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Title:

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Date:

2023-02-01

Citation:

Parr, E. B., Kouw, I. W. K., Wheeler, M. J., Radford, B. E., Hall, R. C., Senden, J. M., Goessens, J. P. B., van Loon, L. J. C. & Hawley, J. A. (2023). Eight-hour time-restricted eating does not lower daily myofibrillar protein synthesis rates: A randomized control trial. *Obesity*, 31 (S1), pp.116-126. <https://doi.org/10.1002/oby.23637>.

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## ORIGINAL ARTICLE

## Time-Restricted Eating

# Eight-hour time-restricted eating does not lower daily myofibrillar protein synthesis rates: A randomized control trial

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**Funding information**

European Society for Clinical Nutrition and Metabolism, Grant/Award Number: Early Career Fellowship (to IWKK)

**Abstract**

**Objective:** This study aimed to assess the impact of time-restricted eating (TRE) on integrated skeletal muscle myofibrillar protein synthesis (MyoPS) rates in males with overweight/obesity.

**Methods:** A total of 18 healthy males (age  $46 \pm 5$  years; BMI:  $30 \pm 2$  kg/m<sup>2</sup>) completed this exploratory, parallel, randomized dietary intervention after a 3-day lead-in diet. Participants then consumed an isoenergetic diet (protein:  $\sim 1.0$  g/kg body mass per day) following either TRE (10:00 AM to 6:00 PM) or an extended eating control (CON; 8:00 AM to 8:00 PM) protocol for 10 days. Integrated MyoPS rates were measured using deuterated water administration with repeated saliva, blood, and muscle sampling. Secondary measures included continuous glucose monitoring and body composition (dual-energy x-ray absorptiometry).

**Results:** There were no differences in daily integrated MyoPS rates (TRE:  $1.28\% \pm 0.18\%$  per day, CON:  $1.26\% \pm 0.22\%$  per day;  $p = 0.82$ ) between groups. From continuous glucose monitoring, 24-hour total area under the curve was reduced following TRE ( $-578 \pm 271$  vs. CON:  $12 \pm 272$  mmol/L  $\times$  24 hours;  $p = 0.001$ ). Total body mass declined (TRE:  $-1.6 \pm 0.9$  and CON:  $-1.1 \pm 0.7$  kg;  $p < 0.001$ ) with no differences between groups ( $p = 0.22$ ). Lean mass loss was greater following TRE compared with CON ( $-1.0 \pm 0.7$  vs.  $-0.2 \pm 0.5$  kg, respectively;  $p = 0.01$ ).

**Conclusion:** Consuming food within an 8-hour time-restricted period does not lower daily MyoPS rates when compared with an isoenergetic diet consumed over 12 hours. Future research should investigate whether these results translate to free-living TRE.

**INTRODUCTION**

Fasting is a common dietary strategy to improve health that has been practiced for decades [1]. However, the links between the feeding-

fasting cycle, the timing of meals, and the effects on circadian biology have only recently been appreciated [2]. One nutritional strategy that alters the feeding-fasting cycle is time-restricted eating (TRE), in which the duration between the first and last energy intake is

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restricted to ~8 to 10 hours during waking (daylight) hours [3]. Different durations of TRE have been investigated from as short as a 4-hour period [4, 5] to as long as a 12-hour period [6, 7], with numerous benefits for metabolic health [8–11]. Compared with other strategies that alter the feeding-fasting cycle (i.e., chronic energy restriction and intermittent fasting) and impart their therapeutic value from restricting energy intake, TRE confines food consumption to specified times of day to play off chronobiology [12, 13]. Weight loss generally accompanies TRE when energy intake is restricted [4, 14–17]. However, several measures of metabolic health are improved when TRE is compared with an energy-balanced extended period of eating (>12 hours), including increased insulin sensitivity, reduced blood pressure, and enhanced 24-hour glucose profiles [8, 9, 11]. However, the impact of isoenergetic TRE on skeletal muscle health is unknown.

Daily muscle protein synthesis (MPS) rates are largely determined by the frequent postprandial-induced increase in MPS following meal ingestion. Considering a single meal-sized amount of protein (~20 g) increases MPS rates for up to ~5 hours in healthy individuals [18, 19], three meals consumed over ~15 h/d would likely maximize MPS [20]. Conversely, restricting energy and protein intake to a shorter (~8 h/d) eating window may compromise the capacity to stimulate MPS. Over a prolonged period, the reduced window of protein intake via TRE may lead to lower lean mass and negatively impact metabolic health [21]. As MPS rates cannot be measured by long-term intervention studies (because of methodological limitations), and as body mass and composition have been previously reported to be altered by TRE interventions in the absence of energy restriction [22], the measurement of body composition of lean and fat mass after such protocols is important. In addition, whether the greater postabsorptive period due to prolonged fasting negatively affects daily MPS rates and, as such, lowers lean body mass has not been assessed.

Previous work from our laboratory demonstrated that regular protein intake (4 × 20 g every 3 hours) maximized daily rates of MPS compared with smaller repetitive (8 × 10 g) or two large bolus (40 g) feedings [20]. Here we conducted the first study, to our knowledge, to determine the effects of isoenergetic, protein-matched (isonitrogenic) TRE (8 hours) versus extended eating (CON; 12 hours) on daily rates of myofibrillar protein synthesis (MyoPS) during a 10-day intervention, with secondary supportive measures of body composition and blood glucose regulation. We hypothesized that isoenergetic, isonitrogenic, 8-hour TRE would reduce rates of MyoPS compared with eating over 12 hours and reduce lean mass.

## METHODS

### Study design

The study was a randomized, parallel-group design, prospectively registered on the Australian New Zealand Clinical Trials Registry (ACTRN12619000757112, [ANZCTR.org.au](http://ANZCTR.org.au)), approved by Australian Catholic University Human Research Ethics Committee (2018-291H),

### Study Importance

#### What is already known?

- Time-restricted eating (TRE) is an effective strategy to reduce energy intake and therefore lower body weight.
- Independent of energy restriction, TRE can improve blood glucose homeostasis.
- TRE may reduce lean mass through a reduced capacity for postprandial stimulation of muscle protein synthesis, although this hypothesis has not been experimentally tested.

