Received: 18 November 2023 | Revised: 11 March 2024 | Accepted: 16 April 2024

DOI: 10.1002/oby.24057

ORIGINAL ARTICLE

Obesity Biology and Integrated Physiology

Obesity A Research Journal | THE OBESITY SOCIETY | WILEY

Time-restricted eating affects human adipose tissue fat mobilization

Carolina Zambrano[1,2] | Elena González-Alvarado[1,2] | Diego Salmerón[2,3,4] | Francisco Javier Ruiz-Ojeda[5,6,7] | Juan Luján[8] | Frank A. J. L. Scheer[9,10,11] | Marta Garaulet[1,2,9]

[1]Department of Physiology, Regional Campus of International Excellence, University of Murcia, Murcia, Spain

[2]Biomedical Research Institute of Murcia (IMIB), University Hospital Virgen Arrixaca, University of Murcia, Murcia, Spain

[3]Department of Health and Social Sciences, University of Murcia, Murcia, Spain

[4]Consortium for Biomedical Research in Epidemiology and Public Health (CIBERESP), Madrid, Spain

[5]Institute of Nutrition and Food Technology "José Mataix," Center of Biomedical Research, University of Granada, Granada, Spain

[6]Department of Biochemistry and Molecular Biology II, School of Pharmacy, University of Granada, Granada, Spain

[7]ibs.GRANADA Biosanitary Research Institute, Granada University Hospital Complex, Granada, Spain

[8]General Surgery Service, Hospital Quirónsalud, Murcia, Spain

[9]Medical Chronobiology Program, Division of Sleep and Circadian Disorders, Brigham and Women's Hospital, Boston, Massachusetts, USA

[10]Broad Institute of Massachusetts Institute of Technology (MIT) and Harvard, Cambridge, Massachusetts, USA

[11]Division of Sleep Medicine, Harvard Medical School, Boston, Massachusetts, USA

Correspondence
Marta Garaulet, Department of Physiology, School of Medicine, Espinardo Campus, University of Murcia, Murcia. Spain.
Email: garaulet@um.es

Frank A. J. L. Scheer, Medical Chronobiology Program, Division of Sleep and Circadian Disorders, Brigham and Women's Hospital, Boston, MA, USA.
Email: fscheer@bwh.harvard.edu

Funding information
Ministerio de Ciencia e Innovación, Grant/Award Number: PID2020-112768RB-I00; National Heart, Lung, and Blood Institute, Grant/Award Number: R01HL140574; National Institute of Diabetes and Digestive and Kidney Diseases, Grant/Award Number: R01DK105072

Abstract

Objective: Time-restricted eating (TRE), a dietary approach that confines food intake to specific time windows, has shown metabolic benefits. However, its impact on body weight loss remains inconclusive. The objective of this study was to investigate the influence of early TRE (eTRE) and delayed TRE (dTRE) on fat mobilization using human adipose tissue (AT) cultures.

Methods: Subcutaneous AT was collected from 21 participants with severe obesity. We assessed fat mobilization by measuring glycerol release in AT culture across four treatment conditions: control, eTRE, dTRE, and 24-h fasting.

Results: TRE had a significant impact on lipolysis (glycerol release [mean (SD)] in micromoles per hour per gram: control, 0.05 [0.003]; eTRE, 0.10 [0.006]; dTRE, 0.08 [0.005]; and fasting, 0.17 [0.008]; $p < 0.0001$). Both eTRE and dTRE increased lipolysis compared with the control group, with eTRE showing higher glycerol mobilization than dTRE during the overall 24-h time window, especially at the nighttime/habitual sleep episode ($p < 0.0001$). Further analysis of TRE based on fasting duration revealed that, independently of the time window, glycerol release increased with

See Commentary, pg. 1609.

fasting duration (in micromoles per hour per gram: 8 h = 0.08 [0.001]; 12 h = 0.09 [0.008]; and 16 h of fasting = 0.12 [0.011]; $p < 0.0001$).

Conclusions: This study provides insights into the potential benefits of TRE on fat mobilization and may guide the design of future dietary strategies for weight management and metabolic health.

INTRODUCTION

Obesity prevalence has steadily risen worldwide over the past few decades, becoming a significant public health concern due to its association with various comorbidities [1–3]. Meal timing and meal frequency can influence obesity, with evidence suggesting that irregular eating patterns and excessive snacking may contribute to weight gain and an increased risk of obesity [4]. In the contemporary world, an alarming trend reveals that over 50% of the population has eating periods that exceed 15 h/day [5]. At the same time, only a minority of the population maintains a fasting duration of at least 12 h [6].

The advantages of fasting on body weight, intestinal function, glucose tolerance, and blood pressure have been underlined in previous research [7]. As a result, eating plans based on extended fasting have become popular. One such plan is time-restricted eating (TRE), which limits energy intake to a specific time window by maintaining fasts of at least 14 to 16 h and often aiming to have mealtimes coincide with biological rhythms [8,9].

One of the primary purposes of opting for TRE is body weight loss. Nevertheless, results are still contradictory; although some cohort studies have observed a body weight reduction with the implementation of TRE [10,11], this effect was not observed in other studies [12]. On the other hand, benefits derived from TRE on metabolism have been observed, especially in glycemic control [13,14]. Several clinical trials have further reported that TRE results in increased insulin sensitivity, reduced blood pressure, and decreased appetite [11,15,16]. There are no studies, to our knowledge, specifically on TRE and its relationship with peripheral clocks. However, it has been shown that mealtime schedules can shift clock gene expression rhythms and can synchronize endogenous circadian rhythms in circulating glucose concentrations (i.e., independent of behavioral and environmental cycles), indicating that human peripheral circadian rhythms can be regulated by eating time [17,18].

Several studies have further investigated the best time for food intake for TRE. New concepts include early TRE (eTRE), i.e., limiting food to early hours, and delayed TRE (dTRE), i.e., eating later in the day [19]. Meta-analyses globally have found no significant weight loss differences between eTRE and dTRE [20]. However, independent studies have shown that eTRE improves blood pressure [11] and glucose tolerance [13] compared with dTRE. More trials and mechanistic studies are needed to determine the impact of eTRE on body weight regulation.

