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Effects of a 12-Week Mediterranean-Type Time-Restricted Feeding Protocol in Patients With Metabolic Dysfunction-Associated Steatotic Liver Disease: A Randomised Controlled Trial—The 'CHRONO-NAFLD Project'

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Received: 16 November 2024 | **Revised:** 17 December 2024 | **Accepted:** 11 February 2025

Handling Editor: Daniel Huang

Funding: The authors received no specific funding for this work.

Keywords: glucose metabolism | insulin resistance | intermittent fasting | liver steatosis | Mediterranean diet | metabolic dysfunction-associated steatotic liver disease | time-restricted feeding

ABSTRACT

Background: The Mediterranean diet (MD) is considered the best dietary approach for patients with metabolic dysfunction-associated steatotic liver disease (MASLD). Recently, time-restricted feeding (TRF) has gained attention for its lifestyle compatibility and health benefits.

Aims: This study aimed to compare the effects of a hypocaloric MD with a 10-h TRF protocol to an unrestricted MD in MASLD patients with overweight/obesity and evaluate differences between early and late TRF.

Methods: This 12-week randomised controlled trial in MASLD patients with overweight/obesity consisted of three groups, all following a hypocaloric Mediterranean-type diet. The control group had no eating time restrictions. The early TRF (eTRF) and late TRF (lTRF) groups had a 10-h eating window, from 8 AM to 6 PM and from 12 PM to 10 PM, respectively. Various health parameters were measured. Compliance was tracked via food diaries, and an 8-week follow-up occurred post-intervention.

Results: Fifty-nine MASLD individuals (27 males; 52.9 years; body mass index 32.1 kg/m^2) completed the trial (control, $n = 19$; eTRF, $n = 20$; lTRF, $n = 20$). All groups showed significant 12-week reductions in body weight, anthropometry and blood pressure. Glycated haemoglobin A_{1c} and insulin resistance, as measured by the Matsuda index, homeostatic model assessment for insulin resistance and fasting glucose-to-insulin ratio, improved in the eTRF group at 12 weeks.

Conclusions: This study corroborates the efficacy of MD in ameliorating cardiometabolic risk factors such as body weight and blood pressure in MASLD patients. The combination with an eTRF protocol may improve glycaemic control (NCT05866744).

Trial Registration: The study is registered at clinicaltrials.gov (NCT05866744)

1 | Introduction

Non-alcoholic fatty liver disease (NAFLD) has a wide clinical spectrum, ranging from simple liver steatosis to liver fibrosis, cirrhosis and hepatocellular carcinoma [1]. The term NAFLD has been replaced with metabolic dysfunction-associated steatotic liver disease (MASLD) [2] and is used to describe the accumulation of fat in liver cells (> 5%) of individuals who do not consume a significant amount and/or no alcohol (< 30 g/day for males and < 20 g/day for females) [3]. Currently, MASLD has spread at alarming rates and is the most common cause of chronic liver disease worldwide [4]. MASLD prevalence, estimated at 30%–32% [5], is increasing exponentially at the same rate as type 2 diabetes and obesity as a result of unhealthy eating habits and a sedentary lifestyle [6]. MASLD is considered the liver manifestation of metabolic syndrome (MetS) and has a close and bidirectional relationship with type 2 diabetes and obesity [7]. Nutritional management of individuals with MASLD aims to reduce body weight (7%–10%) by adopting a low-calorie diet plan based on the Mediterranean Diet (MD) pattern, which is considered the optimal diet [8].

MD has been widely studied for its beneficial effects on overall health, particularly in reducing cardiovascular disease (CVD) risk and improving metabolic conditions [9]. Studies have shown that adherence to MD, characterised by high consumption of fruits, vegetables, whole grains and healthy fats, such as olive oil, can improve liver fat content and reduce liver inflammation in patients with MASLD [10]. The anti-inflammatory and antioxidant properties of MD are thought to play key roles in mitigating the progression of MASLD [10].

Nevertheless, several other nutritional strategies for weight loss and cardiometabolic profile improvement have been suggested for patients with MASLD. One of these strategies is intermittent fasting (IF), which is characterised by alternations between cycles of prolonged fasting and food intake and includes various protocols depending on the duration of fasting [11]. In the context of time-restricted feeding (TRF), an IF protocol, individuals are usually asked to consume all their meals within a specific 'time window', for example, 10 h, with food intake restricted for 14 h (14:10). The most prevalent types of TRF are early TRF (eTRF, restricting feeding early in the day) and late or delayed TRF (lTRF, restricting eating later in the day). These two types do not seem to differ in weight loss rates but may affect other parameters, such as insulin sensitivity, in a different way [12]. Although TRF was first described as an *ad libitum* IF regimen, it has been examined lately in combination with caloric restriction, which seems to result in clinically significant weight loss [13]. Several studies have compared a hypocaloric TRF protocol with a control group (only a hypocaloric diet without time restriction) [14–17]. In contrast, only a few randomised controlled trials (RCTs) have examined the effects of TRF (with or without caloric restriction) on health parameters in patients with MASLD [18–22] and none of the RCTs compared TRF with the gold-standard MD in this population.

The aims of this study were (a) to compare, for the first time, the effects of a hypocaloric Mediterranean-type 14:10 TRF protocol with the gold standard MD in MASLD patients with overweight or obesity and (b) to examine whether there are differences in restricting eating early (eTRF) versus late (lTRF) in this context.

2 | Materials and Methods

2.1 | Study Design

This RCT employed a parallel design (allocation ratio 1:1) with three intervention groups: the control group ($n = 19$), the early TRF (eTRF) group ($n = 20$) and the late TRF (lTRF) group ($n = 20$). All groups adhered to a hypocaloric diet (500 kcal/day below resting energy expenditure) based on MD principles (carbohydrates 45%, protein 20%, fat 35%) and maintained usual physical activity habits [3, 8]. Participants were provided with the same diet plan (foods and macronutrient composition) containing traditional and simple food choices, differing only in caloric intake. The control group consumed meals throughout the day without time restrictions (over 12 h daily). The eTRF group ate within a 10-h window (8 AM to 6 PM), fasting for the remaining 14 h. The lTRF group ate within a 10-h window (12 PM to 10 PM), also fasting for 14 h. Both TRF groups could adjust their eating window by ±1 h but were encouraged to maintain the 10-h duration. During fasting, participants could drink water or zero-calorie beverages.

The study took place at the Laboratory of Dietetics and Quality of Life, Agricultural University of Athens and the Outpatient Hepatology Clinic of 'Laiko' General Hospital of Athens, between December 2022 and July 2024, in accordance with the Declaration of Helsinki. It was approved by the Bioethics Committee of the Agricultural University of Athens (EIDE Reference Number: 40/27.04.2022) and the Scientific Committee of 'Laiko' General Hospital (716/26-11-2022). The study is registered at clinicaltrials.gov (NCT05866744).

2.2 | Study Participants

Individuals > 18 years with confirmed MASLD (abdominal ultrasound indicating liver steatosis per standard criteria [23] and exclusion of other causes [2]) and a body mass index (BMI) ≥ 25 kg/m^2 were eligible. Detailed exclusion criteria are provided in the Methods section in Supporting Information. Enrollment and screening occurred at the Outpatient Hepatology Clinic of the 'Laiko' General Hospital of Athens. Participants were required to maintain a constant medication dose and type throughout the study.

2.3 | Randomisation, Screening and Inclusion of Participants

Eligible individuals were randomly assigned to one of three groups post-screening. Randomisation was performed by a non-participating team member using random.org (accessed 27.11.2022). Due to the intervention's nature, both researchers and participants were aware of group allocations, disclosed the day before the intervention during the education process. Participants received all relevant information and provided written consent.

A total of 133 individuals were screened; 51 (38.4%) did not meet inclusion criteria, and 11 (8.3%) declined participation. Seventy-one (53.3%) were allocated to intervention groups, but 12 (16.9%) did not complete the study due to health issues (unrelated to the

intervention) or protocol adherence difficulties (3 in the first week, 1 in the third week, 3 in the fourth week, 3 in the fifth week and 2 in the sixth week). The final analysis included 19 from the control group, 20 from the eTRF group and 20 from the lTRF group, as shown in Figure 1.

2.4 | Study Protocol and Visits

Participants were required to visit the Laboratory of Dietetics and Quality of Life biweekly for 12 weeks during the intervention and 8 weeks post-intervention (totaling 20 weeks) (Figure 2). Throughout the main study, a research team nutritionist conducted follow-up phone calls to address questions and encourage protocol adherence.

2.5 | Determination of the Metabolic Health Status

We defined type 2 diabetes and prediabetes according to the American Diabetes Association guidelines [24], stratified blood pressure (BP) based on the 2023 European Society of Cardiology guidelines [25], defined dyslipidaemia using the European Society of Cardiology guidelines [26] and identified MetS following the National Cholesterol Education Program (NCEP) Adult

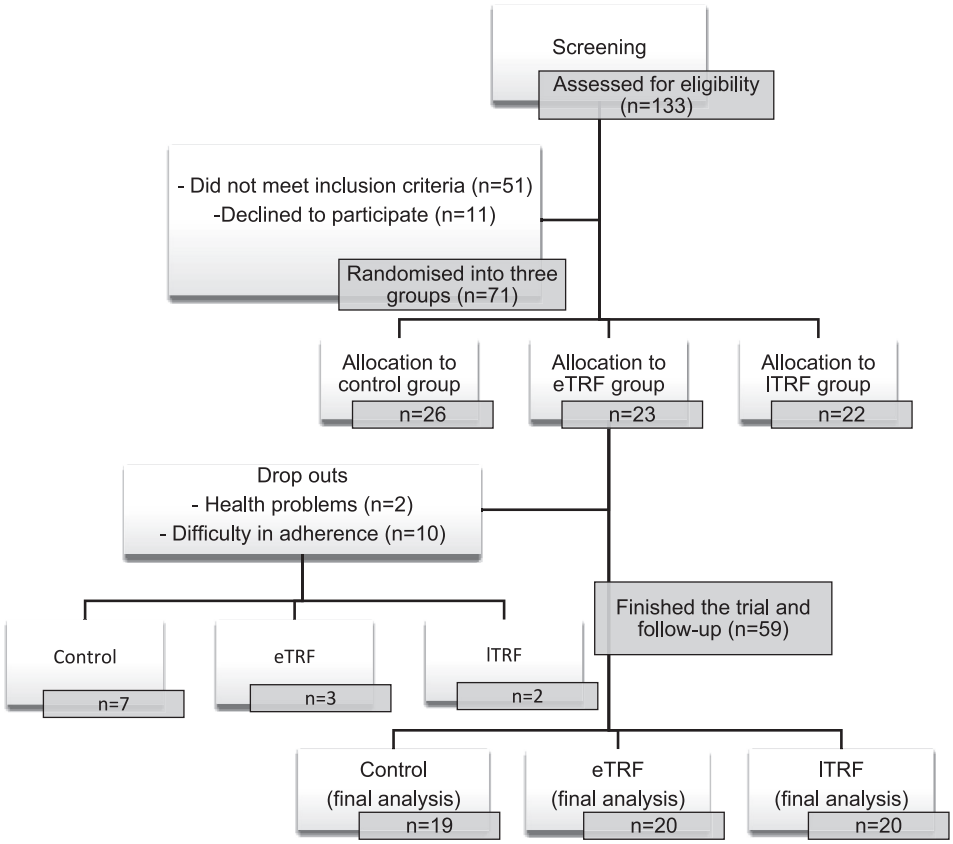

FIGURE 1 | Flow diagram of screening and inclusion of participants. eTRF, early time-restricted feeding; lTRF, late time-restricted feeding.