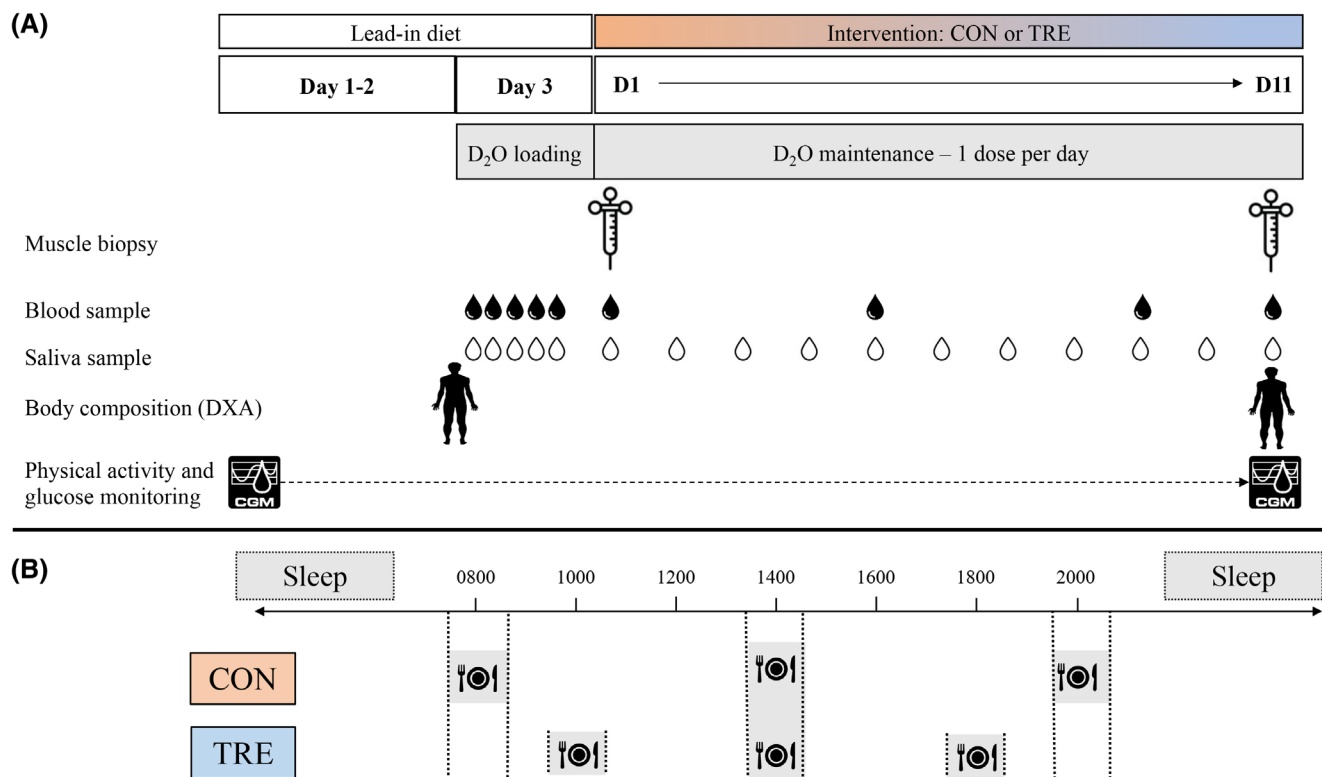
#### What does this study add?

- Short-term TRE (8 h/d) does not impair rates of muscle protein synthesis compared with an extended isoenergetic feeding condition (12 h/d).
- Reducing the daily “eating window” from 12 to 8 h/d, in which breakfast was delayed and dinner was earlier, improved daily blood glucose homeostasis despite the same amount of food being consumed over a 24-hour period.

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

- Short-term TRE does not impair rates of muscle protein synthesis in adults with overweight/obesity. However, long-term interventions are urgently needed to determine whether TRE-induced weight loss can be achieved without compromising muscle health.
- TRE improves daily blood glucose homeostasis and is an effective intervention for glucose management in people with impaired glucose tolerance and/or type 2 diabetes.

and conducted in accordance with the Declaration of Helsinki. The study was completed between May 2019 and March 2020 at Australian Catholic University St Patrick's (Melbourne) campus. This study is part of a large project, in which two groups of 8 to 10 males with overweight or obesity, but no other noncommunicable diseases, were enrolled in this 13-day protocol (Figure 1). Both groups completed a 3-day “lead-in” period, during which on Day 3, they spent 6 hours at the laboratory for a deuterated water loading day to rapidly increase plasma and body water (saliva) enrichments via the deuterated water (D<sub>2</sub>O) protocol [23]. The following day, a resting muscle biopsy was obtained from the vastus lateralis [24]. Participants were then randomized to a dietary intervention group for the subsequent 10 days, during which D<sub>2</sub>O was ingested daily as a sustained dose and a subsequent biopsy on Day 11 was obtained to assess integrated rates of MPS over the 10-day dietary intervention.



**FIGURE 1** (A) Overview of the 13-day study protocol in which participants completed 3 days of a lead-in diet prior to 10 days of provided meals. Integrated myofibrillar protein synthesis rates were measured over the 10-day intervention period using a deuterated water ( $^2\text{H}_2\text{O}$ ) protocol with repeated saliva, blood, and muscle samples for MPS rate calculations. Secondary measures included continuous glucose and physical activity monitoring and body composition (DXA) scans. (B) Three meals per day were consumed in either a TRE (8 hours, 10:00 AM to 6:00 PM) or an extended eating (CON; 12 hours, 8:00 AM to 8:00 PM) protocol. CGM, continuous glucose monitoring; CON, extended eating control; DXA, dual-energy x-ray absorptiometry; TRE, time-restricted eating

## Participants

A total of 18 recreationally active (engaging in sports or structured exercise  $\leq 3$  d/wk and not participating in any structured resistance exercise program and/or  $< 10,000$  steps per day), middle-aged (aged 35–55 years) males with overweight or obesity (BMI: 25–35 kg/m<sup>2</sup>) were recruited to participate via TrialFacts or our established participant database. Participants were randomized to one of four conditions (where only two of these conditions are being reported on in this paper; see Consolidated Standards of Reporting Trials [CONSORT] diagram in Supporting Information Figure S1) in blocks ( $n = 4$ ) using opaque envelopes by a colleague not involved in this project. Owing to the nature of the study, both participants and study staff were unblinded to conditions. COVID-19 restrictions in Victoria, Australia, prevented reaching the target total sample size ( $n = 44$ ), and randomization ceased at  $n = 41$  participants. Participant characteristics are presented in Table 1.

## Pretesting

Participants were initially screened to measure height, weight, and resting energy expenditure (REE; TrueOneRMR, Parvo Medic), as

previously reported [25], to ensure isoenergetic dietary provision. Within 28 days of their REE, participants collected their standardized meals to begin the 3-day lead-in period, at which point they were fitted with a continuous glucose monitor (CGM) sensor (FreeStyle Libre, Abbott GmbH; fitted on triceps area) and an ActiGraph accelerometer (ActiGraph GTX3+; waist-worn during waking hours only). These devices were worn continuously throughout the 13-day study period. Participants were asked to not perform any structured strenuous physical activity throughout the 13-day study period.