Study Importance

What is already known?

- Time-restricted eating (TRE) has become popular in recent years as a novel strategy for weight loss. However, results on weight loss with TRE are conflicting, and the relationship between TRE and weight loss must be better understood.
- This study aims to investigate the impact of TRE on fat mobilization in human adipose tissue (AT) to gain mechanistic insights into its effects.

What does this study add?

- The study demonstrates, in ex vivo explants of human AT, that both early TRE (eTRE) and delayed TRE (dTRE) are more effective in mobilizing fat from AT than a regular dietary pattern.
- eTRE shows higher fat mobilization during the 24-h time window than dTRE. Differences were significant during the nighttime/habitual sleep episode.
- This study, focused on AT culture, provides valuable mechanistic insights into the effects of TRE on AT fat mobilization.

How might these results change the direction of research or the focus of clinical practice?

- The findings suggest that eTRE and dTRE patterns can enhance fat mobilization, but eTRE may offer additional benefits in fat mobilization from AT, particularly during the nighttime.
- Understanding the impact of fasting duration on AT fat mobilization may lead to more personalized TRE recommendations and optimized TRE strategies for better weight loss outcomes and metabolic improvements.

Although adipose tissue (AT) is the main organ affected by TRE [21], there are no mechanistic studies, to our knowledge, in human AT culture regarding the impact of TRE on fat mobilization. Therefore, we aimed to study the impact of TRE on fat mobilization in AT. The hypothesis was that TRE would increase fat mobilization

compared with a more typical eating pattern with meals spread across the 16-h day and fasting during the nighttime. Additionally, we aimed to determine the effect of TRE on body fat mobilization compared with 24 h of continuous fasting. We measured glycerol release, the most direct measure of lipolysis in AT explants [22], from subcutaneous AT culture in individuals with obesity simulating four different conditions: 1) 24-h continuous fasting; 2) a typical dietary pattern with four meals across the day and nighttime fasting (control); 3) eTRE; and 4) dTRE. The goal was to gain insights into the mechanistic aspects of how TRE affects body fat mobilization.

METHODS

Participants

Subcutaneous AT biopsies were obtained from individuals with obesity ($n = 21$) with a mean (SD) age of 39.33 (10.38) years and a mean (SD) body mass index (BMI) of 40.51 (5.56) kg/m^2 and who underwent laparoscopic gastric bypass surgery at the Hospital Quirónsalud (Murcia, Spain). The general characteristics of the population are presented in Table 1.

AT biopsies were obtained from abdominal subcutaneous AT at the end of the surgical procedure, i.e., between 5 p.m. and 8 p.m., for all participants. Anthropometry, meal timing, metabolic syndrome (MetS), and sleep characteristics were assessed in the morning before the surgical procedure. Written informed consent was obtained following the Declaration of Helsinki and the study was approved by the Ethical Committee of the University of Murcia, Spain.

TABLE 1 General characteristics of the participants.

	Total population ($N = 21$)	
	Mean	SD
Sex (% female)	52.4	
Age (y)	39.33	10.38
BMI (kg/m^2)	40.51	5.56
Total body fat (%)	44.17	7.5
MetS traits		
Waist circumference (cm)	121.35	13.97
WHR	0.89	0.067
Glucose (nmol/L)[a]	5.54	1.81
Triglycerides (nmol/L)[a]	1.87	0.79
Diastolic BP (mm Hg)[a]	80	14
Systolic BP (mm Hg)[a]	114	12
MetS score	2.47	1.30
Nighttime sleep habits		
Sleep onset (hh:mm)	23:51	1:51
Sleep offset (hh:mm)	7:17	2:55
Sleep duration (h)	7.92	1.47
Number of nocturnal awakenings/per night	1.78	1.2
Habitual meal timing		
Breakfast onset (hh:mm)	7:58	1:08
Lunch onset (hh:mm)	14:49	0:46
Dinner onset (hh:mm)	21:01	0:39
Nighttime fasting duration (h)	13.41	1.19

Abbreviations: BP, blood pressure; MetS, metabolic syndrome; WHR, waist-hip ratio.
[a]Fasting conditions.

Anthropometry and MetS characteristics

Body weight was determined in participants wearing light clothes while barefoot using a digital electronic weighing scale. Height was determined using a Harpenden digital stadiometer with the participant standing and the head in the Frankfurt plane. The BMI was calculated using weight in kilograms divided by height in meters squared. Total body fat (percent) was measured by bioimpedance with a Tanita TBF-300.

Waist circumference was measured at the umbilicus level, and hip circumference was measured at the widest circumference over the great trochanters [23]. The waist-hip ratio was calculated as the ratio between waist and hip circumferences. Fasting serum concentrations of glucose, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and total cholesterol were determined and obtained at the clinical analysis laboratories of the Hospital Quirónsalud. Arterial systolic and diastolic blood pressure was measured with a mercury sphygmomanometer while the participant was seated for at least 10 min. A MetS score was calculated for each participant based on thresholds for waist circumference (≥80/94 cm), fasting glucose (5.6 mmol/dL), triglycerides (1.7 mmol/L), high-density lipoprotein cholesterol (1.03 mmol/L for men and 1.29 mmol/L for women), and systolic or diastolic blood pressure (130 mm Hg and 85 mm Hg, respectively), with a maximum value of six points [24].

Sleep characteristics, meal timing, and drug therapy

The same interviewer questioned all individuals regarding their habitual food and sleep schedules. The interview included the following sleep-related questions: 1) "At what time do you go to sleep?"; and 2) "What time do you usually awake in the morning?" The sleep duration was determined as the difference between sleep onset and offset. Furthermore, the question "How many times do you usually wake up during the night?" was asked to assess the number of awakenings during nocturnal sleep. None of the participants was a shift worker, and none reported insomnia. Habitual timing of food intake was assessed by asking the following question: "At what time do you usually have breakfast (lunch or dinner)?" Additionally, nighttime fasting duration was estimated using the following formula: the timing of the previous

night's last meal (dinner) minus the timing of the first meal of the next day (breakfast). None of the participants followed a dietary restriction diet except for the preoperative restriction imposed by the surgeon, which was the same for all. Some participants took antihypertensives, antidiabetics, and xanthine oxidase inhibitors.

