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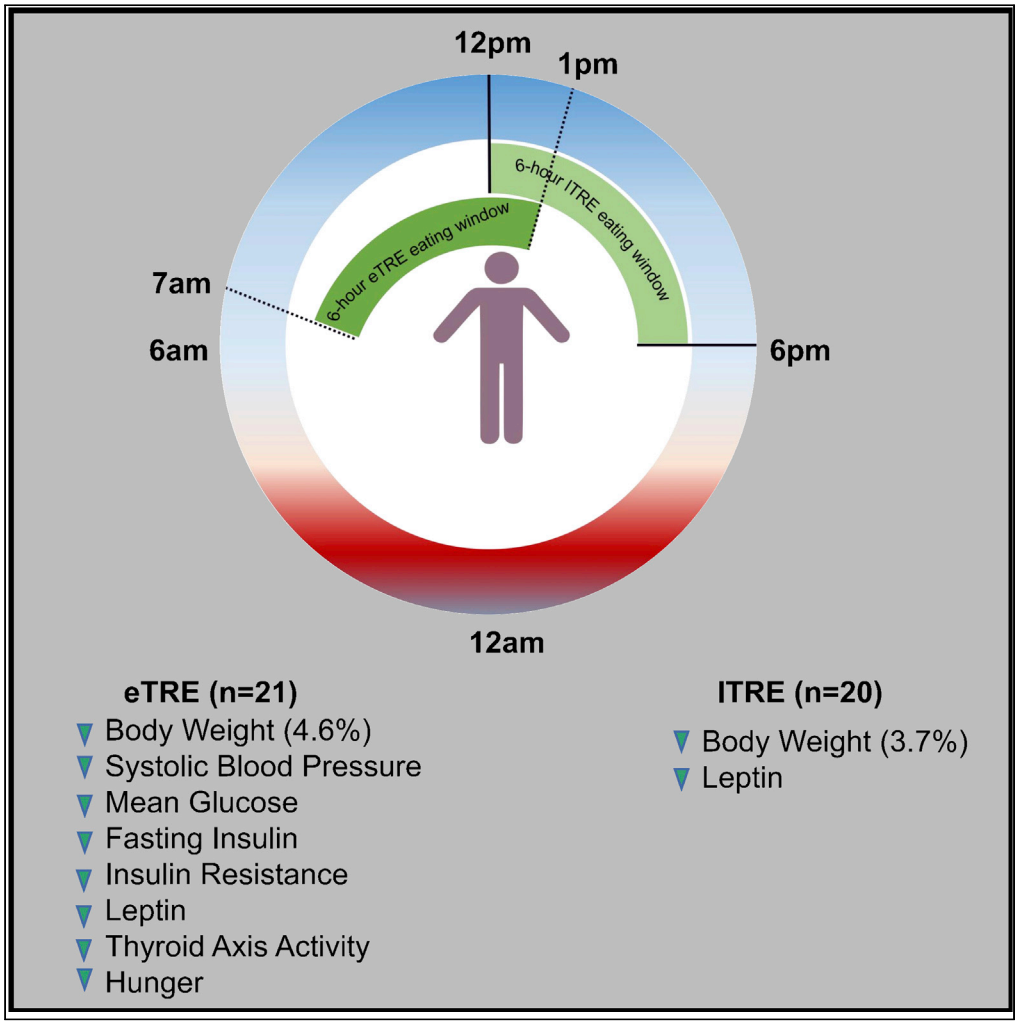

Li-min Zhang, Zhan Liu, Jia-qi Wang, ..., Hui-chen Zhu, Yu-tian Zhou, Yu-xia Ma

mayuxia@hebmu.edu.cn

Highlights

6-h eTRE and ITRE regimens were evaluated in 60 overweight and obese young adults

The duration from the last meal to the measurement for eTRE is much longer than ITRE

Both regimens produce similar body weight loss over the 8 weeks of the study

ETRE, but not ITRE, reduced blood pressure, mean glucose, and insulin resistance

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Randomized controlled trial for time-restricted eating in overweight and obese young adults

Li-min Zhang,[1] Zhan Liu,[1] Jia-qi Wang,[1] Rui-qiang Li,[1] Jing-yi Ren,[1] Xian Gao,[1] Shuai-shuai Lv,[1] Lu-yao Liang,[1] Fan Zhang,[1] Bo-wen Yin,[1] Yan Sun,[1] Hao Tian,[1] Hui-chen Zhu,[1] Yu-tian Zhou,[1] and Yu-xia Ma[1,2,*]

SUMMARY

Time-restricted eating (TRE) is known to improve metabolic health, whereas very few studies have compared the effects of early and late TRE (eTRE and lTRE) on metabolic health. Overweight and obese young adults were randomized to 6-h eTRE (eating from 7 a.m. to 1 p.m.) (n = 21), 6-h lTRE (eating from 12 p.m. to 6 p.m.) (n = 20), or a control group (*ad libitum* intake in a day) (n = 19). After 8 weeks, 6-h eTRE and lTRE produced comparable body weight loss compared with controls. Compared with control, 6-h eTRE reduced systolic blood pressure, mean glucose, fasting insulin, insulin resistance, leptin, and thyroid axis activity, whereas lTRE only reduced leptin. These findings shed light on the promise of 6-h eTRE and lTRE for weight loss. Larger studies are needed to assess the promise of eTRE to yield better thyroid axis modulation and overall cardiometabolic health improvement.

INTRODUCTION

Overweight and obesity have become one of the greatest public health challenges worldwide, with an increasing prevalence (Bessesen and Van Gaal, 2018). According to the World Health Organization report, more than 39% of adults aged 18 years and older were overweight in 2016, of which approximately 13% of adults were obese (WHO). If the upward trend continues, global obesity prevalence is estimated to reach 18% in men and 21% in women by 2025 (NCD Risk Factor Collaboration (NCD-RisC), 2016). Obesity is associated with an increased risk for a variety of chronic diseases, such as diabetes, hypertension, cardiovascular and kidney diseases, musculoskeletal disorders, and some cancers (Guidelines (2013); NCD Risk Factor Collaboration (NCD-RisC), 2016; Berrington de Gonzalez et al., 2010; Lu et al., 2014; Ni Mhurchu et al., 2004; Whitlock et al., 2009; Wormser et al., 2011), decreased quality of life and life expectancy and substantial economic burden (Blüher, 2019; Williams et al., 2015).

The first line of therapy for obesity is lifestyle interventions including increased physical activity and decreased caloric intake (Jensen et al., 2014; Yannakoulia et al., 2019). Recently, intermittent fasting (IF)—the paradigm of alternating periods of eating and fasting—has emerged as a promising dietary strategy for assisting in the treatment not only of obesity but also of metabolic diseases relevant to excess weight (de Cabo and Mattson, 2020; Cioffi et al., 2018; Longo and Mattson, 2014; Mattson et al., 2017; Patterson and Sears, 2017). IF is an umbrella term for several general types of fasting regimens, including fasting 2 days each week, alternate day fasting, and time-restricted eating (TRE). Among the various protocols of IF, TRE has recently received increasing attention from researchers and public health professionals due to its obvious effectiveness, safety, and compliance. TRE, which is characterized by limiting *ad libitum* eating window to a certain number of hours each day (usually 4 to 10 h) and fasting (with zero-calorie beverages) for the remaining hours of the day, is practiced with or without altering diet quality and quantity (Lundell et al., 2020; Sutton et al., 2018).

Most clinical trials (Anton et al., 2019; Chow et al., 2020; Cienfuegos et al., 2020; Gabel et al., 2018, 2020; Gill and Panda, 2015; Peeke et al., 2021; Wilkinson et al., 2020), but not all (Lowe et al., 2020), demonstrated that TRE with the feeding window ranging from 4 h to 10 h may inadvertently decrease energy intake by 20%–30%, producing mild-to-moderate weight loss of 1%–4%. In addition to weight loss, TRE may improve some aspects of cardiometabolic health (Gabel et al., 2018; Jamshed et al., 2019; Martens et al., 2020; Parr et al., 2020; Sutton et al., 2018; Wilkinson et al., 2020). TRE appears to improve glucose, insulin, insulin sensitivity, β cell responsiveness, and blood pressure (Cienfuegos et al., 2020; Gabel et al., 2018; Jamshed

[1]Department of Nutrition and Food Hygiene, School of Public Health, Hebei Medical University, Hebei Key Laboratory of Environment and Human Health, Shijiazhuang 050017, China

[2]Lead contact

*Correspondence: mayuxia@hebmu.edu.cn

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et al., 2019; Martens et al., 2020; Parr et al., 2020; Wilkinson et al., 2020), even independence of weight loss (Sutton et al., 2018). However, the effects of TRE on plasma lipid levels remain unclear. Most studies reported that TRE has no effect on lipid levels (Cienfuegos et al., 2020; Gabel et al., 2018; Lowe et al., 2020; Sutton et al., 2018; Tinsley et al., 2019). Although some studies showed improvements in low-density lipoprotein cholesterol (LDL-C) (Karras et al., 2021; Wilkinson et al., 2020) and triglyceride (TG) (Chow et al., 2020; Zeb et al., 2020), others found a harmful response to lipid metabolism (Jamshed et al., 2019; Parr et al., 2020; Sutton et al., 2018).

The circadian system orchestrates 24 h rhythms in biological, metabolic, and behavioral processes that are continued under constant light/dark, sleep/awakening, and feeding/fasting conditions (Longo and Panda, 2016). It regulates glucose, lipid, and energy metabolism, increasing them at certain times of the day and decreasing them at others. In nocturnal animal models, 6-h eTRF (initiated at lights off) and 6-h dTRF (initiated after a 6-h delay at lights off) groups decreased hepatic lipid accumulation, plasma cholesterol levels, and adipose tissue inflammation compared with the controls (Delahaye et al., 2018). In addition, the dTRF group gained less weight compared with the controls but gained more weight than the eTRF group (Delahaye et al., 2018). Appropriate nutrition, where energy intake is aligned with energy expenditure and clear feeding/fasting cycles, should be synchronized with clock-regulated metabolic changes.

