

## METABOLISM

# Intended isocaloric time-restricted eating shifts circadian clocks but does not improve cardiometabolic health in women with overweight

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Time-restricted eating (TRE) is a promising strategy to improve metabolic outcomes. However, it remains unclear whether TRE has cardiometabolic benefits in an isocaloric setting and whether its effects depend on the eating timing. We conducted a randomized crossover trial in 31 women with overweight or obesity to directly compare the effects of a 2-week early TRE (eTRE; eating from 8:00 to 16:00) and a 2-week late TRE (lTRE; eating from 13:00 to 21:00) on insulin sensitivity, cardiometabolic risk factors, and the internal circadian phase. During the restricted 8-hour eating period, participants were asked to consume their habitual food quality and quantity. Insulin sensitivity did not differ between (−0.07; 95% CI, −0.77 to 0.62;  $P = 0.60$ ) or within (eTRE: 0.31; 95% CI, −0.14 to 0.76;  $P = 0.11$ ; lTRE: 0.19; 95% CI, −0.22 to 0.60;  $P = 0.25$ ) interventions. Twenty-four-hour glucose, lipid, inflammatory, and oxidative stress markers showed no clinically meaningful between- or within-intervention differences. Participants demonstrated high timely adherence (eTRE, 96.5%; lTRE, 97.7%), unchanged dietary composition and physical activity, minor daily calorie deficit (eTRE, −167 kilocalories/day), and weight loss (eTRE, −1.08 kilograms; lTRE, −0.44 kilograms). In lTRE, the circadian phase in blood monocytes (24 minutes; 95% CI, −5 to 54 minutes;  $P = 0.10$ ) and sleep midpoint (15 minutes; 95% CI, 7 to 23 minutes;  $P < 0.001$ ) occurred later compared with eTRE. Overall, in an intended isocaloric setting, neither eTRE nor lTRE improves insulin sensitivity or other cardiometabolic traits, despite a shift of internal circadian clocks.

## INTRODUCTION

Time-restricted eating (TRE) is a form of intermittent fasting characterized by a daily eating window of 10 hours or less and a prolonged fasting period of at least 14 hours over the course of the day (1). TRE is becoming increasingly popular as a simple dietary approach to

controlling body weight and improving metabolic health (2, 3). In rodents, TRE is protective against diet-induced obesity and associated metabolic disturbances (4, 5). Similarly, human trials on TRE have highlighted numerous beneficial cardiometabolic effects, such as improved fasting (3, 6, 7) and mean daily (3, 8) glucose concentrations, insulin resistance (3, 7, 9) or insulin sensitivity (2, 10), triglyceride (6, 8, 11, 12), total (12, 13) and low-density lipoprotein (LDL) cholesterol (13) concentrations, and blood pressure (2, 13, 14), as well as moderate body weight (8, 9, 13, 15, 16) and body fat reduction (7, 9, 13). Therefore, beyond its effects on body weight, TRE represents a promising approach to combating insulin resistance and diabetes.

However, results of TRE trials are inconsistent (17, 18) and require stronger clinical trial evidence (19) to answer several practically relevant questions. It is unclear whether metabolic improvements are induced by the restriction of the daily eating duration itself, by accompanying caloric restriction (and respective weight loss), or by the combination of both factors. Most TRE trials have not carefully monitored energy intake or other potential cofounders. Therefore, we investigated whether 8-hour TRE can improve insulin sensitivity and other cardiometabolic parameters in an intended isocaloric setting, where we precisely controlled calorie intake, timely adherence, dietary composition, and physical activity.

The secondary objective of the trial was to compare the effects of eating early (eTRE) versus eating late (lTRE) in the day during the TRE intervention. Although most clinical studies suggest additional benefits of eTRE (2, 3, 10, 20), which might be explained by circadian rhythms of key metabolic processes (18, 21), trials that directly compare eTRE and lTRE are limited (8, 22, 23). On the basis of previous research, we hypothesized that an isocaloric TRE would

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improve insulin sensitivity and cardiometabolic health compared with baseline and that eTRE would be more effective than ITRE. To investigate this, we conducted a randomized crossover ChronoFast trial including two dietary intervention periods—eTRE and ITRE—which were intended to be isocaloric (17). Because some previously published trials comparing eTRE and ITRE or trials investigating impacts of TRE on glucose metabolism have included exclusively male participants (2, 7, 8, 10) and therefore may not be generalizable to females, our study was conducted exclusively in women with overweight or obesity. Here, we report the trial results on insulin sensitivity and other cardiometabolic outcomes as well as on circadian clocks assessed by circadian phase in blood monocytes and sleep timing.

## RESULTS

### Participant characteristics and study flow

A 10-week randomized crossover ChronoFast trial (NCT04351672) included two 2-week dietary intervention periods: (i) eTRE (8-hour eating window from 8:00 to 16:00) and (ii) ITRE (8-hour eating window from 13:00 to 21:00), preceded by a 2- to 4-week baseline period and separated by a 2-week washout period (Fig. 1A) (17). Between April 2020 to December 2021, we screened 90 and enrolled 31 participants, of whom 15 were allocated to the eTRE-ITRE and 16 were allocated to the ITRE-eTRE study arms (Fig. 1B). Participants had a mean body mass index (BMI) of 30.5 kg/m<sup>2</sup> (±SD of 2.9) and a median age of 62 years [interquartile range (IQR) of 53 to 65]. Eighteen participants showed a normal glucose tolerance (NGT), and 13 participants had an impaired fasting glucose or impaired glucose tolerance (IFG/IGT). All participants were female (26 postmenopausal and 5 premenopausal), white, and of Caucasian ethnicity (Table 1). There were no dropouts after randomization, and all 31 participants completed the study. Final analysis included 31 participants. Thirty adverse events possibly related to the study were reported, but none were serious (table S1).

### Adherence to TRE interventions and study protocol

During the TRE interventions, participants were asked to restrict their eating duration to 8 hours per day but consume their usual kind and amount of food. All participants showed high adherence to the 8-hour eating duration during the TRE interventions as shown by analysis of food records over 14 days during the baseline and both interventions. During the baseline, participants ate within a mean time period of 12:06 hours (±1:35). The eating duration was a mean of 7:09 hours (±0:32) in eTRE and a mean of 6:57 hours (±0:50) in ITRE (Fig. 2A and fig. S1). Adherence to the prescribed eating time was also high, with a mean of 96.5% (±6.3%) of all intervention days in eTRE and a mean of 97.7% (±6.1%) in the ITRE, according to the food record analysis (Fig. 2B). Energy intake remained unchanged in ITRE [−97 kcal; 95% confidence interval (CI), −193 to −2; *P* = 0.06] but decreased minimally in eTRE (−167 kcal; 95% CI, −249 to −86 kcal; *P* < 0.001) compared with the baseline (Fig. 2C). No changes in percentage of carbohydrate, fat, and protein intake, as well as in physical activity assessed by actigraphy, were found in either TRE intervention compared to the baseline (Fig. 2, D and E, and table S2).

### Body weight and composition

Participants showed a minimal weight loss of −1.08 kg (95% CI, −0.77 to −1.40 kg; *P* < 0.001) within eTRE and −0.44 kg (95% CI, −0.74 to −0.13 kg; *P* = 0.01) within ITRE, resulting in a between-

intervention difference of 0.65 kg (95% CI, 0.27 to 1.03 kg; *P* = 0.012) (Table 2). BMI decreased in eTRE (−0.45 kg/m<sup>2</sup>; 95% CI, −0.56 to −0.33 kg/m<sup>2</sup>; *P* < 0.001) and ITRE (−0.12 kg/m<sup>2</sup>; 95% CI, −0.21 to −0.03 kg/m<sup>2</sup>; *P* = 0.01), with a between-diet difference of 0.33 kg/m<sup>2</sup> (95% CI, 0.21 to 0.44 kg/m<sup>2</sup>; *P* < 0.001). Fat mass loss (−0.61 kg; 95% CI, −1.01 to −0.22 kg; *P* = 0.002) and lean mass loss (−0.57 kg; 95% CI, −1.11 to −0.04 kg; *P* = 0.04) were observed within eTRE only, but their percentage to body weight was not changed within or between both interventions (Table 2).