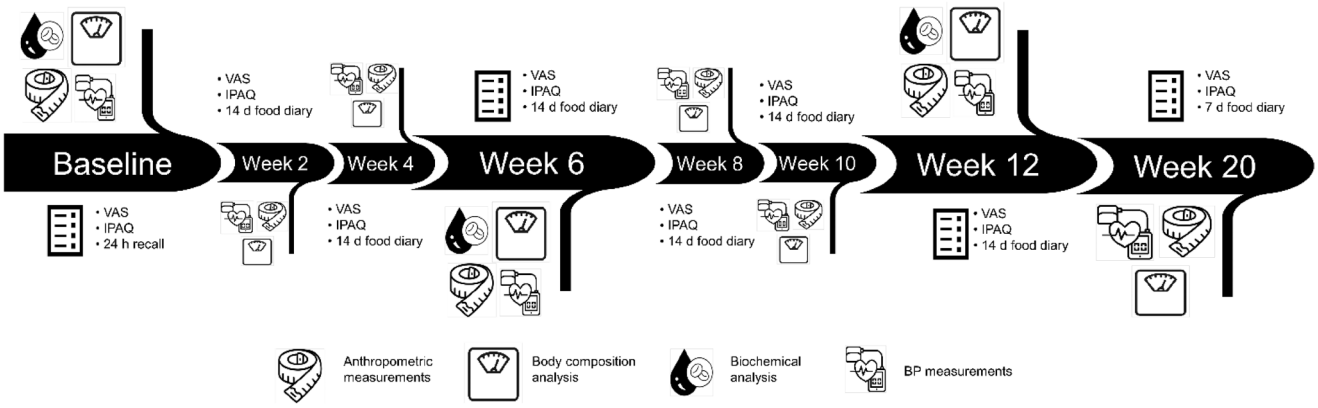

FIGURE 2 | Study's graphical protocol. BP, blood pressure; eTRF, early time-restricted feeding; IPAQ, International Physical Activity Questionnaire; lTRF, late time-restricted feeding; VAS, visual analogue scale.

Treatment Panel III (ATP III) definition [27]. The Methods section in Supporting Information provide comprehensive details regarding these definitions.

2.6 | Anthropometric Measurements and Body Composition Analysis

Anthropometric measures—height, waist circumference (WC), hip circumference (HC), neck circumference (NC), mid-arm circumference (MAC) and calf circumference (CC)—were collected at baseline, biweekly until the 12th week and during the follow-up visit. Body composition was analysed via bioelectrical impedance. For a detailed description, refer to Methods section in Supporting Information.

2.7 | Biochemical Analyses and Oral Glucose Tolerance Test

Blood and urine samples from participants were collected between 7 AM and 9 AM (e.g., 10–16 h of fasting depending on the last eating event and the intervention group) at baseline and at 6 and 12 weeks post-randomisation. Analytes measured included alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), gamma-glutamyl transferase (GGT), total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), fasting plasma glucose (FPG), fasting insulin, glycated haemoglobin A_{1c} (HbA_{1c}), C-reactive protein (CRP), ferritin, albumin, creatinine, uric acid and urine ketone bodies. Calculations included the atherogenic index (AI, LDL-to-HDL ratio) [28, 29], atherogenic index of plasma [AIP, log10(TG to HDL)] [30, 31], coronary risk index (CRI, TC-to-HDL ratio) [28] and TG-to-HDL ratio [31].

Participants also underwent a 2-h oral glucose tolerance test (OGTT) with 75 g of D-glucose, with insulin levels measured at 0, 60 and 120 min, at baseline and 12 weeks. Further details on glucose and insulin calculations can be found in the Methods section in Supporting Information. Insulin resistance (IR) [32] was assessed using homeostatic model assessment for IR (HOMA-IR) [33, 34], Matsuda index [35] and the fasting glucose-to-insulin (FGI) ratio [36].

2.8 | Blood Pressure Measurement

BP was measured in the left arm of seated participants, with the arm supported at heart level. Measurements were taken at baseline, biweekly during the intervention and at the follow-up visit. Further details are available in the Methods section in Supporting.

2.9 | Liver Elastography

FibroScan (Echosens, Paris, France) was used to measure liver stiffness (hardness) [37, 38]. Liver steatosis was assessed using the controlled attenuation parameter (CAP) score (dB/m), representing fat accumulation level [39]. Participants underwent liver elastography with FibroScan and CAP at baseline and at 12 weeks, performed by blinded, specialised physicians.

2.10 | Questionnaires

Participants filled out a medical history questionnaire and a baseline demographic and smoking habits sheet. They also completed the Chrononutrition-Profile Questionnaire to assess chrononutrition parameters [40]. The short form of the International Physical Activity Questionnaire (IPAQ) measured physical activity intensity (low, moderate, high) and sitting time, estimating total physical activity in MET-min/week [41, 42]. The IPAQ was administered biweekly during the study and at the follow-up.

2.11 | Visual Analogue Scale

Participants completed the Visual Analogue Scale (VAS) weekly to rate their subjective appetite [43]. The VAS, a 100-mm horizontal line with verbal descriptors at each end, required participants to mark the point best representing their feelings. Assessed feelings included hunger, fullness, desire to eat, thirst, preoccupation with food, prospective food consumption and pleasure, with responses ranging from 0 (not at all) to 100 (extreme; e.g., hunger). The VAS was completed each morning on an empty stomach before any food or beverage consumption.

2.12 | Dietary Intake and Adherence to the Intervention

A detailed 24-h recall of a typical day was collected from each participant at baseline by a trained nutritionist. Information on food quality and quantity, meal timing and frequency, beverage consumption and supplements was documented. A validated food frequency questionnaire for the Greek population was also completed to assess dietary habits over the past year [44]. Additionally, the Mediterranean diet score was calculated to evaluate adherence to the MD at baseline [45].

During the intervention, participants maintained a 7-day food diary (totaling 12 diaries) to monitor compliance with the diet program (MD plan and macronutrient composition) and eating/fasting window (exact times of first and last eating events). During the follow-up phone calls and the in-person meetings, the research team's nutritionists examined the food diaries for any inaccuracies and, when necessary, used food models and pictures to clarify discrepancies in portion sizes. According to previous studies, compliance was recorded if they adhered to the schedule (both the MD hypocaloric diet and the eating window) for more than 80% of the intervention period [16, 21]. Data for each day were exported and assessed separately for each participant. At follow-up, participants also kept a detailed 7-day food diary before their visit to assess dietary habits and eating windows post-intervention. Moreover, the researchers collected some data concerning the time of waking up and bedtime at baseline, 6, 12 and 20 weeks, and computed the morning latency (time between waking-up and first energy intake) and the evening latency (time between last energy intake and bedtime).

2.13 | Statistical Analysis

The sample size calculation determined that 18 participants per group were needed to achieve 80% power at a 0.05 significance level, to detect a 30 (±50) dB/m reduction in CAP between interventions [22]. To accommodate a dropout rate of at least 15%, more than 21 participants were randomised per group.

All statistical analyses were conducted per the per protocol principle. Normality of quantitative data was assessed using the Kolmogorov–Smirnov test, Shapiro–Wilk test and Q-Q plots. Normally distributed data are presented as mean ± standard error of the mean (SEM), whereas skewed data are shown as median and interquartile range (25th and 75th percentiles). Qualitative data are reported as absolute numbers and frequencies (%). One-way ANOVA tested mean differences in parametric variables, whereas the Kruskal–Wallis test was used for non-parametric data comparisons among three groups; post hoc analysis was conducted with Tukey's test. Paired samples *t*-test compared means of two continuous variables within a group, and the Wilcoxon signed-rank test was applied to non-normal variables. Pearson's chi-square test assessed differences between categorical variables. Moreover, a stratified analysis according to the participants' baseline BMI status (overweight vs. obesity) was conducted to test for further differences in the study's main outcomes. A linear mixed effects model (LMM) was used for baseline to 12 weeks and 12 weeks to follow-up, adjusted for sex, age, MetS, HOMA-IR, BMI and total fat mass, to evaluate within- and between-group differences. The statistical significance was set at $p < 0.05$. Analyses were performed using SPSS v.25.0 (IBM Corporation, Chicago, IL, USA).

3 | Results

3.1 | Baseline Characteristics of Participants

Table 1 outlines participants' baseline characteristics, which did not differ between the three intervention groups. Before the study, 81.4% showed moderate MD adherence. Overweight and obesity were observed in 42.4% and 57.6% of participants, respectively (see Table S1). Moreover, the three groups did not differ in their baseline comorbidities or medications, which remained unchanged until the end of the intervention (Table 1). Additionally, the sample consisted mainly of employers (50.8%), highly educated individuals (66.1%) and married participants (55.9%) (see Table S1). Lastly, the three groups did not differ in their baseline chrononutrition profile (Table 1) and sleeping parameters (see Table S2).

3.2 | Effects on Body Composition and Anthropometric Measurements

At 12 weeks, all three groups exhibited similar body weight loss (7.6%–8.3%) and fat mass loss (14.8%–16.7%) compared to the baseline, with no significant differences between the three groups ($p > 0.05$) (Table 2, Figure 3a,b). This fat reduction, including abdominal fat as assessed by WC, was consistent across all body areas without group differences (Table 2, Figure 3d–h). Muscle mass also decreased uniformly across all groups by 12 weeks (Table 2, Figure 3c). At the 20-week follow-up, body weight/fat mass loss and body circumferences were maintained or further decreased, whereas muscle mass did not decline further, with no significant differences between the groups (Table 2, Figure 3a–h).

3.3 | Effects on Glucose Metabolism

MD improved glucose metabolism without significant intergroup differences (Table 3, Figure 4a–h). Specifically, the control and eTRF groups exhibited enhanced insulinaemic responses (Figure 4b) and IR, as indicated by HOMA-IR, FGI ratio and Matsuda index, at 12 weeks, compared to the baseline, unlike the lTRF group, with no intergroup differences (Figure 4f–h). Notably, only eTRF significantly reduced HbA_{1c} (by 0.3%) at 12 weeks from baseline (Table 3, Figure 4e). Glycaemic responses at 12 weeks showed no significant differences in any group (Figure 4a,c) or between groups (p for all > 0.05).

3.4 | Effects on Lipidaemic Profile

Time restriction resulted in differences in TC, LDL, AI and CRI at 12 weeks between the three groups (p for all < 0.05) (Table 4). Both the control and lTRF groups showed improved lipid markers at 12 weeks from baseline, unlike the eTRF group.

3.5 | Effects on Blood Pressure

All three groups adhering to modified MDs showed significant reductions in systolic BP (SBP) (7%–12%) and diastolic BP (DBP) (5%–12%) at 12 weeks, compared to the baseline (p for all < 0.05), with no intergroup differences (Table 4). Neither SBP nor DBP changed at the 20-week follow-up compared to 12 weeks in any group, with no differences between groups (p for all > 0.05) (Table 4).

3.6 | Effects on Liver Biochemistry and Imaging

MD resulted in reduced liver enzyme levels at 12 weeks, with no significant differences among the three groups (Table 5). The control and eTRF groups showed improved AST and/or ALT levels at 12 weeks compared to the baseline. Additionally, GGT levels decreased in the control and lTRF groups at 12 weeks from baseline. All three groups exhibited reduced liver steatosis (6.5%–9.5%) at 12 weeks compared to the baseline (p for all < 0.05), with no differences among them (Figure 5a). Liver stiffness decreased by 5.9% only in the eTRF group at 12 weeks compared to the baseline, with no significant differences between groups (Figure 5b).