## Dietary intervention

Total daily estimated energy intake (kJ/d) was calculated using REE measured at the first baseline visit  $\times 1.4$  activity factor. All meals were provided to participants, and total energy intake (TEI; 100%) was individualized based on baseline energy requirements (using REE measured at the baseline visit  $\times 1.4$  activity factor), with matched macronutrient profiles (56% carbohydrate, 30% fat, and 14% protein 1.0 g/kg/d) across three meals per day (breakfast: 25% TEI, lunch: 35% TEI, dinner: 40% TEI). Participants consumed the meals at times specific to the randomized condition (Figure 1B), where the menu (see online Supporting Information) altered every second day. As well as

**TABLE 1** Characteristics of the study participants randomized to the CON diet group compared with the TRE group as measured at the end of the 3-day lead-in period

Variable	CON (n = 8)	TRE (n = 10)	p value
Age (y)	42 ± 6	48 ± 3*	0.01
Body composition/anthropometrics			
Total mass (kg)	96.5 ± 7.0	94.2 ± 14.0	0.68
Lean mass (kg)	62.5 ± 6.3	59.0 ± 5.5	0.20
Fat mass (kg)	30.6 ± 5.0	32.0 ± 9.1	0.71
Body fat (%)	32.8 ± 4.7	34.6 ± 4.6	0.43
Height (m)	1.78 ± 0.05	1.77 ± 0.05	0.53
BMI (kg/m <sup>2</sup> )	30.2 ± 2.4	29.9 ± 2.8	0.79
Waist circumference (cm)	101.6 ± 4.3	103.2 ± 9.1	0.36
Hip circumference (cm)	107.2 ± 6.4	105.8 ± 5.7	0.99
Physical activity			
Steps per day	10,505 ± 3540	12,397 ± 3802 <sup>a</sup>	0.31
Time spent sedentary (%)	69 ± 7	65 ± 9 <sup>a</sup>	0.27
Time spent in light PA (%)	27 ± 6	32 ± 9 <sup>a</sup>	0.30
Time spent in MVPA (%)	3 ± 3	4 ± 2 <sup>a</sup>	0.61
Valid wear time (min)	908 ± 48	952 ± 38 <sup>a</sup>	0.05
CGM metrics			
24-Hour total AUC (mmol/L)	7643 ± 810	8340 ± 894	0.10
Mean 24-hour glucose (mmol/L)	5.2 ± 0.6	5.8 ± 0.7	0.10
SD 24-hour glucose (mmol/L)	0.9 ± 0.2	1.1 ± 0.4	0.31
CV 24-hour glucose (%)	18 ± 4	19 ± 6	0.66
Fasting proxy glucose (mmol/L)	4.3 ± 0.5	4.7 ± 0.6	0.16
Fasted blood samples			
HbA <sub>1c</sub> (%)	5.4 ± 0.3	5.5 ± 0.5	0.73
Fasting glucose (mmol/L)	5.4 ± 0.6	5.3 ± 0.5	0.68
Fasting insulin (mIU/mL)	9.2 ± 6.4	11.1 ± 6.4	0.55
HOMA-IR	1.1 ± 0.8	1.3 ± 0.7	0.58
Total cholesterol (mmol/L)	4.8 ± 0.4	5.1 ± 1.1	0.44
LDL (mmol/L)	2.7 ± 0.6	2.8 ± 0.6	0.59
HDL (mmol/L)	1.2 ± 0.5	1.0 ± 0.4	0.57
Triglycerides (mmol/L)	2.1 ± 1.0	2.5 ± 1.8	0.59
Blood pressure			
SBP (mm Hg)	128 ± 6	129 ± 8	0.50
DBP (mm Hg)	81 ± 3	82 ± 5	0.80
Resting HR (beats per minute)	63 ± 7	67 ± 8	0.17

Note: Data are presented as mean ± SD.

Abbreviations: AUC, area under the curve; CGM, continuous glucose monitoring; CON, extended eating control; CV, coefficient of variation; DBP, diastolic blood pressure; HbA<sub>1c</sub>, glycated hemoglobin; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; HR, heart rate; LDL, low-density lipoprotein; MVPA, moderate and vigorous physical activity; PA, physical activity; SBP, systolic blood pressure; TRE, time-restricted eating.

\*Significantly ( $p < 0.05$ ) different to CON using independent samples  $t$  tests, with equal variance.

<sup>a</sup> $n = 9$  due to non-wear of monitor.

being isoenergetic and isonitrogenic, both groups had the same meal timing for the lead-in days (8:00 AM, 2:00 PM, and 8:00 PM). The CON group continued with these mealtimes for the 10-day experimental period. For the TRE group, the timing of breakfast was 2 hours later

and dinner was 2 hours earlier, such that the time window of eating was 4 hours shorter (10:00 AM, 2:00 PM, and 6:00 PM) each day. Participants were provided with a 13-day food diary in a handbook where foods were checked off to monitor compliance.

## Experimental protocol and monitoring

The 11-day D<sub>2</sub>O protocol (Figure 1A) was implemented to determine integrated rates of MPS via the specific measurement of myofibrillar (i.e., contractile) proteins. This protocol involved a “loading day,” on Day 3 of the lead-in diet, during which eight doses of 50 mL of 70 mol% enriched D<sub>2</sub>O (Sigma Aldrich) were ingested to increase body water enrichments and a daily 50-mL maintenance dose was ingested during the subsequent 10 days. Daily saliva samples were collected, and blood samples were taken on Days 1, 4, 8, and 11 of the intervention to assess the rise in <sup>2</sup>H-enrichment in body water and plasma, respectively. On Days 1 and 11, a skeletal muscle biopsy was obtained from the middle region of the medialis vastus lateralis, 15 cm above the patella and ~4 cm below entry through the fascia, using the percutaneous needle biopsy technique to measure muscle protein-bound [<sup>2</sup>H]-amino acid enrichment levels for the calculation of myofibrillar protein fractional synthesis rate (FSR; percent per day, primary outcome). Muscle biopsy samples were dissected and freed from any visible non-muscle material before being immediately frozen in liquid nitrogen and stored at –80 °C until further analysis.

Prior to the biopsy, a whole body dual-energy x-ray absorptiometry scan (GE Lunar iDXA Pro, enCORE software version 18) was conducted when participants were fasted and had voided their bladder. Participants were positioned using aids, as described previously [26], to allow the differentiation between trunk and limb sections. In our laboratory, dual-energy x-ray absorptiometry scans are reproducible with coefficients of variation (CV) of <1.5% (±0.7 kg lean mass, ±0.4 kg fat mass, ±0.8 kg total mass). Participant adherence to the D<sub>2</sub>O loading protocol was assessed via daily saliva samples and return of the empty D<sub>2</sub>O bottles. Also prior to the biopsy, fasted blood samples (6 mL) were collected in EDTA-containing tubes, a small sample of the whole blood was taken for glycated hemoglobin (HbA<sub>1c</sub>) and a lipid panel analysis (Cobas b 101, Roche Diagnostics Ltd), and the remaining sample was centrifuged at 1800g for 10 minutes at 4 °C. Aliquots of plasma were stored at –80 °C for later subsequent analysis. Saliva samples, using a cotton swab (Celluron), were collected before eating or drinking in the morning and stored at –80 °C.

