International Doctoral Thesis / Tesis Doctoral Internacional

WHEN TO EAT: INVESTIGATING THE ROLE OF MEAL TIMING AND INTERMITTENT FASTING IN BODY COMPOSITION AND CARDIOMETABOLIC HEALTH IN HUMANS

CUANDO COMER: INVESTIGANDO EL ROL DEL HORARIO DE LAS COMIDAS Y DEL AYUNO INTERMITENTE EN LA COMPOSICIÓN CORPORAL Y LA SALUD CARDIOMETABÓLICA EN HUMANOS

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CERTIFICA:

Que la Tesis Doctoral titulada **"when to eat: investigating the role of meal timing and intermittent fasting in body composition and cardiometabolic health in humans"** que presenta D. Manuel Dote Montero al superior juicio del Tribunal que designe la Universidad de Granada, ha sido realizado bajo mi dirección durante los años 2019-2023, siendo expresión de la capacidad técnica e interpretativa de su autor en condiciones tan aventajadas que le hacen merecedor del Título de Doctor, siempre y cuando así lo considere el citado Tribunal.

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En Granada, a 20 de mayo de 2023

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El doctorando **D. Manuel Dote Montero** y el director de la tesis Dr. D. Jonatan Ruiz Ruiz:

Garantizamos, al firmar esta Tesis Doctoral, que el trabajo ha sido realizado por el doctorando bajo la dirección del director de la tesis y hasta donde nuestro conocimiento alcanza, en la realización del trabajo, se han respetado los derechos de otros autores al ser citados, cuando se han utilizado sus resultados o publicaciones.

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Doctorando

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En Granada, a 20 de mayo de 2023

El doctorando D. Manuel Dote Montero ha realizado la presente Tesis Doctoral Internacional siendo beneficiaria de un contrato con cargo al programa de Formación de Profesorado Universitario (FPU18/03357) del Ministerio de Ciencia, Innovación y Universidades (actualmente Ministerio de Universidades), por resolución de 4 de octubre de 2018, de la Secretaría de Estado de Universidades, Investigación, Desarrollo e Innovación (BOE-B-2018-48361, publicado el 9 de octubre de 2018).

"If nature is the answer, what was the question?"

"Si la naturaleza es la respuesta, ¿cuál era la pregunta?"

Jorge Wagensberg

❖

A mi madre, ejemplo de constancia y perseverancia

A mi padre, por transmitirme el gozo intelectual

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Research projects and funding

The present International Doctoral Thesis was performed under the framework of the ACTIBATE STUDY (ClinicalTrials.gov:http://bitly.ws/Iqp9) and the EXTREME STUDY (ClinicalTrials.gov: https://bit.ly/3HUyhf9). The ACTIBATE STUDY was funded by the following organizations:

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List of abbreviations

Acc: accelerometry.

ACTIBATE: activating brown adipose tissue thought exercise.

ADF: alternative-day fasting.

ADMF: alternative-day modified fasting.

AEBQ: Adult Eating Behavior Questionnaire.

AEE: activity energy expenditure.

AIDS: Acquired immunodeficiency syndrome.

ALP: alkaline phosphatase.

ALT: alanine aminotransferase.

APOA1: apolipoprotein A.

APOB: apolipoprotein B.

Appendi: appendicular.

BDI-FS: Beck Depression Inventory Fast Screen.

BP: blood pressure.

CGM, continuous-glucose monitoring.

CHO: carbohydrates.

CI: confidence intervals.

Cons.: breakfast consumers.

CONSORT: Consolidated Standards of Reporting Trials.

CR: Caloric restriction.

CVD: cardiovascular.

DASH: dietary approaches to stop hypertension.

DII: dietary inflammatory index.

DQI: dietary quality indices.

DXA: dual-energy X-ray absorptiometry.

ECG: electrocardiogram.

EE: energy expenditure.

EQ-5D-5L: EuroQol 5 dimensions 5 levels.

FCI: Food Craving Inventory.

FFA: free fatty acids.

FPG: Fasting plasma glucose.

GGT: gamma-glutamyltransferase.

GH: grow hormone.

HbA1c: glycated haemoglobin.

HDL-C: high-density lipoprotein cholesterol.

HIV: human immunodeficiency virus.

HOMA-IR: homeostasis model assessment of insulin resistance.

IF: intermittent fasting.

iMUDS: Instituto Mixto Deporte y Salud.

IPAQ: International physical activity questionnaire.

LDL-C: low-density lipoprotein cholesterol.

MCTQ: Munich Chronotype Questionnaire.

MD: mean difference.

MeD-DQI: dietary quality index for the Mediterranean diet.

MeD-P: a priori Mediterranean dietary pattern.

MeD-S: Mediterranean diet score.

MEQ-SA: Horne y Östberg.

MRI: magnetic resonance imaging.

PA: physical activity.

PSQI: Pittsburgh Sleep Quality Index.

PSS: Perceived Stress Scale.

QUICKI: quantitative insulin-sensitivity check index.

RER: respiratory exchange ratio.

RMR: resting metabolic rate.

SEE: sleeping energy expenditure.

SF-36: Rand Short Form 36.

Skip.: breakfast skippers.

SPSS: Statistical Package for the Social Sciences.

STAI: State-Trait Anxiety Inventory.

TD2M: type 2 diabetes mellitus.

TDEE: total daily energy expenditure.

TEF: thermic effect of food.

TRE: time-restricted eating.

UC: usual-care.

VAT: Visceral adipose tissue.

WC: waist circumference.

WL: weight loss.

Abstract

Meal timing and intermittent fasting have emerged as potential dietary interventions for managing obesity. However, their impact on human health is still not fully comprehended.

The objectives of the present International Doctoral Thesis are to summarize the effects of various types of intermittent fasting on body composition and cardiometabolic health in humans, with a particular emphasis on energy metabolism (**Chapter 1**). Additionally, the thesis aims to explore the relationship between meal timing with body composition and cardiometabolic risk factors in young men and women (**Chapter 4**). Furthermore, it seeks to assess the efficacy of three different 8-hour time-restricted eating schedules (early, late and self-selected vs. usual-care) on visceral adipose tissue, body composition and cardiometabolic health in men and women with overweight/obesity and slight metabolic impairments (**Chapter 5**).

The results of this thesis suggest that meal timing is not cross-sectionally associated with body composition in young men and women. However, it was observed that a longer daily eating window and a shorter time from midsleep point to first food intake (i.e., earlier first food intake in a 24-hour cycle) are associated with better cardiometabolic health in young men (**Chapter 4**). The thesis also highlights that self-selected time-restricted eating (TRE) may be an effective strategy for reducing visceral adipose tissue in men. Additionally, implementing an 8-hour TRE, regardless of the timing of the eating window, as part of a usual-care intervention without specific energy restriction resulted in greater body weight loss in both men and women, although no differences between intervention groups were observed in the changes in body composition or cardiometabolic risk factors. It is noteworthy that weight loss exceeding a clinically meaningful threshold (≥5%) was observed in some individuals, leading to improvements in cardiometabolic health (**Chapter 5**).

Based on the findings of this International Doctoral Thesis, an 8-hour TRE can be considered a simple strategy for clinicians to teach their patients during routine care. It leads to greater body weight loss compared to a usual-care intervention.

Resumen

El horario de las comidas y el ayuno intermitente han surgido como posibles intervenciones dietéticas para el tratamiento de la obesidad. Sin embargo, su impacto en la salud humana aún no se comprende completamente.

Los objetivos de esta Tesis Doctoral Internacional son resumir los efectos de varios tipos de ayuno intermitente en la composición corporal y la salud cardiometabólica en humanos, con un énfasis particular en el metabolismo energético (**Capítulo 1**). Además, explora la relación entre el horario de las comidas y la composición corporal y los factores de riesgo cardiometabólico en hombres y mujeres jóvenes (**Capítulo 4**). Asimismo, evalúa la eficacia de tres esquemas diferentes de 8 horas de restricción de la ingesta nutricional (temprano, tardía, autoseleccionado vs. tratamiento habitual) en el tejido adiposo visceral, la composición corporal y la salud cardiometabólica en hombres y mujeres con sobrepeso/obesidad y leves alteraciones metabólicas (**Capítulo 5**).

Los resultados de esta tesis sugieren que el horario de las comidas no está asociado transversalmente con la composición corporal en jóvenes. Sin embargo, una ventana de alimentación más prolongada y un tiempo más corto desde el punto medio del sueño hasta la primera ingesta de alimentos (es decir, una primera ingesta de alimentos más temprana en un ciclo de 24 horas) están relacionados con una mejor salud cardiometabólica en hombres (**Capítulo 4**). La tesis destaca que un esquema autoseleccionado de restricción temporal de la ingesta nutricional puede ser una estrategia efectiva para reducir el tejido adiposo visceral en hombres. Además, implementar una restricción temporal de 8 horas de la ingesta nutricional, independientemente del momento del día, como parte de una intervención estándar sin restricción específica de energía, resultó en una mayor pérdida de peso corporal tanto en hombres como en mujeres. No se observaron diferencias entre los grupos de intervención en los cambios en la composición corporal o los factores de riesgo cardiometabólico. Se observó una pérdida de peso que superó un umbral clínicamente relevante (≥5%) en algunos individuos, lo cual condujo a mejoras en la salud cardiometabólica (**Capítulo 5**).

Basándose en los hallazgos de esta Tesis Doctoral Internacional, una restricción temporal de 8 horas de la ingesta nutricional puede considerarse una estrategia sencilla a implementar en atención primaria y produce a una mayor pérdida de peso corporal que una intervención estándar.


Eat
Fast
16 hrs

GENERAL INTRODUCTION

Chapter 1. Effects of intermittent fasting on cardiometabolic health: An energy metabolism perspective

Introduction

Caloric restriction (CR), a sustained reduction in energy intake while maintaining optimal nutrition, is probably the most effective non-pharmacological intervention to extend healthspan[1,2]. However, prescribing continuous daily CR results in poor long- term adherence[3], which can be attributed to biological (e.g., increased appetite), behavioral (e.g., social events), psychosocial (e.g., elevated food reward), and environmental (e.g., availability of high-caloric palatable foods) factors[1-3]. Over the past decade or so, intermittent fasting (IF) has emerged as a promising alternative to continuous/traditional CR[4]. In simple terms, IF consists of alternating fasting and unrestricted eating periods, which may facilitate adherence[5]. As shown in **Figure 1**, IF can be categorized into six different approaches: religious fasting, alternative-day fasting (ADF), alternative-day modified fasting (ADMF), twice-weekly fasting usually called the "5:2 diet", modified periodic fasting usually called "fasting-mimicking diet", and time-restricted eating (TRE). Religious fasting integrates a myriad of modalities, among which Ramadan is probably the most extended form. While doing Ramadan, Muslims abstain from all forms of food and drink during the daylight hours, thus concentrating all food and liquid intake during the night[6]. ADF consists of alternating 24-hour fast periods with the unrestricted intake of food during the subsequent 24 hours, repeating that for multiple days or weeks. Similarly, the ADMF modality severely limits food intake (usually to 25% of habitual energy needs or ~500 kcal/day) during 24-hour periods, followed by unrestricted access to food for 24-hour. Whereas ADF and ADMF usually alternate fasting and fed days in 48-h cycles, the twice-weekly fasting modality limits food intake for two days a week (consecutive or non-consecutive) with complete fasts or with severely restricted energy intake during these days. The modified periodic fasting consists of consuming a plant-based, very-low-caloric diet during 5 consecutive days followed by at least 10 days of unrestricted eating. Lastly, TRE consists of restricting the daily energy intake to a pre-determined eating window (generally 10-h), fasting for the rest of the day (14-h or more).

Adherence to IF effectively decreases energy intake, producing weight loss in most studies[7]. Moreover, recent studies suggest that IF improves cardiometabolic health even with no reduction in energy intake[8], challenging the dogma that CR is a prerequisite for IF to induce health benefits. In this review, we will summarize the existing evidence in relation to the effects of different types of IF on human cardiometabolic health. We will emphasize how IF impacts energy metabolism, as this is likely mediating, at least in part, the cardiometabolic health benefits of IF.

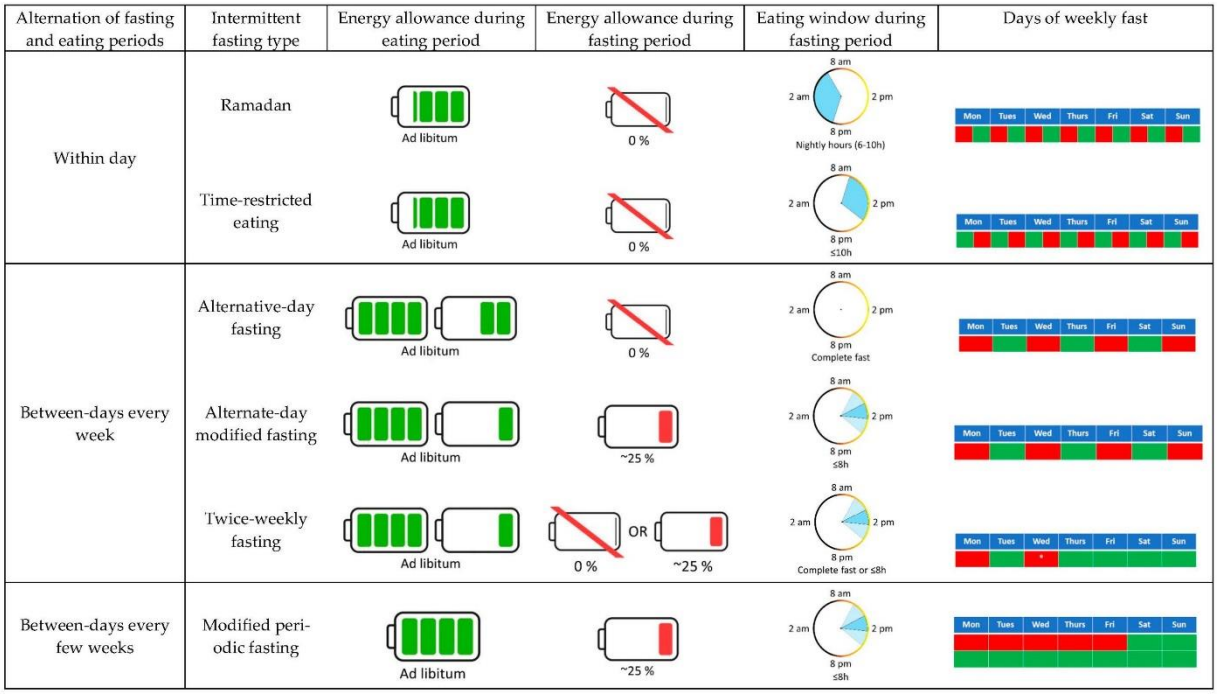

Figure 1. Different types of intermittent fasting. The green and red shading within the battery symbols illustrate the energy intake during the eating and fasting periods, with one battery symbol representing 100% of energy needs. The alternative-day modified fasting, twice-weekly fasting, and modified periodic fasting approaches do not typically restrict food intake to a specific time during the fasting days; therefore, the reduced food intake during these days can be consumed within a single meal (darker blue areas) or various meals (lighter blue areas). The blue areas within the circles indicate the eating window. The areas shaded in red in the calendar tables indicate fasting periods, while the green areas indicate eating periods. * Fast days in the twice-weekly fasting may be consecutive or not.

Acute effect of fasting on energy metabolism

Sustained periods of food scarcity were highly common over the course of human evolution[9,10]. Accordingly, humans have developed numerous behavioral and physiological adaptations that allow them to survive in a food-deprived/fasted state. Contemporary scientific investigation of human starvation began in the late nineteenth and early twentieth centuries[9,11-14]. We focus our analysis on the coordinated metabolic responses of adults to short-term fasting (i.e., 0–72-h), as it is applicable to all IF regimens.

Figure 2 illustrates the dynamic changes of circulating energy substrates and hormones during a 72-h fast, whereas the changes of whole-body substrate utilization and metabolic provenance of energy are illustrated in **Figure 3**. After a meal consumption, an abrupt increase in blood glucose concentration is detected within ~15 minutes peaking 30–60 minutes after the start of the meal[15]. In response to this, beta cells of the pancreatic islets secrete insulin resulting in a drastic increase in systemic insulin (~400–500 pmol/L), while alpha cells decrease the secretion of glucagon. Circulating cortisol and catecholamines concentrations also increase after a meal in response to a macronutrient-dependent stimulation[16,17]. The elevated insulin concentration acts on adipose tissue to inhibit the release of glycerol and free fatty acids (FFA), whose circulating concentration is reduced to 0.1 mol/L. This in turn stops the production of ketones that become undetectable in blood[15]. Hepatic glycogen metabolism switches from breakdown (glycogenolysis) to synthesis (glycogenesis) and muscle metabolism from fatty acids and amino acids oxidation to glucose oxidation and glycogen storage. Consequently, carbohydrate utilization represents 70–75% of energy expenditure after consuming a meal. This finely tuned response results in a decrease in blood glucose concentration to < 7.8 mmol/L two hours after a meal[15]. In contrast to the rapid absorption of glucose or amino acids after the meal, the absorption of dietary triglycerides is much slower. The peak in plasma triglycerides concentration (1.5–2 mmol/L) occurs 3–5h after the meal. Unlike carbohydrate or protein, ingested triglycerides have no or very little influence on their own oxidation and are primarily directed towards deposition in the adipose tissue[18,19]. Food intake induces a 5–15% increase in energy expenditure over a 3–5-h period, known as the thermic effect of food (TEF). TEF is mainly related to the energy cost of digestion, absorption, and storage of the ingested nutrients, although a facultative component of TEF also exists mostly associated with hormonal and autonomic nervous changes[20,21].

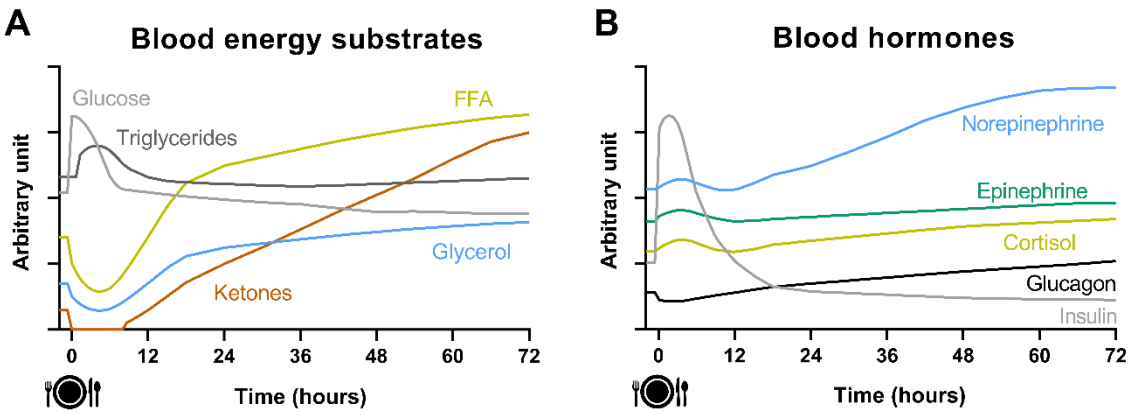

Figure 2. Dynamic changes of blood energy substrates (Panel (A)) and blood hormones (Panel (B)) during a 72-h fasting period after consuming a meal following an overnight fast.

Five or six hours after the meal, the postabsorptive state is set, slowly drifting energy metabolism to a state of overnight fasting (~12-h). At that time, blood insulin concentration is low, typically around 60 pmol/L, although it widely varies between individuals[15]. Blood glucose homeostasis (4.0–5.5 mmol/L) is now maintained by counter-regulatory hormones such as glucagon (20–25 pmol/L), cortisol (138–690 nmol/L), epinephrine (<328 pmol/L) and norepinephrine (709–4019 pmol/L), all slightly elevated[15,22]. In this state, carbohydrate oxidation typically represents ~35% of energy expenditure, whereas lipid oxidation is the major contributor to energy expenditure (~45%)[15,23,24]. The remaining ~20% of energy expenditure is covered by protein oxidation, facilitated by proteolysis. Some of the amino acids released (branched-chain amino acids) are oxidized in the muscle and their amino groups are transferred to pyruvate to produce alanine. Both alanine and glycerol are taken up by the liver as substrates for gluconeogenesis. Simultaneously, low insulin levels and circulating catecholamines promote lipolysis with the release of FFA and glycerol from adipose tissue. The concentrations of blood FFA, glycerol and triglycerides are typically ~0.5 mmol/L, ~0.15 mmol/L and ~1 mmol/L after an overnight fast[15]. At this time, ketone bodies, 3-hydroxybutyrate and acetoacetate, are also produced in the liver and secreted into the circulation, although their concentration remains low, usually, ≤0.5 mmol/L for both ketones combined[15].

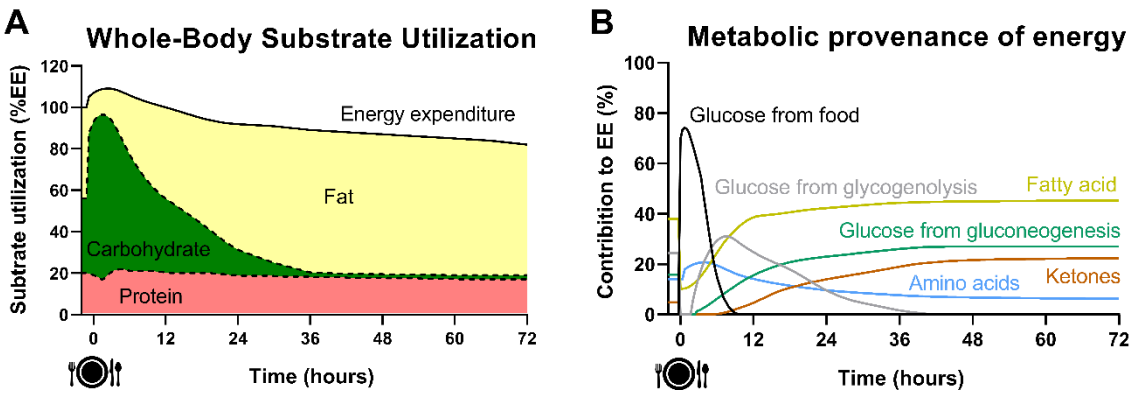

Figure 1. Dynamic changes of whole-body substrate utilization (Panel (A)) and metabolic provenance of energy (Panel (B)) during a 72-h fasting period after consuming a meal following an overnight fast.

Beyond the 12-h fasting state, blood glucose and insulin concentration continue to slowly decline, whereas catabolic hormones, FFA, glycerol, and ketones concentrations continue to rise[15,25]. Liver glycogen content decreases drastically within 24-hour, and its contribution to energy expenditure becomes almost nil after ~36-h of fasting[26]. As glycogenolysis is reduced, increased gluconeogenesis is triggered to avoid a severe decrease in systemic glucose that would seriously impair brain and other glucose-requiring systems (e.g., erythrocytes) functions[14]. A progressive switch from glycogenolysis to gluconeogenesis is necessary to produce glucose within the first 24-hour of fasting[15,26], the time at which gluconeogenesis accounts for ~64% of total glucose production[26]. In parallel, lipolysis is steadily increased, and lipid oxidation becomes the major contributor to energy expenditure. Moreover, the high influx of FFA to the liver in the context of low circulating insulin sharply increases ketogenesis, which is reflected in a continuously increasing circulating concentration of ketones[14,15,25]. Finally, it is worth noting that energy expenditure decreases ~10% after 36–48-h of fasting[27,28] mainly due to the absence of TEF and a low sympathetic tone.

During the first 24–48-hour of fasting the main gluconeogenesis precursors are lactate (~50%) and proteolysis (~40%), while the contribution of glycerol is minimal (~10%)[29]. At this pace, with the brain requiring 100–120 g of glucose per day, the body's stores of protein would be rapidly depleted. Unlike fat stores, body proteins are functionally important, and their progressive depletion leads to major complications and potentially death. Therefore, a series of metabolic adaptations that lead to the sparing of muscle protein kick in from 48–72-h of

fasting onwards[14,15,25]. Blood ketones concentration is similar to FFA (~1.5 mmol/L) after 72-h of fasting and the brain begins to use significant amounts of ketones as a fuel source, reducing the need for glucose production[14,15]. In addition, there is a further decrease in metabolic rate beyond the absence of TEF, which is partially underlined by decreased circulating leptin and thyroid hormones[11,15,25].

The metabolic switch from glycogenolysis to gluconeogenesis, fat oxidation and ketogenesis (see **Figure 3**) seems to be one of the main drivers of the metabolic health benefits provided by IF[30]. TRE extends overnight fasting to 14–20-h, which means that this metabolic switch has just occurred by the end of the fasting period. A slightly more pronounced switch from glycogenolysis to gluconeogenesis occurs with ADMF and twice-weekly fasting since both regimens are characterized by two 14–18-hour fasting periods interspersed with short eating windows. ADF is characterized by a further extension of fasting to 24–36 hours, implying that liver glycogen content is practically depleted, and the metabolic switch was completely achieved. As discussed in Section 5, exercise may advance the occurrence of the metabolic switch[30-32]. Future IF studies should employ new technology such as continuous glucose, FFA and ketone monitoring to better understand the role of the metabolic switch in improvements of metabolic health. As for any other physiological stimuli, the acute metabolic response to fasting is likely to be modified over time after prolonged adaptation to IF and may eventually alter its impact on health and metabolism.

Impact of IF on cardiometabolic health

IF has recently received a lot of attention within the scientific community, as well as among the media and the lay public. Nevertheless, most of the published articles studying the effects of IF on human cardiometabolic health are preliminary studies often with methodological limitations. In this section, we summarize the available evidence for the chronic (2 weeks) effects of IF regimens on body weight and composition, ectopic fat, and accepted cardiometabolic risk factors.

Body weight and composition

A meta-analysis including 85 studies (4176 adults aged 16–80 years) has shown that Ramadan induces a small reduction in body weight (~ 1.0 kg)[33]. This is in line with two other meta-analyses showing that, in the short term (i.e., 3 months), IF regimens cause a moderate decrease in body weight of ~ 3.0 kg compared to ad libitum eating control groups[34,35]. More specific meta-analyses investigating a particular type of IF have shown that both ADF and TRE triggered body weight loss (~ 4.3 kg and ~ 0.9 kg, respectively) and fat mass loss (~ 4.9 kg and ~ 1.6 kg, respectively) compared to ad libitum eating[36,37]. A reduction in fat-free mass was also observed after ADF (~ 1.4 kg)[36]. Interestingly, a secondary analysis of a meta-analysis suggested that TRE might be more effective in reducing body weight in metabolically unhealthy participants than in their metabolically healthy counterparts[37].

Whereas both IF and continuous daily CR seem to be effective in reducing body weight and fat mass, how fat-free mass is affected by these two interventions is still a matter of debate[30,38]. According to several meta-analyses, both IF and CR interventions produced similar changes in body weight, fat mass, fat-free mass, and waist circumference[7,34,35,39-41], provided that the adherence to interventions is similar[7,40,41]. However, a recently published study, imposing a relatively tightly matched degree of energy restriction, has reported that ADF induced lower fat and greater fat-free mass loss (~ 0.74 kg and ~ 0.75 kg, respectively) than an isocaloric continuous daily CR (~ 1.75 kg and ~ 0.03 kg, respectively) during 3 weeks in 12 lean healthy adults[42]. Further research with a larger sample size and implementing isocaloric interventions in people with overweight/obesity is needed to unravel the effects of IF regimens on body composition, as compared to continuous daily CR.

Ectopic fat

Beyond the total amount of body fat, its distribution plays a key role in the pathogenesis of cardiometabolic diseases. Ectopic fat depositions, defined as the accumulation of triglycerides within cells (or sometimes around) of non-adipose tissues (liver, skeletal muscle, beta cells . . .) are at the center of metabolic health derangements[43]. Only two studies have investigated the effect of IF on ectopic fat assessed by magnetic resonance imaging and/or spectroscopy. Trepanowski et al.,[44] observed a ~0.4 kg reduction in visceral fat mass after 6 and 12 months

of AMDF, as compared to a non-food restricted control group. However, this change was comparable to a continuous daily CR group. Likewise, Holmer et al.,[45] found that 12 weeks of twice-weekly fasting or low-carbohydrate high-fat diet were superior to the standard of care intervention in reducing hepatic steatosis (~ 6.1% and ~ 7.2%, respectively) in patients with non-alcoholic fatty liver disease, without differences between the twice-weekly fasting and low-carbohydrate high-fat diets. Studies estimating visceral fat mass by dual X-ray absorptiometry have shown conflicting results, with some studies reporting reductions of visceral fat by ADF and 8-hour TRE and others showing no differences between ADF, ADMF, 4-hour TRE, 6-hour TRE, 8-hour TRE, and standard nutrition regimen control groups[42,46-48]. The cumulative evidence is still very preliminary and future research is warranted to better understand the effects of IF on different ectopic fat depots (e.g., visceral fat, liver fat, intramuscular fat, pancreatic fat) and how it compares to the effects of matched continuous daily CR.