### Insulin sensitivity and glucose homeostasis

Insulin sensitivity assessed by Matsuda index showed no differences between the TRE interventions (−0.07; 95% CI, −0.77 to 0.62; *P* = 0.60 for ITRE versus eTRE) or within eTRE (0.31; 95% CI, −0.14 to 0.76; *P* = 0.11) and ITRE (0.19; 95% CI, −0.22 to 0.60; *P* = 0.25) (Fig. 3A). Similarly, the post hoc analysis of the oral glucose insulin sensitivity (OGIS) index revealed no changes within (eTRE: −0.35; 95% CI, −15.2 to 14.5; *P* = 0.54; ITRE: −2.68; 95% CI, −15.9 to 10.6; *P* = 0.80) or between interventions (−2.32; 95% CI, −23.3 to 18.7; *P* = 0.67). Area under the curve (AUC) glucose in oral glucose tolerance test (OGTT) increased within eTRE (1636; 95% CI, 798 to 2475; *P* = 0.001), and its changes differed between interventions (−2175; 95% CI, −3088 to 1262; *P* = 0.002) (fig. S2, A and B). This minor decline in OGTT-derived glucose tolerance within eTRE might be explained by a decrease in insulin secretion relative to glucose, as indicated by an insulinogenic index (−0.43; 95% CI, −0.69 to −0.16; *P* < 0.001; fig. S2, C to E). This was also reflected by a decrease in the disposition index (−1.58; 95% CI, −2.75 to −0.41; *P* = 0.01; fig. S2F) and an increase in free fatty acids (fig. S2, K and L) and aligns with a decrease in glucagon within eTRE as derived in OGTT (fig. S2, I and J). Despite minor changes in glucose tolerance in OGTT, mean 24-hour glucose in continuous glucose monitoring (CGM) showed no differences between and no changes within eTRE and ITRE compared to baseline (Fig. 3, B and C, and table S3). Glucose variability in CGM showed increased intraday variation and prolonged time in the high-glucose range within eTRE, whereas interday variation decreased within ITRE (table S3).

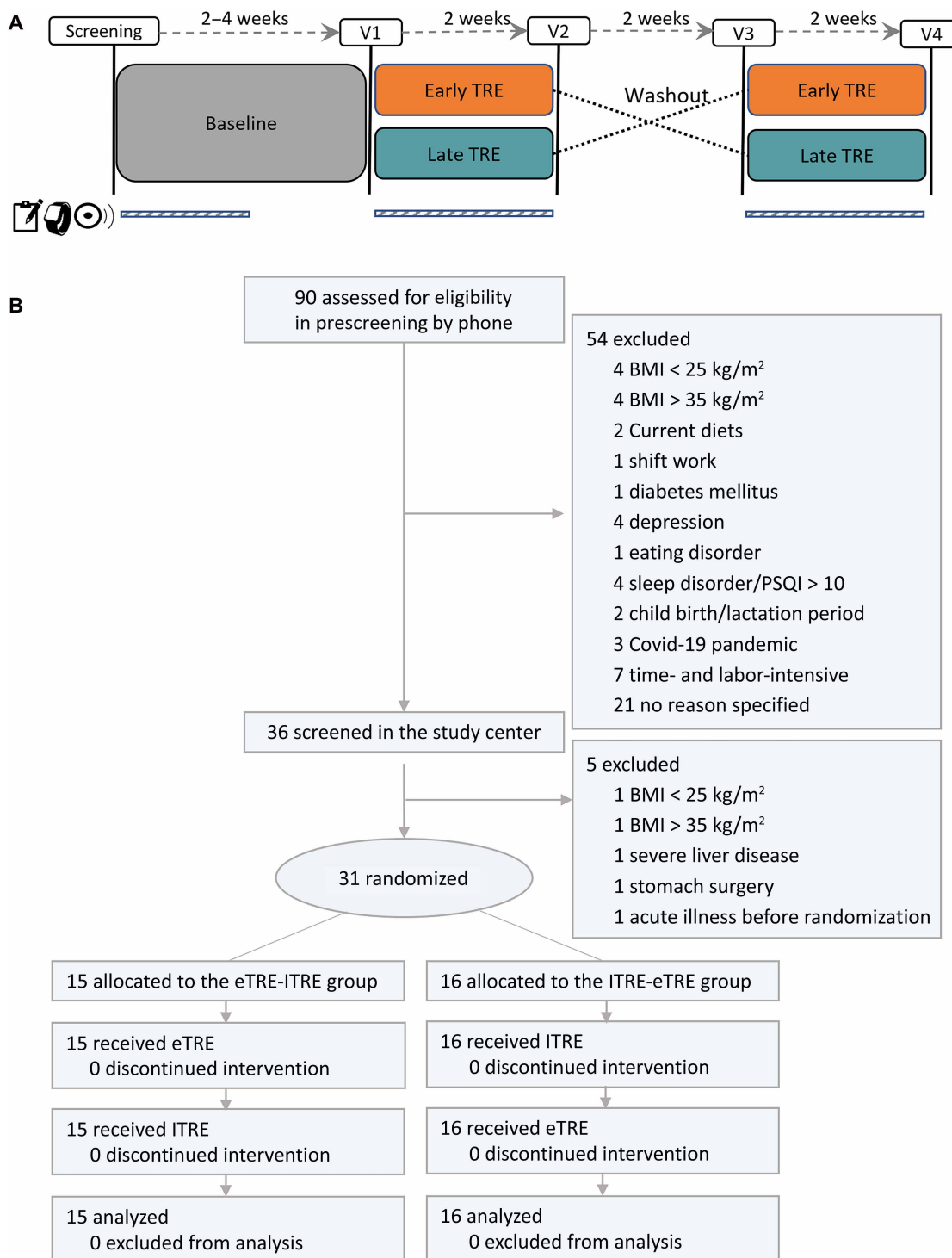
Additional analysis in a subcohort of individuals with impaired glucose metabolism [IFG/IGT] revealed no differences in the insulin sensitivity and mean 24-hour glucose between or within TRE interventions. Other secondary glycemic outcomes also showed effects similar to the whole cohort (tables S6 and S7).

### Cardiometabolic parameters

None of the TRE interventions affected systolic and diastolic blood pressure, total cholesterol, LDL cholesterol, or triglyceride concentrations (Table 2). High-density lipoprotein (HDL) cholesterol declined within both eTRE (−0.10 mM; 95% CI, −0.14 to −0.06 mM; *P* < 0.001) and ITRE (−0.07 mM; 95% CI, −0.11 to −0.03 mM, *P* = 0.003), with no difference between the interventions. Both the eTRE (−3.58 U/liter; 95% CI, −5.60 to −1.56 U/liter; *P* < 0.001) and the ITRE (−3.46 U/liter; 95% CI −6.14 to −0.79 U/liter; *P* = 0.001) induced a decrease in  $\gamma$ -glutamyltransferase (GGT) but not in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Table 2).

### Adipokines, inflammatory markers, and oxidative stress markers

We further investigated concentrations of adipokines leptin and adiponectin, as well as inflammatory markers interleukin-6 (IL-6),



**Fig. 1. Study design and participant flow diagram.** The ChronoFast study was a 10-week randomized crossover trial (A) including two 2-week dietary intervention periods: eTRE (8-hour eating window from 8:00 and 16:00) and ITRE (8-hour eating window from 13:00 and 21:00), preceded by a 2- to 4-week baseline period and separated by a 2-week washout period. Eligible participants were randomly allocated to the eTRE-ITRE or ITRE-eTRE study arms on the basis of their BMI and age. During the study center visits (V1 to V4) before and after each intervention, glycemic and other cardiometabolic parameters were assessed in a fasting state and in an OGTT. During the 14 days of the baseline and both TRE intervention periods, CGM, actigraphy, food and sleep diaries were conducted. (B) CONSORT flow diagram describing the number of participants throughout the study, from enrollment to completion, is shown. PSQI, Pittsburgh Sleep Quality Index.

**Table 1. Baseline characteristics of study participants.** Shown are demographic characteristics, anthropometric parameters, data on glucose homeostasis, cardiometabolic parameters, and lifestyle (eating, physical activity, and sleep habits) of the study participants at the baseline. Data are presented as mean with  $\pm$ SD or median with IQR (25th to 75th percentiles).

