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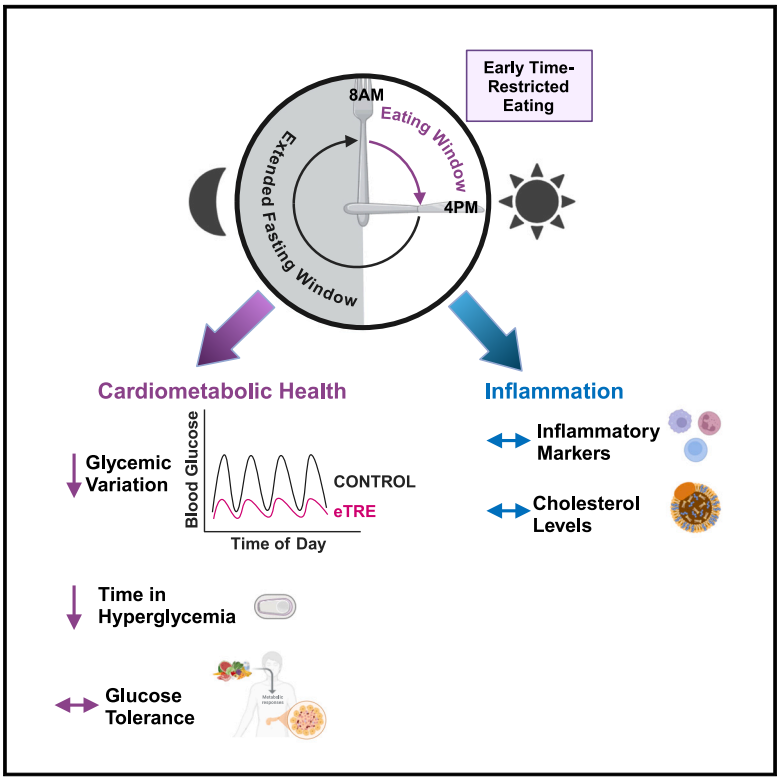
Weight-neutral early time-restricted eating improves glycemic variation and time in range without changes in inflammatory markers

Graphical abstract



Highlights

- Weight-neutral eTRE reduces glycemic variation and time in hyperglycemia
- Short-term eTRE does not affect levels of circulating inflammatory markers
- eTRE may be an effective dietary strategy for management of dysglycemia

Authors

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In brief

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Weight-neutral early time-restricted eating improves glycemic variation and time in range without changes in inflammatory markers

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SUMMARY

Early time-restricted eating (eTRE) is a dietary strategy that restricts caloric intake to the first 6–8 h of the day and can effect metabolic benefits independent of weight loss. However, the extent of these benefits is unknown. We conducted a randomized crossover feeding study to investigate the weight-independent effects of eTRE on glycemic variation, multiple time-in-range metrics, and levels of inflammatory markers. Ten adults with prediabetes were randomized to eTRE (8-h feeding window, 80% of calories consumed before 14:00 h) or usual feeding (50% of calories consumed after 16:00 h) for 1 week followed by crossover to the other schedule. Using continuous glucose monitoring, we showed that eTRE decreased glycemic variation (mean amplitude of glycemic excursion) and time in hyperglycemia greater than 140 mg/dL without affecting inflammatory markers (erythrocyte sedimentation rate and C-reactive protein). These data implicate eTRE as a candidate dietary intervention for the weight-independent management of dysglycemia in high-risk individuals.

INTRODUCTION

There is an increasing body of evidence that circadian misalignment caused by changes in sleeping and eating behaviors is a significant contributor to obesity and cardiometabolic disease.[1,2] Higher proportional caloric intake in the evening is associated with obesity,[3,4] weight loss inhibition,[5] and cardiometabolic disease risk factors such as dysglycemia, hyperlipidemia, and increased markers of systemic inflammation.[6–12] While the mechanisms underlying these correlations are not well understood, these effects are most likely due to suboptimal timing of caloric intake against diurnal variations in hormone and metabolite activity,[1,2,13–16] supporting the use of feeding strategies that optimize not only timing of the feeding window but also higher proportionate caloric intake to earlier in the day in order to coincide with certain circadian patterns.

Time-restricted eating (TRE) is a meal timing strategy that restricts the daily eating window to a short period of consecutive hours in the day, while extending the fasting period between the final meal of the day and first meal the following morning. In lay communities, TRE has been touted as an increasingly popular approach to weight loss, and most, but not all, clinical trials of TRE have supported it as an effective dietary strategy for weight loss.[17–21] While TRE regimens do not place explicit limits on calorie consumption, evidence shows that shortening the eating window to 6 to 8 h per day results in fewer calories consumed, explaining any weight loss benefits.[19,22,23]

In addition to reducing body weight, trial data have suggested that TRE has additional beneficial metabolic effects including improved insulin sensitivity, reductions in plasma glucose levels, decreased blood pressure, improved lipid profiles, and reduced inflammatory markers.[24–28] Timing of the feeding window seems to be critical for these observed effects, with earlier eating windows appearing to be more effective than later eating windows at inducing metabolic change.[18,29–33] This suggests that the effects of TRE are not only subsequent to weight loss but also due to synchronization of nutrient intake with an individual's chronotype.[34] A publication by Sutton et al.[24] sought to test this hypothesis by comparing the effects of early time-restricted eating (eTRE; 6 h feeding window with dinner before 15:00 h) to a control feeding schedule (12 h feeding window) on various markers of cardiometabolic health in a supervised controlled feeding trial. They reported that eTRE improved insulin sensitivity, β cell responsiveness, blood pressure, oxidative stress, and evening satiety under weight-neutral conditions, showing for the first time that certain benefits of TRE are inherent to the feeding strategy itself.

Glycemic variability, the measurement of fluctuations of glucose levels over a specified time period, is associated with

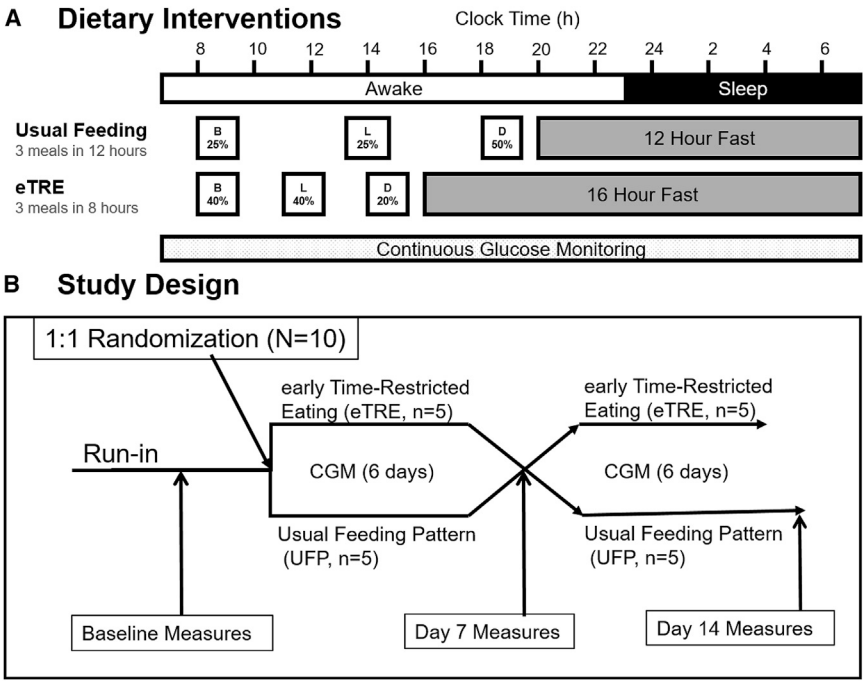

Figure 1. Study protocol: An example meal timing schedule

(A) On the usual feeding pattern (UFP) arm, participants were provided with 3 meals with 50% of calories consumed after 16:00. On the early time-restricted eating (eTRE) arm, participants were provided with 3 meals between 08:00 and 16:00 with 80% of calories consumed prior to 14:00. Participants were allowed to elect when to eat so long as it fell within these parameters. Continuous glucose monitors were placed on day 1 and worn throughout the study.

