007). Although fewer trials have examined the effects of IF on oxidative stress markers, both our and Johnson et al. (2007)'s data suggest that IF may affect oxidative stress levels more than inflammatory markers. Because eTRF reduces lipid peroxidation, it may, in turn, reduce the risk of atherosclerosis.

eTRF Reduces Appetite in the Evening

As shown in Figure 5, there were no differences in subjective measures of appetite in the morning ($p \geq 0.20$). However, eTRF substantially reduced the desire to eat (Δ = −22 ± 7 mm; p = 0.007) and the capacity to eat (Δ = −23 ± 6 mm; p = 0.001) in the evening and non-significantly decreased hunger levels (Δ = −9 ± 6 mm; p = 0.15). Participants also reported that eTRF dramatically increased sensations of fullness in the evening (Δ = 31 ± 6 mm; $p < 0.0001$) and nearly significantly increased sensations of a full stomach (Δ = 10 ± 5 mm; p = 0.07).

As an exploratory analysis to support self-reported appetite ratings, we also measured metabolic hormones in the morning. As shown in Table S2, eTRF decreased morning fasting values of the satiety hormone PYY by 23 ± 7 pg/mL (p = 0.003). However, it did not affect morning fasting levels of the hunger hormone ghrelin (Δ = −5.7 ± 6.6 pg/mL; p = 0.41), the incretin GLP-1 (Δ = −1.2 ± 1.0 pmol/mL; p = 0.26), or the adipokines leptin (Δ = −0.6 ± 1.0 ng/mL; p = 0.54) and high-molecular weight adiponectin (Δ = 408 ± 765 ng/mL; p = 0.61).

Thus, despite the longer daily fasting duration for the eTRF schedule, eTRF does not increase hunger—at least, not when food intake is calorie matched to the control arm. On the contrary, eTRF decreased the desire and capacity to eat and increased feelings of fullness in the evening. eTRF may therefore help curb food intake in the evening and, in turn, facilitate weight loss. This is consistent with rodent studies, which have reported that both eTRF and other forms of TRF reduce appetite hormones and body weight (Belkacemi et al., 2010, 2011; Chaix et al., 2014; Chung et al., 2016; Duncan et al., 2016; García-Luna et al., 2017; Hatori et al., 2012; Kudo et al., 2004; Manzanero et al., 2014; Olsen et al., 2017; Park et al., 2017; Philippens et al., 1977; Sherman et al., 2011, 2012; Sundaram and Yan, 2016; Wu et al., 2011; Zarrinpar et al., 2014). In contrast, previous studies on mid-day and late TRF in humans report conflicting results for hunger (Gill and Panda, 2015; Stote et al., 2007), food intake (Gill and Panda, 2015; Tinsley et al., 2017), and body weight (Gill and Panda, 2015; Moro et al., 2016; Stote et al., 2007; Tinsley et al., 2017). It remains to be determined whether these discrepancies were due to limitations or differences in the study design (e.g., no control group or only measuring hunger at one time of day) or the timing of food intake.