	All participants (n = 31)	eTRE-ITRE group (n = 15)	ITRE-eTRE group (n = 16)
<i>Demographic characteristics</i>			
Age, median (IQR), years	62 (53–65)	62 (53–65)	60 (52–65)
Sex, female:male	31:0	15:0	16:0
Race, white:other	31:0	15:0	16:0
Ethnicity, Caucasian:other	31:0:0	15:0:0	16:0:0
<i>Anthropometric parameters and body composition</i>			
Weight, means ( $\pm$ SD), kg	82.5 ( $\pm$ 8.4)	83.2 ( $\pm$ 8.8)	81.9 ( $\pm$ 8.2)
Fat mass, means ( $\pm$ SD), kg	34.1 ( $\pm$ 6.6)	35.0 ( $\pm$ 6.4)	33.3 ( $\pm$ 6.9)
Lean mass, means ( $\pm$ SD), kg	48.4 ( $\pm$ 5.4)	48.2 ( $\pm$ 4.7)	48.6 ( $\pm$ 6.1)
Waist circumference, means ( $\pm$ SD), cm	99 ( $\pm$ 9)	101 ( $\pm$ 9)	98 ( $\pm$ 9)
BMI, means ( $\pm$ SD), kg/m <sup>2</sup>	30.5 ( $\pm$ 2.9)	30.7 ( $\pm$ 2.9)	30.2 ( $\pm$ 3.0)
<i>Glucose homeostasis</i>			
Insulin sensitivity, means ( $\pm$ SD)*	3.95 ( $\pm$ 2.14)	3.96 ( $\pm$ 2.47)	3.94 ( $\pm$ 1.86)
Fasting glucose, median (IQR), mg/dl	90 (87–96)	88 (82–96)	91 (89–102)
HbA1c, median (IQR), % <sup>†</sup>	5.50 (5.22–5.70)	5.41 (5.16–5.68)	5.50 (5.23–5.70)
MSG, median (IQR), mM	5.31 (4.93–5.62)	5.38 (4.79–5.70)	5.27 (4.95–5.57)
Glycemic state by OGTT, NGT:IFG/IGT	18:13	9:6	9:7
<i>Cardiometabolic parameters</i>			
Systolic blood pressure, median (IQR), mmHg	117 (110–135)	120 (112–132)	116 (108–137)
Diastolic blood pressure, median (IQR), mmHg	75 (68–79)	75 (70–79)	74 (66–80)
Total cholesterol, means ( $\pm$ SD), mM	5.63 ( $\pm$ 0.92)	5.60 ( $\pm$ 0.89)	5.66 ( $\pm$ 0.97)
HDL cholesterol, median (IQR), mM	1.46 (1.33–1.65)	1.51 (1.31–1.76)	1.46 (1.34–1.63)
LDL cholesterol, means ( $\pm$ SD), mM	3.47 ( $\pm$ 0.82)	3.41 ( $\pm$ 0.80)	3.54 ( $\pm$ 0.86)
Triglycerides, means ( $\pm$ SD), mM	1.35 ( $\pm$ 0.62)	1.30 ( $\pm$ 0.52)	1.40 ( $\pm$ 0.72)
AST, means ( $\pm$ SD), U/liter	32.2 ( $\pm$ 6.6)	31.2 ( $\pm$ 5.8)	33.1 ( $\pm$ 7.4)
ALT, median (IQR), U/liter	22.1 (19.8–27.6)	22.2 (17.7–26.3)	22.0 (19.8–28.3)
GGT, median (IQR), U/liter	21.2 (18.0–25.3)	19.5 (15.8–24.1)	23.8 (19.6–35.9)
hsCRP, median (IQR), mg/liter	1.30 (0.80–2.60)	1.30 (0.90–2.10)	1.55 (0.55–3.28)
<i>Eating habits and physical activity</i>			
Eating duration, means ( $\pm$ SD), hour:minute	12:06 ( $\pm$ 1:35)	11:52 ( $\pm$ 1:28)	12:20 ( $\pm$ 1:43)
Eating start time, means ( $\pm$ SD), hour:minute	8:30 ( $\pm$ 1:03)	8:27 ( $\pm$ 0:49)	8:33 ( $\pm$ 1:15)
Eating end time, means ( $\pm$ SD), hour:minute	20:38 ( $\pm$ 1:11)	20:08 ( $\pm$ 0:56)	21:06 ( $\pm$ 1:14)
Energy intake, means ( $\pm$ SD), kcal	1997 ( $\pm$ 308)	1927 ( $\pm$ 231)	2057 ( $\pm$ 359)
Protein intake, means ( $\pm$ SD), EN%	15.5 ( $\pm$ 2.0)	15.3 ( $\pm$ 1.7)	15.6 ( $\pm$ 2.2)
Carbohydrate intake, means ( $\pm$ SD), EN%	43.2 ( $\pm$ 4.2)	44.0 ( $\pm$ 4.2)	42.4 ( $\pm$ 4.2)
Fat intake, means ( $\pm$ SD), EN%	39.5 ( $\pm$ 3.9)	38.8 ( $\pm$ 4.0)	40.1 ( $\pm$ 3.7)
MET, means ( $\pm$ SD) <sup>‡</sup>	1.59 ( $\pm$ 0.15)	1.63 ( $\pm$ 0.13)	1.57 ( $\pm$ 0.14)
<i>Internal clocks and sleep timing</i>			
Chronotype (MSFsc), early:intermediate:late	18:11:2	11:3:1	7:8:1
Sleep duration, means ( $\pm$ SD), hour:minute	7:53 ( $\pm$ 0:51)	7:59 ( $\pm$ 0:58)	7:47 ( $\pm$ 0:46)
Sleep onset, means ( $\pm$ SD), hour:minute	23:29 ( $\pm$ 0:54)	23:14 ( $\pm$ 0:56)	23:44 ( $\pm$ 0:49)
<i>(Continued)</i>			

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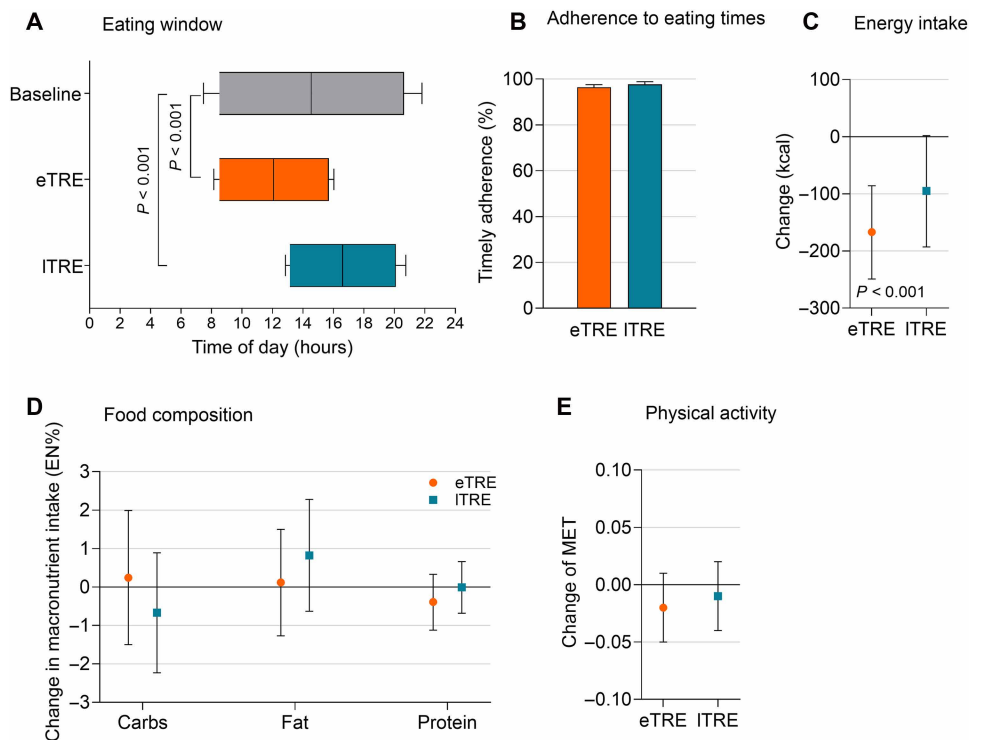
(Continued)

	All participants (n = 31)	eTRE-ITRE group (n = 15)	ITRE-eTRE group (n = 16)
Sleep offset, means (±SD), hour:minute	7:23 (±0:40)	7:14 (±0:39)	7:32 (±0:39)

\*Assessed by Matsuda index in OGTT during visit 1. †One baseline value in group B (ITRE-eTRE) was not measured because of loss of the sample. ‡Two baseline values for MET were excluded in all groups because of defective actigraphs.