Cardiometabolic risk factors

A recent meta-analysis including 91 studies has shown that Ramadan produces a small but significant reduction in serum triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL-C), diastolic blood pressure, and an increase in high-density lipoprotein cholesterol (HDL-C)[49]. Two other meta-analyses have shown that IF regimens reduced total cholesterol and systolic blood pressure compared to unrestricted eating, although the clinical relevance of the magnitude of these reductions may be debatable[34,40]. Meta-analyses considering only ADF and TRE studies were also conducted. On the one hand, ADF reduced total cholesterol, LDL-C, triglycerides, and blood pressure, but did not modify HDL-C, fasting glucose or insulin sensitivity[36]. On the other hand, TRE decreased systolic blood pressure, triglycerides, and fasting glucose, but not diastolic blood pressure, LDL-C and HDL-C, which might be dependent on the baseline metabolic health status of study participants[37,50]. When compared to continuous daily CR, IF regimens were equally effective in improving plasma glucose, glycated hemoglobin, triglycerides, total cholesterol, LDL-C, HDL-C, systolic and diastolic blood pressure, or C-reactive protein[34,40]. Nevertheless, one study conducted in our lab found that eucaloric (i.e., energy intake equals energy needs, and thus in absence of weight loss) early TRE impressively improved blood pressure while still improving oxidative stress, insulin sensitivity, and β cell responsiveness in men with prediabetes[8]. Overall, different modalities of IF

were shown to improve the cardiometabolic risk profiles of human adults. Future larger studies will probably elucidate whether IF has weight-loss independent effects on cardiometabolic risk factors, how it compares to continuous daily CR, and whether different IF modalities exert different beneficial effects.

The majority of IF studies have measured only fasting biomarkers of cardiometabolic risk. However, assessing energy metabolism during the post-prandial state is key for understanding the effect of an intervention on the overall cardiometabolic risk as humans in modern societies spend most of the day in a postprandial state[51]. Indeed, post-prandial glycemia, insulinemia and lipidemia are all implicated in the etiology of chronic cardiometabolic diseases[52,53]. Importantly, Antoni et al.,[54] showed that twice-weekly fasting was superior to continuous daily CR in reducing postprandial triglycerides following matched weight loss of 5%. Similarly, Templeman et al.,[42] have recently reported that 3 weeks of ADF producing a ~25% energy deficit reduced postprandial triglycerides in lean healthy adults, whereas an increase was observed by continuous daily CR producing the same energy deficit. However, no significant differences were detected in postprandial glucose, insulin, FFA and glycerol in both studies[42,54]. Similar results were observed between 2-week interventions of either a twice-weekly fasting or continuous daily CR in normal-weight, young adults[55]. Lastly, a 4-day crossover study carried out in our lab found that eucaloric (i.e., energy intake equals energy needs, and thus in absence of weight loss) 6-hour early TRE decreased mean 24-hour and nocturnal glucose levels[56]. In the same vein, Parr et al.,[57] conducted a 5-days crossover study in isocaloric conditions, showing that early TRE reduced nocturnal glucose concentrations. In contrast, another 7-day crossover study found no effect of early TRE and late TRE in the mean 24-hour glucose levels[58]. Similarly, 10-hour and 8-hour self-selected TRE did not improve mean glucose levels[59,60] after 12 weeks of intervention. Therefore, it appears that only early TRE improves glycemic control, although further studies assessing postprandial metabolism and including continuous glucose monitoring are needed to confirm this hypothesis.

Impact of IF on cardiometabolic health: conclusion and gaps

Overall, IF regimens seem to improve body composition, ectopic fat, and classic cardiometabolic risk factors compared to ad libitum eating control groups. However, IF does not seem to provide additional benefits when compared to continuous daily CR, suggesting that if a net energy deficit is achieved, it

becomes the main driver of the cardiometabolic health benefits. Therefore, energy balance needs to be matched and tightly controlled (e.g., food being provided and/or energy intake being objectively measured by methods such as the intake-balance method using doubly-labeled water) to allow a proper comparison of both interventions and really isolate the effects of fasting and energy restriction on cardiometabolic health, which was performed only in a few studies[8,42]. However, preliminary studies suggest that IF might be beneficial in the absence of weight loss[8]. Consequently, IF may be useful to enhance cardiometabolic health of weight stable individuals, for instance, after a weight loss intervention.

The underlying mechanisms of fasting are thought to be mediated, at least in part, by the metabolic switch from carbohydrate utilization to fat and ketones oxidation that happens during fasting (see **Figure 3**)[30]. The capacity to rapidly adjust substrate oxidation to substrate availability and energy demand, known as metabolic flexibility, is considered central in the prevention of ectopic fat accumulation, which in turn induces peripheral insulin resistance[61,62]. Therefore, future studies need to be designed to test whether IF regimens improve metabolic flexibility leading to a greater loss (or less accumulation) of ectopic fat depots, thus enhancing biomarkers of cardiometabolic risk. However, a potential side effect of the metabolic switch associated with IF regimens may be elevated proteolysis to supply gluconeogenesis during the fasting periods, causing excessive fat-free mass loss which is known to impair physical function and cardiometabolic health and favor weight regain and increased fatness[42,63,64]. It is therefore important to determine whether fat-free mass loss differs between IF regimens and continuous daily CR. Lastly, future IF studies should employ wearable technologies such as continuous glucose, FFA and ketone monitoring, accelerometers to assess physical activity and sleep, skin temperature sensors, and smartphone apps to assess dietary intake. Such data will provide a better understanding of the effects of IF on cardiometabolic health.

Effects of IF on energy metabolism: Energy expenditure and substrate oxidation

Energy metabolism (i.e., energy expenditure and the substrates oxidized to sustain energy expenditure) is crucially linked to cardiometabolic health. For instance, a low energy expenditure, adjusted for body size and composition, and a reduced-fat oxidation rate predict future weight gain[65-67]. Likewise, changes in

energy expenditure and substrate oxidation in response to perturbations of energy intake were shown to be determinants of weight gain, and weight loss during controlled dietary interventions or in free-living conditions[68,69]. As discussed in Section 2, fasting acutely impacts energy metabolism. Therefore, it is plausible that adaptations in energy metabolism are part of the underlying mechanisms by which IF exerts benefits on cardiometabolic health. In this section, we summarize the existing evidence on the effects of different modalities of IF on energy expenditure and substrate oxidation.

Ramadan

Despite the numerous studies conducted during Ramadan, only three have documented its impact on energy expenditure and/or substrate oxidation (**Table 1**)[70-73]. These studies have reported no changes in resting metabolic rate (RMR) after Ramadan[71-73]. Nonetheless, Lessan et al.,[72] found that RMR, but not total daily energy expenditure (TDEE) assessed by doubly-labeled water, was significantly decreased during the 3 last weeks of the 4-week Ramadan period. Regarding substrate oxidation, one study observed similar fasting respiratory exchange ratio (RER) values before and after Ramadan[73], whereas Lessan et al.,[72] detected a significant reduction in fasting RER (from 0.88 to 0.80) after Ramadan.

Alternate-fay fasting (ADF)

A total of seven studies have assessed the effects of ADF on energy expenditure and/or substrate oxidation (**Table 2**)[42,74-78]. Studies analyzing the effects of ADF on RMR have yielded inconsistent results. In general, short ADF interventions (8 weeks) do not seem to affect RMR[42,74,76,78]. Only one 2-week ADF intervention study has shown a reduction in RMR even in the absence of body mass loss[77]. Two studies have compared the changes in RMR induced by ADF and continuous daily CR. Templeman et al.,[42] found no differences between both interventions, whereas Catenacci et al.,[74] detected that RMR (adjusted for changes in fat-free mass and fat mass) decreased after 8-week of continuous daily CR (~ 111 kcal/day), but not after ADF (~ 16 kcal/day). On the other hand, fasting substrate oxidation seems not to be affected by short-term ADF interventions[42,75,78]. However, Heilbronn et al.,[76] observed that substrate oxidation during the fed days was comparable to baseline values, but fasting carbohydrate oxidation was decreased, and fat oxidation increased during the

fasting days. Lastly, Templeman et al.,[42] observed greater postprandial fat oxidation in two ADF groups (with and without CR) compared to continuous daily CR after 3 weeks of intervention. Clearly, the currently available evidence is limited and further, properly powered, long-term, controlled clinical research is needed to really understand the effects of ADF on energy expenditure and substrate oxidation.

Alternate-day modified fasting (ADMF)

We identified three studies in which energy expenditure and/or substrate oxidation were measured in response to ADMF (**Table 2**)[79-81]. All of them found no significant impact of ADMF on RMR or adjusted 24-hour energy expenditure, adjusted activity energy expenditure, and adjusted sleeping energy expenditure between ADMF and continuous daily CR. Moreover, Coutinho et al.,[80] observed similar fasting substrate oxidation between ADMF and continuous daily CR after 12 weeks of intervention.

Twice-weekly fasting

Three studies comparing the effects of twice-weekly fasting versus continuous daily CR reported no significant differences on RMR and/or RER in adults with overweight or obesity[54,82] or in healthy individuals with normal weight (**Table 2)**[55]. The concordance between the results of studies investigating ADF and the twice-weekly fasting can be partly attributed to the similarity in study designs as well as the matching of energy deficit and weight loss in both ADMF and twice-weekly fasting interventions. No studies have compared the effects of ADMF or twice-weekly fasting versus ad libitum eating on energy expenditure and/or substrate oxidation.

Table 1. Chronic effects of Ramadan on energy expenditure and substrate oxidation.

Study	Population	Design and Intervention	Assessment	No Change	Increase	Decrease
(71)	Normal weight n = 16 (0M/16F)	4-week Observational (1) Ramadan	15 h period Metabolic chamber	RMR and nigh EE Day and evening protein ox.	Fat ox. from 2PM–11PM 11PM	RER and CHO ox. from 11AM–11PM EE from 11AM–5PM
(73)	(1) Healthy n = 7 (7M/0F) (2) TD2M n = 5 (3M/2F)	3-week Observational (1) Ramadan (2) Ramadan	Overnight fasting Metabolic cart	RMR and RER		
(72)	Healthy normal/overweight n = 29 (13M/16F) for RMR n = 10 (5M/5F) for TDEE	4-week Observational (1) Ramadan	Overnight fasting Metabolic cart (Quark, Cosmed) Doubly-labeled water during 14 days	RMR and adjusted RMR * TDEE		RER

No change indicates no significant difference to pre-intervention values; increase indicates significantly higher than pre-intervention values; and decrease indicates significantly lower than pre-intervention values. * RMR results adjusted for sex, age, weight, and the number of hours since suhoor. Metabolic cart brands and models are provided when they are available. Abbreviations: EE, energy expenditure; RER, respiratory exchange ratio; RMR, resting energy expenditure; TDEE, total daily energy expenditure; TD2M, type 2 diabetes mellitus.

Table 2. Acute and chronic effects of alternative-day fasting, alternative-day modified fasting, and twice-weekly fasting on energy expenditure and substrate oxidation.

Study	Population	Design	Fasting Regimen	Eating Regimen	Assessment	No Change	Increase	Decrease
Acute alternative-day fasting								
(27)	Healthy normal weight/obesity *n* = 14 (14M/0F)	48-hour Cross-over (a) Control (b) ADF	(2) 48-hour fasting	(1) 100% Energy needs	Metabolic chamber	AEE		24-hour EE and SEE 24-hour RER 24-hour EE
(83)	Healthy normal weight/obesity *n* = 64 (51M/13F)	36-hour Cross-over (a) Control (b) ADF	(2) 36-hour fasting	(1) 100% Energy needs	Metabolic chamber	SEE	24-hour Fat ox.	24-hour RER and sleep RER 24-hour CHO ox. 24-hour Protein ox.
Chronic alternative-day fasting								
(75)	Healthy normal/overweight *n* = 8 (8M/0F)	2-week Single-arm (1) ADF	20 h fasting every other day (From 10PM to 6PM)	Ad libitum	Overnight fasting Metabolic cart (Oxycon Pro, Jaeger)	RER		
(76)	Healthy normal/overweight *n* = 16 (8M/8F)	22-day Single-arm (1) ADF	24-hour fasting every other day	Ad libitum	Overnight fasting Metabolic cart (DeltaTrac, SensorMedics)	RMR and adjusted RMR * in fed and fast days RER, CHO and Fat ox. In fed day	Fat ox. In fast day	RER and CHO ox. In fast day
(77)	Healthy normal/overweight *n* = 8 (8M/0F)	2-week Cross-over (a) Control (b) ADF	(2) 20 h fasting every other day (From 10PM to 6PM)	1 and (2) 100% Energy needs	Overnight fasting Metabolic cart	RER, CHO and Fat ox.		RMR
(74)	Obesity (1) *n* = 12 (3M/9F) (2) *n* = 13 (3M/10F)	8-week RCT (1) CR (2) ADF	(2) 24-hour fasting every other day	(1) Prescribed CR −400 kcal/day. Measure 28% CR (2) 100% Energy needs + ad libitum access to 5–7 snacks (200 kcal/serve) during eating days. Measure 47% CR	Overnight fasting Metabolic cart (TrueOne 2400, Parvo Medics)	RMR and adjusted RMR *		
(78)	Healthy normal/overweight (1) *n* = 29 (12M/17F) (2) *n* = 28 (11M/17F)	4-week RCT (1) Control (2) ADF	(2) 24-hour fasting every other day	(1) Ad libitum. Measured 8% CR (2) Ad libitum. Measured 37% CR	Overnight fasting Metabolic cart (MetaMax 3b, Cortex)	RMR		
(78)	Healthy normal/overweight (1) *n* = 60 (24M/36F) (2) *n* = 30 (14M/16F)	Observational (1) Control (2) ADF performed >24 weeks	(2) 24-hour fasting every other day	(1) Ad libitum. Measured 0% CR (2) Ad libitum. Measured 29% CR	Overnight fasting Metabolic cart (MetaMax 3b, Cortex)	RMR		

(42[ı])	Healthy normal/overweight (1) *n* = 12 (5M/7F) (2) *n* = 12 (3M/9F) (3) *n* = 12 (7M/5F)	3-week RCT (1) CR (2) ADF without CR (3) ADF + CR	2 and (3) 24-hour fasting every other day	(1) Daily 25% CR (2) 200% Energy needs in fed days, 0% net CR (3) 150% Energy needs in fed days, 25% net CR	Overnight fasting and 3-h postprandial period Metabolic cart	RMR and adjusted RMR [¥] Fasting CHO, Fat and Protein ox. Postprandial CHO and Protein ox.	Postprandial Fat ox. in both ADF groups vs. CR
Chronic alternative-day modified fasting							
(81)	Overweight/obesity (1) *n* = 10 (0M/10F) (2) *n* = 10 (0M/10F) (3) *n* = 10 (0M/10F)	4-week Cross-over (a) CR (b) Bread ADMF (c) ADMF	(2) Ad libitum bread, coffee and tea (3) 50% CR	(1) Daily 50% CR (2) 100% Energy needs (3) 100% Energy needs	Metabolic chamber	Adjusted 24-hour EE [¥] and AEE Adjusted SEE [¥] in CR vs. ADMF	Adjusted SEE [¥] in ADMF vs. bread ADMF
(80)	Obesity (1) *n* = 14 (4M/10F) (2) *n* = 14 (2M/12F)	12-week RCT (1) CR (2) ADMF	(2) EI of 660 and 550 kcal/day for men and women, respectively, on 3 non-consecutive days/week	(1) Daily 25% CR (2) 100% Energy needs	Overnight fasting Metabolic cart (Vmax Encore 29N, Care Fusion)	RMR and adjusted RMR [α] RER	
(79)	Overweight/obesity (1) *n* = 18 (0M/18F) (2) *n* = 12 (0M/12F)	RCT ≥ 5% WL within 12 weeks (1) CR (2) ADMF	(2) 75% CR every other day	(1) Daily 25% CR (2) Ad libitum	Overnight fasting Metabolic cart (GEMNutrition)	RMR	
Chronic twice-weekly fasting (2-WF)							
(54)	Overweight/obesity (1) *n* = 12 (6M/6F) (2) *n* = 15 (7M/8F)	RCT 5% WL target (1) CR (2) 2-WF	(2) 75% CR on two consecutive days/week	(1) Daily 33% CR (2) Ad libitum	Overnight fasting Metabolic cart [Φ] (ISGEM319, GEMNutrition)	RMR and adjusted RMR [£] RER	
(82)	Adults with central obesity (1) *n* = 22 (6M/16F) (2) *n* = 21 (6M/15F)	4-week RCT (1) CR (2) 2-WF	(2) EI of 600 kcal on two consecutive days/week	(1) Daily 500 kcal CR (2) Energy-controlled diet to target the same weekly energy deficit as the CR	Overnight fasting Metabolic cart (FitMate, Cosmed)	RMR	
(55)	Healthy normal weight (1) *n* = 8 (4M/4F) (2) *n* = 8 (4M/4F)	2-week RCT (1) CR (2) 2-WF	(2) 70% CR on two non-consecutive days/week	(1) Daily 20% CR (2) 100% Energy needs	Overnight fasting Metabolic cart (Quark CPFT, Cosmed)	RMR RER	

No change indicates no significant difference to pre-intervention or control group values; increase indicates significantly higher than pre-intervention or control group values; and decrease indicates significantly lower than pre-intervention or control group values. * RMR results adjusted for fat-free mass and fat mass; ¥ RMR, 24-hour EE or SEE results adjusted for body weight or fat-free mass; α RMR results adjusted for fat-free mass; £ RMR results adjusted for metabolically active mass (fat-free mass + 18 kg); Φ Sample size n = 10 and n = 13 for CR and twice-weekly fasting (2-WF), respectively. Metabolic cart brands and models are provided when they are available. Abbreviations: ADF, alternate-day fasting; ADMF, alternative-day fasting modified fasting; AEE, activity energy expenditure; CR daily calorie restriction; EE, energy expenditure; RER, respiratory exchange ratio; RMR, resting energy expenditure; SEE, sleeping energy expenditure; WL, weight loss.

Time-restricted eating (TRE)

TRE is the most studied IF modality when it comes to energy metabolism. This is due not only to the increased popularity of TRE over the past few years, probably fueled by the potential to be widely applicable in clinical settings but also to older studies investigating topics such as breakfast skipping and meal frequency, extending nocturnal fasting and confining all daily eating events to a 4–10-hour window. A detailed description of the studies investigating the acute (i.e., 72 hours) and chronic effects of TRE on energy expenditure and substrate oxidation is provided in **Table 3**.

We identified eight studies in which energy expenditure was measured in response to acute TRE[84-91]. TRE can be categorized based on when the eating window occurs, that is early TRE (eating earlier in the day), midday TRE (eating in the middle of the day) and late TRE (eating later in the day). It is hypothesized that early TRE may be the most effective schedule to enhance cardiometabolic health since it aligns with metabolism circadian rhythms[8,92]. However, mixed findings between early, midday and late TRE were observed when it comes to energy expenditure. Overall, TRE, independently of the time of the eating window, does not seem to acutely affect energy expenditure[84-88,91,93]. The study of Nas et al.,[90] was the only one that reported that both 6-hour early and 6-hour late TRE led to a small but significant increase 24-hour energy expenditure (91 kcal/day and 41 kcal/day, respectively) as compared to an isocaloric 12-hour control schedule. Three other studies have found an increase in RMR[85], sleeping metabolic rate[84] or night energy expenditure[91], but not in 24-hour energy expenditure as compared to isocaloric control conditions.

A total of seven studies reported substrate oxidation in response to acute TRE[84-90], providing mixed findings. In general, TRE, regardless of the time of the eating window, does not appear to acutely affect 24-hour substrate oxidation[84-88,93]. However, Nas et al.,[90] have shown that 6-hour late TRE decreased 24-hour carbohydrate oxidation and increased 24-hour fat oxidation in comparison to 12-hour control. No differences were observed between 6-hour early TRE and 12-hour control, which might contrast with the hypothesis that early TRE is the most effective schedule[8,92]. Nonetheless, it is important to highlight that the duration of the intervention was only 24-hour and that there were no significant differences between 6-hour early and 6-hour late TRE. Lastly, Munsters and Saris[85] noted an increase in protein oxidation in response to 9 h early TRE versus

a 13-houreating window (14 meals per day) without differences in carbohydrate or fat oxidation.

Although the acute response to TRE provides valuable knowledge, longer studies are needed to really understand the influence of TRE on energy expenditure and substrate oxidation. We identified 10 studies that have measured energy expenditure in response to TRE interventions ranging from 4 days to 13 weeks[48,94-102]. Overall, the length of the eating window and the circadian timing of food intake did not impact 24-hour energy expenditure or RMR, which is consistent with findings from acute TRE studies. Only a few studies have assessed the substrate oxidation response to chronic TRE. Ogata et al.,[101] found no significant differences in 24-hour substrate oxidation between 5:30 h late TRE and 11-hour control. Similarly, fasting RER was not altered by a 12-week 8-hour late TRE compared to three ad libitum meals per day (~16-hour of eating window)[48]. However, we observed an increased 24-hour protein oxidation and decreased 24-hour nonprotein RER, especially at nighttime, during a 6-hour early TRE intervention, which is indicative of elevated fat oxidation[102]. Therefore, it appears that the circadian timing of the eating window may affect substrate oxidation, although long-term and well-powered trials are needed to test this hypothesis.

Table 3. Acute and chronic effects of time-restricted eating on energy expenditure and substrate oxidation.

Study	Population	Design and Intervention	Assessment	No Change	Increase	Decrease
Acute time-restricted eating						
(88)	Healthy normal/overweight *n* = 13 (2M/11F)	48-hour Cross-over (a) 13-hour Control (7:30AM–8:30PM, 7 meals/day, 100% energy needs) (b) 6-hour TRE (12PM–6PM, 2 meals/day, 100% energy needs)	Metabolic chamber	24-hour EE 24-hour RER Fat ox. from 6PM–9PM	Fat ox. from 9AM–12PM CHO ox. from 6PM–9PM	CHO ox. from 9AM–12PM
(91)	Overweight/Obesity *n* = 10 (0M/10F)	48-hour Cross-over (a) 10-hour Control (9AM–7PM, 6 meals/day,1000 kcal/day) (b) 8-hour TRE (11AM–7PM, 2 meals/day, 1000 kcal/day)	Metabolic chamber	24-hour EE	Night EE	
(87)	Healthy normal weight *n* = 14 (0M/14F)	36-hour Cross-over (a) 8:30-hour TRE (8AM–4:30PM, 3 meals/day, 100% energy needs) (b) 8:30-hour TRE (8AM–4:30PM, 2 meals/day, 100% energy needs)	Metabolic chamber	24-hour EE, SMR, TEF and AEE 24-hour Fat, 24-hour CHO and 24-hour protein balance	24-hour Fat ox. in 3 meals/day vs. 2 meals/day	24-hour and night RER in 3 meals/day vs. 2 meals/day 24-hour CHO ox. in 3 meals/day vs. 2 meals/day
(85)	Healthy normal weight *n* = 12 (12M/0F)	36-hour Cross-over (a) 13-hour Control (8AM–9PM, 14 meals/day, 100% energy needs) (b) 9-hour TRE (8AM–5PM, 3 meals/day, 100% energy needs)	Metabolic chamber	24-hour EE, SMR, TEF and AEE 24-hour RER, 24-hour CHO and 24-hour Fat ox.	RMR 24-hour Protein ox.	
(84)	Healthy normal/overweight *n* = 8 (8M/0F)	24-hour Cross-over (a) 11-hour Control (8AM–7PM, 3 meals/day, 100% energy needs) (b) 7-hour TRE (12PM–7PM, 2 meals/day, 100% energy needs)	Metabolic chamber	24-hour EE, RMR and TEF 24-hour RER, 24-hour CHO, 24-hour Fat and 24-hour Protein ox.	SMR Morning Fat ox. Sleeping CHO ox.	Morning CHO ox. Sleeping Fat ox.
(86)	Healthy normal/overweight *n* = 15 (7M/8F)	24-hour Cross-over (a) 12:30 h Control (9AM–9:30PM, 6 meals/day, 100% energy needs) (b) 10-hour TRE (9AM–7PM, 3 meals/day, 100% energy needs)	Metabolic chamber	24-hour EE 24-hour RER and 24-hour Fat ox.		
(90)	Healthy normal/overweight *n* = 17 (8M/9F)	24-hour Cross-over (a) 12-hour control (7AM–7PM, 3 meals/day, 100% energy needs) (b) 6-hour TRE (7AM–1PM, 2 meals/day, 100% energy needs)	Metabolic chamber [Φ]	24-hour Protein ox. and balance in both 6-hour TRE No significant differences between both 6-hour TRE	24-hour EE in both 6-hour TRE 24-hour Fat ox. and 24-hour CHO balance in 1PM–7PM TRE	24-hour RER, 24-hour CHO ox. and Fat balance in 1PM–7PM TRE

(89)	Healthy normal weight *n* = 12 (2M/10F)	(c) 6-hour TRE (1PM–7PM, 2 meals/day, 100% energy needs) 72-hour Cross-over (a) 13-hour Control (8AM–9PM, 4 meals/day, 100% energy needs) (b) 10-hour TRE (8AM–6PM, 4 meals/day, 100% energy needs)	Overnight fasting and 4-h postprandial period Douglas bags	RMR and RER		RER 30 and 60 minutes after a meal
			Chronic time-restricted eating			
(94)	Healthy normal/overweight *n* = 8 (8M/0F)	2-week Cross-over (a) 10-hour Control (9AM–7PM, 6 meals/day, 100% energy needs) (b) 8-hour TRE (11AM–7PM, 2 meals/day, 100% energy needs)	Metabolic chamber	24-hour EE and RMR	Night EE	Day EE
(96)	Healthy normal/overweight *n* = 10 (10M/0F)	7-day Cross-over (a) 13-hour Control (7:30AM–8:30PM, 7 meals/day, 100% energy needs) (b) 6-hour TRE (12PM–6PM, 2 meals/day, 100% energy needs)	Metabolic chamber Doubly-labeled water during 7 days	24-hour EE, TDEE, RMR, TEF and AEE		
(95)	Overweight/Obesity *n* = 14 (0M/14F)	4-week RCT (1) 13:30-hour CR (7:30AM–9PM, 3–5 meals/day, 1000 kcal/day) (2) 6-hour TRE (12PM–6PM, 2 meals/day, 1000 kcal/day)	Metabolic chamber	24-hour EE, SEE and TEF		
(97)	Healthy normal/overweight (1) *n* = 16 (6M/10F) (2) *n* = 17 (6M/11F)	6-week RCT (1) Control (Ad libitum energy intake starting within 2 h of waking) (2) TRE (Ad libitum energy intake starting after 12 PM)	Overnight fasting and 2-h postprandial period Metabolic cart	RMR		TEF
(98)	T2DM *n* = 54 (29M/25F)	12-week Cross-over (a) CR (6 meals/day, measured −380 kcal/day) (b) 10-hour TRE + CR (6AM–4PM, measured −420 kcal/day)	Overnight fasting Metabolic cart [a] (VMAX, SensorMedics)	RMR		
(99)	(1) Normal weight *n* = 9 (4M/5F) (2) Overweight/obesity *n* = 10 (3M/7F) (3) Normal weight *n* = 9 (5M/4F)	7-day Cross-over (a) Control (Ad libitum energy intake starting within 2 h of waking) (b) TRE (Ad libitum with breakfast skipping)	Overnight fasting and 2-h postprandial period Douglas bag	RMR and 2 h TEF		

	(4) Overweight/obesity n = 9 (4M/5F)					
(100)	Obesity (1) n = 16 (6M/10F) (2) n = 17 (6M/11F)	6-week RCT (1) Control (Ad libitum energy intake starting within 2 h of waking) (2) TRE (Ad libitum energy intake starting after 12 PM)	Overnight fasting and 2-h postprandial period Metabolic cart	RMR and TEF		
(101)	Healthy normal/overweight n = 9 (9M/0F)	6-day Cross-over (a) 11-hour Control (7AM–6PM, 3 meals/day, 100% energy needs) (b) 5:30 h TRE (12:30PM–6PM, 2 meals/day, 100% energy needs)	Metabolic chamber	24-hour EE and 7-hour SEE 24-hour RER, 24-hour CHO, 24-hour Fat and 24-hour Protein ox.		TEF
(102)	Overweight/Obesity n = 10 (6M/4F)	4-day Cross-over (a) 12-hour control (8AM–8PM, 3 meals/day, 100% energy needs) (b) 6-hour TRE (8AM–2PM, 3 meals/day, 100% energy needs)	Metabolic chamber	24-hour EE and RMR Day RER	Day EE and TEF 24-hour Protein ox.	Nigh EE and sleep EE 24-hour RER, night, rest and sleep RER
(48)	Overweight/Obesity (1) n = 25 (15M/10F) (2) n = 25 (13M/12F)	13-week RCT (1) ~16-hour Control (6–10AM to 5–10PM, 3 meals/day, ad libitum) (2) 8-hour TRE (12PM–8PM, ad libitum)	Overnight fasting Metabolic cart (TrueOne 2400, Parvo Medics) Doubly-labeled water during 7 days	RMR, RER and TDEE		

No change indicates no significant difference to pre-intervention or control group values; increase indicates significantly higher than pre-intervention or control group values; and decrease indicates significantly lower than pre-intervention or control group values. Φ Sample size n = 15; α Sample size n = 52. Metabolic cart brands and models are provided when they are available. Abbreviations: AEE, activity energy expenditure; CR daily calorie restriction; EE, energy expenditure; RER, respiratory exchange ratio; RMR, resting energy expenditure; SEE, sleeping energy expenditure; TEF, thermic effect of food; TRE, time-restricted eating.