Study design

The study design is represented in Figure 1. On day 1, the circadian rhythm of all explants was synchronized with dexamethasone between 6 a.m. and 7 a.m. to guarantee uniform synchrony among all cells and explants [25]. To mitigate the potential influence of dexamethasone on lipolysis, a thorough 1-h post-synchronization washout was carried out. The four treatment conditions were the following: control, simulating a more typical dietary pattern with four daily meals (every 4 h) and nighttime fasting (from midnight to 8 a.m.); eTRE, simulating eTRE with the eating window between 8 a.m. and 12 p.m.; dTRE, simulating dTRE with the eating window between 4 p.m. and 8 p.m.; and fasting, simulating continuous 24 h of fasting. On day 1, the explants were incubated according to the treatment, and, on day 2, the same treatment guidelines were maintained while the explants and culture medium were collected every 4 h at different circadian times (CTs; i.e., CT0 [hh:mm; 08:00], CT4 [noon, i.e., 12:00], CT8 [16:00], CT12 [20:00], CT16 [midnight, i.e., 00:00], CT20 [04:00], and CT24 [08:00]). In total, in each individual, four AT samples were cultured (one for each condition, i.e., control, fasting, eTRE, and dTRE) at each CT (CT0–CT24) for a total of 28 samples of AT per individual. Because the experiment was planned to be performed in 21 individuals for every condition, the expected number of samples was $21 \times 28 = 588$. However, we did not have enough AT for the control condition in one participant; therefore, the final number was $588 - 7 = 581$ AT samples. To keep the culture medium constant and to be able to compare the media among different conditions, the media were refreshed every 4 h for all conditions. Furthermore, in the control condition, glucose was added during the day every 4 h to keep glucose constant during the eating condition.

AT culture

After surgery, AT biopsies (30–40 g of AT/patient) were cut into small pieces of 1 to 2 mm^3 to enhance the contact of the AT with the culture medium. The fragments were combined in each well to obtain an approximate weight of 400 mg for each CT (no technical replicates). The explants were incubated in a total of 400 µL of culture medium. Different media were used for each treatment: DMEM, which was supplemented with 10% fetal bovine serum (FBS) and glucose (4.5 g/L) to simulate eating (Eating-Medium), and DMEM without glucose and without FBS to simulate fasting (Fasting-Medium). All media were supplemented with a penicillin–streptomycin-glutamine mixture.

On day 0 (the day of surgery), subcutaneous AT was cut and washed with phosphate-buffered saline (PBS). AT explants were distributed in seven wells (400 mg of AT for each well), one for each CT, and this was repeated for each condition (four conditions). These points were chosen to coincide with the usual start of the first meal in the morning, i.e., CT0 = 8 a.m., and the rest of the CTs were chosen

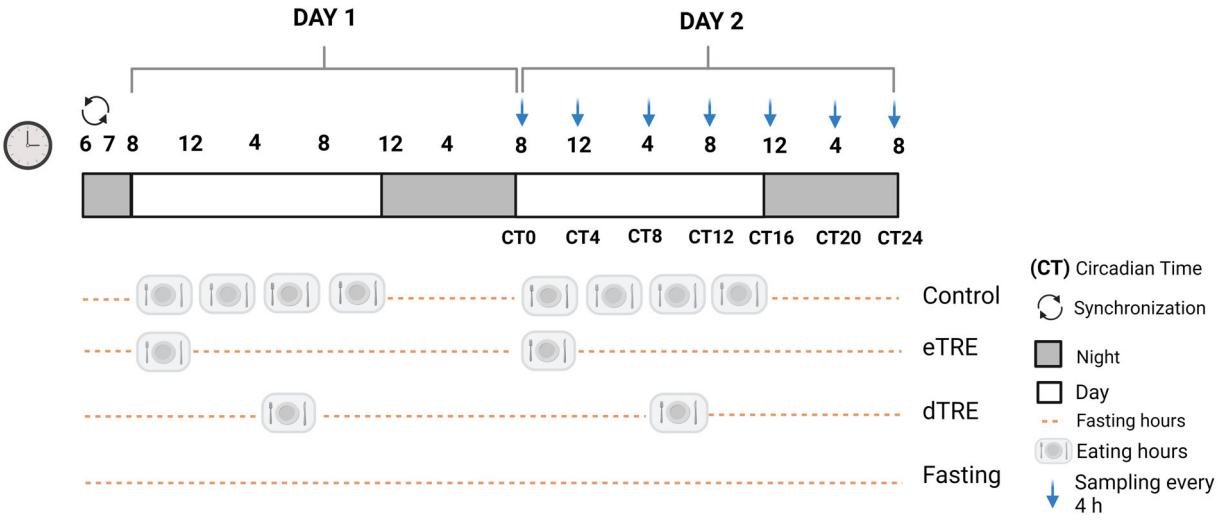

FIGURE 1 Study design. Treatment conditions: control, simulating a regular dietary pattern with four daily meals and nighttime fasting from midnight to 8 a.m. of the following day; early time-restricted eating (eTRE), eTRE between 8 a.m. and noon; delayed TRE (dTRE), dTRE between 4 p.m. and 8 p.m.; and fasting (F), 24 h of continuous fasting. All the explants were synchronized between 6 and 7 a.m. On day 1, the explants were incubated according to the treatment, and, on day 2, the same treatment guidelines were maintained while the explants and culture medium were collected every 4 h. Horizontal, orange, dotted lines indicate fasting hours, the gray rectangles with the plates allude to simulated mealtimes, approximate habitual nighttime sleep episodes of the patients from whom the adipose tissue was obtained are represented by gray rectangles, the awake day is represented by white rectangles, and blue arrows point to the explant sampling moments. [Color figure can be viewed at wileyonlinelibrary.com]

every 4 h to represent regular intervals throughout the 24-h day/night cycle, including the meal schedule. All explants were incubated with Eating-Medium until midnight on day 0. At midnight, explants were washed with PBS, and the culture medium was changed to Fasting-Medium to simulate overnight fasting. At 6 a.m., all explants were synchronized with dexamethasone (Fasting-Medium +1 μM dexamethasone) for 1 h, washed with PBS, and, between 7 and 8 a.m., re-incubated with Fasting-Medium.

