Habe *et al. Nutrition & Metabolism* (2025) 22:85
https://doi.org/10.1186/s12986-025-00984-3

Nutrition & Metabolism

RESEARCH Open Access

Comparing the influence of early and late time-restricted eating with energy restriction and energy restriction alone on cardiometabolic markers, metabolic hormones and appetite in adults with overweight/obesity: per-protocol analysis of a 3-month randomized clinical trial

Bernarda Habe[1], Tanja Črešnovar[1], Ana Petelin[1], Saša Kenig[1], Nina Mohorko[1] and Zala Jenko Pražnikar[1*]

Abstract

Background It remains unclear whether adding time-restricted eating (TRE) to energy restriction (ER) offers additional cardiometabolic benefits, particularly in metabolic hormone regulation, and insulin sensitivity. This per-protocol analysis assessed whether early TRE (eTRE) or late TRE (lTRE), when combined with ER, additionally improves insulin resistance indexes, and cardiovascular and liver biomarkers compared to ER alone.

Methods We analysed per-protocol data of 90 participants, 31 from the eTRE with ER (eTRE + ER) group, 28 from the lTRE with ER (lTRE + ER) group and 31 from the ER group. As chronotype-adapted diets have already been shown to produce better outcomes than non-adapted ones and in line with real-life behaviour, randomisation was performed on the basis of the individuals' chronotype. Anthropometric and biochemical measurements for analysis were taken at baseline, and after first and third month of intervention. The primary outcome was mean change in body mass, while the secondary outcomes were mean changes in glycaemic markers (fasting glucose, fasting insulin), indexes of insulin resistance, cardiovascular and liver markers and metabolic hormones (adiponectin, ghrelin, leptin, leptin/ghrelin ratio). Additionally, participant's subjective appetite was also assessed at baseline and in third month of the intervention.

Results We confirmed that participants who adhered to eTRE + ER for 3 months showed greater improvements in % of fat mass, BMI, and fasting glucose compared to those in the lTRE + ER and/or ER group. These greater reductions in % of the fat mass and BMI were accompanied by more pronounced decreases in leptin levels, with eTRE + ER showing larger leptin reductions than lTRE + ER or ER. Additionally, the eTRE group showed a significantly greater decrease in

*Correspondence:
Zala Jenko Pražnikar
zala.praznikar@upr.si

Full list of author information is available at the end of the article

desire for food and greater reduction in capacity to eat than ER. However, insulin levels, insulin resistance indexes, lipid profiles, adiponectin, ghrelin, visceral fat indexes, and liver enzymes showed similar changes across all groups.

Conclusions This analysis showed that eTRE + ER is more effective weight management strategy, while eTRE + ER, lTRE + ER and ER are comparable effective on cardiovascular, liver and insulin resistance markers.

Trial registration https://clinicaltrials.gov/study/NCT05730231 (NCT05730231, registered on February 6, 2023).

Keywords Time-restricted eating, Energy restriction, Metabolic health, Insulin resistance, Leptin

Introduction

The global prevalence of overweight and obesity, particularly abdominal obesity, is increasing at an alarming rate and represents a significant burden on healthcare systems worldwide [1]. The development of obesity and obesity-related comorbidities is closely linked to the proliferation of ectopic adipose tissue, excessive fat accumulation and metabolic dysregulation. In addition, hypertrophic adipocytes often become dysfunctional and secrete inflammatory markers and chemotactic factors that promote immune cell infiltration and activation. This leads to systemic metabolic and immunological dysfunction, establishing obesity as a chronic, low-grade inflammatory disease [2, 3]. Therefore, early-stage obesity is frequently associated with comorbidities such as hyperglycaemia, insulin resistance (IR) and hypertension. And, if obesity progresses further, it directly contributes to the development of type 2 diabetes mellitus (T2DM), metabolic dysfunction-associated steatotic liver disease (MASLD), cardiovascular diseases (CVDs) and an increased risk of all-cause mortality [4]. Moreover, dysregulation of leptin, ghrelin and other peptides that regulate energy homeostasis is often observed in obesity, leading to leptin resistance and elevated ghrelin levels, which promote overeating and weight gain in long term [5].

Lifestyle factors are critical to the aetiology of obesity and associated comorbidities [6]. Consequently, lifestyle modification, particularly body weight reduction, especially reduction of body fat mass, is widely recognized as a fundamental strategy for managing obesity and metabolic disorders. Weight loss interventions, including energy restriction (ER) and intermittent fasting, have been shown to enhance metabolic health [6, 7] and to modulate leptin and ghrelin levels, contributing to improved appetite control and weight loss [8, 9]. In animal models, intermittent fasting has numerous benefits: it reduces body weight and adiposity, enhances insulin sensitivity, lowers blood glucose levels, reduces blood pressure, improves cardiometabolic health, slows cellular ageing, slows the progression of cancer, improves cognitive function, mitigates the progression of neurodegenerative diseases and even extends lifespan [10–13].

Among the various intermittent fasting regimens, time-restricted eating (TRE) has emerged as a prominent dietary strategy and has attracted considerable interest from researchers and the general public. TRE involves restricting food intake to a daily window of 10 h or less, followed by a fasting period of 14 h or more. TRE can be categorized into various subtypes based on different time windows for restricting food intake. Early TRE (eTRE) means that the first meal is eaten in the morning (before 10:00 am), while late TRE (lTRE) means that meals are restricted to the afternoon or evening. This dietary pattern aligns with the body's circadian rhythms, which govern several physiological processes, including the regulation of the body's energy homeostasis and several metabolic pathways [14–16]. In a few human studies, TRE has been reported to improve cardiometabolic outcomes, including insulin sensitivity, blood pressure, and oxidative stress, even when energy intake remains identical to that of the control group [16–19]. However, findings from larger and longer-duration studies [20–22] suggest that these effects are comparable to those achieved by traditional ER, and thus current evidence does not clearly support a superior benefit of TRE over ER. Indeed, although studies on circadian rhythms have confirmed that insulin sensitivity and β-cell function are more responsive in the morning [17, 23, 24], a recently published meta-analysis showed that although the addition of TRE to ER resulted in greater weight loss and improvements in cardiometabolic risk factors, including IR, in some studies compared to ER alone, the majority of studies failed to demonstrate additional benefits of TRE in overweight and obese adults [25]. It is therefore not yet clear whether the addition of TRE to ER provides additional benefit in terms of cardiometabolic parameters, including the regulation of metabolic hormones, glucose metabolism, liver function, and insulin sensitivity.

Considering our findings of our parent study that eTRE combined with ER is more effective in reducing % of fat mass, diastolic blood pressure and fasting glucose levels than lTRE combined with ER or ER alone [26], we conducted a secondary per-protocol analysis to determine whether the addition of TRE, particularly eTRE, to ER provides additional benefits for peptides involved in energy homeostasis, insulin resistance-related indexes, and specific cardiovascular and liver biomarkers compared to ER alone.

Methods

Study design

This study is a secondary analysis of a three-arm, parallel, 3-month randomized clinical trial conducted between March and June 2023, following a recruitment and screening period that began in February 2023 at the University of Primorska, Faculty of Health Sciences. The detailed protocol and primary results have been published previously [26]. The study protocol was approved by the Slovenian National Medical Ethics Committee (No. 0120–557/2017/4; Ministry of Health, Republic of Slovenia) and registered at ClinicalTrials.gov (NCT05730231), and all participants signed a written informed consent form prior to participation.

Study participants

Participants were recruited via online, radio and public television adverts, social media and local newspapers. The inclusion and exclusion criteria were previously described in detail [26]. In brief, eligible participants were individuals aged 18 to 60 years with a body mass index (BMI) between 25 kg/m^2 and 35 kg/m^2 who met at least one additional component of the metabolic syndrome, based on established criteria [27]. Participants were excluded if they were taking medication for hypertension and hypercholesterolaemia, had an average daily eating window of less than 11 h/day, were pregnant or breastfeeding, smoked, were on a weight loss programme, had a chronic disease (e.g. cardiovascular disease, gastrointestinal, oncological, haematological diseases, etc.), a diagnosed eating disorder or a history of an eating disorder, shift work, alcohol consumption > 2 servings/day for men and > 1 serving/day for women, or were using dietary supplements that could influence the main results of the study.

Intervention

Intervention protocol was previously described [26]. Briefly, participants were allocated to three groups: eTRE + ER who consumed their food within an 8-hour window between 8:00 and 16:00, lTRE + ER who consumed their food eating within an 8-hour window between 12:00 and 20:00 and ER group who consumed their food eating within a 12-hour window between 8:00 and 20:00. In line with real-life settings, allocation was based on the individual chronotype, as assessed by the Morningness-Eveningness Questionnaire (MEQ) [28]. The participants with a total MEQ score > 58 were randomized to the eTRE + ER or ER group in a 2:1 ratio, while participants with a total MEQ score ≤ 58 were randomized either to the lTRE + ER or ER group, in a 2:1 ratio. Consequently, eTRE + ER consisted of morning and intermediate types (average MEQ score: 64), lTRE + ER consisted of evening and intermediate types (average MEQ score: 54), whereas ER consisted of morning, intermediate and evening types (average MEQ score: 58). Randomization with stratification was performed using the free open-source desktop application MinimPy (https://sourceforge.net/projects/minimpy) [29]. The stratified variables were sex (male, female), age (18–39 and 40–60 years) and BMI (25–30 kg/m^2 and 30–35 kg/m^2). As previously reported, we screened 300 participants and enrolled 108 participants. Ultimately, 93 participants completed the entire 3-month intervention [30]. However, there was missing data about peptides that regulate energy homeostasis for 3 participants, therefore in the secondary analysis, we analysed per-protocol data of 90 participants, 31 from the eTRE + ER group, 28 from the lTRE + ER group and 31 from the ER group (Fig. 1).