(B) Ten participants were randomized 1:1 to either a UFP or eTRE diet for 6 days and then crossed over to the other arm for 6 days. Measurements were obtained at baseline, on day 7, and on day 14. CGM, continuous glucose monitor.

poor outcomes in individuals with metabolic disease including diabetic retinopathy, diabetic kidney disease, and diabetic peripheral neuropathy.[35–38] While the pathogenic mechanism underlying these correlations has yet to be identified, there is growing evidence that increased glycemic variability drives increased oxidative stress leading to inflammation at both the systemic and tissue level.[35,38–41] Systemic inflammation has been identified as a major contributor to obesity-related comorbidities and is associated with the progression of type 2 diabetes, cardiovascular disease, and certain cancers.[42] Studies evaluating the effects of intermittent fasting and TRE on inflammatory markers have yielded variable results, potentially related to differences in study populations and intermittent fasting/TRE protocols.[43,44] For example, work from Xie et al.,[30] comparing eTRE to mid-day TRE, showed that in healthy, non-obese subjects eTRE reduces inflammatory markers, total body mass, and adiposity in healthy individuals, whereas mid-day TRE does not, suggesting that timing of the eating window may be a limiting factor in evaluating certain metabolic outcomes. While animal studies have shown weight loss-independent effects of TRE on ameliorating inflammation in high fat diet-fed mice,[45,46] this has not been investigated in humans.

Understanding the full scope of weight loss-independent metabolic benefits of eTRE will inform on its use as a preventative or therapeutic strategy for mitigating metabolic disease and may also provide insight into novel therapeutic targets that exploit the involved pathways. Accordingly, we performed a randomized, crossover, supervised feeding trial comparing the effects of 6 days of eTRE with calorie compression into the earlier half of the day (feeding window 08:00–16:00 h with 80% of calories consumed prior to 14:00 h) to 6 days of a usual feeding pattern (UFP; 50% of calories consumed after 16:00 h) in individuals with prediabetes and overweight or obesity on inflammation and glycemia. The primary endpoints of the study were glycemic variation as measured by the mean amplitude of glycemic excursion (MAGE) and plasma inflammatory marker concentration (erythrocyte sedimentation rate [ESR] and C-reactive protein [CRP]). We hypothesized that eTRE with greater proportional caloric intake would improve glycemic variability and reduce inflammatory markers even with weight neutrality.

RESULTS

We performed a 6-day, randomized, crossover, eucaloric, supervised feeding study in a metabolic ward at The Rockefeller University Hospital testing the effects of eTRE compared to a UFP in 10 individuals with prediabetes and a body mass index (BMI) >25 kg/m^2. Participants were randomized 1:1 to either 6 days of an eTRE feeding schedule (daily feeding window from 08:00–16:00 h, with 80% of total daily calories consumed prior to 14:00 h) or a control feeding schedule (UFP, 50% of total daily calories consumed after 16:00 h) followed by a testing day (Figures 1 and S1; Data S1). After this initial assigned dietary arm, participants crossed over to the alternate arm for the subsequent week of the study. Food was provided according to the assigned dietary arm, and participants were required to only consume food provided by the study staff. Study menus were designed according to participant dietary preferences with efforts made to match their self-reported pre-intervention macronutrient percentages and to ensure that participants would consume enough calories to maintain their baseline weight (Table S1). Continuous glucose monitors (CGMs) were placed by trained study staff and worn by study participants for the entirety of the two-week study period.

Trial population and adherence

Ten individuals were enrolled in and completed the study. Participant demographics and baseline clinical data are summarized in Table 1. In our study population, the mean age was 57.7 ± 10.1 years, 50% were female and 80% were Black. Mean baseline hemoglobin A1c (HbA1c) was 5.79% ± 0.12%. Mean BMI was 37.29 ± 5.4 kg/m^2; 1 participant had a BMI ≤29.99 kg/m^2, 3

Table 1. Baseline characteristics of enrolled participants both overall and after initial randomization

Variables	Overall (n = 10)	UFP arm 1 (n = 5)	eTRE arm 1 (n = 5)	*p* value
Age — years	57.70 (10.1)	56.8 (9.9)	58.6 (11.4)	0.79
Female sex, *n* (%)	5 (50%)	2 (40%)	3 (60%)	0.57
Race-ethnicity, *n* (%) †	–	–	–	0.99
White	2 (20%)	1 (20%)	1 (20%)	–
Black	8 (80%)	4 (80%)	4 (80%)	–
Weight (kg)	107.48 (17.0)	111.28 (12.4)	103.68 (21.5)	0.51
Body mass index (kg/m^2)	37.29 (5.4)	38.10 (4.1)	36.48 (6.8)	0.68
≤29.99	1 (10%)	0 (0%)	1 (20%)	–
30–34.99	3 (30%)	2 (40%)	1 (20%)	–
≥35	6 (60%)	3 (60%)	3 (60%)	–
Waist circumference (cm)	116.4 (14.8)	117.2 (11.2)	115.6 (19.2)	0.87
Lean body mass (%)	57.9 (5.08)	57.4 (6.6)	58.6 (2.9)	0.75
Fasting glucose (mg/dL)	92.9 (7.8)	93.8 (7.6)	92 (8.8)	0.74
Hemoglobin A1c (%)	5.79 (0.12)	5.78 (0.04)	5.80 (0.2)	0.81
Lipids (mg/dL)				
Total C holesterol	182.9 (26.7)	189 (18.3)	176.8 (34.2)	0.50
LDL	116.72 (25.3)	127.1 (15.2)	103.27 (27.9)	0.15
HDL	46.43 (13.6)	47.4 (14.3)	45.89 (13.5)	0.59
TG	85.31 (38.7)	74.7 (19.3)	94.11 (50.5)	0.28
C-reactive protein (mg/L)	0.66 (0.5)	0.46 (0.08)	0.83 (0.6)	0.28

Data are shown as mean ± standard deviation for continuous variables and count for categorical variables. †, patient-reported race-ethnicity; eTRE, early time-restricted eating; UFP, usual feeding practice.

participants had BMIs between 30 and 34.99 kg/m^2, and 6 participants had BMI ≥35 kg/m^2. Baseline mean lipid levels and CRP levels were in normal ranges. As shown in Table 1, there were no significant baseline differences in age, gender, ethnicity, BMI, HbA1c, lipid levels, or CRP levels between those who were randomized initially to the UFP arm or the eTRE arm.

Participants were exceptionally compliant. All enrolled participants completed the trial. Within each 6-day arm, participants were observed in the metabolic ward at The Rockefeller University Hospital for the initial 4 days (Tuesday through Friday) and returned home for the final 2 weekend days as per The Rockefeller University Hospital's standard protocol. Their compliance with eating the provided meals according to their assigned study arm was observed to be 100% while they remained inpatient, and they endorsed 100% compliance during the time they spent at home. Body weight was stable throughout the study (106.6 ± 16.8 kg at baseline vs. 106.1 ± 16.6 kg at study completion; $p = 0.08$), and there were no significant differences between baseline weight and post-intervention weights for either of the two dietary arms ($p = 0.68$ for baseline vs. UFP, $p = 0.09$ for baseline vs. eTRE) (Table S2).