On day 1, at 8 a.m., the eating window started for the control and eTRE groups. The control group was incubated for 16 h, refreshing the medium every 4 h until midnight with Eating-Medium to simulate a regular dietary pattern with four daily meals (every 4 h). During the nighttime, AT was maintained in fasting with Fasting-Medium (from midnight to 8 a.m.) to simulate nighttime fasting. On the other hand, eTRE was incubated for only 4 h with Eating-Medium until noon, remaining the rest of the day and the night in fasting. The dTRE group was incubated for 4 h with Eating-Medium between 4 and 8 p.m., and it was maintained with Fasting-Medium in the hours before and after the eating window and during the night. Finally, the fasting group was continuously incubated during the 24 h with Fasting-Medium.

On day 2, the same eating/fasting pattern was maintained, and explants and culture medium were collected every 4 h, as shown in Figure 1.

All cultures were kept at 37°C in a modified atmosphere of 7% carbon dioxide (CO_2). AT explants were collected and immediately frozen in liquid nitrogen at −195.8°C and preserved in freezers at −80°C in cryotubes for subsequent glycerol analysis.

Lipolysis in cultured AT explants

Lipolysis was evaluated by measuring the glycerol released (micromoles per hour per gram), a direct product of lipolysis in AT explants [22]. Glycerol was determined by a Glycerol Standard solution and Free Glycerol Reagent according to the manufacturer's instructions. Finally, the absorbance at 540 nm was detected with CLARIOstar plate readers to measure the released glycerol.

Statistical methods

The characteristics of the participants are expressed as mean and SD in Table 1. The glycerol release (micromoles per hour per gram) did not follow a normal distribution; therefore, it was log-transformed. A linear mixed model was developed to determine potential differences in glycerol release among different treatment conditions, and the following were considered as fixed factors: treatment (control, eTRE, dTRE, and fasting) and CT (each point of the circadian cycle). The individual was considered the random effect to analyze the correlations among data of the same patient. The response variable was the logarithm of the glycerol release (micromoles per hour per gram). We considered the interactions between treatment and circadian timing.

To understand whether fasting duration per se influenced fat mobilization, a secondary mixed model was developed. In this model, we analyzed differences between eTRE and dTRE in 24-h average glycerol release after matching, by fasting duration, both eTRE and dTRE. Fasting duration and treatment were considered as fixed factors. In order to understand whether differences in fasting duration depended on the time window, we considered the interactions between fasting duration and treatment. All of the analyses were further repeated after adjusting by different covariates such as age, sex, drug therapy, and individual chronotype (Morning-Evening Questionnaire [MEQ]), and significance was maintained. Statistical analyses were conducted using IBM SPSS Statistics for Windows. All statistical tests and hypotheses' significance level were $p < 0.05$. The standard error of the mean (SEM) glycerol release was calculated using Bootstrap Stata. GraphPad Prism software version 8.0.2 was used to obtain the figures.

RESULTS

The general characteristics of the participants ($n = 21$) are shown in Table 1. Participants had class 2 or 3 obesity (BMI ≥ 35 kg/m^2). Waist circumference and triglycerides concentrations were above the normal ranges according to the International Diabetes Federation (IDF), whereas blood pressure and serum glucose concentrations were within the normal ranges [26].

The population average glycerol release of the 21 participants' AT cultures for every CT (i.e., each point of the circadian cycle, every 4 h) in each treatment condition is represented in Figure 2. Statistical results from the linear mixed model showed that there were significant independent differences in glycerol release (micromoles per hour per gram) for the following: 1) the treatment conditions (control, eTRE, dTRE, or fasting; $p < 0.0001$); and 2) the circadian timing ($p < 0.0001$). Additionally, significant interactions were found between the treatment and the circadian timing for glycerol release ($p < 0.0001$), suggesting that the effect of the treatment on glycerol release changes depending on the circadian timing.

Further exploratory analyses with the same mixed models for each circadian time showed significant differences between control and eTRE at CT0, CT4, CT12, CT16, CT20, and CT24 and between control and dTRE at CT4, CT8, CT20, and CT24, as indicated in Figure 2 by the asterisks and hash symbols. The control condition was significantly different at every CT compared with the 24-h continuous fasting condition ($p < 0.0001$). Furthermore, significant differences were also found between eTRE and the fasting condition at every CT except CT0 and CT24 (8 a.m.) and between dTRE and the fasting condition at every CT except CT4 (noon) and CT8 (4 p.m.), with higher glycerol release in the fasting condition. Symbols for significance between fasting and any condition are not represented in Figure 2 to avoid confusion.

Figure 3A represents the 24-h average glycerol release in the four treatment conditions. As expected, significant differences were found between control and the fasting condition ($p < 0.0001$), with significantly higher glycerol release in the fasting condition. Significant

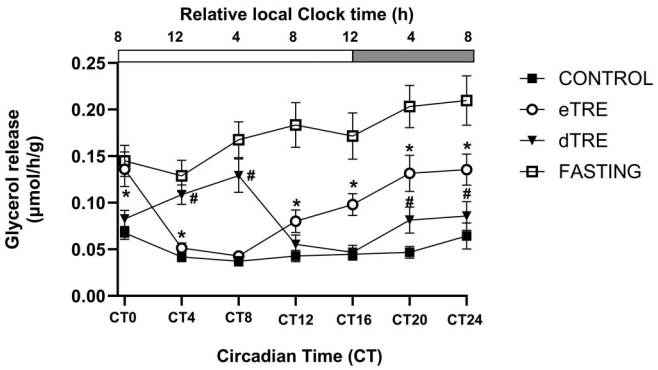

FIGURE 2 Population average glycerol release every 4 h during the 24-h time window according to the treatment. Data are represented as black squares for control, white squares with black borders for fasting, white circles with black borders for early time-restricted eating (eTRE), and black triangles for delayed TRE (dTRE). All SEM values are represented as vertical lines. Asterisks and hash symbols indicate statistical significance ($p < 0.0001$). Asterisks were included when differences were significant between control and eTRE at the circadian time (CT) points and hash symbols when differences were significant between control and dTRE. The control condition was significantly different at every point compared with the 24-h continuous fasting condition ($p < 0.0001$) for every CT. Furthermore, significant differences were also found between eTRE and the fasting condition at CT4, CT8, CT12, CT16, and CT20 and between dTRE and the fasting condition for CT0, CT12, CT16, CT20, and CT24 (symbols for significance are not represented to avoid confusion). The x-axes are represented in relative local clock time (hours; top x-axis) and CT (bottom x-axis). On the top x-axis, the approximate habitual sleep episode of the patients from whom the adipose tissue was obtained is represented with a gray horizontal bar, and the awakening day is represented with a white horizontal bar. Data are presented as mean ± SEM.