3.7 | Effects on Other Parameters

MD did not significantly alter uric acid, creatinine, ferritin or CRP levels after 12 weeks in any group, with no differences between groups (p for all > 0.05) (Table 4).

TABLE 1 | Baseline characteristics of participants.

	Total ($n = 59$)	Control ($n = 19$)	eTRF ($n = 20$)	lTRF ($n = 20$)	p value
Age, years	52.9 ± 1.6	49.9 ± 3.8	54.7 ± 1.9	54.1 ± 2.1	0.408
Sex, *n* male (%)	27 (45.8)	9 (47.4)	9 (45.0)	9 (45.0)	0.986
Metabolic syndrome, *n* yes (%)	36 (61.0)	9 (47.4)	15 (75.0)	12 (60.0)	0.208
Dyslipidaemia, *n* yes (%)	43 (72.9)	12 (63.2)	15 (75.0)	16 (80.0)	0.480
Hypertension, *n* yes (%)	26 (44.1)	8 (42.1)	10 (50.0)	8 (40.0)	0.799
Prediabetes, *n* yes (%)	20 (33.9)	6 (31.6)	6 (30.0)	8 (40.0)	0.774
Type 2 diabetes, *n* yes (%)	20 (33.9)	7 (36.8)	8 (40.0)	5 (25.0)	0.573
Lifestyle parameters					
Physical activity, MET-min/week	1869.1 ± 222.6	1682.5 ± 374.8	1942.3 ± 392.2	1973.2 ± 404.1	0.849
Physical activity level, *n* (%)					
Low	20 (33.9)	7 (36.8)	6 (30.0)	7 (35.0)	0.968
Moderate	22 (37.3)	6 (31.6)	8 (40.0)	8 (40.0)	
High	17 (28.8)	6 (31.6)	6 (30.0)	5 (25.0)	
Mediterranean diet score	30.9 ± 0.8	32.2 ± 1.4	30.7 ± 1.5	29.8 ± 1.2	0.459
Mediterranean diet score (level), *n* (%)					
Low (1–17)	1 (1.7)	0 (0.0)	1 (5.0)	0 (0.0)	0.251
Moderate (18–36)	48 (81.4)	15 (78.9)	14 (70.0)	19 (95.0)	
High (37–55)	10 (16.9)	4 (21.1)	5 (25.0)	1 (5.0)	
Energy intake, kcal	2107.4 ± 98.8	2116.8 ± 205.6	2096.4 ± 138.7	2109.5 ± 174.9	0.996
Eating window, hh:mm	12:46 ± 00:20	12:50 ± 00:38	12:56 ± 00:19	12:33 ± 00:43	0.900
Fasting window, hh:mm	11:13 ± 00:20	11:10 ± 00:38	11:03 ± 00:19	11:13 ± 00:20	0.900
Weekdays wake-up time, hh:mm	07:09 ± 00:11	07:18 ± 00:28	06:56 ± 00:09	07:14 ± 00:24	0.707
Free days fall-asleep time, hh:mm	23:30 (23:00, 00:30)	00:00 (23:00, 00:30)	23:30 (23:00, 00:00)	23:00 (23:00, 01:00)	0.791
Breakfast consumption, days/week	5.46 ± 0.305	5.84 ± 0.509	5.05 ± 0.564	5.50 ± 0.521	0.579
Snacking after last meal, days/week	3.39 ± 0.344	3.95 ± 0.516	2.85 ± 0.559	3.40 ± 0.694	0.438
Anthropometric measurements					
Body weight, kg	92.7 ± 2.1	93.9 ± 3.7	88.2 ± 3.4	96.2 ± 3.7	0.274
Body mass index, kg/m^2	32.1 ± 0.6	32.4 ± 0.9	31.4 ± 1.1	32.7 ± 1.2	0.682
Waist circumference, cm	107.7 ± 1.5	109.0 ± 2.9	104.0 ± 2.1	110.1 ± 2.6	0.191
Hip circumference, cm	113.9 ± 2.1	111.6 ± 5.8	112.7 ± 2.3	117.4 ± 2.1	0.506
Mid-arm circumference, cm	35.6 ± 0.5	35.5 ± 0.9	35.3 ± 1.0	36.0 ± 0.8	0.834
Calf circumference, cm	41.9 ± 0.5	42.7 ± 0.8	41.2 ± 0.9	41.9 ± 0.8	0.457
Neck circumference, cm	38.8 ± 0.5	38.5 ± 1.0	38.9 ± 0.8	38.9 ± 1.0	0.944
Body composition					
Body fat percentage, %	37.1 ± 1.2	37.3 ± 2.1	36.5 ± 1.9	37.5 ± 2.1	0.927
Total fat mass, kg	34.5 ± 1.5	34.9 ± 2.5	32.3 ± 2.3	36.4 ± 2.8	0.504
Total body water, kg	42.9 ± 1.2	43.3 ± 2.3	41.1 ± 1.9	44.3 ± 1.2	0.522
Body water percentage, %	46.0 ± 0.9	46.0 ± 1.6	46.7 ± 1.4	45.4 ± 1.5	0.841

(Continues)

TABLE 1 | (Continued)

	Total ($n = 59$)	Control ($n = 19$)	eTRF ($n = 20$)	lTRF ($n = 20$)	p value
Fat-free mass, kg	58.0 ± 1.6	58.5 ± 3.0	55.9 ± 2.6	59.8 ± 2.7	0.605
Total muscle mass, kg	32.3 ± 1.0	32.5 ± 1.9	31.3 ± 1.6	33.3 ± 1.7	0.718
Liver parameters					
Aspartate aminotransaminase, U/L	22.0 (17.0, 32.0)	23.8 (19.0, 33.0)	22.5 (17.0, 27.8)	21. (15.5, 35.3)	0.498
Alanine aminotransaminase, U/L	27.0 (19.0, 42.0)	32.0 (20.0, 51.0)	27.0 (19.8, 37.5)	22.5 (16.0, 45.8)	0.578
Gamma-glutamyl transferase, U/L	27.0 (20.0, 72.0)	26.0 (15.0, 70.0)	30.5 (22.8, 67.5)	26.0 (20.3, 76.5)	0.589
Albumin, g/dL	5.2 ± 0.7	4.4 ± 0.1	6.6 ± 2.1	4.6 ± 0.1	0.392
Liver steatosis, dB/m	287.8 ± 5.5	292.9 ± 9.0	292.1 ± 10.3	280.1 ± 9.6	0.670
Liver stiffness, kPa	5.2 (4.5, 5.8)	5.4 (4.2, 6.6)	5.1 (4.6, 5.5)	5.3 (4.6, 5.9)	0.736
Cardiometabolic factors					
Glucose metabolism					
OGTT					
Fasting plasma glucose, mg/dL	102.8 ± 2.0	103.1 ± 4.2	105.8 ± 3.6	99.4 ± 2.7	0.424
60-min glucose, mg/dL	169.3 ± 7.5	166.9 ± 12.7	182.2 ± 13.6	158.7 ± 12.5	0.427
120-min glucose, mg/dL	123.0 ± 6.6	124.7 ± 13.8	139.0 ± 12.0	105.3 ± 7.1	0.108
Fasting insulin, mIU/L	10.5 (7.3, 15.9)	10.5 (7.0, 16.2)	11.7 (6.7, 19.3)	10.2 (7.5, 14.4)	0.906
60-min insulin, mIU/L	83.7 (47.0, 116.3)	71.2 (46.7, 134.7)	82.5 (34.5, 148.5)	89.6 (46.0, 105.5)	0.977
120-min insulin, mIU/L	47.1 (27.2, 88.8)	35.6 (36.7, 72.0)	59.1 (27.3, 131.7)	48.1 (23.9, 86.5)	0.559
HbA_{1c}, %	5.5 (5.3, 5.9)	5.5 (5.4, 5.9)	5.7 (5.3, 6.0)	5.4 (5.2, 5.7)	0.392
Lipidaemic profile					
Total cholesterol, mg/dL	190.9 ± 4.9	183.0 ± 7.9	188.5 ± 8.6	200.9 ± 8.7	0.310
Triglycerides, mg/dL	119.3 ± 6.7	111.0 ± 11.0	134.0 ± 12.9	106.8 ± 9.7	0.085
LDL, mg/dL	113.4 ± 4.3	104.8 ± 7.2	111.6 ± 7.0	123.4 ± 8.0	0.206
HDL, mg/dL	52.1 ± 1.7	56.4 ± 3.7	48.0 ± 2.5	52.1 ± 2.6	0.147
Blood pressure					
Systolic blood pressure, mmHg	129.5 ± 1.6	127.6 ± 2.7	130.8 ± 2.9	130.0 ± 3.1	0.716
Diastolic blood pressure, mmHg	79.9 ± 1.2	76.5 ± 1.9	83.8 ± 1.7	79.1 ± 2.5	0.055
Other parameters					
Uric acid, mg/dL	5.6 ± 0.2	5.3 ± 0.3	5.7 ± 0.3	5.7 ± 0.3	0.661
Creatinine, mg/dL	0.8 ± 0.02	0.8 ± 0.04	0.8 ± 0.03	0.9 ± 0.04	0.169
C-reactive protein, mg/L	1.5 (0.6, 2.8)	1.5 (0.8, 2.7)	0.9 (0.5, 1.5)	2.0 (0.7, 3.7)	0.100
Ferritin, ng/mL	93.0 (48.5, 172.0)	79.2 (39.8, 163.0)	83.1 (47.3, 216.3)	107.8 (66.1, 179.5)	0.521
Urine ketone bodies, *n* yes (%)	2 (3.4)	1 (5.3)	1 (5.0)	0 (0.0)	0.394

Note: Normally and non-normally distributed variables are shown as mean ± standard error of the mean (SEM) and median and interquartile range (25th, 75th), respectively, and categorical variables as absolute numbers (frequencies, %). p value: Pearson's chi-square test for categorical variables, one-way ANOVA and Kruskal-Wallis for parametric and non-parametric variables, respectively.

Abbreviations: eTRF, early time-restricted feeding; HbA_{1c}, glycated haemoglobin A_{1c}; lTRF, late time-restricted feeding; OGTT, oral glucose tolerance test.

TABLE 2 | Results on body composition and anthropometric measurements from baseline to 12 and 20 weeks.