At baseline, participants in all three groups met one-on-one with a registered dietitian and were instructed to reduce their energy intake by approximately 2093 kJ/500 kcal based on individual resting metabolic rate and daily physical activity level. Moreover, all participants had the same macronutrient distribution according to their individual energy requirements: 45–50% of energy from carbohydrates, 15–20% from protein and 30–35% from fat. Their daily energy intake was evenly distributed over three meals: 30% for breakfast, 40% for lunch and 30% for dinner. Snacks between meals were not allowed. Outside of meals, only water and herbal infusions without added sugar were allowed. In addition, participants had monthly motivational online meeting with a dietitian to help them adhere to the prescribed nutrition plan. Participants self-reported their daily adherence to the time window for food intake, any deviations from energy intake and the number of meals using a dietary calendar. To evaluate the dietary strategy, participants also completed a 3-day food diary after 2 months of the intervention. Dietary data were analyzed using the Open Platform for Clinical Nutrition (OPEN), which is accessible via the website http://opkp.si/ (accessed on 1st March 2024).

Per-protocol analysis criteria

The per-protocol criteria were pre-defined in the study's plan. Per-protocol analysis included participants who: (a) adhered to their assigned TRE window (within a ± 30 min tolerance per day) and ER; (b) consumed 3 meals per day and; (c) completed all primary and secondary outcome assessments. At the introductory meeting, the participants were informed that if they did not follow the protocol for four consecutive days, they would be excluded from the study.

Measurements

All measurements were conducted in the morning after 12–15 h of fasting. Blood sampling in the eTRE + ER

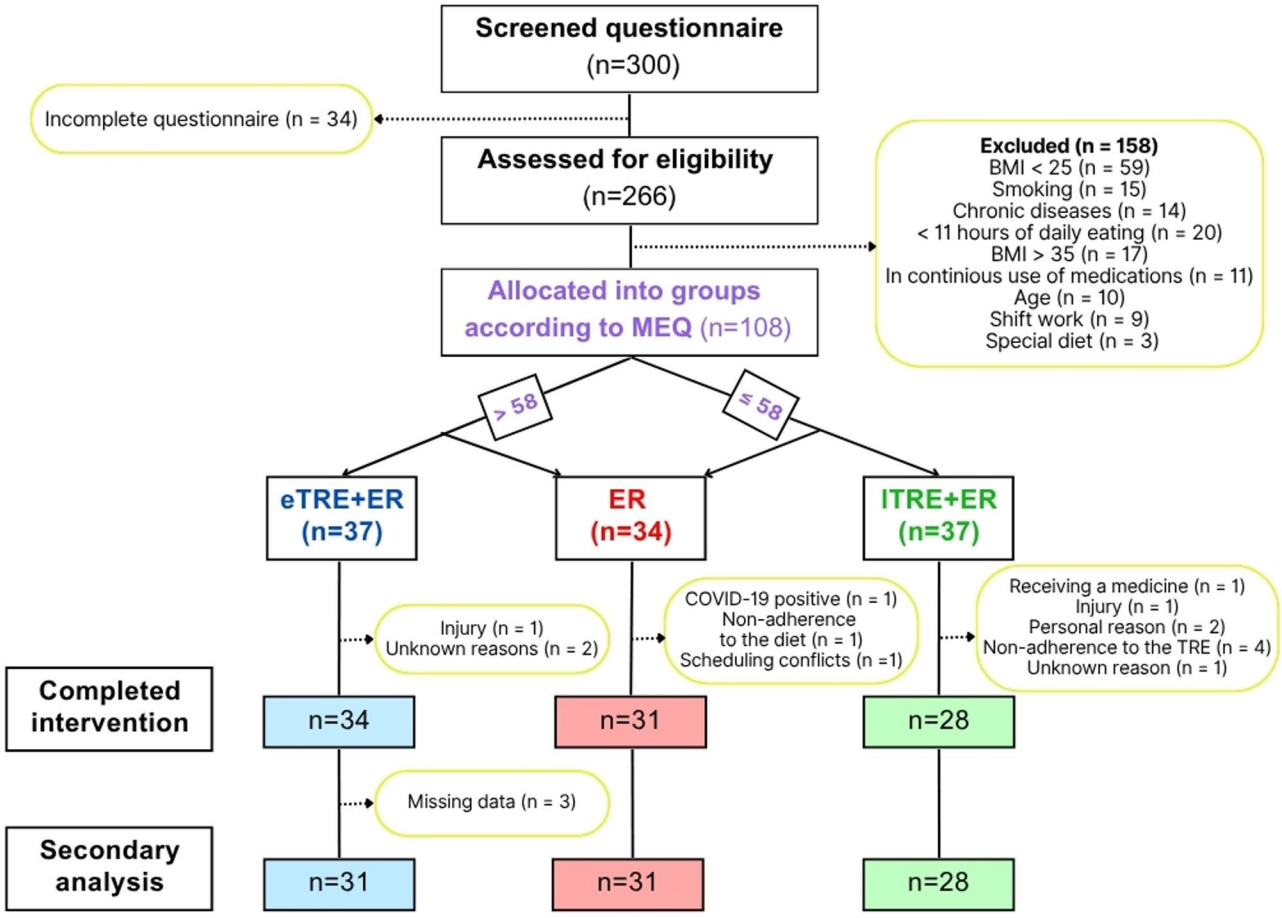

Fig. 1 Flow diagram of participants. Abbreviations: eTRE + ER, early time restricted eating with energy restriction; ER, energy restriction; lTRE + ER, late time restricted eating with energy restriction; MEQ, Morningness-eveningness questionnaire

group was performed between 5:30 and 7:00 a.m., in the lTRE + ER between 10:00 and 11:00 a.m., while in ER group between 9:00 and 11:00 a.m. Body mass and body composition were measured using the Tanita MC-980MA bioelectrical impedance analyser (BIA) (Tanita Corporation, Arlington Heights, IL, USA) and dedicated software (GMON Pro-Tanita). Waist circumference was measured at the mid-axillary line.

Venous blood samples were collected in 9 mL vacuum serum test tubes and 6 mL vacuum EDTA test tubes (Greiner Bio-One). The serum and plasma were separated by centrifugation at 2000 x g for 10 min, frozen and stored at -80 °C until analysis. Serum concentrations of fasting glucose, triglycerides (TG), total cholesterol, low density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured with a Cobas® c111 analyser (Roche, Basel, Switzerland). Serum insulin, adiponectin, leptin and plasma acylated ghrelin were measured using the ELISA immunoenzymatic assay (BioVendor, Brno, Czech Republic). Assay sensitivity was 0.2 ng/mL for leptin, 26 ng/mL for adiponectin, 4 pg/mL for acylated ghrelin and 0.17 μIU/mL for insulin. Assays inter-assay and intra-assay CVs were typically < 10%.

In addition to measuring peptides that regulate appetite, also participants' subjective appetite was assessed with visual analogue scale (VAS) at baseline and in third month of the intervention. VAS consisted of four dimensions (hunger, fullness, stomach fullness, desire to eat and capacity to eat) and scales (0–100 mm) were anchored with the words "not at all" (left hand end) and "extremely" (right hand end) [30].

Moreover, different metabolic indexes were calculated. Homeostatic model assessment of insulin resistance (HOMA-IR) and homeostatic model assessment of β-cell function (HOMA-β) were calculated using the following formulas [31, 32]: HOMA-IR = fasting serum insulin (μU/mL) × fasting plasma glucose (mmol/L) / 22.5, HOMA-β = 20 × fasting insulin (μU/mL) / [fasting plasma glucose (mmol/L)– 3.5]. Metabolic score for insulin resistance (METS-IR) was calculated according to [33]: METS-IR = ln [2 × fasting plasma glucose (mg/dL) + fasting triglycerides (mg/dL)] × BMI (kg/m^2) / ln

[HDL-cholesterol (mg/dL)], whereas the triglyceride glucose body mass index (TyG-BMI) as follows: TyG-BMI = ln [TG (mg/dL) × fasting plasma glucose (mg/dL)/2] × BMI (kg/m^2) [34]. The lipid accumulation product (LAP) and visceral adiposity index (VAI) were calculated using sex-specific formulas [35, 36]: LAP (male) = [waist circumference (cm)– 65] × [fasting triglycerides (mmol/L)] and LAP (female) = [waist circumference (cm) – 58] × [fasting triglycerides (mmol/L)], while VAI (male) = [waist circumference (cm) / 39.68 + [1.88 × BMI (kg/m^2)]] × [fasting triglycerides (mmol/L) / 1.03] × [1.31 / HDL-cholesterol (mmol/L)] and VAI (female) = [waist circumference (cm) / 36.58 + [1.89 × BMI (kg/m^2)]] × [triglycerides (mmol/L) / 0.81] × [1.52 / HDL-cholesterol (mmol/L)].

Outcomes measures

Anthropometric and biochemical measurements for secondary analysis were taken at baseline, and after first and third month of intervention. The primary outcome was mean change in body mass. The secondary outcomes were mean change in glycaemic markers (fasting glucose, fasting insulin), indexes of insulin resistance (HOMA-IR, HOMA-β, METS-IR, TyG– BMI), cardiovascular markers (lipid profile, TG/HDL-ratio), liver markers (AST, ALT, VAI and LAP indexes), metabolic hormones (adiponectin, ghrelin, leptin, leptin/ghrelin ratio) and participant's subjective appetite.

Statistical analysis

For the secondary analysis, the statistical procedure was performed using SPSS version 29.0 (IBM Corp., Armonk, NY, USA). All analysis were per-protocol. The normality of variables was tested using the Shapiro–Wilk test. The one-way ANOVA test for normal distribution or Kruskal-Wallis test for non-normal distribution were used to evaluate differences in baseline characteristics and nutrition intervention at two time points between participants in all three groups, while within-group evaluations of changes in outcome variables were performed with paired t-test for normally distributed variables or paired Wilcoxon tests for abnormally distributed variable. Measurements at multiple time points was analysed using linear mixed model, with adjustment for age, sex, baseline BMI and baseline values. Moreover, the Bonferroni post hoc test was used to detect significant differences between groups in linear mix model and one-way ANOVA test. A significance level of 5% ($P < 0.05$) was defined for all statistical tests. Values are reported as mean (95% confidence intervals), except the baseline characteristics are reported as mean ± SD.