differences were also found between control and both TRE conditions (eTRE and dTRE), with significantly higher glycerol release in the TRE conditions ($p < 0.0001$) during the 24-h period. Statistical results from the linear mixed model showed a higher fat mobilization in eTRE than in dTRE during the 24-h period ($p = 0.019$).

Furthermore, after dividing the day between the daytime/habitual waking episode and the nighttime/habitual sleeping episode, significant differences were found between both TRE timing windows (eTRE and dTRE) only during the nighttime/habitual sleeping episode (from midnight to 8 a.m. of the following day; $p = 0.000007$; Figure 3C), whereas no significant differences were found during the daytime/habitual active episode ($p = 0.976$; Figure 3B).

Further exploratory analyses matched both eTRE and dTRE according to the fasting duration (Figure S1). Results from the linear mixed model showed that, independent of the time window (early or delayed), glycerol release increased with fasting duration. At 8 h of fasting, the glycerol release was 0.08 [0.001] µmol/h/g, whereas at 12 h of fasting it was 0.09 [0.008] µmol/h/g, and at 16 h of fasting it was 0.12 [0.011] µmol/h/g ($p < 0.0001$), with no significant interactions between the duration of fasting and the early or delayed time window ($p = 0.988$). All of the analyses were further repeated after adjusting for age, sex, drug therapy, and individual chronotype, and significance was maintained.

DISCUSSION

The study aimed to investigate the impact of TRE on fat mobilization in subcutaneous AT culture from individuals with class 2 and 3 obesity. The outcome measure was glycerol release, which serves as an indicator of lipolysis or fat mobilization from the AT explants. The study design allowed for a comprehensive evaluation of the effects of different simulated eating patterns on fat mobilization, including eTRE and dTRE, along with continuous fasting (fasting condition) and a typical dietary pattern with several meals across the day and nighttime fasting (control condition).

Results showed significant differences in glycerol release among the four treatment conditions and across the circadian time points, indicating a dynamic influence of both treatment and circadian timing on lipolysis. The exploratory analyses also revealed that eTRE and dTRE showed significantly higher glycerol release than the control condition, suggesting that both TRE patterns can enhance fat mobilization from AT. Importantly, eTRE showed higher glycerol mobilization than dTRE, which would suggest that concentrating eating in the morning could produce additional benefits regarding lipolysis and fat breakdown than concentrating eating during the evening. Eating in the morning may be better aligned with the body's natural circadian rhythm because, during the morning hours, the body shows higher diet-induced thermogenesis [27], faster gastric emptying [28], higher glucose tolerance [29], increased beta cell function [30], and lower insulin resistance [31–33] than during the evening. The exploratory analyses comparing the duration of fasting between eTRE and dTRE showed that longer fasting durations were linked to increased glycerol release, irrespective of the time window.

TRE has become popular as a novel strategy to lose weight and combat metabolic diseases in part because it does not require tedious and time-consuming methods such as calorie counting [34]. The present study aims to provide a greater understanding of the role of TRE in body fat mobilization in human AT. To our knowledge, this is the first study to evaluate dietary strategies of TRE directly in explants of human AT ex vivo, in which circadian time and fasting duration are taken into account. The study focuses on human AT culture, which is particularly valuable because it offers mechanistic insights into the effects of TRE at a cellular level. Furthermore, this approach is relevant because AT plays a fundamental role in fat mobilization and, subsequently, in weight loss [35].

The observed increase in glycerol release during both eTRE and dTRE, compared with the control condition, suggests that TRE enhances lipolysis and fat breakdown compared with a regular eating pattern with meals every 4 h during the day and fasting during the nighttime, which might be linked to improved insulin sensitivity and reduced adiposity, as has been seen in previous clinical trials [12,36,37]. Animal studies have also revealed that limiting the feeding schedule to a specific time window, compared with a regular