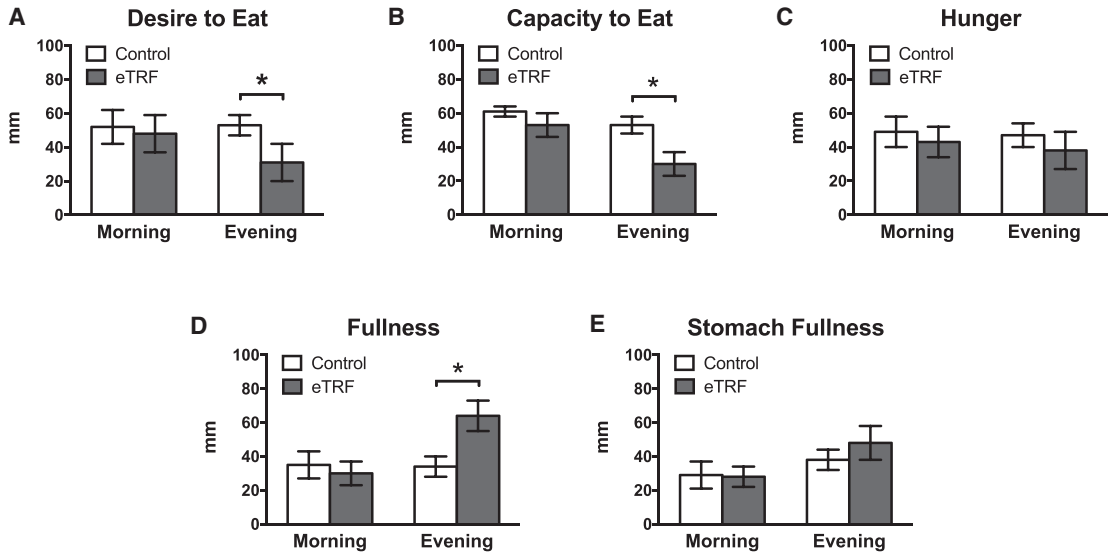


Figure 5. Subjective Appetite

Participants rated their appetite on a 0–100-mm visual analog scale, ranging from "Not at All" (0 mm) to "Extremely" (100 mm). eTRF did not affect appetite in the morning. In the evening, eTRF reduced desire to eat (A) and capacity to eat (B) and increased feelings of fullness (D). Changes in evening levels of hunger (C) and stomach fullness (E) did not quite reach statistical significance. All data are paired, with n = 8 completers in each arm. Data are presented as least-squares mean ± SEM. *$p \leq 0.05$.

See also Table S2 and Figure S3.

Feasibility and Acceptability

Although our study was an efficacy trial, we also collected preliminary data on feasibility and acceptability. As shown in Figure S3, participants reported that it took 12 ± 10 days (range: 2–35 days) to adjust to the eTRF schedule, and all but one participant adjusted within about 2 weeks. Participants also reported that the challenge of eating within 6 hr each day was more difficult than the challenge of fasting for 18 hr per day (difficulty scores: 65 ± 20 versus 29 ± 18 mm; p = 0.009). In fact, all but one participant reported that it was not difficult or only moderately difficult (<50 mm on a 100-mm scale) to fast for 18 hr daily. Based on their experiences in adhering to eTRF, participants thought that eating within a 7.8 ± 1.8-hr daily period (range: 4–10 hr) would be feasible for most people. At the end of the study, seven out of eight participants were willing to eat dinner earlier, based on their subjective experiences in the study, while all eight said they were willing to do so if it improved their health. Thus, while fasting for 18 hr per day is well tolerated and not difficult, the feasting aspect of eTRF is more difficult for participants, so TRF interventions with an 8-hr or longer eating period may be a better target for future effectiveness trials.

Limitations

This study has several limitations. First, our trial included only eight men. Although our sample size is similar to other extremely well-controlled or inpatient circadian trials, our results need to be replicated in a larger trial that also includes women. Second, we did not match the fasting duration prior to testing, which may have underestimated the improvements in insulin sensitivity and also likely explains the increase in triglycerides and total cholesterol. Although we suspect that the elevation in fasting triglycerides is a transient byproduct of eTRF's extended daily fasting, future trials that measure lipid levels across the 24-hr day and/or that image plaque and ectopic fat depots are needed to confirm that this phenomenon is not pathophysiologic. Third, our trial did not measure glucose levels over a 24-hr period, so we were unable to investigate whether eTRF, by virtue of shifting the timing of lunch and dinner to earlier during the day, lowers mean 24-hr glucose levels as would be expected based on prior research (Poggiogalle et al., 2018). Along similar lines, since we did not measure blood pressure across the 24-hr day, measuring only morning fasting values may overestimate eTRF's effects on blood pressure. Finally, since our trial was an efficacy trial designed to isolate and measure the physiologic effects of eTRF, our study does not provide any insight into feasibility. Future trials are needed to determine the optimal length and timing of the feeding period and whether eTRF is feasible and effective in the general population.

Conclusion

In conclusion, 5 weeks of eTRF improved insulin levels, insulin sensitivity, β cell responsiveness, blood pressure, and oxidative stress levels in men with prediabetes—even though food intake was matched to the control arm and no weight loss occurred. Our trial was the first randomized controlled trial to show that IF has benefits independent of food intake and weight loss in humans. Our study was also the first clinical trial to test eTRF in humans and to show that eTRF improves some aspects of cardiometabolic health. Our trial tested eTRF in men with prediabetes—a population at great risk of developing diabetes—and indicates that eTRF is an efficacious strategy for treating both prediabetes and likely also prehypertension. We speculate that eTRF—by virtue of combining daily intermittent fasting and eating in alignment with circadian rhythms in metabolism—will prove to be a particularly efficacious form of IF. In light of these

promising results, future research is needed to better elucidate the mechanisms behind both intermittent fasting and meal timing, to determine which forms of IF and meal timing are efficacious, and to translate them into effective interventions for the general population.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures and two tables and can be found with this article online at https://doi.org/10.1016/j.cmet.2018.04.010.