## Plasma and muscle analyses

Body water enrichment was analyzed using the saliva samples collected on Days 1, 4, 8, and 11, as previously described [23]. Plasma amino acid enrichments were determined by gas chromatography–mass spectrometry analysis (Agilent 5975C MSD & 7890A GC) using samples collected on Day 1 and 11, as previously described [23]. Measurement of [<sup>2</sup>H]-alanine enrichment in the myofibrillar protein pools was done using ~60 mg of wet muscle that was freeze dried. From thawed plasma samples, glucose concentrations were measured in duplicate (YSI 2900 analyzer, YSI Life Sciences), and insulin concentrations were measured by enzyme-linked immunosorbent assay (ELISA) (Alpco Ltd.), with mean CV of 1.1% and 5.7%, respectively. Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using fasting glucose and insulin concentrations [27].

## Calculations

Myofibrillar protein FSR was determined using the incorporation of [<sup>2</sup>H]-alanine into muscle proteins and either mean free [<sup>2</sup>H]-alanine enrichment in plasma or mean body water (sampled from saliva) deuterium enrichment, corrected by a factor of 3.7 based on the deuterium labeling during de novo alanine synthesis. FSR was calculated using the standard precursor-product method:

$$\text{FSR (percent/day}^{-1}\text{)} = (E_{m2} - E_{m1} E_{\text{precursor}} \times t) \times 100\%.$$

Where  $E_{m1}$  and  $E_{m2}$  are the myofibrillar protein-bound enrichments on Days 1 and 11, respectively;  $E_{\text{precursor}}$  represents either mean free [<sup>2</sup>H]-alanine enrichment in plasma or body water deuterium enrichment corrected by a factor of 3.7 based on the deuterium labeling of alanine during de novo synthesis; and  $t$  represents the time between biopsies on Days 1 and 11.

## Data and statistical analyses

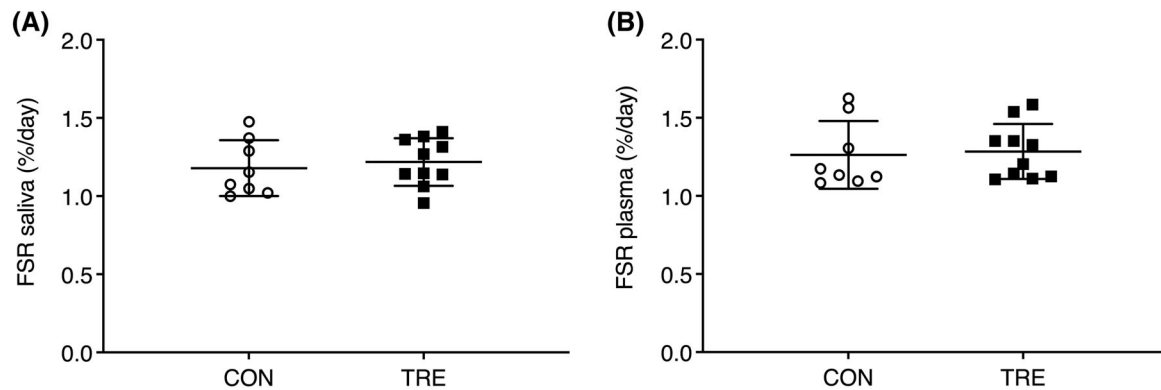
Analysis of FSL CGM data was performed using the continuously stored data (i.e., scanned data and the day of monitor insertion were not included) with the *GLU* package in R (version 4.0.5, *Shake and Throw*) [28] (<https://github.com/MRCIEU/GLU>). Only full days of data were included in the daily (24 hours) analysis, whereby other day imputation within condition (i.e., CON or TRE) was used for days with less than 6 hours of missing data (in periods of no more than 2 hours of missing data) [28]. Mean, SD, and CV were calculated using the 24-hour imputed data set. Total area under the curve (AUC) was calculated for 24-hour periods using the trapezoid method with a baseline of 0. A proxy of fasting glucose was measured using the lowest 30-minute average glucose concentration during the night (defined as 11:00 PM to 6:30 AM) period [28]. For the postprandial CGM data, meals were included when the glucose data was complete for the 2-hour period after the mealtime, using the prescribed mealtime for each condition. Postprandial incremental 1- and 2-hour AUC was calculated using the trapezoid method (using premeal glucose concentration as baseline). Details of the physical activity monitor analysis are in ESM.

The power calculations used to determine sample size were for the comparison of the continuous energy restriction (as described in the clinical trial registration) and CON conditions (online Supporting Information). The TRE arm was added, as a prespecified and prospective secondary comparison, to use and directly compare with the CON group. Statistical analyses were performed using SPSS Statistics (version 25, IBM Corp.). Baseline variables and the primary outcome, of muscle protein FSR, were compared using independent samples *t* tests, using equal variances based on nonsignificant Levene *F* tests to assess differences between groups. The change score, between the intervention and lead-in period, generated for each secondary or supporting measure was used to compare between conditions (i.e., CON vs. TRE) using ANCOVA, with baseline data as the