Results

Baseline participants characteristic

The baseline characteristics of the ninety participants assigned to the three different groups are shown in Table 1. At baseline, there were no significant differences between eTRE + ER, lTRE + ER and ER groups in terms of sex, age, body mass and BMI, confirming that randomisation was maintained in this per-protocol analysis. In addition, there were also no significant differences at baseline in waist circumference, various body composition parameters (% of fat mass, and fat-free mass), various glycaemic markers and insulin resistance indexes (fasting glucose, fasting insulin, HOMA-IR, HOMA-β, TyG-BMI, METS-IR), cardiovascular markers (triglycerides, total cholesterol, HDL, TG/HDL ratio), liver markers (AST, ALT, VAI, LAP) and metabolic hormones (adiponectin, ghrelin, leptin, leptin/ghrelin ratio). Subjective appetite, eating windows as well as energy and macronutrient intake were also similar in all three groups at the beginning of the intervention study. On the other hand, significant differences were found between the groups at baseline for LDL cholesterol levels ($P = 0.030$). In fact, participants in lTRE + ER had significantly higher LDL cholesterol levels than participants in eTRE + ER ($P = 0.036$).

Effects of eTRE + ER, lTRE + ER and ER intervention on eating time and energy intake

Mean changes in the daily eating window, energy intake, and macronutrient consumption, along with differences between groups, are reported in Table 2. There was a significant reduction in eating time in the eTRE + ER group (-4.8 h, 95% CI, -5.1, -4.5) and the ITRE + ER group (-4.5 h, 95% CI, -4.8, -4.2) from baseline to the end of the intervention period, emphasizing the effective implementation of the time-restricted eating protocols. Interestingly, a significant reduction in the eating window was also observed in the ER group, albeit less pronounced (-0.9 h, 95% CI, -1.1, -0.6,). As expected, significant differences were found in changes in eating time between the ER and the two TRE + ER groups eTRE + ER vs. ER: (-3.9 h, 95% CI, -4.4, -3.5, $P < 0.001$) and ITRE + ER vs. ER: (-3.6 h, 95% CI, -4.1, -3.1, $P < 0.001$).

Analysis of the 3-day dietary records showed that participants adhered well to the prescribed dietary plan throughout the study in terms of energy intake, meal frequency and the proportion of macronutrients. This adherence was reflected in significant reductions in total energy intake (kJ/day) for all groups: -2820 kJ/day (95% CI, -3330, -2310) for eTRE + ER, -2138 kJ/day (95% CI, -2824, -1565) for ITRE + ER, and – 2732 kJ/day (95% CI, -3138, -2326) for ER, with no significant differences among them ($P = 0.318$). Similarly, participants in all three groups significantly reduced carbohydrate (g/day)

Table 1 Baseline participants characteristic

Variables	eTRE + ER M ± SD	lTRE + ER M ± SD	ER M ± SD	*P* value
N (F/M)	31 (24/7)	28 (18/10)	31 (19/12)	0.651
Age (years)	45 ± 9	46 ± 11	46 ± 6	0.776
Anthropometric parameters				
Body mass (kg)	85.2 ± 12.8	86.8 ± 9.3	88.2 ± 16.0	0.661
BMI (kg/m^2)	29.1 ± 3.1	29.1 ± 2.1	29.3 ± 3.6	0.716
Waist circumference (cm)	95.2 ± 9.7	98.0 ± 6.9	97.6 ± 13.6	0.532
Fat mass (%)	29.3 ± 6.1	28.1 ± 5.6	28.9 ± 8.6	0.829
Fat-free mass (kg)	55.9 ± 11.3	58.7 ± 10.6	59.4 ± 11.9	0.452
Glycaemic markers and insulin resistance indexes				
Fasting glucose (mmol/L)	5.2 ± 0.6	5.4 ± 0.5	5.5 ± 0.7	0.472
Fasting insulin (μIU/mL)	9.0 ± 4.0	7.8 ± 3.7	9.6 ± 7.3	0.431
HOMA– IR	2.1 ± 1.1	1.9 ± 1.0	2.3 ± 2.2	0.643
HOMA– β (%)	111 ± 55	86 ± 36	117 ± 70	0.187
TyG - BMI	265 ± 37	272 ± 33	275 ± 41	0.542
METS-IR	41 ± 8	42 ± 6	42 ± 8	0.788
Cardiovascular markers, liver markers and fat accumulation indexes				
Triacylglycerols (mmol/L)	1.3 ± 1.5	1.6 ± 1.6	1.4 ± 0.7	0.670
Total cholesterol (mmol/L)	5.1 ± 1.2	5.7 ± 1.1	5.2 ± 0.9	0.065
LDL cholesterol (mmol/L)	3.6 ± 1.0	4.3 ± 1.3	3.7 ± 0.9	**0.030**[a]
HDL cholesterol (mmol/L)	1.6 ± 0.5	1.6 ± 0.4	1.6 ± 0.4	0.843
TG/HDL - ratio	1.1 ± 2.4	1.3 ± 2.0	1.0 ± 0.6	0.830
AST (IU/L)	20 ± 7	21 ± 5	21 ± 7	0.678
ALT (IU/L)	25 ± 22	26 ± 11	25 ± 14	0.987
VAI	1.9 ± 3.4	2.1 ± 2.5	1.7 ± 1.1	0.824
LAP	51 ± 66	62 ± 57	53 ± 33	0.711
Metabolic hormones				
Adiponectin (μg/mL)	9.3 ± 3.7	8.9 ± 3.4	8.7 ± 4.0	0.871
Ghrelin (pg/mL)	24 ± 24	16 ± 15	17 ± 23	0.280
Leptin (ng/mL)	96 ± 63	77 ± 48	71 ± 46	0.176
Leptin/ghrelin ratio	13,568 ± 30,802	11,395 ± 16,919	12,665 ± 24,603	0.964
Visual analogue scale for subjective appetite				
Hunger (1–10 mm)	5.5 ± 2.2	5.6 ± 1.4	5.3 ± 1.6	0.827
Fullness (1–10 mm)	6.0 ± 2.1	6.2 ± 1.5	5.6 ± 1.5	0.366
Stomach fullness (1–10 mm)	5.1 ± 2.6	5.2 ± 2.2	3.9 ± 2.7	0.077
Desire to eat (1–10 mm)	5.7 ± 2.2	5.2 ± 2.4	5.1 ± 2.0	0.477
Capacity to eat (1–10 mm)	5.8 ± 2.3	5.1 ± 2.4	4.8 ± 1.9	0.220
Dietary intake and eating window				
Eating time (h/day)	12.9 ± 0.9	12.5 ± 1.2	12.9 ± 1.1	0.738
Energy intake (kJ/day)	9757 ± 2029	9443 ± 3556	9652 ± 2360	0.904
Carbohydrate intake (g/day)	261 ± 63	248 ± 96	253 ± 61	0.792
Protein intake (g/day)	97 ± 23	95 ± 41	92 ± 28	0.840
Fat intake (g/day)	95 ± 27	93 ± 38	98 ± 34	0.824
Macronutrients distribution				
% Carbohydrates	44.9 ± 6.8	43.8 ± 5.1	44.3 ± 7.1	0.807
% Protein	16.8 ± 3.1	16.9 ± 3.1	16.1 ± 2.9	0.475
% Fat	36.3 ± 5.6	36.9 ± 4.2	37.7 ± 6.4	0.590

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; BMI, body mass index; ER, energy restriction; eTRE, early time restricted eating; F, female; HOMA-β, homeostatic model assessment of β-cell function; HOMA-IR, homeostatic model assessment of insulin resistance; lTRE, late time restricted eating; kg, kilograms; M, male; M, mean; METS-IR, metabolic score for insulin resistance; N, total number; SD, standard deviation; TyG-BMI, triglyceride-glucose index combined with body mass index; LAP, lipid accumulation product index; VAI, visceral adiposity index. P value denotes significant ($P < 0.05$) difference between eTRE + ER, lTRE + ER and ER group using ANOVA for normal distribution, Kruskal-Wallis test for non-normal distribution. The Bonferroni post hoc test was used to detect significant differences between groups Pa– denotes significant ($P < 0.05$) difference between eTRE + ER and lTRE + ER groups

Table 2 Effects of eTRE + ER, lTRE + ER and ER intervention on eating time and energy intake

Variables	Mean change from baseline (95% CI)[a]			Mean difference between groups (95% CI)[b]			
	eTRE + ER	lTRE + ER	ER	eTRE + ER vs. lTRE + ER	eTRE + ER vs. ER	lTRE + ER vs. ER	*p*-value
Eating time (h/day)	-4.8 (-5.1, -4.5)*	-4.5 (-4.8, -4.2)*	-0.9 (-1.1, -0.6)*	-0.3 (-0.8, 0.1)	**-3.9 (-4.4, -3.5)**	**-3.6 (-4.1, -3.1)**	< 0.001
Energy intake (kJ/day)	-2820 (-3330, -2310)*	-2138 (-2824, -1452)*	-2732 (-3138, -2326)*	-682 (-1607, 243)	-88 (-987, 812)	594 (-331, 1518)	0.160
Energy intake (%/day)	-26.8 (-30.9, -22.5)*	-19.8 (-25.1 -14.5)*	-26.9 (-30.3, -23.5)*	-6.9 (-14.3, 0.5)	0.1 (-7.1, 7.4)	7.0 (-0.4, 14.5)	0.063
Carbohydrates (g/day)	-73 (-88, -58)*	-62 (-81, -43)*	-72 (-86, -59)*	-11 (-38, 16)	-1 (-27, 26)	10 (-17, 37)	0.574
Protein (g/day)	-13 (-19, -7)*	-10 (-19, -1)	-3 (-9, 2)	-3 (-15, 9)	-9 (-21, 2)	-6 (-18, 5)	0.132
Fats (g/day)	-34 (-41, -27)*	-22 (-33, -12)*	-36 (-43, -30)*	-11 (-25, 2)	3 (-10, 16)	**14 (0, 27)**	0.035
Macronutrient distribution (mean change from baseline (95% CI)[a] and mean values ± SD during the intervention)							
Fats (Δ%)	-3.0 (-4.6, -1.4)*	-1.2 (-3.6, 1.2)	-4.5 (-6.3, -2.7)*	-1.8 (-5.2, 1.5)	1.5 (-1.8, 4.8)	3.3 (0.0, 6.7)	0.061
Carbohydrates (Δ %)	-0.3 (-1.5, 2.1)	-1.1 (-3.4, 1.1)	-0.3 (-2.0, 1.4)	1.4 (-1.9, 4.8)	0.6 (-2.6, 3.8)	-0.8 (-4.1, 2.5)	0.574
Protein (Δ %)	3.5 (2.6, 4.4)*	2.8 (1.7, 3.9)*	5.4 (4.5, 6.3)*	0.7 (-0.9, 2.4)	**-1.9 (-3.5, 0.3)**	**-2.6 (-4.2, -0.9)**	< 0.001