There were no serious adverse events. There was one mild adverse event identified as possibly related to the study, as one participant experienced diarrhea and bloating the day after a 2-h oral glucose tolerance test (OGTT) that resolved after antacid treatment. Additionally, one participant was incidentally found to be hypokalemic on baseline labs (K 3.3 mEq/dL; normal range = 3.5–5.1 mEq/dL); this resolved without intervention and was likely unrelated to the study intervention.

eTRE improves glycemic variation and reduces time in hyperglycemia

Study participants wore CGMs throughout the study. CGM data were downloaded and analyzed after study completion (Table 2). There were no statistically significant differences in mean glucose levels ($p = 0.11$) or in standard deviation of measured glucose levels ($p = 0.08$) between the two arms. Glycemic variation was assessed by calculating daily MAGE, which is the mean of blood glucose levels exceeding one standard deviation from the 24-h mean blood glucose. Daily MAGE values from the 6 day period on each dietary arm were then averaged together to determine the overall MAGE for the study period. MAGE was decreased in the eTRE arm relative to UFP (eTRE = 2.4 ± 0.2 mmol/L vs. UFP = 2.9 ± 0.3 mmol/L; $p = 0.001$).

The 2017 International Consensus on CGM Metrics identified 14 core metrics for assessing CGM data, among which were time below range ($TBR_{<70}$, percentage of readings and time with glucose <70 mg/dL), time in range ($TIR_{70\text{-}180}$, percentage of readings and time with glucose between 70 and 180 mg/dL), and time above range ($TAR_{>180}$, percentage of readings and time with glucose >180 mg/dL).[47] There were no statistically significant differences in any of these parameters between the eTRE and UFP arms ($p = 0.24$, $p = 0.26$, and $p = 0.62$, respectively) (Table 2). However, these metrics were developed based on CGM data from individuals with type 1 or insulin-treated type 2 diabetes. Recent studies have suggested that glycemic targets for the prediabetes population may be better captured by estimating time in 70–140 mg/dL, 140–180 mg/dL, and >140 mg/dL to capture glycemic excursions outside of the normal range (greater than 140 mg/dL).[48–50] When we analyzed the percentage of readings and time with glucose between 140 and 180 mg/dL ($TIR_{140\text{-}180}$), we found that participants spent a significantly lower percentage of time in this hyperglycemic blood glucose range during the eTRE arm than they did on the control arm (eTRE = 2.7% ± 1.2% vs. UFP = 5.6% ± 1.4%, $p = 0.01$) (Table 2). This significant difference was maintained when we extended our analysis to include any measured glucose level above 140 mg/dL ($TAR_{>140}$) (eTRE = 3.0% ± 1.3% vs. UFP = 5.8% ± 1.5%, $p = 0.03$) (Table 2). Representative CGM tracings for two study participants on each of the dietary arms are shown in Figure 2 and demonstrate the reductions in glycemic variability and $TAR_{>140}$ that were observed in participants on the eTRE arm.

Table 2. eTRE reduces (A) glycemic variation and (B) overall time in hyperglycemia

A	UFP (N = 10) mean (SEM)	eTRE (N = 10) mean (SEM)	p value
MAGE (mmol/L)	2.9 (0.3)	2.4 (0.2)	0.001*
Mean glucose (mmol/L)	5.4 (0.16)	5.2 (0.18)	0.11
SD	1.08 (0.1)	0.99 (0.08)	0.08

B	UFP (N = 10) mean (SEM) (95% CI)	UFP (N = 10) median (IQR)	eTRE (N = 10) mean (SEM) (95% CI)	eTRE (N = 10) median (IQR)	p value
$TBR_{<70}$ (%)	3.51 [1.50] (0.56–6.45)	1.16 [3.94]	7.00 [2.72] (1.67–12.32)	2.78 [7.75]	0.22
$TIR_{70\text{-}140}$ (%)	90.59 [1.88] (86.90–94.28)	89.93 [6.54]	90.05 [2.45] (85.24–94.86)	90.51 [5.96]	0.81
$TIR_{70\text{-}180}$ (%)	96.11 [1.70] (92.79–99.44)	98.84 [3.94]	92.80 [2.66] (87.58–98.02)	97.22 [6.37]	0.25
$TIR_{140\text{-}180}$ (%)	5.56 [1.50] (2.62–8.49)	8.91 [9.84]	2.74 [1.21] (0.37–5.11)	1.5 [6.19]	0.01*
$TAR_{>140}$ (%)	5.90 [1.56] (2.85–8.96)	9.72 [11.52]	2.95 [1.33] (0.34–5.56)	1.5 [6.54]	0.02*
$TAR_{>180}$ (%)	0.38 [0.31] (−0.22 - 0.99)	0.00 [0.35]	0.21 [0.13] (−0.04 - 0.46)	0.00 [0.35]	0.62

Continuous glucose monitor analysis. All data are paired, with N = 10 completers in each arm. Data are shown as mean ± standard error of the mean. Metrics that deviated from normality are also reported as median (interquartile range [IQR]). *$p \leq 0.05$. eTRE, early time-restricted eating; UFP, usual feeding practice; MAGE, mean amplitude of glycemic excursion; SD, standard deviation; SEM, standard error of the mean.

To determine whether these effects were due to increased fasting hypoglycemia or decreased postprandial glycemic excursions, we calculated the daytime $TIR_{140\text{-}180}$ and the overnight $TBR_{<70}$, with the assumption that the daytime $TIR_{140\text{-}180}$ would be a proxy for measuring changes in post-meal glucose excursions whereas the overnight $TBR_{<70}$ would be a proxy for measuring changes in fasting glucose excursions (Table S3).[49,50] Participants had lower daytime $TIR_{140\text{-}180}$ on the eTRE arm compared to the UFP arm (eTRE = 3.2% ± 1.3% vs. UFP = 7.2% ± 1.9%; p = 0.01). However, there were no statistically significant differences between the nighttime $TBR_{<70}$ between the two arms (eTRE = 12.3% ± 5.8% vs. UFP = 6.1% ± 2.5%, p = 0.28).

Finally, we performed a linear mixed effects model to examine if the differences in both overall and daytime $TIR_{140\text{-}180}$ between arms persisted after adjusting for potential confounders including age, self-reported sex, BMI, and order of intervention. This analysis showed that the eTRE arm still had significantly lower daytime $TIR_{140\text{-}180}$ by 4.31% (95% confidence interval [CI]: (0.8%, 7.8%); p = 0.017) compared to UFP, indicating improve daytime glycemic control with eTRE (Table S4). We found similar results for overall $TIR_{140\text{-}180}$ where again the eTRE arm had lower $TIR_{140\text{-}180}$ compared to UFP by 3.3% (95% CI: (0.7%, 5.9%); p = 0.012) (Table 3). In both models, we also observed that self-reported sex had a significant association with $TIR_{140\text{-}180}$, with males having lower daytime $TIR_{140\text{-}180}$ by 7.1% (95% CI: (1.5%, 12.6%); p = 0.013) (Table S4) as well as lower overall $TIR_{140\text{-}180}$ by 5.9% (95% CI: (1.1%,10.8%); p = 0.017) (Table 3) compared to females. Univariate analysis revealed no correlation between overall or daytime $TIR_{140\text{-}180}$ and the primary endpoints of CRP (p = 0.53 and p = 0.54, respectively) and soluble circulating RAGE (sRAGE) (p = 0.72 and p = 0.66, respectively). Conversely, both overall and daytime $TIR_{140\text{-}180}$ were highly correlated with MAGE. Thus, these parameters were not integrated into our linear mixed effects model in order to avoid over-manipulation and redundancy within the model.