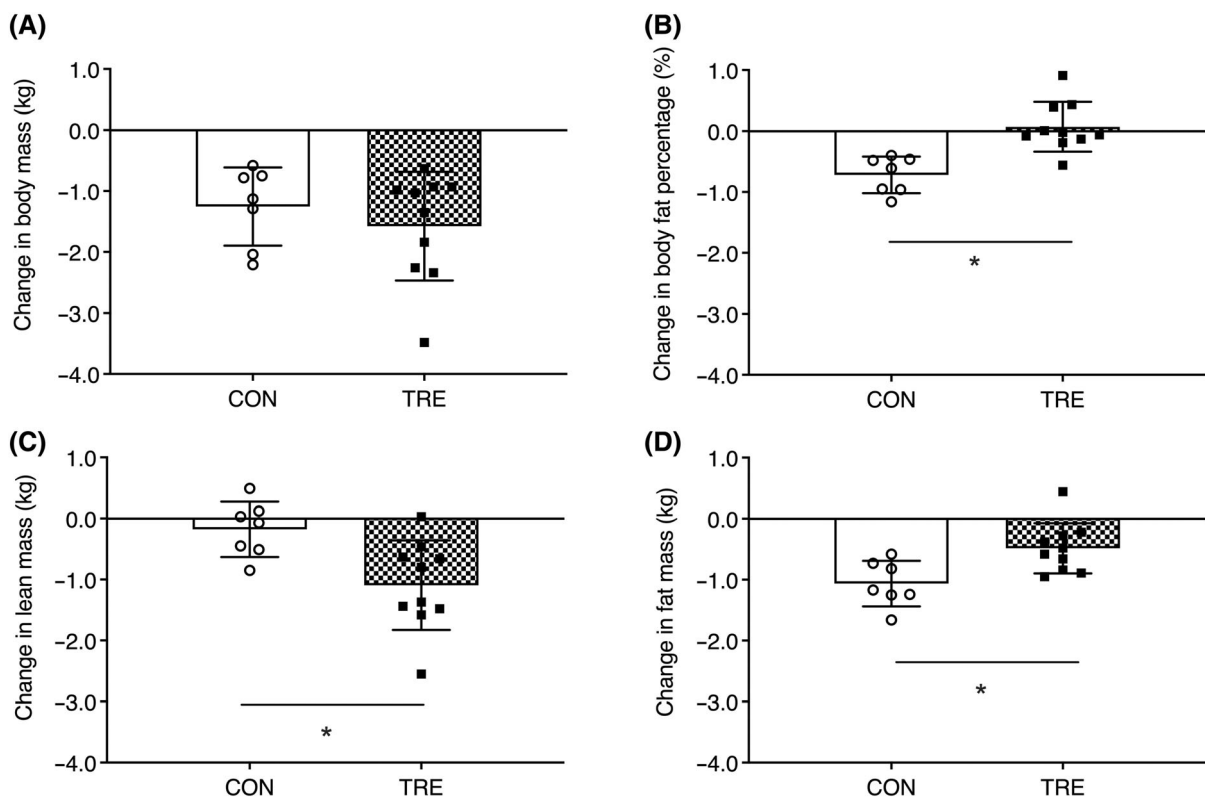
covariate for each measure. When multiple time points were measured, that is, the saliva and plasma precursor enrichments, repeated measures ANOVA of condition by time was conducted. Significance was set at  $p < 0.05$  and all data are presented as mean  $\pm$  SD, with mean difference and 95% confidence interval (CI) of differences reported when appropriate.

## RESULTS

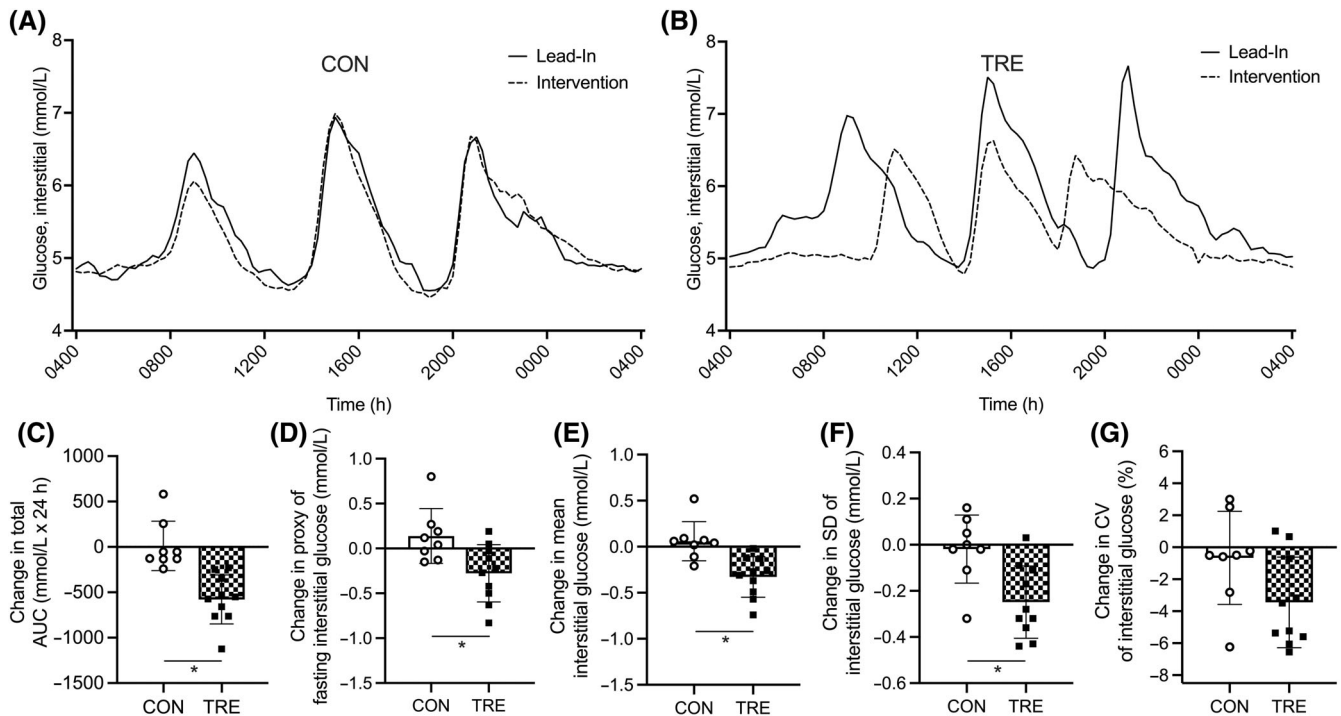
There were no differences in baseline metrics between conditions except for age, where the participants randomized to TRE were older (+6 years, 95% CI: 2-10 years,  $p = 0.01$ ; Table 1). Levels of physical activity were consistent between the lead-in and intervention periods



**FIGURE 2** Myofibrillar protein FSR, measured via (A) saliva and (B) plasma, was assessed in males after 10 days of consuming meals in either a CON (8:00 AM to 8:00 PM; white circles) or TRE (10:00 AM to 6:00 PM; black squares) pattern. Data are mean  $\pm$  SD, with individual data points. No differences between conditions were observed using independent samples *t* tests. CON, extended eating; FSR, fractional synthetic rates; TRE, time-restricted eating



**FIGURE 3** Dual-energy x-ray absorptiometry-derived estimates of (A) body mass, (B) body fat percentage, (C) lean mass, and (D) fat mass after 10 days of following a CON (8:00 AM to 8:00 PM;  $n = 7$ ; white bars and circles) or TRE (10:00 AM to 6:00 PM;  $n = 10$ ; black bars and squares) protocol in males with overweight or obesity. Data are mean  $\pm$  SD, with individual data points. Analyzed using ANCOVA with baseline as the covariate: \*significantly different between conditions, TRE versus CON,  $p < 0.01$ . CON, extended eating control; TRE, time-restricted eating