Optimizing IF by combining it with exercise

Metabolic switching: The role of endurance exercise

As previously mentioned, the metabolic switches from glycogenolysis to gluconeogenesis and from carbohydrate to fat oxidation and ketogenesis (see **Figure 3**) are thought to be key factors promoting some of the health benefits of IF[30]. Importantly, this metabolic switch largely depends on the state of hepatic glycogen content and, to a lesser extent, on muscle glycogen content. In sedentary conditions, the liver glycogen content severely de- creases within 24-hour of fasting and it is almost depleted after 36–48 hours [15,26]. However, liver glycogen content is substantially depleted within 90–120 minutes of moderate- to high-intensity exercise if it is not compensated by ingesting carbohydrates[31]. Therefore, exercise ap- pears to be an effective strategy to advance, and perhaps potentiate, the metabolic switch from glycogenolysis to gluconeogenesis, fat oxidation and ketogenesis[30-32].

To the best of our knowledge, there are no studies explicitly investigating the combined effects of IF and exercise on energy expenditure and substrate oxidation assessed by whole- room indirect calorimetry. However, several studies have been conducted to investigate the effects of exercise on 24-hour energy metabolism under energy balance conditions. Some of these studies concluded that a short session (1 h) of moderate- or vigorous-intensity exercise performed in the postprandial state does not influence 24-hour fat oxidation[103]. In contrast, Schrauwen et al.,[104] reported that high-intensity interval exercise until exhaustion (i.e., glycogen-depleting exercise) performed 2 h after eating increased fat oxidation in the subsequent 24 hours. Therefore, the intensity of exercise and the resulting glycogen depletion seem to be key factors in modulating 24-hour fat oxidation. On the other hand, 24-hour fat oxidation was consistently shown to be increased when moderate-intensity exercise (60–100 minutes) is performed before breakfast, i.e., in a fasted state[105-107]. Although the circadian regulation of energy metabolism cannot be disregarded, the combined effect of an overnight fast and exercise on glycogen depletion and the release of FFAs seem to be the underlying mechanism[105-107]. Therefore, glycogen-depleting exercise performed during the fasting periods of IF regimes may be the most efficacious combination to boost the metabolic switch from glycogenolysis to gluconeogenesis, fat oxidation and ketogenesis[30-32,104,105].

A potential side effect of ADF and TRE is a behavioral compensatory decrease in low- to moderate-intensity physical activity[42,97,100]. Indeed, the energy expended via physical activity is the most fluctuating component of energy expenditure and hence has the greatest potential to undermine an imposed energy deficit. Consequently, performing structured endurance exercise and/or physical activity may be an effective strategy to avoid an IF-induced reduction in total daily energy expenditure.

Optimizing IF by combining it with resistance exercise

Based on the existence of non-caloric deprived periods, some have hypothesized that IF regimens may decrease fat mass while retaining larger amounts of fat-free mass, as compared with continuous CR[30,38]. In contrast, some studies have reported greater fat-free mass loss with IF regimens than with continuous daily CR[42,63,108], which can be attributed to increased proteolysis to supply substrates for gluconeogenesis during fasting periods (see **Figure 3**). Fat-free mass loss is known to impair physical functionality, cardiometabolic health and may be a risk factor for weight regain and increased fatness[42,63,64]. This side effect of IF may be counteracted, at least in part, by exercise, in particular, resistance training since it is known to increase skeletal muscle mass[109,110].

Indeed, a recent systematic review and meta-analysis including eight studies and 221 participants, have shown that IF regimens combined with resistance exercise reduced fat mass (~ 1.3 kg) with preservation of fat-free mass compared to non-CR control diets[111]. Therefore, more longer-term, and high-quality studies are needed to examine whether resistance exercise can mitigate the fat-free mass loss associated with each type of IF. Moreover, these effects may be dependent on the time at which exercise is performed (i.e., fasting versus eating periods), hence it is important to further clarify the most effective and feasible exercise timing in the context of IF.

Perspectives of the usefulness of IF on cardiometabolic health: Gaps and future directions

Energy expenditure is decreased in parallel to weight loss, which is in part, but not completely due to the loss of metabolically active tissues. A further reduction in energy expenditure beyond what can be predicted by changes in body weight and composition (i.e., metabolic adaptation or adaptive thermogenesis) has been

consistently documented[112]. Metabolic adaptation is believed to progressively counteract further weight loss and con- tribute to weight regain[112]. Therefore, preventing metabolic adaptation will likely contribute to a more pronounced and sustainable weight loss. Whether reducing body weight by IF instead of continuous CR prevents metabolic adaptation is still unknown. Some preliminary studies suggest that there are no differences in the change in RMR between the two intervention modes. Nonetheless, not all studies have reported these findings[74,77], and well-controlled studies are needed to determine whether the decreases in the different components of energy expenditure are comparable between IF regimens and continuous daily CR.

Although TRE does not seem to impact 24-hour energy expenditure when compared to larger eating windows (12 hours)[113], the few studies performed using room indirect calorimetry suggest that IF regimens may affect substrate oxidation, increasing protein and fat oxidation. In normal conditions, carbohydrate and protein oxidation are closely regulated to match their intake, whereas the gap between energy intake and expenditure is buffered by fat balance[114,115]. Furthermore, a reduced-fat oxidation rate in energy balance conditions or in response to acute overfeeding is predictive of long-term weight gain and is thought to determine ectopic fat accumulation[61,66,68]. Therefore, increasing fat oxidation by TRE with or without exercise might prevent weight gain and ectopic fat deposition. Long-term and well-powered trials are needed to ascertain whether IF regimens increase fat oxidation and if this ultimately leads to better weight management and metabolic health than continuous daily CR.

The cardiometabolic health benefits of IF regimens may be optimized, and its undesirable effects attenuated, by combining it with exercise[111]. For instance, adding exercise training to an IF approach may advance by several hours, and probably reinforce, the metabolic switch from glycogenolysis to gluconeogenesis, fat oxidation and ketogenesis[30,32]. Studies suggest that moderate- to high-intensity exercise for 60 minutes performed in the fasted state may be the most effective combination to induce this metabolic switch[30-32,104,105]. Moreover, implementing structured endurance exercise may be an effective strategy to avoid the reduction in low- to moderate-intensity activity energy expenditure observed with IF regimens[42,97,100]. Another undesirable effect of IF is the potential loss of fat-free mass, which might be even more worrisome than in continuous

daily CR[42,63]. This undesirable effect is likely counteracted, at least in part, by resistance exercise.

The study of IF in humans is still in its infancy. To date, pioneer studies have shown that IF regimens slightly decrease body weight and fat mass and improve cardiometabolic risk factors compared to unrestricted eating, especially in metabolic unhealthy study participants[33-37,49,50]. According to the current evidence, IF regimens and continuous daily CR seem to be equally effective to enhance body composition and cardiometabolic health[34,40], which is partly explained by the similar adherence to both interventions[7,40,41]. However, one crossover study (5 weeks for each intervention) in our lab found that eucaloric (i.e., energy intake equals energy needs, and thus in absence of weight loss) early TRE improved appetite regulation, blood pressure, oxidative stress, insulin sensitivity, and β cell responsiveness in men with prediabetes[8]. These observations suggest that IF regimens may improve cardiometabolic health independently of weight loss. Little is known about the effects of IF regimens on ectopic fat accumulation and postprandial metabolism, two important determinants of metabolic health. The few studies conducted reported no differences between IF regimens and continuous daily CR except for the reduction in postprandial triglycerides, which seems to be enhanced in IF regimens[42,54]. Together, preliminary evidence from human trials suggests that the cardiometabolic health benefits of IF are mediated, at least in part, by the mentioned adaptations in energy metabolism. Nonetheless, properly powered, long-term well-controlled clinical research is still needed to unravel the underlying mechanisms of IF and its role on energy metabolism including energy expenditure and fat oxidation.

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Eat
Fast
16 hrs

AIMS AND HYPOTESHIS

Aims

The overall aim of this International Doctoral Thesis is to investigate the role of meal timing and IF on body composition and cardiometabolic health in humans. This overall aim is addressed by three different specific objectives:

- **Specific aim I:** To describe the rationale, design, and methodology of the EXTREME multicenter randomized controlled trial (**Chapter 3***).*
- **Specific aim II:** To investigate the association of meal timing with body composition and cardiometabolic risk factors in young men and women (**Chapter 4**).
- **Specific aim III:** To determine the efficacy of three different 8-hour TRE schedules on visceral adipose tissue (VAT), body composition and cardiometabolic health in men and women with overweight/obesity (**Chapter 5**).

Hypothesis

The main hypothesis of this International Doctoral thesis is that meal timing plays an important role in body composition and cardiometabolic health in humans. Specifically, we hypothesize that:

(i) A shorter daily eating window, earlier food intake schedule and less variability in meal timing between non-working days and working days would be cross-sectionally associated with healthier body composition and cardiometabolic risk factors.

(ii) An earlier 8-hour eating window during TRE would result in greater improvements in body composition and cardiometabolic health than usual-care (UC) treatment, late or self-selected 8-hour eating window, following a 12-week intervention.


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MATERIAL AND METHODS

This section includes two chapters:

(i) **Chapter 2** presents the ACTIBATE randomized controlled trial, which contributed the baseline data for the analysis conducted in **Chapter 4** of this International Doctoral Thesis.

(ii) **Chapter 3** provides an overview of the rationale, design, and methodology of the EXTREME multicenter randomized controlled trial, which served as the foundation for **Chapter 5**.

Chapter 2. Methodological overview of the ACTIBATE study

The ACTivating Brown Adipose Tissue through Exercise (ACTIBATE) study is a randomized controlled trial (ClinicalTrials.gov ID: NCT02365129) designed to investigate the effects of a 24-week supervised exercise intervention on brown adipose tissue volume and activity (primary outcome) in a cohort of 145 young sedentary adults[1]. The exercise intervention combined endurance and resistance training, and the study also assessed several secondary outcomes including body composition, cardiometabolic risk factors, dietary assessment, sleep parameters, sedentary time, and physical activity levels[1]. The study design, protocols, and informed consent procedure were approved by the Human Research Ethics Committee of both the University of Granada (nº 924) and Servicio Andaluz de Salud (Centro de Granada, CEI-Granada), and all participants provided written informed consent. Participants were randomly allocated to the usual care (control), moderate-intensity exercise or vigorous-intensity exercise groups and were followed for 6 months during the exercise interventions. The baseline and follow-up examinations were conducted in the same setting [Instituto Mixto Deporte y Salud (iMUDS) at the University of Granada and Hospital Universitario Virgen de las Nieves, Granada, Spain] by the same investigators. The study was performed following the ethical guidelines of the Declaration of Helsinki, last modified in 2013. The baseline evaluations were conducted between October and November 2015 (n≈60 participants) and 2016 (n≈90 participants).

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Eat
Fast
16 hrs

Chapter 3. The rationale, design and methodology of the EXTREME multicenter randomized controlled trial

Abstract

Objective: To investigate the efficacy and feasibility of three different 8h time-restricted eating (TRE) schedules (i.e., early, late, and self-selected) compared to each other and to a usual-care (UC) intervention on visceral adipose tissue (VAT) and cardiometabolic health in adults.

Methods: Anticipated 208 adults (50% women) aged 30-60 years, with overweight/obesity ($25 \leq BMI < 40$ kg/m^2) and with mild metabolic impairments will be recruited for this parallel-group, multicenter randomized controlled trial. Participants will be randomly allocated (1:1:1:1) to one of four groups for 12 weeks: UC, early TRE, late TRE or self-selected TRE. The UC group will maintain their habitual eating window and receive, as well as the TRE groups, healthy lifestyle education for weight management. The early TRE group will start eating not later than 10:00, and the late TRE group not before 13:00. The self-selected TRE group will select an 8h eating window before the intervention and maintain it over the intervention. The primary outcome is changes in VAT, whereas secondary outcomes include body composition and cardiometabolic risk factors.

Conclusions: This study will determine whether the timing of the eating window during TRE impacts its efficacy on VAT, body composition and cardiometabolic risk factors and provide insights about its feasibility.

Introduction

Obesity has nearly tripled since 1975 and currently there are more than 1.9 billion adults with overweight or obesity worldwide according to the World Health Organization[1]. The obesity epidemic is a major contributor to the global burden of disability and chronic disease such as diabetes, cardiovascular disease, certain cancers, kidney disease, and obstructive sleep apnea[1,2]. Consequentially, obesity results in a significant economic impact on health care systems[1,3].

Energy restricted diets reduce body weight and improve cardiometabolic health. However, these approaches are still not a standard public health strategy owing to their limited long-term adherence, even in highly motivated patients. Over the past decade, IF has emerged as a promising dietary strategy for the treatment of obesity and its comorbidities[4-6]. In simple terms, IF consists of alternating consistent fasting and eating periods[4-6]. IF is an umbrella term for several protocols of fasting regimens[5,6]. Among the various types of IF, TRE has recently received increasing attention from researchers since it seems to be a safe and feasible fasting regimen for most people[5,6]. TRE consists of restricting the daily energy intake to a pre-determined eating window (generally ≤10 hours) and fasting for the rest of the day (14 hours or more)[5,6]. During the eating window, individuals are not required to count calories or monitor food intake in any way[5,6].

Human trial findings show that TRE reduces body weight by 1–4% in short term (i.e., ≤3 months) in individuals with overweight/obesity relative to controls with no energy and meal timing restrictions[5,6]. This weight loss results from unintentional reductions in energy intake (10-30% or ~300-500 kcal/day) that occurs when participants confine their eating windows to 4-10 h/day[5,6]. Nonetheless, the impact of TRE on cardiometabolic risk parameters is still uncertain, and while some studies have demonstrated improvements in blood pressure, insulin resistance, lipid profile and markers of oxidative stress, others have shown no benefit on these parameters[5-7]. One of the factors that may explain these discrepancies among studies may be the timing of the eating window during TRE[8].

Most studies of TRE have used an arbitrary clock time to characterize the timing of food intake (e.g., 12:00-20:00). However, this approach does not consider meal timing in relation to the internal circadian time. Accumulating evidence suggests

that the body is optimized for food intake in the morning[9]. That is, insulin sensitivity, beta cell responsiveness, and thermic effect of food are all higher in the morning than in the afternoon or evening[9]. Therefore, it has been hypothesized that earlier eating windows during TRE may produce superior metabolic benefits than later eating windows. Indeed, it has been shown that early TRE (6-hour eating window, with dinner before 15:00) enhanced cardiometabolic health compared with controls (12-hour eating window) after 5 weeks even in the absence of weight loss[10]. Recently, a meta-analysis has shown that early TRE was more effective in improving insulin resistance compared to late TRE[8]. Additionally, only early TRE demonstrated significant benefits in fasting blood glucose and diastolic blood pressure compared to controls (unrestricted eating time)[8]. Nevertheless, no significant differences between early and late TRE were found for body weight loss, fasting blood glucose, blood pressure, and lipid profiles[8]. Therefore, it remains unclear whether there are differences in the effectiveness of early and late TRE on body weight and cardiometabolic risk factors.

Previous studies present important limitations, such as short duration, small sample size to study sex differences and the absence of a self-selected TRE group. There is a lack of evidence on whether the effects of TRE interventions on cardiometabolic health in people at risk are different in women and men mainly because studies are no powered to conduct the analysis separately for men and women, and therefore the results are presented jointly for both sexes. Taking an individual's schedule and personal preference into consideration, and allowing participants to choose their own TRE window, may be important factors to improve adherence, acceptability, and resulting efficacy. Lastly, there is no evidence about the effects of the timing of TRE on different ectopic fat depots including VAT, subcutaneous adipose tissue, hepatic fat, and intermuscular adipose tissue despite being important determinants of metabolic health and mortality[11]. Therefore, whether the timing of the eating window (early, late or self-selected) during TRE impacts weight loss and cardiometabolic risk factors is still largely unknown.

The overall aim of the present study is to investigate the efficacy and feasibility of three different 8h TRE schedules (i.e., early, late, and self-selected) compared to each other and to a UC intervention over 12 weeks on VAT, body composition and cardiometabolic health in adults with overweight/obesity.

Methods

Study design

The experimental protocol was approved by the Servicio Andaluz de Salud (Comité Ético de Investigación Provincial de Granada) and the Comité Ético de Investigación Clínica de Navarra (PI_2021/119). Participants will provide written informed consent prior to study participation (see Online Supporting Information). The present study is registered at the US National Institutes of Health (ClinicalTrials.gov), identifier: NCT05310721. Adults with overweight/obesity will be recruited in Granada (southern of Spain) and Pamplona (northern Spain). This study will therefore be a multicenter randomized controlled trial, which will certainly increase the generalizability and external validity of results by providing a broader basis for generalizations across institutions. The study design is illustrated in **Figure 1**.

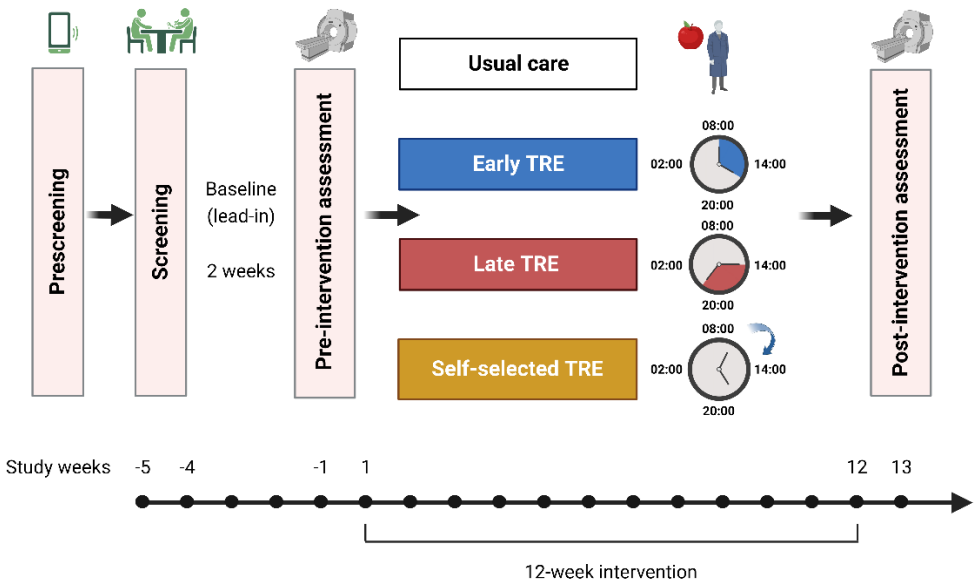

Figure 1. Study design. Prescreening of potential participants will be performed by phone calls, and potential participant will be scheduled for a screening visit. The randomized controlled trial includes a 2-week baseline (lead-in period) and 12-week dietary intervention. TRE, time-restricted eating.

Participants and eligibility criteria

The study population will be men and women (50%) with overweight/obesity and with at least one cardiometabolic risk factor impaired. The inclusion and exclusion criteria are listed in **Table 1**. Eligibility of study participants will be based on the results of screening medical history, vital signs, and clinical laboratory tests (Screening phase, **Figure 1**). The nature of any conditions present at the time of the physical examination and any pre-existing conditions will be documented.

Recruitment and Screening

In Granada, potential participants will be recruited through advertisements on newspapers and the Endocrinology and Nutrition service of the Hospital Universitario Clínico San Cecilio and Virgen de las Nieves. In Pamplona, potential participants will be recruited through advertisements on newspapers and in the Endocrinology and Nutrition service of the University Hospital of Navarra. A pre-screening (**Figure 1**) will be performed as a telephone interview to determine the eligibility of potential participants and check their interest in the study. Potential participants who will be eligible based on the pre-screening will receive written information about the study and will be scheduled for a screening visit (**Figure 1**). At the screening visit, potential participants will provide oral and written informed consent; in addition, body weight, anthropometry, and blood pressure will be measured. Afterwards, the endocrinologists, by telephone interview and consulting the patient medical records, will assess the medical history and check inclusion and exclusion criteria (**Table 1**). Subsequently, potential participants will be scheduled to have fasting blood sampling to confirm that they present at least one of the metabolic impairments (see inclusion criteria, **Table 1**).

Lead-in period

There will be a 2-week lead-in period (before baseline measurements and group allocation; **Figure 1**) in which potential participants will continue with their habitual nutritional and physical activity habits. Potential participants will record daily their eating time and sleep and any adverse event using a phone app. This data will be used to confirm that their eating window is ≥12 hours (see inclusion criteria, **Table 1**).

Table 1. Eligibility criteria of the study.

Inclusion criteria	Exclusion criteria
• Aged 30-60 years. • Body mass index ≥25.0 and <40 kg/m² and abdominal obesity (waist circumference ≥95 cm in men and ≥82 cm in women). • Weight stability (within 3% of screening weight) for >3 months prior to study entry. • Inactive lifestyle (<150 min/week of moderate-vigorous intensity exercise) for >3 months prior to study entry. • Habitual eating window ≥12 hours. • At least one of the following metabolic impairments: - High-density lipoprotein cholesterol (HDL-C) concentration <50 mg/dL for females and <40 mg/dL for males. - Low-density lipoprotein cholesterol (LDL-C) levels >100 mg/dL (or on medication to treat elevated LDL-C cholesterol levels). - Serum triglycerides concentration ≥150 mg/dL or on medication to treat elevated triglycerides. - Systolic blood pressure >130 mm Hg and/or diastolic blood pressure >85 mm Hg or already being treated with anti-hypertension medications. - Impaired glucose tolerance defined as at least one of the following: - Fasting plasma glucose (FPG) ≥100 mg/dL and ≤125 mg/dL. - Hemoglobin A1c between ≥5.7% and <6.5%. - Insulin resistance as determined by the Homeostatic Model Assessment of Insulin Resistance (HOMA2-IR) >1.8.	• History of a major adverse cardiovascular event (acute myocardial infarction, ischemic or haemorrhagic stroke, peripheral arterial ischemia), kidney failure, chronic liver disease, or HIV / AIDS. Active endocrinological disease (Cushing's syndrome, Acromegaly, Adrenocortical insufficiency, GH deficiency), Innate errors of metabolism, Myopathies, Epilepsy. Patients who have undergone bariatric surgery surgical techniques or used for the treatment of other pathologies (Example: "Roux Y"). • Rheumatoid arthritis, Parkinson's disease, active cancer treatment in the past year, type 1 or 2 diabetes mellitus, or another medical condition where fasting is contraindicated. • Use of medications that may affect the results of the study; for example, drugs for glycaemic control (e.g., antidiabetic, steroids, beta-blockers, antibiotics). • The consumption of prebiotics, probiotics, and symbiotics as drugs or dietary supplements. • Metal or electrical prosthesis. • Foreign bodies in the eyes. • Diagnosis of major sleep or eating disorders. • Active tobacco or illicit drug use or a history of alcohol abuse treatment (this is moderate or severe alcoholism). • Participating in a weight loss or a weight-management program. • Pregnancy and lactation or planned pregnancy (within the study period). • Caregiver for a dependent requiring frequent nocturnal care/sleep interruption. • Shift workers with nocturnal hours. • Frequent travel over time zones during the study period. • Fear of needles or claustrophobia to magnetic resonance imaging (MRI). • Being unable to understand and to accept the instructions or the study objectives and protocol. • Not having or being able to use a smartphone with Apple iOS or Android OS. • Are deemed unsuitable by the investigator for any other reason.

Measurements

All the measurements will be conducted at baseline and at 12-week post-intervention (± 3 days) by a consistent team of trained personnel. Table 2 offers a comprehensive summary of the study's outcomes.

Table 2. Overview of the study's outcomes.

Outcomes	Baseline	12-week post intervention
Primary outcome		
Visceral adipose tissue (i.e., MRI)	✔	✔
Secondary outcomes		
Body weight and anthropometry	✔	✔
Body composition (i.e., DXA)	✔	✔
Ectopic fat depots (i.e., MRI)	✔	✔
Cardiometabolic risk factors	✔	✔
Glycemic control (i.e., CGM)	✔	✔*
Gut microbiota	✔	✔*
Sleep and physical activity (i.e., Acc)	✔	✔*
Psychosocial assessment	✔	✔
Eating behaviour assessment	✔	✔
Dietary habits	✔	✔
Quality of life	✔	✔

*Assessment will be conducted during the last two weeks of the intervention. Abbreviation: Acc, accelerometry; CGM, continuous-glucose monitoring; DXA, dual-energy X-ray absorptiometry; MRI, magnetic resonance imaging.

Primary outcome

Visceral adipose tissue

VAT (primary outcome) will be quantified by magnetic resonance imaging (Siemens 3T Magnetom Vida in both Granada and Pamplona).

Secondary outcomes

Body weight, anthropometry, and body composition

Body weight and height will be measured using a stadiometer and scale (Seca model 799, Electronic Column Scale, Hamburg, Germany in both Granada and Pamplona) without shoes and with light clothing. Neck, waist, and hip circumferences will be measured following the ISAK procedures[12]. Bone mineral density, fat mass, and fat-free mass will be assessed using a dual-energy X-ray absorptiometry scan (DXA; QDR Discovery Wi Hologic, Inc., Bedford, MA, USA in Granada and Horizon Wi Hologic, Inc., Bedford, MA, USA in Pamplona).

Ectopic fat depots

Abdominal subcutaneous and intermuscular adipose tissue, as well as hepatic fat fraction will be quantified by magnetic resonance imaging (Siemens 3T Magnetom Vida). Semiautomatic software for tissue segmentation will be used to calculate visceral, subcutaneous, and intermuscular abdominal adipose tissue variables in all 3D abdominal volume: volume, cross-sectional area at selected levels, and mean/median fat fraction. These image markers will be derived from a standard 6 echo Dixon series. At L3 and L5 levels, we will obtain these image markers: cross-sectional area, muscular tissue, and intramuscular fat fraction. We will also quantify with a 6 echoes Dixon series, the fat fraction in all lumbar vertebral bodies, from a mid-sagittal image. The segmentation of these areas will be manually edited. At last, we will measure image markers from mid-thigh. Again, using a 6 echoes Dixon series, we will obtain cross-sectional area, muscular tissue, intermuscular adipose tissue, fat fraction, subcutaneous adipose tissue, and bone marrow fat fraction. The segmentation for all these structures will be done with a semiautomatic proprietary algorithm.

Cardiometabolic risk factors.

Fasting blood samples will be collected to analyze:

- Glucose, insulin, and hemoglobin A1c, and we will calculate simple insulin resistance surrogates such as the homeostatic model assessment for insulin resistance (HOMA-IR) and the quantitative insulin-sensitivity check index (QUICKI).
- Lipid profile (e.g., total cholesterol, HDL-C, LDL-C, triglycerides, apolipoprotein A1 and B).
- Bone metabolism profile (e.g., Vitamin D, alkaline phosphatase, calcium).
- Liver and kidney function markers (e.g., alanine transaminase, gamma-glutamyl transferase, bilirubin, creatinine, estimated glomerular filtration rate).
- Steroid hormones (e.g., estradiol, progesterone, testosterone).
- Thyroid hormones (e.g., thyrotropin, thyroxine, triiodothyronine).
- Blood count and biochemistry markers (e.g., iron, ferritin, folic acid).
- Blood samples will be also stored at -80°C for future analysis. The systolic and diastolic blood pressure will be measured with an automatic

monitor (M3-Comfort, Omron Healthcare Europe B.V. Hoofddorp, The Netherlands, in both Granada and Pamplona) following the 2021 European Society of Hypertension practice guidelines[13].

Glycemic control.

Participant will wear continuous-glucose monitoring (FreeStyle LibrePro, Abbott, in Granada and FreeStyle 2, Abbott, in Pamplona) during two weeks before the intervention (lead-in period) and during the last two weeks of the intervention. From the continuous-glucose monitoring data several variables of glycemic control will be calculated according to the last international consensus statement[14].

Gut microbiota

Faecal samples will be collected at baseline and during the last two weeks of the intervention to extract genomic DNA and thereafter, to perform the metagenomics analysis (16S rRNA gene amplicon sequencing – shotgun methodology will be considered pending the final budget) to obtain a complete description of gut microbioma diversity and composition. Besides, a faecal metabolomic fingerprint analysis will be carried out in order to establish the metabolic profile in the different groups of patients by liquid chromatography method coupled to mass spectrometry (LC-MS).

Sleep and physical activity assessment.

Sleep, chronotype, and physical activity will be subjectively assessed using validated questionnaires including the Pittsburgh Sleep Quality Index (PSQI), Munich Chronotype Questionnaire (MCTQ), Horne y Östberg (MEQ-SA), and the International physical activity questionnaire (IPAQ). Sleep and physical activity levels will be also objectively assessed using accelerometers; concretely, participant will wear a triaxial accelerometer (ActiGraph GT3X+, Pensacola, FL, USA, in both Granada and Pamplona) on the non-dominant wrist during two weeks before the intervention (lead-in period) and during the last two weeks of the intervention.

Psychosocial assessment.

Participants will complete validated questionnaires regarding several psychosocial dimensions: Beck Depression Inventory Fast Screen (BDI-FS), Perceived Stress Scale (PSS), and State-Trait Anxiety Inventory (STAI).

Eating behaviour assessment.

Eating behaviour will be assessed using validated questionnaires: Food Craving Inventory (FCI) and the Adult Eating Behavior Questionnaire (AEBQ).

Dietary habits assessment.

Participants will complete a validated questionnaire to assess their adherence to the Mediterranean dietary pattern. Moreover, three non-consecutive 24-hour dietary recalls (two of working days and one of non-working day) will be recorded in a face-to-face or telephone interview by qualified and trained research dietitians.

Quality of life.