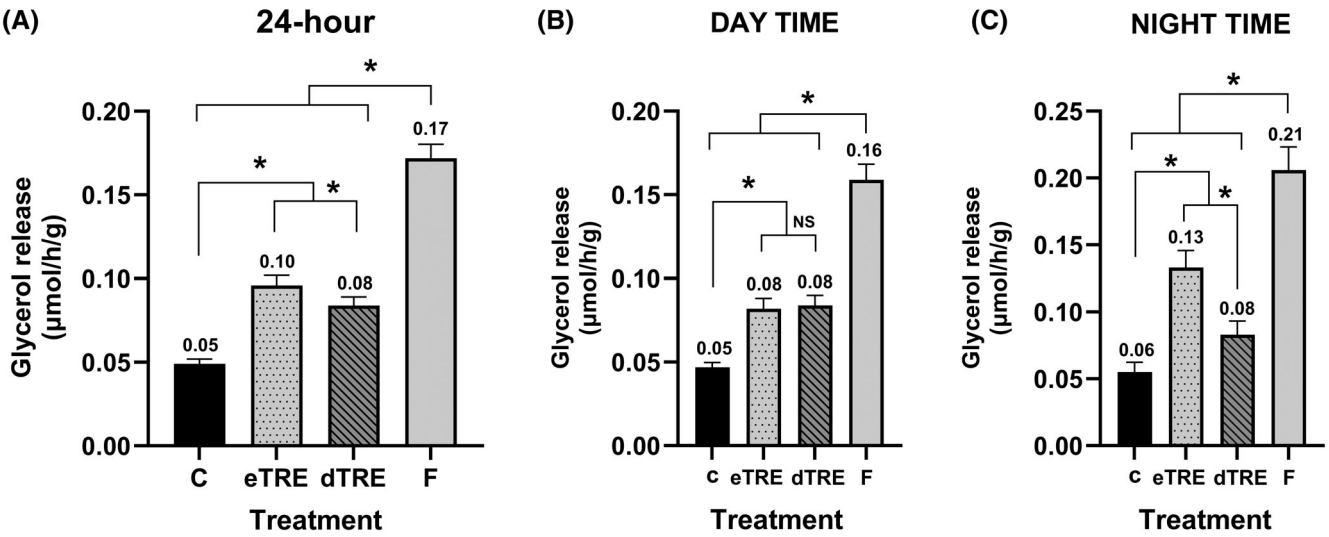

FIGURE 3 Average glycerol release in different treatment conditions. Control (C; black bar), $n = 20$; early time-restricted eating (eTRE; dotted bar), $n = 21$; delayed TRE (dTRE; striped bar), $n = 21$; and fasting (F; gray bar), $n = 21$. (A) 24-h glycerol release is divided in two periods: (B) the daytime/awake period (from 8 a.m. to midnight) and (C) the nighttime/sleeping period (from midnight to 8 a.m. of the following day). All SEM are represented as vertical lines. Data are presented as mean ± SEM. *Indicates statistical significance ($p < 0.0001$). NS, not significant.

feeding pattern, leads to significant weight loss, decreased adipocyte size, increased β-oxidation, and browning of white AT by increased expression of uncoupling protein 1 (UCP-1) [38,39] and also decreased expression of cell death–inducing DNA fragmentation factor-like effector C (*CIDEC*), an inhibitory gene for lipolysis [40].

TRE has been proposed as a potential therapeutic strategy for weight management in adults with obesity [41–43]. However, there is still debate in the scientific community regarding the efficacy of TRE as a dietary strategy for weight loss [44]. Indeed, several studies have suggested that TRE is no more effective for weight loss than diets without a time restriction [45,46]. This controversy could be related to the fact that the recommended duration of the eating window for weight loss needs to be clarified. In humans, the most frequently recommended eating window ranges from 6 to 10 h, which has been shown to produce weight loss and metabolic improvements in several studies [11,47]. In the current experiment, to test our hypothesis in extreme fasting conditions that have already been used in humans [16], we have simulated what would have happened in AT with an eating window of 4 h. Results show that this short 4-h eating window (eTRE or dTRE) produces more significant fat mobilization than a regular eating pattern with several meals during the daytime and fasting during the nighttime. Cienfuegos et al. [16] observed in humans that 4 h of TRE mobilized more fat than no schedule restriction, which supports our results.

Another confounding factor that may impact the disparity among studies is the timing of the eating window, i.e., whether the eating window during TRE occurs in the morning or the evening. Human studies have shown that consuming most calories earlier in the day and limiting food intake in the evening have potential health benefits such as improved lipid levels, glycemic control, weight loss, and reduced hunger [37,43,48,49]. Our results show significant differences in 24-h glycerol release between the early and delayed timing window toward a higher glycerol mobilization in eTRE than in dTRE, which was particularly relevant during the nighttime/habitual sleeping episode, whereas no significant differences were found during the daytime or habitual waking episode within both TRE timing windows. The findings suggest that eTRE, which initiates the eating window early in the day, may promote more efficient fat mobilization during the nighttime, increasing lipolysis [50] to meet energy needs during nighttime fasting [35]. Although our results show significant differences between eTRE and dTRE glycerol release during the 24 h (Figure 3A), previous meta-analyses have found no differences in body weight between eTRE and dTRE [20]. Indeed, mechanisms underlying the effects of meal timing on health outcomes may be multifactorial.

Among this study's strengths is the fact that it is the first, to our knowledge, to evaluate the direct effect of TRE on an ex vivo circadian pattern in human AT. One of the limitations of the present work is that the study was conducted on people with class 2 and 3 obesity to ensure a sufficient quantity of AT for explant culture; therefore, results may not be directly transferable to the general population. Furthermore, the study was only performed in subcutaneous AT and not in visceral AT. Even though age, sex, medications, and individual chronotype did not significantly impact the results, further studies in larger populations should test whether there may be differences dependent on sex, age, and chronotype that were too small to be detected in the current study. In addition, we only determined data every 4 h during the 24-h time window due to limitations in the amount of AT collected, and we only tested a 4-h eating window, which may be difficult to apply in today's society. Further studies should be performed testing different eating windows of longer duration to test whether the effects of TRE are similar.

In summary, we have shown that, when simulating eTRE and dTRE in human AT, fat mobilization was higher than when simulating a regular dietary pattern with four daily meals (every 4 h) and nighttime fasting

(from midnight to 8 a.m., i.e., a 16/8 pattern). Moreover, between eTRE and dTRE, differences were found in the amount of glycerol released during the 24 h of the day, with increased glycerol released during the night period in the eTRE compared with the dTRE condition. These results may lead to a better understanding of the mechanistic factors involved in these novel dietary strategies that use TRE for weight loss. Nevertheless, in order to translate these conclusions to general practice, it is essential to compare different time window durations and to consider the habitual meal timing of the individuals and other factors that may be involved in the effectiveness of these treatments, such as age; sex; individual chronotype; or genetic, physiological, psychological, and environmental factors.O

AUTHOR CONTRIBUTIONS

Marta Garaulet conceived and designed the study, acquired funding, designed the research, managed the project, and wrote the final version of the paper. Frank A. J. L Scheer conceived and designed the study and reviewed the final version of the manuscript. Carolina Zambrano performed research, contributed to writing the first draft of the manuscript, analyzed data, and designed all figures and tables. Elena González-Alvarado performed research (initial experiments). Diego Salmerón analyzed data, contributed to writing the statistical methods, and reviewed the paper. Francisco Javier Ruiz-Ojeda contributed to methodological advice and reviewed the paper. Juan Luján contributed to recruitment of patients and adipose tissue sampling. All authors contributed to manuscript revision and read and approved the submitted version.