**FIGURE 4** Summary data from FreeStyle Libre continuous glucose monitors (measuring interstitial glucose concentrations [mmol/L]) displaying mean 24-hour glucose profiles between the lead-in (solid lines) and intervention (dotted lines) periods for (A) the CON condition ( $n = 8$ ) and (B) the TRE condition ( $n = 10$ ); and the change in (C) total AUC (mmol/L  $\times$  24 hours), (D) a proxy for fasting glucose (lowest 30-minute concentration between 11:30 PM and 6:30 AM; mmol/L), (E) mean interstitial glucose concentration (mmol/L), (F) SD of interstitial glucose concentrations (mmol/L), and (G) CV of interstitial glucose concentrations, between the extended eating (CON, 8:00 AM to 8:00 PM;  $n = 8$  day; white bars and circles) and TRE (10:00 AM to 6:00 PM;  $n = 10$ , black bars and squares) intervention periods in males with overweight or obesity. Data are mean  $\pm$  SD, with individual data points. Significant differences observed between conditions were measured using ANCOVA with baseline measures as the covariate, \*significantly different from CON ( $p < 0.05$ ). AUC, area under the curve; CON, extended eating control; CV, coefficient of variation; TRE, time-restricted eating

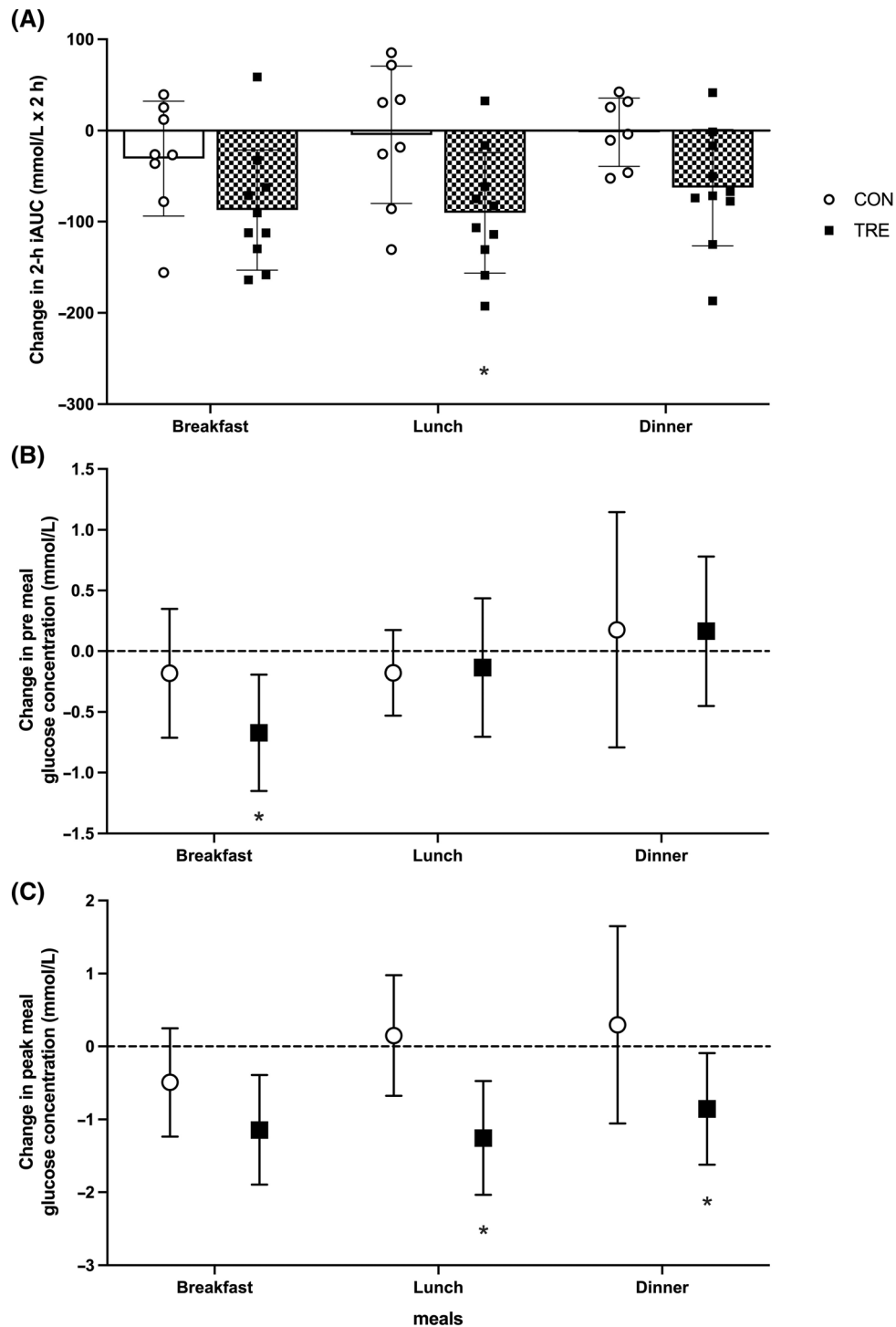
for both conditions (Supporting Information Table S1). There were no differential changes between groups for fasting circulating metabolite concentrations (glucose, insulin, cholesterol, triglycerides, or HbA<sub>1c</sub>) or blood pressure (Supporting Information Table S1). Adherence to the D<sub>2</sub>O protocol and meal schedule was 100% with all food checklists completed, and no side effects were reported.

Myofibrillar protein FSR was not different between the TRE and CON conditions for either saliva (TRE:  $1.18\% \pm 0.18\%$  per day vs. CON:  $1.22\% \pm 0.15\%$  per day,  $p = 0.62$ ; Figure 2A) or plasma (TRE:  $1.28\% \pm 0.18\%$  per day vs. CON:  $1.26\% \pm 0.22\%$  per day,  $p = 0.82$ ; Figure 2B) free [<sup>2</sup>H]-alanine enrichments as precursors. There were no differences between conditions for the precursor measures of body water deuterium enrichment (TRE:  $0.69\% \pm 0.09\%$  vs. CON:  $0.67 \pm 0.07\%$ ,  $p = 0.57$ ; Supporting Information Figure S2A) or plasma [<sup>2</sup>H]-alanine enrichment (TRE:  $2.44\% \pm 0.29\%$  vs. CON:  $2.33\% \pm 0.25\%$ ,  $p = 0.41$ ; Supporting Information Figure S2B), but there were significant effects of time ( $p < 0.001$ ). No differences between conditions were measured for myofibrillar protein-bound alanine enrichments (Supporting Information Figure S2C).