Abbreviations; CI, confidence interval; ER, energy restriction; eTRE, early time restricted eating, lTRE, late time restricted eating. [a] Within-group evaluations of changes in outcome variables were performed with paired t-test for normally distributed variables or paired Wilcoxon tests for abnormally distributed variables. Asterix (*) indicates significant differences ($P < 0.05$) from baseline within each group. [b] Between group comparisons were conducted using one-way ANOVA and post hoc Bonferroni tests. Bolded values indicate significant differences ($P < 0.05$) between groups

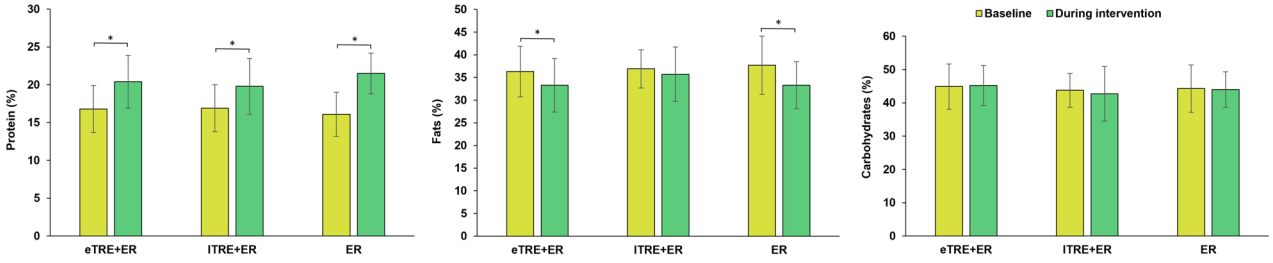

Fig. 2 Macronutrient distribution (energy %) before and during the intervention (mean ± SD). eTRE, early time-restricted eating; lTRE, late time-restricted eating; ER, energy restriction; SD, standard deviation. Estimates of changes in outcome variables within the group were performed using a paired t-test for normally distributed variables or a paired Wilcoxon test for abnormally distributed variables. An asterisk (*) indicates statistically significant differences ($P < 0.05$) from baseline within each group

and protein (g/day) intake, with no significant differences among groups ($P = 0.550$ and $P = 0.132$, respectively). On the other hand, the between-group analysis showed a significantly ($P = 0.042$) smaller decrease in fat intake (g/day) in the lTRE + ER group compared to the ER group (mean difference: 14 g, 95% CI, 0, 27) and a significantly higher increase in the proportion of protein intake in the ER group compared to the eTRE + ER group (-1.9%, 95% CI, -3.5, 0.3, $P = 0.016$) and the lTRE + ER group (-2.6%, 95% CI, -4.2, -0.9, $P < 0.001$). Despite these differences, the macronutrient distribution (in energy %) was comparable between the groups during the intervention (Fig. 2). Moreover, there were also no significant between-group differences in meal frequency per day, as all participants consumed in average 3 meals per day.

Effects of eTRE + ER, lTRE + ER and ER intervention on anthropometric parameters

The mean changes in body mass and parameters of the body composition are shown in Table 3. There was a significant time main effect ($P < 0.001$) indicating a decrease in body mass at the end of the 3-month intervention, with a mean loss of -5.1 kg (95% CI, -5.7, -4.6) for the eTRE + ER group, -4.5 kg (95% CI, -5.1, -3.9) for the lTRE + ER and − 4.3 kg (95% CI, -4.8, -3.7) for the ER group, with no significant difference among groups ($P = 0.321$). Similarly, there was a significant time effect on fat-free mass ($P = 0.043$) and waist circumference ($P < 0.001$) at the end of the 3-month intervention for all three groups, with no significant difference among them ($P = 0.140$ and $P = 0.094$, respectively). In addition, there was also a significant time effect on BMI and % of fat mass (both Ps < 0.001) with significant differences after 3-month intervention between the eTRE + ER group and the ER group in the mean change in BMI − 0.4 (95% CI, -0.7, -0.03, $P = 0.026$), and % of fat mass − 0.9% (95% CI, -1.6, -0.2, $P = 0.009$). Moreover, at the end of the 3-month intervention, there was also a significant difference between the eTRE + ER and the lTRE + ER in the mean change in % of fat mass − 1.0% (95% CI, -1.7, -0.3, $P = 0.003$). Overall, all three interventions (eTRE + ER, lTRE + ER and ER) were effective weight loss strategies,

Table 3 Effects of eTRE + ER, lTRE + ER and ER interventions on anthropometric parameters

Variables	Mean change from baseline (95% CI)			Time effect	Mean difference between groups (95% CI)					
	eTRE + ER	lTRE + ER	ER	*P*-value	eTRE + ER vs. lTRE + ER	*P*value	eTRE + ER vs. ER	*P*-value	lTRE + CR vs. ER	*P*-value
Anthropometric parameters										
Body mass (kg)	-5.1 (-5.7, -4.6)	-4.5 (-5.1, -3.9)	-4.3 (-4.8, -3.7)	< 0.001	-0.6 (-1.6, 0.3)	0.349	-0.8 (-1.8, 0.13)	0.114	-0.2 (-1.2, 0.8)	1.000
BMI (kg/m^2)	-1.8 (-2.0, -1.6)	-1.5 (-1.7, -1.3)	-1.4 (-1.6, -1.2)	< 0.001	-0.3 (-0.6, 0.1)	0.190	**-0.4 (-0.7, -0.03)**	0.026	-0.1 (-0.4, 0.2)	1.000
Fat mass (%)	-2.5 (-2.9, -2.1)	-1.5 (-1.9, -1.1)	-1.6 (-2.1, -1.2)	< 0.001	**-1.0 (-1.7, -0.3)**	0.003	**-0.9 (-1.6, -0.2)**	0.009	-0.1 (-0.6, -0.9)	1.000
Fat-free mass (kg)	-1.4 (-1.7, -1.1)	-1.8 (-2.1, -1.5)	-1.5 (-1.9, -1.3)	0.043	-0.4 (-0.1, 0.9)	0.190	0.2 (-0.3, 0.7)	1.000	-0.2 (-0.8, 0.3)	0.841
Waist circumference (cm)	-3.7 (-4.3, -3.2)	-3.2 (-3.7, -2.6)	-3.0 (-3.5, -2.4)	< 0.001	-0.6 (-1.5, 0.3)	0.396	-0.8 (-1.7, 0.1)	0.107	-0.2 (-1.1, 0.7)	1.000

Abbreviations: BMI, body mass index; CI, confidence interval; ER, energy restriction; eTRE, early time restricted eating; lTRE, late time restricted eating. Means were estimated using linear mix model with Bonferroni post hoc tests and with adjustment for BMI, age and sex and baseline values for all variables. P values for the main effect of the time are listed. Bolded values indicate statistically significant differences ($P < 0.05$) between groups

Table 4 Effects of eTRE + ER, lTRE + ER and ER intervention on glycaemic markers and insulin resistance

Variables	Mean change from baseline (95% CI)			Time effect	Mean difference between groups (95% CI)					
	eTRE + ER	lTRE + ER	ER	*P*-value	eTRE + ER vs. lTRE + ER	*P*-value	eTRE + ER vs. ER	*P*-value	lTRE + CR vs. ER	*P*-value
Fasting glucose (mmol/L)	-0.5 (-0.6, -0.4)	-0.1 (-0.3, 0.0)	-0.2 (-0.3, 0.0)	0.854	**-0.4 (-0.6, -0.1)**	0.002	**-0.3 (-0.5, -0.1)**	0.007	0.1 (-0.2, 0.3)	1.000
Fasting insulin (µIU/mL)	-2.8 (-4.0, -1.6)	-1.7 (-2.9, -0.4)	-3.3 (-4.5, -2.1)	0.307	-1.1 (-3.3, 1.0)	0.596	-0.5 (-1.6, 2.6)	1.000	1.6 (-05, 3.8)	0.596
HOMA - IR	-0.8 (-1.1, -0.5)	-0.4 (-0.8, -0.1)	-0.9 (-1.2, -0.5)	0.429	-0.4 (-1.0, 0.2)	0.426	0.1 (-0.5, 0.7)	1.000	0.4 (-0.2, 1.1)	0.251
HOMA– β (%)	-4 (-19, 11)	-7 (-23, 9)	-21 (-37, -6)	0.039	3 (-24, 30)	1.000	17 (-9, 44)	0.333	14 (-13, 41)	0.618
TYG - BMI	-23 (-27, -19)	-20 (-24, -16)	-18 (-22, -15)	0.005	-3 (-10, 4)	0.898	-4 (-11, 2)	0.342	-1 (-8, 5)	1.000
METS-IR	-3 (-3, -2)	-2 (-3, -2)	-3 (-3, -2)	< 0.001	-1 (-2, 1)	0.620	0 (-1, 1)	1.000	0 (-1, 1)	1.000

Abbreviations: CI, confidence interval; ER, energy restriction; eTRE, early time restricted eating; HOMA-β, homeostatic model assessment of β-cell function; HOMA-IR, homeostatic model assessment of insulin resistance; lTRE, late time restricted eating; METS-IR, metabolic score for insulin resistance; TyG-BMI, triglyceride-glucose index combined with body mass index. Means were estimated using linear mix model with Bonferroni post hoc tests and with adjustment for BMI, age and sex and baseline values for all variables. P values for the main effect of the time are listed. Bolded values indicate statistically significant differences ($P < 0.05$) between groups

but dietary regime eTRE + ER was significantly more effective in reducing % of fat mass than lTRE + ER and ER and significantly more effective in reducing BMI than ER alone after 3-month intervention.