FUNDING INFORMATION

This work has been supported in part by grant PID2020 112768RB-I00 funded by Ministerio de Ciencia, Innovacion y Universidades/Agencia estatal de investigación (MCIN/AEI)/10.13039/501100011033 to Marta Garaulet and by National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) R01DK105072 to Marta Garaulet. Frank A. J. L. Scheer was supported in part by NIDDK R01DK105072 and National Heart, Lung, and Blood Institute (NHLBI) R01HL140574.

CONFLICT OF INTEREST STATEMENT

Frank A. J. L. Scheer served on the Board of Directors for the Sleep Research Society and has received consulting fees from the University of Alabama at Birmingham and Morehouse School of Medicine. The interests of Frank A. J. L. Scheer were reviewed and managed by Brigham and Women's Hospital and Partners HealthCare following their conflict of interest policies. Frank A. J. L. Scheer's consultancies are not related to the current work. The other authors declared no conflicts of interest.

ORCID

Frank A. J. L. Scheer https://orcid.org/0000-0002-2014-7582
Marta Garaulet https://orcid.org/0000-0002-4066-3509

REFERENCES

1. Dikaiou P, Björck L, Adiels M, et al. Obesity, overweight and risk for cardiovascular disease and mortality in young women. *Eur J Prev Cardiol*. 2021;28(12):1351-1359.
2. Avgerinos KI, Spyrou N, Mantzoros CS, Dalamaga M. Obesity and cancer risk: emerging biological mechanisms and perspectives. *Metabolism*. 2019;92:121-135.
3. Larsson SC, Burgess S. Causal role of high body mass index in multiple chronic diseases: a systematic review and meta-analysis of Mendelian randomization studies. *BMC Med*. 2021;19(1):320.
4. Barrington WE, Beresford SAA. Eating occasions, obesity and related behaviors in working adults: does it matter when you snack? *Nutrients*. 2019;11(10):2320.
5. Gupta NJ, Kumar V, Panda S. A camera-phone based study reveals erratic eating pattern and disrupted daily eating-fasting cycle among adults in India. *PLoS One*. 2017;12(3):e0172852.
6. Kant AK. Eating patterns of US adults: meals, snacks, and time of eating. *Physiol Behav*. 2018;193(Pt B):270-278.
7. Regmi P, Heilbronn LK. Time-restricted eating benefits, mechanisms, and challenges in Translation. *iScience*. 2020;23(6):101161.
8. Varady KA, Cienfuegos S, Ezpeleta M, Gabel K. Clinical application of intermittent fasting for weight loss: progress and future directions. *Nat Rev Endocrinol*. 2022;18(5):309-321.
9. Queiroz JDN, Macedo RCO, Tinsley GM, Reischak-Oliveira A. Time-restricted eating and circadian rhythms: the biological clock is ticking. *Crit Rev Food Sci Nutr*. 2021;61(17):2863-2875.
10. Gill S, Panda S. A smartphone app reveals erratic diurnal eating patterns in humans that can be modulated for health benefits. *Cell Metab*. 2015;22(5):789-798.
11. Gabel K, Hoddy KK, Haggerty N, et al. Effects of 8-hour time restricted feeding on body weight and metabolic disease risk factors in obese adults: a pilot study. *Nutr Healthy Aging*. 2018;4(4):345-353.
12. Sutton EF, Beyl R, Early KS, Cefalu WT, Ravussin E, Peterson CM. Early time-restricted feeding improves insulin sensitivity, blood pressure, and oxidative stress even without weight loss in men with prediabetes. *Cell Metab*. 2018;27(6):1212-1221.e3.
13. Hutchison AT, Regmi P, Manoogian ENC, et al. Time-restricted feeding improves glucose tolerance in men at risk for type 2 diabetes: a randomized crossover trial. *Obesity (Silver Spring)*. 2019;27(5):724-732.
14. Andriessen C, Fealy CE, Veelen A, et al. Three weeks of time-restricted eating improves glucose homeostasis in adults with type 2 diabetes but does not improve insulin sensitivity: a randomised crossover trial. *Diabetologia*. 2022;65(10):1710-1720.
15. Tacad DKM, Tovar AP, Richardson CE, et al. Satiety associated with calorie restriction and time-restricted feeding: peripheral hormones. *Adv Nutr*. 2022;13(3):792-820.
16. Cienfuegos S, Gabel K, Kalam F, et al. Effects of 4- and 6-h time-restricted feeding on weight and cardiometabolic health: a randomized controlled trial in adults with obesity. *Cell Metab*. 2020;32(3):366-378.e3.
17. Wehrens SMT, Christou S, Isherwood C, et al. Meal timing regulates the human circadian system. *Curr Biol*. 2017;27(12):1768-1775.e3.
18. Chellappa SL, Qian J, Vujovic N, et al. Daytime eating prevents internal circadian misalignment and glucose intolerance in night work. *Sci Adv*. 2021;7(49):eabg9910.
19. Aragon AA, Schoenfeld BJ. Does timing matter? A narrative review of intermittent fasting variants and their effects on bodyweight and body composition. *Nutrients*. 2022;14(23):5022.
20. Liu J, Yi P, Liu F. The effect of early time-restricted eating vs later time-restricted eating on weight loss and metabolic health. *J Clin Endocrinol Metab*. 2023;108(7):1824-1834.
21. Chaix A, Zarrinpar A. The effects of time-restricted feeding on lipid metabolism and adiposity. *Adipocyte*. 2015;4(4):319-324.
22. Schweiger M, Eichmann TO, Taschler U, Zimmermann R, Zechner R, Lass A. Measurement of lipolysis. *Methods Enzymol*. 2014;538:171-193.
23. Garaulet M, Hernandez-Morante JJ, Tebar FJ, Zamora S. Anthropometric indexes for visceral fat estimation in overweight/obese women attending to age and menopausal status. *J Physiol Biochem*. 2006;62(4):245-252.