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AUTHOR CONTRIBUTIONS

C.M.P. conceived of, designed, and lead the clinical trial, with input from E.R. and W.T.C. C.M.P., E.F.S., K.S.E., and W.T.C. conducted the investigation. R.B. performed the statistical analyses, and C.M.P. and E.F.S. drafted the manuscript. All authors helped interpret the data, revised the manuscript for critical content, and approved the final version of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Critical Commercial Assays		
Glucagon-like peptide-1 (Active) ELISA	EMD Millipore	EGLP-35K
Human Leptin Radioimmunoassay	EMD Millipore	HL-81K
Human Ghrelin (Active) Radioimmunoassay	EMD Millipore	GHRA-88HK
Human Adiponectin Radioimmunoassay	EMD Millipore	HADP-61HK
Human PYY (3-36) Specific Radioimmunoassay	EMD Millipore	PYYT-67HK
SMC Human Interleukin-6 (IL-6) Immunoassay Kit	EMD Millipore	03-0089-01
8-Isoprostane ELISA Kit	Cayman Chemical	516351
Software and Algorithms		
SphygmoCor, v.8	AtCor Medical	http://atcormedical.com/healthcare-professionals/products/
SAS, v.9.4	SAS Institute	https://www.sas.com/; RRID: SCR_008567

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Dr. Courtney Peterson (cpeterso@uab.edu). For the specific cases of biospecimen and data sharing requests, such requests will require a Material Transfer Agreement and/or a Data Use Agreement and will be managed by the University of Alabama at Birmingham's Material Transfer Office, which abides by the Uniform Biological Material Transfer Agreement (UBMTA).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

This clinical trial was conducted at Pennington Biomedical Research Center (PBRC; Baton Rouge, LA), approved by the center's Institutional Review Board, and conducted in accordance with the Helsinki Declaration of 1975. Prior to enrolling participants, the trial was preregistered on clinicaltrials.gov (NCT01895179). Participants were recruited from the Greater Baton Rouge area between October 2013 and January 2016 via emails, flyers, social media, local radio and TV appearances, and website advertisements. The study population comprised overweight and obese (BMI between 25-50 kg/m^2) adult males aged 35-70 with prediabetes. To qualify as having prediabetes, participants needed to exhibit both elevated levels of HbA1c (5.5%–6.4%) and impaired glucose tolerance (IGT), defined as a glucose level between 140-199 mg/dL at the end of a 2-hr oral glucose tolerance test (OGTT). Potential participants were excluded if they performed overnight shift work more than once a week; regularly fasted (defined as fasting $\geq$ 16 hr per day or having completed twelve 24-hr fasts within the past year); regularly consumed more than 2 servings/day of alcohol; regularly performed heavy physical activity; had gastrointestinal surgery or impaired nutrient absorption; took anti-diabetes medications, steroids, beta blockers, adrenergic-stimulating agents, or other medications that could affect the study endpoints; or were afflicted with diabetes or a significant cardiovascular, renal, cardiac, liver, lung, or nervous system disease. All participants provided both verbal and written informed consent prior to enrolling in the study. Because this was an efficacy trial, participants were continuously enrolled until eight individuals completed the trial (see "Statistical Power" in the section "Quantification and Statistical Analysis").

METHOD DETAILS

Study Design

The trial was conducted as a randomized, crossover, controlled feeding study. Participants were randomized to initially follow either a control schedule (~12-hr eating window with 12 hr of daily fasting) or an eTRF schedule (~6-hr eating period with 18 hr of daily fasting) for 5 weeks. Thereafter, they completed an approximately 7-week washout period before crossing over to the other arm. The eating schedules were modestly customized by allowing each participant to choose a habitual time to start eating breakfast every day (all started breakfast between 06:30 - 08:30 hr). Their two subsequent meals (lunch and dinner) were spaced by 6 hr for the control schedule versus 3 hr for the eTRF schedule. For example, for a breakfast time of 07:00 hr, lunch and dinner would be at 10:00 hr and 13:00 hr in the eTRF arm but at 13:00 hr and 19:00 hr in the control arm (Figure 1A). Regardless of their chosen breakfast

time, all participants were scheduled to *finish* eating dinner by mid-afternoon (≤15:00 hr) while following the eTRF schedule. Participants were instructed to maintain consistent physical activity and sleep patterns throughout the entire 4-month study.