**Fig. 2. Adherence to eTRE and ITRE regimes.**

(A) Times of day when participants began eating (left end of box shows a mean, and left whisker shows SD) and stopped eating (right end of box shows a mean, and right whisker shows SD) in the baseline phase and eTRE and ITRE intervention phases are presented. The vertical lines within the boxes indicate the midpoint of the eating window (averaged across all participants). *N* = 31 for each study period. *P* < 0.001 for eTRE and ITRE compared with the baseline by paired Student's *t* test. (B) Timely adherence defined as a percentage of all intervention days when participants ate within prescribed time windows (eTRE, 8:00 to 16:00, ±30 min; ITRE, 13:00 to 21:00, ±30 min) is presented. Bars show the means of adherence values, and whiskers show the SD. *N* = 31 for both interventions. (C) Changes in energy intake within eTRE and ITRE interventions compared with the baseline are displayed. *N* = 29 for both interventions. *P* < 0.001 for eTRE versus baseline. (D) Changes in food composition within eTRE and ITRE interventions compared with the baseline are shown for carbohydrate (carbs), fat, and protein intake. *N* = 29 for both interventions. (E) Changes in physical activity measured by actigraphy and defined as MET are plotted. *N* = 29 for both interventions. [(C) to (E)] Data are shown as mean differences with 95% CI. Statistical analysis was performed using paired Student's *t* test or Wilcoxon test as appropriate.



tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), monocyte chemoattractant protein 1 (MCP-1), and high-sensitive complement-reactive protein (hsCRP), which contribute to obesity and diabetes pathogenesis. Leptin concentration declined within both eTRE (-8080 pg/ml; 95% CI, -15,995 to -166 pg/ml; *P* = 0.02) and ITRE (-10,763 pg/ml; 95% CI, -19,035 to -2492 pg/ml; *P* = 0.001) without differences between interventions. Adiponectin concentrations were also reduced within eTRE (-0.94 pg/ml; 95% CI, -1.54 to 0.34 pg/ml; *P* = 0.003) without differences between interventions (fig. S3, A and B). The inflammatory markers were not affected by either eTRE or ITRE intervention (Table 2 and fig. S3, C to E). Concentrations of oxidative stress markers malondialdehyde, 3-nitrotyrosine, and protein carbonyls were not changed in any TRE intervention (table S4). In a subcohort with IFG/IGT, cardiometabolic, inflammatory, and oxidative stress outcomes showed effects similar to the whole cohort (table S7).

**Hunger and satiety**

Participants showed lower desire to eat, hunger, and capacity to eat during the ITRE in the morning but not in the evening compared with the eTRE, whereas satiety did not differ at any time of day (table S5). In agreement with this, changes in the satiety hormone peptide YY (PYY) differed between interventions (34.7 pg/ml; 95% CI, 18.2 to 51.3 pg/ml; *P* < 0.001), showing a decrease within eTRE (-13.0 pg/ml; 95% CI, -24.5 to -1.5 pg/ml; *P* = 0.01) and increase within ITRE (22.5 pg/ml; 95% CI, 13.1 to 31.9 pg/ml; *P* < 0.001). Concentrations of the hunger hormone ghrelin remained unchanged in both TRE interventions (table S5).

**Peripheral blood mononuclear cell gene expression**

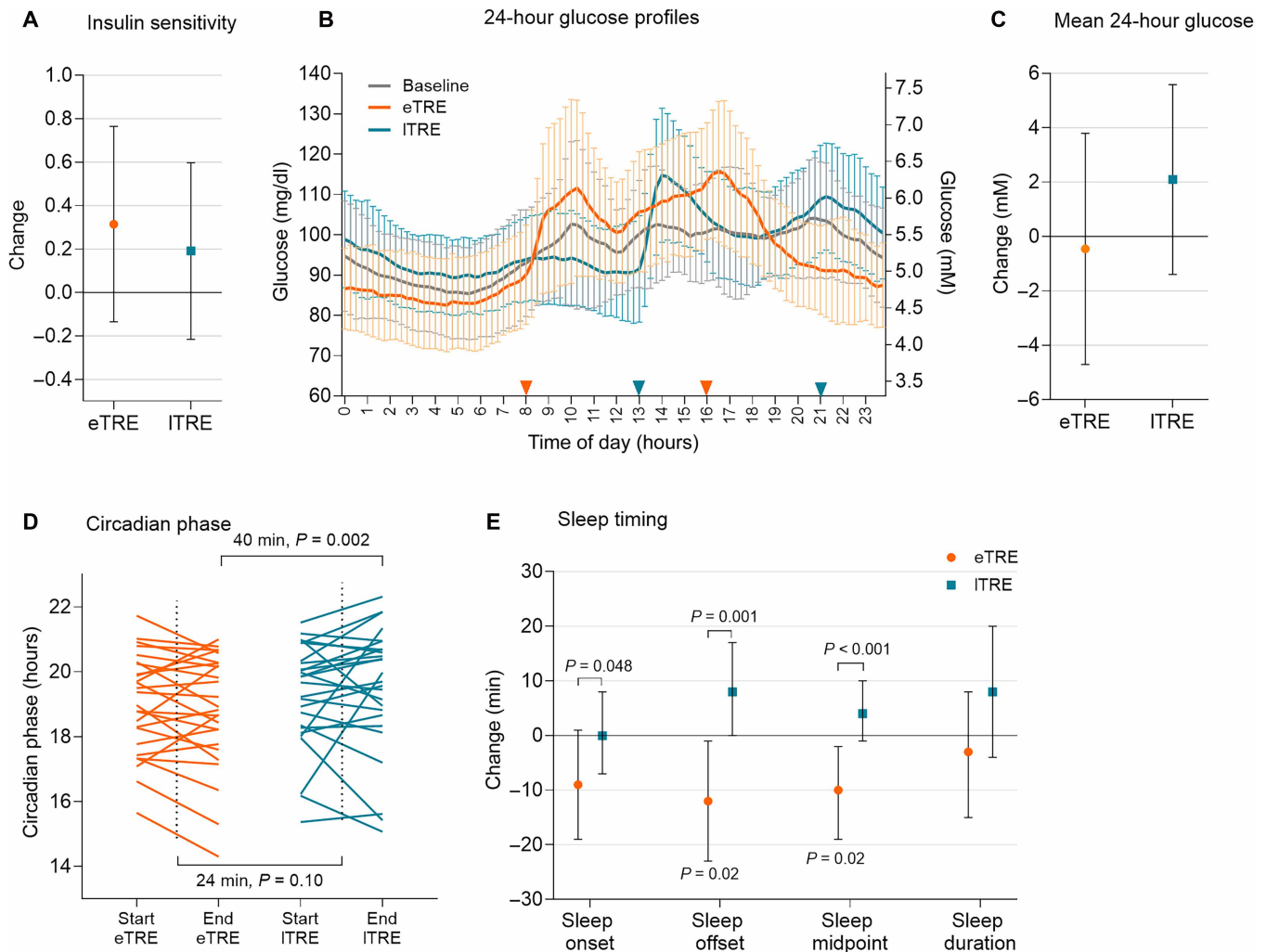
To elucidate the molecular pathway potentially induced by TRE, we then measured the expression of genes coding inflammatory markers

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**Table 2. TRE effects on body composition, glucose homeostasis, and cardiometabolic parameters.** Shown are changes in anthropometric parameters, glucose homeostasis, and cardiometabolic parameters of study participants within and between eTRE and ITRE interventions. Data are presented as mean difference with 95% CI.