Quality of life will be evaluated using the EuroQol 5 dimensions 5 levels (EQ-5D-5L), Rand Short Form 36 (SF-36), an adverse events questionnaire and menstrual questionnaire.

Randomization and blinding

The method used for randomization will be the stratified permuted block randomization. A total of ~208 patients will be randomized using both stratification and permuted blocks with random block sizes. Randomization will be stratified at each site (Granada and Pamplona) based on sex (men-women); with a total of two strata for each site. For this randomization scheme, a randomization list will be generated prior to the start of the trial; one randomization list being generated for each site and strata. A sequence of block sizes will be randomly generated where allowable block sizes will be 4 and 8. Within each block, each quarter of assignments will be randomly selected to be to one of the four possible groups (UC, early TRE, late TRE or self-selected TRE) using a parallel design (1:1:1:1 allocation ratio). As each participant is randomized into the trial, the participant will receive the next sequential

assignment on the randomization list specific to his/her site and strata. The use of a random block size ensures that the next randomization assignment cannot be guessed. Because this will be a multicenter trial with two sites (Granada and Pamplona), randomization within each site will ensure that a site discontinuing participation in the trial or enrolling poorly would not affect the overall balance of the treatment groups. Stratifying by sex ensures that intervention groups are balanced on this important characteristic. Personnel in charge of the evaluations of the primary outcome (VAT and other ectopic MRI derived fat deports), fasting blood samples and statistical analysis will be blinded to the group assignment, whereas personnel in charge of the other measures as well as the intervention will be not blinded to the group assignment (open label).

Time-restricted eating intervention

Participant will be randomly assigned to one of the following four groups:

- UC. Participants in the UC group will continue with their dietary eating time schedule and will receive, as well as the participants in the TRE groups, an educational program for weight management and cardiovascular health promotion based on Mediterranean dietary pattern[15] and physical activity recommendations from the World Health Organization[16].
- Early TRE. Participants will select an early 8h eating window (i.e., starting not later than 10:00) before the intervention and will maintain the same 8h eating window during the 12-week intervention.
- Late TRE. Participants will select a late 8h eating window (i.e., starting not before 13:00) before the intervention and will maintain the same 8h eating window during the 12-week intervention.
- Self-selected TRE. Participants will select their preferred 8h eating window before the intervention and will maintain the same 8h eating window during the 12-week intervention.

No calorie-containing food or beverage intake will be allowed outside the eating window for the TRE groups. Only water, coffee, and tea without sugar or artificial sweeteners are allowed outside the eating window for the TRE groups. Participants in the TRE groups will be instructed to perform the TRE intervention every day of the week (i.e., seven days).

Usual-care intervention

Intervention meetings for all groups will take place every two weeks by experienced dietitians. It will be voluntary, but attendance to the meetings will be recorded. There will be 6 topics to be addressed in each intervention meeting: (i) healthy lifestyle based on Mediterranean dietary pattern and physical activity recommendations; (ii) organization and planning of food intake; (iii) control of hunger and satiety; (iv) nutritional labelling; (v) nutritional myths; and (vi) healthy snacks. After each meeting, experienced research dietitians will answer the doubts/questions of the participants.

Assessment of adherence and adverse events

Every day during the 12-week intervention, all participants will record their time of sleep, eating, and any potential adverse event using a phone app. The Study Coordinators will record the frequency of adverse events and report them to the Principal Investigators weekly. If a serious adverse event or unanticipated problem is reported, the Study Coordinators will immediately notify the Principal Investigators and Medical Monitor, who will determine if it is necessary to inform the ethics committee and determine the appropriate course of action to address the event.

Participant retention

Every effort will be made by the Principal Investigators and study team to ensure participants complete each study visit and the study overall. We will use the following strategies to help to maximize retention and minimize loss to follow-up:

Following a proactive plan for retention, building participant relations and participant satisfaction. Including asking participants how they are doing during the intervention meetings.

Giving participants and their families the opportunity to ask questions and express concerns pertaining to their condition throughout the study.

Enhancing participant's understanding of the study's objectives and the protocol by reminding the participant of the study aim during study visits or having question and answer sessions after each visit, if needed.

Assessing each participant's drop-out potential and intervening as needed to keep participants interested in continuing to participate.

Data management

The majority of data will be recorded directly into REDCap, which is a secure web-based platform for building and managing online research-related databases and surveys. Any data not recorded in REDCap will be stored securely on university computers under strict access control to ensure confidentiality and data integrity. To ensure data quality and integrity, we will perform regular data quality control checks that may identify potential data anomalies, such as missing data or forms, out-of-range or erroneous data, inconsistent and illogical dates over time, data inconsistency across forms and visits, and incomplete fields on completed forms without a reason for missing data provided. Any identified issues will be reviewed and resolved by the research team promptly.

Sample size

A recent systematic review and meta-analysis indicate that a VAT reduction of 6.1% is considered a clinically meaningful change even in the absence of weight loss[17]; previous exercise and nutrition intervention trials also showing changes in VAT ranging from 10 to 28%[18,19]. The sample size calculations for VAT assume the study will be able to detect a mean difference of 10% in each intervention group from baseline to intervention endpoint, relative to the UC group. Therefore, assuming a standard deviation of 7% in VAT[18], the enrolment of 21 participants per arm will provide a statistical power of 90% at an alpha level of 0.008 (controlling for multiple group comparisons) to detect a minimum effect size of 10% in VAT. Considering subgroup analyses by sex and a maximum dropout rate of ~20%, we will recruit ~52 participants for each trial group; the total sample size being of ~208 participants (~104 in each study site).

Statistical analysis

Intervention effects on primary and secondary outcomes at 3 months after the intervention will be assessed based on repeated-measures linear mixed-effects

multilevel models, which will include random cluster (site) effects[20]. Individual measures of change will therefore be modelled as the function of randomly assigned group, site, assessment time, and their interaction terms. Model-based estimations will be performed with an intention-to-treat approach (primary analyses) using the restricted maximum-likelihood method; the model assuming that missing values are missing-at-random. Analyses and estimations will also be performed with a per-protocol approach and an attrition propensity will be calculated using a logistic model predicting attrition with baseline values of site, allocation group, age, sex and BMI.

It should also be noted that the intervention effect assessments will not only be based on statistical and practical significance (as usually done), but also on a practical benefit approach emphasizing and reporting unadjusted values that are intuitive to human judgment and readily replicable considering the design and methodology of this project.

Potential impact of the study

There is increasing evidence indicating that not only what, but also when we eat is crucial to prevent fat accumulation and the development of cardiometabolic diseases[21]. TRE is an emerging dietary approach that has gained enormous attention and expectation among the scientific and public community[4,5]. Indeed, many people around the globe are putting it into practice despite the lack of robust and reliable scientific evidence. This study will provide strong scientific evidence to overcome the shortcomings found in this field. Firstly, we will show whether TRE is a feasible strategy in a Mediterranean country where people usually have breakfast at ~8:00 and dinner at ~22:00[22]. Moreover, we will determine whether early TRE is more effective than late and self-selected TRE in terms of VAT and cardiometabolic risk factors reduction, an important question that is still unsolved. The assessment of other ectopic fat depots such as hepatic fat and intermuscular will also be important in determining the effectiveness of early, late, and self-selected TRE in reducing cardiometabolic risk factors. Excessive hepatic fat accumulation has been strongly associated with insulin resistance, type 2 diabetes, and cardiovascular disease[23-25], while intermuscular fat has been linked to increased insulin resistance[26] and to the presence and severity of hepatic steatosis[27,28]. We will also evaluate changes in other markers of metabolic health such as glucose levels over day and night with the use of , continuous-glucose monitoring 14d/24-hour glucose, insulin, and lipid levels to

gain a comprehensive understanding of the effects of TRE on overall cardiometabolic health. Whereas the majority of previous studies have been conducted on men, we will determine the differential benefits of TRE in both men and women. We strongly believe that taking the existing sex-dimorphic into serious consideration will lead to a better understanding of sex differences in obesity and related comorbidities and will aid in achieving sex-specific personalized treatments and therapies. The final goal is to examine the potential health benefits of a novel and pragmatic intervention for the treatment of obesity and related cardiometabolic risk factors; an approach readily adaptable to real-world practice settings, easy for clinicians to deliver, and intuitive for patients to implement and maintain in their lives.

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Eat
Fast
16 hrs

RESULTS AND DISCUSSION

Chapter 4. Association of meal timing with body composition and cardiometabolic risk factors in young men and women

Abstract

Purpose: To investigate the association of meal timing with body composition and cardiometabolic risk factors in young adults.

Methods: In this cross-sectional study participated 118 young adults (82 women; 22 ± 2 years old; BMI: 25.1 ± 4.6 kg/m^2). Meal timing was determined via three non-consecutive 24-hour dietary recalls. Sleep outcomes were objectively assessed using accelerometry. The eating window (time between first and last caloric intake), caloric midpoint (local time at which ≥50% of daily calories are consumed), eating jetlag (variability of the eating midpoint between non-working and working days), time from the midsleep point to first food intake, and time from last food intake to midsleep point were calculated. Body composition was determined by dual-energy x-ray absorptiometry. Blood pressure and fasting cardiometabolic risk factors (i.e., triglycerides, total cholesterol, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, and insulin resistance) were measured.

Results: Meal timing was not associated with body composition ($p>0.05$). The eating window was negatively related to HOMA-IR and cardiometabolic risk score in men ($R^2=0.348$, $\beta=-0.605$; $R^2=0.234$, $\beta=-0.508$; all $p\leq0.003$). The time from midsleep point to first food intake was positively related to HOMA-IR and cardiometabolic risk score in men ($R^2=0.212$, $\beta=0.485$; $R^2=0.228$, $\beta=0.502$; all $p=0.003$). These associations remained after adjusting for confounders and multiplicity (all $p\leq0.011$).

Conclusions: Meal timing seems unrelated to body composition in young adults. However, a longer daily eating window and a shorter time from midsleep point to first food intake (i.e., earlier first food intake in a 24-hour cycle) are associated with better cardiometabolic health in young men.

Introduction

The obesity epidemic is one of the leading contributors to the global burden of cardiometabolic diseases and disability, resulting in a significant economic impact on health care systems[1,2]. However, despite the known consequences of excess body weight, the prevalence of obesity continues to rise, and currently there are more than 1.9 billion adults with overweight or obesity worldwide[1]. Body weight regulation and obesity are highly influenced by several factors such as genetics, physiology, and socioeconomic factors[3]. For instance, in recent years, emerging evidence has shown that the timing of food intake may be a relevant risk factor for obesity and cardiometabolic diseases[4,5].

The importance of when we eat is tied to our circadian system, which temporally orchestrates numerous metabolic processes across the body over a 24-hour period[6,7]. The circadian system is constituted by a central clock located in the suprachiasmatic nuclei of the hypothalamus and a series of peripheral clocks placed in virtually all cells and tissues of the body[6,7]. The central clock is primarily controlled by external light via direct connection to the retina, whereas peripheral clocks are regulated by the central clock and external time cues, such as eating, physical activity and sleep among other behavioural tasks[6,7]. Since different stimuli impact the central and peripheral clocks, the two clock systems become misaligned whenever their respective time cues are out of synchrony[6,7]. The modern lifestyle promotes this circadian misalignment by allowing exposure to artificial light at night and by allowing access to food all day long[6-8].

Epidemiological data have shown that eating late in the day is associated with greater energy intake, adiposity and worst cardiometabolic health[9-16]. Furthermore, considerable variability in meal timing between non-working days and working days, coined eating jet lag, has been linked to higher body mass index (BMI)[17]. Clinical trials have also revealed that reducing the daily eating window (i.e., the period of time between the first and the last caloric intake) and/or shifting dietary energy intake earlier in the day results in body weight loss or optimized cardiometabolic health in adults with overweight or obesity[9,18-22]. Nonetheless, one of the main limitations of meal timing studies is the use of clock time to illustrate the timing of food intake, which fails to correctly characterize meal timing in relation to the internal circadian time[10,23]. Because obtaining measures of dim-light melatonin is not practical in large clinical trials, it has been proposed that the timing of food intake should be assessed in relation

to the sleep/wake cycle as a proxy of circadian time[10,23,24]. Only a few studies have followed this methodology, indicating that a longer time period from dinner to midsleep point or bedtime is associated with lower adiposity and BMI, respectively[10,23]. However, these studies also present important limitations, such as the self-reported assessment of sleep timing, solely including anthropometric variables or fat mass measured by non-state-of-the-art methods and the absence of various cardiometabolic risk. Moreover, although sex is known to influence several aspects of biology, physiology and psychology previous studies have not investigated if there are sex differences in the relationship between meal timing with body composition and cardiometabolic risk factors.

Our study was aimed at elucidating the association of meal timing [i.e., eating window, caloric midpoint (the time at which ≥50% of daily calories are consumed), eating jet lag, time from midsleep point to first food intake, and time from last food intake to midsleep point] with anthropometry [i.e., body weight, BMI and waist circumference], body composition [i.e., fat mass, lean mass, and VAT mass] and cardiometabolic risk factors [i.e., blood pressure, triglycerides, total cholesterol, high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), insulin resistance] in young adults. Special attention was paid to the influence of sex on these associations. We hypothesized that a shorter daily eating window, earlier food intake schedule and less variability in meal timing between non-working days and working days would be associated with healthier body composition and cardiometabolic risk factors.

Methods

Participants

A total of 118 young adults (n= 82 women; see Flow Chart, **Figure S1**) took part in this cross-sectional study which includes the baseline measurements of the ACTIBATE study (ClinicalTrials.gov NCT02365129)[25] and follows the STROBE-nut guidelines (**Table S1**)[26]. All assessments were performed in Granada (Spain) during September-December in 2015 and 2016. The inclusion/exclusion criteria included: (i) being 18–25 years, (ii) having a BMI ranging from 18 to 35 kg/m2, (iii) being non-smokers, (iv) not being enrolled in a weight loss program, (iii) having a stable body weight (body weight changes <3 kg over 3 months), (iv) not being physically active (<20 min on <3 days/week), (v) not taking any medication or drugs, and (vi) not suffering from any acute or chronic illness.

Outcome Measurements

Anthropometry and Body Composition

Body weight and height were determined with participants wearing light clothing and without shoes using a SECA scale and stadiometer (model 799; Electronic Column Scale, Seca GmbH, Hamburg, Germany). The BMI was calculated as weight (kilograms) divided by height squared (square meters). Waist circumference (centimetres) was assessed midway between the lowest rib and the top of the iliac crest. Alternatively, when participants showed abdominal obesity, waist circumference was determined in a horizontal plane above the umbilicus after exhalation.

Body composition was measured by DXA (QDR Discovery Wi Hologic, Inc., Bedford, MA, USA) strictly following the manufacturer's instructions, obtaining fat mass, lean mass, and VAT mass. The lean mass index was calculated as lean mass (kilograms) divided by height squared (square meters).

Cardiometabolic profile

The systolic and diastolic blood pressure were measured on three different days with an automatic monitor (Omron Healthcare Europe B.V. Hoofddorp, The Netherlands), and the average was used in later analyses. We calculated the mean blood pressure as diastolic blood pressure + 1/3(systolic blood pressure – diastolic blood pressure). Blood samples were collected from the antecubital area in the morning after fasting for >10 hours. All samples were centrifuged, and aliquots of serum were stored at -80ºC until analysis. All participants were requested to abstain from drugs and caffeine and to avoid moderate-intensity physical activity and vigorous-intensity activity for 24-hour and 48-hour before testing, respectively. Serum glucose, total cholesterol, HDL-C and triglycerides were assessed following standard methods using an AU5832 automated analyzer (Beckman Coulter Inc., Brea CA, USA). LDL-C was then estimated[27]. Serum insulin was measured using the Access Ultrasensitive Insulin Chemiluminescent Immunoassay Kit (Beckman Coulter Inc., Brea, CA, USA). The HOMA-IR was calculated.

Cardiometabolic risk scores were calculated for each sex based on variables included in the diagnosis of Metabolic Syndrome[28]: Waist circumference, blood pressure, plasma glucose, HDL-C, and triglyceride concentrations. A Z-score was

calculated for each variable. The HDL-C standardized values were multiplied by -1 to be directly proportional to the cardiometabolic risk. The final score was determined as the average of the 5 Z-Scores. Thus, the cardiometabolic risk score is a continuous variable with a mean of 0 and a standard deviation of 1; higher scores indicating higher risk.

Sleep parameters, sedentary time and physical activity assessment

Participants were instructed to wear a triaxial accelerometer (ActiGraph GT3X+, Pensacola, FL, USA) on the non-dominant wrist 24-hour a day for seven consecutive days, removing them only when swimming or bathing, and to record information on sleep onset and wakeup times each day in a sleep diary. The midsleep point and other sleep-related outcomes were objectively assessed using an algorithm guided by the participants' reported times. The raw data were processed in R [version 3.1.2, https://www.R-project.org/] using the GGIR package [version 1.5-12, https://cran.r-project.org/web/packages/GGIR/]. Actigraphy recordings were used to determine: (i) sleep onset (time at which the subject fell asleep); (ii) sleep offset (time at which the subject woke up); and (iii) sleep duration (time between falling asleep and waking up). Daytime naps were not considered. Those participants registering less than 16 h/day of wear time for more than four days and/or not having data from at least one weekend day were excluded from the final analysis. We used these sleep variables to calculate the midsleep point (the middle time point between sleep onset and sleep offset) as follows: midsleep point (local time) = (sleep duration/2) + sleep onset[24]. We determined the midsleep point for non-working days and working days separately. Subsequently, the midsleep point for the week was calculated as a weighted mean, which was used in later analyses. Lastly, we computed social jetlag (a proxy of the discrepancy between social and biological time) by subtracting each participant's midsleep point for working days from non-working days[24].

We estimated the time spent in sedentary behavior and different physical activity intensities (i.e., light, moderate, vigorous, and moderate to vigorous) using age-specific cut points[29].

Dietary assessment and meal timing

Dietary intake and meal timing were recorded using three non-consecutive 24-hour dietary recalls (one of them for a non-working day) distributed over three weeks with the participants unaware of when their diets were going to be recorded. In face-to-face interviews performed by trained dietitians, participants were asked to recall all food consumed on the previous day, using photographs of portion sizes[30], as well as the time of the meal event. Data recorded in the interviews were independently introduced by two dieticians in the EvalFINUT® software When the coefficient of variance between the two datasets was >5%, a third dietician reintroduced the data obtained from the 24-hour recalls in the EvalFINUT® software and the mean of the two datasets with the best agreement (i.e., lower coefficient of variance) was used. From these data, we calculated the following meal timing variables:

The eating window (i.e., the period of time between the first and the last caloric intake) was calculated for each day[31]. The caloric midpoint is defined as the time at which ≥50% of daily calories are consumed and is expressed in local time[12]. We determined the eating window and the caloric midpoint for non-working days and working days. Subsequently, the value for the week was calculated as a weighted mean, which was used in later analyses. Eating jetlag (i.e., the variability of the eating midpoint between non-working days and working days) was determined as[24]: eating midpoint for non-working days - eating midpoint for working days. The time from the midsleep point to first food intake and the time from last food intake to the midsleep point were also calculated.

Dietary patterns and quality were determined by analysing the participants' data from the 24-hour recalls and a validated food frequency questionnaire[32]. Three Mediterranean dietary patterns[33-35] were computed: the *a priori* Mediterranean dietary pattern[33], the Mediterranean diet score, and the dietary quality index for a Mediterranean diet[34,35]. Adherence to the Dietary Approaches to Stop Hypertension guidelines was also calculated. The diet quality index[36] and the dietary inflammatory index[37], were also determined. Breakfast consumers were defined as consuming breakfast in all of the three non-consecutive 24-hour dietary recalls, whereas breakfast skippers were defined as not consuming breakfast on at least one of the three non-consecutive 24-hour dietary recalls.

Statistical analyses

The distribution of the variables was verified using the Shapiro-Wilk test, skewness and kurtosis values, visual checking of histograms, and Q-Q and box plots. The descriptive parameters are reported as mean and standard deviation when normally distributed, or medians (interquartile range) when a Gaussian distribution was not found. Triglycerides, total cholesterol, HDL-C, LDL-C, and HOMA-IR were log10-transformed to bring their distributions closer to normal and used in subsequent analyses. A one-way analysis of variance was used to compare baseline characteristics between men and women. We conducted simple linear regression models to examine the association of meal timing (i.e., eating window, caloric midpoint, eating jetlag, time from midsleep point to first food intake, and time from last food intake to midsleep point) with anthropometry (i.e., weight, BMI and waist circumference), body composition (i.e., fat mass percentage, lean mass index, and VAT mass) and cardiometabolic risk factors (i.e., mean blood pressure, triglycerides, total cholesterol, HDL-C, LDL-C, HOMA-IR, and cardiometabolic risk score). We also conducted multiple linear regression models to examine these associations adjusting for potential confounders (i.e., sex, a priori Mediterranean diet pattern, light physical activity, midsleep point or sleep duration, and BMI). Differences between breakfast skippers and consumers in anthropometry, body composition, cardiometabolic risk factors and potential confounders were determined by the Welch's t-test. Some statistical differences were observed; therefore, additional analyses were performed. Concretely, the simple linear regression models were repeated to examine the association of meal timing with anthropometry, body composition and cardiometabolic risk factors only in breakfast consumers.

The main analyses were corrected for multiple comparison errors (familywise error rate [Hochberg procedure])[38]. The Statistical Package for the Social Sciences (SPSS) v.25.0 (IBM Corporation, Chicago, IL, USA) was used for all analyses. GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA) was used for plots. Significance was set at $P < 0.05$.

Results

Table 1 shows the main characteristics of the study participants.

Meal timing and anthropometry/body composition

The caloric midpoint was negatively associated with fat mass percentage in men (R^2=0.089, β=-0.342, p=0.048; **Table 2**), but became non-significant after adjusting for multiplicity (p>0.05). No further associations were found between meal timing and anthropometry/body composition (all p≥0.068; **Table 2**).

Meal timing and cardiometabolic risk factors

The eating window was negatively related to HOMA-IR in all participants and in men (R^2=0.100, β=-0.328; R^2=0.348, β=-0.605, respectively; all p≤0.001; **Table 3**, **Figure 1** and **S2**). The eating window was also negatively associated with cardiometabolic risk score in all participants and in men (R^2=0.079, β=-0.296; R^2=0.234, β=-0.508, respectively; all p≤0.003; **Table 3**, **Figure 1** and **S2**). A negative association between the eating window and triglycerides was observed in all participants (R^2=0.044, β=-0.229, p=0.013; **Table 3** and **Figure S2**). The eating window was positively related to HDL-C in all participants and in men (R^2=0.048, β=0.238; R2=0.162, β=0.431, respectively; all p≤0.010; **Table 3** and **Figure S2**). No associations were found between caloric midpoint and eating jetlag with cardiometabolic risk factors (all p≥0.150; **Table 3**). The time from midsleep point to first food intake was positively related to HOMA-IR in all participants and in men (R^2=0.078, β=0.294; R2=0.212, β=0.485, respectively; all p≤0.003; **Table 3**, **Figure 1** and **S2**). The time from midsleep point to first food intake was also positively associated with cardiometabolic risk score in all participants and in men (R^2=0.081, β=0.300; R^2=0.228, β=0.502, respectively; all p≤0.003; **Table 3**, **Figure 1** and **S2**). A positive association between the time from midsleep point to first food intake and mean blood pressure was found in all participants (R^2=0.030, β=0.197, p=0.037; **Table 3** and **Figure S2**). The time from midsleep point to first food intake was negatively associated with HDL-C in all participants and in men (R^2=0.045, β=-0.231; R^2=0.137, β=-0.403, respectively; all p≤0.016; **Table 3** and **Figure S2**). A significant positive association between the time from last food intake to midsleep point and triglycerides was found in women (R^2=0.087, β=0.314, p=0.005; **Table 3**). Several variables of meal timing were significantly associated with potential confounders such as a priori

Mediterranean diet pattern, light physical activity and midsleep point (all p≤0.05; **Figure S3**). Of note is that only the associations of eating window and time from midsleep point to first food intake with HOMA-IR and cardiometabolic risk score - in all participants and men - remained after adjusting for potential confounders and false discovery rate (all p≤0.011; **Table 2-3** and **S2-S3**).

Differences between breakfast skipper and consumer

Significant differences between breakfast skipper and consumer were found for fasting blood glucose, midsleep point, dietary patterns and quality, eating window, caloric midpoint, eating jetlag, and time from midsleep point to first food intake (all p≤0.045; **Table S4**). Breakfast consumption influenced the relationship between meal timing and body composition in women observing that the eating window was positively associated with BMI and VAT mass in women breakfast consumers (all p≤0.036; **Figure S4**). Breakfast consumption did not modify the relationship shown by meal timing with cardiometabolic risk factors (**Figure S5**).

Table 1. Descriptive characteristics of participants.

		All		Men		Women
Age (years)	118	22.2 (2.2)	36	22.5 (2.3)	82	22.1 (2.1)
Anthropometry and body composition						
Height (cm)	116	168.0 (8.5)*	34	176.3 (6.4)	82	164.5 (6.7)
Weight (kg)	116	71.2 (16.8)*	34	85.5 (18.5)	82	65.2 (11.7)
Body mass index (Kg/m^2)	116	25.1 (4.6)*	34	27.4 (5.4)	82	24.1 (3.9)
Fat mass (kg)	116	25.6 (9.0)	34	27.0 (11.6)	82	25.0 (7.6)
Fat mass (%)	116	36.4 (7.3)*	34	31.1 (7.7)	82	38.6 (5.9)
Lean mass (Kg)	116	41.6 (9.8)*	34	53.7 (7.5)	82	36.6 (5.0)
Lean mass index (Kg/m^2)	116	14.6 (2.4)*	34	17.3 (2.2)	82	13.5 (1.4)
Visceral adipose tissue mass (g)	116	346.0 (187.3)*	34	446.7 (188.7)	82	304.3 (171.2)
Waist circumference (cm)	114	80.8 (14.5)*	34	91.4 (16.4)	80	76.3 (10.9)
Cardiometabolic risk factors						
Systolic blood pressure (mmHg)	116	116.2 (12.4)*	36	126.0 (12.5)	80	111.8 (9.6)
Diastolic blood pressure (mmHg)	116	70.7 (7.9)*	36	73.0 (10.1)	80	69.7 (6.6)
Mean blood pressure (mmHg)	116	85.9 (8.1)*	36	90.7 (8.8)	80	83.7 (6.7)
Triglycerides (mg/dl)	117	70.0 (52.5, 97.5)	36	74.0 (57.8, 105.8)	81	68.0 (52.0, 89.5)
Total cholesterol (mg/dl)	117	158.0 (143.0, 179.0)	36	153.0 (140.0, 174.8)	81	164.0 (143.0, 183.5)
High-density lipoprotein cholesterol (mg/dl)	117	51.0 (45.5, 58.0)*	36	46.5 (39.3, 51.0)	81	53.0 (47.5, 63.0)
Low-density lipoprotein cholesterol (mg/dl)	117	92.0 (78.5, 109.0)	36	91.0 (81.3, 108.8)	81	92.0 (75.5, 109.0)
Glucose (mg/dl)	117	87.0 (83.0, 92.0)*	36	89.5 (82.3, 97.0)	81	86.0 (83.0, 90.0)
Insulin (μIU/ml)	117	7.2 (5.5, 10.1)	36	7.3 (5.2, 11.4)	81	7.1 (5.5, 9.8)
HOMA-IR	117	1.5 (1.1, 2.2)*	36	1.6 (1.2, 2.5)	81	1.5 (1.1, 2.1)
Chronobiology						
Sleep onset (hh:mm)[a]	113	01:13 (01:13)	34	01:15 (01:20)	79	01:12 (01:10)
Sleep offset (hh:mm)[a]	113	08:52 (01:02)	34	09:00 (01:12)	79	08:48 (00:57)
Sleep duration (h) [b]	113	7.85 (1.2)	34	7.98 (1.25)	79	7.8 (1.17))
Midsleep point (hh:mm)[a]	113	05:04 (01:05)	34	05:10 (01:15)	79	05:02 (01:00)
Social jetlag (h)[b]	113	1.42 (1.23)	34	1.40 (1.10)	79	1.42 (1.30)
Meal timing						
Eating window (h)[b]	117	12.10 (1.5)	36	12.00 (1.70)	81	12.20 (1.40)
Caloric midpoint (hh:mm)[a]	117	15:54 (01:36)	36	15:48 (01:36)	81	15:54 (01:42)
Eating jetlag (h)[b]	117	1.20 (1.10)	36	1.20 (1.33)	81	1.20 (1.00)
Time from midsleep point to first food intake (h)[b]	113	4.87 (1.40)	34	4.87 (1.72)	79	4.87 (1.23)
Time from last food intake to midsleep point (h)[b]	113	7.05 (1.08)	34	7.22 (1.23)	79	6.97 (1.02)

Data are presented as sample size, mean (standard deviation) when normally distributed, or medians (interquartile range) when not. [a] Time shown in local time. [b] Time shown in decimal format. * P≤0.05 for sex comparisons by a one-way analysis of variance (all cardiometabolic risk factors except for blood pressure variables were log10-transformed to bring their distributions closer to normal). HOMA-IR, homeostasis model assessment of insulin resistance.

Table 2. Association of meal timing with body composition in young adults.