Total body mass was reduced across the intervention ( $-1.4 \pm 0.8$  kg,  $p < 0.001$ ), but the changes in total body mass were not different between conditions ( $p = 0.22$ ; Figure 3A). One participant was excluded

from the body composition analysis owing to measurement error; therefore, there were  $n = 7$  in CON for the following body composition measures. Total fat mass was reduced across the intervention ( $-0.7 \pm 0.5$  kg,  $p = 0.01$ ), but to a greater extent in the CON ( $-0.6$  kg, 95% CI:  $-0.2$  to  $-1.0$  kg,  $p = 0.01$ ) compared with the TRE condition (Figure 3D). Total lean mass was reduced across the intervention ( $-0.7 \pm 0.7$  kg,  $p = 0.01$ ), with TRE participants losing more lean mass ( $-1.0$  kg, 95% CI:  $-0.3$  to  $-1.7$  kg,  $p = 0.01$ ) than the CON participants (Figure 3C). Lean mass loss in the appendicular (arm + leg) regions was not different between conditions ( $p = 0.51$ ), but there was greater trunk lean mass loss in the TRE group ( $-0.7$  kg, 95% CI:  $+0.3$  to  $-1.2$  kg,  $p < 0.01$ ; Supporting Information Figure S3). Subsequently, the change in body fat percentage was different between groups: CON decreased body fat percentage to a greater extent ( $-0.8\%$ , 95% CI:  $-0.4$  to  $-1.2\%$ ,  $p = 0.001$ ; Figure 3B) than the TRE condition.

A total of 197 (84%) 24-hour days were included in the CGM analysis. The grouped average CGM interstitial glucose concentrations over 24 hours (Figure 4A,B) show that the dietary protocols were well adhered to in both conditions. Total 24-hour AUC was reduced to a greater extent in the TRE group compared with CON ( $-579$  mmol/L  $\times$  24 hours [ $-7\%$ ], 95% CI:  $-270$  to  $-888$  mmol/L  $\times$  24 hours,  $p = 0.001$ ; Figure 4C). The CGM-measured proxy for fasting glucose



**FIGURE 5** Postprandial (2 hours) data from FreeStyle Libre continuous glucose monitors (measuring interstitial glucose concentrations [mmol/L]) displaying the change in (A) 2-hour iAUC (mmol/L  $\times$  2 hours), (B) premeal interstitial glucose concentration (mmol/L), and (C) peak interstitial glucose concentration (mmol/L), between the CON (12 hours; breakfast = 8:00 AM [ $n = 8$ ], lunch = 2:00 PM [ $n = 8$ ], dinner = 8:00 PM [ $n = 7$ ]; white bars and circles) and TRE (8 hours; breakfast = 10:00 AM [ $n = 10$ ], lunch = 2:00 PM [ $n = 10$ ], dinner = 6:00 PM [ $n = 10$ ]; black bars and squares) intervention periods in males with overweight or obesity. Data are mean  $\pm$  SD. Significant differences observed between conditions, within meal, were measured using ANCOVA with baseline measures as the covariate, \*significantly different from CON ( $p < 0.05$ ). CON, extended eating control; iAUC, incremental area under the curve; TRE, time-restricted eating

was also reduced in TRE compared with the CON condition ( $-0.3$  mmol/L, 95% CI:  $-0.005$  to  $-0.6$  mmol/L,  $p = 0.05$ ; Figure 4D). The change in mean and SD of 24-hour glucose concentrations

was lower in the TRE (mean:  $-0.3$  mmol/L, 95% CI:  $-0.1$  to  $-0.6$  mmol/L,  $p < 0.01$ , Figure 4E; SD:  $-0.2$  mmol/L, 95% CI:  $-0.05$  to  $-0.3$  mmol/L,  $p = 0.01$ , Figure 4F) than the CON condition. There

was a tendency for CV to also be lower following the TRE intervention compared with CON ( $-2.4\%$ , 95% CI:  $+0.1\%$  to  $-5.0\%$ ,  $p = 0.06$ ; Figure 4G).

The TRE intervention also modified the 2-hour postprandial responses (Figure 5). Specifically, delaying breakfast in TRE reduced the premeal glucose concentration compared with CON ( $-0.3$  mmol/L, 95% CI:  $-0.01$  to  $-0.6$  mmol/L,  $p = 0.04$ ; Figure 5B). In response to the lunch (second) meal, 2-hour incremental AUC (iAUC) and peak glucose concentration were reduced by the TRE intervention compared with CON (2-hour iAUC:  $-74$  mmol/L  $\times$  2 hours, 95% CI:  $-10$  to  $-138$  mmol/L  $\times$  2 hours,  $p = 0.03$ ; peak:  $-1.1$  mmol/L, 95% CI:  $-0.4$  to  $-1.8$  mmol/L,  $p < 0.01$ ; Figure 5A,C). The postprandial peak glucose concentration after the dinner meal was also reduced by the TRE intervention compared with CON ( $-0.8$  mmol/L, 95% CI:  $-0.06$  to  $-1.6$  mmol/L,  $p = 0.04$ ; Figure 5C). No changes were observed in the mean 2-hour postprandial glucose concentration or time to peak glucose, and the 1-hour iAUC mirrored the 2-hour iAUC outcomes (Supporting Information Figure S4).

## DISCUSSION

To our knowledge, this is the first study to determine the effects of short-term, isoenergetic, 8-hour TRE on rates of MPS in males with overweight or obesity. Contrary to our hypothesis, we report no negative impact of TRE on rates of MyoPS when all meals were consumed within 8 hours compared with an isoenergetic 12-hour eating window. Although both groups lost total body mass over the 10 days, TRE induced greater declines in total lean mass, via loss of trunk lean mass, and led to improvements in glycemic control over 24 hours and in the postprandial periods.

Daily MPS rates are maximized when protein intake is evenly spread across multiple servings throughout the day [20]. As such, we hypothesized that reducing the eating window would negatively impact MPS rates. The current study provides evidence that, in the short term, 8-hour TRE is a safe and practical intervention that does not impair rates of MPS. It is highly likely that the isonitrogenic protein intake (1.0 g/kg body mass per day), which was relatively evenly spread across three meals, contributed to the lack of differences in MPS rates between the 8- and 12-hour eating window. In the present study we aimed to assess the impact of TRE per se, as opposed to the impact of TRE in a setting of overall dietary intake restriction. Therefore, whether overall MPS rates are maintained in a free-living TRE environment when energy and protein restriction may be a result of TRE, and over longer periods (i.e., several weeks to several months), requires further investigation. The effect of a shorter eating window, such as 4- to 6-hour TRE, is likely to reduce MPS rates, although this needs to be determined. Under the conditions of the present study, the redistribution of daily energy and protein intake did not reduce average daily MPS rates (Figure 2). Although we hypothesized that the restricted eating window would lower daily rates of MPS during a 10-day dietary intervention, it is possible that the more condensed eating time frame allows the postprandial period to be extended,


thereby offsetting any impact on the acute anabolic response to the various meals. The latter may result in the absence of obvious differences in MPS rates when assessed over multiple days of an intervention.