	Control ($n=19$)					eTRF ($n=20$)					lTRF ($n=20$)							Interaction (0–12)		Interaction (12–20)	
	Baseline	12 weeks	20 weeks	p^a	p^b	Baseline	12 weeks	20 weeks	p^a	p^b	Baseline	12 weeks	20 weeks	p^a	p^b	p_{12}	p_{20}	p time	p time × intervention	p time	p time × intervention
Body weight, kg	93.9 ± 3.7	86.1 ± 3.7	85.0 ± 3.6	**< 0.001**	0.059	88.2 ± 3.4	81.0 ± 3.3	80.7 ± 3.3	**< 0.001**	0.457	96.2 ± 3.7	89.1 ± 3.7	88.9 ± 3.6	**< 0.001**	0.733	0.266	0.251	**< 0.001**	0.862	0.917	0.918
BMI, kg/m^2	32.4 ± 0.9	29.5 ± 1.0	29.1 ± 1.0	**< 0.001**	**0.034**	31.4 ± 1.1	28.8 ± 1.0	28.7 ± 1.0	**< 0.001**	0.441	32.7 ± 1.2	30.2 ± 1.2	30.1 ± 1.1	**< 0.001**	0.547	0.615	0.576	**< 0.001**	0.676	0.902	0.877
Waist circumference, cm	109.0 ± 2.9	100.2 ± 3.0	99.6 ± 3.1	**< 0.001**	0.163	104.0 ± 2.1	96.6 ± 1.9	97.2 ± 1.9	**< 0.001**	0.228	110.1 ± 2.6	102.1 ± 2.8	102.7 ± 2.5	**< 0.001**	0.363	0.308	0.296	**< 0.001**	0.807	0.844	0.758
Hip circumference, cm	111.6 ± 5.8	110.0 ± 2.2	108.8 ± 2.1	0.805	0.054	112.7 ± 2.3	106.6 ± 2.1	106.5 ± 2.0	**< 0.001**	0.805	117.4 ± 2.1	111.7 ± 2.2	111.0 ± 2.1	**< 0.001**	0.214	0.247	0.313	**0.010**	0.941	0.601	0.858
WHR	1.34 ± 0.4	0.91 ± 0.02	0.91 ± 0.02	0.312	0.489	0.93 ± 0.02	0.91 ± 0.02	0.91 ± 0.01	**0.024**	0.064	0.94 ± 0.02	0.92 ± 0.02	0.93 ± 0.01	**0.005**	0.107	0.967	0.827	0.827	0.992	0.537	0.807
Mid-arm circumference, cm	35.5 ± 0.9	33.4 ± 0.9	33.2 ± 0.9	**< 0.001**	0.316	35.3 ± 1.0	32.8 ± 0.8	33.1 ± 0.9	**< 0.001**	0.435	36.0 ± 0.8	33.9 ± 0.8	33.8 ± 0.7	**< 0.001**	0.827	0.660	0.809	**< 0.001**	0.976	0.986	0.981
Calf circumference, cm	42.7 ± 0.8	41.2 ± 0.8	40.7 ± 0.8	**< 0.001**	**0.008**	41.2 ± 0.9	39.5 ± 0.8	39.3 ± 0.8	**< 0.001**	0.134	41.9 ± 0.8	40.5 ± 0.8	40.4 ± 0.8	**< 0.001**	0.599	0.234	0.403	**< 0.001**	0.991	0.883	0.911
Neck circumference, cm	38.5 ± 1.0	36.9 ± 1.0	37.0 ± 1.0	**< 0.001**	0.716	38.9 ± 0.8	37.2 ± 0.8	37.3 ± 0.9	**< 0.001**	0.383	38.9 ± 1.0	37.4 ± 0.9	37.5 ± 0.9	**0.004**	0.470	0.944	0.934	**< 0.001**	0.944	0.643	0.964
NCtH	22.6 ± 0.5	21.6 ± 0.4	21.7 ± 0.4	**< 0.001**	0.701	23.2 ± 0.3	22.2 ± 0.3	22.2 ± 0.4	**< 0.001**	0.374	22.6 ± 0.5	21.7 ± 0.4	21.8 ± 0.4	**0.004**	0.492	0.578	0.580	**< 0.001**	0.845	0.617	0.961
NCtW	0.416 ± 0.01	0.436 ± 0.01	0.442 ± 0.01	**0.001**	**0.033**	0.448 ± 0.01	0.462 ± 0.01	0.465 ± 0.01	**0.046**	**0.044**	0.412 ± 0.01	0.427 ± 0.01	0.429 ± 0.01	**0.002**	0.434	0.115	0.091	**0.008**	0.805	0.702	0.961
Body fat percentage, %	37.3 ± 2.1	33.8 ± 2.4	33.0 ± 2.4	**< 0.001**	0.057	36.5 ± 1.9	32.9 ± 2.0	32.7 ± 1.9	**< 0.001**	0.622	37.5 ± 2.1	34.5 ± 2.3	34.0 ± 2.2	**0.004**	0.220	0.879	0.900	**< 0.001**	0.926	0.630	0.905
Total fat mass, kg	34.9 ± 2.5	29.2 ± 2.5	28.3 ± 2.6	**< 0.001**	**0.029**	32.3 ± 2.3	26.9 ± 2.2	26.6 ± 2.1	**< 0.001**	0.496	36.4 ± 2.8	31.0 ± 2.8	30.6 ± 2.7	**< 0.001**	0.278	0.506	0.516	**< 0.001**	0.841	0.762	0.946
FMI	12.1 ± 0.9	10.2 ± 1.0	9.9 ± 1.0	**< 0.001**	**0.021**	11.0 ± 0.7	9.7 ± 0.9	9.6 ± 0.8	0.055	0.485	12.6 ± 1.1	10.8 ± 1.1	10.6 ± 1.0	**< 0.001**	0.242	0.732	0.728	**< 0.001**	0.852	0.713	0.900
Right arm fat mass, kg	3.0 ± 0.4	2.3 ± 0.3	2.2 ± 0.3	**< 0.001**	0.088	2.6 ± 0.3	2.0 ± 0.3	2.0 ± 0.3	**< 0.001**	0.566	3.4 ± 0.5	2.6 ± 0.4	2.3 ± 0.3	**< 0.001**	0.246	0.472	0.654	**< 0.001**	0.491	0.286	0.483
Left arm fat mass, kg	3.1 ± 0.4	2.4 ± 0.3	2.3 ± 0.3	**< 0.001**	**0.038**	2.7 ± 0.3	2.1 ± 0.3	2.0 ± 0.3	**< 0.001**	0.551	3.4 ± 0.5	2.7 ± 0.4	2.4 ± 0.3	**< 0.001**	0.266	0.517	0.698	**< 0.001**	0.448	0.262	0.470
Body fat mass, kg	16.8 ± 1.4	15.0 ± 1.3	14.4 ± 1.2	0.216	**0.019**	16.8 ± 1.0	14.1 ± 1.1	14.0 ± 1.1	**< 0.001**	0.437	19.3 ± 1.2	16.5 ± 1.4	15.8 ± 1.3	**< 0.001**	**0.028**	0.452	0.514	**< 0.001**	0.622	0.556	0.858
Right leg fat mass, kg	4.9 ± 0.4	4.1 ± 0.4	4.1 ± 0.4	**< 0.001**	0.225	4.4 ± 0.4	3.7 ± 0.3	3.7 ± 0.3	**< 0.001**	0.752	4.9 ± 0.3	4.2 ± 0.3	4.3 ± 0.4	**< 0.001**	0.257	0.538	0.462	**< 0.001**	0.556	0.719	0.673
Left leg fat mass, kg	4.8 ± 0.4	4.1 ± 0.4	4.0 ± 0.4	**< 0.001**	0.212	4.4 ± 0.4	3.7 ± 0.3	3.6 ± 0.3	**< 0.001**	0.640	4.9 ± 0.3	4.1 ± 0.3	4.2 ± 0.4	**< 0.001**	0.250	0.548	0.458	**< 0.001**	0.478	0.816	0.686
Total body water, kg	43.3 ± 2.3	42.5 ± 2.3	41.7 ± 2.1	**0.015**	0.807	41.1 ± 1.9	39.7 ± 1.8	39.8 ± 1.9	**0.001**	0.887	44.3 ± 1.2	43.3 ± 2.1	42.9 ± 2.0	**0.002**	0.190	0.602	0.535	0.220	0.995	0.856	0.977
Body water percentage, %	46.0 ± 1.6	48.6 ± 1.8	49.2 ± 1.8	**< 0.001**	**0.043**	46.7 ± 1.4	49.3 ± 1.5	50.0 ± 1.3	**< 0.001**	0.341	45.4 ± 1.5	48.1 ± 1.7	48.5 ± 1.6	**< 0.001**	0.120	0.863	0.789	**< 0.001**	0.919	0.574	0.927
FFM, kg	58.5 ± 3.0	56.9 ± 3.1	56.7 ± 2.9	**0.016**	0.707	55.9 ± 2.6	54.1 ± 2.5	54.1 ± 2.5	**0.001**	0.916	59.8 ± 2.7	58.1 ± 2.8	58.4 ± 2.8	**0.004**	0.340	0.590	0.537	0.210	0.993	0.898	0.982
FFMI	19.8 ± 0.5	19.2 ± 0.6	19.2 ± 0.5	**0.026**	0.849	19.7 ± 0.6	19.1 ± 0.5	19.1 ± 0.5	**0.001**	0.957	20.1 ± 0.5	19.4 ± 0.5	19.6 ± 0.5	**0.006**	0.298	0.891	0.786	**0.019**	0.967	0.750	0.948
Total muscle mass, kg	32.5 ± 1.9	30.7 ± 1.7	31.6 ± 1.7	0.115	0.392	31.3 ± 1.6	30.1 ± 1.5	30.1 ± 1.6	**0.001**	0.533	33.3 ± 1.7	32.5 ± 1.7	32.6 ± 1.7	**0.002**	0.487	0.573	0.538	0.094	0.801	0.580	0.834
Right arm muscle mass, kg	3.5 ± 0.2	3.3 ± 0.2	3.2 ± 0.2	**0.006**	0.490	3.3 ± 0.2	3.2 ± 0.2	3.1 ± 0.2	**0.004**	0.186	3.6 ± 0.2	3.5 ± 0.2	3.6 ± 0.3	**0.020**	0.369	0.744	0.404	0.144	0.851	0.455	0.443
Left arm muscle mass, kg	3.4 ± 0.2	3.2 ± 0.2	3.2 ± 0.2	**0.002**	0.895	3.3 ± 0.2	3.1 ± 0.2	3.1 ± 0.2	**0.003**	0.356	3.5 ± 0.2	3.4 ± 0.2	3.5 ± 0.3	**0.004**	0.300	0.699	0.332	0.154	0.974	0.359	0.334
Body muscle mass, kg	26.9 ± 1.3	25.4 ± 1.3	25.3 ± 1.3	**< 0.001**	0.297	25.9 ± 1.2	24.9 ± 1.1	24.7 ± 1.2	**0.001**	0.187	27.6 ± 1.1	26.9 ± 1.1	26.4 ± 1.1	**0.002**	0.530	0.622	0.582	0.102	0.911	0.843	0.994
Right leg muscle mass, kg	9.1 ± 0.5	8.9 ± 0.5	8.7 ± 0.4	0.082	0.597	8.3 ± 0.4	8.0 ± 0.4	8.0 ± 0.4	**0.004**	0.781	9.3 ± 0.5	9.0 ± 0.5	8.9 ± 0.5	**0.003**	0.655	0.336	0.300	0.261	0.986	0.937	0.990
Left leg muscle mass, kg	9.0 ± 0.5	8.8 ± 0.5	8.7 ± 0.4	**0.043**	0.272	8.2 ± 0.4	8.1 ± 0.4	8.0 ± 0.4	**0.003**	0.766	9.1 ± 0.5	8.9 ± 0.5	8.8 ± 0.5	**0.009**	0.560	0.363	0.345	0.342	0.978	0.942	0.987
Conicity index	1.35 ± 0.02	1.30 ± 0.02	1.30 ± 0.02	**< 0.001**	0.817	1.32 ± 0.01	1.28 ± 0.01	1.29 ± 0.01	**< 0.001**	0.073	1.35 ± 0.01	1.30 ± 0.02	1.31 ± 0.01	**< 0.001**	0.149	0.596	0.566	**0.001**	0.912	0.705	0.752

Note: Parametric and non-parametric variables are shown as mean ± standard error of the mean (SEM) and median and interquartile range (25th, 75th), respectively. p^a: difference in each group from baseline to 12 weeks, p^b: difference in each group from 12 to 20 weeks. Differences were tested using the paired samples t-test and the Wilcoxon test for parametric and non-parametric variables, respectively. p_{12}: difference between the three groups at 12 weeks, p_{20}: difference between the three groups at 20 weeks. Differences were tested using one-way ANOVA and Kruskal–Wallis tests for parametric and non-parametric variables, respectively. p time, p time × intervention: derived through comparisons from baseline to 12 weeks (0–12) and from 12 to 20 weeks (12–20) between the three groups adjusted for sex, age, metabolic syndrome, homeostatic model assessment for insulin resistance, BMI, and total fat mass by using linear mixed effects model. Significant p values are bold.