Body composition	All				Men				Women			
	N	R^2	β	P-value	N	R^2	β	P-value	N	R^2	β	P-value
Body mass index (Kg/m²)												
Eating window (h)	116	-0.004	-0.070	0.452	34	0.030	-0.243	0.166	82	-0.005	0.088	0.433
Caloric midpoint (h)	116	-0.008	0.029	0.759	34	-0.009	-0.146	0.410	82	0.008	0.143	0.201
Eating jetlag (h)	116	-0.009	-0.007	0.945	34	-0.026	-0.073	0.681	82	-0.012	0.012	0.917
Time from midsleep point to first food intake (h)	113	-0.001	0.091	0.339	34	0.030	0.243	0.166	79	-0.012	-0.024	0.833
Time from last food intake to midsleep point (h)	113	-0.008	-0.032	0.737	34	-0.031	-0.010	0.956	79	-0.001	-0.110	0.334
Fat mass (%)												
Eating window (h)	116	-0.009	0.003	0.979	34	-0.015	-0.125	0.482	82	-0.012	0.016	0.886
Caloric midpoint (h)	116	-0.007	-0.037	0.691	34	0.089	-0.342	0.048	82	-0.006	0.083	0.460
Eating jetlag (h)	116	-0.006	-0.054	0.563	34	-0.031	-0.013	0.943	82	-0.009	-0.055	0.625
Time from midsleep point to first food intake (h)	113	-0.006	0.053	0.578	34	-0.013	0.131	0.459	79	-0.013	0.005	0.968
Time from last food intake to midsleep point (h)	113	-0.002	-0.082	0.390	34	-0.031	-0.014	0.938	79	-0.010	-0.054	0.634
Lean mass index (Kg/m²)												
Eating window (h)	116	0.001	-0.099	0.289	34	0.072	-0.316	0.068	82	0.004	0.128	0.250
Caloric midpoint (h)	116	-0.007	0.041	0.659	34	-0.031	0.022	0.901	82	0.006	0.135	0.228
Eating jetlag (h)	116	-0.008	0.027	0.776	34	-0.023	-0.087	0.625	82	-0.010	0.052	0.642
Time from midsleep point to first food intake (h)	113	-0.003	0.074	0.434	34	0.054	0.287	0.100	79	-0.010	-0.052	0.651
Time from last food intake to midsleep point (h)	113	-0.008	0.034	0.724	34	-0.030	0.029	0.873	79	0.002	-0.120	0.292
Visceral adipose tissue mass (g)												
Eating window (h)	116	0.004	-0.111	0.236	34	0.037	-0.257	0.142	82	-0.012	0.002	0.989
Caloric midpoint (h)	116	-0.008	0.027	0.771	34	-0.014	-0.130	0.464	82	0.000	0.112	0.315
Eating jetlag (h)	116	-0.006	-0.049	0.600	34	-0.030	-0.034	0.848	82	-0.005	-0.087	0.440
Time from midsleep point to first food intake (h)	113	0.002	0.107	0.261	34	0.051	0.282	0.106	79	-0.013	0.006	0.960
Time from last food intake to midsleep point (h)	113	-0.009	0.002	0.982	34	-0.029	-0.046	0.798	79	-0.012	-0.028	0.809
Waist circumference (cm)												
Eating window (h)	114	-0.001	-0.088	0.352	34	0.049	-0.279	0.111	80	-0.001	0.109	0.337
Caloric midpoint (h)	114	-0.008	-0.028	0.770	34	0.020	-0.224	0.203	80	-0.003	0.101	0.375
Eating jetlag (h)	114	-0.005	-0.065	0.491	34	-0.022	-0.095	0.594	80	-0.002	-0.105	0.352
Time from midsleep point to first food intake (h)	111	-0.004	0.072	0.450	34	0.007	0.192	0.277	77	-0.013	-0.018	0.876
Time from last food intake to midsleep point (h)	111	-0.009	0.016	0.866	34	-0.019	0.110	0.536	77	0.008	-0.147	0.203

Adjusted R^2, β standardized regression coefficients and P values are obtained from single linear regressions. Neither association remained statistically significant after applying false discovery rate correction (Benjamini-Hochberg).

Table 3. Association of meal timing with cardiometabolic risk factors in young adults.

Cardiometabolic risk factors	All				Men				Women			
	N	R^2	β	P-value	N	R^2	β	P-value	N	R^2	β	P-value
Mean blood pressure (mmHg)												
Eating window (h)	116	0.015	-0.153	0.100	36	0.051	-0.280	0.099	80	-0.011	-0.036	0.749
Caloric midpoint (h)	116	-0.006	0.054	0.562	36	-0.007	0.149	0.387	80	-0.012	0.033	0.773
Eating jetlag (h)	116	-0.009	-0.015	0.871	36	-0.029	-0.013	0.940	80	-0.010	-0.056	0.624
Time from midsleep point to first food intake (h)	112	0.030	0.197	0.037	35	0.031	0.245	0.156	77	0.021	0.185	0.107
Time from last food intake to midsleep point (h)	112	-0.006	-0.052	0.585	35	-0.030	0.006	0.973	77	0.017	-0.174	0.129
Triglycerides												
Eating window (h)	117	0.044	-0.229	0.013	36	0.040	-0.259	0.127	81	0.030	-0.204	0.068
Caloric midpoint (h)	117	-0.008	0.032	0.730	36	-0.005	0.154	0.371	81	-0.012	-0.022	0.844
Eating jetlag (h)	117	-0.005	0.063	0.502	36	0.032	0.245	0.150	81	-0.010	-0.051	0.650
Time from midsleep point to first food intake (h)	113	0.005	0.119	0.209	35	0.072	0.315	0.066	78	-0.012	-0.026	0.823
Time from last food intake to midsleep point (h)	113	0.015	0.154	0.103	35	-0.017	-0.113	0.516	78	0.087	0.314	0.005
Total cholesterol												
Eating window (h)	117	-0.006	-0.052	0.577	36	-0.012	-0.130	0.451	81	-0.012	-0.017	0.878
Caloric midpoint (h)	117	-0.009	-0.013	0.892	36	-0.020	0.096	0.579	81	-0.007	-0.073	0.516
Eating jetlag (h)	117	-0.001	0.088	0.346	36	-0.009	0.142	0.409	81	-0.009	0.058	0.604
Time from midsleep point to first food intake (h)	113	-0.005	0.063	0.505	35	0.057	0.291	0.090	78	-0.003	-0.101	0.380
Time from last food intake to midsleep point (h)	113	-0.009	0.008	0.936	35	-0.003	-0.164	0.347	78	0.007	0.141	0.218
High-density lipoprotein cholesterol												
Eating window (h)	117	0.048	0.238	0.010	36	0.162	0.431	0.009	81	0.008	0.141	0.208
Caloric midpoint (h)	117	-0.006	0.054	0.560	36	0.013	0.202	0.237	81	-0.012	-0.021	0.855
Eating jetlag (h)	117	-0.009	0.004	0.964	36	0.004	-0.179	0.296	81	-0.003	0.099	0.379
Time from midsleep point to first food intake (h)	113	0.045	-0.231	0.014	35	0.137	-0.403	0.016	78	0.021	-0.184	0.106
Time from last food intake to midsleep point (h)	113	-0.008	-0.027	0.774	35	-0.030	-0.007	0.966	78	-0.011	0.041	0.721
Low-density lipoprotein cholesterol												
Eating window (h)	117	-0.001	-0.088	0.345	36	-0.001	-0.167	0.329	81	-0.011	-0.039	0.730
Caloric midpoint (h)	117	-0.008	-0.020	0.830	36	-0.029	-0.008	0.963	81	-0.012	-0.027	0.812
Eating jetlag (h)	117	0.000	0.094	0.313	36	-0.003	0.161	0.349	81	-0.010	0.053	0.637
Time from midsleep point to first food intake (h)	113	0.011	0.140	0.139	35	0.077	0.323	0.058	78	-0.013	0.007	0.953
Time from last food intake to midsleep point (h)	113	-0.007	-0.041	0.665	35	-0.008	-0.146	0.401	78	-0.012	0.032	0.784
HOMA-IR												
Eating window (h)	117	0.100	-0.328	<0.001*	36	0.348	-0.605	<0.001*	81	-0.002	-0.101	0.369
Caloric midpoint (h)	117	-0.002	0.079	0.399	36	-0.029	0.015	0.935	81	0.005	0.131	0.245
Eating jetlag (h)	117	-0.002	0.080	0.393	36	-0.022	0.085	0.623	81	-0.007	0.076	0.499
Time from midsleep point to first food intake (h)	113	0.078	0.294	0.002*	35	0.212	0.485	0.003	78	0.003	0.126	0.271
Time from last food intake to midsleep point (h)	113	-0.006	0.053	0.579	35	-0.017	0.115	0.511	78	-0.013	-0.019	0.867
Cardiometabolic risk score												
Eating window (h)	110	0.079	-0.296	0.002*	33	0.234	-0.508	0.003*	77	0.008	-0.144	0.210
Caloric midpoint (h)	110	-0.007	0.050	0.604	33	-0.031	-0.041	0.823	77	-0.003	0.100	0.388
Eating jetlag (h)	110	-0.009	0.010	0.914	33	-0.024	0.090	0.618	77	-0.011	-0.045	0.700
Time from midsleep point to first food intake (h)	107	0.081	0.300	0.002*	33	0.228	0.502	0.003*	74	0.003	0.131	0.267
Time from last food intake to midsleep point (h)	107	-0.009	0.021	0.832	33	-0.032	0.002	0.993	74	-0.013	0.032	0.787

Adjusted R^2, β standardized regression coefficients and P values are obtained from single linear regressions. All cardiometabolic risk factors (except for mean blood pressure and cardiometabolic risk score) were log10-transformed to bring their distributions closer to normal. Cardiometabolic risk score was calculated for each sex based on waist circumference, blood pressure, plasma glucose, high-density lipoprotein cholesterol, and triglyceride concentrations (see methods for further details). Symbol * these associations remained statistically significant after applying false discovery rate correction (Benjamini-Hochberg). Abbreviations: HOMA-IR, homeostasis model assessment of insulin resistance.

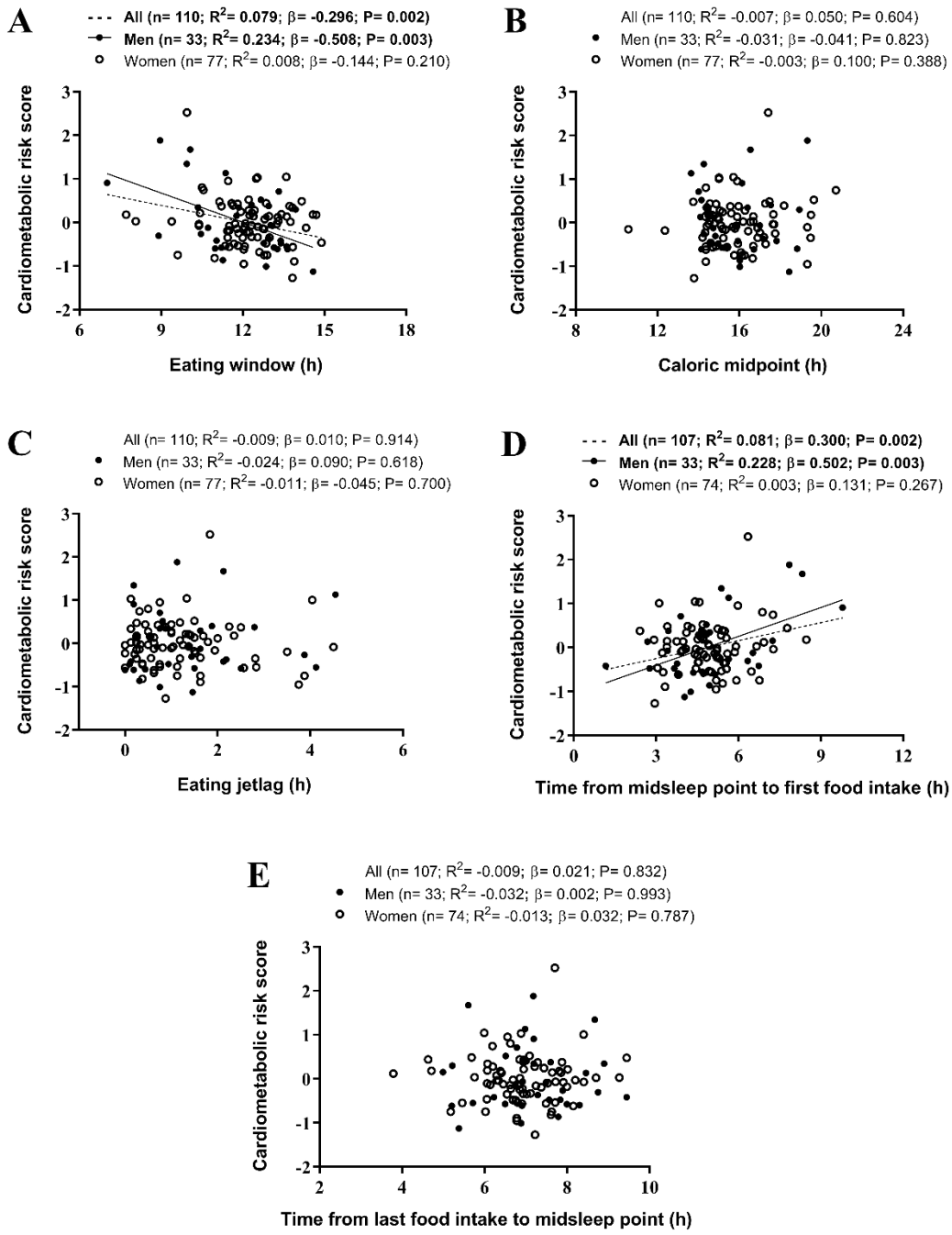

Figure 1. Scatterplots of the associations of meal timing with cardiometabolic risk score (calculated for each sex based on waist circumference, blood pressure, plasma glucose, high-density lipoprotein cholesterol, and triglyceride concentrations, see methods for further details) in young adults. Adjusted R^2, β standardized regression coefficients and P values are obtained from single linear regressions.

Discussion

Our findings suggest that meal timing is not related to anthropometry or body composition parameters in young adults. Similarly, caloric midpoint, eating jetlag and the time from last food intake to midsleep point are not associated with cardiometabolic risk factors. On the other hand, our results show that a longer daily eating window and a shorter time from midsleep point to first food intake (i.e., earlier first food intake in a 24-hour cycle) are associated with a healthier cardiometabolic profile (i.e., lower HOMA-IR and cardiometabolic risk score) in young men. These results refute our previous hypothesis, by which a shorter eating window would be associated with a healthier status. However, these findings confirm previous evidence that eating early in alignment with circadian rhythms may play an important role in cardiometabolic health.

The habitual daily eating window in adults in modern societies is ≥12 hours which is abnormal from an evolutionary perspective[4,31]. The eating pattern of our hunter-gatherer ancestors was characterized by eating sporadically with inter-meal intervals that depend upon the availability of food sources which included extended fasting period[4]. Moreover, clinical trials have also revealed that reducing the daily eating window results in body weight loss and improvement in cardiometabolic health in adults with overweight or obesity[20-22]. Therefore, we hypothesized that a shorter daily eating window would be associated with better body composition and cardiometabolic health in our cohort of young adults. However, interpreting the cross-sectional relationship between meal timing and health status is a complex task, as it can be influenced by several factors. Indeed, contradictory findings have been reported[9-14,17,23,31,39,40]. For instance, we found that the daily eating window is not related to body composition, which could be partly explained by the low variability (standard deviation of 1.5 hours) of the daily eating window in our sample. Our results concur with other studies in young adults[12], middle-aged adults[31] and adults with prediabetes[39]. In contrast, others reported that a longer daily eating window is related to a lower BMI[40] and lower fat mass percentage in adults[11]. A confounding factor may be the consumption of breakfast; epidemiological data have constantly shown that breakfast consumption is associated with lower BMI and adiposity[41-43]. Greater energy intake, lower diet quality, lower physical activity levels and misalignment of circadian rhythms are thought to be the physiological mechanisms behind these associations[41-43]. In our cohort of young adults, we observed that adherence to the Mediterranean diet and Mediterranean

diet quality was lower in breakfast skippers than in consumers in women. Furthermore, breakfast consumption influences the relationship between meal timing and body composition in women. Specifically, a longer daily eating window was associated with worst body composition (i.e., higher BMI and VAT mass) in women breakfast consumers which is in line with our previous hypothesis and with other study findings in middle-aged women[16] and in older adults[14].

The daily eating window does not take into account the time distribution of food intake, which may be a more important factor than its duration. Indeed, Xiao et al.,[14] observed that when a short daily eating window occurs early in the day is associated with a reduced likelihood of being overweight or obese in older adults; while, when a short daily eating window occurs late in the day is related to a higher likelihood of being overweight or obese. For this reason, other meal timing variables have been proposed. One of them is the caloric midpoint, that links meal timing to clock time. A study conducted in Spanish middle-aged adults found that late eaters (i.e., caloric midpoint after 3 pm) had higher BMI, fat mass percentage and waist circumference than early eaters[9], findings that partially concur with those observed in Brazilian young adults[13]. In our study, the caloric midpoint was negatively associated with fat mass percentage in men but became non-significant after adjusting for multiplicity. Another meal-timing variable is eating jetlag, which indicates the variability of the eating midpoint between working and non-working days, being informative about the circadian misalignment. For example, Zerón-Rugerio et al.,[17] showed that a greater eating jet lag is related to higher BMI in Spanish and Mexican young adults. Conversely, we observed no association between eating jetlag and body composition in our cohort of Spanish young adults.

Nonetheless, the caloric midpoint and eating jetlag use clock time to illustrate the timing of food intake, which fails to correctly characterize meal timing in relation to the internal circadian time[10,23]. In this sense, McHill et al.,[12] found that a long time from caloric midpoint to melatonin onset is associated with lower BMI and fat mass percentage. However, obtaining measures of dim-light melatonin is not practical in large clinical trials; thus, it has been proposed to measure the timing of food intake relative to the sleep/wake cycle as a proxy for circadian time[10,23,24]. Using this methodology, two studies observed that a longer time period from dinner to midsleep point or bedtime is associated with lower adiposity and BMI, respectively[10,23]. In contrast, we found no relationship between the time from the

midsleep point to first food intake or time from last food intake to midsleep point with anthropometry or body composition parameters, which concurs with another study[14]. These discrepancies could be partially explained by the sample size and the assessment of meal and sleep timing.

Regarding cardiometabolic health, we found that a longer daily eating window and a shorter time from midsleep point to first food intake are associated with healthier cardiometabolic profile in men, which agree with previous studies. Concretely, a longer daily eating window has been related to decreased insulin, total cholesterol, LDL-C and increased HDL-C in middle-aged adults [44]. In addition, in concordance with our results, previous studies have found that late eating is related to worst cardiometabolic health[9-16]. Circadian misalignment between the central clock (controlled by external light) and peripheral clocks (regulated by eating, physical activity and sleep among other factors) is one of the mechanisms that may explain these results[6,7]. In addition, glucose tolerance is higher in the biological morning, which appear to be driven by diurnal variations in β-cell responsiveness, peripheral insulin sensitivity, insulin clearance, and glucose effectiveness[6,7]. Skeletal muscle fatty acid oxidation and the thermic effect of food are also higher in the biological morning or around noon, which implicates earlier in the daytime is optimal for eating whereas nighttime is better for fasting and sleep[6,7]. Our findings could also be partly explained by the consumption of breakfast; epidemiological data have systematically reported that breakfast skipping (i.e., shorter and later daily eating window) is associated with worst cardiometabolic health[41,42]. As mentioned above, this association could be driven in part by lower diet quality and lower physical activity levels[41-43]. Indeed, in our cohort of young men, we observed that breakfast skippers had a more inflammatory diet and tended to be less physically active. Lastly, others[16] and we did not observe a relationship between meal timing and cardiometabolic risk factors in women, which is intriguing and requires further investigation.

Our findings should be interpreted with caution because the current study suffers from several limitations. Firstly, the cross-sectional design prevents establishing causal relationships. Secondly, we measured the clock timing of food intake via three non-consecutive 24-hour recalls. Thirdly, the study population comprised young and healthy adults, limiting the generalizability of the results to older or metabolically compromised individuals. Lastly, the statistical power of the study may have been insufficient to comprehensively

investigate potential sex differences in the relationship between meal timing with body composition and cardiometabolic risk factors. Despite these limitations, this study is one of the pioneers in investigating the relationship between meal timing (characterized relative to the sleep/wake cycle) and cardiometabolic risk factors. Further well-designed long-term prospective studies and randomized controlled trials are needed to elucidate the effects of meal timing on body composition and cardiometabolic health.

Conclusions

Meal timing is not related to either anthropometry or body composition parameters in young adults. Similarly, caloric midpoint, eating jetlag and the time from last food intake to midsleep point are not associated with cardiometabolic risk factors. Nonetheless, a longer daily eating window and a shorter time from midsleep point to first food intake are associated with better cardiometabolic health in men. These results confirm previous evidence that eating early in alignment with circadian rhythms may improve cardiometabolic health. Nutrition strategies aimed to improve cardiometabolic health may contemplate advancing the timing of food intake. Further well-designed studies are needed to confirm these findings and unravel potential mechanisms.

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Supplementary material

Table S1. Reporting Table for STROBE-nut: An extension of the STROBE statement for nutritional epidemiology.

Item	Item Nº	STROBE recommendations	Extension for Nutritional Epidemiology studies (STROBE-nut)	Reported on page
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract. (b) Provide in the abstract an informative and balanced summary of what was done and what was found.	nut-1 State the dietary/nutritional assessment method(s) used in the title, abstract, or keywords.	91
Introduction				
Background rationale	2	Explain the scientific background and rationale for the investigation being reported.		92-93
Objectives	3	State specific objectives, including any pre-specified hypotheses.		93
Methods				
Study design	4	Present key elements of study design early in the paper.		93-94
Settings	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection.	nut-5 Describe any characteristics of the study settings that might affect the dietary intake or nutritional status of the participants, if applicable.	93-94
Participants	6	a) Cohort study—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up. Case-control study—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls. Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants. (b) Cohort study—For matched studies, give matching criteria and number of exposed and unexposed. Case-control study—For matched studies, give matching criteria and the number of controls per case.	nut-6 Report particular dietary, physiological or nutritional characteristics that were considered when selecting the target population.	93-94
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable.	nut-7.1 Clearly define foods, food groups, nutrients, or other food components. nut-7.2 When using dietary patterns or indices, describe the methods to obtain them and their nutritional properties.	94-96
Data sources - measurements	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group.	nut-8.1 Describe the dietary assessment method(s), e.g., portion size estimation, number of days and items recorded, how it was developed and administered, and how quality was assured. Report if and how supplement intake was assessed. nut-8.2 Describe and justify food composition data used. Explain the procedure to match food composition with consumption data. Describe the use of conversion factors, if applicable. nut-8.3 Describe the nutrient requirements, recommendations, or dietary guidelines and the evaluation approach used to compare intake with the dietary reference values, if applicable. nut-8.4 When using nutritional biomarkers, additionally use the STROBE Extension for Molecular Epidemiology (STROBE-ME). Report the type of biomarkers used and their usefulness as dietary exposure markers. nut-8.5 Describe the assessment of nondietary data (e.g., nutritional status and influencing factors) and timing of the assessment of these variables in relation to dietary assessment. nut-8.6 Report on the validity of the dietary or nutritional assessment methods and any internal or external validation used in the study, if applicable.	94-96
Bias	9	Describe any efforts to address potential sources of bias.	nut-9 Report how bias in dietary or nutritional assessment was addressed, e.g., misreporting, changes in habits as a	-

			result of being measured, or data imputation from other sources	
Study Size	10	Explain how the study size was arrived at.		93-94
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why.	nut-11 Explain categorization of dietary/nutritional data (e.g., use of N-tiles and handling of nonconsumers) and the choice of reference category, if applicable.	93-94
Statistical Methods	12	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions. (c) Explain how missing data were addressed. (d) Cohort study—If applicable, explain how loss to follow-up was addressed. Case-control study—If applicable, explain how matching of cases and controls was addressed. Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy. (e) Describe any sensitivity analyses.	nut-12.1 Describe any statistical method used to combine dietary or nutritional data, if applicable. nut-12.2 Describe and justify the method for energy adjustments, intake modeling, and use of weighting factors, if applicable. nut-12.3 Report any adjustments for measurement error, i.e,. from a validity or calibration study.	97
Results				
Participants	13	(a) Report the numbers of individuals at each stage of the study—e.g., numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analyzed. (b) Give reasons for non-participation at each stage. (c) Consider use of a flow diagram.	nut-13 Report the number of individuals excluded based on missing, incomplete or implausible dietary/nutritional data.	98
Descriptive data	14	(a) Give characteristics of study participants (e.g., demographic, clinical, social) and information on exposures and potential confounders (b) Indicate the number of participants with missing data for each variable of interest (c) Cohort study—Summarize follow-up time (e.g., average and total amount)	nut-14 Give the distribution of participant characteristics across the exposure variables if applicable. Specify if food consumption of total population or consumers only were used to obtain results.	98
Outcome data	15	Cohort study—Report numbers of outcome events or summary measures over time. Case-control study—Report numbers in each exposure category, or summary measures of exposure. Cross-sectional study—Report numbers of outcome events or summary measures.		98-99
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included. (b) Report category boundaries when continuous variables were categorized. (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period.	nut-16 Specify if nutrient intakes are reported with or without inclusion of dietary supplement intake, if applicable.	98-99
Other analyses	17	Report other analyses done—e.g., analyses of subgroups and interactions and sensitivity analyses.	nut-17 Report any sensitivity analysis (e.g., exclusion of misreporters or outliers) and data imputation, if applicable.	99
Discussion				
Key results	18	Summarize key results with reference to study objectives.		104
Limitation	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	nut-19 Describe the main limitations of the data sources and assessment methods used and implications for the interpretation of the findings.	106-107
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	nut-20 Report the nutritional relevance of the findings, given the complexity of diet or nutrition as an exposure.	107
Generalizability	21	Discuss the generalizability (external validity) of the study results.		107
Other information				

Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based.		-
Ethics			nut-22.1 Describe the procedure for consent and study approval from ethics committee(s).	93-94
Supplementary material			nut-22.2 Provide data collection tools and data as online material or explain how they can be accessed.	113-124

Table S2. Association of meal timing with body composition after adjusting for potential confounders in young adults.

Body composition	P-value														
	All (N=116)					Men (N=34)					Women (N=82)				
	M0	M1	M2	M3	M4	M0	M1	M2	M3	M4	M0	M1	M2	M3	M4
Body mass index (Kg/m²)															
Eating window (h)	0.452	0.617	0.574	0.516	0.493	0.166	-	0.121	0.116	0.105	0.433	-	0.410	0.293	0.280
Caloric midpoint (h)	0.759	0.662	0.643	0.972	0.811	0.410	-	0.369	0.373	0.412	0.201	-	0.205	0.594	0.353
Eating jetlag (h)	0.945	0.815	0.848	0.780	0.839	0.681	-	0.783	0.787	0.723	0.917	-	0.933	0.855	0.984
Time from midsleep point to first food intake (h)[a]	0.339	0.313	0.277	0.351	0.185	0.166	-	0.111	0.117	0.015	0.833	-	0.825	0.531	0.388
Time from last food intake to midsleep point (h)[a]	0.737	0.474	0.482	0.689	0.464	0.956	-	0.925	0.929	0.418	0.334	-	0.334	0.412	0.477
Fat mass (%)															
Eating window (h)	0.979	0.677	0.663	0.665	0.719	0.482	-	0.373	0.493	0.424	0.886	-	0.809	0.766	0.550
Caloric midpoint (h)	0.691	0.535	0.539	0.527	0.816	0.048	-	0.037	0.047	0.063	0.460	-	0.505	0.652	0.333
Eating jetlag (h)	0.563	0.682	0.686	0.688	0.837	0.943	-	0.918	0.949	0.922	0.625	-	0.572	0.520	0.695
Time from midsleep point to first food intake (h)[a]	0.578	0.528	0.512	0.519	0.502	0.459	-	0.332	0.317	0.127	0.968	-	0.972	0.866	0.513
Time from last food intake to midsleep point (h)[a]	0.390	0.691	0.694	0.704	0.702	0.938	-	0.899	0.626	0.302	0.634	-	0.612	0.658	0.965
Lean mass index (Kg/m²)															
Eating window (h)	0.289	0.514	0.462	0.380	0.330	0.068	-	0.058	0.029	0.032	0.250	-	0.268	0.160	0.234
Caloric midpoint (h)	0.659	0.334	0.316	0.759	0.713	0.901	-	0.920	0.997	0.979	0.228	-	0.205	0.684	0.570
Eating jetlag (h)	0.776	0.949	0.996	0.897	0.856	0.625	-	0.653	0.681	0.704	0.642	-	0.608	0.820	0.816
Time from midsleep point to first food intake (h)[a]	0.434	0.261	0.222	0.307	0.098	0.100	-	0.082	0.088	0.009	0.651	-	0.695	0.387	0.419
Time from last food intake to midsleep point (h)[a]	0.724	0.562	0.572	0.892	0.510	0.873	-	0.884	0.617	0.878	0.292	-	0.306	0.384	0.292
Visceral adipose tissue mass (g)															
Eating window (h)	0.236	0.342	0.312	0.272	0.285	0.142	-	0.077	0.095	0.077	0.989	-	0.926	0.784	0.685
Caloric midpoint (h)	0.771	0.669	0.653	0.997	0.743	0.464	-	0.384	0.418	0.481	0.315	-	0.343	0.821	0.516
Eating jetlag (h)	0.600	0.467	0.485	0.439	0.539	0.848	-	0.959	0.975	0.920	0.440	-	0.402	0.261	0.369
Time from midsleep point to first food intake (h)[a]	0.261	0.233	0.207	0.260	0.178	0.106	-	0.046	0.047	0.008	0.960	-	0.997	0.704	0.548
Time from last food intake to midsleep point (h)[a]	0.982	0.721	0.728	0.951	0.855	0.798	-	0.744	0.602	0.296	0.809	-	0.791	0.923	0.945
Waist circumference (cm)[b]															
Eating window (h)	0.352	0.541	0.525	0.472	0.463	0.111	-	0.077	0.078	0.087	0.337	-	0.293	0.209	0.283
Caloric midpoint (h)	0.770	0.842	0.847	0.570	0.738	0.203	-	0.179	0.187	0.178	0.375	-	0.407	0.808	0.443
Eating jetlag (h)	0.491	0.292	0.292	0.287	0.328	0.594	-	0.687	0.689	0.722	0.352	-	0.317	0.243	0.337
Time from midsleep point to first food intake (h)[a]	0.450	0.393	0.373	0.421	0.188	0.277	-	0.200	0.206	0.048	0.876	-	0.838	0.595	0.607
Time from last food intake to midsleep point (h)[a]	0.866	0.722	0.727	0.895	0.564	0.536	-	0.565	0.500	0.989	0.203	-	0.196	0.335	0.175

P values are obtained for Model 0 (single linear regression), then the analyses were adjusted for: sex (only in all, Model 1); sex and a priori Mediterranean diet pattern (MeD-P) (Model 2); sex, a priori Mediterranean diet pattern and light physical activity (min/day)[c] (Model 3); sex, a priori Mediterranean diet pattern, light physical activity (min/day) and midsleep point (h)[d] (Model 4). Sex was included only when men and women were analysed together (i.e., all). In time from midsleep point to first food intake and time from last food intake to midsleep point model 4 included sleep duration (h) instead of midsleep point. Neither association remained statistically significant after applying false discovery rate correction (Benjamini-Hochberg). Some specific outcomes had missing data for all and women: [a,d]3 missing participants, [b]2 missing participants, and [c]1 missing participant. Abbreviation: M, Model.