However, whether the timing of the eating window (early versus late in the day) during TRE influences weight reduction and cardiometabolic health in humans remains unclear. Few studies have examined the impact of eating time window on cardiometabolic outcomes (Hutchison et al., 2019b; Wijayatunga et al., 2020). However, those studies were conducted in small numbers of subjects, which might have limited their ability to detect statistically significant effects. Therefore, we performed a randomized controlled clinical trial to evaluate the effects of 6-h eTRE (*ad libitum* intake from 7 a.m. to 1 p.m.) and 6-h lTRE (*ad libitum* intake from 12 p.m. to 6 p.m.) versus a control group (*ad libitum* intake in a day) on body weight loss and comprehensive cardiometabolic outcomes in young adults with overweight and obesity. We also investigated the compliance and feasibility of 6-h eTRE and lTRE and the occurrence of adverse events. We hypothesized that the 6-h eTRE group would produce greater body weight loss and improvements in cardiometabolic parameters compared with the 6-h lTRE group and controls.

RESULTS AND DISCUSSION

We performed a 10-week randomized controlled clinical trial to compare the effects of 6-h eTRE and 6-h lTRE versus controls on body weight and cardiometabolic parameters in 60 overweight and obese young adults. In brief, the trial comprised a 2-week baseline period and an 8-week TRE intervention period. During the 8-week intervention, the 6-h eTRE group was directed to eat *ad libitum* from 7:00 a.m. to 1:00 p.m. daily and completely fast from 1:00 p.m. to 7:00 a.m. the following day (Figure 1A). The 6-h lTRE group was directed to eat *ad libitum* from 12:00 p.m. to 6:00 p.m. daily and completely fast from 6:00 p.m. to 12:00 p.m. the following day. During the 6-h eating windows, participants were not demanded to supervise caloric intake without constraints on the quality or quantity of foods consumed. Only water and energy-free beverages (black tea, coffee, and diet sodas) were allowed outside of the eating widow. Controls were directed to maintain their eating or physical activity habits throughout the trial without receiving food or dietary counseling. The primary outcome was change in body weight, whereas the secondary outcomes were cardiometabolic parameters, metabolic hormone, and markers of inflammatory cytokines and oxidative stress.

Participants

As shown in Figures 1B, 89 individuals expressed interest in attempting the trial, among which 26 were excluded from the study based on the inclusion criteria. Three subjects withdrew from the study in the baseline period, among which two were hospitalized and one reported muscle discomfort associated with implantation and use of continuous glucose monitor (CGM). A total of 60 participants were included in the final analysis. Table 1 displays the baseline characteristics of the 6-h eTRE, 6-h lTRE, and control groups. At baseline, no significant differences were observed between the 6-h eTRE, 6-h lTRE, or control groups for the primary outcome measure (body weight) or any secondary outcome measure.

Impact of 6-h eTRE and 6-h lTRE on caloric intake, physical activity, and sleep

The 6-h eTRE group (−554.1 kcal/day [95% confidence interval [CI]: −695.3 to −412.9]) and 6-h lTRE group (−407.0 kcal/day [95%CI: −548.5 to −265.5]) experienced greater reductions in energy intake than the

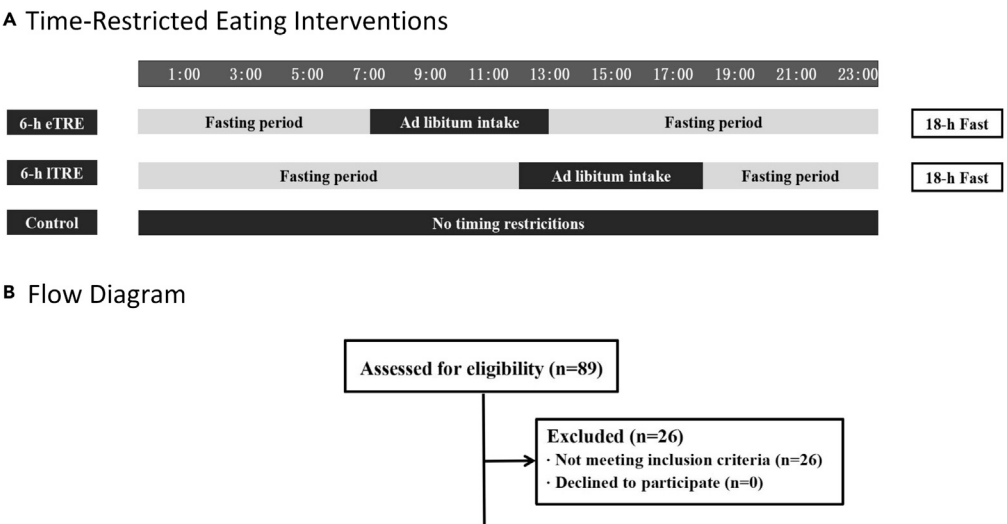

Figure 1. Time-restricted eating (TRE) interventions and CONSORT flow diagram

(A) TRE interventions. Participants were randomized to a control group (*ad libitum* intake), 6-h eTRE group (*ad libitum* intake from 7:00 a.m. to 13:00 p.m.), or 6-h lTRE group (*ad libitum* intake from 12:00 p.m. to 18:00 p.m.).

(B) CONSORT flow diagram describing the process of participant enrollment, intervention, and data analysis.

control group (−112.1 kcal/day [95%CI: −253.4 to 29.2]), with no significant difference between the intervention groups (Figure S1A). Participants seem to spontaneously consume less energy during TRE, although there were no recommendations to change dietary quantity or quality, which is consistent with other trials (Antoni et al., 2018; Cienfuegos et al., 2020; Gabel et al., 2018; Gill and Panda, 2015). The carbohydrate intake reduced in 6-h eTRE group more than the 6-h lTRE and control groups (Figure S1B). The 6-h eTRE group had more protein reduction than the control group, whereas the 6-h lTRE group had more fat reduction than the control group (Figures S1C and S1D). Carbohydrate reduction was correlated with fasting interval change ($r = -0.331$, $p = 0.012$), but protein and fat reductions were not significantly correlated with fasting interval change. Changes in physical activity and PSQI showed no significant difference across the three groups (Figures S1E and S1F).

Both 6-h eTRE and 6-h lTRE decreased body weight

At the end of the study, there was a trend for a greater reduction in body weight in 6-h eTRE group (−4.6% [95%CI: −5.5 to −3.8]) compared with the 6-h lTRE group (−3.7% [95%CI: −4.6 to −2.9]), but this was not statistically significant (Figure 2A). The degree of body weight loss observed in our study is on par with the effects of TRE in other studies, which reported weight loss of 1%–4% (Anton et al., 2019; Antoni et al., 2018; Arnason et al., 2017; Chow et al., 2020; Cienfuegos et al., 2020; Gabel et al., 2018, 2020; Gill and Panda, 2015; Hutchison et al., 2019a; Kesztyüs et al., 2019; Wilkinson et al., 2020). The differences in body weight loss were marginally correlated with the changes in energy intake ($r = 0.227$, $p = 0.089$) and carbohydrate ($r = 0.254$, $p = 0.057$), suggesting that the differences in body weight loss between the intervention groups may be partly ascribed to the differences in energy intake and carbohydrate. Greater

Table 1. Baseline characteristics

	Control (n = 19)	eTRE (n = 21)	lTRE (n = 20)	p Value
Basic Information				
Age (years)	22.1 ± 0.4	23.8 ± 0.6	23.2 ± 0.5	0.154
Gender (men)	10 (52.6)	12 (57.1)	11 (55.0)	0.960
Weight and Body Composition				
Weight (kg)	77.1 ± 3.4	78.0 ± 2.9	83.5 ± 4.1	0.468
Waist circumference (cm)	89.2 ± 2.7	91.0 ± 1.9	92.9 ± 2.6	0.551
Hip circumference (cm)	105.3 ± 1.3	105.1 ± 1.4	107.7 ± 1.5	0.338
Waist/Hip	0.8 ± 0.02	0.9 ± 0.01	0.9 ± 0.02	0.595
BMI (kg/m^2)	27.8 ± 0.8	27.1 ± 0.7	28.5 ± 0.8	0.326
Lean mass (kg)	48.5 ± 2.7	49.0 ± 2.4	51.8 ± 3.0	0.619
Body fat (kg)	25.7 ± 1.5	25.6 ± 1.4	28.3 ± 1.7	0.373
Percent body fat (%)	33.6 ± 1.6	33.0 ± 1.3	34.1 ± 1.3	0.851
Visceral fat area (cm^2)	117.7 ± 8.4	118.9 ± 7.4	131.3 ± 8.8	0.452
Cardiometabolic Factors				
Fasting blood glucose (mg/dL)	80.6 ± 1.3	82.3 ± 1.4	82.8 ± 1.9	0.793
Mean blood glucose (CGM) (mg/dL)	87.9 ± 1.3	90.1 ± 1.1	90.0 ± 2.8	0.306
HbA1c (%)	5.3 ± 0.05	5.5 ± 0.04	5.5 ± 0.10	0.055
C-P (ng/mL)	2.8 ± 0.2	3.4 ± 0.3	3.3 ± 0.2	0.164
Fasting insulin (mIU/mL)	10.3 ± 1.6	12.4 ± 1.6	12.2 ± 1.7	0.589
HOMA-IR	2.1 ± 0.4	2.6 ± 0.4	2.6 ± 0.4	0.546
Metabolic syndrome score	−0.2 ± 0.8	1.2 ± 0.6	1.2 ± 0.9	0.338
Systolic BP (mmHg)	118.5 ± 3.4	122.0 ± 3.4	121.1 ± 2.9	0.738
Diastolic BP (mmHg)	72.3 ± 2.1	73.7 ± 1.9	69.9 ± 1.9	0.394
Pulse pressure (mmHg)	46.3 ± 2.1	48.4 ± 2.3	51.2 ± 2.5	0.324
Heart rate (bpm)	73.5 ± 1.7	73.5 ± 3.3	72.4 ± 2.2	0.797
TC (mg/dL)	178.3 ± 5.4	164.7 ± 7.3	164.0 ± 6.4	0.230
TG (mg/dL)	88.1 ± 11.8	84.2 ± 7.6	92.8 ± 11.3	0.928
LDL-C (mg/dL)	105.2 ± 3.8	97.5 ± 5.0	95.9 ± 4.4	0.307
HDL-C (mg/dL)	45.7 ± 2.0	39.8 ± 1.3	41.1 ± 2.0	0.058
Leptin (ng/mL)	17.7 ± 3.2	16.0 ± 2.7	17.0 ± 2.3	0.872
Adiponectin (μg/mL)	5.2 ± 0.7	4.1 ± 0.7	4.8 ± 1.0	0.167