Abbreviations: BMI, body mass index; FFM, fat-free mass; FFMI, fat-free mass index; FMI, fat mass index; NCtH, neck-to-height ratio; NCtW, neck-to-weight ratio; WHR, waist-to-hip ratio.

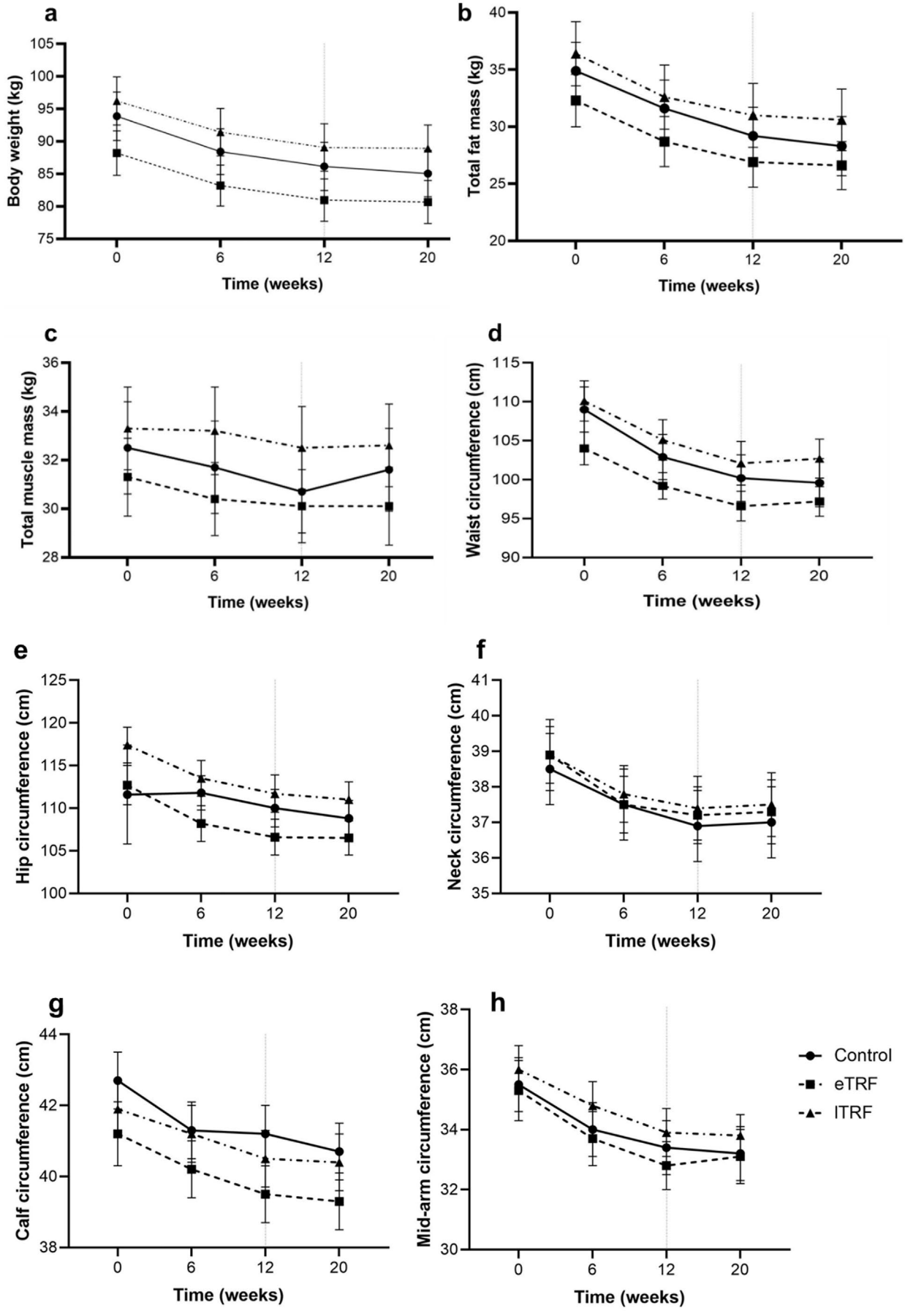

FIGURE 3 | Legend on next page.

FIGURE 3 | Changes in body composition and body circumferences from baseline to 12 weeks and follow-up (20 weeks): (a) body weight, (b) total fat mass, (c) total muscle mass, (d) waist circumference, (e) hip circumference, (f) neck circumference, (g) calf circumference and (h) mid-arm circumference. The vertical lines indicate the end of the intervention. Differences between groups were tested using one-way ANOVA. The paired sample t-test was used to test the differences within each group. Data are represented as mean ± standard error of the mean (SEM). eTRF, early time-restricted feeding; lTRF, late time-restricted feeding.

3.8 | Effects on Subjective Appetite

There were no differences among the three groups in VAS scores at 12 or 20 weeks compared to the baseline (Figure 6a–g). Perceived fullness increased at 12 weeks compared to the baseline in both the control and eTRF groups ($p < 0.001$ and $p = 0.017$, respectively) (Figure 6b). Additionally, the eTRF group had higher thirst rates at follow-up than at 12 weeks ($p = 0.025$) (Figure 6d). The lTRF group showed a reduction in prospective food consumption at 12 weeks compared to the baseline ($p = 0.027$) (Figure 6f).

3.9 | Compliance With the Intervention

Adherence to the intervention (both time schedule and MD diet plan and composition), based on 12 collected 7-day food diaries, was 91.7% (77 of 84 days) for the control group, 95.1% (79.9 of 84 days) for the eTRF group and 94% (79 of 84 days) for the lTRF group, with no significant compliance differences between groups ($p = 0.305$). Notably, participants in both TRF groups continued a fasting window exceeding 12 h daily post-intervention without prompting, as shown in Table 6. Physical activity levels remained consistent from baseline to 12 weeks and from 12 weeks to follow-up across all groups, with no significant differences (p for all > 0.05) (Table 6, Figure 7).

The three groups did not differ in their main sleeping characteristics (wake-up time, bedtime and sleeping duration), which also remained unchanged during the intervention in all groups (p for all > 0.05); however, there were differences in the morning and evening latency at 12 and 20 weeks (see Table S2). The morning latency changed in both the eTRF and lTRF groups at 12 weeks compared to the baseline, while the evening latency changed in the eTRF from baseline to 12 weeks and from 12 weeks to 20 weeks (see Table S2). These results are in agreement with the nature of the intervention.

4 | Discussion

This is the first RCT to investigate the effects of a hypocaloric MD-type 14:10 TRF protocol in patients with MASLD, using unrestricted MD as a control, and to compare early and late hypocaloric Mediterranean-type 14:10 TRF in this population. Our findings confirmed the beneficial effects of MD on weight loss and cardiometabolic risk factors [9]. MD improved body composition, anthropometric indices, and BP across all groups by the end of the 12-week study. Notably, MD combined with early eating enhanced glucose metabolism, indicated by HbA_{1c} and IR indices improvements, while the unrestricted MD reduced TC, LDL and all liver enzyme (AST, ALT, GGT) levels at 12 weeks. Interestingly, all modified MD interventions were equally accepted over time, with no differences between them, and all groups maintained their weight loss for 2 months post-study.

Our previous systematic review demonstrated that TRF significantly contributes to weight loss when combined with a hypocaloric diet [13]. In our current study, both the TRF and control groups achieved over 5% weight loss at 12 weeks, corroborating our earlier findings and indicating that TRF does not provide any further favourable effects on weight loss in the context of a hypocaloric MD diet. Literature indicates lower body weight in *ad libitum* TRF studies compared to controls [18, 20, 22], with no significant weight loss differences between eTRF and lTRF [21] or between eTRF and control groups on hypocaloric diets [19]. Similar trends were observed for WC [19–22] and fat mass [19–21] in MASLD patients. However, in the previous studies [18–22], MD was not used as a control, while TRF groups did not adhere to a hypocaloric MD diet. Additionally, waist-to-hip ratios reduction in both TRF groups suggests a lowered risk of weight-related conditions like type 2 diabetes or CVD [46]. Recent studies link measurements of NC, neck-to-height (NCtH) ratio and neck-to-weight (NCtW) ratio to liver fat and upper-body adiposity [47]. MD intervention reduced NC and NCtH and increased NCtW across all three groups, indicating effective MASLD management [47].

Skipping or moving dinner earlier can improve glycaemic responses and reduce IR [48]. In our study, a hypocaloric Mediterranean-type eTRF protocol and an unrestricted MD improved fasting insulin, HOMA-IR and Matsuda index, whereas HbA_{1c} and FGI ratio were ameliorated only in the eTRF group. Starting eating earlier in the day, as happened in the eTRF and the unrestricted MD groups, is in alignment with the circadian rhythms of insulin sensitivity, which may explain the effects on IR, while increasing the fasting window in this context may lead to further favourable effects on glucose metabolism [48]. Although late eating has been associated with worsened glucose levels [48], in our study, lTRF did not negatively affect glucose metabolism, indicating the favourable effects of MD. A network meta-analysis ranked MD as the most effective dietary approach for improving postprandial hyperglycaemia and IR [49]. Results partially agree with Wei et al. and Deng et al., who revealed improvements in FPG, HbA_{1c}, fasting insulin levels and HOMA-IR in eTRF and lTRF groups under calorie-restricted conditions [19, 21]. The three groups did not differ in any glycaemic parameter at 12 weeks, as observed in other RCTs [18–20, 22].

The unrestricted MD group showed lower TC and LDL levels and AI at 12 weeks, indicating a reduced risk of atherosclerosis and coronary heart disease [28, 29]. Kord-Varkaneh et al. showed that the TRF group (isocaloric low-sugar diet)

TABLE 3 | Results on glucose metabolism parameters from baseline to 12 weeks.