Table S3. Association of meal timing with cardiometabolic risk factors after adjusting for potential confounders in young adults.

Cardiometabolic risk factors	P-value																	
	All (N=117)						Men (N=36)						Women (N=81)					
	M0	M1	M2	M3	M4	M5	M0	M1	M2	M3	M4	M5	M0	M1	M2	M3	M4	M5
Mean blood pressure (mmHg)[a]																		
Eating window (h)	0.100	0.140	0.185	0.156	0.160	0.232	0.099	-	0.099	0.118	0.158	0.472	0.749	-	0.902	0.990	0.770	0.554
Caloric midpoint (h)	0.562	0.441	0.489	0.705	0.817	0.925	0.387	-	0.385	0.386	0.421	0.200	0.773	-	0.919	0.533	0.513	0.328
Eating jetlag (h)	0.871	0.684	0.577	0.542	0.330	0.339	0.940	-	0.934	0.890	0.715	0.847	0.624	-	0.503	0.340	0.263	0.235
Time from midsleep point to first food intake (h)[b]	0.037	0.026	0.037	0.050	0.011	0.033	0.156	-	0.166	0.176	0.059	0.333	0.107	-	0.141	0.260	0.147	0.078
Time from last food intake to midsleep point (h)[b]	0.585	0.281	0.260	0.376	0.200	0.289	0.973	-	0.974	0.989	0.696	0.905	0.129	-	0.111	0.183	0.107	0.133
Triglycerides																		
Eating window (h)	0.013	0.015	0.014	0.014	0.032	0.044	0.127	-	0.105	0.166	0.150	0.374	0.068	-	0.074	0.069	0.220	0.149
Caloric midpoint (h)	0.730	0.704	0.705	0.586	0.620	0.636	0.371	-	0.409	0.362	0.314	0.163	0.844	-	0.804	0.810	0.700	0.594
Eating jetlag (h)	0.502	0.508	0.506	0.471	0.450	0.405	0.150	-	0.120	0.140	0.222	0.146	0.650	-	0.617	0.651	0.599	0.611
Time from midsleep point to first food intake (h)[b]	0.209	0.207	0.210	0.178	0.042	0.078	0.066	-	0.047	0.044	0.012	0.072	0.823	-	0.777	0.819	0.846	0.723
Time from last food intake to midsleep point (h)[b]	0.103	0.126	0.129	0.155	0.315	0.230	0.516	-	0.506	0.299	0.141	0.221	0.005	-	0.006	0.006	0.012	0.009
Total cholesterol																		
Eating window (h)	0.577	0.519	0.540	0.564	0.410	0.457	0.451	-	0.423	0.440	0.234	0.544	0.878	-	0.946	0.890	0.951	0.874
Caloric midpoint (h)	0.892	0.848	0.834	0.973	0.769	0.784	0.579	-	0.606	0.601	0.388	0.210	0.516	-	0.470	0.635	0.671	0.724
Eating jetlag (h)	0.346	0.337	0.351	0.337	0.199	0.186	0.409	-	0.377	0.389	0.343	0.239	0.604	-	0.645	0.577	0.469	0.477
Time from midsleep point to first food intake (h)[b]	0.505	0.509	0.210	0.469	0.288	0.356	0.090	-	0.062	0.063	0.039	0.210	0.380	-	0.346	0.410	0.510	0.455
Time from last food intake to midsleep point (h)[b]	0.936	0.800	0.129	0.901	0.898	0.952	0.347	-	0.337	0.252	0.184	0.288	0.218	-	0.234	0.260	0.322	0.347
High-density lipoprotein cholesterol																		
Eating window (h)	0.010	0.011	0.012	0.012	0.007*	0.009	0.009	-	0.012	0.034	0.015	0.050	0.208	-	0.208	0.217	0.214	0.051
Caloric midpoint (h)	0.560	0.665	0.646	0.713	0.989	0.956	0.237	-	0.272	0.321	0.419	0.580	0.855	-	0.865	0.923	0.763	0.942
Eating jetlag (h)	0.964	0.918	0.875	0.917	0.889	0.971	0.296	-	0.349	0.412	0.669	0.543	0.379	-	0.371	0.345	0.401	0.384
Time from midsleep point to first food intake (h)[b]	0.014	0.006*	0.006*	0.005*	0.008*	0.020	0.016	-	0.023	0.013	0.010	0.066	0.106	-	0.106	0.120	0.179	0.046
Time from last food intake to midsleep point (h)[b]	0.774	0.796	0.783	0.722	0.996	0.764	0.966	-	0.931	0.523	0.538	0.761	0.721	-	0.719	0.757	0.978	0.802
Low-density lipoprotein cholesterol																		
Eating window (h)	0.345	0.344	0.384	0.398	0.212	0.262	0.329	-	0.328	0.377	0.363	0.412	0.730	-	0.814	0.778	0.835	0.787
Caloric midpoint (h)	0.830	0.828	0.799	0.910	0.639	0.663	0.963	-	0.963	0.989	0.697	0.452	0.812	-	0.742	0.845	0.896	0.932
Eating jetlag (h)	0.313	0.315	0.347	0.332	0.173	0.142	0.349	-	0.345	0.367	0.331	0.213	0.637	-	0.693	0.646	0.450	0.448
Time from midsleep point to first food intake (h)[b]	0.139	0.141	0.151	0.132	0.102	0.164	0.058	-	0.046	0.045	0.038	0.235	0.953	-	0.990	0.957	0.932	0.889
Time from last food intake to midsleep point (h)[b]	0.665	0.696	0.686	0.609	0.581	0.667	0.401	-	0.399	0.257	0.248	0.385	0.784	-	0.822	0.844	0.854	0.829
HOMA-IR																		
Eating window (h)	<0.001*	<0.001*	<0.001*	<0.001*	0.001*	0.001*	<0.001*	-	<0.001*	<0.001*	<0.001*	0.002	0.369	-	0.459	0.541	0.659	0.367
Caloric midpoint (h)	0.399	0.372	0.392	0.431	0.553	0.539	0.932	-	0.042	0.845	0.915	0.411	0.245	-	0.299	0.737	0.674	0.896
Eating jetlag (h)	0.393	0.398	0.434	0.429	0.520	0.418	0.623	-	0.579	0.670	0.873	0.685	0.499	-	0.573	0.719	0.668	0.615
Time from midsleep point to first food intake (h)[b]	0.002*	0.001*	0.002*	0.002*	<0.001*	0.001*	0.003	-	0.002*	0.001*	<0.001*	0.004	0.271	-	0.320	0.481	0.340	0.160
Time from last food intake to midsleep point (h)[b]	0.579	0.679	0.699	0.644	0.804	0.493	0.511	-	0.520	0.928	0.871	0.618	0.867	-	0.806	0.930	0.787	0.946
Cardiometabolic risk score[c]																		
Eating window (h)	0.002*	0.002*	0.002*	0.002*	0.002*	<0.001*	0.003*	-	0.002*	0.003*	0.003*	0.011	0.210	-	0.233	0.257	0.347	0.041
Caloric midpoint (h)	0.604	0.604	0.619	0.776	0.639	0.654	0.823	-	0.815	0.885	0.963	0.521	0.388	-	0.421	0.852	0.682	0.931

Eating jetlag (h)	0.914	0.926	0.962	0.938	0.889	0.843	0.618	-	0.604	0.687	0.741	0.473	0.700	-	0.655	0.574	0.667	0.531
Time from midsleep point to first food intake (h)[b]	0.002*	0.002*	0.002*	0.002*	<0.001*	<0.001*	0.003*	-	0.002*	0.002*	<0.001*	0.006	0.267	-	0.285	0.464	0.223	0.018
Time from last food intake to midsleep point (h)[b]	0.832	0.841	0.857	0.749	0.987	0.638	0.993	-	0.992	0.815	0.589	0.965	0.787	-	0.819	0.584	0.843	0.622

P values are obtained for Model 0 (single linear regression), then analyses were adjusted for: sex (only in all, Model 1); sex and a priori Mediterranean diet pattern (MeD-P) (Model 2); sex, a priori Mediterranean diet pattern and light physical activity (min/day)[d] (Model 3); sex, a priori Mediterranean diet pattern, light physical activity (min/day) and midsleep point (h)[e] (Model 4); sex, a priori Mediterranean diet pattern, light physical activity (min/day), midsleep point (h) and body mass index (kg/m2)f (Model 5). Sex was included only when men and women were analysed together (i.e., all). In time from midsleep point to breakfast and time from dinner to midsleep point model 4 and 5 included sleep duration (h) instead of midsleep point. All cardiometabolic risk factors (except for mean blood pressure and cardiometabolic risk score) were log10-transformed to bring their distributions closer to normal. Cardiometabolic risk score was calculated for each sex based on waist circumference, blood pressure, plasma glucose, high-density lipoprotein cholesterol, and triglyceride concentrations (see methods for further details). Symbol * these associations remained statistically significant after applying false discovery rate correction (Benjamini-Hochberg). Some specific outcomes had missing data for all: [a,d,f]1 missing participant, [b,e]4 missing participants, and [c]7 missing participants; for men: [c]3 missing participants and [e,f]1 missing participant; and for women: [a,d]1 missing participant, [c]4 missing participants and [b,e]3 missing participants. Abbreviations: HOMA-IR, homeostasis model assessment of insulin resistance; M, Model.

Table S4. Differences between breakfast skipper and consumer in body composition, cardiometabolic risk factors, physical activity, sleep patterns, dietary patterns, and energy and macronutrients intake.

	All		Men		Women	
	Skip. (N)= 29; Cons. (N)= 88		Skip. (N)= 10; Cons. (N)= 26		Skip. (N)= 19; Cons. (N)= 62	
	MD (95% CI)	P-value	MD (95% CI)	P-value	MD (95% CI)	P-value
Body mass index (Kg/m^2)[a]	0.7 (-1.7, 3.1)	0.551	1.3 (-4.3, 6.9)	0.619	0.3 (-2.1, 2.7)	0.816
Fat mass (kg)[a]	1.3 (-3.1, 5.7)	0.564	2.4 (-8.2, 13)	0.630	0.6 (-4.1, 5.4)	0.780
Fat mass (%)[a]	-0.3 (-3.6, 3.0)	0.856	0.8 (-5.0, 6.7)	0.768	-0.4 (-4.1, 3.3)	0.810
Lean mass index (Kg m^2)[a]	0.4 (-0.8, 1.6)	0.697	0.6 (-1.7, 3.0)	0.569	0.1 (-0.6, 0.9)	0.897
Visceral adipose tissue mass (g)[a]	23.9 (-73.8, 121.6)	0.494	41.5 (-155.1, 238.2)	0.650	9.0 (-102.0, 120.1)	0.868
Waist circumference (cm)[b]	1.4 (-6.2, 9.1)	0.702	3.7 (-12.8, 20.2)	0.634	-0.6 (-7.6, 6.5)	0.872
Systolic blood pressure (mmHg)[c]	0.9 (-4.6, 6.5)	0.734	0.3 (-10.5, 11.2)	0.948	-0.2 (-4.9, 4.5)	0.926
Diastolic blood pressure (mmHg)[c]	2.6 (-0.9, 6.2)	0.141	4.2 (-3.9, 12.3)	0.284	1.5 (-2.1, 5.2)	0.387
Mean blood pressure (mmHg)[c]	2.1 (-1.7, 5.9)	0.275	2.9 (-5.1, 10.9)	0.445	1.0 (-2.6, 4.5)	0.587
Triglycerides (mg/dl)	19.7 (-12.8, 52.2)	0.227	42.6 (-25.6, 110.8)	0.196	7.5 (-30.4, 45.4)	0.684
Total cholesterol (mg/dl)	7.1 (-9, 23.1)	0.378	24.3 (-8.8, 57.5)	0.136	-0.9 (-19.9, 18.0)	0.920
High-density lipoprotein cholesterol (mg/dl)	-0.5 (-5.7, 4.7)	0.848	-2.9 (-9.2, 3.4)	0.343	1.5 (-4.7, 7.6)	0.629
Low-density lipoprotein cholesterol (mg/dl)	6.5 (-6.3, 19.4)	0.311	20.3 (-3.2, 43.7)	0.085	-0.3 (-16.2, 15.6)	0.968
Glucose (mg/dl)	5.2 (1.9, 8.4)	0.003	9.1 (2.2, 16.0)	0.014	3.0 (-0.1, 6.0)	0.060
Insulin (μIU/ml)	3.5 (-0.3, 7.3)	0.068	8.3 (-2.2, 18.7)	0.108	1.0 (-1.4, 3.3)	0.045
HOMA-IR	1.0 (0.0, 2.0)	0.057	2.4 (-0.6, 5.3)	0.100	0.3 (-0.3, 0.9)	0.324
Cardiometabolic risk score[d]	0.2 (-0.1, 0.6)	0.233	0.5 (-0.2, 1.3)	0.150	0.1 (-0.4, 0.5)	0.792
Physical activity (ENMO/day)[e]	0.1 (-1.8, 2.0)	0.924	-2.7 (-5.5, 0.2)	0.066	1.5 (-0.8, 3.9)	0.196
Sedentary time (min/day)[e]	-7.3 (-33.1, 18.5)	0.571	3 (-35.5, 41.5)	0.875	-13.8 (-48.4, 20.7)	0.419
Cardiorespiratory fitness (ml/kg/min)	1.1 (-3.1, 5.2)	0.604	-0.6 (-9.1, 8.0)	0.894	1.5 (-3.2, 6.3)	0.517
Sleep duration (h)[f]	0.0 (-0.5, 0.5)	0.866	0.3 (-0.6, 1.2)	0.524	-0.2 (-0.8, 0.4)	0.427
Midsleep point (h)[f]	0.4 (-0.1, 1.0)	0.102	0 (-1.1, 1.1)	0.998	0.6 (0.0, 1.3)	0.045
Social jet lag (h)[f]	-0.3 (-0.8, 0.2)	0.246	-0.5 (-1.3, 0.2)	0.141	-0.1 (-0.8, 0.5)	0.655
MeD-P	-2.4 (-4.3, -0.5)	0.013	-1.5 (-4.8, 1.7)	0.336	-2.7 (-5.1, -0.3)	0.028
MeD-S[g]	-0.6 (-1.3, 0.0)	0.050	-0.7 (-2.3, 0.9)	0.374	-0.6 (-1.2, 0.0)	0.068
MeD-DQI[h]	1.3 (0.4, 2.3)	0.007	1.2 (-0.7, 3)	0.208	1.3 (0.2, 2.4)	0.024
DASH[i]	-1.4 (-3.3, 0.4)	0.129	-1.4 (-5.4, 2.5)	0.452	-1.4 (-3.2, 0.4)	0.132
DQI	0.6 (-0.4, 1.6)	0.215	0.5 (-1.3, 2.3)	0.553	0.6 (-0.6, 1.8)	0.330
DII	0.5 (-0.1, 1.1)	0.087	1.2 (0.3, 2.2)	0.015	0.2 (-0.6, 1.0)	0.581
Energy intake (kcal/day)	-24.5 (-230.2, 181.2)	0.812	-210.2 (-588.0, 167.6)	0.262	38.6 (-209.6, 286.7)	0.752
Energy density (kcal/g)	0.1 (-0.1, 0.2)	0.542	0.0 (-0.3, 0.3)	0.797	0.1 (-0.1, 0.3)	0.383
Carbohydrates (% energy)	-1.4 (-4.1, 1.2)	0.292	-0.2 (-5.0, 4.6)	0.925	-1.8 (-5.0, 1.5)	0.283
Protein (% energy)	-0.2 (-1.4, 1.0)	0.718	-1.3 (-3.6, 1.0)	0.244	0.2 (-1.2, 1.6)	0.784
Fat (% energy)	2.1 (-0.5, 4.7)	0.104	2.8 (-2.4, 8.1)	0.273	1.7 (-1.3, 4.7)	0.261
Eating window (h)	-2.0 (-2.6, -1.3)	<0.001	-2.2 (-3.5, -0.9)	0.004	-1.9 (-2.7, -1.1)	<0.001
Caloric midpoint (h)	1.3 (0.5, 2.1)	0.002	0.4 (-1.1, 1.8)	0.588	1.7 (0.8, 2.7)	0.001
Eating jetlag (h)	0.9 (0.3, 1.5)	0.003	1.0 (-0.2, 2.2)	0.095	0.9 (0.2, 1.6)	0.018
Time from midsleep point to first food intake (h)	1.8 (1.2, 2.3)	<0.001	2.2 (0.9, 3.5)	0.003	1.6 (1.0, 2.1)	<0.001
Time from last food intake to midsleep point (h)	0.3 (-0.2, 0.8)	0.224	-0.1 (-0.9, 0.8)	0.885	0.5 (-0.2, 1.1)	0.150

Values obtained from Welch's t-test (breakfast skippers – consumers). Cardiometabolic risk score was calculated for each sex based on waist circumference, blood pressure, plasma glucose, high-density lipoprotein cholesterol, and triglyceride concentrations (see methods for further details). A higher MeD-P and MeD-S represents greater adherence to the Mediterranean diet, whereas the higher the MeD-DQI score, the lower the Mediterranean diet quality. A higher DASH score represents greater adherence to the DASH guidelines. A lower DQI score represents a higher diet quality. The higher the DII score, the more inflammatory the diet. Some specific outcomes had missing data for all: [a]2 missing participants (one skipper and one consumer), [b]4 missing participants (two skippers and two consumers), [c]3 missing participants (one skipper and two consumers), [d]7 missing participants (three skippers and four consumers), [e]1 missing subject (one consumer), [f]4 missing participants (one skipper and three consumers), [g]3 missing participants (one skipper and two consumers), [h]2 missing participants (consumers), and [i]4 missing participants (two skippers and two consumers); for men: [a,b,g]2 missing participants (one skipper and one consumer), [c,f,h]1 missing subject (one consumer), [d]3 missing participants (one skipper and two consumers), and [i]3 missing participants (two skippers and one consumer); and for women: [b,c]2 missing participants (one skipper and one consumer), [d]4 missing participants (two skippers and two consumers), [e, g,h,i]1 missing subject (one consumer), and [f]3 missing participants (one skipper and two consumers). Abbreviations: CI, confidence intervals; Cons., breakfast consumers; HOMA-IR, homeostasis model assessment index; MD, mean difference; MeD-P, a priori Mediterranean dietary pattern; MeD-S, Mediterranean diet score; MeD-DQI, dietary quality index for the Mediterranean diet; DASH, dietary approaches to stop hypertension; DQI, dietary quality indices; DII, dietary inflammatory index; Skip., breakfast skippers

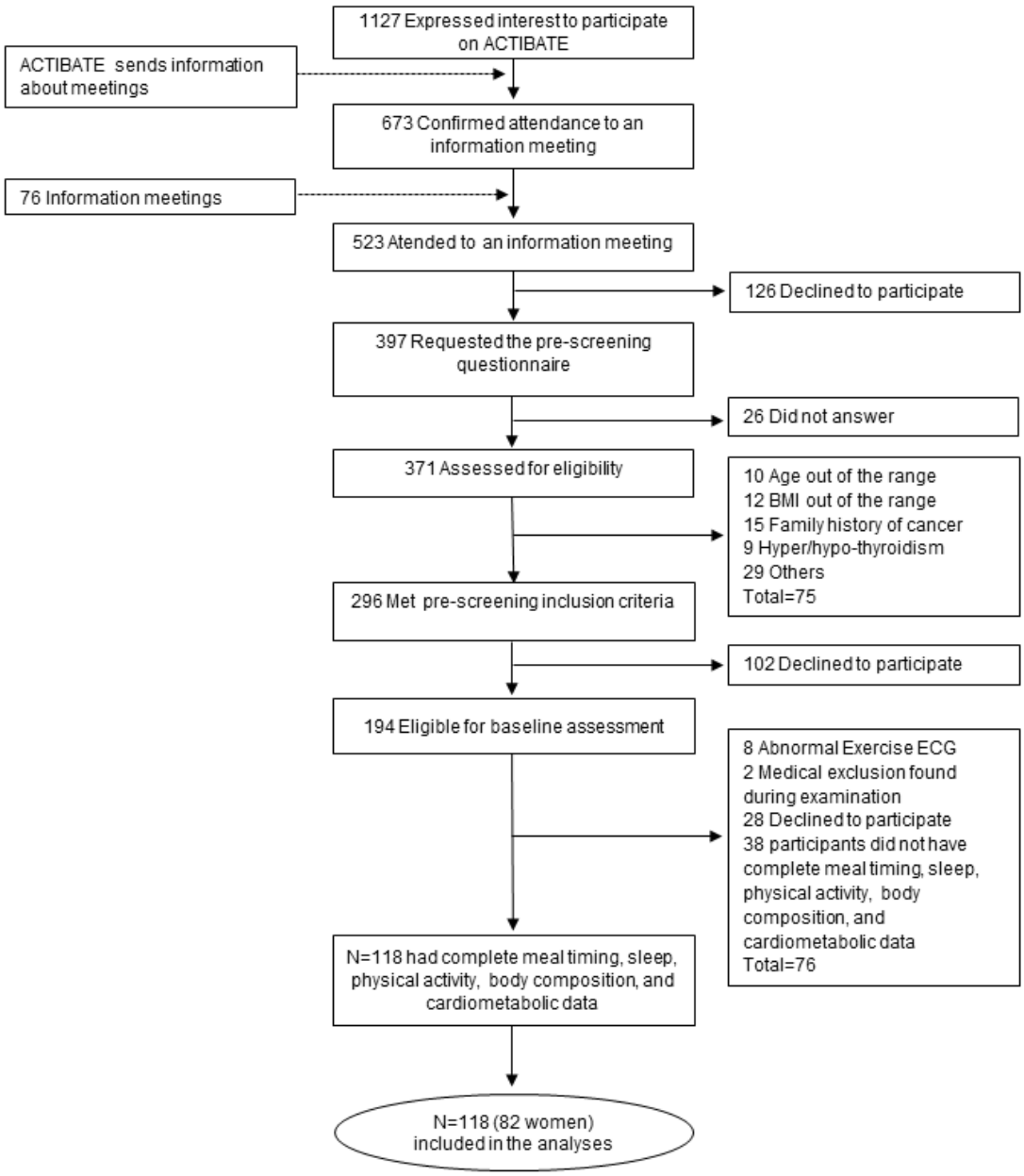

Figure S1. Flow-chart for subject enrolment. BMI: body mass index, ECG: electrocardiogram.

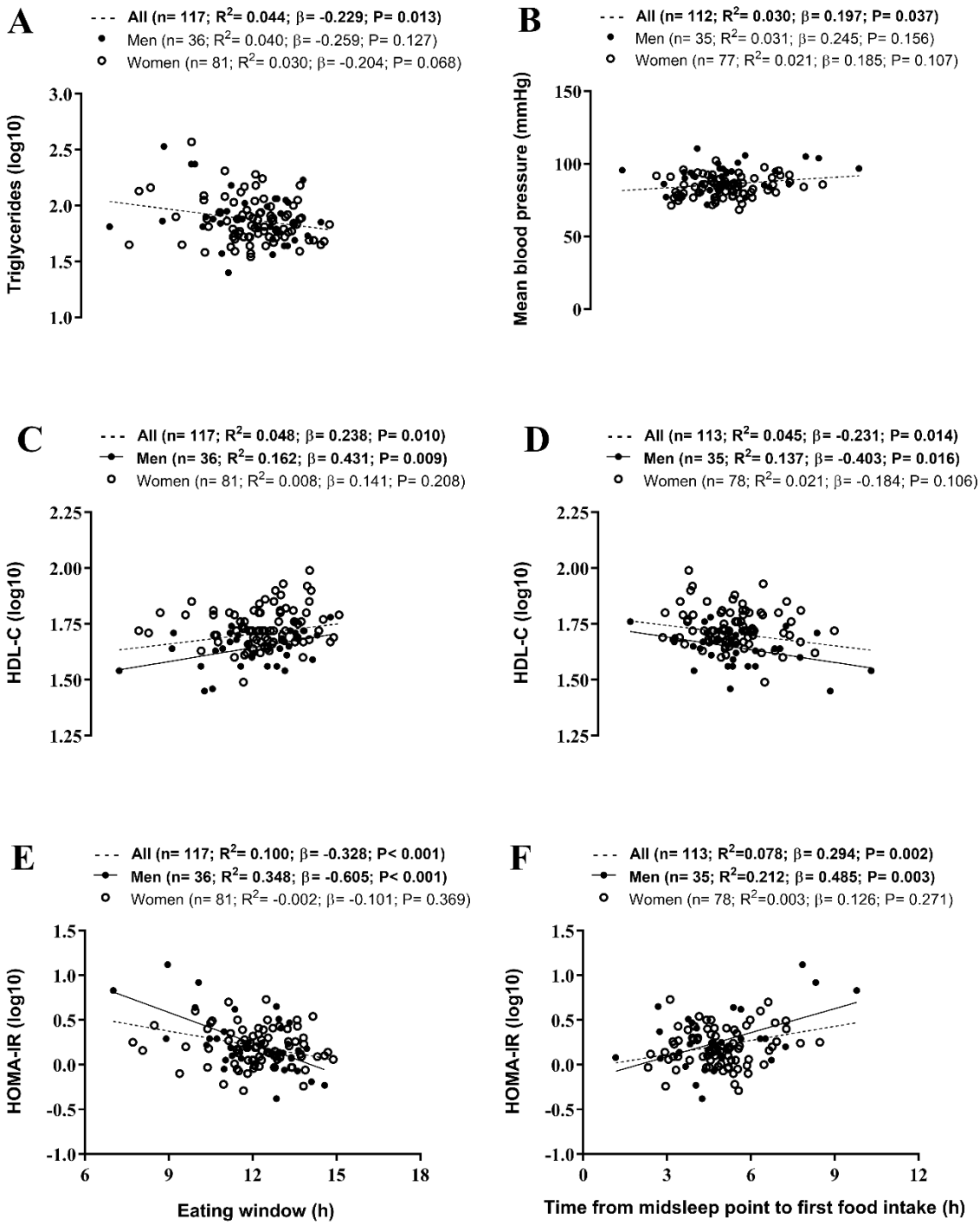

Figure S2. Scatterplots of the associations of eating window and time from midsleep point to first food intake with cardiometabolic risk factors (only significant associations from **Table 3** are shown) in young adults. Adjusted R2, β standardized regression coefficients and P values are obtained from single linear regressions. All cardiometabolic risk factors (except for mean blood pressure) were log10-transformed to bring their distributions closer to normal. Abbreviations: HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance.