24. Zimmet P, Magliano D, Matsuzawa Y, Alberti G, Shaw J. The metabolic syndrome: a global public health problem and a new definition. *J Atheroscler Thromb*. 2005;12(6):295-300.
25. Friedrichs M, Kolbe I, Seemann J, et al. Circadian clock rhythms in different adipose tissue model systems. *Chronobiol Int*. 2018;35(11):1543-1552.
26. Alberti KG, Zimmet P, Shaw J, IDF Epidemiology Task Force Consensus Group. The metabolic syndrome-a new worldwide definition. *Lancet*. 2005;366(9491):1059-1062.
27. Morris CJ, Garcia JI, Myers S, Yang JN, Trienekens N, Scheer FA. The human circadian system has a dominating role in causing the morning/evening difference in diet-induced thermogenesis. *Obesity (Silver Spring)*. 2015;23(10):2053-2058.
28. Goo RH, Moore JG, Greenberg E, Alazraki NP. Circadian variation in gastric emptying of meals in humans. *Gastroenterology*. 1987;93(3):515-518.
29. Morris CJ, Yang JN, Garcia JI, et al. Endogenous circadian system and circadian misalignment impact glucose tolerance via separate mechanisms in humans. *Proc Natl Acad Sci USA*. 2015;112(17):E2225-E2234. doi:10.1073/pnas.1418955112
30. Qian J, Dalla Man C, Morris CJ, Cobelli C, Scheer FA. Differential effects of the circadian system and circadian misalignment on insulin sensitivity and insulin secretion in humans. *Diabetes Obes Metab*. 2018;20(10):2481-2485. doi:10.1111/dom.13391
31. Ravussin E, Acheson KJ, Vernet O, Danforth E, Jequier E. Evidence that insulin resistance is responsible for the decreased thermic effect of glucose in human obesity. *J Clin Invest*. 1985;76(3):1268-1273.
32. Van Cauter E, Blackman JD, Roland D, Spire JP, Refetoff S, Polonsky KS. Modulation of glucose regulation and insulin secretion by circadian rhythmicity and sleep. *J Clin Invest*. 1991;88(3):934-942.
33. Carrasco-Benso MP, Rivero-Gutierrez B, Lopez-Minguez J, et al. Human adipose tissue expresses intrinsic circadian rhythm in insulin sensitivity. *FASEB J*. 2016;30(9):3117-3123.
34. Christensen RAG, Kirkham AA. Time-restricted eating: a novel and simple dietary intervention for primary and secondary prevention of breast cancer and cardiovascular disease. *Nutrients*. 2021;13(10):3476.
35. Kersten S. The impact of fasting on adipose tissue metabolism. *Biochim Biophys Acta Mol Cell Biol Lipids*. 2023;1868(3):159262.
36. Xie Z, Sun Y, Ye Y, et al. Randomized controlled trial for time-restricted eating in healthy volunteers without obesity. *Nat Commun*. 2022;13(1):1003.
37. Ravussin E, Beyl RA, Poggiogalle E, Hsia DS, Peterson CM. Early time-restricted feeding reduces appetite and increases fat oxidation but does not affect energy expenditure in humans. *Obesity (Silver Spring)*. 2019;27(8):1244-1254.
38. Bushman T, Lin TY, Chen X. Depot-dependent impact of time-restricted feeding on adipose tissue metabolism in high fat diet-induced obese male mice. *Nutrients*. 2023;15(1):238.
39. Aouichat S, Chayah M, Bouguerra-Aouichat S, Agil A. Time-restricted feeding improves body weight gain, lipid profiles, and atherogenic indices in cafeteria-diet-fed rats: role of browning of inguinal white adipose tissue. *Nutrients*. 2020;12(8):2185.
40. Hatori M, Vollmers C, Zarrinpar A, et al. Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. *Cell Metab*. 2012;15(6):848-860.
41. Huang L, Chen Y, Wen S, et al. Is time-restricted eating (8/16) beneficial for body weight and metabolism of obese and overweight adults? A systematic review and meta-analysis of randomized controlled trials. *Food Sci Nutr*. 2023;11(3):1187-1200.
42. Charlot A, Hutt F, Sabatier E, Zoll J. Beneficial effects of early time-restricted feeding on metabolic diseases: importance of aligning food habits with the circadian clock. *Nutrients*. 2021;13(5):1405.
43. Garaulet M, Gomez-Abellan P, Alburquerque-Bejar JJ, Lee YC, Ordovas JM, Scheer FA. Timing of food intake predicts weight loss effectiveness. *Int J Obes (Lond)*. 2013;37(4):604-611.
44. Elortegui Pascual P, Rolands MR, Eldridge AL, et al. A meta-analysis comparing the effectiveness of alternate day fasting, the 5:2 diet, and time-restricted eating for weight loss. *Obesity (Silver Spring)*. 2023;31(suppl 1):9-21.
45. Lowe DA, Wu N, Rohdin-Bibby L, et al. Effects of time-restricted eating on weight loss and other metabolic parameters in women and men with overweight and obesity: the TREAT randomized clinical trial. *JAMA Intern Med*. 2020;180(11):1491-1499.
46. Liu D, Huang Y, Huang C, et al. Calorie restriction with or without time-restricted eating in weight loss. *N Engl J Med*. 2022;386(16):1495-1504.
47. Wilkinson MJ, Manoogian ENC, Zadourian A, et al. Ten-hour time-restricted eating reduces weight, blood pressure, and atherogenic lipids in patients with metabolic syndrome. *Cell Metab*. 2020;31(1):92-104 e5.
48. Ruddick-Collins LC, Morgan PJ, Fyfe CL, et al. Timing of daily calorie loading affects appetite and hunger responses without changes in energy metabolism in healthy subjects with obesity. *Cell Metab*. 2022;34(10):1472-1485.e6.
49. Vujović N, Piron MJ, Qian J, et al. Late isocaloric eating increases hunger, decreases energy expenditure, and modifies metabolic pathways in adults with overweight and obesity. *Cell Metab*. 2022;34(10):1486-1498.e7. doi:10.1016/j.cmet.2022.09.007
50. Grabner GF, Xie H, Schweiger M, Zechner R. Lipolysis: cellular mechanisms for lipid mobilization from fat stores. *Nat Metab*. 2021;3(11):1445-1465.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Zambrano C, González-Alvarado E, Salmerón D, et al. Time-restricted eating affects human adipose tissue fat mobilization. *Obesity (Silver Spring)*. 2024;32(9):1680-1688. doi:10.1002/oby.24057