BMI, body mass index; CGM, continuous glucose monitor; HbA1c, hemoglobin A1c; C-P, C-peptide; HOMA-IR, homeostasis model assessment-insulin resistance [calculated as (fasting glucose*fasting insulin)/405]; BP, blood pressure; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. Values are expressed as raw mean ± SEM. p value between groups for "all participants:" ANOVA (Gaussian distribution) or Kruskal–Wallis test (non-Gaussian distribution) for continuous variables and McNemar's test or Fisher test for categorical variables.

weight loss is associated with reduced carbohydrate diets (Bogardus et al., 1981; Hall et al., 2015; Rabast et al., 1981).

The 6-h eTRE and lTRE resulted in greater reduction in fat mass and visceral fat area by week 8 compared with the control group (Figures 2B and 2C; Table 2). Some of the fat mass loss was from the abdomen because we observed a significant decrease in waist circumference (Table 2) and visceral fat area. Excessive visceral adiposity, considered to be lipotoxic, is associated with cardiometabolic risk factors and clinical cardiovascular disease (Kang et al., 2011; Kelli et al., 2017). The observed changes in the visceral fat area indicate that TRE can reduce the risk of future adverse cardiovascular events even in healthy young adults. Lean mass is associated with physical and nutritional condition, and the extent of lean mass loss during weight loss has been positively correlated with weight regain (Pasiakos et al., 2013). Lean mass loss in

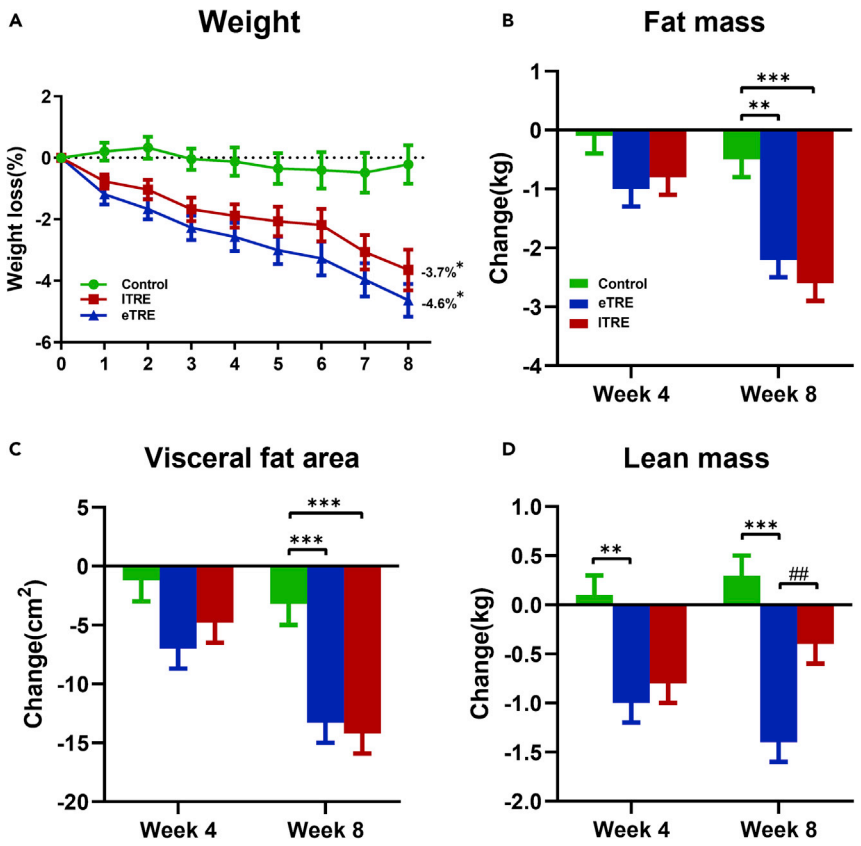

Figure 2. Weight and body composition

(A–D) Changes in percentage weight loss (A), fat mass (B), percent body fat (C), and lean mass (D) after 4 and 8 weeks of intervention. Data are presented as least squares mean ± SEM; *p < 0.05 versus control group, **p < 0.01 versus control group, ***p < 0.001 versus control group, ##p < 0.01 versus eTRE group. See also Figure S1 and Tables S2 and S4.

the eTRE group was greater than that in lTRE group at week 8 (p = 0.008) (Figure 2D; Table 2). In present study, the measurement of lean mass, which is predominantly composed of muscle mass and body water, is affected by fluid and electrolyte distribution (Jayanama et al., 2018; Ritz et al., 2007). Weight loss in obese people often improves lymphatic circulation and results in loss of fluid mass. Therefore, the differences in lean mass loss between the two intervention groups may be partly explained by the differences in fluid mass reduction. Cienfuegos et al. reported that the 6-h TRE group lost significantly more lean mass than the control group and the 4-h group, but the cause remains unknown (Cienfuegos et al., 2020). The proportion of lean mass loss in the 6-h eTRE group (approximately 40% of total weight loss) exceeded the normal range of 20%–30% from other studies (Bradley et al., 2012; Magkos et al., 2016; Santanasto et al., 2011; Verreijen et al., 2015). The proportion of lean mass loss in another study was approximately 65% (Lowe et al., 2020). Future studies should explore the normal range of reduction in lean mass caused by TRE and the influence of protein intake on lean mass.

6-H eTRE, but not 6-h lTRE, reduced mean glucose, fasting insulin, and insulin resistance

Changes in fasting glucose and hemoglobin A1c (%) were not significantly different across the three groups at weeks 4 and 8 (Figure 3A; Table 2). By contrast, the mean glucose significantly decreased in the 6-h eTRE group (−2.3 mg/dL [95% CI: −4.3 to −0.3]), whereas it increased in the 6-h lTRE (2.5 mg/dL [95% CI: 0.4 to 4.7]) and control group (2.5 mg/dL [95% CI: 0.4 to 4.6]) (Figure S2D; Table 2). At the end of the study, 6-h eTRE, but not lTRE, resulted in greater reductions in fasting insulin, C-peptide, and insulin resistance compared with the control group (Figure 3A; Table 2).

Eight weeks of 6-h eTRE and lTRE did not improve fasting glucose levels, which is consistent with previous reports in other human trials (Anton et al., 2019; Cienfuegos et al., 2020; Gabel et al., 2018; Karras

Table 2. Changes in body composition and cardiometabolic risk factors from baseline to end of 4 and 8 weeks of time-restricted eating