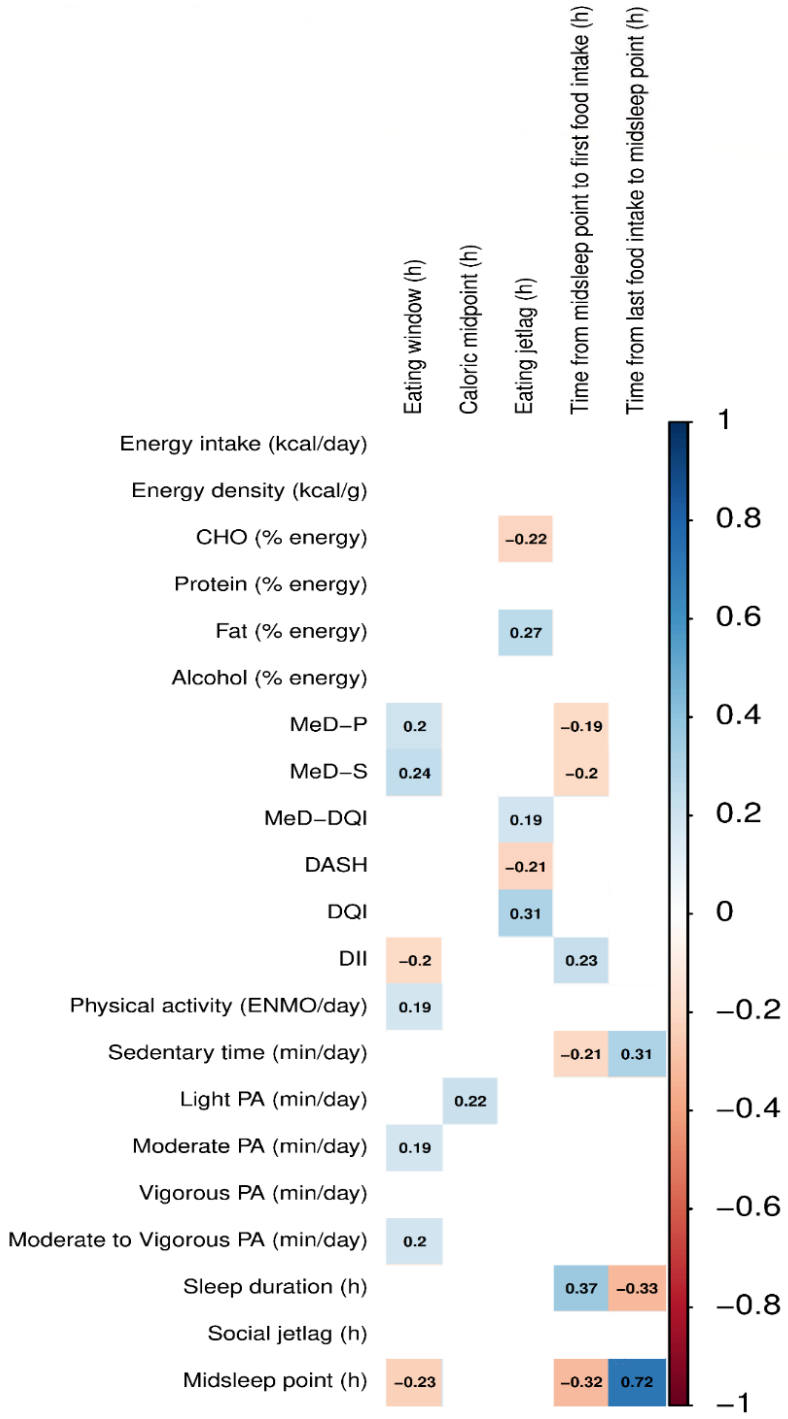

Figure S3. Pearson correlation of meal timing with energy and macronutrients intake, dietary patterns, physical activity and sleep patterns in young adults. Boxes only represent the statistically significant (P≤0.05) correlations and the value within the boxes show the Pearson correlation coefficient. Blue boxes indicate positive correlation whereas red squares indicate negative correlation. Abbreviation: CHO, carbohydrates; MeD-P, a priori Mediterranean dietary pattern; MeD-S, Mediterranean diet score; MeD-DQI, dietary quality index for the Mediterranean diet; DASH, dietary approaches to stop hypertension; DQI, dietary quality indices; DII, dietary inflammatory index; PA, physical activity.

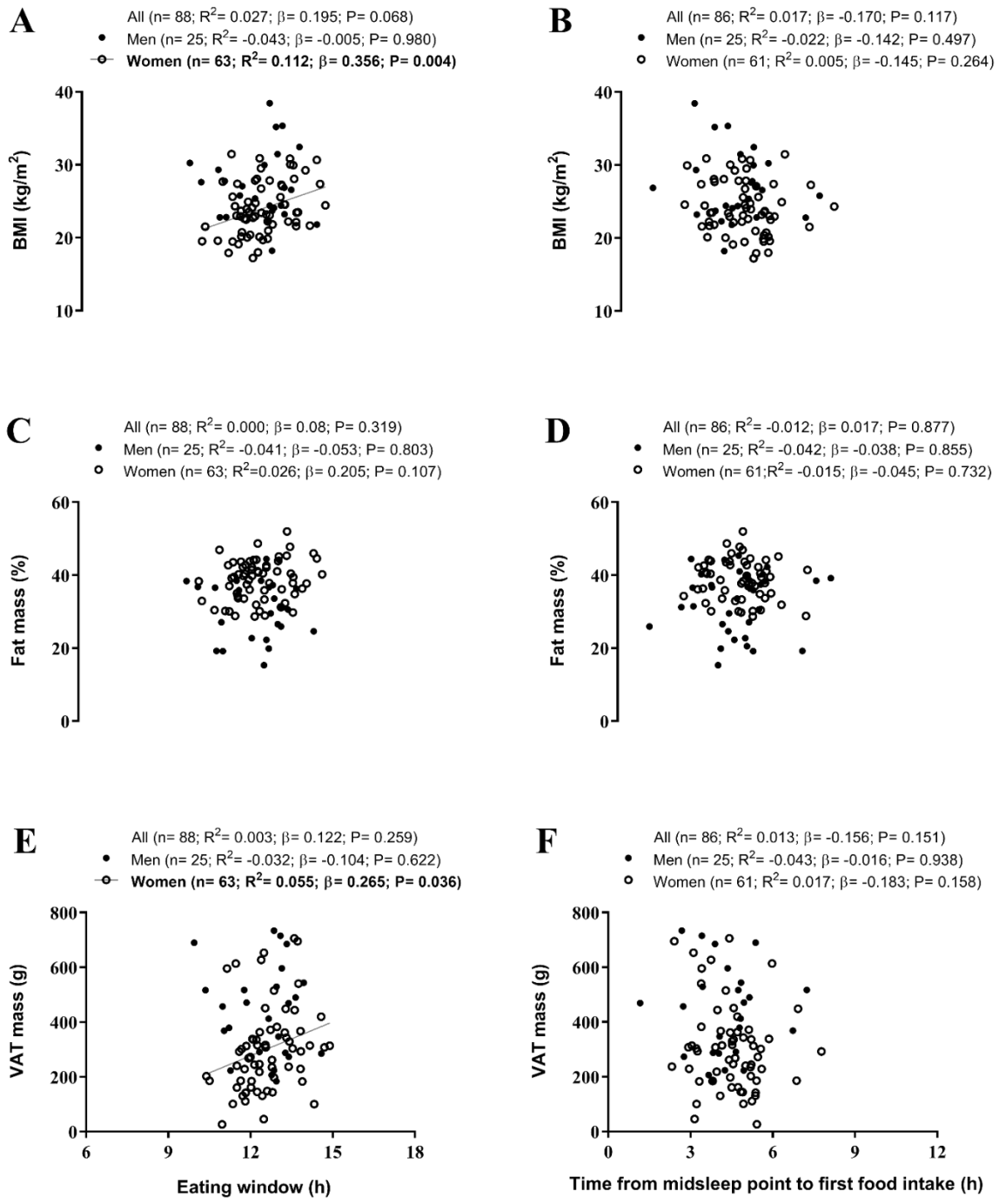

Figure S4. Scatterplots of the associations of eating window and time from midsleep point to first food intake with body composition in breakfast consumers. R2 adjusted, β standardized regression coefficients and P values are showed from single linear regressions. Abbreviations: BMI, body mass index; VAT, visceral adipose tissue

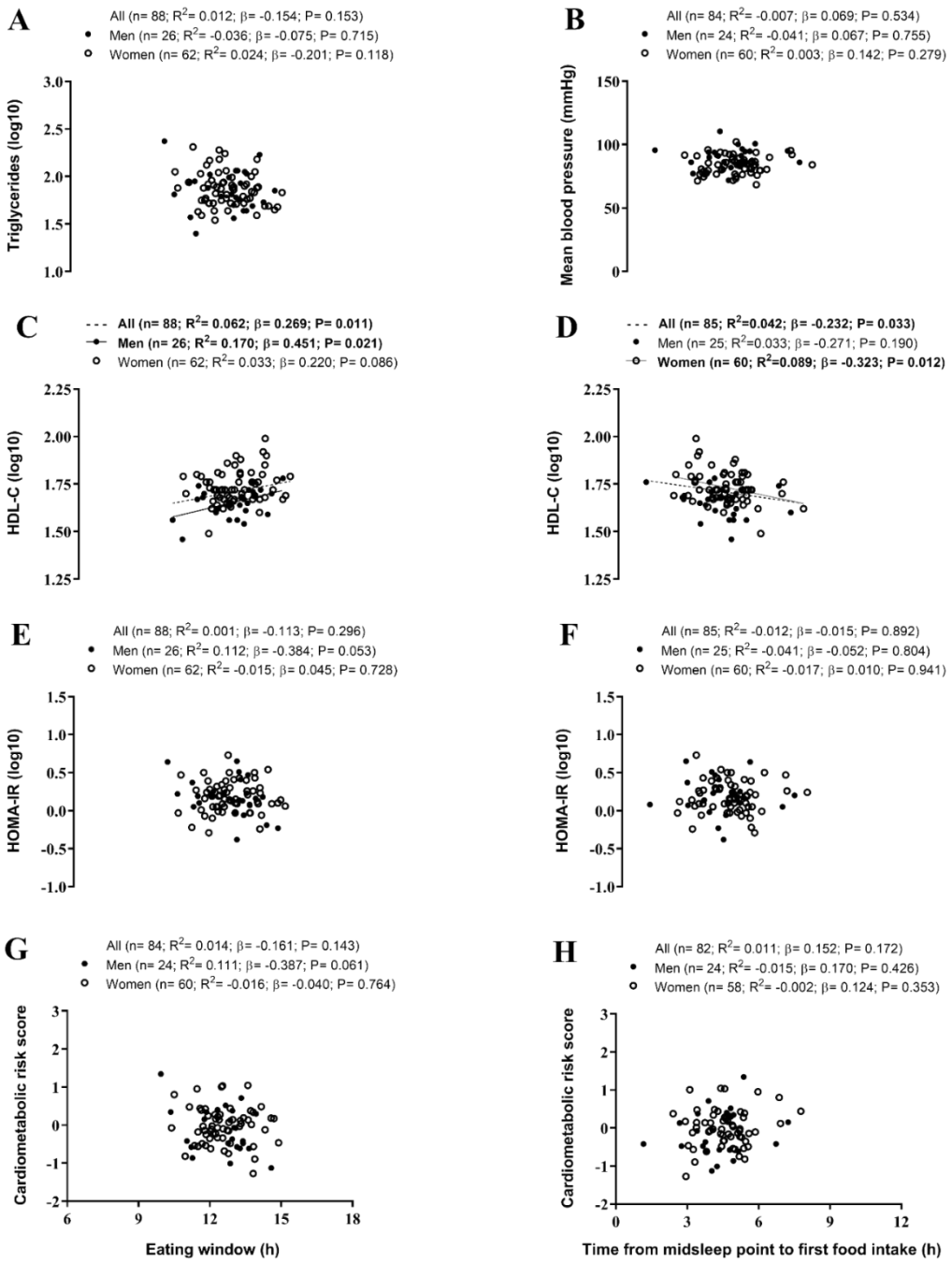

Figure S5. Scatterplots of the associations of eating window and time from midsleep point to first food intake with cardiometabolic risk factors (only those associations which were significant in **Table 3** are shown) in breakfast consumers. R2 adjusted, β standardized regression coefficients and P values are showed from single linear regressions. All cardiometabolic risk factors (except for mean blood pressure and cardiometabolic risk score) were log10-transformed to bring their distributions closer to normal. Cardiometabolic risk score was calculated for each sex based on waist circumference, blood pressure, plasma glucose, high-density lipoprotein cholesterol, and triglyceride concentrations (see methods for further details). Abbreviations: HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment index.


Eat
Fast
16 hrs

Chapter 5. Effects of three 8-hour time-restricted eating schedules on visceral adipose tissue, body composition and cardiometabolic health in men and women with overweight/obesity: A multicenter randomized controlled trial

Abstract

Importance: Time-restricted eating (TRE) has emerged as a promising dietary intervention for treating obesity and related comorbidities; however, the optimal timing of the eating window during TRE and its effects on visceral adipose tissue (VAT), body composition and cardiometabolic health remain uncertain.

Objective: To investigate the effects of three 8-hour TRE schedules (i.e., early, late, and self-selected) compared to each other and to a usual-care (UC) 12-week intervention on VAT, body composition and cardiometabolic health.

Design, setting and participants: 197 adults (50% women) aged 46.8 (6.3) years, with overweight/obesity [BMI 32.9 (3.5) kg/m^2] and with mild metabolic impairments were recruited for this parallel-group, multicenter (Granada and Pamplona, Spain) randomized clinical trial, between February 7, 2022, to March 6, 2023.

Interventions: Participants were randomly allocated to UC, early TRE, late TRE, or self-selected TRE. The UC group maintained their habitual eating window and received, as well as the TRE groups, healthy lifestyle education for weight management for 12 weeks. The early TRE group started eating not later than 10:00 and the later TRE group not earlier than 13:00. The self-selected TRE group selected an 8-hour eating window before the intervention and maintain it over the 12-week intervention.

Measurement: Outcomes included changes in VAT mass, body weight and composition, and cardiometabolic risk factors.

Results: A total of 197 adults participated in the study, including 98 women. Randomization resulted in 49 (24 women), 49 (24 women), 52 (25 women), and

47 (25 women) individuals assigned to the UC, early, late, and self-selected TRE groups, respectively. In men, the self-selected TRE group exhibited a significantly greater reduction in VAT mass compared to the UC group (mean difference: -129.7 g; 95% CI, -249.0 to -10.5; P=0.027). No significant differences in the change of VAT mass were observed among the intervention groups in women. Regarding body weight loss, both men and women in the early TRE group achieved a significantly greater decrease (men: mean difference: -3.4 kg; 95% CI, -6.1 to -0.7; P=0.007; women: mean difference: -2.4 kg; 95% CI, -4.5 to -0.3; P=0.021), as did men in the self-selected TRE group and women in the late TRE group (men: mean difference: -4.4 kg; 95% CI, -7.1 to -1.6; P=0.001; women: mean difference: -2.8 kg; 95% CI, -5.0 to -0.7; P=0.004), when compared to the UC group. No significant differences were found in the change of fat-free mass, fat mass, or cardiometabolic risk factors among the intervention groups in both men and women.

Conclusions and relevance: Self-selected TRE may be considered a targeted strategy for reducing VAT in men. Furthermore, implementing an 8-hour TRE, irrespective of the timing of the eating window, within a usual-care intervention led to significantly greater body weight loss in both sexes. Similar changes in body composition and cardiometabolic risk factors were observed among interventions groups.

Introduction

Obesity is a global health concern[1] and is associated with chronic conditions such as diabetes, cardiovascular disease, and cancer[1,2], imposing a significant strain on healthcare systems[1,3]. Although energy-restricted diets are effective in improving body weight and cardiometabolic health, their clinical application is limited by poor long-term adherence[4]. TRE has emerged as a promising dietary intervention for treating obesity[5-7]. TRE limits the daily energy intake to a pre-determined eating window (≤10 hours) and fasting for the rest of the day (≥14 hours)[5-7]. Current literature suggests that TRE is well-tolerated, has high adherence rates with minimal side effects, and results in modest reductions in body weight and slight improvements in cardiometabolic health in individuals with overweight/obesity compared to controls with no restrictions on energy and meal timing[6-8].

Important questions about the effects of TRE remain to be addressed. For instance, its impact on ectopic fat deposition[6], particularly VAT[9,10], which is a significant risk factor for cardiometabolic morbidity and mortality[11]. Additionally, women tend to preferentially deposit fat subcutaneously, particularly in the gluteofemoral region, while men show a propensity for fat accumulation in the visceral compartment[11]. However, despite the evidence indicating sex disparities in fat accumulation[11] and obesity-related comorbidities[12], as well as the recent emphasis on considering sex as a biological variable in research[13], there is a lack of comprehensive examination of sex differences in the effects of TRE. Another important question regarding TRE is whether the timing of the eating window affects its efficacy[6]. While previous TRE studies have typically used arbitrary clock times to determine the timing of food intake, some pilot clinical studies have suggested that earlier eating windows during TRE may yield better cardiometabolic benefits[14-16], despite similar boy weight loss[14-19]. However, drawing definitive conclusions from existing studies comparing early versus late TRE is challenging due to factors such as their relatively short duration (≤8 weeks), small sample sizes, or lack of randomization[16-19]. Furthermore, there is a notable gap in the literature as no studies have directly compared an early or late TRE with a self-selected TRE, which is of great interest. Allowing participants to choose a self-selected TRE window that aligns with their personal preferences and schedule has the potential to improve adherence, acceptability, and ultimately enhance efficacy. Therefore, further research is needed to provide more conclusive evidence on the

optimal timing of the eating window during TRE and its effects on VAT, body composition and cardiometabolic health.

The present study aimed to determines the effects of three TRE schedules (i.e., early, late, and self-selected) versus a UC 12-week intervention on VAT, body composition and cardiometabolic health in men and women with overweight/obesity.

Methods

Study design

This study was an investigator-initiated, parallel-group, multicenter randomized clinical trial. The clinical trial rationale, design, and methods have been described in Chapter 3. The study was registered and approved by all regulatory authorities and ethics committees of each center in Spain (Servicio Andaluz de Salud, Comité Ético de Investigación Provincial de Granada, and the Comité Ético de Investigación Clínica de Navarra). The study is registered at the US National Institutes of Health (ClinicalTrials.gov; identifier: NCT05310721) and followed the Consolidated Standards of Reporting Trials (CONSORT) reporting guideline for randomized clinical trials. All participants provided written informed consent.

Participants and eligibility criteria

Eligible participants were men and women (50%) aged 30 to 60 years with overweight/obesity (BMI ≥25.0 and <40 kg/m^2), abdominal obesity (waist circumference ≥95 cm in men and ≥82 cm in women), weight stability, sedentary lifestyle, habitual eating window ≥12 hours, and with at least one cardiometabolic risk factor for metabolic syndrome. Major exclusion criteria were participation in a weight loss program, cardiovascular or chronic diseases, pregnancy and lactation, and shift workers with nocturnal hours. Details about eligibility criteria and assessments used to ensure inclusion feasibility are provided in Chapter 3.

Study Recruitment, Enrollment, and Randomization

Recruitment, enrollment, and randomization of participants were performed among 9 consecutive sets of 10-13 participants in each center from February 7,

2022, to December 5, 2022, with a study completion date of March 6, 2023. Participants were recruited through advertisements in newspapers and from the Endocrinology and Nutrition services at the Hospital Universitario Clinico San Cecilio and Virgen de las Nieves in Granada, and the University Hospital of Navarra in Pamplona. Before enrollment, potential participants received clinical and physical examinations measurements to ensure inclusion feasibility. If eligibility criteria were met, participants completed baseline assessments. After that, enrolled participants were randomly assigned to one of four groups (UC, early TRE, late TRE, or self-selected TRE) using both stratification and permuted blocks with random block sizes. Randomization was stratified at each site (Granada and Pamplona) based on sex (men-women); with a total of two strata for each site. For this randomization scheme, a randomization list was generated prior to the start of the trial; one randomization list being generated for each site and strata. A sequence of block sizes was randomly generated where allowable block sizes were 4 and 8. Within each block, each quarter of assignments were randomly selected to be to one of the four possible groups (UC, early TRE, late TRE or self-selected TRE) using a parallel design (1:1:1:1 allocation ratio). As each participant was randomized into the trial, the participant received the next sequential assignment on the randomization list specific to his/her site and strata. The use of a random block size ensures that the next randomization assignment cannot be guessed. Because this was a multicenter trial with two sites (Granada and Pamplona), randomization within each site ensured that a site discontinuing participation in the trial or enrolling poorly would not affect the overall balance of the treatment groups. Stratifying by sex ensured that intervention groups were balanced on this important characteristic. Personnel in charge of the evaluations of the primary outcome (VAT and other ectopic MRI derived fat deports), fasting blood samples and statistical analysis were blinded to the group assignment, whereas personnel in charge of the other measures as well as the intervention were not blinded to the group assignment (open label).

Study Assessments and Outcomes

The assessments were conducted at baseline and 12 weeks post-intervention. The primary outcome was VAT quantified by magnetic resonance imaging (Siemens 3T Magnetom Vida). Secondary outcomes included the assessment of other ectopic fat depots such as intermuscular and intramuscular adipose tissue and hepatic fat fraction were also quantified by the same procedure , body weight and height measured using a stadiometer and scale (Seca model 799, Electronic

Column Scale, Hamburg, Germany) and waist circumference measured according to ISAK procedures [20]. Fat mass, fat-free mass, appendicular lean mass, and VAT mass were assessed using a DXA scan (QDR Discovery Wi Hologic, Inc., Bedford, MA, USA in Granada and Horizon Wi Hologic, Inc., Bedford, MA, USA in Pamplona). Blood pressure were measured with an automatic monitor (M3-Comfort, Omron Healthcare Europe B.V. Hoofddorp, The Netherlands) following the 2021 European Society of Hypertension practice guidelines [21]. Fasting blood samples were collected to analyze cardiometabolic risk factors (including blood glucose, lipid metabolism, and liver function). The adherence to the Mediterranean diet was assessed using the 14-item Mediterranean Diet Adherence Screener[22].

Study Intervention and Control Condition

The UC group continued with their usual eating schedule and received an educational program for weight management and cardiovascular health promotion based on Mediterranean dietary pattern[23] and the World Health Organization physical activity recommendations[24], similar to the TRE groups. Participants in the early TRE chose an 8-hour eating window starting no later than 10:00, late TRE participants chose an 8-hour window starting no earlier than 13:00, and participants in the self-selected TRE group selected their preferred 8-hour eating window before the intervention. TRE groups were instructed to maintain the same eating window throughout the 12-week intervention, and to follow the intervention every day of the week (i.e., seven days). Calorie-containing food or beverage intake outside the eating window was not allowed for the TRE groups. Only water, coffee, and tea without sugar or artificial sweeteners were allowed outside the eating window for the TRE groups.

Statistical analysis

The sample size and power of the clinical trial were estimated based on previous studies synthetized in a systematic review and meta-analysis[25]. Assuming a standard deviation of 7% in VAT[26], the enrolment of 21 participants per arm provide a statistical power of 90% at an alpha level of 0.008 (controlling for multiple group comparisons) to detect a minimum effect size of 10% in VAT. Considering the analyses by sex, and a maximum dropout rate of ~20%, we decided to recruit ~52 participants for each trial group (50% women).

Intervention effects on primary and secondary outcomes at 3 months after the intervention were assessed based on repeated-measures linear mixed-effects multilevel models, which included random cluster (site) effects[27]. Individual measures of change were therefore modelled as the function of randomly assigned group, site, assessment time, and their interaction terms. Model-based estimations were performed with an intention-to-treat approach using the restricted maximum-likelihood method; the model assuming that missing values were missing-at-random. Pearson's chi-square test was conducted to examine the association between the intervention group and the achievement of a clinically meaningful weight loss (≥5%). A simple linear regression analysis was conducted to examine the relationship between changes in adherence to the Mediterranean diet and body weight loss. A t-test was conducted to determine the changes in cardiometabolic risk factors following the intervention in participants who achieved a clinically meaningful weight loss and to compare them with those who did not achieve it. All analyses were conducted using R software, version 4.1.2 (R Foundation for Statistical Computing); linear mixed-effects models were performed using the lme4 package for R software.

Results

Study participants

Among 2598 participants initially screened for participation, 197 adults were enrolled and randomized to either UC (25 men, 24 women), early TRE (25 men, 24 women), late TRE (27 men, 25 women), or self-selected TRE groups (22 men, 25 women) (**Figure 1**). Overall, 14 participants (3, 2, 4, and 5 participants for the UC, early, late, and self-selected TRE groups, respectively) were unable to end the intervention for several reasons. A total of 197 (98 women) participants were thus included in the intention-to-treat analysis.

Table 1 displays the baseline participant characteristics. All participants were of Spanish ethnicity, with men having a mean (standard deviation) age of 47.3 (6.5) years and a BMI of 32.8 (3.3) kg/m^2, while women had a mean (standard deviation) age of 46.3 (6.1) years and a BMI of 33.2 (3.7) kg/m^2.

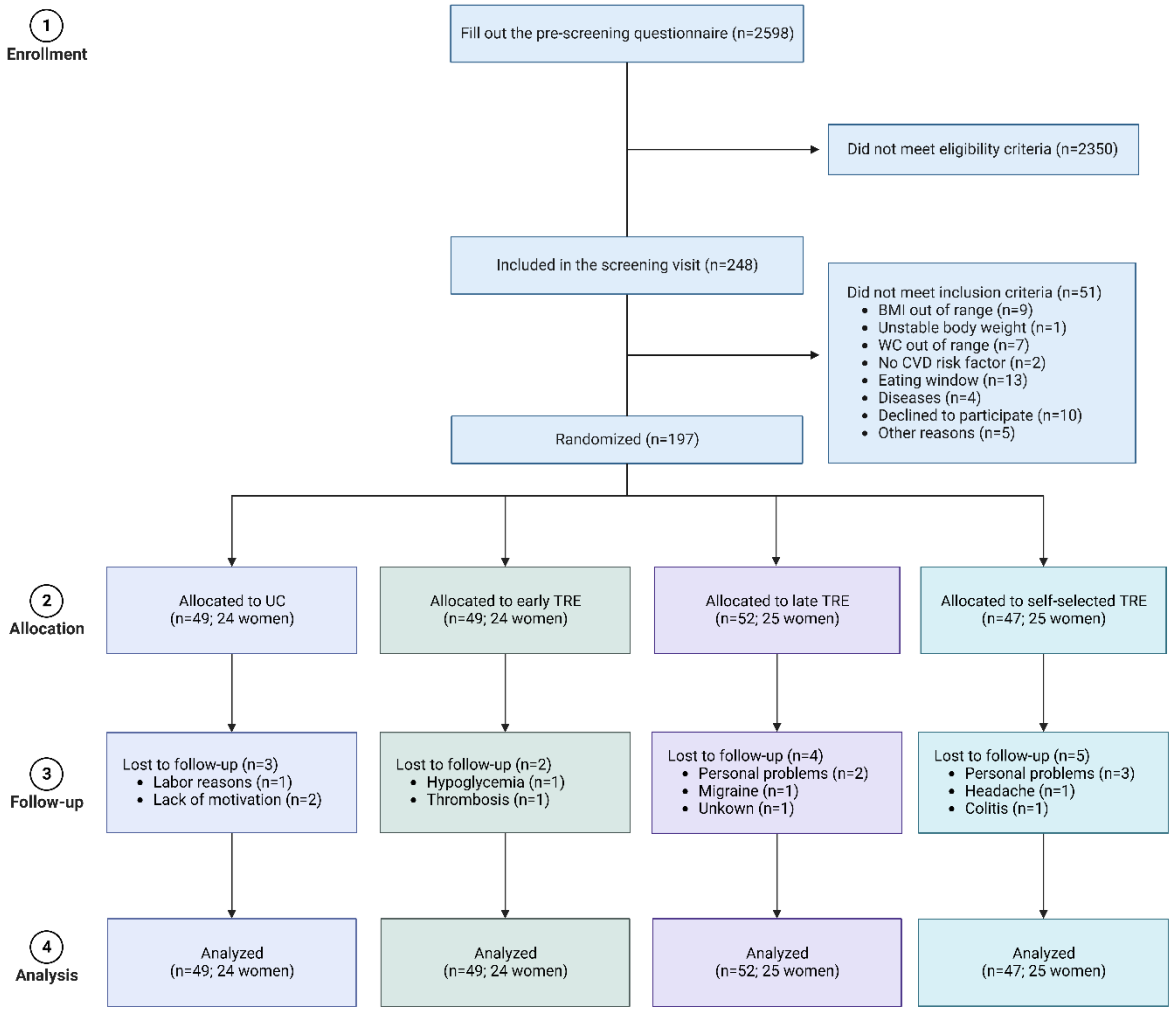

Figure 1. Study flow diagram. Usual-care (UC), early time-restricted eating (TRE), late TRE, or self-selected TRE groups. WC, waist circumference; CVD, cardiovascular.

Table 1. Baseline participant characteristics.