	Control ($n=19$)			eTRF ($n=20$)			lTRF ($n=20$)				Interaction (0–12)	
	Baseline	12 weeks	p^a	Baseline	12 weeks	p^a	Baseline	12 weeks	p^a	p_{12}	p time	p time × intervention
Fasting plasma glucose, mg/dL	103.1 ± 4.2	97.8 ± 2.9	0.060	105.8 ± 3.6	98.9 ± 3.0	0.068	99.4 ± 2.7	94.5 ± 2.2	0.096	0.484	0.379	0.997
60-min glucose, mg/dL	166.9 ± 12.7	161.3 ± 11.9	0.473	182.2 ± 13.6	174.3 ± 11.9	0.477	158.7 ± 12.5	144.5 ± 12.6	0.106	0.225	0.936	0.806
120-min glucose, mg/dL	124.7 ± 13.8	124.1 ± 13.8	0.951	139.0 ± 12.0	124.0 ± 9.0	0.146	105.3 ± 7.1	101.8 ± 8.4	0.618	0.233	0.802	0.965
Fasting insulin, mIU/L	10.5 (7.0, 16.2)	9.9 (6.6, 14.5)	**0.040**	11.7 (6.7, 19.3)	8.1 (4.3, 11.5)	**0.002**	10.2 (7.5, 14.4)	9.8 (5.6, 12.0)	0.102	0.361	0.195	0.552
60-min insulin, mIU/L	71.2 (46.7, 134.7)	56.3 (34.9, 107.4)	**0.019**	82.5 (34.5, 148.5)	68.5 (36.0, 128.3)	0.277	89.6 (46.0, 105.5)	58.5 (38.6, 111.6)	0.286	0.878	0.477	0.522
120-min insulin, mIU/L	35.6 (36.7, 72.0)	42.1 (27.9, 56.9)	0.557	59.1 (27.3, 131.7)	57.9 (26.8, 99.9)	0.102	48.1 (23.9, 86.5)	35.2 (11.9, 66.6)	0.177	0.289	0.442	0.485
Peak glucose, mg/dL	69.2 ± 10.8	68.5 ± 10.3	0.923	79.4 ± 12.2	75.4 ± 10.1	0.659	61.1 ± 11.2	55.0 ± 11.2	0.387	0.380	0.902	0.907
Peak time for glucose, min	66.3 ± 6.3	69.5 ± 6.9	0.578	63.0 ± 6.9	60.0 ± 0.0	0.666	51.0 ± 4.9	60.0 ± 7.5	0.267	0.431	0.970	0.465
iAUC for glucose, mg*min/dL	4600.6 ± 819.0	4724.2 ± 804.0	0.858	5678.5 ± 881.9	5319.5 ± 785.8	0.605	4041.1 ± 746.7	3181.5 ± 785.4	0.206	0.241	0.974	0.824
Peak insulin, mIU/L	74.1 (30.1, 157.8)	51.6 (29.1, 107.5)	0.124	78.2 (29.8, 177.4)	68.3 (34.7, 114.7)	0.286	79.0 (37.3, 110.7)	55.3, (34.9, 102.4)	0.616	0.879	0.225	0.600
Peak time for insulin, min	60.0 (60.0, 120.0)	60.0 (60.0, 120.0)	1.000	120.0 (60.0, 120.0)	61.0 (60.0, 120.0)	0.414	60.0 (60.0, 75.0)	60.0 (60.0, 120.0)	0.564	0.521	0.472	0.583
iAUC for insulin, mIU*min/L	4993.5 (2520.0, 9045.0)	3591.3 (2408.6, 7585.2)	0.193	5502.0 (2598.0, 12366.0)	5483.4 (2616.0, 8070.6)	0.349	6021.0 (2881.5, 7950.0)	4558.5 (2340.8, 7028.3)	0.327	0.725	0.443	0.663
HbA_{1c}, %	5.5 (5.4, 5.9)	5.5 (5.4, 5.7)	0.444	5.7 (5.3, 6.0)	5.4 (5.2, 5.7)	**0.001**	5.4 (5.2, 5.7)	5.6 (5.2, 5.7)	0.190	0.469	0.669	0.826
HOMA-IR	3.1 (1.6, 3.8)	2.4 (1.6, 3.8)	**0.024**	2.9 (1.8, 5.1)	1.7 (1.1, 3.0)	**0.001**	2.6 (1.7, 3.7)	2.2 (1.1, 3.1)	0.094	0.407	0.138	0.683
Fasting glucose to insulin ratio	10.3 ± 1.2	10.8 ± 1.1	0.615	10.6 ± 1.4	16.6 ± 2.8	**0.011**	10.3 ± 0.9	12.3 ± 1.6	0.118	0.133	**0.014**	0.143
Matsuda index	4.0 ± 0.5	5.4 ± 0.8	**0.014**	3.9 ± 0.6	5.4 ± 0.7	**0.021**	4.2 ± 0.6	5.3 ± 0.8	0.056	0.721	**0.021**	0.918

Note: Parametric and non-parametric variables are shown as mean ± standard error of the mean (SEM) and median and interquartile range (25th, 75th), respectively. p^a: difference in each group from baseline to 12 weeks. Differences were tested using the paired samples *t*-test and the Wilcoxon test for parametric and non-parametric variables, respectively. p_{12}: difference between the three groups at 12 weeks. Differences were tested using one-way ANOVA and Kruskal–Wallis tests for parametric and non-parametric variables, respectively. p time, p time × intervention: derived through comparisons from baseline to 12 weeks (0–12) between the three groups adjusted for sex, age, metabolic syndrome, homeostatic model assessment for insulin resistance, body mass index and total fat mass by using linear mixed effects model. Significant p values are bold.

Abbreviations: eTRF, early time-restricted feeding; HbA_{1c}, glycated haemoglobin A_{1c}; HOMA-IR, homeostatic model assessment for insulin resistance; iAUC, incremental area under curve; lTRF, late time-restricted feeding.

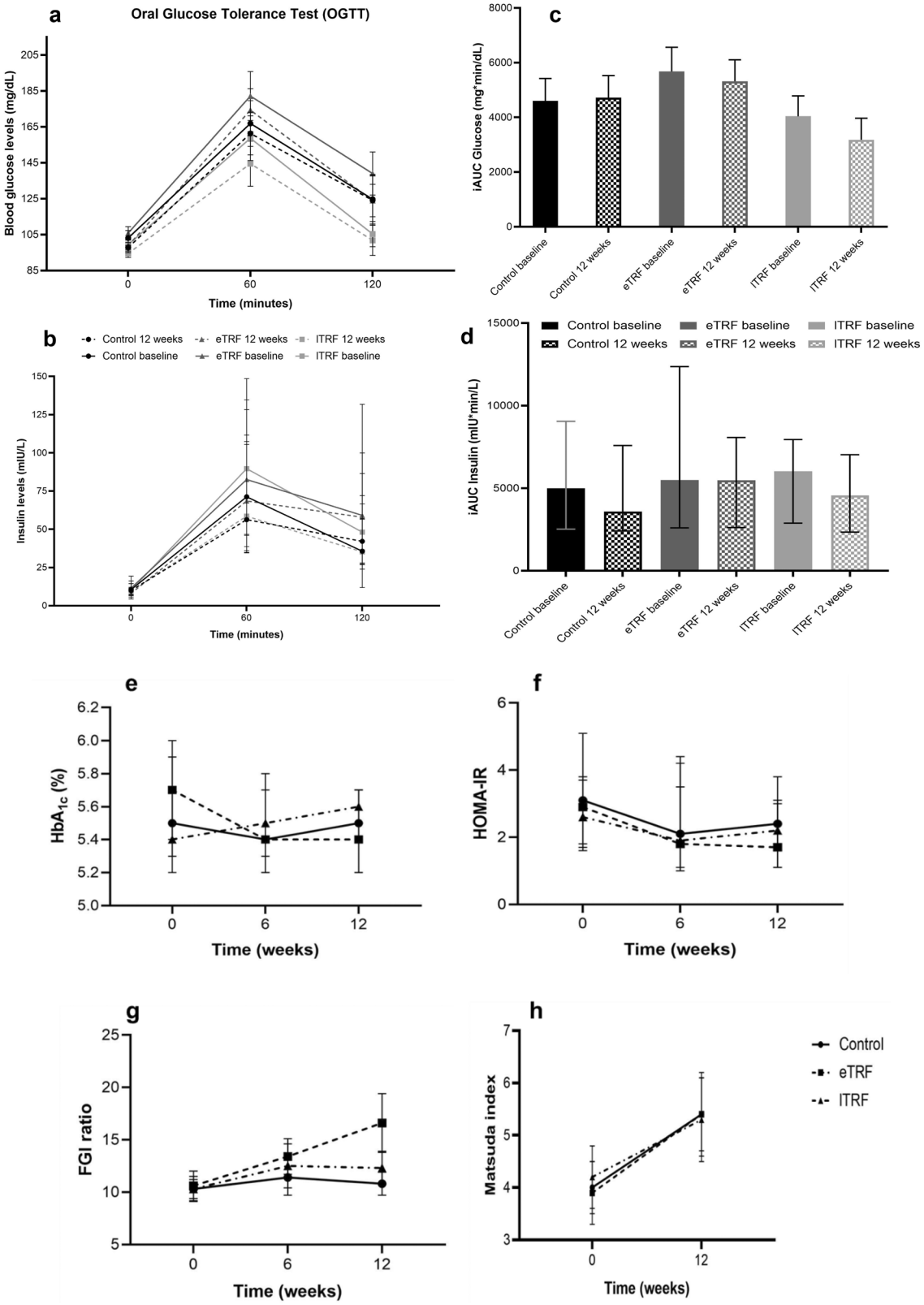

FIGURE 4 | Changes in glycaemic parameters from baseline to 12 weeks: (a) blood glucose levels and (b) insulin levels after the OGTT, incremental AUC for (c) glucose and (d) insulin, (e) HbA_{1c}, (f) HOMA-IR, (g) fasting glucose-to-insulin ratio and (h) Matsuda index. Differences between groups were tested using one-way ANOVA for normally distributed data and with Kruskal–Wallis for skewed data at all time points. The paired samples *t*-test and the Wilcoxon test were used to test differences within each group for normal and skewed data, respectively. Normally and non-normally distributed variables are represented as mean ± standard error of the mean (SEM) and median and interquartile range (25th, 75th), respectively. eTRF, early time-restricted feeding; FGI, fasting glucose-to-insulin; HbA_{1c}, glycated haemoglobin A_{1c}; iAUC, incremental area under curve; lTRF, late time-restricted feeding; OGTT, oral glucose tolerance test.

TABLE 4 | Results on lipidemic profile, blood pressure and other biochemical parameters from baseline to 12 and 20 weeks.