Effects of eTRE + ER, lTRE + ER and ER intervention on glycaemic markers and insulin resistance indexes

Furthermore, we compared the effects of eTRE + ER, lTRE + ER and ER on glycaemic markers and indicators of insulin resistance (Table 4; Fig. 3). The effect of time was not significant for fasting glucose and insulin levels. However, for glucose, the observed mean reductions of -0.5 mmol/L (95% CI, -0.6, -0.4) for the eTRE + ER group, -0.1 mmol/L (95% CI, -0.3, 0.0) for the lTRE + ER group, and – 0.2 mmol/L (95% CI, -0.3, 0.0) for the ER group, were significantly different between eTRE + ER and lTRE + ER and between eTRE+ER and ER ($P = 0.002$ and $P = 0.007$, respectively). There were no significant differences between groups for fasting insulin changes.

There was a significant time effect for HOMA-β ($P = 0.039$), TYG-BMI ($P = 0.005$), and METS-IR ($P = 0.001$), with no significant time effect observed for HOMA-IR (Table 4). No significant differences between groups were observed in mean changes of HOMA-IR ($P = 0.180$), HOMA-β ($P = 0.079$), TyG-BMI ($P = 0.273$) and METS-IR ($P = 0.430$) after 3 months of intervention. Although all three interventions had similar, non-significant effects on insulin levels and HOMA-IR, we have to point out that HOMA-IR values of all participants who were insulin resistant at baseline ($N = 11$ (eTRE + ER:7, lTRE + ER:2, ER:2), HOMA-IR > 2.77) fell below 2.77 [37] after 3-month intervention.

Figure 3 shows the absolute changes in fasting glucose, insulin, HOMA-β and METS-IR after one and three months of the intervention. After one month, only the eTRE + ER group showed a significant decrease in fasting blood glucose, but all groups showed significant reductions after three months. Fasting insulin and METS-IR decreased significantly in all groups at both time points, while HOMA-β decreased significantly only after three months of the intervention, and equally in all groups.

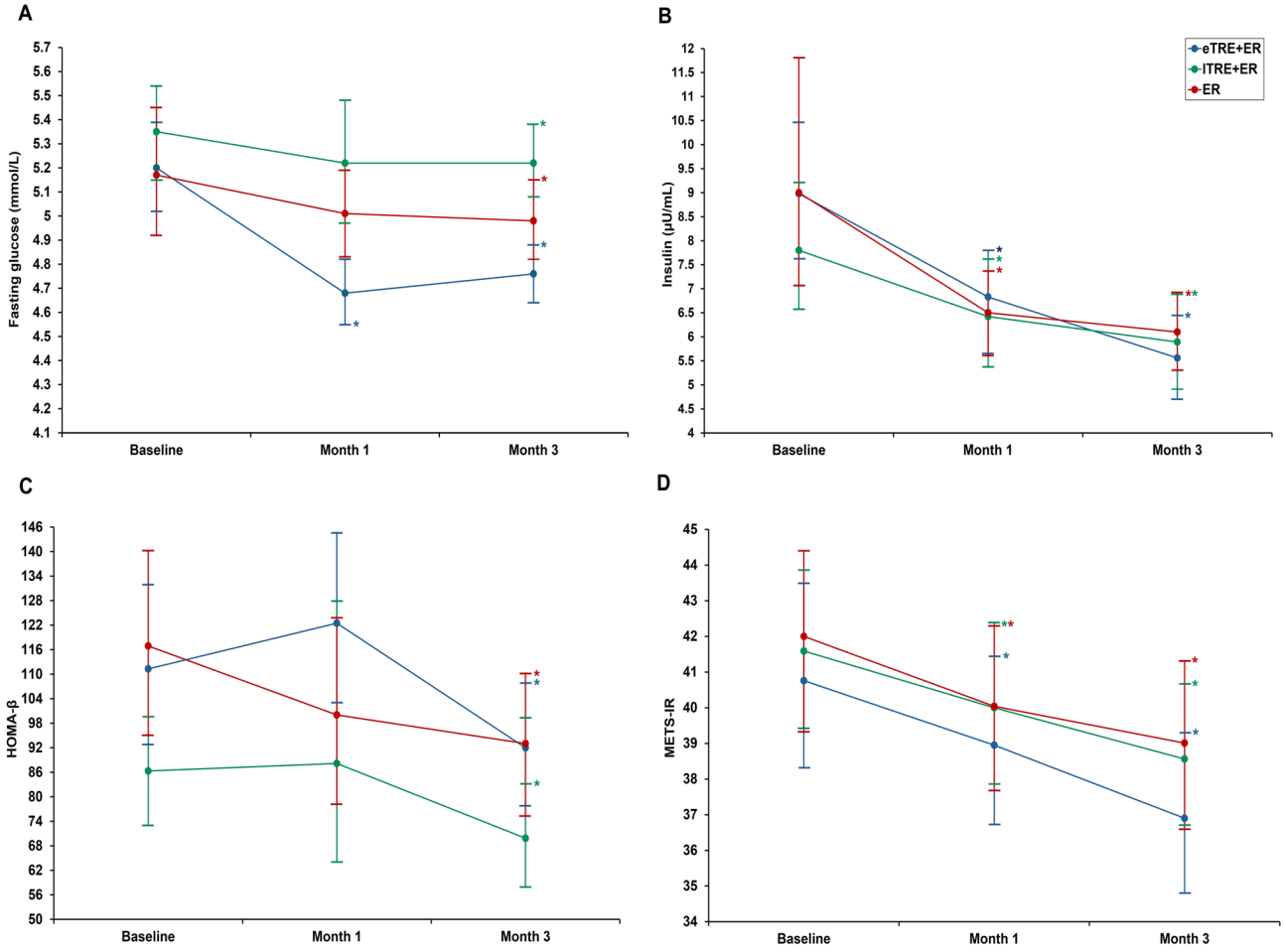

Fig. 3 A change after one and three months for **(A)** fasting glucose, **(B)** insulin, **(C)** HOMA-β, **(D)** METS-IR. Values are presented as mean (95% CI). eTRE, early time-restricted eating; lTRE, late time-restricted eating; ER, energy restriction; SD, standard deviation. Within-group evaluations of changes in outcome variables were performed with paired t-test for normally distributed variables or paired Wilcoxon tests for abnormally distributed variables. Asterisk (*) indicates significant differences ($P < 0.05$) from baseline within each group

Effects of eTRE + ER, lTRE + ER and ER intervention on cardiovascular markers, hepatic markers and visceral fat accumulation indexes

We further evaluated the effects of eTRE + ER, lTRE + ER, and ER on cardiovascular markers, liver markers, and visceral fat accumulation indexes (Table 5). A significant time effect was observed only for total cholesterol ($P = 0.040$), with mean reductions of -0.4 mmol/L (95% CI, -0.5, -0.2) for the eTRE + ER group, -0.2 mmol/L (95% CI, -0.4, -0.1) for the lTRE + ER group, and − 0.3 mmol/L (95% CI, -0.5, -0.2) for the ER group with no significant differences among groups. No between-groups differences were found also in regard to triglycerides, total cholesterol, HDL cholesterol levels and TG/HDL ratio.

Regarding liver enzymes, no significant time effect was detected overall ($P = 0.064$ for AST and $P = 0.067$ for ALT); however, there was a significant time effect for AST only in the ER group ($P = 0.040$) and for ALT in the ER ($P = 0.011$) and lTRE + ER ($P = 0.014$) groups, with no statistically significant differences among groups.

Lastly, no significant time effect or differences among groups were found for the VAI and LAP indexes. The VAI index showed mean reductions of -0.2 (95% CI, -0.4, 0.0) in the eTRE + ER group, -0.3 (95% CI, -0.4, 0.0) in the lTRE + ER group, and − 0.3 (95% CI, -0.5, 0.0) in the ER group. The LAP index decreased similarly across groups, with mean changes of -11 (95% CI, -17, -5) in both the eTRE + ER and ER groups, and − 15 (95% CI, -21, -8) in the lTRE + ER group.

Effects of eTRE + ER, lTRE + ER and ER intervention on peptides that regulate appetite and on subjective appetite

Table 6; Fig. 4 illustrate the effects of eTRE + ER, lTRE + ER, and ER interventions on metabolic hormones, including adiponectin, ghrelin, leptin, and the leptin/ghrelin ratio. There was a significant time effect for ghrelin ($P < 0.001$), with mean changes showing a significant

Table 5 Effects of eTRE + ER, lTRE + ER and ER intervention on cardiovascular markers, hepatic markers and visceral fat accumulation indexes