	4 weeks change				8 weeks change			
	Control (n = 19)	eTRE (n = 21)	lTRE (n = 20)	p value	Control (n = 19)	eTRE (n = 21)	lTRE (n = 20)	p value
Weight (kg)	−0.01 (−0.7 to 0.7)	−1.9 (−2.6 to −1.3)[a]	−1.5 (−2.1 to −0.8)[a]	0.0003	−0.2 (−0.9 to 0.5)	−3.5 (−4.2 to −2.9)[a]	−2.9 (−3.6 to −2.3)[a]	< 0.0001
Waist circumference (cm)	−0.5 (−2.1 to 1.1)	−4.2 (−5.7 to −2.7)[a]	−1.9 (−3.5 to −0.4)	0.004	−1.4 (−3.0 to 0.1)	−5.6 (−7.1 to −4.1)[a]	−3.9 (−5.4 to −2.4)	0.001
Hip circumference (cm)	0.6 (−0.5 to 1.8)	−1.9 (−2.9 to −0.8)[a]	−1.6 (−2.7 to −0.5)[a]	0.004	−0.6 (−1.7 to 0.6)	−3.0 (−4.1 to −2.0)[a]	−2.3 (−3.4 to −1.2)	0.007
Waist/Hip ratio	−0.01 (−0.03 to 0.004)	−0.02 (−0.04 to −0.01)	−0.01 (−0.02 to 0.01)	0.195	−0.01 (−0.03 to 0.003)	−0.03 (−0.04 to −0.01)	−0.02 (−0.03 to −0.005)	0.249
BMI (kg/m^2)	−0.02 (−0.3 to 0.2)	−0.7 (−0.9 to −0.5)[a]	−0.5 (−0.8 to −0.3)[a]	0.0005	−0.1 (−0.3 to 0.2)	−1.2 (−1.5 to −1.0)[a]	−1.0 (−1.3 to −0.8)[a]	< 0.0001
Lean mass (kg)	0.1 (−0.4 to 0.6)	−0.9 (−1.4 to −0.5)[a]	−0.7 (−1.1 to −0.2)	0.014	0.3 (−0.2 to 0.8)	−1.3 (−1.7 to −0.8)[a]	−0.3 (−0.8 to 0.2)[b]	0.0001
Fat mass (kg)	−0.1 (−0.7 to 0.5)	−1.0 (−1.6 to −0.4)	−0.8 (−1.4 to −0.2)	0.119	−0.5 (−1.1 to 0.1)	−2.2 (−2.8 to −1.6)[a]	−2.6 (−3.2 to −2.0)[a]	< 0.0001
Percent body fat (%)	−0.2 (−0.8 to 0.4)	−0.6 (−1.2 to 0.0)	−0.4 (−1.0 to 0.2)	0.700	−0.7 (−1.3 to −0.1)	−1.5 (−2.1 to −0.9)	−2.1 (−2.7 to −1.5)[a]	0.010
Visceral fat area (cm^2)	−1.2 (−4.7 to 2.4)	−7.0 (−10.4 to −3.7)	−4.8 (−8.3 to −1.3)	0.064	−3.2 (−6.8 to 0.4)	−13.3 (−16.7 to −10.0)[a]	−14.2 (−17.7 to −10.8)[a]	< 0.0001
Fasting blood glucose (mg/dL)	1.4 (−0.9 to 3.6)	−1.2 (−3.4 to 1.1)	0.3 (−1.9 to 2.5)	0.287	2.5 (0.2–4.7)	1.5 (−0.6 to 3.7)	3.4 (1.2–5.6)	0.475
Mean blood glucose (mg/dL)	–	–	–	–	2.5 (0.4–4.6)	−2.3 (−4.3 to −0.3)[a]	2.5 (0.4–4.7)[b]	0.005
HbA1c (%)	–	–	–	–	−0.1 (−0.2 to −0.1)	−0.3 (−0.3 to −0.2)	−0.2 (−0.3 to −0.1)	0.104
C-P (ng/mL)	0.1 (−0.1 to 0.4)	−0.6 (−0.8 to −0.3)[a]	−0.4 (−0.6 to −0.1)[a]	0.001	−0.1 (−0.4 to 0.2)	−0.6 (−0.8 to −0.3)[a]	−0.4 (−0.6 to −0.1)	0.031
Fasting insulin (mIU/mL)	1.4 (−0.4 to 3.2)	−2.7 (−4.4 to −1.0)[a]	−1.4 (−3.1 to 0.3)	0.005	0.4 (−1.4 to 2.1)	−3.4 (−5.1 to −1.8)[a]	−1.6 (−3.3 to 0.1)	0.012
HOMA-IR	0.4 (−0.04 to 0.8)	−0.6 (−1.0 to −0.2)[a]	−0.3 (−0.8 to 0.1)[a]	0.006	0.2 (−0.3 to 0.6)	−0.7 (−1.1 to −0.3)[a]	−0.2 (−0.7 to 0.2)	0.014
Metabolic syndrome score	1.9 (1.1–2.6)	0.6 (−0.1 to 1.3)[a]	1.4 (0.7–2.1)	0.050	−0.1 (−0.8 to 0.7)	−1.0 (−1.7 to −0.3)	−0.5 (−1.2 to 0.3)	0.237
Systolic BP (mmHg)	1.3 (−1.4 to 4.1)	−1.5 (−4.1 to 1.1)	0.4 (−2.3 to 3.0)	0.299	0.9 (−1.8 to 3.7)	−5.5 (−8.1 to −2.9)[a]	−1.6 (−4.3 to 1.0)	0.004
Diastolic BP (mmHg)	−1.4 (−3.3 to 0.6)	−2.6 (−4.5 to −0.8)	0.5 (−1.4 to 2.4)	0.073	−4.2 (−6.2 to −2.3)	−4.9 (−6.8 to −3.1)	−2.0 (−3.9 to −0.1)	0.078
Pulse pressure (mmHg)	2.5 (−0.2 to 5.2)	1.2 (−1.3 to 3.8)	−0.1 (−2.7 to 2.5)	0.389	5.0 (2.3–7.7)	−0.4 (−3.0 to 2.1)[a]	0.4 (−2.3 to 3.0)[a]	0.012
Heart rate (bpm)	−2.7 (−6.0 to 0.6)	−0.4 (−3.5 to 2.8)	0.2 (−3.1 to 3.5)	0.425	−0.3 (−3.6 to 3.1)	1.8 (−1.4 to 5.0)	1.9 (−1.3 to 5.2)	0.564
TC (mg/dL)	9.3 (2.7–15.8)	10.2 (3.9–16.6)	11.8 (5.4–18.2)	0.856	3.1 (−3.4 to 9.7)	9.1 (2.9–15.3)	11.7 (5.3–18.0)	0.175
TG (mg/dL)	19.0 (5.1–33.0)	26.8 (13.2–40.3)	17.8 (4.2–31.4)	0.602	10.9 (−3.0 to 24.9)	13.6 (0.4–26.9)	2.5 (−11.1 to 16.1)	0.484
HDL-C (mg/dL)	−4.8 (−6.5 to −3.1)	−3.2 (−4.8 to −1.6)	−3.7 (−5.3 to −2.1)	0.392	2.7 (1.1–4.4)	2.8 (1.2–4.3)	3.6 (2.0–5.2)	0.679
LDL-C (mg/dL)	−7.6 (−11.5 to −3.6)	−4.6 (−8.4 to −0.7)	−2.3 (−6.2 to 1.6)	0.179	12.5 (8.5–16.5)	19.2 (15.4–23.0)[a]	19.7 (15.8–23.5)[a]	0.021
Leptin (ng/mL)	1.6 (−0.8 to 3.9)	−7.8 (−10.0 to −5.6)[a]	−3.2 (−5.4 to −0.9)[ab]	<0.0001	0.2 (−2.1 to 2.6)	−7.0 (−9.2 to −4.8)[a]	−5.4 (−7.7 to −3.1)[a]	0.0001
Adiponectin (μg/mL)	0.1 (−0.5 to 0.7)	0.4 (−0.2 to 0.9)	0.0 (−0.6 to 0.6)	0.592	1.0 (0.5–1.6)	0.5 (−0.05 to 1.0)	0.4 (−0.2 to 0.9)	0.222
HMWA (μg/mL)	0.0004 (−0.1 to 0.1)	0.04 (−0.03 to 0.1)	0.01 (−0.1 to 0.1)	0.687	0.1 (0.1–0.2)	0.2 (0.1–0.2)	0.1 (−0.01 to 0.1)	0.130
Leptin/adiponectin ratio	−0.3 (−1.5 to 0.9)	−2.9 (−4.0 to −1.8)[a]	0.4 (−0.7 to 1.6)[b]	0.0003	−0.8 (−2.0 to 0.4)	−2.5 (−3.7 to −1.4)	−2.0 (−3.2 to −0.9)	0.108

BMI, body mass index; HbA1c, hemoglobin A1c; C-P, C-peptide; HOMA-IR, homeostasis model assessment-insulin resistance [calculated as (fasting glucose*fasting insulin)/405]; BP, blood pressure; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HMWA, high molecular weight adiponectin. Values are expressed as least square means (means adjusted for baseline) with 95% CI. p value between groups for "all participants:" one-way ANOVA with Tukey post hoc (continuous variables). [a]$p < 0.05$ compared with control group. [b]$p < 0.05$ compared with eTRE group. See also Figures S1 and S2 and Tables S1, S2, S4, and S5.