Outcome	Change (95% CI)				Difference between ITRE versus eTRE interventions (95% CI)	P value <sup>†</sup>
	eTRE (n = 31)	P value*	ITRE (n = 31)	P value*		
<i>Anthropometric parameters and body composition</i>						
Weight, kg	-1.08 (-1.40 to -0.77)	9.1 × 10 <sup>-8</sup>	-0.44 (-0.74 to -0.13)	0.01	0.65 (0.27 to 1.03)	0.012
Fat mass, kg <sup>‡</sup>	-0.61 (-1.01 to -0.22)	0.002	0.02 (-0.36 to 0.39)	0.92	0.65 (0.19 to 1.11)	0.53
Fat mass, % <sup>‡</sup>	-0.24 (-0.81 to 0.32)	0.44	0.18 (-0.35 to 0.70)	0.47	0.42 (-0.18 to 1.02)	0.82
Lean mass, kg <sup>‡</sup>	-0.57 (-1.11 to -0.04)	0.04	-0.28 (-0.78 to 0.23)	0.27	0.25 (-0.31 to 0.81)	0.11
Lean mass, % <sup>‡</sup>	0.24 (-0.33 to 0.81)	0.45	-0.18 (-0.70 to 0.35)	0.47	-0.42 (-1.02 to 0.19)	0.81
Total body water, % <sup>‡§</sup>	0.09 (-0.37 to 0.55)	0.69	-0.07 (-0.49 to 0.34)	0.72	-0.19 (-0.79 to 0.41)	0.59
Waist circumference, cm	-0.36 (-1.65 to 0.92)	0.57	0.48 (-0.97 to 1.92)	0.51	0.84 (-1.21 to 2.89)	0.40 <sup>¶</sup>
BMI, kg/m <sup>2</sup>	-0.45 (-0.56 to -0.33)	5.4 × 10 <sup>-9</sup>	-0.12 (-0.21 to -0.03)	0.01	0.33 (0.21 to 0.44)	6.8 × 10 <sup>-4</sup>
<i>Glucose homeostasis</i>						
Fasting glucose, mg/dl	-0.16 (-0.31 to -0.01)	0.04	0.08 (-0.02 to 0.18)	0.07	0.24 (0.06 to 0.42)	0.006
Fasting insulin, mU/liter	-2.25 (-3.33 to -1.17)	3.1 × 10 <sup>-4</sup>	-1.54 (-3.77 to 0.69)	0.23	0.71 (-1.86 to 3.27)	0.74
HbA1c, %	-0.01 (-0.05 to 0.03)	0.73	-0.02 (-0.06 to 0.01)	0.22	-0.01 (-0.06 to 0.03)	0.82
<i>Cardiometabolic parameters</i>						
Systolic blood pressure, mmHg	0.61 (-3.41 to 4.62)	0.76	4.23 (-0.05 to 8.51)	0.05	2.04 (-3.46 to 7.54)	0.45
Diastolic blood pressure, mmHg	0.29 (-2.68 to 3.25)	0.84	1.10 (-1.92 to 4.12)	0.69	0.11 (-3.93 to 4.15)	0.63
Total cholesterol, mM	0.13 (-0.13 to 0.39)	0.30	-0.16 (-0.48 to 0.15)	0.30	-0.29 (-0.68 to 0.10)	0.14 <sup>¶</sup>
HDL cholesterol, mM	-0.10 (-0.14 to -0.06)	6.0 × 10 <sup>-5</sup>	-0.07 (-0.11 to -0.03)	0.003	0.03 (-0.04 to 0.09)	1.00
LDL cholesterol, mM	0.21 (-0.15 to 0.57)	0.24	0.22 (-0.10 to 0.55)	0.17	0.01 (-0.54 to 0.56)	0.34
Triglycerides, mM	0.06 (-0.08 to 0.21)	0.27	-0.01 (-0.15 to 0.13)	0.56	-0.07 (-0.22 to 0.08)	0.56
AST, U/liter	-0.55 (-1.77 to 0.68)	0.37	-0.66 (-2.31 to 0.99)	0.42	-0.12 (-2.51 to 2.27)	0.50 <sup>¶</sup>
ALT, U/liter	-1.74 (-3.71 to 0.22)	0.13	-2.08 (-5.43 to 1.28)	0.72	-0.34 (-4.33 to 3.66)	0.53
GGT, U/liter	-3.58 (-5.60 to -1.56)	4.0 × 10 <sup>-5</sup>	-3.46 (-6.14 to -0.79)	0.001	0.11 (-2.02 to 2.24)	0.24
hsCRP, mg/liter	0.08 (-2.02 to 2.18)	0.59	-0.07 (-0.38 to 0.24)	0.61	-0.15 (-2.29 to 1.98)	0.36
β-Hydroxybutyrate, mM	0.03 (0.00 to 0.05)	0.02	0.00 (-0.02 to 0.03)	0.83	-0.02 (-0.05 to 0.00)	0.33

\*Comparison by paired Student's *t* test or Wilcoxon test. †Comparison of changes between eTRE and ITRE by the linear mixed model. ‡One eTRE and three ITRE datasets (pre and post) were excluded because of invalid BIA. §Assessed in relation to body weight. ¶Showed a period effect. #Showed a carryover effect; therefore, only the data from the first intervention are included in analysis.



**Fig. 3. Effects of eTRE and ITRE on glucose homeostasis and circadian time.** (A) Changes in insulin sensitivity within eTRE and ITRE compared with the baseline are plotted. Data are shown as mean differences with 95% CI.  $N = 31$  for eTRE and  $N = 30$  for ITRE. (B) Twenty-four-hour glucose profiles assessed by CGM over 14 days in the baseline phase and eTRE and ITRE intervention phases are plotted. Solid lines and error bars represent the mean glucose and SD, respectively, summarizing all assessed days for all participants.  $N = 30$  for the baseline and both interventions. Colored arrows on the x axis show start and end of the prescribed eating windows in eTRE (orange) and ITRE (petrol blue). (C) Changes in mean 24-hour glucose values assessed by CGM over 14 days within eTRE and ITRE compared with the baseline are plotted.  $N = 30$  for both interventions. (D) Changes in the individual circadian phase before and after eTRE (orange lines) and ITRE (petrol blue lines) as assessed in blood monocytes. The circadian phase was determined on the basis of the biomarker transcripts by the BodyTime assay. The  $P$  value bar below the lines shows a between-intervention difference (24 min,  $P = 0.01$ ). The  $P$  value bar above the lines shows comparison of samples collected after both interventions (40 min,  $P = 0.002$ ).  $N = 26$  for both interventions. (E) Changes in sleep timing within eTRE and ITRE compared with the baseline are presented for sleep onset, sleep offset, sleep midpoint, and sleep duration.  $N = 30$  for both interventions.  $P = 0.048$  for sleep onset,  $P = 0.001$  for sleep offset,  $P < 0.001$  for sleep midpoint in the between-intervention comparison, and  $P = 0.02$  for sleep offset and sleep midpoint within eTRE compared with the baseline. [(A) and (C) to (E)] Data are shown as mean differences with 95% CI. Statistical analysis was performed using paired Student's  $t$  test or Wilcoxon test as appropriate.

(*interleukin-6*, *tumor necrosis factor- $\alpha$* , *C-C motif chemokine ligand 2*, and *interleukin-10*) and key metabolic genes (*carnitine palmitoyltransferase 1A*, *pyruvate dehydrogenase kinase 4*, *sirtuin 1*, *fatty acid synthase*, and *lipoprotein lipase*) and clock genes [*clock circadian regulator*, *basic helix-loop-helix ARNT like 1*, *period circadian regulator 1* (*PER1*), *period circadian regulator 2*, *nuclear receptor subfamily 1 group D member 1* (*NR1D1*), *cryptochrome circadian regulator 1*, *cryptochrome circadian regulator 2*, and *RAR-related orphan receptor A*] in peripheral blood mononuclear cell (PBMC) samples (table S8). Core clock genes *PER1* ( $-0.30$ ; 95% CI,  $-0.57$  to  $-0.03$ ;  $P = 0.03$ ) and *NR1D1* ( $-0.24$ ; 95% CI,  $-0.48$  to  $-0.01$ ;  $P = 0.02$ ), which are strongly involved in

metabolic regulation (24), declined in expression within eTRE without between-intervention difference. Other genes showed no TRE-induced expression changes (fig. S4).

### Sleep timing and circadian phase

Considering the previous data on the effect of food intake on circadian clocks (23, 25–27) and observed changes in the PBMC gene expression, we tested whether the eating timing during the TRE affected internal circadian phase. Circadian phase was defined by the dim-light melatonin onset (DLMO) predicted on the basis of transcript biomarkers in a single blood monocyte sample and determined

by a BodyTime assay (28). The change in eating times between interventions was associated with a trend toward a later circadian phase, with a mean difference of 24 min (95% CI, -5 to 54 min;  $P = 0.10$ ) in lTRE compared with the eTRE. When comparing samples collected at the end of both interventions, the circadian phase after lTRE was 40 min later than that after eTRE (95% CI, 18 to 62 min;  $P = 0.002$ ) (Fig. 3D). In agreement with this, the self-reported sleep timing parameters—sleep onset (9 min; 95% CI, 0 to 18 min;  $P = 0.048$ ), offset (20 min; 95% CI, 9 to 32 min;  $P = 0.001$ ), and midpoint (15 min; 95% CI, 7 to 23 min;  $P < 0.001$ )—occurred later in lTRE as compared with eTRE. Within eTRE, the sleep timing advanced showing earlier sleep offset (-12 min; 95% CI, -23 to -1 min;  $P = 0.02$ ) and sleep midpoint (-10 min; 95% CI, -19 to -2 min;  $P = 0.02$ ) compared with the baseline. Sleep duration was not altered by eTRE and lTRE (Fig. 3E).