Daily MAGE was calculated with a significant difference between the eTRE vs. UFP arm as early as intervention day 2 (eTRE = 2.4 ± 1.1 mmol/L vs. UFP = 8.0 ± 2.1 mmol/L; p = 0.003) and nearing significance on intervention day 1 (p = 0.07) (Figure 3A). Participants underwent 2-h OGTTs in the morning at baseline and post-intervention for each study arm. As shown in Figure 3, there were no significant differences in glucose levels at any time point during the 2-h OGTT (Figure 3B) or in change in fasting glucose levels from baseline (Δ UFP = −6.4 mg/dL vs. Δ eTRE = −7.1 mg/dL; p = 0.84, Figure 3D) between the UFP and eTRE arms. Accordingly, area under the curve (AUC) was not statistically different between the two groups (UFP = 16126.5 vs. eTRE = 16392; p = 0.73, Figure 3C).

Short-term eTRE does not affect lipid levels or inflammatory marker concentrations

Fasting concentrations of total cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were measured at baseline and post-intervention for each studyarm. There were no significant differences between post-intervention total cholesterol (UFP = 181.9 mg/dL vs. eTRE = 182.8 mg/dL; p = 0.77), triglycerides (UFP = 74.8 mg/dL vs. eTRE = 77.4 mg/dL; p = 0.42), HDL (UFP = 46.8 mg/dL vs. eTRE = 46.6 mg/dL; p = 0.93), or LDL levels (UFP = 119.6 mg/dL vs. eTRE = 116.2 mg/dL; p = 0.26) (Figure 4A). Additionally, there was no difference in change from baseline for any of these parameters (Figure S2A; p = 0.77, p = 0.42, p = 0.93, and p = 0.26, respectively). eTRE had no effect on CRP (UFP = 0.95 mg/dL vs. eTRE = 1.13 mg/dL; p = 0.23, Figure 4B) or ESR levels (UFP = 21 mm/h vs. eTRE = 21.3 mm/h; p = 0.89, Figure 4C), or in change from baseline of either of these two inflammatory markers (Figures S2B and S2C; p = 0.38 and p = 0.133, respectively).

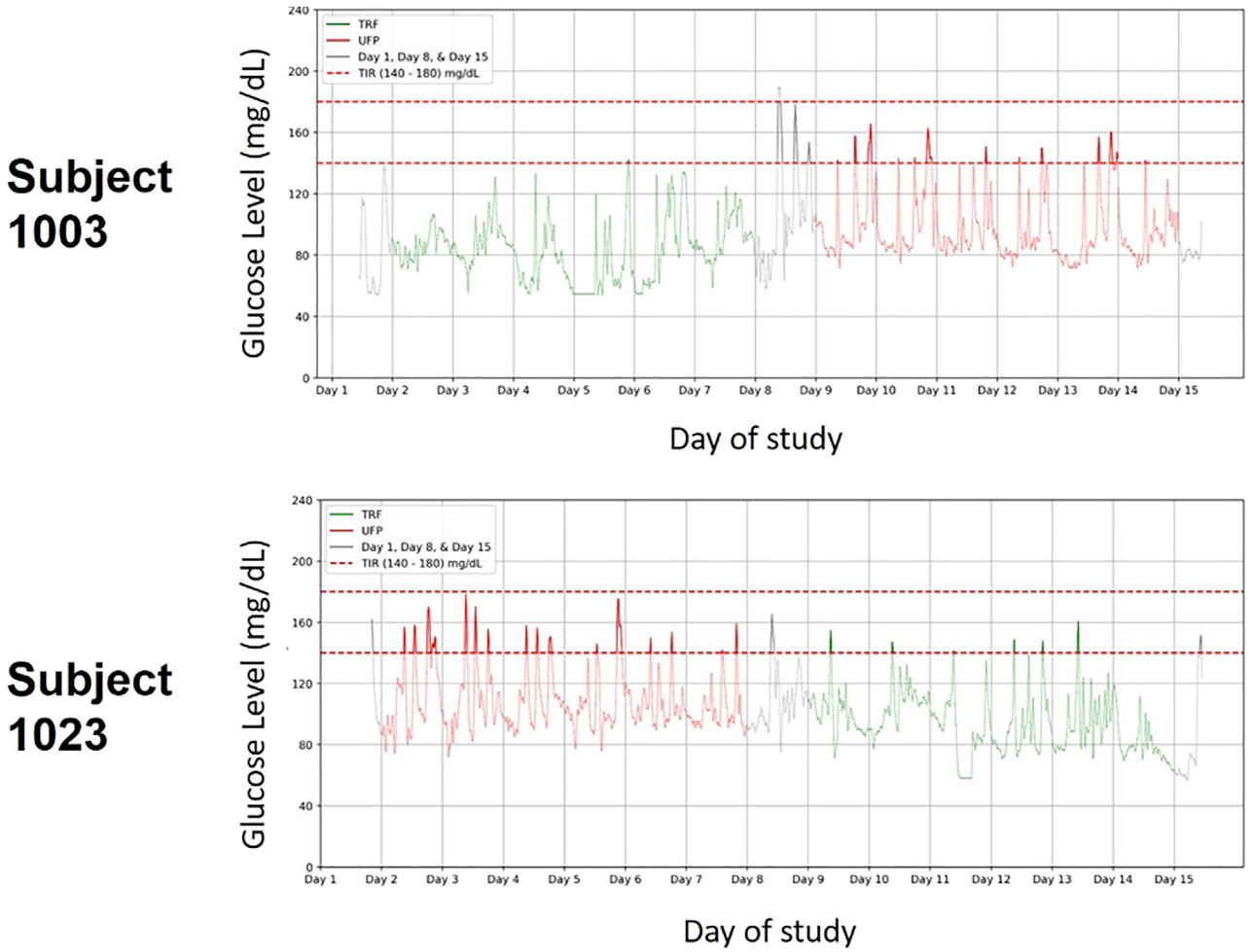

Figure 2. Continuous glucose monitoring confirms intervention compliance

Representative continuous glucose monitor tracings from two participants during the study period. Glucose readings from the eTRE phase in green; UFP phase in red; and baseline, washout, and final day in gray. Glucose readings in the 140–180 mg/dL range are highlighted using darker lines while those outside this range are shown with a lighter color. eTRE, early time-restricted eating; UFP, usual feeding practice.

Advanced glycation end products (AGEs) and their receptor (RAGE) are important mediators of inflammation and associated complications in obesity and diabetes.[51–54] sRAGE is believed to act as a decoy receptor inhibiting the interaction between RAGE and its ligands, thereby protecting against these dysglycemia-associated pathologies.[54] To determine whether the glycemic improvements observed with eTRE translated to this metabolic pathway, we measured fasting plasma sRAGE levels at baseline and post-intervention. There were no statistically significant differences in sRAGE levels between the two dietary arms (UFP = 889.9 pg/mL vs. eTRE = 848.9 pg/mL; $p = 0.48$, Figure 4D), nor were there any significant changes from baseline ($p = 0.48$; Figure S2D).

eTRE does not affect hunger, satiety, or fullness

Feasibility of compliance with eTRE practice is a major concern for real-world applications of this dietary intervention. To address this, visual analog scales were used to rate subject reflection on their average hunger, satiety, and fullness levels at baseline and after one week on each dietary arm. Participants were asked to report on their experience of these parameters over the preceding week. There were no differences in subject-reported hunger, satiety, or fullness with eTRE compared to baseline or the UFP arm (Figures 5A–5C). However, after a week on the UFP arm participants, reported significantly decreased fullness compared to baseline (Figure 5C).