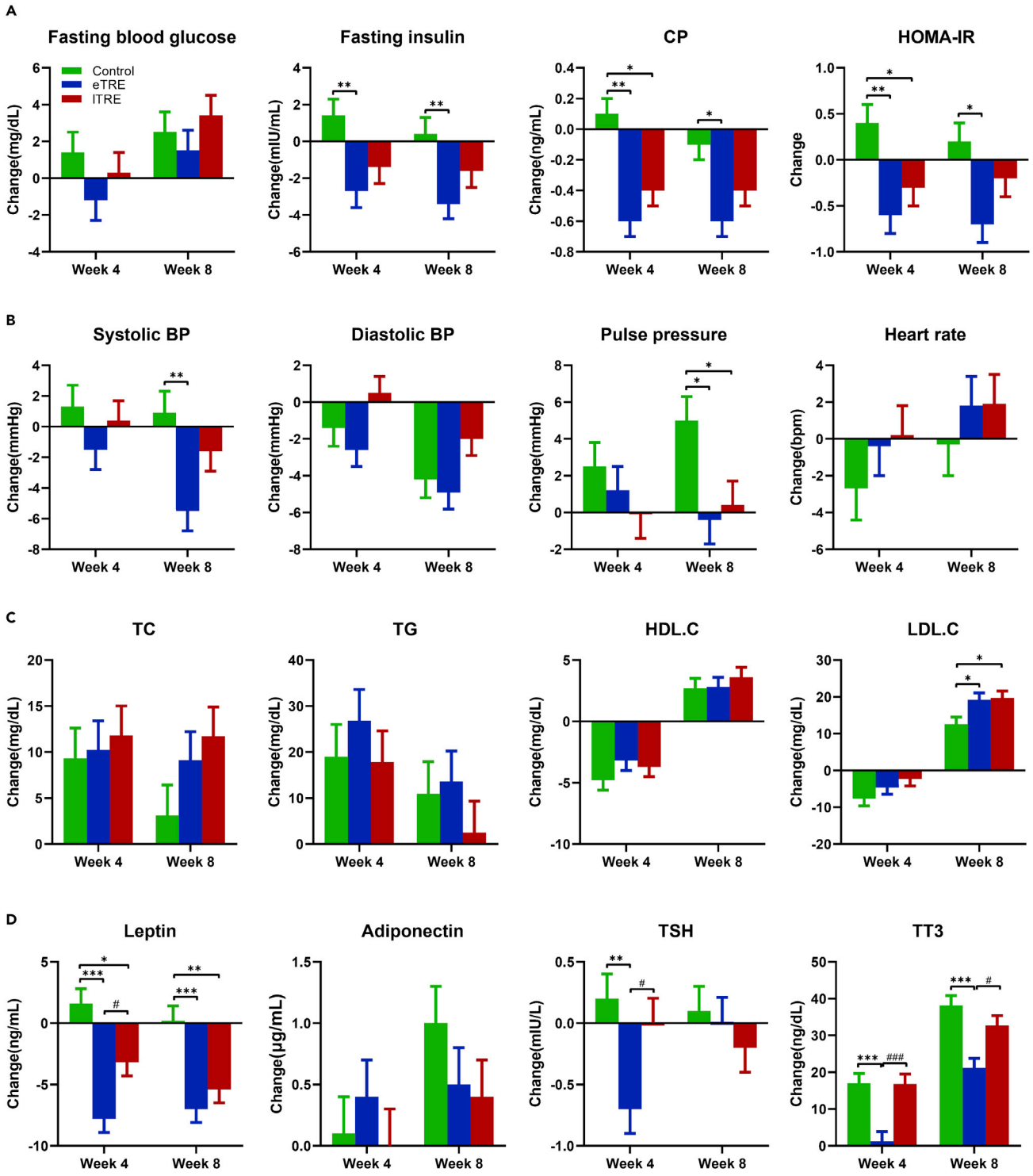

Figure 3. Cardiometabolic risk markers

(A) Glucose regulation. C-P, C-peptide; HOMA-IR, homeostasis model assessment insulin resistance.

(B) Blood pressure. BP, blood pressure.

(C) Lipid profile. TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

(D) Hormones. TSH, thyroid-stimulating hormone; TT3, triiodothyronine. Data are presented as least squares mean ± SEM; *p < 0.05 versus control group, **p < 0.01 versus control group, ***p < 0.001 versus control group, $^{\#\#\#}$p < 0.001 versus eTRE group.

See also Figure S2 and Tables S1, S2, S4, and S5.

et al., 2021; Tinsley et al., 2019; Wilkinson et al., 2020). This result is expected, because almost all studies were conducted in healthy adults, but not in adults with elevated fasting glucose. A recent study reported similar results, in which fasting glucose was not improved when it was ≤99 mg/dL at baseline period, but a significant decrease was observed among subjects with fasting glucose levels >99 mg/dL at baseline period (Wei et al., 2017). The outcomes are limited if glucose levels are measured only in the morning (Cienfuegos et al., 2020; Sutton et al., 2018). Therefore, we measured the mean glucose levels by CGM and found that 6-h eTRE decreased mean 24-h glucose compared with the lTRE and controls. The mean fasting glucose by CGM was lower in the 9-h eTRE group but not in the 9-h lTRE group compared with baseline (Hutchison et al., 2019b). Circadian rhythms influence glucose regulation, manifested by insulin sensitivity and glucose tolerance peak shortly after waking (Gabel and Varady, 2020; Poggiogalle et al., 2018), and human studies report that the incremental glucose area under the curve is up to 2-fold higher in the evening relative to the morning (Van Cauter et al., 1997). Indeed, the significantly time discrepancy on interstitial glucose between 6-h eTRE and lTRE was observed in the evening and while sleeping (from 6:30 p.m. to 11:00 p.m.) but not in the morning (Figure S2C).

Although 6-h eTRE did not improve fasting glucose, it markedly decreased fasting insulin and insulin resistance in our study. This result is comparable with other human trials, suggesting that TRE may be more effective at decreasing insulin levels and insulin resistance than reducing fasting glucose levels (Cienfuegos et al., 2020; Sutton et al., 2018). Continuously high levels of glucose and insulin reduce the capacity of muscle, fat, and liver cells to use and store glucose, contributing to insulin resistance. Insulin resistance is the main contributing factor to the development of type 2 diabetes, coronary heart disease, and certain forms of cancer (Calle et al., 1999; Kraemer and Ginsberg, 2014). Reduced insulin resistance could have beneficial effects not only in decreasing the risk of cardiovascular disease but also in the primary and secondary prevention of some common cancers.

In sum, compared with 6-h lTRE, 6-h eTRE may result in better improvement on glucose regulation by shifting the feasting window to earlier in the day when the circadian system may promote better glucose tolerance.

6-H eTRE, but not 6-h lTRE, reduced systolic blood pressure

Changes in systolic blood pressure and pulse pressure were significantly different across the three groups at week 8 ($p = 0.004$ and $p = 0.012$, respectively) (Figure 3B; Table 2). At week 8, systolic blood pressure was significantly decreased in the 6-h eTRE group (−5.5 mmHg [95% CI: −8.1 to −2.9]), whereas it was not changed in the 6-h lTRE group (−1.6 mmHg [95% CI: −4.3 to 1.0]) and control group (0.9 mmHg [95% CI: −1.8 to 3.7]) (Figure 3B; Table 2). No significant differences were observed in diastolic blood pressure and heart rate among the three groups at 4 and 8 weeks (Figure 3B; Table 2). Elevated blood pressure is an important risk factor for the development of cardiovascular diseases such as myocardial infarction, stroke, and heart failure. Data from observational studies involving more than 1 million people showed that the mortality risk from coronary heart disease and stroke doubles for every 20 mmHg increase in systolic blood pressure or 10 mmHg increase in diastolic blood pressure (Lewington et al., 2002). A 5-mmHg reduction in systolic or diastolic blood pressure is clinically significant following a lifestyle intervention (Wing et al., 2011). Our findings are consistent with previous reports. For instance, 6-h eTRE caused significant decreases in systolic blood pressure (−11 mmHg) and diastolic blood pressure (−10 mmHg) (Sutton et al., 2018), 8-h TRE resulted in dramatic reduction in systolic blood pressure (−7 mmHg) (Gabel et al., 2018), and 10-h TRE reduced systolic blood pressure (−5 mmHg) and diastolic blood pressure (−6 mmHg) (Wilkinson et al., 2020). The mechanisms mediating this rapid reduction in blood pressure induced by TRE are not completely clear. These conditions could be caused by TRE-mediated reduction in insulin levels considering that increased insulin levels may directly contribute to increased blood pressure (Biston et al., 1996; Persson, 2007). In addition, our trial found that 6-h eTRE reduced systolic blood pressure, possibly because eTRE facilitates natriuresis by shifting salt consumption earlier in the daytime when sodium excretion is upregulated by the circadian system (Johnston et al., 2016).

6-H eTRE and 6-h lTRE did not affect TC, TGs, or HDL-C

Changes in total cholesterol (TC), TG, and high-density lipoprotein cholesterol (HDL-C) were not significantly different among the three groups at 4 and 8 weeks (Figure 3C; Table 2). In contrast, both 6-h eTRE and lTRE groups produced greater increases in LDL-C than the control group at week 8 ($p = 0.045$ and $p = 0.033$, respectively), whereas no difference was observed between the intervention groups

(Figure 3C; Table 2). Jamshed et al. reported that eTRE increased morning fasting levels of LDL-C by 9 mg/dL (Jamshed et al., 2019), Martens et al. reported that TRE increased LDL-C by 11 mg/dL compared with the controls (Martens et al., 2020), Stote et al. (2007) and Carlson et al. (2007) found that LDL-C increased after the subjects were required to consume all of their energy needs for the day within a 4-h period in the early evening over the course of 8 weeks. TRE increased LDL-C possibly because of the prolonged fasting period and greater reliance on fat oxidation (Ravussin et al., 2019). Therefore, whether the mild increase in LDL-C in the morning is pathologic remains to be further explored.

Cardiovascular disease is also related to nonalcoholic fatty liver disease, which is characterized by elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST), reflecting underlying liver pathology. In the present study, neither 6-h eTRE nor 6-h lTRE caused dramatic changes in ALT and AST (Table S1). Uric acid is a very sensitive indicator of renal function, and its elevation reflects the malfunction of tubulointerstitial and renal tubules to some extent. The change in uric acid showed no significant difference across the three groups in our study (Table S1).

6-H eTRE, but not 6-h lTRE, reduced metabolic hormones

The 6-h eTRE and 6-h lTRE groups produced greater reductions of serum leptin by weeks 4 and 8 compared with the control group, and the reduction in 6-h eTRE group was greater than that in 6-h lTRE group at week 4 (Figure 3D; Table 2). However, the changes of adiponectin both in total and high-molecular weight did not significantly differ across the three groups at 4 and 8 weeks (Figure 3D; Table 2). Leptin and adiponectin are released from adipose tissues and exert defined responses in target organs. Leptin has long been implicated in overweight and obesity. A higher concentration of leptin in the serum has been associated with a higher percentage of body fat (Kord-Varkaneh et al., 2018). In healthy conditions in humans and rodents, the level of circulating leptin decreases sharply during prolonged fasting and more gradually with diminished body fat mass (Friedman and Halaas, 1998; Leal-Cerro et al., 1998; Maffei et al., 1995). During TRE, we observed a reduction in fat mass and a parallel reduction in leptin, which is consistent with previous weight-loss studies (Moro et al., 2016; Neseliler et al., 2019; Redman et al., 2018). In the fasted state, the principal leptin-reduction mechanism is the sympathetic nervous system acting on adipocyte β-adrenergic receptors (Caron et al., 2018; Mantzoros et al., 1996). The elevated plasma concentration of adiponectin is correlated with improved metabolism and loss in fat mass (Moro et al., 2016). Some human trials have reported that adiponectin levels increase after TRE intervention, and these results are correlated with weight loss (McAllister et al., 2020; Moro et al., 2016, 2020). However, our trial did not find an increase in adiponectin levels, which is consistent with other trials (Stratton et al., 2020; Sutton et al., 2018). Adiponectin and leptin have opposing effects in many biological pathways, and leptin/adiponectin ratio is a risk factor for cardiovascular disease. In the present study, the 6-h eTRE group resulted in greater decreases in leptin/adiponectin ratio by week 4 compared with the 6-h lTRE and control groups (Table 2).