	Control ($n=19$)					eTRF ($n=20$)					lTRF ($n=20$)							Interaction (0–12)		Interaction (12–20)	
	Baseline	12 weeks	20 weeks	p^a	p^b	Baseline	12 weeks	20 weeks	p^a	p^b	Baseline	12 weeks	20 weeks	p^a	p^b	p_{12}	p_{20}	p time	p time × intervention	p time	p time × intervention
Total cholesterol, mg/dL	183.0 ± 7.9	163.4 ± 6.4		**0.002**		188.5 ± 8.6	186.7 ± 6.5		0.835		200.9 ± 8.7	186.1 ± 7.3		**0.009**		**0.029**		0.222	0.453		
Triglycerides, mg/dL	111.0 ± 11.0	97.9 ± 11.9		0.245		134.0 ± 12.9	125.9 ± 14.9		0.280		106.8 ± 9.7	94.4 ± 8.1		0.176		0.133		0.326	0.538		
LDL, mg/dL	104.8 ± 7.2	89.2 ± 6.0		**0.002**		111.6 ± 7.0	111.0 ± 5.9		0.921		123.4 ± 8.0	114.6 ± 7.1		0.078		**0.015**		0.313	0.651		
HDL, mg/dL	56.4 ± 3.7	53.4 ± 3.0		0.235		48.0 ± 2.5	49.1 ± 2.8		0.483		52.1 ± 2.6	49.8 ± 2.1		0.290		0.474		0.618	0.818		
Atherogenic index	2.0 ± 0.2	1.8 ± 0.2		**0.046**		2.5 ± 0.3	2.4 ± 0.2		0.646		2.5 ± 0.2	2.4 ± 0.2		0.399		**0.018**		0.414	0.852		
Coronary risk index	3.4 ± 0.2	3.2 ± 0.2		0.142		4.1 ± 0.3	4.0 ± 0.3		0.516		4.0 ± 0.3	3.9 ± 0.2		0.321		**0.028**		0.433	0.795		
Triglycerides to HDL ratio	2.2 ± 0.3	2.0 ± 0.3		0.540		3.2 ± 0.4	3.0 ± 0.5		0.348		2.3 ± 0.3	2.0 ± 0.2		0.367		0.114		0.498	0.695		
Atherogenic index of plasma	0.3 ± 0.1	0.2 ± 0.1		0.332		0.4 ± 0.1	0.4 ± 0.1		0.125		0.3 ± 0.1	0.3 ± 0.1		0.386		0.202		0.404	0.677		
Systolic blood pressure, mmHg	127.6 ± 2.7	118.2 ± 3.2	118.2 ± 2.9	**0.001**	0.998	130.8 ± 2.9	115.1 ± 1.8	117.1 ± 1.9	**< 0.001**	0.286	130.0 ± 3.1	119.8 ± 2.6	124.0 ± 2.8	**0.001**	0.067	0.429	0.128	**< 0.001**	0.801	0.277	0.723
Diastolic blood pressure, mmHg	76.5 ± 1.9	70.5 ± 1.7	71.5 ± 2.1	**0.001**	0.505	83.8 ± 1.7	73.7 ± 2.1	73.5 ± 2.5	**< 0.001**	0.883	79.1 ± 2.5	75.0 ± 2.1	75.4 ± 2.5	**0.037**	0.822	0.245	0.520	**< 0.001**	0.494	0.632	0.960
Uric acid, mg/dL	5.3 ± 0.3	5.4 ± 0.4		0.761		5.7 ± 0.3	5.4 ± 0.3		0.197		5.7 ± 0.3	5.5 ± 0.3		0.231		0.981		0.831	0.969		
Creatinine, mg/dL	0.83 ± 0.04	0.83 ± 0.04		0.935		0.78 ± 0.03	0.808 ± 0.04		0.249		0.88 ± 0.04	0.85 ± 0.04		0.121		0.716		0.799	0.754		
C-reactive protein, mg/L	1.5 (0.8, 2.7)	1.0 (0.4, 1.4)		0.214		0.9 (0.5, 1.5)	0.5 (0.2, 2.1)		0.170		2.0 (0.7, 3.7)	1.0 (0.3, 3.3)		0.074		0.662		0.773	0.759		
Ferritin, ng/mL	79.2 (39.8, 163.0)	64.3 (28.0, 139.0)		0.314		83.1 (47.3, 216.3)	108.6 (48.0, 208.5)		0.627		107.8 (66.1, 179.5)	99.0 (53.3, 172.4)		0.550		0.602		0.638	0.614		
Urine ketone bodies, % yes/% no	5.3/94.7	10.5/89.5		**< 0.001**		5.0/95.0	0.0/100.0		1.000		0.0/100.0	5.0/95.0		1.000		0.517					

Note: Parametric and non-parametric variables are shown as mean ± standard error of the mean (SEM) and median and interquartile range (25th, 75th), respectively. Categorical values are shown as frequencies (%). p^a: difference in each group from baseline to 12 weeks, p^b: difference in each group from 12 to 20 weeks. Differences were tested using the paired samples *t*-test and the Wilcoxon test for parametric and non-parametric variables, respectively. p_{12}: difference between the three groups at 12 weeks, p_{20}: difference between the three groups at 20 weeks. Differences were tested using one-way ANOVA and Kruskal–Wallis tests for parametric and non-parametric variables, respectively. *p* time, *p* time × intervention: derived through comparisons from baseline to 12 weeks (0–12) and from 12 to 20 weeks (12–20) between the three groups adjusted for sex, age, metabolic syndrome, homeostatic model assessment for insulin resistance, body mass index and total fat mass by using linear mixed effects model. Significant *p* values are bold.

Abbreviations: eTRF, early time-restricted feeding; HDL, high-density lipoprotein; lTRF, late time-restricted feeding; LDL, low-density lipoprotein.

TABLE 5 | Results on liver biochemistry and imaging from baseline to 12 weeks.

	Control ($n=19$)			eTRF ($n=20$)			lTRF ($n=20$)				Interaction (0–12)	
	Baseline	12 weeks	p^a	Baseline	12 weeks	p^a	Baseline	12 weeks	p^a	p_{12}	p time	p time × intervention
Aspartate aminotransaminase, U/L	23.8 (19.0, 33.0)	18.8 (15.0, 23.0)	**0.019**	22.5 (17.0, 27.8)	20.0 (17.0, 24.8)	0.434	21.0 (15.5, 35.3)	22.5 (18.3, 25.8)	0.309	0.396	**0.041**	0.266
Alanine aminotransaminase, U/L	32.0 (20.0, 51.0)	19.0 (15.4, 29.0)	**0.010**	27.0 (19.8, 37.5)	24.0 (18.3, 27.0)	**0.024**	22.5 (16.0, 45.8)	22.5 (17.8, 32.0)	0.235	0.476	**0.008**	0.143
Gamma-glutamyl transferase, U/L	26.0 (15.0, 70.0)	20.0 (14.0, 33.0)	**0.001**	30.5 (22.8, 67.5)	21.5 (18.0, 41.5)	0.073	26.0 (20.3, 76.5)	20.5 (15.3, 49.8)	**0.004**	0.595	0.083	0.882
Albumin, g/dL	4.4 ± 0.1	4.4 ± 0.1	0.918	6.6 ± 2.1	4.6 ± 0.1	0.343	4.5 ± 0.1	4.5 ± 0.1	0.374	0.465	0.219	0.263
Liver steatosis, dB/m	292.9 ± 9.0	264.6 ± 11.9	**0.001**	292.1 ± 10.3	264.4 ± 8.0	**< 0.001**	280.1 ± 9.6	261.5 ± 8.6	**0.037**	0.981	**0.001**	0.733
Liver stiffness, kPa	5.4 (4.2, 6.6)	4.8 (4.4, 5.7)	0.066	5.1 (4.6, 5.5)	4.8 (4.3, 5.7)	**0.030**	5.3 (4.6, 5.9)	5.2 (4.4, 5.5)	0.074	0.870	0.325	0.391

Note: Parametric and non-parametric variables are shown as mean ± standard error of the mean (SEM) and median and interquartile range (25th, 75th), respectively. p^a: difference in each group from baseline to 12 weeks. Differences were tested using the paired samples t-test and the Wilcoxon test for parametric and non-parametric variables, respectively. p_{12}: difference between the three groups at 12 weeks. Differences were tested using one-way ANOVA and Kruskal–Wallis tests for parametric and non-parametric variables, respectively. p time, p time × intervention: derived through comparisons from baseline to 12 weeks (0–12) between the three groups adjusted for sex, age, metabolic syndrome, homeostatic model assessment for insulin resistance, body mass index and total fat mass by using linear mixed effects model. Significant p values are bold.
Abbreviations: eTRF, early time-restricted feeding; lTRF, late time-restricted feeding.

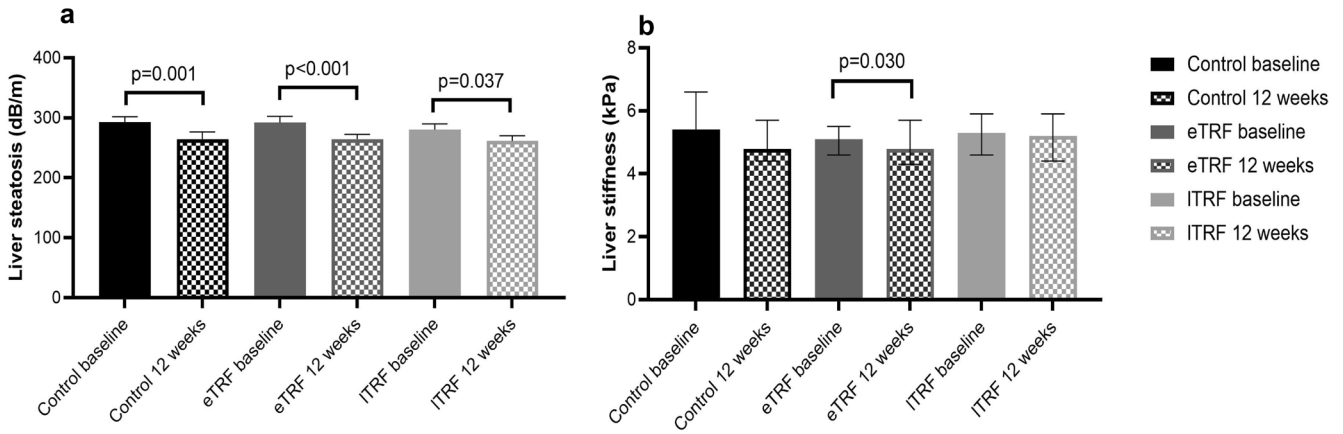

FIGURE 5 | Changes in liver steatosis (a) and liver stiffness (b) from baseline to 12 weeks. Differences between groups were tested using one-way ANOVA for normally distributed data and with Kruskal–Wallis for skewed data at all time points. The paired samples *t*-test and the Wilcoxon test were used to test differences within each group for normal and skewed data, respectively. Normally and non-normally distributed variables are represented as mean ± standard error of the mean (SEM) and median and interquartile range (25th, 75th), respectively. eTRF, early time-restricted feeding; lTRF, late time-restricted feeding.

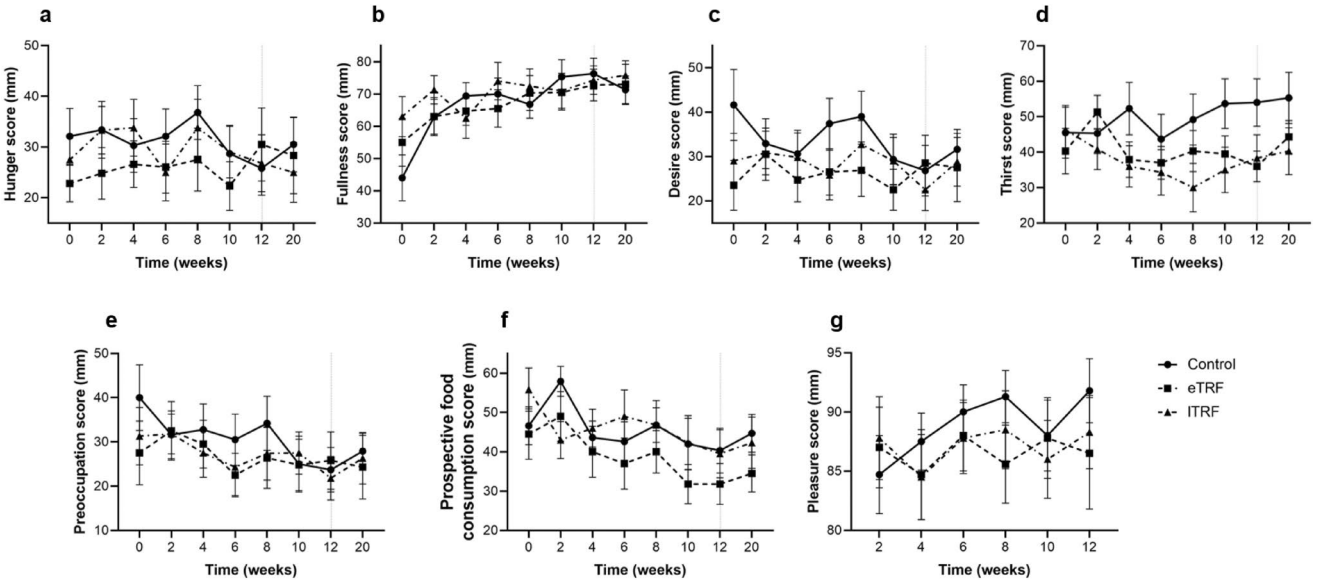

FIGURE 6 | Changes in subjective appetite VAS scores during the intervention and follow-up: (a) hunger, (b) perceived fullness, (c) desire to eat, (d) thirst, (e) preoccupation with food, (f) prospective food consumption and (g) pleasure due to the intervention. All scores were recorded in the morning. Pleasure due to the intervention was not assessed during follow-up visits. The vertical lines indicate the end of the intervention. Differences between groups were tested using one-way ANOVA. The paired samples *t*-test was used to test differences within each group. Data are represented as mean ± standard error of the mean (SEM), $n = 59$. eTRF, early time-restricted feeding; lTRF, late time-restricted feeding; VAS, Visual Analogue Scale.

had ameliorated TG and LDL levels compared with the control group (isocaloric typical diet) [20]. The eTRF group showed no significant change in lipid markers, while the lTRF group showed decreased TC levels in our study. These results partially disagree with other studies in MASLD patients, where TRF groups showed reduced TG [18–20], TC [19, 20] and LDL [19, 20] levels and increased HDL levels [19, 21] post-intervention, compared to the baseline, with or without hypocaloric diets. The differences between early TRF and control and/or late TRF groups in lipid markers may be due to different pre-testing fasting [12]. Longer durations of fasting may contribute to the re-esterification of TG after lipolysis and in the hepatic and intramuscular storage of TG (a short-term adverse reaction to dietary changes) [50–53]. However, the data are limited, and the studies usually are not powered, like ours, to detect statistically significant differences in lipids after a TRF protocol [52].