DISCUSSION

This 6-day randomized crossover supervised feeding trial demonstrates that weight-neutral eTRE reduces glycemic variability by 17% and time in hyperglycemia (140–180 mg/dL) by 52% compared to usual feeding in adults with prediabetes and obesity. These glycemic changes are observed as early as day 2 on the intervention arm. To date, the small body of work evaluating the weight loss-independent effects of eTRE in humans has shown improved insulin sensitivity and β cell responsiveness without changes in glucose tolerance, reduced mean 24-h

Table 3. eTRE reduces overall time in hyperglycemia independent of intervention order

Predictors	Coeff (SEM)	95% CI	*p* value
Intercept	5.192 (2.047)	[1.2,9.2]	0.011
Intervention arm (ref: eTRE) – UFP	3.333 (1.328)	[0.7,5.9]	0.012*
Intervention order (ref: TU) – UT	1.040 (2.579)	[−4.0,6.1]	0.687
Arm (ref: eTRE):order (ref: TU) – UFP: UT	−1.042 (1.879)	[−4.7,2.6]	0.579
Age	0.124 (0.124)	[−0.1,0.4]	0.319
Self-reported sex (ref: female) – male	−5.938 (2.834)	[−10.8,-1.1]	0.017*
BMI	0.143 (0.236)	[−0.3,0.6]	0.605

Linear mixed effects model for overall $TIR_{140\text{-}180}$. TU, eTRE followed by UFP; UT, UFP followed by eTRE. *$p \leq 0.05$. eTRE, early time-restricted eating; UFP, usual feeding practice.

glucose levels, and improved response to a mixed-meal tolerance test with this feeding strategy.[24,31,55] Our findings expand on this body of literature by dissociating the glycemic and inflammatory benefits of TRE, and our study is only the second study to evaluate the weight-independent benefits of eTRE in a highly controlled inpatient setting.[56] We replicate the findings of *Jamshed* et al.[31] that eTRE reduces glycemic variation and add a detailed glucometric analysis using CGM data to reveal that eTRE improves glycemic variation and $TIR_{>140}$ within the first 48 h of intervention through reductions in postprandial hyperglycemia. Though there are an abundance of studies showing cardiometabolic benefits of TRE, many report accompanying weight loss making it difficult to differentiate those effects that are a direct result of timed feeding from those that are subsequent to reduced body mass. Our study mitigates this by our weight-neutral, eucaloric approach. Study diets were individualized to a participant's reported food preferences and caloric intake and designed to ensure sufficient caloric intake for weight maintenance. Additionally, the 3-day rotating menu was identical in content between the two study arms for a given subject with the only difference being the timing of when the food was eaten. Participants were strongly encouraged to consume the provided food in its entirety. Thus, the glycemic changes that we identified with eTRE were not due to differences in dietary content or caloric consumption between the two arms. An added variable in TRE trials is timing of the feeding window, with early feeding windows appearing to be more effective at eliciting metabolic changes with this dietary intervention.[19,21,24,30–32] Despite this, few TRE studies have specified an early feeding window. This may explain some of the null results that have been reported in TRE trials that use a later feeding window or allow for patient selection of the feeding window in order to promote adherence to the study protocol.[18,30,57,58] Our study design is unique in that, in addition to specifying feeding windows, we also delineated differences in daily calorie distribution between the two arms, a concept that is referred to as calorie compression. Whereas most eTRE studies evenly distribute caloric intake throughout the feeding window, we chose to restrict higher proportionate caloric intake to the earlier hours of the feeding window in the eTRE arm and the latter half of the day in the UFP arm, mimicking the typical western feeding pattern. By maximizing the differences in food timing between the two arms in this way, we likely enhanced our ability to identify inter-arm differences despite our relatively short intervention period and small study population.

Using CGMs to closely track subject glycemia throughout each treatment arm, we report that eTRE reduces glycemic variation as measured by MAGE, a finding that has been reported in only one other study.[31] Our work expands on this literature through detailed analysis of daily glucose trends, showing that the glycemic benefits of eTRE begin as early as 48 h post-intervention and that eTRE reduces $TAR_{>140.}$ These results are driven by reductions in $TIR_{140\text{-}180}$ as $TAR_{>180}$ was not significantly different between interventional arms. This is not surprising as the participants were prediabetic and had very few glycemic excursions greater than 180 mg/dL. A linear mixed effects model determined that treatment order had no impact on the glycemic response to eTRE, which was of particular concern given the lack of washout period between the study arms. OGTTs indicated no change in glucose tolerance over the short study period, consistent with what has been previously reported.[24] Most eTRE trials to date are too short to evaluate effects of eTRE on HbA1c, which would require at least 3 months study duration. Further research is needed to determine whether the reduced $TAR_{>140}$ observed here translates to reductions in HbA1c over the long term, though this would be expected.

A concern about implementing eTRE and other protocols that require prolonged fasting in individuals with metabolic disease is that they might provoke hypoglycemia. However, we did not observe an increase in $TBR_{<70}$ in the eTRE arm. We stratified the data to distinguish whether the observed glycemic improvements were due to decreased daytime hyperglycemia or increased nighttime hypoglycemia and found that there was a significant decrease in daytime $TIR_{140\text{-}180}$ in the eTRE group but no difference in nighttime hypoglycemia compared to the control arm. Taken together, these data suggest that eTRE improves glycemia by minimizing postprandial glycemic excursions leading to decreased glycemic variability and reduced time in hyperglycemia rather than by increasing overnight hypoglycemia.

Recent advances in continuous glucose monitoring technology have enabled the measurement of short-term glycemic variability as an indicator of daily glycemic fluctuation.[35] This may more accurately assess dysregulation of glucose homeostasis than HbA1c or OGTT, the current gold standards for assessing glycemic control.[59] While this is a newer metric, short-term glycemic variability has already been associated with diabetic complications and may be an independent driver of increased oxidative stress and inflammation in individuals with metabolic dysfunction.[36,39–41] Jamshed et al. (2019) also found that eTRE reduces MAGE compared to control feeding. However, their analysis differs from ours in that they only analyzed CGM data from the final day of each dietary intervention, whereas we looked at daily MAGE to determine time to treatment effect in