The 6-h eTRE group induced decreases in thyroid-stimulating hormone (TSH) and triiodothyronine (TT3) compared with the 6-h lTRE and control groups, with no significant difference between the 6-h lTRE group and the control group (Figure 3D; Table S1). There were no significant differences in free triiodothyronine (FT3), thyroxine (TT4), and free thyroxine (FT4) among the three groups at week 8 (Table S1). Fasting-induced inhibition of the hypothalamic–pituitary–thyroid axis is an adaptive response to reduce energy expenditure during food deprivation (Vella et al., 2011). Indeed, humans who experience weight reduction or caloric restriction undergo decreased circulating TSH and thyroid hormone levels (Rosenbaum et al., 2002, 2005). Low levels of TSH and thyroid hormone levels, without an impaired thyroid gland function, have been related to longevity in humans (Rozing et al., 2010). Previous studies in humans and rodents have shown that the decrease in leptin levels during caloric restriction controls thyroid hormone levels (Rosenbaum et al., 2002; Vella et al., 2011). In our study, only 6-h eTRE contributed to a decrease in TSH and TT3 levels although the levels of leptin were reduced in both intervention groups, which is likely due to the difference in fasting duration preceding testing (19 h in eTRE versus 14 h in the lTRE arm) or reflects that thyroid hormone levels may be regulated by other pathways besides leptin levels. The aforementioned phenomenon is worthy of further study, particularly in a trial that matches the fasting duration and leptin levels prior to testing.

The caloric restriction may compromise immune function and impose an adverse influence on bone mineral density (BMD), which is accompanied by a reduction in the number of white blood cells (Villareal et al., 2016). However, changes in white blood cell, red blood cell, hemoglobin, hematocrit, and platelet by weeks

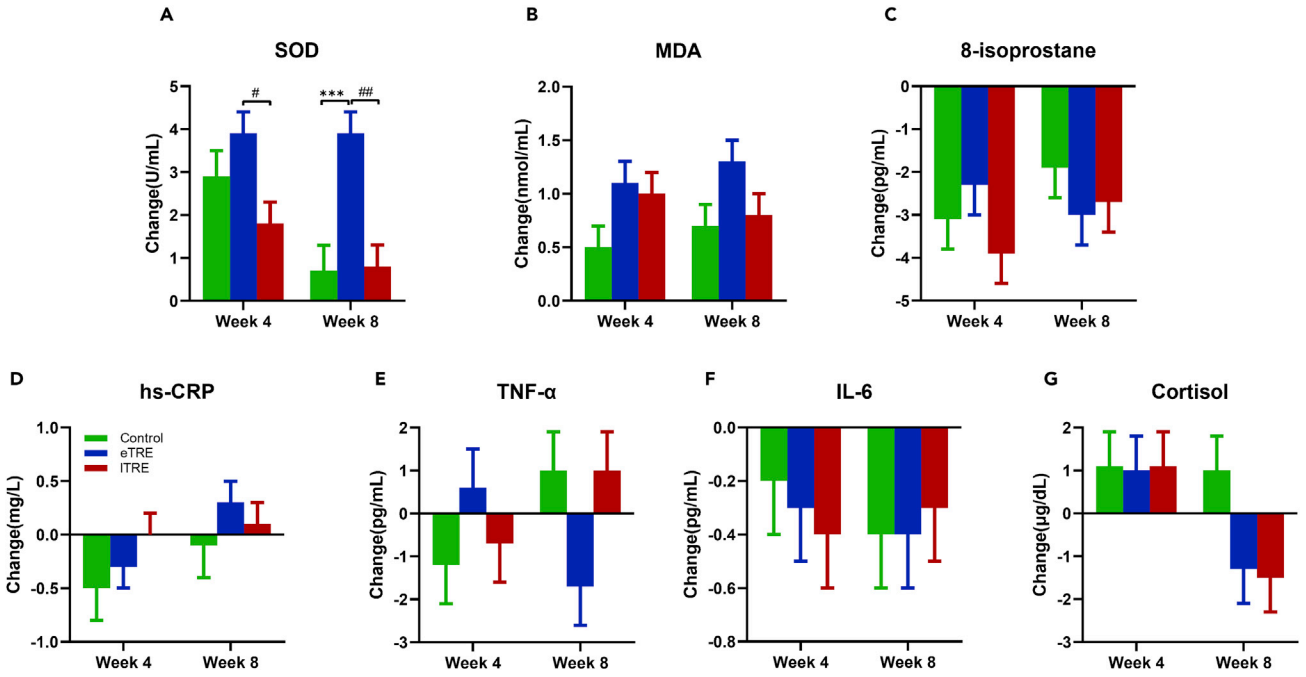

Figure 4. Oxidative stress markers and inflammatory

(A–G) Changes in superoxide dismutase (SOD) (A), malondialdehyde (MDA) (B), 8-isoprostane (C), high-sensitivity C-reactive protein (hs-CRP) (D), tumor necrosis factor alpha (TNF-α) (E), interleukin-6 (IL-6) (F), and cortisol (G) levels after 4 and 8 weeks of intervention. Data are presented as least squares mean ± SEM; ***$p < 0.001$ versus control group, $^{\#}p < 0.05$ versus eTRE group, $^{\#\#}p < 0.01$ versus eTRE group. See also Table S2.

4 and 8 showed no difference across the three groups (Table S1). These findings are on par with those from other studies in humans, which also did not find significant changes in blood cell counts with TRE (Chaix et al., 2019; Gabel et al., 2019). The changes in speed of sound (SOS) and T-score by weeks 4 and 8 showed no difference across the three groups (Table S1). TRE did not cause a negative effect in white blood cell count or BMD, as it was reported for constant caloric restriction (Meydani et al., 2016; Schafer, 2016; Villareal et al., 2016), which makes TRE intervention a suitable alternative to continuous caloric restriction.

6-H eTRE and 6-h lTRE did not affect inflammatory markers, but 6-h eTRE increased antioxidant potential

At week 8, the 6-h eTRE group (3.9 U/mL [95% CI: 2.8 to 4.9]) experienced greater increase in superoxide dismutase (SOD) than the 6-h lTRE group (0.7 U/mL [95% CI: −0.4 to 1.8]) and control group (0.8 U/mL [95% CI: −0.3 to 1.9]), and no difference was observed between the 6-h lTRE and control groups (Figure 4A). Changes in malondialdehyde (MDA), 8-isoprostane, high-sensitivity C-reactive protein (hs-CRP), tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and cortisol by weeks 4 and 8 were not significantly different among the three groups (Figures 4B–4G). 8-Isoprostane and MDA are lipid-specific markers of oxidative stress. In an 8-week trial of 4-h and 6-h TRE, circulating 8-isoprostane levels decreased by 37% and 34% in obese adults, respectively (Cienfuegos et al., 2020). In a 5-week trial of 6-h TRE, circulating 8-isoprostane level did not change in TRE but increased in the control arm (Sutton et al., 2018). Our results demonstrated that 6-h eTRE can increase antioxidant potential, which may be associated with reduced insulin resistance. Further studies on the effects of eTRE on serum antioxidant and oxidative stress biomarkers are needed. In terms of inflammatory markers, our findings are in line with most human trials, which reported that IF does not influence hs-CRP, IL-6 and TNF-α (Bhutani et al., 2013; Cienfuegos et al., 2020; Harvie et al., 2013; Sutton et al., 2018; Trepanowski et al., 2017; Wei et al., 2017).

6-H eTRE reduced hunger in the midday

The 6-h eTRE group induced decreases in the sensation of hunger compared with the 6-h lTRE and control groups ($p = 0.013$ and $p = 0.045$, respectively), with no difference between the 6-h lTRE and control groups (Figure 5A). Therefore, 6-h eTRE may be beneficial to curb food intake in the midday and thus facilitate weight reduction. Previous studies about the effects of TRE on hunger are contradictory, with some

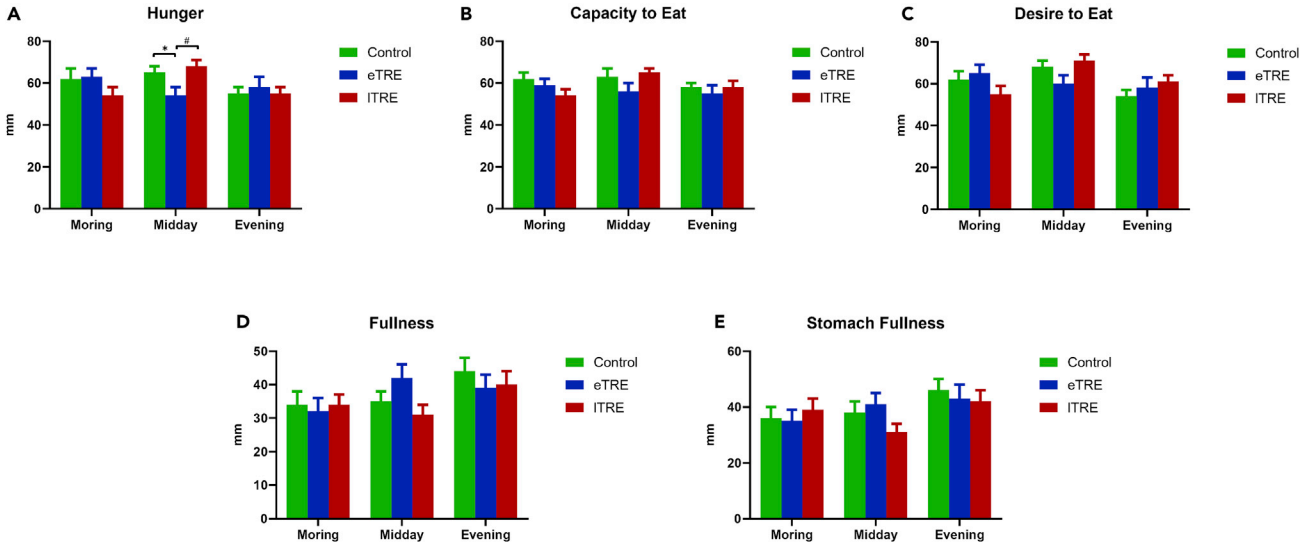

Figure 5. Subjective appetite

Participants rated their appetite on a 0–100 mm visual analog scale, ranging from "Not at All" (0 mm) to "Extremely" (100 mm).