Studies consistently show positive effects of TRF (with or without caloric restriction) on BP in individuals with MASLD [18, 19, 22], corroborating our findings. All groups in our study exhibited clinically significant improvements in both SBP and DBP. Kandzari et al. define a meaningful improvement as a decrease of 5–10 mmHg for SBP and 3–5 mmHg for

TABLE 6 | Results on physical activity and eating window from baseline to 12 (intervention) and 20 (follow-up) weeks.

	Control ($n = 19$)						eTRF ($n = 20$)						lTRF ($n = 20$)								Interaction (0–12)		Interaction (12–20)	
	Baseline	Intervention	Follow-up	p^a	p^b	p^c	Baseline	Intervention	Follow-up	p^a	p^b	p^c	Baseline	Intervention	Follow-up	p^a	p^b	p^c	p_{12}	p_{20}	p time	p time × intervention	p time	p time × intervention
Physical activity, MET-min/week	1682.5 ± 374.8	1844.6 ± 333.9	2269.0 ± 683.8	0.537	0.396	0.396	1942.3 ± 392.2	1975.0 ± 397.0	2065.8 ± 541.6	0.948	0.839	0.839	1973.2 ± 404.1	1775.0 ± 317.3	2455.7 ± 683.1	0.556	0.343	0.343	0.919	0.910	0.602	0.835	0.260	0.914
Eating window, hh:mm	12:50 ± 0:38	12:02 ± 00:20	12:11 ± 00:18	0.058	0.793	0.341	12:56 ± 00:19	09:32 ± 00:06	10:59 ± 00:16	**< 0.001**	**< 0.001**	**0.002**	12:33 ± 00:43	09:27 ± 00:10	10:15 ± 00:28	**< 0.001**	0.090	**0.005**	**< 0.001**	**0.002**	**< 0.001**	**0.001**	**0.003**	0.167
Fasting window, hh:mm	11:09 ± 00:38	11:57 ± 00:20	11:48 ± 00:18	0.058	0.792	0.343	11:03 ± 00:19	14:27 ± 00:06	13:00 ± 00:16	**< 0.001**	**< 0.001**	**0.002**	11:26 ± 00:43	14:32 ± 00:10	13:44 ± 00:28	**< 0.001**	0.090	**0.005**	**< 0.001**	**0.002**	**< 0.001**	**0.001**	**0.003**	0.166
Time of first meal, hh:mm	09:01 ± 00:33	08:54 ± 00:17	08:34 ± 00:14	0.512	0.614	0.500	08:01 ± 00:15	08:41 ± 00:06	09:03 ± 00:15	**0.012**	0.311	**0.027**	08:48 ± 00:31	11:37 ± 00:25	10:56 ± 00:25	**< 0.001**	0.169	**0.003**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	0.637	0.132
Time of last meal, hh:mm	21:41 ± 00:17	21:21 ± 00:16	20:45 ± 00:15	0.175	**0.023**	**< 0.001**	20:57 ± 00:14	18:16 ± 00:04	20:03 ± 00:19	**< 0.001**	**< 0.001**	**0.024**	20:10 ± 01:07	21:05 ± 00:26	21:12 ± 00:10	0.483	0.749	0.364	**< 0.001**	**0.010**	0.232	**0.011**	**0.034**	**0.001**

Note: Data are shown as mean ± standard error of the mean (SEM). p^a: difference in each group from baseline to 12 weeks, p^b: difference in each group from 12 to 20 weeks, p^c: difference in each group from baseline to 20 weeks. Differences were tested using the paired samples *t*-test. p_{12}: difference between the three groups at 12 weeks, p_{20}: difference between the three groups at 20 weeks. Differences were tested using one-way ANOVA. *p* time, *p* time × intervention: derived through comparisons from baseline to 12 weeks (0–12) and from 12 to 20 weeks (12–20) between the three groups adjusted for sex, age, metabolic syndrome, homeostatic model assessment for insulin resistance, body mass index and total fat mass by using a linear mixed effects model. Significant *p* values are bold.

Abbreviations: eTRF, early time-restricted feeding; lTRF, late time-restricted feeding.

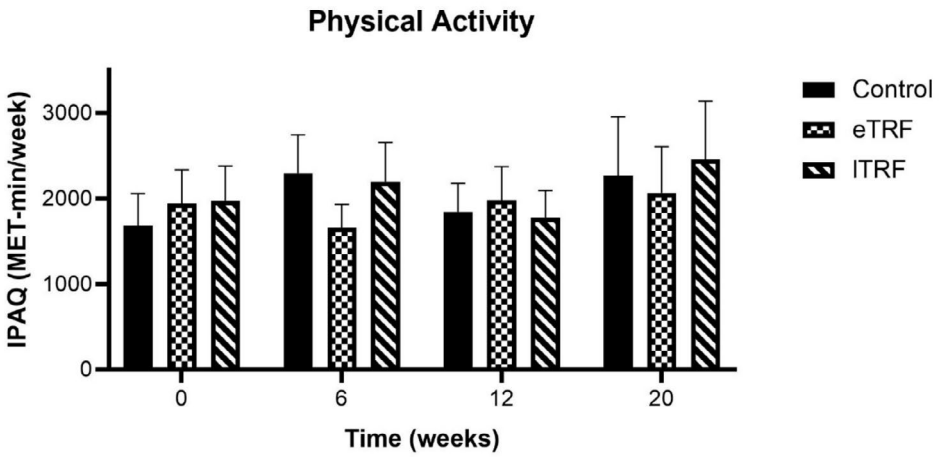

FIGURE 7 | Changes in physical activity from baseline to 6, 12 and 20 weeks (follow-up). Differences between groups were tested using one-way ANOVA at all time points, and paired samples *t*-test was used to test differences within each group (p for all >0.05). Data are presented as mean ± standard error of the mean (SEM), $n=59$. eTRF, early time-restricted feeding; IPAQ, International Physical Activity Questionnaire; lTRF, late time-restricted feeding.

DBP [54], with a 7 mmHg reduction in SBP potentially lowering major CVD events [25]. Weight loss likely moderates BP improvement [55].

Research indicates a 5% body weight reduction alleviates liver steatosis, whereas a 7%–10% loss is needed to reduce inflammation and histological disease activity [3]. All groups in our study achieved clinically significant weight loss and reduced liver steatosis. Studies on TRF, whether *ad libitum* or hypocaloric, in MASLD patients, report improvements in CAP measurements and intrahepatic fat [19–22]. Liver stiffness was improved only in the eTRF group, with no significant differences between groups at 12 weeks, suggesting the result may be statistically, not clinically, significant due to the study's short duration. Most studies also found no difference in liver stiffness between intervention groups [18, 19, 21, 22], except Wei et al.'s study, which noted improvements in both eTRF and control groups, but this was observed after a 12-month caloric restriction [19].

Our findings on liver enzymes are mixed and not entirely consistent with the existing literature. TRF and caloric restriction alone generally improve AST, ALT and GGT levels in MASLD patients [19–22]. However, in our study, eTRF improved only ALT levels, and lTRF decreased GGT levels, with no differences among the three groups at the intervention's end, consistent with most studies [19, 21, 22]. Additionally, we found that the unrestricted MD group improved AST, ALT and GGT levels, contrary to a recent meta-analysis of 10 RCTs by Del Bo et al., which showed no effect of MD on ALT and GGT levels [56]. Nevertheless, the liver enzyme levels do not adequately reflect the severity of liver damage [57].

The study has notable strengths. Few studies have compared early and late TRF, particularly in conjunction with a Mediterranean-type diet in MASLD individuals. This is the first study to measure glycaemic and insulinaemic responses after an OGTT in the MASLD population and to conduct an 8-week follow-up post-intervention. However, the study had limitations. Although participants were diagnosed with MASLD, indicating liver steatosis and at least one cardiometabolic risk factor [3], there was significant variability in these factors among participants. Future studies should address each factor individually to determine whether these dietary regimens are more beneficial, enabling more structured and individualised clinical practice for MASLD patients. Moreover, we did not measure circadian clock genes to evaluate changes in metabolic parameters considering circadian rhythms, which would be of great interest. Further RCTs with longer durations and larger samples are needed to validate these findings, analysed by both per protocol and intention-to-treat principles, and provide more evidence on the role of TRF and/or MD in modulating MASLD-related metabolic disorders.

5 | Conclusion

This 12-week RCT validated the positive effects of MD, the standard treatment for MASLD patients, on weight loss and cardiometabolic risk factors. Notably, combining MD with eTRF reduced HbA_{1c} and IR levels. However, late eating within the MD framework did not impair glucose metabolism or cardiometabolic profile, highlighting the importance of a balanced diet for those who eat late due to lifestyle. Our findings suggest that a 14:10 TRF protocol with a Mediterranean hypocaloric diet may be an effective, accepted and well-tolerated alternative nutritional treatment for MASLD individuals.

Author Contributions

Sofia Tsitsou: investigation, formal analysis, writing – original draft, methodology. **Triada Bali:** investigation. **Magdalini Adamantou:** investigation. **Aristi Saridaki:** investigation. **Kalliopi-Anna Poulia:** writing – review and editing. **Dimitrios S. Karagiannakis:** investigation. **Emilia Papakonstantinou:** conceptualization, supervision, writing – review and editing, methodology. **Evangelos Cholongitas:** conceptualization, project administration, writing – review and editing, methodology, investigation.

Acknowledgements

The authors are grateful to all the volunteers in this study for their participation. Some of the data were presented as an oral presentation at the 60th Annual Meeting of the European Association for the study of Diabetes (EASD) in 2024 and as a poster presentation at the 14th European Nutrition Conference (FENS) in 2023.

Ethics Statement

This study was conducted in accordance with the Declaration of Helsinki. It was approved by the Bioethics Committee of the Agricultural University of Athens (EIDE Reference Number: 40/27.04.2022) and the Scientific Committee of 'Laiko' General Hospital (716/26-11-2022).

Consent

Participants received all relevant information and provided written consent.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authorship

Guarantors of the article: Emilia Papakonstantinou and Evangelos Cholongitas.

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Supporting Information

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