Variables	Mean change from baseline (95% CI)			Time effect	Mean difference between groups (95% CI)					
	eTRE + ER	lTRE + ER	ER	*P* value	eTRE + ER vs. lTRE + ER	*P* value	eTRE + ER vs. ER	*P* value	lTRE + CR vs. ER	*P* value
Triacylglycerols (mmol/L)	-0.2 (-0.3, 0.0)	-0.3 (-0.4, -0.1)	-0.2 (-0.3, -0.1)	0.857	0.1 (-0.1, 0.4)	0.765	0.5 (-0.2, 0.3)	1.000	-0.1 (-0.3, 0.2)	1.000
Total cholesterol (mmol/L)	-0.4 (-0.5, -0.2)	-0.2 (-0.4, -0.1)	-0.3 (-0.5, -0.2)	0.040	-0.1 (-0.4, 0.1)	0.474	-0.0 (-0.3, 0.2)	1.000	0.1 (-0.1, 0.4)	0.916
LDL cholesterol (mmol/L)	-0.3 (-0.4, -0.2)	-0.1 (-0.3, 0.0)	-0.3 (-0.4, -0.1)	0.261	-0.2 (-0.4, 0.1)	0.243	-0.2 (-0.2, 0.1)	1.000	0.2 (-0.1, 0.4)	0.332
HDL cholesterol (mmol/L)	-0.1 (-0.1, 0.0)	-0.1 (-0.1, 0.0)	-0.1 (-0.1, 0.0)	0.334	-0.0 (-0.1, 0.1)	1.000	-0.0 (-0.1, 0.0)	0.664	-0.0 (-0.1, 0.1)	0.943
TG/HDL	-0.1 (-0.3, 0.2)	-0.2 (-0.3, 0.0)	-0.2 (-0.3, 0.0)	0.351	-0.0 (-0.2, 0.3)	1.000	-0.0 (-0.2, 0.3)	1.000	-0.0 (-0.3, 0.3)	1.000
AST (IU/L)	-2 (-3,– 1)	-4 (-5,– 2)	-4 (-5,– 2)*	0.064	2 (0,– 5)	0.072	2 (1,– 4)	0.391	-1 (-4, 2)	1.000
ALT (IU/L)	-4 (-7,– 1)	-7 (-9,– 4)*	-7 (-9,– 4)*	0.067	3 (-2, 8)	0.602	3 (-2, 8)	0.547	0 (-5, 5)	1.000
VAI	-0.2 (-0.4, 0.0)	-0.3 (-0.4, 0.0)	-0.3 (-0.5, 0.0)	0.308	0.5 (-0.3, 0.4)	1.000	0.5 (-0.3, 0.4)	1.000	0.0 (-0.4, 0.4)	1.000
LAP	-11 (-17, -5)	-15 (-21, -8)	-11 (-17, -5)	0.257	3 (-7, 19)	1.000	0 (-10, 10)	1.000	-3 (-14, 7)	1.000

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; CI, confidence interval; ER, energy restriction; eTRE, early time restricted eating; lTRE, late time restricted eating; LAP, lipid accumulation product index; VAI, visceral adiposity index. Means were estimated using linear mix model with Bonferroni post hoc tests and with adjustment for BMI, age and sex and baseline values for all variables. P values for the main effect of the time are listed. Asterix (*) indicates statistically significant differences ($P < 0.05$) from baseline to the end of the intervention within each group

increase in the ITRE + ER group (3 pg/mL, 95% CI, -1, 8) and in the ER group (2 pg/mL, 95% CI, -3, 6), while a non-significant decrease was observed in the eTRE + ER group (-2 pg/mL, 95% CI, -6, 2). However, there were no statistically significant differences among the groups for mean changes in ghrelin levels after 3-month intervention. On the other hand, no significant time effects were observed for adiponectin, leptin and leptin/ghrelin ratio.

Although the decrease in leptin levels across all groups was not significant, with the eTRE + ER group showing a mean reduction of -46 ng/mL (95% CI, -53, -39), the ITRE + ER group – 29 ng/mL (95% CI, -36, -22), and the ER group – 26 ng/mL (95% CI, -34, -19), significant differences among groups were detected, with eTRE + ER demonstrating a significantly greater reduction in leptin levels compared to both ITRE + ER (-17 (95% CI, -29, -4.)) and ER (-19 (95% CI, -31, -7)). Moreover, significant associations between reduction in leptin levels and reduction in BMI ($r = 0.36$, $P < 0.001$) and body mass ($r = 0.26$, $P = 0.006$) were observed in all participants ($N = 90$).

Lastly, although the leptin/ghrelin ratio showed a tendency to decrease in the eTRE + ER group and increase in the ITRE + ER group after 3-month intervention, there were no significant differences in mean changes among the groups.

Additionally, Fig. 4 shows the absolute changes in leptin, ghrelin, adiponectin and the leptin/ghrelin ratio after one and three months of the intervention. Leptin levels decreased significantly in all groups at both time points. Ghrelin only decreased in the lTRE + ER group after one month, but increased in the lTRE + ER and ER groups after three months of the intervention. Adiponectin and the leptin/ghrelin ratio showed no significant changes in either group at either time point.

Because the significant time effect on ghrelin was observed and because we noted significant differences in leptin changes between groups, we were also interested in assessing subjective appetite. The effects of the eTRE + ER, ITRE + ER, and ER interventions on subjective appetite, as assessed by the VAS, are shown in Table 6. No significant time effects were observed for subjective feelings of hunger, fullness, stomach fullness, desire to eat, but the time effect was significant for the capacity to eat. Pairwise comparison showed a statistically significant difference in reduction in desire to eat between eTRE + ER and ER group (-1.7 mm (95% CI, -3.2, -0.1)). Moreover, the eTRE + ER group also showed a significantly greater reduction in capacity to eat compared to the ER group with a mean difference of -1.4 mm (95% CI, -2.8, -0.1).

Discussion

Given our recently published findings that eTRE + ER is more effective in reducing percent of fat mass, diastolic blood pressure, and fasting glucose levels than lTRE + ER or ER alone [26], we conducted a secondary per-protocol analysis to find out if the addition of TRE, especially eTRE, to ER would yield additional benefits on peptides that mediate energy homeostasis, insulin indexes and specific cardiovascular and liver biomarkers compared to ER alone. We used data from a relatively large randomized controlled weight-loss trial ($N = 108$) comparing TRE with an 8-hour eating window (eTRE and lTRE) versus 12-hour eating period. Importantly, the participants in the present study, allocated to three different groups (eTRE + ER, lTRE + ER, ER), had similar energy deficit (approximately– 500 kcal/day) and similar distribution of macronutrients over three meals during the eating window. As previous studies have shown that the timing

Table 6 Effects of eTRE + ER, lTRE + ER and ER intervention on peptides that regulate appetite and on subjective appetite

	Mean change from baseline (95% CI)			Time effect	Mean difference between groups (95% CI)				
	eTRE + ER	lTRE + ER	ER	*P* value	eTRE + ER vs. lTRE + ER	*P* value	eTRE + ER vs.ER	*P* value	lTRE + ER vs. ER	*P* value
Adiponectin (µg/mL)	-0.5 (-1.0, 0.1)	-0.6 (-1.1, 0.0)	-0.4 (-1.0, 0.2)	0.312	0.1 (-0.9, 1.1)	1.000	-0.1 (-1.0, 0.9)	1.000	-0.2 (-1.1, 0.8)	1.000
Ghrelin (pg/mL)	-2 (-6, 2)	3 (-1, 8)*	2 (-3, 6)*	< 0.001	-5 (-13, 2)	0.271	-4 (-11, 3)	0.602	1 (-6, 9)	0.721
Leptin (ng/mL)	-46 (-53, -39)	-29 (-36, -22)	-26 (-34, -19)	0.752	**-17 (-29, -4)**	0.005	**-19 (-31, -7)**	< 0.001	-3 (-15, 10)	1.000
Leptin/ghrelin ratio	-7778 (-16996,1439)	2997 (-7162,13157)	94 (-9352, 9539)	0.234	-10,776 (-27589, 6038)	0.368	-7872 (-24047, 8304)	0.721	2904 (-14099, 19907)	1.000
Visual analogue scale for appetite										
Hunger (1–10 mm)	-1.5 (-2.3, -0.6)	-1.4 (-2.3, -0.5)	-0.9 (-1.8, -0.1)	0.145	-0.1 (-1.6, 1.4)	1.000	-0.5 (-2.0, 0.9)	1.000	-0.4 (-1.9, 1.0)	1.000
Fullness (1–10 mm)	0.5 (-0.2, 1.2)	0.4 (-0.3, 1.1)	-0.5 (-0.2, 1.2)	0.315	0.0 (-1.2, 1.3)	1.000	-0.1 (-1.2, 1.1)	1.000	-0.1 (-1.3, 1.1)	1.000
Stomach fullness (1–10 mm)	-0.5 (-1.1, 0.1)	-0.5 (-1.1, 0.2)	0.2 (-0.5, 0.8)	0.107	0.0 (-1.2, 1.1)	1.000	-0.7 (-1.8, 0.4)	0.372	-0.7 (-1.8, 0.5)	0.461
Desire to eat (1–10 mm)	-2.6 (-3.5, -1.7)	-2.0 (-2.9, -1.1)	-0.9 (-1.8, 0.0)	0.806	-0.6 (-2.2, 1.0)	1.000	**-1.7 (-3.2, -0.1)**	0.031	-1.1 (-2.7, 0.5)	0.324
Capacity to eat (1–10 mm)	-2.0 (-2.8, -1.2)	-0.9 (-1.8, -0.1)	-0.6 (-1.3, 0.2)	0.029	-1.1 (-2.4, 0.3)	0.203	**-1.4 (-2.8, -0.1)**	0.037	-0.4 (-1.8, 1.0)	1.000

Abbreviations: CI, confidence interval; ER, energy restriction; eTRE, early time restricted eating; lTRE, late time restricted eating. Means were estimated using linear mix model with Bonferroni post hoc tests and adjustment for BMI, age and sex. P values for the main effect of the time are listed. Bolded values indicate statistically significant differences ($P < 0.05$) between groups (columns on the right side), while asterix (*) indicates statistically significant differences ($P < 0.05$) from baseline to the end of the intervention within each group

of food intake, tailored to an individual's chronotype, is important for the success and maintenance of dietary strategies [38–40], we randomized participants as closely as possible to their individual chronotype, thus ensuring that the participants' eating time corresponded to their lifestyle.

In comparison to the parent study, the sample here was somewhat smaller, due to the fact that for some participants all relevant outcome measures were not available. We have therefore first controlled that the main results of the parent study are also true for this sample. Indeed, we confirmed that participants who adhered to eTRE + ER for 3 months showed greater improvements in percent of fat mass, BMI, and fasting glucose compared to those in the lTRE + ER and/or ER groups. These greater reductions in percent of fat mass and BMI were accompanied by more pronounced decreases in leptin levels, with eTRE + ER showing larger leptin reductions than lTRE + ER or ER. Additionally, the eTRE+ER group showed a significantly greater decrease in desire for food and greater reduction in capacity to eat than ER. On the other hand, this per-protocol analysis indicated that eTRE + ER, lTRE + ER and ER induced comparable effects on insulin levels, HOMA-IR, HOMA-β, TYG-BMI, METS-IR, lipid profile, adiponectin, ghrelin, VAI, LAP and liver enzymes after 12 weeks of intervention.