(A–E) Hunger (A), capacity to eat (B), desire to eat (C), fullness (D), and stomach fullness (E) in the morning, midday, and evening in 8 weeks. Data are presented as mean ± SEM; *$p < 0.05$ versus control group, $^{\#}p < 0.05$ versus eTRE group.

reporting either a decrease (Gill and Panda, 2015; Ravussin et al., 2019), increase (Stote et al., 2007), or no change (Jamshed et al., 2019; Sutton et al., 2018). Whether these discrepancies were due to differences in the time of day of measurement, the feeding window duration (single meal versus extended window), the time of food consumption (eTRE versus lTRE), study design (controlled feeding versus free eating), subject characteristics, and other factors remains to be elucidated. The desire to eat, capacity to eat, fullness, and stomach fullness at week 8 were not significantly different across the three groups (Figures 5B–5E).

Adverse events

No serious adverse events were observed in both intervention groups throughout the trial relative to the control group (Figures S3A–S3O). Surprisingly, occurrences of inability to stay asleep at weeks 1, 2, 3, and 7 in the 6-h lTRE group were lower than those in the control group (Figure S3N). A 6-h eTRE intervention study reported several mild adverse events, such as vomiting, headaches, increased thirst, and diarrhea (Sutton et al., 2018). Another 4-h and 6-h TRE trial reported that mild adverse effects, including dizziness, nausea, headaches, and diarrhea, may occur at the beginning of TRE and then disappear when participants adapt to the diet (Cienfuegos et al., 2020).

Change in eating window and compliance

The average eating window decreased by approximately 5.3 and 3.4 h/day in the 6-h eTRE and 6-h lTRE participants, respectively (Figures 6A and 6B). Almost all subjects were able to maintain a daily feasting window during the TRE throughout the 8-week intervention period (Figures 6A and 6B). Interstitial glucose in the 6-h eTRE group did not increase in the afternoon and evening, whereas that in the 6-h lTRE did not increase in the morning, indicating that participants abstained from caloric intake during their fasting windows (Figure S2B). On average, self-reported compliance with the feasting window was higher in the 6-h eTRE group (89.0% ± 2.9%) than in the 6-h lTRE group (77.9% ± 1.4%), and a downward trend was observed in the level of compliance over the course of the study in both groups (Figure 6C). At week 8, the results of body weight and cardiometabolic parameters may have been better if adherence had not dropped.

Post hoc analyses

In a post hoc analysis, the main effects of intervention (eTRE + lTRF) group compared with the control group were tested, and the results were not materially changed (Table S2).

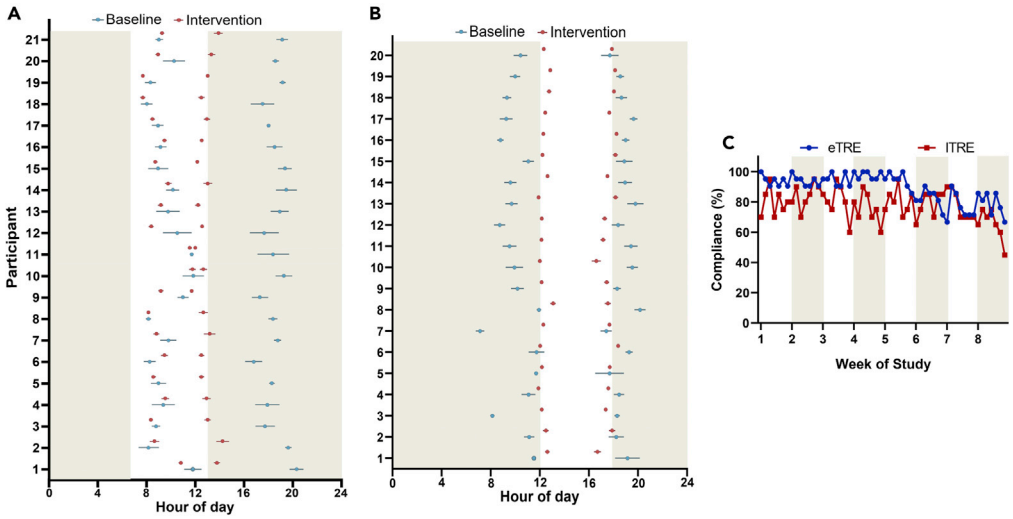

Figure 6. Change in eating window and compliance

(A and B) Mean time and SD for the participant started and stopped eating at baseline (red) and intervention (blue) in the 6-h eTRE (A) and 6-h lTRE group (B) y axis: each blue/red combination represents an individual participant. x axis: clock hour for eating event.

(C) Daily compliance of the dietary regimen for the 6-h eTRE and lTRE groups during the 8-week intervention period. See also Figure S3.

Conclusion

Overall, this study provides further evidence regarding the influence of timing of the eating window (early versus late in the day) on body weight loss and cardiometabolic health in overweight and obese young adults. 6-h eTRE and lTRE produced comparable body weight loss compared with controls. Furthermore, compared with control, 6-h eTRE reduced systolic blood pressure, mean glucose, fasting insulin, insulin resistance, leptin, and thyroid axis activity, whereas lTRE only reduced leptin. Self-reported compliance with the feasting window was higher in the 6-h eTRE group than in the 6-h lTRE group. These findings shed light on the promise of 6-h eTRE and lTRE for weight loss. Larger studies are needed to assess the promise of eTRE to yield better thyroid axis modulation and overall cardiometabolic health improvement.

Limitations of the study

This study has several limitations. First, our sample size was small, and although many outcomes were measured in our study, only changes in weight, lean mass, and leptin were adequately powered (i.e., had at least 80% power). All other outcomes were underpowered (range of 10%–79% power) and have elevated risk of type II error (i.e., false negatives). Neither was this study powered for superiority testing (i.e., eTRE vs lTRE effects), so assertions about mean effect differences cannot be made. We measured many outcomes and did not correct for family wise error, which increases risk of type I error (i.e., false positives). Thus, the findings should be interpreted with caution and need to be replicated in larger trials. Second, we did not match the fasting duration prior to testing, which may underestimate differences and improvements in certain secondary outcome measures across groups. Third, the CGM and electronic pedometer may have acted as "reminders" to participants that they were taking part in a health study and therefore might have altered their behavior. Fourth, the regimens to the recruitment of subjects could have introduced a selection bias toward participants who were already knowledgeable and/or interested in TRE. Finally, although electronic scales were provided for each participant, estimates of energy and macronutrient intake in this study may have limited accuracy.

STAR★METHODS

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

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2022.104870.

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AUTHOR CONTRIBUTIONS

Conceptualization, Y.X.M. and L.M.Z.; Methodology, Y.X.M., L.M.Z., and Z.L.; Formal Analysis, J.Q.W.; Investigation, L.M.Z., Z.L., J.Q.W., R.Q.L., J.Y.R., and X.G.; Resources, J.Q.W., R.Q.L., J.Y.R., and X.G.; Data Curation, J.Q.W., Y.S., and Y.T.Z.; Writing—Original Draft, L.M.Z. and Z.L.; Writing—Review & Editing, Y.X.M., L.M.Z., Z.L., S.S.L., L.Y.L., F.Z., and B.W.Y.; Visualization, R.Q.L., H.T., and H.C.Z.; Funding Acquisition, Y.X.M. and L.M.Z.; Supervision, Y.X.M. and L.M.Z.

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		
leptin ELISA kit	Crystal Chem	Catalog#: 80968
adiponectin ELISA kit	Crystal Chem	Catalog#: 80671
HMW adiponectin ELISA kit	Mlbio	Catalog#: ml063723
IL-6 ELISA kit	Mlbio	Catalog#: ml058097
TNF-α ELISA kit	Mlbio	Catalog#: ml077385
8-Isoprostane ELISA kit	Cayman Chemical	Catalog#: 516351
Software and algorithms		
R Version 4.0.5	R Foundation	https://www.r-project.org/

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Yu-xia Ma (mayuxia@hebmu.edu.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

We conducted the trial between October 2020 and December 2020 at the Nutrition and Food Hygiene Laboratory of Hebei Medical University, China. A total of 89 participants were originally recruited from Hebei Medical University via posters around campus and word of mouth. Among the 89 participants, 26 were excluded from the study based on the inclusion criteria. Three subjects withdrew from the study in the baseline period, among which two were hospitalized and one reported muscle discomfort associated with implantation and use of continuous glucose monitor (CGM). A total of 60 participants were included in the final analysis (Figure 1B). The study comprised 60 individuals with 55% male and 45% female adults. The influence of gender on the results of the study was not explicitly measured. The age of participants ranged from 19 to 29 years old. This randomized controlled clinical trial was approved by the Medical Ethics Committee of Hebei Medical University (protocol number: 2020204), and all participants provided written informed consent.