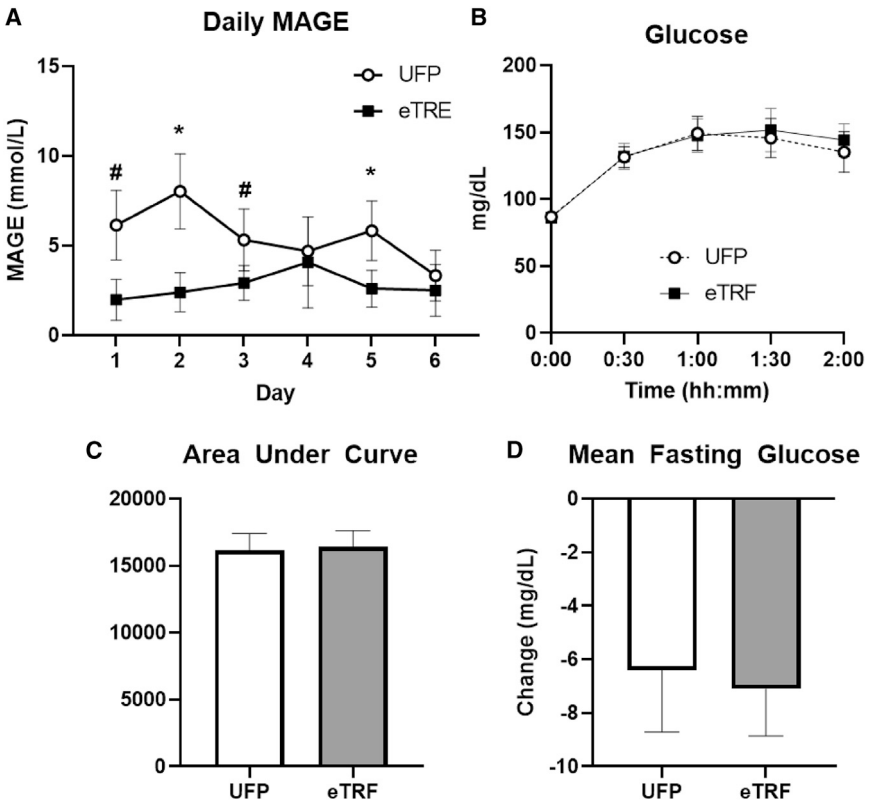

Figure 3. eTRE lowers glycemic variability without affecting glucose tolerance during a 2-h OGTT

(A) Daily MAGE levels for participants during 1 week on UFP and eTRE dietary arms.

(B) OGTT results for participants after 1 week on UFP and eTRE dietary arms.

(C) Quantification of OGTT area under the curve (AUC).

(D) Change from baseline of mean fasting glucose levels after 1 week on UFP and eTRE dietary arms. Values are mean ± SEM. #$p = 0.07$, *$p \leq 0.05$. eTRE, early time-restricted eating; UFP, usual feeding practice; OGTT, oral glucose tolerance test; MAGE, mean amplitude of glycemic excursions.

addition to pooling the data from the entire six days of our study.[31] Comparatively, without a run-in or washout period, our analysis begins on the first day of each interventional arm, likely underestimating the effects of eTRE on glycemic variability. Despite significant reductions in glycemic variation, we did not identify any effects of short-term eTRE on CRP or ESR, which are important circulating plasma markers of inflammation. Consistent with our findings, a recent meta-analysis of 5 human trials of TRE revealed no effect of TRE on key circulating inflammatory markers, including CRP, tumor necrosis factor alpha, and interleukin-6.[44] Given its unique position as a biomarker of disease risk linking glycemia to inflammatory pathway activation, we hypothesized that eTRE would alter levels of circulating sRAGE.[54,60] However, sRAGE levels were not significantly different between the two dietary arms. Changes in sRAGE levels have not been evaluated before in intermittent fasting trials, and it may be that a longer period of glycemic improvement is needed to effect change in this signaling pathway. Though not tested in our study, other work has reported improved markers of oxidative stress with intermittent fasting.[24,61] Further research is required to determine the pathways that serve as the link between reduced glycemic variation and improved cardiometabolic outcomes.

Very few adverse events were reported over the course of the trial. Only one was identified as being potentially related to the study. On review, it was more likely related to the glucose load from the OGTT than to the study intervention itself. A major concern in eTRE trials is whether there is real-world feasibility of this dietary intervention given the preponderance of social behaviors that favor late-night eating. While it is difficult to obtain evidence for or against real-world feasibility in a highly controlled supervised feeding trial such as ours, our participants did not report any subjective differences in hunger, satiety, or fullness after one week of eTRE compared to baseline or after one week on the control arm. Thus, at least these indices of dietary satisfaction would likely not impact real-world adherence. Many questions remain as to whether individuals need to be fully compliant with this dietary practice to reap the metabolic benefits or whether some degree of noncompliance is acceptable. Recent studies have reported metabolic benefits with 5.0–6.4 days/week adherence[19,21,22] indicating that there likely is some flexibility, though more rigorous trials correlating daily adherence to metabolic outcomes need to be pursued.

Strengths of this study include the demographics of our participant pool, which included equal numbers of males and females and an ethnically diverse study population. The crossover study design enhanced statistical power even with a relatively small subject volume. The limitations include the short study duration and small sample size. We opted not to include a washout period in our study design in order to keep the study duration within one CGM cycle (14 days) to reduce costs, mitigate inter-device discrepancies, and enhance participant retention. Additionally, there was no run-in period, and, while efforts were made to match the study diet with usual dietary composition, baseline timing of food consumption was not assessed. For these reasons, the UFP arm may not have been fully reflective of participants' usual eating practices. Though similar to other supervised feeding trials, these results need to be replicated in larger trials of longer duration to determine the true impact of the eTRE intervention. While the highly controlled supervised nature of our study enabled confidence in dietary compliance, it did not allow for understanding of real-world feasibility of eTRE, which still needs to be determined. We did not measure differences in energy expenditure at baseline or between arms, so we cannot

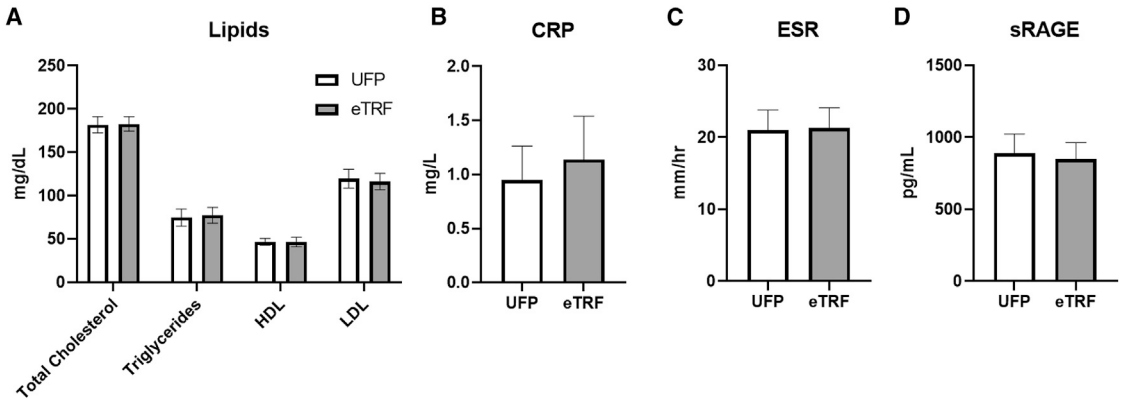

Figure 4. Impact of eTRE on Cholesterol and Inflammatory Markers

eTRE does not affect (A) lipid levels or the inflammatory markers (B) CRP, (C) ESR, or (D) sRAGE. Values are mean ± SEM. eTRE, early time-restricted eating; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; sRAGE, soluble receptor for advanced glycation end products.

speak to whether differences in this may have contributed to changes in glycemia. However, there were no differences in participants' step count between eTRE and UFP periods, indicating similar activity levels during the two dietary arms (Table S5). Additionally, a linear mixed effects model identified self-reported sex as having a significant association with improved glycemia independent of dietary intervention, with males having lower overall and daytime $TIR_{140\text{-}180}$ than females. There were no significant baseline differences in markers of metabolic health (HbA1c, fasting glucose) or inflammation (CRP, ESR, sRAGE) between the two sexes (data not shown) that would explain this phenomenon. Possible confounders that could have led to this observed effect include significant differences in activity level or sleep habits between genders, inaccurate reporting of baseline diets resulting in significant differences in macronutrient or caloric content between the study diet and participants' home diet, or other differences in baseline characteristics that were not identified at study outset.