## DISCUSSION

We conducted a randomized controlled crossover trial comparing 8-hour eTRE versus lTRE in an intended isocaloric setting. We achieved high adherence to both interventions including successful reductions of the eating window to under 8 hours and a corresponding prolongation of fasting by about 5 hours. We also achieved timely adherence of greater than 96% of all intervention days, with unchanged dietary composition and physical activity, which allowed us to exclude these potential sources of bias.

The main finding of this study is that neither eTRE nor lTRE improved insulin sensitivity or induced other clinically meaningful changes in cardiometabolic and inflammatory traits under nearly isocaloric conditions. This contradicts our study hypothesis and most published data on TRE, which show beneficial effects on insulin sensitivity (2, 3, 7, 9, 10, 29), glucose (3, 6–8), and lipid (6, 8, 11–13) concentrations, as well as body weight and body fat (7–9, 13, 15, 16), whereas eTRE is suggested to be more effective compared with the late or mid-day TRE (2, 8, 22, 23, 30). In contrast, long eating windows (15) and late evening (15, 31) and night (32, 33) eating, which are common in modern society, are associated with a risk of obesity, diabetes, and other metabolic diseases, at least partly because of the desynchronization of circadian clocks (34, 35).

The beneficial cardiometabolic effects described previously might be induced by TRE-mediated calorie restriction and not by the shortening of the eating window itself. In this nearly isocaloric trial, no improvements in metabolic parameters were observed after 2 weeks of TRE. In particular, despite the small decrease in energy intake in eTRE, which could not be avoided despite efforts to maintain isocaloric intake, and minimal weight loss within both interventions (more pronounced in eTRE), insulin sensitivity assessed by Matsuda index, the primary outcome in this trial, showed no between- or within-intervention difference. The post hoc analysis of another index of insulin sensitivity, OGIS, confirmed this result. This finding aligns with a recent study in individuals with type 2 diabetes, which reported no improvement in insulin sensitivity after 3 weeks of 10-hour self-selected TRE (34). However, it contradicts another study that demonstrated an improvement in insulin sensitivity in eight men with prediabetes after 5 weeks of eTRE (2), although weight loss was minor in both studies (1.4 and 1.0 kg in the eTRE and control schedule, respectively) and similar to our trial (1.08 kg in eTRE and 0.44 kg in lTRE). Thus, timing or duration of the eating window and differences in study populations may contribute to the data heterogeneity.

To note, the Matsuda insulin sensitivity index, which is calculated using glucose and insulin data from the OGTT, might be compromised by different fasting periods before the OGTT start. In our study, the OGTT was conducted at the same time of day (9:30) to avoid diurnal variation of glucose tolerance (36, 37). However, this resulted in 5-hour longer fasting before the OGTT in eTRE compared with lTRE, which can reduce insulin secretion (38, 39) and partially explain the decrease in glucose tolerance within eTRE. We observed an increase in glucose AUC within eTRE, and these changes differed between interventions. This agrees with the published research (40) that estimated an effect size at a 1.7% increase in 2-hour plasma glucose for every additional hour of fasting. Further, in our study, we observed a decrease in insulin secretion relative to glucose, as indicated by the insulinogenic index. Thus, the differing fasting durations before the OGTT might mask the true changes in insulin sensitivity assessed by the Matsuda index and potentially explain the absence of observed effects. This also emphasizes the importance of planning for similar fasting durations before OGTT in studies assessing glycemic control, including TRE trials.

Despite potential OGTT data-related bias, the mean CGM glucose and other cardiovascular parameters were not meaningfully affected by eTRE or lTRE. Twenty-four-hour mean glucose concentration, which reflected glycemic control under real-life conditions over 14 days, did not show any differences within or between the TRE interventions. Last, the absence of TRE-induced changes in most lipid, inflammatory, and oxidative stress markers (as confirmed at the transcriptional level in PBMCs) supports the idea that calorie restriction, but not shortening of the eating timing itself, is crucial to induce positive metabolic effects of TRE.

Because our trial observed no effects of TRE on most analyzed parameters in a nearly isocaloric setting, we were unable to compare effects of eTRE and lTRE. When calorie intake is spontaneously reduced, metabolic effects of eTRE are apparently more beneficial compared with lTRE (3, 7, 9, 10, 18, 20, 41), but few trials directly compared eTRE and lTRE (8, 22, 23). Xie *et al.* (22) compared early and mid-day TRE in a parallel-arm study and revealed that early eating is more effective for improvement of insulin sensitivity, fasting glucose, body mass, and inflammation. Similarly, Zhang *et al.* (23) reported improvements in mean glucose, fasting insulin, and insulin resistance after the eTRE, whereas leptin was reduced after both eTRE and lTRE. Our trial observed similar effects on leptin, confirming TRE's influence on adipose tissue. In contrast, a single published crossover study comparing 7-day eTRE and lTRE (8) found no difference in improvements in postprandial glucose and fasting triglycerides between the two eating windows. Recent research suggests that the most beneficial eating timing may vary for individuals on the basis of chronotype, genetic, social, and other personal factors (42–44), highlighting the need for further investigation.

The second key finding of our study suggests that alterations of eating timing shift internal circadian time. We made three observations supporting this postulate. First, shifting the eating window by 5 hours from eTRE to lTRE was associated with a later circadian phase, as estimated by transcript biomarkers in blood monocytes [BodyTime assay, which strongly correlates with DLMO (28)]. Second, it resulted in expression changes in core clock genes *PER1* and *NR1D1* at a single time point (which could also be caused by the clock phase shift). Third, an advance in sleep timing, which is also controlled by the circadian system (45), was observed in eTRE compared with the baseline, which resulted in between-intervention sleep

timing differences between eTRE and lTRE. These findings support the idea that food intake in humans acts as a zeitgeber for circadian clocks as shown in multiple animal studies (25, 26) and few human trials (27, 46, 47). The study of Koppold-Liebscher *et al.* (27), which used the same monocyte assay as our study, observed transient shifts of circadian phase after religious intermittent fasting, characterized by eating at an unusual time before sunrise and after the sunset; this effect disappeared 3 months after the return to usual eating time. Wehrens *et al.* (46) demonstrated a delay of *PER2* mRNA rhythms in adipose tissue by about 1 hour after the 5-hour delay in meal times, whereas our study observed a 40-min shift in samples collected after the lTRE versus eTRE. Last, a recent trial showed that eTRE advanced sleep in late sleepers by moving sleep onset, offset, and midpoint earlier after 2 weeks of eTRE compared with the control group (47), which aligns with our observations. The regulation of internal circadian clocks by the timing of food intake may be mediated by postprandial changes in nutrients, metabolites, and hormones (48), with insulin apparently playing an essential role (49). Together, our predicted circadian phase data suggested that eating timing can shift internal circadian clocks in humans, using single-time point sampling. However, to draw a conclusion about whether eating timing-based strategies can resynchronize or restore circadian rhythms in individuals with circadian rhythm disturbances, such as shift workers or people with metabolic diseases, further studies using sampling around the clock and DLMO assessment should be conducted.

Last, this trial elucidated an altered hunger regulation on the basis of the eating timing during the TRE. In eTRE, participants felt more hunger, desire, and capacity to eat in the morning than in lTRE. This can be explained by longer fasting after the last meal in eTRE at the day before the visit and by a habituation effect to the early eating window such that the body expects the food intake early in the day (50). Our study also suggests a hormonal mechanism that might contribute to hunger regulation on the basis of the TRE timing. Whereas the ghrelin concentration did not differ, the morning concentration of the anorectic hormone PYY increased within lTRE and declined in eTRE, suggesting a role of PYY in the observed lower hunger in the morning after the lTRE. Whether the eating/fasting timing during TRE alters the circadian rhythm of PYY secretion (51, 52) in humans and how it can be related to possible changes in the gut microbiome (53) need to be investigated in the future.