METHOD DETAILS

Experimental design

This study is a randomized parallel-arm controlled clinical trial consisting of a 2-week baseline period and an 8-week TRE intervention period. Participants were randomized to the control group, 6-h eTRE group, or 6-h lTRE group. During the 2-week baseline period, all participants were required to maintain stable weight by keeping their usual diet and physical activity habits. During the 8-week intervention period, the 6-h eTRE group was directed to eat *ad libitum* from 7:00 a.m. to 1:00 p.m. daily and completely fast from 1:00 p.m. to 7:00 a.m. the following day (Figure 1A). The 6-h lTRE group was directed to eat *ad libitum* from 12:00 p.m. to 6:00 p.m. daily and completely fast from 6:00 p.m. to 12:00 p.m. the following day. During the 6-h eating

windows, participants were not demanded to supervise caloric intake without constraints on the quality or quantities of foods consumed. Only water and energy-free beverages (e.g., black tea, coffee, and diet sodas) were allowed outside the eating widow. Controls were directed to maintain their eating or physical activity habits throughout the trial without receiving food or dietary counseling.

Participant selection

The inclusion criteria were as follows: 1) healthy young adults aged 18–30 years old; 2) body mass index (BMI) $\geq$24 kg/m^2; 3) body weight stable for 3 months before the start of the study (gain or loss <10% current weight); 4) able to give written informed consent. The following participants were excluded from the study: 1) used medications or supplements that could influence study outcomes; 2) current smokers; 3) regularly consuming alcohol exceeding 2 servings a day; 4) with history of any cardiometabolic, neurological, or musculoskeletal diseases; 5) currently participating in a weight loss or weight management program; 6) pregnant, breast-feeding, or trying to become pregnant; and 7) with irregular menstrual cycles (if female).

Anthropometric measurements

Body weight and body composition were evaluated in the morning before eating or drinking every weekend using Inbody-720 Body Composition Analyzer. Height was measured during the baseline period using a stable stadiometer (Seca, Germany) with a 0.1 cm precision. BMI was calculated using the weight in kilograms divided by the height in meters squared. Waist and hip circumference were measured using a telescopic ruler with a 0.1 cm precision at the baseline period and at weeks 4 and 8 of the intervention period.

Blood pressure and heart rate

Blood pressure and heart rate were measured thrice using an automated electronic sphygmomanometer (HBP-1300, Omron Corporation, Japan) after 10 min rest in a seated position, and the average of the three measurements was calculated and used for analyses.

Blood sampling and storage

Blood samples were collected in the morning (8:00–9:30 a.m.) after an overnight fast at baseline period and at weeks 4 and 8 of the intervention period. All blood draws were performed at the Nutrition and Food Hygiene Laboratory of Hebei Medical University. Samples were collected as serum, plasma, and whole blood and either analyzed immediately after the blood drawing for clinical testing (e.g., chemistry, endocrinology, and immunotyping) or stored at −80°C until the measurements of inflammation, oxidative stress, and metabolic hormones.

Biochemical measurements

Glucose, total cholesterol (TC), Triglycerides (TGs), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), alanine aminotransferase (ALT), aspartate aminotransferase (AST), high-sensitivity C-reactive protein (hs-CRP), and uric acid were measured on an AU5800 instrument (Beckman Coulter, Inc., USA). Free triiodothyronine (FT3), free thyroxine (FT4), triiodothyronine (TT3), thyroxine (TT4), thyroid-stimulating hormone (TSH), cortisol, and insulin were measured on a DXI800 instrument (Beckman Coulter, Inc. USA). Hemoglobin A1c (HbA1c) was measured on an HA8180 instrument (ARKRAY, Japan). C-peptide (C-P) and 25(OH)vitamin D were measured on 4000 Plus (Snibe, China). Fasting levels of leptin, adiponectin, high molecular weight adiponectin (HMWA), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), and 8-isoprostane were measured using ELISA kits (Crystal Chem, USA; Mlbio, China; Cayman Chemical Company, Ann Arbor, MI, respectively) on a SpectraMax M2e microplate reader (Molecular Devices, USA). The superoxide dismutase (SOD) activities and malondialdehyde (MDA) concentrations were determined using commercial colorimetric assay kits (Nanjing Jiancheng, China).

Subjective appetite

Participants were asked to rate their appetite across five dimensions, namely, desire to eat, hunger, capacity to eat, fullness, and stomach fullness, by using visual analog scales (VAS; 0–100 mm scale). VAS surveys were administered before breakfast (the 6-h lTRE group at 7:00 a.m.), lunch, and dinner (the 6-h eTRE group at 6:00 p.m.) on the last day of the intervention.

CGMs

All participants wore a FreeStyle Libre H CGM (Abbott Diabetes Care Inc., USA) for 14 days at baseline period and week 7 of the intervention period. According to the manufacturer's instructions, an electrochemical sensor based on glucose oxidase was inserted under the skin on the arm. The sensor updated the interstitial fluid glucose measurements every minute and stored it every 15 min.

Bone densitometry

The measurement of bone density in the radius was carried out by a single trained technologist using the BMD-A5 ultrasonic bone densitometry system (Pinyuan, China) at baseline period and weeks 4 and 8 of the intervention period. The probe, coated with standard ultrasound gel, was parallel to the long axis of the radius and fully coupled to the skin. The sound waves were transmitted, and then the speed of sound (SOS, m/s) was recorded and stored in a matched computer. The final results were shown as the T-value.

Compliance monitoring

Adherence to the 6-h eTRE and 6-h lTRE windows was assessed by asking participants to complete a daily adherence log, which recorded the time of day the participant started and stopped eating each day. Based on the log, if the participant ate within the 6-h eTRE and 6-h lTRE windows, the day was marked as "adherent.", whereas if food was consumed outside of the 6-h eTRE and 6-h lTRE windows, the day was marked as "non-adherent." Adherence to the TRE regimen was evaluated as the rate of daily compliance.

Dietary intake and physical activity

All participants completed a 7-day food record (for 7 consecutive days) at baseline period and week 8 of the intervention period. During the baseline period, a dietitian provided each participant with a 15-min instruction about completing the food records. Participants received an SF-400A electronic compact scale and were asked to measure the weight of foods consumed. The timing of food consumption (for each beverage or food item) was also documented in the food record. The energy content of each meal was estimated using China Food Composition Database (Yang et al., 2009). All participants were asked to maintain their level of physical activity throughout the entire trial. Step counts and total energy expenditure were measured over 7 days using an AM-120 multi-function electronic pedometer (TANITA, Japan) at the baseline period and week 8 of the intervention period.

Adverse events

Neurological (dizziness, headache, fatigue, and irritability) and gastrointestinal issues (vomiting, nausea, diarrhea, constipation, dry mouth, and bad breath) and sleep disturbances (inability to fall asleep, inability to stay asleep) were evaluated by an adverse events questionnaire at baseline and each week of the intervention period.

QUANTIFICATION AND STATISTICAL ANALYSIS

Power and sample size

Assuming an alpha value of 5 and 80% of power that the difference in body weight loss would be 3% between the 6-h TRE intervention and control groups by week 8 (Cienfuegos et al., 2020), 17 participants were required per group. We anticipated a dropout rate of 20%. Thus, we initially aimed to recruit 63 participants (n = 21 per group), assuming that 54 participants (n = 17 per group) would complete the trial. This study was powered for weight change. Power calculations were performed using PASS 15.0 and showed that only changes in weight, lean mass, and leptin were adequately powered for our study design, i.e., above the standard 80% power cutoff. Underpowered outcomes have elevated risk of type II error. See supplemental table (Table S3). Further, this study was not powered for superiority testing. Thus, comparisons of eTRE and lTRE outcomes to each other cannot yield statistically reliable statements or conclusions.

Randomization procedures

Participants were randomly assigned to the control group, 6-h lTRE group, or 6-h eTRE group (before the 2-week baseline period) in a 1:1:1 ratio using SPSS21.0. Randomization was carried out though a stratified random sampling procedure by gender and BMI (24.0–27.9 kg/m^2/≥28.0 kg/m^2).

Statistical analyses

Statistical analyses were conducted as two-sided tests in R software (version 4.0.5) with a significance threshold of $\alpha = 0.05$ for the type I error rate. Baseline data are presented in the text as raw mean $\pm$ SEM, while the treatment effects or change scores among arms are presented as least squares mean with 95% confidence intervals (95% CI). The baseline characteristics of participants in the three groups were compared using ANOVA (Gaussian distribution) or Kruskal–Wallis test (non-Gaussian distribution) for continuous variables and McNemar's test or Fisher test for categorical variables. Post hoc analyses were performed using the Tukey test (Gaussian distribution) or Holm's method (non-Gaussian distribution). Differences between treatment arms were evaluated as change scores using linear mixed models with compound symmetry structure, in which participants served as the random effect and the treatment, time, baseline data, and interaction with treatment and time were treated as fixed effects. Post hoc analyses were performed using the Tukey test. Missing data were considered missing at random. Error bars in the figures are presented as SEMs for visual clarity. In post hoc analyses, the main effects of intervention (eTRE + lTRE) group versus the control group were performed using linear mixed models.

ADDITIONAL RESOURCES

Prior to enrolling participants, the study was registered on the Chinese Clinical Trail Registry (ChiCTR2000039115).