Taken together, our data support the use of eTRE for improved glycemia and as a weight-neutral candidate dietary strategy for diabetes prevention in at-risk individuals including those with prediabetes and metabolic disease. Current recommendations for diabetes prevention involve a multimodal approach of intensive lifestyle behavior change to induce a weight loss of 7%–10% of initial body weight.[62] While this is achievable using a wide range of available treatment modalities, long-term studies show that a majority of patients experience weight regain and progression or recurrence of their metabolic disease.[63–66] Our results indicate that the glycemic benefits induced by eTRE do not require weight loss or changes in dietary composition, positioning it as an attractive adjuvant or alternative approach that can be added to the therapeutic armamentarium for the management and prevention of dysglycemia. Future studies should be aimed at elucidating the mechanisms driving the beneficial effects of early meal timing on metabolic health and on translating the current body of work into real-world studies that can inform on the feasibility and generalizability for the treatment of metabolic disease in the general population.

Limitations of the study

This study had several limitations that must be addressed in the future. First, these results need to be replicated in a larger trial of longer duration to determine the true impact of eTRE on dysglycemia. Second, it does not address the real-world feasibility of eTRE, which needs to be assessed further.

RESOURCE AVAILABILITY

Lead contact

Requests for further information and resources should be directed to and will be fulfilled by the lead contact, Joanne Bruno (joanne.bruno@nyulangone.org).

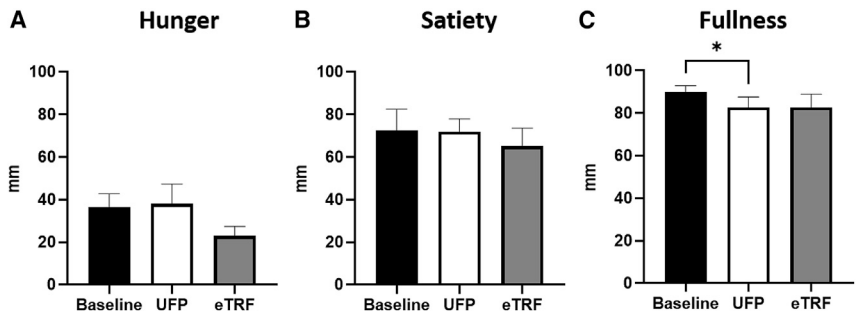

Figure 5. eTRE does not impact participants' feeding motivation measures

Participants rated their (A) hunger, (B) satiety, and (C) fullness at baseline and after 6 days on the UFP and eTRE dietary arms on a 0–100 mm visual analog scale, ranging from "Not at All" (0 mm) to "Extremely" (100 mm). Values are mean ± SEM. *$p \leq 0.05$. eTRE, early time-restricted eating; UFP, usual feeding practice.

Materials availability

This study did not generate unique reagents.

Data and code availability

- No original code was generated for this study.
- All code used for analysis was properly cited in the STAR Methods.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

J.B.: investigation, writing – original draft, writing – review and editing, and visualization. J.M.W.: conceptualization, investigation, screening, clinical management, resources, and writing – review and editing. S.N.: investigation and writing – review and editing. D.U.: software, formal analysis, visualization, and writing – review and editing. S.M.V.: investigation, resources, and writing – review and editing. C.J.P.: investigation and writing – review and editing. S.B.: conceptualization, investigation, software, formal analysis, visualization, and writing – review and editing. J.O.A.: conceptualization, methodology, investigation, resources, writing – review and editing, visualization, supervision, project administration, management and coordination responsibility for the research activity planning and execution, and funding acquisition.

DECLARATION OF INTERESTS

J.O.A. is currently acting as a consultant for Novo Nordisk and formerly served as a chair on Novo Nordisk's Data and Safety Monitoring Board without compensation.

STAR★METHODS

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

- KEY RESOURCES TABLE
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
- METHOD DETAILS
  - Patient recruitment
  - Study design
  - Diets
  - Compliance monitoring
  - OGTTs
  - Continuous glucose monitor analysis
  - Human sRAGE measurement
- QUANTIFICATION AND STATISTICAL ANALYSES
  - Power and sample size
  - Randomization procedures
  - Statistical analysis
- ADDITIONAL RESOURCES

SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Critical commercial assays		
Human RAGE ELISA Kit - Quantikine	R&D Systems	DRG00
Software and algorithms		
easyGV	Hill et al.[67]	https://www.phc.ox.ac.uk/research/resources/easygv
iGLU	Brol et al.[68]	https://irinagain.github.io/iglu/
Other		
Freestyle Libre Pro Sensor Kit	Abbott	7156201

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

This was a single-center study performed at The Rockefeller University Hospital between June 2021 and August 2022. A total of 30 participants were originally recruited from the community through advertising or participation in The Rockefeller University Hospital registry of participants previously screened for research studies. Among the 30 participants, 20 were excluded from the study based on the inclusion criteria. A total of 10 participants were included in the final analysis. Participant randomization was done by the research pharmacist and the investigator, and participants were blinded to randomization prior to study initiation. There were 5 females and 5 males. Throughout the text, when sex is mentioned, the term sex assigned at birth is meant. The influence of sex or gender on the study was not explicitly measured. Age ranged from 42 to 69 years old. Eight subjects identified as African American and two as Caucasian. Other racial or ethnic information was not registered. More details are presented in Table 1. The Rockefeller University Institutional Review Board approved the study (Data S1, protocol number: JAL-1007) and written consent was obtained from all participants.

METHOD DETAILS

Patient recruitment

Eligible participants were adults aged 18 to 75 with a BMI > 25 kg/m^2 and prediabetes, defined as hemoglobin A1c between 5.7-6.4%. We excluded participants with diabetes; HIV positivity; hepatitis; self-reported autoimmune diseases; smokers; those currently taking metformin, systemic steroids, weight loss medication, any medication with significant weight effects (i.e., tricyclic antidepressants, certain SSRIs, lithium, antipsychotics, etc), or other medications that could affect study endpoints; those with allergies to adhesive tape; current pregnancy; irregular sleep schedules; or who endorsed adherence to any intermittent feeding diet within the prior 2 weeks. The Institutional Review Board approved the study and written consent was obtained from all participants (Data S1). Participants were compensated $1500 upon completion of the study. As shown in Figure S1, thirty participants were screened. Of these, most were excluded due to not meeting the hemoglobin A1c criteria. Ten participants meeting all inclusion criteria were evaluated, enrolled, and completed the study. Participants underwent a complete medical examination, standard blood and urine tests, and an electrocardiogram. All were found to be healthy prior to study enrollment.

Study design

The trial was conducted as a randomized, crossover, supervised feeding study. Participants were randomized to initially follow either a usual feeding pattern (UFP; 50% of calories consumed after 16:00h) or an eTRE feeding pattern (8 hour feeding window from 08:00-16:00h; 80% of calories consumed prior to 14:00h) for six days. Afterwards they completed a day of testing before crossing over to the other arm. Participants were provided with three meals per day and all meals were prepared in a metabolic kitchen in order to ensure adherence to the calorie distribution requirements of the assigned dietary arm. Participants were allowed some customization of meal timing within the study constraints and were instructed to consume all of the food provided. For instance, on the UFP arm, participants could choose when to eat breakfast and lunch, however the dinner meal was designed to be 50% of the total daily caloric intake and had to be consumed after 16:00h. On the eTRE arm, breakfast and lunch were designed to be 80% of the total daily caloric intake and had to be consumed prior to 14:00h while dinner had to be consumed between 14:00-16:00h.