The fact that eTRE + ER provided more benefit in losing fat mass compared with lTRE + ER and ER group both in our parent [26] and in the present study is in line with recent meta-analyses where eTRE + ER significantly reduced body mass and fat mass compared with ER alone [41]. As the circulatory levels of adipokines are dependent upon fat mass [42], the current study evaluated levels of two adipokines, leptin and adiponectin, which reflect the secretory function of adipocytes, mediate the energy homeostasis and play a critical role in obesity-related cardiovascular diseases [43]. We found that changes in leptin levels were positively related to BMI and body weight changes ($r = 0.36$, $P < 0.001$; $r = 0.29$, $P = 0.006$) and that in eTRE + ER, where specific anthropometric parameters were reduced to a greater extent, also leptin levels reduction was more pronounced than in lTRE + ER or ER groups. Recently it was shown that a significant reduction in leptin induced a higher degree of leptin sensitivity in hypothalamic neurons [44]. The authors proposed that a reduction of leptin levels is a necessary prerequisite for substantial weight loss, and partial leptin reduction is a viable strategy to treat obesity. In line with our results, fluctuations in leptin levels were concurrently observed with total fat mass loss and particularly visceral fat mass loss [45]. This was previously confirmed in a study [46], which found that after 12 months of TRE, leptin decreased by 25%, correlating with both body weight and fat mass. However, another study

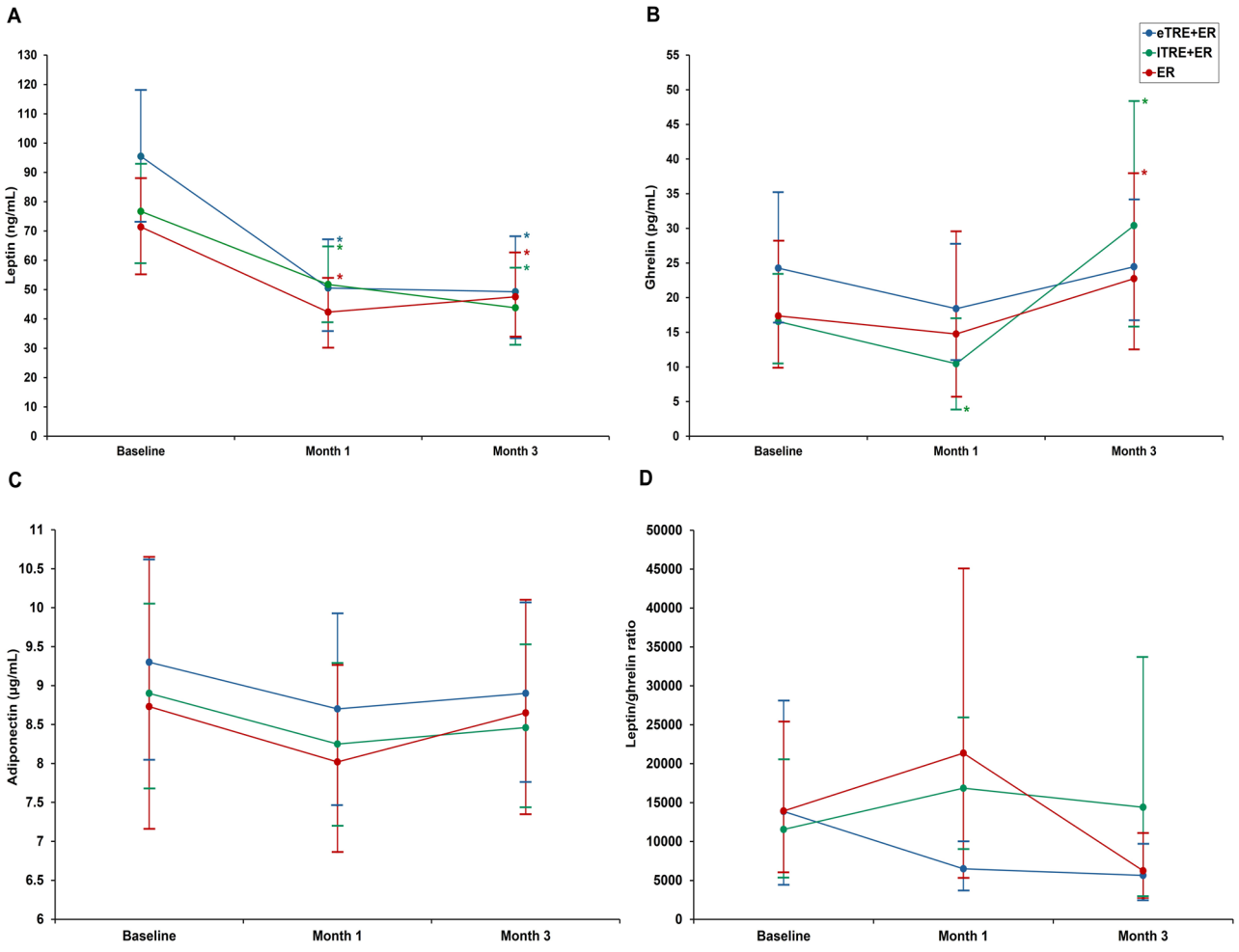

Fig. 4 Absolute change after one and three months for **(A)** leptin, **(B)** ghrelin, **(C)** adiponectin, **(D)** leptin/ghrelin ratio. Values are presented as mean (95% CI). eTRE, early time-restricted eating; lTRE, late time-restricted eating; ER, energy restriction; SD, standard deviation. Within-group evaluations of changes in outcome variables were performed with paired t-test for normally distributed variables or paired Wilcoxon tests for abnormally distributed variables. Asterisk (*) indicates significant differences ($P < 0.05$) from baseline within each group

found that eTRE reduced leptin to a greater extent than lTRE despite similar total body fat and visceral fat area losses [47], indicating that TRE with different meal timing may also impact leptin through other mechanisms. In healthy adults, a 24-h pattern of levels of leptin can be observed under conditions of energy balance, with relatively lower levels most of the waking day and higher levels at night [48, 49]. Indeed, leptin levels peak usually between midnight and early morning [50]. This nighttime spike in leptin helps regulate energy balance during sleep. However, under constant routine conditions, a very small amplitude circadian rhythm occurs in leptin [51]. But, disruptions to this natural rhythm, such as eating late into the night, can interfere with normal leptin secretion and increase the risk of metabolic disorders [50, 52]. In the present study, it seems that reduced BMI and body weight had major effect on leptin levels, but it is possible that also eTRE (meal timing) had an impact on circulatory leptin levels. On the other hand, neither intervention significantly increased adiponectin. We previously proposed [26] that one mechanism by which eTRE + ER could affect adipose tissues and increase fat mass loss in greater extent than lTRE + ER or ER is by increasing lipolysis and beta-oxidation [53, 54] through the secretion of adiponectin [55, 56], but this was not the case. The present findings in regard to adiponectin are therefore in line with a previous systematic review and meta-analyses suggesting that fasting and energy-restricted strategies have no effects on adiponectin concentrations [45, 47, 57].

Additionally, as eTRE + ER had stronger impact on leptin than lTRE + ER and ER, and as circadian rhythms influence also the levels of other appetite hormones, we aimed to assess the effect of the interventions on ghrelin levels. In healthy adults, the ghrelin levels increase between meals, decrease after meals, increase before and

during the first few hours of sleep, and decrease during the second half of the sleep episode [48, 58]. Moreover, it has been shown that prolonged fasting (72 h) is not associated with elevated levels of ghrelin, thereby suggesting that the meal-related pattern observed in plasma concentrations of ghrelin in the aforementioned studies [48, 51, 58] might be driven more by the postprandial reduction rather than by pre-prandial increases [59]. Furthermore, hypocaloric diets that induce moderate weight loss are not necessarily associated with elevated ghrelin [60]. In line with these observations, ghrelin was not significantly affected by eTRE + ER and no between-group differences were observed. However, the findings of the present study revealed that ghrelin was significantly affected by lTRE + ER and ER. In both groups ghrelin levels significantly increased after 3-month intervention, which was also accompanied by significantly lower reduction in the desire to eat and capacity to eat in the ER group than in the eTRE + ER group. A study [18] also investigated the effects of eTRE and lTRE in adults with overweight or obesity on ghrelin levels and appetite. After 7 days of following their assigned TRE protocol, fasting ghrelin levels were significantly lower in the eTRE group compared with the lTRE group. Decrease in ghrelin levels in the eTRE + ER group, although not significant, may be therefore connected to the alignment of mealtimes with the natural circadian rhythm, which may lead to better metabolic synchronisation [61]. Previous research [9] found that eTRE reduced hunger, desire to eat and increased fullness. Furthermore, in line with two previous studies [17, 62], we can confirm that eTRE does not increase hunger despite a longer period of daily fasting. In contrast, the observed increase in ghrelin in the lTRE + ER and ER groups may be partially explained by the adaptive physiological response to ER, where the body attempts to counterbalance energy deficit by increasing appetite-stimulating hormones like ghrelin [63]. This significant increase in ghrelin in lTRE + ER and ER groups may be related to the smaller decrease in fasting glucose levels after the 3-month intervention compared with the eTRE + ER. Indeed, ghrelin has been shown to affect fasting glucose levels by several mechanisms, including stimulating hepatic gluconeogenesis and reducing insulin secretion [64].