Each interventional arm of the trial lasted 37 days and was structured as follows. On Day 1 (run-in period), all participants ate three meals over a 10-hr period, starting at their chosen breakfast time and with the meals spaced every 5 hr. The purpose of the one-day run-in period was to ensure that all participants ate the same diet at the same meal times on the day prior to baseline testing. On Day 2 (baseline testing), a 3-hr OGTT and applanation tonometry were performed, and blood was collected to measure fasting levels of lipids and of metabolic, hormonal, and oxidative stress markers. On Days 2-36, participants followed their assigned meal timing schedule. On Day 36, participants' appetite levels were measured using visual analog scales. Finally, all baseline tests were repeated on Day 37. All physiologic tests were performed starting at each participant's habitual breakfast time.

Diets

Calories, meal frequency (3 meals/day), and food composition were matched on a ''meal-by-meal'' basis in both arms of the trial to eliminate any confounding effects from differences in food intake; the only difference between the two arms was the timing of meals. All food was prepared by the PBRC Research Kitchen using a 5-day rotating menu (Figure 1B). Diets were formulated to contain 50% carbohydrate, 35% fat, and 15% protein, and each meal provided approximately one-third of each participants' daily energy requirements. To determine whether there are intrinsic benefits to eTRF—''independent of weight loss''—participants were intentionally fed enough food to maintain their weight using the equation (in kcal/day): 2189 + 19.6 × (weight in kg) – 17.6 × (age in years) (Redman et al., 2009). To ensure that participants maintained their weight, each participant was weighed daily during Days 1-14 and weekly thereafter of the first arm of the study, and any changes in weight were counterbalanced by adjusting calorie intake in ± 100 kcal increments. Participants were required to eat all provided meals and were not allowed to eat any non-study foods; any rare protocol deviations (such as sick days) were calculated and matched in the second arm of the study.

Compliance Monitoring

To ensure compliance, participants were required to eat all meals at our research clinic or to be supervised in real-time via remote video monitoring by Skype (Peterson et al., 2016). The start and stop time of every meal eaten in the study was logged. Participants were instructed to start eating each meal within ± 30 min of the scheduled time and to finish eating each meal within 45 min. At the end of the trial, dietary compliance was quantified in two ways: (1) compliance with eating the provided foods and (2) compliance with the meal timing schedules. Compliance with eating the provided foods was quantified as the percent of provided meals that were eaten while being monitored, while compliance with the meal timing schedules was quantified as the percent of meals eaten within 1 hr of the scheduled time. Due to both the nature of the intervention and the monitoring of compliance, neither study participants nor study staff could be blinded.

OGTTs

Intravenous lines were inserted into participants' arm veins, and fasting blood samples were collected. Participants then consumed 75 g loads of glucose (Azer Scientific; Morgantown, PA) within 5 min. For the 3-hr OGTTs administered at baseline and post-intervention, the ingestion of glucose was timed to start at each participant's habitual breakfast time. Blood was subsequently collected at 30, 60, 90, 120, and 180 min after glucose ingestion to measure both glucose and insulin. The primary outcomes were mean glucose and insulin levels, which were calculated as the 180-min AUC value divided by the 180-min duration of the OGTT. β cell responsiveness was estimated using the insulinogenic index, which was calculated as the change in insulin divided by the change in glucose during the first 30 min. Insulin resistance was estimated using the incremental AUC ratio (Conn et al., 1956), which was calculated as the ratio of the incremental AUC values for insulin and glucose, or equivalently, as (mean insulin – fasting insulin) / (mean glucose – fasting glucose).

Applanation Tonometry

Applanation tonometry, which measures arterial stiffness, was performed immediately before each 3-hr OGTT. For the test, participants rested in a supine position, while a 3-lead EKG was placed on their wrists, leg, and/or chest to monitor the cardiac cycle. At the end of a 20-min rest period, the tonometer (AtCor Medical; Itasca, IL) was lightly applied at the wrist to sample radial artery pressure waveforms. The pressure waveform data were processed using SphygmoCor software (Version 8.0; AtCor Medical; Itasca, IL). The outcome variables were augmentation index and pulse wave velocity (m/s). Peripheral and central augmentation indices were calculated as a percentage based on the difference in the second systolic peak and diastolic pressure, divided by the difference between the first systolic peak and diastolic pressure (Wilkinson et al., 1998).