Each arm of the trial lasted 7 days (Monday-Sunday). On Day 1 participants underwent baseline testing including a 2-hour OGTT, and collection of blood to measure fasting lipids and inflammatory markers. Fasting labs were obtained at 8AM after an overnight fast;

duration of fast varied between UFP and eTRE arms due to differences in timing of the dinner meal the day prior. Continuous glucose monitors (Abbott Freestyle Libre Pro) were placed on all participants. Participants wore pedometers throughout the study and daily step counts are reported. On Days 2-7, participants followed their assigned dietary schedule. Baseline tests were repeated on Day 8. Participants subsequently crossed over to the other study arm for days 9-14. Baseline tests were repeated on Day 15, at which point continuous glucose monitors were removed. CGM data were downloaded nightly to ensure continued functionality of the devices. On Days 1, 8, and 15 participants' hunger, satisfaction, and fullness levels over the preceding week were assessed using visual analogue scales. Participants remained inpatient at The Rockefeller University Hospital for Days 1-5 and 8-12 of the study. They were allowed to return home on Days 6-7 and 13-14 with all food provided to them.

Fasting and OGTT blood samples were analyzed in the Clinical Pathology Laboratory of Memorial Sloan-Kettering Cancer Center for electrolyte levels, liver function, renal function, lipid profile, hemoglobin A1c, plasma glucose, CRP levels, and ESR levels. Aliquots of plasma, serum, urine, and stool were stored at -80°C for future analysis including sRAGE measurement as described below. Baseline body composition was measured by air-displacement plethysmography using the BodPod system (COSMED, Italy).

Diets

Prior to study onset, participants were interviewed regarding their dietary history via the Vioscreen Food Frequency Questionnaire (FFQ)[69] and study diets were engineered to align with an individual's home diet macronutrient content and food preferences as closely as possible. All food was prepared by The Rockefeller University Hospital metabolic kitchen using a 3-day rotating menu that was selected by participants from a set of defined options and then edited by the Bionutrition team to ensure alignment with the macronutrient profile and total daily caloric content of the subject-reported home diet that was described in the FFQ. Participants received three meals every day regardless of dietary arm. Food composition was matched between trial arms so that the only difference between the two arms was meal and caloric timing as described above. Participants were fed at least enough calories for weight maintenance as calculated using the Harris-Benedict formula and daily calorie intake was typically in excess of what would be expected for weight maintenance in order to conform to baseline patient-reported daily caloric intake. Participants were instructed to eat all meals and were not allowed to eat any non-study foods or deviate from meal timing as outlined in the study protocol. To ensure weight maintenance and avoid any confounding effects of weight loss on study results, participants were weighed daily for the duration of the study.

Compliance monitoring

To promote compliance, study participants spent the first four days of each study arm at The Rockefeller University Hospital where their dietary intake and meal timing was supervised. Participants returned home for the final two days of each study arm and were provided with all of the food that they would need to consume over this time period along with detailed instructions on when they should consume it. These instructions were reinforced by the study bionutritionist prior to discharge. Self-reported compliance was assessed by study staff on return and CGM data was downloaded on return.

OGTTs

Blood samples were collected from participants after an overnight fast. Participants then consumed 75 grams of glucose in 10 oz of liquid (Glucola) within 5 minutes. For the 2-hour OGTTs administered at baseline and on study testing days, the ingestion of glucose occurred between 09:00-10:00h. Blood was collected at 30, 60, 90, and 120 minutes post glucose ingestion. AUC was calculated for each plasma glucose curve.

Continuous glucose monitor analysis

Participants wore Freestyle Libre Pro continuous glucose monitors (Abbott, Abbott Park, IL, USA) for 2 weeks. Participants were blinded to CGM data for the duration of the study. No glucometer calibration was required. CGM data were downloaded nightly to ensure continued device functionality, and at study completion. These data were used to compute the following measures: mean glucose level, standard deviation of glucose, mean amplitude of glycemic excursion, time spent in hypoglycemic range (percent time in range < 70 mg/dL), time spent in euglycemic range (percent time in range 70-140 mg/dL), time spent in mild hyperglycemic range (percent time in 140-180 mg/dL), and time spent in severe hyperglycemic range (percent time > 180 mg/dL). Mean amplitude of glycemic excursion was calculated using an open-source Excel enabled workbook called EasyGV.[67] To confirm accuracy of these calculations, MAGE was also calculated using the R package iGlu and results were highly correlated with EasyGV calculated values (Spearman correlation coefficient 0.899).[68] We further stratified the time in 140-180 mg/dL range by daytime (6:00-23:59h) and overnight (00:00-05:59h) components to examine if any differences in hyperglycemia between the study arms was driven by postprandial glucose or fasting glucose. Using linear interpolation, all CGM readings for a given day were interpolated to readings at 15-minute intervals starting at midnight until midnight the following day. This was done to ensure standardized comparison of daytime, nighttime, and overall time in range measures across participants.

Human sRAGE measurement

Plasma total sRAGE concentrations were measured using a commercially available ELISA assay (R&D Systems, DRG00). Each unique sample was analyzed in duplicate (50 μL/replicate). The mean minimum detectable dose was 4.12 pg/mL, with an assay range

of 78-5000 pg/mL. The intra-assay precision (CV) was ~5.9% and the inter-assay precision (CV) was ~7.7%. Standards were included in each experiment as per the manufacturer's instructions.

QUANTIFICATION AND STATISTICAL ANALYSES

Power and sample size

The primary end points of this study were to determine whether the intervention would significantly alter glycemic variation (measured by mean amplitude of glycemic excursion, MAGE) and inflammatory marker concentrations (sRAGE and CRP). A sample size of 10 subjects was prospectively determined to be sufficient to detect a 20% between-group difference in MAGE at 80% power and significance (alpha) level less than 0.05, consistent with previous reports.[24,31] This sample size also allowed for detection of a difference in sRAGE levels of approximately 182 pg/ml with 80% power assuming an alpha level of 0.05 and SD of 200 pg/ml.

Randomization procedures

Participants were randomly assigned to the UFP and eTRE groups in a 1:1 ratio. Participant randomization was done by the research pharmacist and the investigator, and participants were blinded to randomization prior to study initiation. However, given the nature of the intervention the assigned study arm was obvious upon commencement.

Statistical analysis

Patient demographics and metabolic profiles are presented as mean ± standard deviation for continuous variables and count for categorical variables in Table 1. Reported metrics were evaluated for normality and those with non-normal distribution are reported as mean ± standard deviation as well as median [IQR]. We performed pairwise comparisons of CGM-derived measures, OGTT values, lipid levels, inflammatory marker levels, and feeding motivation measures between eTRE and UFP arms using a paired t-test. We used linear mixed-effects models to estimate the relationship between the intervention and the time in 140-180 mg/dL range outcomes (overall and daytime) adjusted for age, self-reported sex, baseline BMI, and order of intervention. Participant ID was treated as a random effect. We also included an interaction term between the intervention arm and order of intervention to examine whether the order of the intervention modulated the association between the intervention arm and the time in range outcomes. Error bars in the figures are presented as SEMs.

ADDITIONAL RESOURCES

The study was approved by the institutional review board at The Rockefeller University and registered under ClinicalTrials.gov identifier NCT04884659.