The circadian system plays a crucial role in regulating glucose and lipid metabolism [65]. Epidemiological studies in humans highlight the positive impact of morning energy intake on preventing metabolic disorders, particularly those related to glucose regulation, such as glucose intolerance and dyslipidaemia [15]. Indeed, eTRE + ER has been shown to result in improvements in glucose homeostasis in our parent [26] and in the present study and also in other published studies [14, 66]. Moreover, previous data suggest that eTRE may be more effective at reducing fasting glucose when compared to lTRE or ER alone [14, 18, 67, 68]. Studies on circadian rhythms have confirmed that the insulin sensitivity is better in the morning, so on this basis, eTRE could improve glucose metabolism [17, 23, 24]. Indeed, eating within a restricted window reduces the frequency of insulin spikes, allowing the body to maintain lower levels of insulin throughout the day. This is relevant because chronic high insulin levels are associated with increased IR. Therefore, in the present study we were interested if the addition of TRE can amplify the effect of ER, especially on insulin sensitivity and IR, measured with different indexes. Consistent with the studies [22, 46, 69, 70] we observed a trend towards a reduction in HOMA-IR and fasting insulin in all three groups (eTRE + ER, lTRE + ER, ER), but no statistically significant differences within or between groups were found. Similarly, a study by Cienfuegos and colleagues [71] found that most studies do not show significant improvements in insulin levels. However, the significant effect on insulin levels is found in studies where eating window is very short (4 h to 6 h). On the other hand, we detected significant decreases in HOMA-β in eTRE + ER and ER groups and in two IR indexes, METS-IR and TyG-BMI in all three groups (eTRE + ER, lTRE + ER, ER), with no between-group differences. While HOMA-β decreased in the eTRE + ER and ER groups, this change occurred in parallel with reductions in IR. This suggests a physiological down-regulation of insulin secretion in response to improved peripheral insulin action, rather than impaired β-cell function [72]. The decrease in HOMA-β is consistent with previous findings [73], which reported a decrease in HOMA-β in all groups (eTRE + ER, lTRE + ER, ER), with no significant differences among them. However, this interpretation may not hold for the lTRE + ER group. At baseline, this group exhibited the highest fasting glucose but the lowest HOMA-β, despite relatively low HOMA-IR—indicating that insulin demand was elevated but not adequately met, likely due to limited β-cell secretory capacity rather than enhanced insulin sensitivity. Following the intervention, fasting glucose levels remained largely unchanged, while HOMA-β declined further—suggesting a potential deterioration in β-cell function rather than an adaptive decrease in insulin output. These findings may reflect underlying β-cell stress or functional decline, as supported by emerging preclinical data [74], and highlight the need for future studies employing gold-standard assessments of β-cell function. In regard to IR; in recently published meta-analysis [25], HOMA-IR was tested in five studies, whereas most TRE + ER interventions resulted in improvements in HOMA-IR levels versus baseline [7, 75, 76], while, in accordance with our study, no between-group differences were detected in all five studies testing HOMA-IR [25]. However, it

should be noted that the participants in this study, who were divided into three different groups (eTRE + ER, lTRE + ER, ER), in addition to having a similar ER, also had the same prescribed number of three meals within their eating time window. Therefore, the frequency of insulin spikes, and consequently IR, decreased in all three groups. In addition, we included participants without diabetes but with other risk factors for metabolic syndrome, including overweight or obesity. If there is an effect of TRE on insulin sensitivity or β-cell function, as suggested by small studies [17], it is likely that this effect will only be seen in those participants at highest risk (i.e. participants who have already developed prediabetes or diabetes).

Regarding cardiovascular and liver markers, after 3 months of intervention, we found a significant improvement in total cholesterol levels across all three groups. However, there were no significant differences between the groups in total cholesterol, triglycerides, HDL, LDL, or the TG/HDL ratio. This is consistent with findings from our parent study [26] and a recently published meta-analysis, which showed that neither eTRE + ER nor lTRE + ER had a significant impact on lipid profiles compared to ER alone [24, 25]. In terms of liver biomarkers, slight reductions in LAP and VAI indexes were observed in all three groups, whereas no significant differences were found either within or between the groups. These results suggest that although both TRE and ER may help reduce visceral adiposity, their effects appear to be modest and may not differ significantly when combined. On the other hand, we observed a significant reduction in AST levels in the ER group, and a significant reduction in ALT levels in both the lTRE + ER and ER groups, with no significant differences between the groups. Other studies report mixed results. For instance, a study [69] found only a trend toward reductions in AST and ALT in participants with metabolic syndrome who followed a 10-hour TRE window. Conversely, another study [77] reported a significant reduction in both AST and ALT in the TRE group compared to a control group without ER. A further study [67] observed a significant reduction in AST only in the eTRE group, while no differences in mean changes in ALT were found between the eTRE, lTRE, and ER groups. Moreover, a study [78] found that both TRE + ER and ER were effective strategies in participants with MASLD for weight loss, with marked reductions in both liver stiffness and intrahepatic triglyceride. In this study, but also in our study, the stringent ER targets might mask the relatively subtle benefits of TRE. Although no between-group differences have been observed in liver biomarkers in the present study, small to moderate improvements in ALT and AST have been detected only after lTRE + ER and ER 3-month intervention. Importantly, most participants had normal liver biomarkers at baseline. Nonetheless, modest improvements within the normal range are still important, as these values are strongly associated with the risk of metabolic diseases [79] and mortality [80]. Therefore, the modest changes induced by ER likely reflect benefits for liver function and overall cardiometabolic health, while the additional effects of TRE were not confirmed. Recently, accumulating evidence has demonstrated that the timing of energy intake may play a key role in the risk of MASLD, including night-time eating and irregular meal patterns [81]. It is therefore surprising that lTRE + ER influenced liver health, whereas eTRE + ER did not. Further studies are needed to understand the mechanism.

Limitations

Several limitations should be acknowledged. First, the intervention was relatively short, and further long-term research is needed to assess whether the TRE strategy is also effective during the weight maintenance phase. Second, the timing of fasting measurements differed slightly between groups, which may have introduced bias. Due to the nature of the TRE windows, fasting blood samples were collected at different morning hours between groups. Although all samples were taken after the defined fasting period (on average after 13.5–14.5 h of fasting), this time difference may have influenced certain circadian-regulated metabolic parameters such as glucose tolerance and hormone levels. Third, the sample of subjects was predominantly female (80%), which may limit the generalizability of the findings. In addition, metabolic hormone levels were measured only in the fasting state, without pre- and post-meal measurements. Additionally, food intake was recorded for only 3 days, which may not provide a comprehensive assessment of dietary intake. Moreover, in the ER group, the eating window was also significantly decreased after intervention, and the increase in percentage of protein intake was significantly higher than in the eTRE + ER and lTRE + ER groups. Indeed, this could affect the results, as suggested by de Carvalho et al. (2020) that among individuals with overweight or obesity, higher dietary protein intake may influence appetite sensations by enhancing fullness or satiety [82]. However, the fact that there were no other major differences in the macronutrient distribution, supports the hypothesis that the observed changes are indeed due to TRE and not due to the changed macronutrient intakes. Finally, differences in chronotype distribution may have influenced the results, as evening chronotypes tend to have less success in weight loss interventions [40]. A recently published systematic review [40] found that subjects with the evening chronotype were also more likely to have higher HOMA-IR levels, higher plasma ghrelin levels, higher BMI, and a greater propensity to engage in unhealthy behaviours and eating patterns. However,

we have to stress out that at baseline no between-group differences were observed in BMI, % of fat mass, fasting glucose, insulin, HOMA-IR, ghrelin, suggesting that participants in all three groups were comparable in regard to anthropometric and biochemical measures. Additionally, participants in all three groups received similarly prescribed dietary plan. As chronotype-adapted diets have already been shown to produce better outcomes than non-chronotype-adapted diets [40] and in line with real-life settings, randomisation was performed on the basis of the individuals' chronotype.

Conclusion

In summary, eTRE + ER resulted in greater reductions in fat mass, BMI, and fasting glucose compared to lTRE + ER or ER alone. These improvements were accompanied by more pronounced reductions in leptin levels and appetite measures in the eTRE + ER group. Despite these benefits, insulin resistance markers and other cardiometabolic indicators remained comparable across all groups. In addition, this per-protocol analysis showed that eTRE + ER, lTRE + ER and ER had comparable effects on lipid profile, adiponectin, ghrelin, VAI, LAP and liver enzymes after 12 weeks of intervention. However, further research is needed to better understand the long-term metabolic effects of eTRE + ER beyond weight loss.

Abbreviations

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMI	Body mass index
CI	Confidence interval
CV	Coefficient of variation
ER	Energy restriction
eTRE	Early time restricted eating
HDL	High density lipoprotein
HOMA-IR	Homeostatic model assessment of insulin resistance
HOMA-β	Homeostatic model assessment of β-cell function
IR	Insulin resistance
LAP	Lipid accumulation product
LDL	Low density lipoprotein
lTRE	Late time restricted eating
MASLD	Metabolic dysfunction-associated steatosis liver disease
METS-IR	Metabolic score for insulin resistance
NAFLD	Non-alcoholic fatty liver disease
T2DM	Type 2 diabetes mellitus
TRE	Time restricted eating
TG	Triglycerides
TyG-BMI	Triglyceride glucose body mass index
VAI	Visceral adiposity index
VAS	Visual analogue scale

Acknowledgements

The authors thank the study subjects for their participation.

Author contributions

Z.J.P., A.P., N.M., T.Č., and B.H. conceived and designed the study; T.Č. and B.H. collected the data; A.P., Z.J.P., T.Č. and B.H. analysed anthropometric data, carried out the biochemical experiments and performed statistical analysis; B.H., T.Č., S.K., N.M., A.P. and Z.J.P. contributed to the interpretation of the results; B.H. wrote the paper in consultation with A.P., T.Č. and Z.J.P.; All authors read and approved the final manuscript.

Funding

This research was financially supported by the Slovenian Research and Innovation Agency (Programme P1-0386, Infrastructure Programme I0-0035 and grant number 56962 for junior researcher Tanja Črešnovar).

Data availability

Data of this article will be made available upon reasonable request from the corresponding author.

Declarations

Ethics approval and consent to participate

The present study was approved by the Slovenian National Medical Ethics Committee (No. 0120–557/2017/4; Ministry of Health, Republic of Slovenia). All participants completed an informed consent form.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Faculty of Health Science, University of Primorska, Polje 42, Izola, Slovenia

Received: 22 April 2025 / Accepted: 16 July 2025
Published online: 29 July 2025

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