Serum Chemistry

All serum samples were analyzed in duplicate. Glucose, cholesterol, and triglycerides were measured on a DXC600 instrument (Beckman Coulter; Brea, CA) either using standard reagents, or in the case of HDL cholesterol, using an immunoinhibition assay (Trinity Biotech USA; Jamestown, NY and WAKO Chemicals USA; Richmond, CA). LDL cholesterol was determined using the Friedewald equation. Insulin and hs-CRP were measured using chemiluminescent immunoassays on an Immulite 2000 instrument (Siemens Corporation; Washington, DC). Fasting leptin, active ghrelin, high-molecular weight adiponectin, and peptide YY (PYY) levels were assayed using radioimmunoassay kits (EMD Millipore Corporation; Billerica, MA) on a gamma counter (Wizard 2470;

PerkinElmer; Waltham, MA). Fasting levels of glucagon-like peptide-1 (GLP-1) and 8-isoprostane were assayed using ELISA kits (EMD Millipore Corporation; Billerica, MA, and Cayman Chemical Company; Ann Arbor, MI, respectively) on a Bio Rad Microplate reader (Bio-Rad Laboratories; Hercules, CA). The inflammatory cytokines IL-1β, IL-6, MCP-1, and TNF-α were measured by immunoassay with fluorescent detection on a Luminex instrument (EMD Millipore; Billerica, MA). Unfortunately, the values for IL-6 were undetectable in most participants, and the coefficients of variation for the within-sample measurements of MCP-1 and TNF-α were excessively high, so these data were not included.

Subjective Appetite

Participants rated their appetite across five dimensions—hunger, fullness, stomach fullness, desire to eat, and capacity to eat—using Visual Analog Scales (VAS; a 0-100 mm scale). VAS surveys were administered immediately before breakfast and 12 hr after breakfast (which was immediately before dinner in the control arm) on the last day of the intervention (Day 36). Participants rated their appetite levels based how they habitually felt at that time of day during the previous week.

Exit Survey

On the last day of the study (Day 37 of arm 2), all participants completed an exit survey that assessed how many days they felt it took to adjust to the eTRF eating schedule; the difficulty of adhering to the eating versus fasting periods of eTRF (using a VAS rating system); whether they would be willing to eat earlier in the day based on their experiences in the study; and how long they thought the eating period should be in order to be feasible for the general public.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical Power

The statistical power analysis indicated that for a crossover trial, eight completers were required to have 80% power (two-sided test, $\alpha = 0.05$) to detect a 12 mg/dl difference in glucose levels during an OGTT (the primary endpoint), assuming r = 0.3 and a within-subjects standard deviation of $\sigma = 10$ mg/dl.

Randomization

The randomization code was generated using an online random number generator based on atmospheric noise (https://www.random.org/). Since we continued to enroll participants until the planned eight individuals completed the intervention, randomization was performed with replacement in a 1:1 allocation and using a block size of 8 to ensure equal numbers of participants completed each of the two possible sequences. Allocations were concealed from study participants until after they enrolled in the trial.

Statistical Analyses

Statistical analyses were performed as two-sided tests in SAS (version 9.4; Cary, NC) using a significance threshold of $\alpha = 0.05$ for the Type I error rate. Since this was an efficacy study—designed to isolate and measure the physiologic effects of eTRF uncontaminated by non-adherence—data were analyzed for completers only. All collected data for the eight completers were included in the analysis; one participant had unusual pulsatile insulin secretion patterns, but no data were excluded. Differences between treatment arms were evaluated at baseline and as change scores using linear mixed models with heterogeneous compound symmetry, where participants served as the random effect; the treatment, sequence, and period were treated as fixed effects; and the Satterthwaite method was used for calculating degrees of freedom. Three endpoints—mean insulin levels, β cell responsiveness, and 8-isoprostane—had statistically significant sequence and/or period effects, which are reported in the main text; all other statistically significant endpoints did not. All data are presented as least-squares mean ± SEM, with the exceptions of the baseline data and the exit survey data, which are presented as raw mean ± SD, and the individual time point data for the OGTT (Figures 2A, 2C, and S2), which are graphically presented as raw mean ± SEM for visual clarity.

ADDITIONAL RESOURCES

Clinical Trial Registration URL: https://www.clinicaltrials.gov/ct2/show/NCT01895179.