Our study has several limitations that were not mentioned above. First, although the longer intervention period might induce more pronounced metabolic changes, in the ChronoFast study, we decided for the short intervention duration of 2 weeks. This planning was based on published TRE trials demonstrating improvement of glycemic control or insulin sensitivity even after 1 or 2 weeks of intervention (3, 8, 10), and our previous isocaloric nutritional trials showed metabolic improvements after a similarly short intervention duration (54, 55). Moreover, in planning the 2-week intervention, we aimed to achieve the maximally accurate 24-hour monitoring of food intake, glucose concentrations, physical activity, and sleep and to ensure isocaloric conditions through intensive dietary counseling. The short TRE duration aimed to mitigate the spontaneous small reduction in energy intake that, despite all efforts, still occurred within eTRE. This can happen, for example, if participants skip high-fat or sweet snacks often consumed in the evening. Despite this small caloric reduction within eTRE (equivalent to approximately two boiled eggs per day) and the minimal weight loss, we did not observe metabolic improvements in both interventions. A second limitation is that we

enrolled exclusively women to ensure cohort homogeneity, which limits the generalizability of our findings to men. In this context, menstrual cycle changes may confound the results and should be considered. However, this factor may not be relevant for our study including only 5 premenopausal and 26 postmenopausal women. Third, because of COVID-19 hygienic restrictions, we could not use indirect calorimetry to assess energy expenditure or the BodPod method to measure the body composition. Because the dual-energy x-ray absorptiometry machine was not available, the assessment of body composition was conducted by the bioelectrical impedance analysis (BIA), which has some limitations because the BIA accuracy can be affected by factors such as hydration status, food intake, electrode placement, temperature sensitivity, device calibration, and population specificity. However, in our study, we used highly standardized measurement conditions, trained staff, and a homogenous population. In addition, for the percentage of total body water (in relation to body weight), we found no differences within or between interventions (Table 2). Last, sleep timing assessment was based on the self-reported data using sleep diaries.

Our carefully controlled study showed that, in a nearly isocaloric setting, neither eTRE nor lTRE improved insulin sensitivity or induced other clinically meaningful changes in cardiometabolic traits in a short-time intervention despite a shift of internal circadian clocks. Our findings suggest the importance of calorie restriction for metabolic improvements in TRE. Whether the timing of eating under the hypocaloric conditions can additionally contribute to metabolic changes and whether the optimal eating timing differs between individuals warrant investigation in future studies.

## MATERIALS AND METHODS

### Study design

The ChronoFast study was a 10-week randomized crossover trial including two 2-week dietary intervention periods: (i) eTRE and (ii) lTRE, preceded by a 2- to 4-week baseline (run-in) period and separated by a 2-week washout period (Fig. 1A). Participants were randomly allocated to the eTRE-lTRE or lTRE-eTRE study arms based on their BMI and age using the computed minimization method (MinimPy software) (56). Participants visited the clinical study center at the German Institute of Human Nutrition Potsdam-Rehbruecke for the initial screening as well as before and after each dietary intervention (eTRE and lTRE). Visit days began at 8:30 after an overnight fast and included anthropometrical measurements, fasting blood collection, an OGTT, and nutritional instruction by a dietician. During the 14 days of the baseline and both TRE intervention periods, CGM and actigraphy were performed, and food, sleep, and weight diaries were maintained.

Participants and intervention staff were unblinded. Before randomization, all outcomes were collected blinded. About 70% of the postintervention data were assessed blinded. Specifically, all blood parameters, CGM, physical activity, and gene expression measurements were performed blinded, whereas anthropometric measurements, food intake, and chronotype assessments were not. The dietician who analyzed food records and estimated TRE adherence was not blinded to group assignments. Other investigators and statisticians were blinded during the study procedures and were unblinded only after all data had been analyzed.

The trial was approved by the Ethical Committee of the University of Potsdam, Germany (EA no. 8/2019). All participants provided

written informed consent before the study participation. The trial protocol was registered at ClinicalTrials.gov (NCT04351672) on 17 April 2020, published previously (17). We reported study results using the CONSORT 2025 checklist guideline for reporting randomized trials (57).

### Participant recruitment, eligibility criteria, and sample size calculation

Participants with overweight or obesity from the Berlin-Brandenburg area, Germany, were recruited at the German Institute of Human Nutrition Potsdam-Rehbruecke between April 2020 and December 2021 through flyers, posters, newspaper advertisements, and ads websites. Participants were excluded when they had a male sex; did not meet the age range between 18 and 70 years; had a BMI lower than 18 kg/m<sup>2</sup> or higher than 35 kg/m<sup>2</sup>; did shift work; traveled over more than one time zone in the month before the study start; reported weight changes of more than 5% in the past 3 months before the study start; were on weight loss medication; were pregnant or breastfeeding; had severe intestinal diseases; practiced special diets, such as intermittent fasting; had meaningful sleep disturbances [Pittsburgh Sleep Quality Index (58) higher than 10]; had diabetes type 1 or 2 or other endocrinological diseases; reported severe renal and liver diseases; reported a stroke in the 6 months before the study start; had cancer in the 2 years before the study start; were on medication with glucocorticoids; had coagulation disorders; were on anticoagulant medication; reported severe anemia or systemic infections; and had psychiatric diseases, addictive diseases, or depression. In the case of using other medication or food allergies, the study doctor individually considered whether study participation was possible.

Sample size calculation was completed using the G\*Power software v.3.1 (59) for the primary end point of change in insulin sensitivity. The calculation was based on the difference in insulin sensitivity observed in the meal tolerance tests conducted in the morning and in the evening in participants with IFG/IGT in our previous study (36). The trial was statistically powered to detect a difference in insulin sensitivity of 12.8% ± 7.1% at a significance of 0.05 and a statistical power of 80%. Assuming a 10% dropout rate, an estimated 33 enrollees were needed to ensure that 30 individuals completed the trial. Recruitment stopped once the target sample size was exceeded ( $n = 31$ ).

### TRE interventions

During the baseline period, the participants followed their usual eating habits, including their habitual eating times. During the TRE interventions, participants had to restrict their eating duration to 8 hours/day but consume their usual kind and amount of food. In the eTRE intervention, participants had to consume food between 8:00 and 16:00 and between 13:00 and 21:00 in the ITRE. During the daily 16-hour fasting period, participants had to consume noncaloric drinks only, such as water, black coffee, or tea, as well as sugar-free chewing gums or mints. Participants were free to divide their meals within the predetermined eating windows. Participants received a copy of their individual 14-day food diaries from the baseline period, which they were asked to follow in both interventions to maintain their habitual daily calorie intake and food composition and minimize body weight changes. Within the washout period, participants were requested to return to their habitual eating behavior, including their usual eating timing. Participants were also counseled to maintain their habitual physical activity and sleep times throughout the entire trial duration.

### Food intake, hunger, and body weight documentation

Food documentation was conducted within the baseline period and both TRE intervention periods for 14 consecutive days. Participants were asked to digitally document their food selection, amount, and time of consumption using the free smartphone app Fddb Extender (<https://fddb.info/>) (60). Participants who were not familiar with using a smartphone completed paper-based dietary records, which were then transferred to the Fddb Extender app by a study assistant. Before the baseline period, all participants received detailed instructions for digital or handwritten food documentation. They were requested to weigh their food whenever possible (or use household measures, such as glasses, cups, teaspoons), add supplemental information (such as brand names), and record the time of food consumption. The Fddb food database (Fddb Internetportale GmbH, <https://fddb.info/>) was used to analyze eating timing, energy intake, and macronutrient composition as validated previously (60). Hunger and satiety were assessed on the last day of both TRE interventions as described in Supplementary Materials and Methods.

Participants were also asked to maintain a constant body weight throughout the study. They were instructed to record their weight daily (in the Fddb app or handwritten) and report any weight fluctuation of ≥700 g on 2 consecutive days. Participants were called by the dietician after 7 days of the baseline period and each TRE intervention period to promote adherence to the assigned intervention and answer questions. The Fddb Extender app enabled accurate and simple real-time monitoring (60) of adherence, including given time frames and potential weight changes. Accessing the nutrition log during the intervention allowed a dietician to intervene rapidly and contact the study individuals in case of adherence problems. TRE intervention adherence was assessed as described in Supplementary Materials and Methods.