Weight loss is the most common primary outcome measure in free-living TRE interventions [15, 17, 22, 29] and a desirable outcome for many individuals with overweight or obesity. Although unintentional, the present isoenergetic intervention did result in a small but significant loss of body mass (approximately  $-1.4$  kg,  $-1.5\%$ ) in both groups with no changes in physical activity levels. The loss of body mass likely indicates that there was a mismatch between habitual energy intake and the estimates of total energy requirements, from measurements of REE and an activity factor of 1.4, as we failed to achieve energy balance. Changes in body composition due to weight loss have been infrequently measured across TRE investigations to date [17, 22, 30, 31] and, because of the isoenergetic TRE employed in the current study, the assessments of body mass and composition were ancillary measures. The decline in body mass was, at least partly, attributed to fat mass loss, with a greater loss of fat mass in the CON treatment ( $-0.5$  kg TRE,  $-1.0$  kg CON). We also observed a small but significant decline in lean mass in the TRE group over 10 days, aligning with previous reports over a 12-week TRE intervention [22]. Whether the decline in lean mass after TRE reflects skeletal muscle mass is questionable, as we observed differences only in trunk lean mass loss (TRE:  $-0.7 \pm 0.5$  vs. CON:  $0.0 \pm 0.3$  kg) and not appendicular lean mass (TRE:  $-0.4 \pm 0.6$  vs. CON:  $-0.3 \pm 0.6$  kg). Although dietary intake was controlled, fluid intake was not controlled, it is possible that participants limited their fluid intake to when they were eating, and thus, the TRE conditions induced unintended fluid intake reductions. Therefore, our data indicate that the observed differences in lean mass loss likely reflect changes in fluid, organ, and/or digestive system mass.

Clearly, isoenergetic 8-hour TRE does not seem to lower daily MPS rates and, as such, may not compromise muscle health. Several well-conducted energy matched TRE studies have previously demonstrated a number of metabolic health benefits (improved insulin sensitivity, lower blood pressure, reduced 24-hour glucose concentrations) independent of weight loss [8, 9, 11]. Most of the TRE interventions to date that have demonstrated benefits to 24-hour glucose control have been early TRE [8, 9], in which the eating window is altered by changing the timing of the last (evening) meal to earlier than 3:00 PM. In the present study, and our previous work [11], breakfast was delayed by 2 hours (to 10:00 AM) to enable a socially acceptable dinner time ( $\sim 6:00$  PM) and still promote beneficial glycemic control outcomes measured using both venous samples and interstitial glucose via CGM. The possible mechanisms for how the same meals, consumed over a 4-hour shorter time window, improve total AUC, mean glucose, and reduced glycemic variability (i.e., lower SD) are likely due to persistent elevations in circulating insulin concentrations remaining elevated after breakfast for the subsequent meals. Consequentially, elevated insulin concentrations after breakfast have reduced the postprandial glucose AUC at lunch and lowered peak glucose concentrations at lunch and dinner. The reduced fasting and prebreakfast

interstitial glucose concentrations may be due to the extended time since waking allowing cortisol-driven endogenous glucose to be used prior to exogenous sources appearing in the circulation. Together with previous controlled TRE investigations [9, 11], the collective improved interstitial glucose metrics reported here support TRE as an effective strategy for improved glycemic management.

There are several strengths and limitations of this study. First, by providing all meals to participants over the intervention period, we have conducted the longest investigation of TRE and MPS to date using the novel deuterated water and muscle biopsy sampling methodology. Second, although we provided meals based on total energy intake calculated from REE, our participants lost body mass and therefore were likely to have been in negative energy balance. Finally, the study included males only; therefore, these results cannot be translated to both sexes. Important in the comparison with ad libitum TRE, our results are proof of concept of what occurs when energy and macronutrient intakes are matched and total protein intakes are slightly above recommended dietary intake levels. When applying TRE in a setting of energy restriction, as is common in ad libitum TRE, and therefore reduced total protein intake, it is possible that this may lower MPS and reduce appendicular lean mass. However, in this study we highlight that 8-hour TRE does not compromise MPS with an isoenergetic, isonitrogenic diet when compared with extended intake over 12 hours.

In conclusion, this exploratory study found that consuming food within an 8-hour time-restricted window did not lower daily MyoPS rates compared with when an isoenergetic amount of food was consumed following a more conventional (12 hours) daily eating window. Whether the lack of change to MPS is evident in free-living TRE environments that, by nature, may inadvertently reduce total energy and protein intakes, requires further research. TRE can improve daily glycemic control and lower postprandial glycemic excursions over a 24-hour period, supporting the widespread use of time-restricted eating to align dietary intake with circadian rhythms. 

#### AUTHOR CONTRIBUTIONS

Evelyn B. Parr, Imre W.K. Kouw, Luc J.C. van Loon, and John A. Hawley conceived the experiments. Evelyn B. Parr, Imre W.K. Kouw, Michael J. Wheeler, Bridget E. Radford, and Rebecca C. Hall carried out the experiments. Evelyn B. Parr, Imre W.K. Kouw, Michael J. Wheeler, Joan M. Senden, and Joy P. B. Goessens analyzed and interpreted the data. Evelyn B. Parr and Imre W.K. Kouw wrote the first draft of the manuscript. All authors were involved in reviewing and editing the paper and had final approval of the submitted and published versions.

#### ACKNOWLEDGMENTS

We would like to thank our study participants, Dr. Andrew Garnham (Australian Catholic University) for his expertise and skill in performing the muscle biopsy sampling, Dr. Rich Johnson for his statistical advice, and the volunteer placement students for their contribution to this work. Individual deidentified data used for this article (text, tables, and

figures), study protocol, statistical analysis plan, and analytic code are available upon a reasonable request to the corresponding author. Open access publishing facilitated by Australian Catholic University, as part of the Wiley - Australian Catholic University agreement via the Council of Australian University Librarians.

#### CONFLICT OF INTEREST

The authors declared no conflict of interest.

#### CLINICAL TRIAL REGISTRATION

Australian New Zealand Clinical Trials Registry  
ACTRN12619000757112.

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## SUPPORTING INFORMATION

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

**How to cite this article:** Parr EB, Kouw IWK, Wheeler MJ, et al. Eight-hour time-restricted eating does not lower daily myofibrillar protein synthesis rates: A randomized control trial. *Obesity (Silver Spring)*. 2023;31(Suppl. 1):116-126. doi:[10.1002/oby.23637](https://doi.org/10.1002/oby.23637)