### Outcome measures

The primary outcome was insulin sensitivity assessed by the Matsuda index in OGTT. Secondary outcomes were concentrations of glucose and glucose metabolism hormones in OGTT, mean 24-hour glucose, blood pressure, concentrations of lipids, liver enzymes, adipokines, and cytokines, anthropometric parameters, hunger and satiety scores, parameters of intervention adherence, physical activity, and sleep. Exploratory outcomes were the gene expression in PBMCs and the internal circadian phase. Outcomes were assessed before and after eTRE and ITRE interventions or during baseline, eTRE, and ITRE periods.

### Anthropometric measurements, body composition, and blood pressure

Body weight was measured with a digital scale, and body height was measured with a stadiometer. Waist and hip circumferences were measured using a metric tape. Body composition was assessed by BIA (Quantum S, Akern), with fat and lean mass (in kilograms) calculated using the Bodygram software (Akern). Blood pressure was measured as an average of three measurements on the left upper arm after at least a 3-min rest.

### OGTTs and OGTT indices

Fasting blood samples were collected after an overnight fast. For the OGTT, participants consumed a syrup (ACCU-CHEK Dextrose O.G.-T., Roche Diabetes Care) containing 75 g of glucose at 9:30

within a 5-min time frame. Blood samples were collected after 30, 60, 90, and 120 min using EDTA and serum monovettes (Sarstedt) through an intravenous catheter. To analyze incretins, aprotinin (10 µg/ml; Carl ROTH) and 50 µM dipeptidyl peptidase-4 inhibitor (Merck Millipore) were added to the blood samples. Serum and plasma samples were centrifuged at 1800g for 10 min at 4°C and stored at –80°C until analysis. In OGTT, indices of insulin sensitivity (Matsuda index), insulin secretion in response to glucose challenge (insulinogenic index), and β cell function (disposition index, which characterizes insulin secretion in combination with insulin sensitivity) were calculated on the basis of fasting and postprandial glucose and insulin concentrations using the online calculator at <https://mmatsuda.diabetes-smc.jp/MIndex.html>. The disposition index is often used as a predictor for the development of type 2 diabetes (61). For glucose, free fatty acids, and hormone secretion in OGTT, AUC values were determined using the trapezoidal method. In the post hoc analysis, the OGIS index was additionally assessed as described (62) using the online calculator at <http://webmet.pd.cnr.it/ogis/>.

### CGM and calculation of CGM indices

The 24-hour interstitial glucose profile was examined using a FreeStyle Libre 2 sensor (Abbott) during the baseline and TRE intervention periods for 14 consequent days. A 24-hour mean sensor glucose (MSG), as well as intraday and interday indices of glycemic variability (GV), was assessed using the EasyGV software version 9.0.R2 (63), as described in Supplementary Materials and Methods.

### Biochemical measurements

Measurement of routine laboratory parameters [AST, ALT, GGT, hsCRP, glucose, hemoglobin A1c (HbA1c), total cholesterol, HDL cholesterol, and nonesterified free fatty acids] were performed using ABX Pentra (HORIBA ABX SAS). LDL cholesterol was determined using the Friedewald equation. Circulating concentrations of insulin (10-1113-01), C-peptide (10-1136-01), and glucagon (10-1271-01, all from Mercodia Inc.), MCP-1 [DCP00, Quantikine enzyme-linked immunosorbent assay (ELISA), R&D Systems Inc.], IL-6 (HS600C) and TNF-α (HSTA00E, both Quantikine ELISA HS, R&D Systems Inc.), PYY (EZHPYYT66K, Merck Millipore), adiponectin (RD195023100, BioVendor), and leptin (DLP00, Quantikine ELISA, R&D Systems Inc.) were quantified using commercial ELISA kits using a DSX 4-Plate ELISA Processing System (Dynex Technologies GmbH). Ghrelin ELISA (RA194063500R, BioVendor) was conducted using a BioTek Eon plate reader (BioTek Instruments). Oxidative stress markers were assessed as described (64) in Supplementary Materials and Methods.

### Assessment of physical activity and metabolic equivalent

Participants were asked to maintain their habitual physical activity throughout the entire trial duration. Twenty-four-hour physical activity was monitored using a blinded accelerometer (ActiGraph wGT3X-BT, ActiGraph Corporation), which was placed on the wrist of the nondominant arm for 14 days during the baseline phase and both TRE interventions. The device was to be removed only during bathing or swimming, and participants were asked to record nonwear times in a protocol. The analysis of amounts of light, moderate, and sedentary physical activity and the metabolic equivalent of task (MET) was conducted using ActiLife software version 6.13.4 (ActiGraph Corporation).

### Chronotype assessment and sleep timing

Individual chronotypes were assessed using the Munich Chronotype Questionnaire (MCTQ) (65) and the Horne-Östberg Morningness-Eveningness Questionnaire (MEQ) (66). For chronotype classification, the MSFsc value (midsleep on free days corrected for the sleep debt over the working days) was used. An MSFsc of <4 was defined as an early chronotype, an MSFsc of >5 was defined as a late chronotype, and intermediate values were defined as an intermediate chronotype. Classification in MEQ was also based on standardized criteria with a range from 59 to 69 for early chronotypes, 31 to 41 for late chronotypes, and intermediate values for intermediate chronotypes. Extreme values in MEQ outside the mentioned ranges were counted as early and late chronotypes and not classified as extreme chronotypes. In case of heterogenous results in both questionnaires, the MCTQ was used for the final chronotype classification. Participants were asked to maintain their habitual sleep times throughout the trial. Sleep timing (sleep onset and offset) was monitored using a sleep diary for 14 days during the baseline and both intervention periods.

### Gene expression and circadian phase in blood monocytes

PBMCs were isolated from fasting EDTA blood collected between 8:30 and 11:00 using BD Vacutainer CPT (BD Biosciences). The gene expression analysis in PBMCs was conducted using quantitative real-time polymerase chain reaction and specific primers (table S2).

The circadian phase was defined by DLMO, which typically occurs about 2 hours before habitual bedtime, and was assessed using the recently developed BodyTime assay. This assay requires only a single blood sample to objectively and accurately determine the phase of the circadian clock of an individual as validated previously (28). For the analysis, monocytes were purified from PBMC samples using CD14 microbeads (Miltenyi Biotech) by positive magnetic sorting. Total RNA was isolated from monocytes using TRIzol reagent (Invitrogen). One microgram of RNA was used to analyze the expression of 20 biomarker genes using the NanoString nCounter platform (NanoString Technologies) as described (28).

### Statistical analysis

Data analyses were performed using SPSS 28.0 software (SPSS) using two-sided tests at  $\alpha = 0.05$ . All analyses were intention to treat. All data were initially checked for missing values, cleaned, and inspected to determine ranges, identify outliers, and assess data distribution using the Shapiro-Wilk test. Absolute values were expressed as mean with  $\pm$ SD when normally distributed or expressed as median with IQR (25th to 75th percentiles) when not normally distributed. Changes within intervention and differences between intervention were expressed as mean difference with 95% CI. Between-intervention (parameter changes in ITRE versus changes in eTRE) comparisons for anthropometric parameters and biochemical measurements were assessed by linear mixed models. The model included cardiometabolic parameters as dependent variables, treatment (eTRE or ITRE), period (first or second), and residual effect of the first experimental period over the second period (carryover effect) as fixed factors and participants as a random factor (36). In the whole cohort, four outcomes—waist circumference, AUC glucagon, AST, and 3-nitrotyrosine—had period effects, which are reported in table and figure legends; all other outcomes did not. Total cholesterol showed

a carryover effect and was therefore analyzed for the first intervention only. Within-intervention comparisons (values after/during the intervention versus values before the intervention) and between-intervention (parameter changes in ITRE versus changes in eTRE) comparisons for all other outcomes were assessed using paired Student's *t* test for normally distributed data or the Wilcoxon test for nonnormally distributed data. Comparisons of two independent groups (values in NGT versus IFG/IGT groups) were conducted using Student's unpaired *t* test for normally distributed data or the Mann-Whitney *U* test for nonnormally distributed data. Our analyses did not adjust *P* values for multiple comparisons when analyzing cardiometabolic outcomes. The visualization of the data was performed using GraphPad Prism software version 10.2.3 (GraphPad Prism Inc.).

## Supplementary Materials

### The PDF file includes:

Materials and Methods

Figs. S1 to S4

Tables S1 to S8

Data file S1

References (67, 68)

### Other Supplementary Material for this manuscript includes the following:

MDAR Reproducibility Checklist

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