Men	**UC (n=25)**	**Early TRE (n=25)**	**Late TRE (n=27)**	**Self-selected TRE (n=22)**
Age (years)	47.7 (6.4)	47.8 (7.5)	47.8 (6.6)	45.7 (5.3)
Anthropometry				
Weight (kg)	101.8 (14.9)	106.3 (13.6)	101.6 (14.8)	99.8 (12.5)
Height (cm)	176.6 (5.8)	176.5 (7.1)	176.3 (6.8)	176.7 (6.2)
Body mass index (kg/m^2)	32.5 (3.2)	34.0 (2.9)	32.6 (3.6)	31.9 (3.0)
Body composition				
Fat-free mass (kg)	63.2 (8.3)	65.8 (7.6)	63.2 (7.2)	63.3 (6.5)
Appendi lean mass (kg)	25.7 (3.9)	26.9 (4.0)	25.4 (3.7)	26.0 (3.7)
Fat mass (kg)	37.4 (7.5)	39.5 (8)	37.6 (9.4)	35.3 (7.1)
Fat mass (%)	37.0 (3.9)	37.3 (4.1)	36.8 (5.1)	35.6 (4.0)
VAT mass (g)	1039.6 (266.7)	1042.4 (230.4)	1041.6 (298.7)	986.0 (213.5)
Blood pressure				
Systolic BP (mmHg)	130.3 (12.7)	131.6 (15.5)	124.7 (11.2)	125.7 (13.8)
Diastolic BP (mmHg)	84.8 (9.9)	84.3 (8.3)	81.4 (7.9)	81.9 (8.6)
Glucose metabolism				
Fasting glucose (mg/dL)	92.0 (89.0 – 101.0)	93.0 (90.0 – 101.5)	96.0 (90.0 – 105.0)	95.0 (89.8 – 101.3)
Insulin (mU/L)	12.6 (8.9 – 14)	11.3 (8 – 13.7)	10.2 (8.0 – 16.0)	9.4 (7.4 – 16.2)
HOMA-IR	2.9 (2.1 – 3.4)	2.5 (2 – 3.5)	2.5 (1.8 – 4.1)	2.3 (1.7 – 3.7)
HbA1c (%)	5.4 (5.3 – 5.6)	5.3 (5 – 5.6)	5.4 (5.1 – 5.6)	5.2 (5.1 – 5.5)
Lipids metabolism				
Total cholesterol (mg/dL)	222.0 (194.5 –235.0)	201 (186 – 224)	222.0 (185.0 – 253.0)	196.0 (182.8 – 228.3)
HDL-C (mg/dL)	53.0 (42.5 – 66.0)	47 (43 – 56)	54.0 (41.0 – 60.0)	50.0 (44.0 – 53.3)
LDL-C (mg/dL)	141.0 (122.0 – 156.0)	126 (119 – 147)	140.0 (116.0 – 170.0)	124.0 (102.8 – 149.5)
Triglycerides (mg/dL)	140.0 (84.0 – 163.0)	120 (82 – 173.5)	129.0 (80.0 – 159.0)	128.5 (96.0 – 200.0)
APOA1 (mg/dL)	155.0 (144.0 – 184.0)	141 (122 – 161.5)	141.0 (127.0 – 173.0)	161.5 (127.8 – 179.3)
APOB (mg/dL)	110.0 (92.0 – 123.5)	100 (96 – 110.5)	110.0 (97.0 – 128.0)	97.5 (86.3 – 120.0)
Liver markers				
ALT (U/L)	32 (26.5 – 42.5)	30.0 (23.5 – 39.0)	33.0 (21.0 – 47.0)	32.5 (26.5 – 40.0)
GGT (U/L)	43.0 (29.5 – 66.0)	28.0 (23.0 – 42.0)	35.0 (25.0 – 54.0)	36.5 (24.0 – 45.3)
ALP (U/L)	63.0 (51.5 – 76.5)	57.0 (52.0 – 72.0)	67.0 (57.0 – 79.0)	63.0 (50.8 – 71.3)
Women	**UC (n=24)**	**Early TRE (n=24)**	**Late TRE (n=25)**	**Self–selected TRE (n=25)**
Age (years)	45.7 (5.6)	46.5 (4.5)	48.2 (7.3)	44.8 (6.3)
Anthropometry				
Weight (kg)	90.3 (11.4)	88.9 (11.7)	85.2 (11.1)	88.2 (12.7)
Height (cm)	162.2 (5.1)	162.7 (5.4)	162.2 (6.0)	163.8 (5.3)
Body mass index (kg/m^2)	34.3 (4.0)	33.5 (3.8)	32.3 (3.2)	32.8 (3.6)
Body composition				
Fat–free mass (kg)	47.5 (6.9)	46.4 (5.4)	44.1 (4.9)	45.9 (5.8)
Appendi lean mass (kg)	18.1 (3.2)	17.7 (3.1)	17.1 (2.7)	17.4 (2.5)
Fat mass (kg)	42.0 (7.9)	41.4 (7.8)	40.5 (7.3)	41.7 (7.9)
Fat mass (%)	46.8 (5.2)	46.9 (4.2)	47.7 (4.1)	47.3 (4.0)
VAT mass (g)	842.0 (226.0)	866.2 (237.1)	911.2 (209.4)	873.0 (233.9)
Blood pressure				
Systolic BP (mmHg)	120.3 (12)	119.9 (15.4)	117.6 (13.4)	120.3 (18.2)

Diastolic BP (mmHg)	77.3 (10.8)	79.5 (13.0)	77.2 (8.9)	77.9 (11.3)
Glucose metabolism				
Fasting glucose (mg/dL)	93.0 (88.0 – 102.5)	92.0 (83 – 95.8)	94.0 (89.0 – 97.5)	89.0 (86.0 – 95.0)
Insulin (mU/L)	11.0 (8.5 – 15.8)	9.9 (7.2 – 12.9)	10.5 (9.3 – 14.1)	8.8 (6.5 – 14.0)
HOMA–IR	2.5 (2.2 – 4.0)	2.2 (1.5 – 3.1)	2.5 (2.1 – 3.1)	2.0 (1.4 – 3.3)
HbA1c (%)	5.3 (5.2 – 5.6)	5.4 (5.1 – 5.5)	5.4 (5.2 – 5.5)	5.3 (5.1 – 5.6)
Lipids metabolism				
Total cholesterol (mg/dL)	196.5 (174.3 – 213.5)	209.0 (179.8 – 237.8)	204.0 (187.5 – 239.0)	213.0 (183.5 – 233.0)
HDL–C (mg/dL)	57.5 (46.8 – 62.0)	55.5 (47.3 – 61.0)	57.0 (48.5 – 67.0)	53.0 (47.5 – 62.5)
LDL–C (mg/dL)	108.5 (94.3 – 131.0)	128.0 (104.5 – 157.5)	131.0 (113.5 – 146.0)	133.0 (117.5 – 156.5)
Triglycerides (mg/dL)	114.5 (81.8 – 146.3)	108.0 (85.8 – 151.5)	103.0 (86.5 – 124.0)	103.0 (72.0 – 134.5)
APOA1 (mg/dL)	167.0 (143.8 – 195.5)	157.5 (142.3 – 169.8)	166.0 (135.0 – 193.5)	166.0 (140.5 – 176.5)
APOB (mg/dL)	94.5 (73.3 – 104.0)	92.5 (81.5 – 114.5)	101.0 (86.0 – 118.0)	95.0 (83.5 – 115.5)
Liver markers				
ALT (U/L)	20.0 (16.0 – 24.5)	17.0 (15.0 – 22.0)	19.0 (16.0 – 28.0)	19.0 (13.5 – 23.5)
GGT (U/L)	23.0 (16.5 – 29.8)	19.0 (14.0 – 24.5)	19.0 (14.0 – 25.5)	17.0 (14.0 – 23.5)
ALP (U/L)	62.5 (53 – 75.5)	65.5 (57.8 – 86.0)	63.0 (54.5 – 79.0)	67.0 (58.0 – 80.0)

Data are presented as mean (standard deviation) when normally distributed, or medians (interquartile range) when not. ALP, alkaline phosphatase; ALT, alanine aminotransferase; APOA1, apolipoprotein A; APOB, apolipoprotein B; Appendi, appendicular; BP, blood pressure; GGT, gamma-glutamyltransferase; HbA1c, glycated haemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; MG, mean glucose; TRE, time-restricted eating; UC, usual-care; VAT, visceral adipose tissue.

Adverse events

No deaths or serious adverse events were reported during the trial. Mild adverse events were observed, including hypoglycemia and thrombosis in the early TRE group, migraine in the late TRE group, and headache and colitis in the self-selected TRE group. These mild adverse events led to the discontinuation of the affected participants from the study.

Primary outcome: VAT

Among men, VAT mass remained similar after the 12-week intervention in the UC group (-38.2 g; 95% confidence intervals [CI], -100.8 to 24.3; P=0.228). However, it decreased in the early TRE (-99.5 g; 95% CI, -160.9 to -38.1; P=0.002), late TRE (-125.7 g; 95% CI, -185.8 to -65.6; P<0.001), and self-selected TRE (-167.9 g; 95% CI, -233.4 to -102.5; P<0.001) groups (**Table S1**). Significant differences were observed only between the UC group and self-selected TRE group (mean difference: -129.7 g; 95% CI, -249.0 to -10.5; P=0.027; **Figure 2** and **Table 2**). Among women, VAT mass decreased only in the late TRE group (-84.8 g; 95% CI, -135.3 to -34.2; P=0.001; **Table S2**), with no significant differences across intervention groups (**Figure 2** and **Table 2-3**).

Body composition

In the UC group, body weight decreased by an average of 1.9 kg (95% CI, -3.3 to -0.4; P=0.012) in men and 1.7 kg (95% CI, -2.8 to -0.5; P=0.005) in women. The early TRE group exhibited an average weight loss of 5.3 kg (95% CI, -6.8 to -3.9; P<0.001) in men and 4.1 kg (95% CI, -5.1 to -3.0; P<0.001) in women. Men in the late TRE group showed a reduction of 3.9 kg (95% CI, -5.3 to -2.4; P<0.001), while in women, it was 4.5 kg (95% CI, -5.7 to -3.4; P<0.001). The self-selected TRE group exhibited a weight loss of 6.3 kg (95% CI, -7.8 to -4.7; P<0.001) in men and 3.4 kg (95% CI, -4.5 to -2.2; P<0.001) in women (**Table S1** for men, **Table S2** for women). Significant differences in body weight loss were observed between the UC group and the early TRE group in both men (mean difference: -3.4 kg; 95% CI, -6.1 to -0.7; P=0.007) and women (mean difference: -2.4 kg; 95% CI, -4.5 to -0.3; P=0.021). Similarly, significant differences were found between the UC group and the self-selected TRE group in men (mean difference: -4.4 kg; 95% CI, -7.1 to -1.6; P=0.001) as well as between the UC group and the late TRE group in women (mean difference: -2.8 kg; 95% CI, -5.0 to -0.7; P=0.004). No statistically significant differences were found among the other group comparisons (**Figure 2** and **Table 2-3**). The data on body weight loss percentage are presented in **Figure 3-4**, **Figure S1**, **Table S1** for men, and **Table S2** for women, which illustrates the same results. Interestingly, the proportion of individuals achieving a clinically meaningful weight loss (≥5%) differed significantly across the intervention groups (P=0.024 for men and P=0.017 for women). In the UC group, only 16% of men and 8% of women achieved such weight loss, compared to 40% and 42% in the early TRE group, 41% and 48% in the late TRE group, and 59% and 28% in the self-selected TRE group, respectively.

In men, fat-free mass decreased by -1.5 kg (95% CI, -2.8 to -0.3; P=0.014) in the early TRE group and by -1.7 kg (95% CI, -3.0 to -0.4; P=0.010) in the self-selected TRE group (**Table S1**), with no significant differences among the intervention groups (P=0.224; **Figure 2** and **Table 2-3**). Among women, fat-free mass remained unchanged across all groups after the 12-week intervention (P=0.192; **Figure 2** and **Table 2-3**). In men, appendicular lean mass showed a decrease of -1.5 kg (95% CI, -2.4 to -0.7; P<0.001) in the early TRE group, -0.8 kg (95% CI, -1.6 to 0.0; P=0.044) in the late TRE group, and -1.6 kg (95% CI, -2.4 to -0.7; P<0.001) in the self-selected TRE group (**Table S1**). No significant differences were observed among the intervention groups (P=0.191; **Table 2-3**). Among women, appendicular lean mass decreased by -1.1 kg (95% CI, -1.8 to -0.4; P=0.002) in the

early TRE group and -1.4 kg (95% CI, -2.1 to -0.7; P<0.001) in the late TRE group (**Table S2**), with no significant differences among the intervention groups (P=0.579; **Table 2-3**).

Fat mass decreased in all intervention groups for both men and women. In the UC group, men experienced a decrease of 1.7 kg (95% CI, -3.0 to -0.4; P=0.014), while women had a decrease of 2.2 kg (95% CI, -3.3 to -1.1; P=0.001). The early TRE group showed a decrease of 3.7 kg (95% CI, -5.0 to -2.4; P<0.001) for men and 2.9 kg (95% CI, -3.9 to -1.9; P<0.001) for women. Men in the late TRE group had a decrease of 3.3 kg (95% CI, -4.5 to -2.0; P<0.001), and women had a decrease of 3.8 kg (95% CI, -4.8 to -2.7; P<0.001). The self-selected TRE group had a decrease of 3.9 kg (95% CI, -5.3 to -2.5; P<0.001) for men and 2.8 kg (95% CI, -3.9 to -1.8; P<0.001) for women (**Table S1** for men and **Table S2** for women). However, there were no significant differences in the change of fat mass among the intervention groups in both men and women (**Figure 2** and **Table 2-3**). Consistent results were observed in the changes in fat mass percentage, as shown in **Table 2-3**, **Figure S1**, **Table S1** for men, and **Table S2** for women.

Blood pressure

In men, both systolic and diastolic blood pressure showed reductions in the UC, early TRE, and self-selected TRE groups. For systolic blood pressure, the reductions were -8.5 mmHg (95% CI, -13.0 to -4.1; P<0.001), -6.5 mmHg (95% CI, -10.9 to -2.2; P=0.004), and -4.6 mmHg (95% CI, -9.0 to -0.1; P=0.047; **Table S1**), respectively, with no significant differences between the intervention groups (P=0.087; **Table 2-3**). Similarly, for diastolic blood pressure, the reductions were -3.2 mmHg (95% CI, -6.2 to -0.1; P=0.041), -4.4 mmHg (95% CI, -7.3 to -1.4; P=0.004), and -3.8 mmHg (95% CI, -6.9 to -0.7; P=0.017; **Table S1**), respectively, with no significant differences between the intervention groups (P=0.899; **Table 2-3**). In women, there were no changes in systolic or diastolic blood pressure within or between the intervention groups (P=0.161 and P=0.357, respectively; **Table 2-3** and **Table S2**).

Glucose metabolism

In both men and women, fasting glucose levels showed significant reductions only in the early TRE group: by -5.9 mg/dL (95% CI, -9.7 to -2.1; P=0.002) in men and by -4.9 mg/dL (95% CI, -8.6 to -1.3; P=0.008) in women (**Table S1** for men and

Table S2 for women). These reductions were significantly different when compared to the late TRE group, with a mean difference of 8.4 mg/dL (95% CI, 1.5 to 15.3; P=0.010) in men and 7.4 mg/dL (95% CI, 0.6 to 14.2; P=0.027) in women (**Figure 5** and **Table 3**). Insulin levels exhibited a significant decrease in the self-selected TRE group (-2.4 mU/L; 95% CI, -4.5 to -0.4; P=0.019) among men and in the late TRE group (-1.9 mU/L; 95% CI, -3.6 to -0.1; P=0.037) among women (**Table S1** for men, **Table S2** for women). No significant differences were observed between the intervention groups, neither in men (P=0.263) nor in women (P=0.476) (**Figure 5** and **Table 2-3**). Among men, only those in the self-selected TRE group demonstrated a significant reduction in HOMA-IR (-0.6; 95% CI, -1.1 to -0.1; P=0.024; **Table S1**), with no differences between groups (P=0.390; **Figure 5** and **Table 2-3**). No changes in HOMA-IR were observed within or between the intervention groups in women (P=0.554; **Figure 5**, **Table 2-3**, and **Table S2**). Similarly, no changes in glycated haemoglobin levels were observed within or between the intervention groups in both men and women (P=0.165 and P=0.172, respectively; **Figure 5**, **Table 2-3**, **Table S1** for men, and **Table S2** for women).

Lipid metabolism

Total cholesterol levels did not change within or between intervention groups in both men and women (P=0.728 and P=0.089, respectively; **Figure 6**, **Table 2-3**). In men, HDL-C decreased in the UC group by -3.9 mg/dL (95% CI, -7.2 to -0.6; P=0.021; **Table S1**) with no differences between groups (P=0.353; **Figure 6**, **Table 2-3**). In women, HDL-C decreased in the UC (-3.1 mg/dL; 95% CI, -5.5 to -0.6; P=0.014), early TRE (-4.0 mg/dL; 95% CI, -6.4 to -1.7; P=0.001), and late TRE (-3.4 mg/dL; 95% CI, -5.8 to -1.0; P=0.005) groups (**Table S2**), with no significant differences between groups (P=0.213; **Figure 6**, **Table 2-3**). LDL-C levels did not significantly change within or between intervention groups in men (P=0.707; **Figure 6**, **Table 2-3,** and **Table S1**). Among women, LDL-C increased in the late TRE by 11.7 mg/dL (95% CI, 2.6 to 20.8; P=0.012) and in the self-selected TRE by 13.3 mg/dL (95% CI, 4.0 to 22.6; P=0.005) groups (**Table S2**), with no differences between intervention groups (P=0.071; **Figure 6**, **Table 2-3**). Triglyceride levels showed significant reductions in men who underwent the early TRE (-38.9 mg/dL; 95% CI, -64.8 to -13.1; P=0.004) and self-selected TRE (-37.3 mg/dL; 95% CI, -64.4 to -10.2; P=0.007) interventions (**Table S1**). No significant differences were observed between the intervention groups (P=0.669; **Figure 6**, **Table 2-3**). No significant changes in triglyceride levels were observed within or between

intervention groups in women (P=0.971; **Figure 6**, **Table 2-3**, and **Table S2**). Apolipoprotein A1 levels remained stable within and between intervention groups for both men and women (P=0.612 and P=0.932, respectively, **Table 2-3, Table S1** for men, and **Table S2** for women). In men, apolipoprotein B levels also showed no changes within or between intervention groups (P=0.654; **Table 2-3** and **Table S1**). However, in women, apolipoprotein B increased by 8.8 mg/dL (95% CI, 1.3 to 16.4; P=0.022; **Table S2**) in the late TRE group, with no significant differences compared to the other intervention groups (P=0.084, **Table 2-3**).

Liver markers

Alanine aminotransferase levels decreased in men in the late TRE (-6.1 U/L; 95% CI, -12.0 to -0.1; P=0.046) and self-selected TRE (-7.4 U/L; 95% CI, -13.9 to -0.9; P=0.026) groups (**Table S1**). In women, alanine aminotransferase levels decreased in the UC group (-4.3 U/L; 95% CI, -8.5 to 0.0; P=0.048; **Table S2**). However, there were no significant differences compared to the other intervention groups in both men and women (P=0.086 and P=0.838, respectively; **Table 2-3**). Gamma-glutamyl transferase levels remained stable within and between intervention groups in both men and women (P=0.996 and P=0.906, respectively; **Table 2-3, Table S1** for men, and **Table S2** for women). Alkaline phosphatase levels increased in the early TRE group in men (5.8 U/L; 95% CI, 1.8 to 9.7; P=0.005; **Table S1**), but there were no significant differences compared to the other intervention groups (P=0.304; **Table 2-3**). Alkaline phosphatase levels remained stable within and between intervention groups in women (P=0.928; **Table 2-3** and **Table S2**).

Adherence to the Mediterranean diet

The adherence to the Mediterranean diet showed improvement in the UC and self-selected TRE groups among men (all P<0.017; data not shown), although there were no significant differences observed between the intervention groups (P=0.846; data not shown). In women, the adherence to the Mediterranean diet improved in the late and self-selected TRE groups (all P<0.036; data not shown), and there was a significant difference between the early TRE and late TRE groups (P=0.047; data not shown). Changes in adherence to the Mediterranean diet were positively associated with body weight loss in the UC group among men (R^2= 0.169, P=0.032; data not shown) and in the late TRE group among women (R^2= 0.265, P=0.010; data not shown). A trend towards significance was observed in

the self-selected TRE group among men (R^2= 0.133, P=0.064; data not shown). Similar results were found when considering body weight loss percentage.

Clinically meaningful weight loss and cardiometabolic risk factors

Men who achieved a clinically meaningful weight loss (≥5%) demonstrated significant improvements in adherence to the Mediterranean diet, insulin, HOMA-IR, lipid profile (including total cholesterol, triglycerides, LDL-C, apolipoprotein A1, and apolipoprotein B), alanine aminotransferase, and blood pressure (systolic and diastolic) (all P<0.05; data not shown). Similarly, women with a clinically meaningful weight loss showed significant improvements in adherence to the Mediterranean diet, insulin, HOMA-IR, triglycerides, alanine aminotransferase, gamma-glutamyl transferase, and blood pressure (systolic and diastolic) (all P<0.05; data not shown). These improvements were accompanied by significant differences compared to individuals who did not achieve a clinically meaningful weight loss. In men, there were significant differences in insulin (-3.9 mU/L; 95% CI, -5.7 to -2.0; P<0.001), HOMA-IR (-0.9; 95% CI, -1.4 to -0.5; P<0.001), LDL-C (-11.2 mg/dL; 95% CI, -21.2 to -1.2; P=0.033), apolipoprotein A1 (-13.1 mg/dL; 95% CI, -25.3 to -1.0; P=0.021), apolipoprotein B (-8.9 mg/dL; 95% CI, -16.4 to -1.4; P=0.020), alanine aminotransferase (-12.1 U/L; 95% CI, -18 to -6.1; P<0.001), systolic blood pressure (-5.2 mmHg; 95% CI, -9.6 to -0.7; P=0.015), and diastolic blood pressure (-4.4 mmHg; 95% CI, -7.3 to -1.5; P=0.002) (**Figure 7**). For women, there were significant differences in total cholesterol (-11.7 mg/dL; 95% CI, -23.3 to -0.1; P=0.048) and alanine aminotransferase (-4.8 U/L; 95% CI, -9.2 to -0.4; P=0.016) (**Figure 7**)

Table 2. Changes in body composition, blood pressure, glucose metabolism, lipid metabolism, and liver markers end points in the time–restricted eating groups compared to the usual–care group after the 12–week intervention.

Men			
End point	**UC vs. Early TRE**	**UC vs. Late TRE**	**UC vs. Self–selected TRE**
	Mean difference (95% CI)	Mean difference (95% CI)	Mean difference (95% CI)
Body composition			
Weight (kg)	-3.4 (-6.1, -0.7)*	-2.0 (-4.6, 0.7)	-4.4 (-7.1, -1.6)*
Weight (%)	-3.1 (-5.7, -0.6)*	-1.8 (-4.3, 0.7)	-4.3 (-6.9, -1.6)*
Fat–free mass (kg)	-1.3 (-3.6, 1.0)	-0.4 (-2.7, 1.9)	-1.5 (-3.9, 0.9)
Appendi lean mass (kg)	-1.1 (-2.6, 0.5)	-0.4 (-1.9, 1.2)	-1.1 (-2.7, 0.5)
Fat mass (kg)	-2.0 (-4.4, 0.5)	-1.6 (-4.0, 0.9)	-2.2 (-4.8, 0.3)
Fat mass (%)	-0.6 (-2.6, 1.4)	-0.7 (-2.7, 1.3)	-0.9 (-3.0, 1.2)
VAT mass (g)	-61.2 (-176.7, 54.2)	-87.5 (-201.7, 26.8)	-129.7 (-249.0, -10.5)*
Blood pressure			
Systolic BP (mmHg)	2.0 (-6.2, 10.2)	7.3 (-0.8, 15.3)	3.9 (-4.5, 12.3)
Diastolic BP (mmHg)	-1.2 (-6.8, 4.4)	1.3 (-4.2, 6.8)	-0.7 (-6.4, 5.0)
Glucose metabolism			
Fasting glucose (mg/dL)	-5.8 (-12.9, 1.3)	2.6 (-4.3, 9.6)	-1.0 (-8.3, 6.2)
Insulin (mU/L)	-0.2 (-3.8, 3.4)	0.3 (-3.3, 3.8)	-1.9 (-5.6, 1.8)
HOMA–IR	-0.2 (-1.1, 0.7)	0.2 (-0.7, 1.1)	-0.5 (-1.4, 0.5)
HbA1c (%)	0.0 (-0.3, 0.2)	0.0 (-0.3, 0.2)	-0.1 (-0.4, 0.1)
Lipids metabolism			
Total cholesterol (mg/dL)	6.5 (-17.6, 30.7)	9.0 (-14.7, 32.7)	2.3 (-22.4, 27.0)
HDL–C (mg/dL)	3.2 (-3.0, 9.3)	4.0 (-2.0, 10.0)	1.9 (-4.3, 8.2)
LDL–C (mg/dL)	8.5 (-10.1, 27.1)	5.7 (-12.5, 24.0)	3.7 (-15.3, 22.8)
Triglycerides (mg/dL)	-19.9 (-69.0, 29.2)	5.5 (-42.6, 53.7)	-18.3 (-68.5, 31.9)
APOA1 (mg/dL)	6.7 (-16.0, 29.4)	3.3 (-19.0, 25.5)	-4.1 (-27.3, 19.1)
APOB (mg/dL)	5.0 (-9.1, 19.1)	5.5 (-8.4, 19.3)	2.3 (-12.2, 16.7)
Liver markers			
ALT (U/L)	-6.5 (-18.2, 5.1)	-6.8 (-18.2, 4.7)	-8.1 (-20.0, 3.8)
GGT (U/L)	1.7 (-20.1, 23.4)	1.9 (-19.4, 23.2)	-0.2 (-22.4, 22.1)
ALP (U/L)	4.3 (-3.2, 11.7)	-0.3 (-7.6, 7.0)	-1.6 (-9.3, 6.0)
Women			
End point	**UC vs. Early TRE**	**UC vs. Late TRE**	**UC vs. Self–selected TRE**
	Mean difference (95% CI)	Mean difference (95% CI)	Mean difference (95% CI)
Body composition			
Weight (kg)	-2.4 (-4.5, -0.3)*	-2.8 (-5.0, -0.7)*	-1.7 (-3.9, 0.5)
Weight (%)	-2.8 (-4.9, -0.6)*	-3.4 (-5.6, -1.2)*	-1.7 (-3.9, 0.5)
Fat–free mass (kg)	-1.0 (-2.6, 0.6)	-0.8 (-2.5, 0.8)	-1.0 (-2.7, 0.7)
Appendi lean mass (kg)	-0.7 (-2.1, 0.6)	-1.0 (-2.4, 0.3)	-0.2 (-1.6, 1.1)
Fat mass (kg)	-0.7 (-2.7, 1.2)	-1.6 (-3.6, 0.4)	-0.7 (-2.7, 1.3)
Fat mass (%)	0.3 (-1.4, 2.0)	-0.4 (-2.2, 1.3)	0.3 (-1.5, 2.0)
VAT mass (g)	-25.7 (-121.6, 70.3)	-65.1 (-162.8, 32.6)	-25.7 (-124.4, 73.0)
Blood pressure			
Systolic BP (mmHg)	0.0 (-7.2, 7.3)	1.2 (-6.2, 8.5)	3.8 (-3.6, 11.2)
Diastolic BP (mmHg)	-0.7 (-6.2, 4.8)	0.3 (-5.2, 5.9)	1.7 (-3.9, 7.3)
Glucose metabolism			

Fasting glucose (mg/dL)	-5.7 (-12.6, 1.1)	1.7 (-5.3, 8.6)	-0.4 (-7.4, 6.6)
Insulin (mU/L)	0.0 (-3.3, 3.2)	-0.8 (-4.1, 2.5)	-0.7 (-4.1, 2.7)
HOMA–IR	-0.2 (-1, 0.6)	-0.2 (-1, 0.6)	-0.2 (-1, 0.6)
HbA1c (%)	0.0 (-0.2, 0.2)	0.0 (-0.2, 0.1)	-0.1 (-0.3, 0.1)
Lipids metabolism			
Total cholesterol (mg/dL)	0.9 (-19.7, 21.5)	10.2 (-10.6, 30.9)	11.1 (-9.9, 32.0)
HDL–C (mg/dL)	-1.0 (-5.4, 3.5)	-0.3 (-4.8, 4.1)	2.0 (-2.5, 6.6)
LDL–C (mg/dL)	-0.6 (-17.6, 16.4)	8.0 (-9.2, 25.1)	9.6 (-7.8, 26.9)
Triglycerides (mg/dL)	11.9 (-25.7, 49.6)	-0.4 (-38.3, 37.5)	3.9 (-34.4, 42.1)
APOA1 (mg/dL)	4.2 (-17.7, 26.1)	-0.4 (-22.5, 21.6)	0.8 (-21.5, 23.1)
APOB (mg/dL)	3.8 (-10.4, 17.9)	9.7 (-4.6, 23.9)	7.9 (-6.4, 22.3)
Liver markers			
ALT (U/L)	4.3 (-3.5, 12.1)	1.0 (-6.9, 8.8)	1.8 (-6.1, 9.7)
GGT (U/L)	0.3 (-8.5, 9.1)	-1.7 (-10.6, 7.1)	0.3 (-8.6, 9.2)
ALP (U/L)	0.8 (-6.5, 8.2)	-3.0 (-10.4, 4.4)	1.6 (-5.9, 9.1)

Sample size: usual–care (UC) = 25 men and 24 women; early time–restricted eating (TRE) = 25 men and 24 women; late TRE = 27 men and 25 women; self-selected TRE = 22 men and 25 women. ALP, alkaline phosphatase; ALT, alanine aminotransferase; APOA1, apolipoprotein A; APOB, apolipoprotein B; Appendi, appendicular; BP, blood pressure; CI, confident interval; GGT, gamma-glutamyltransferase; HbA1c, glycated haemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; MG, mean glucose; VAT, visceral adipose tissue.*Represent significant differences between groups as determined by post hoc Tukey analysis ($P<0.05$).

Table 3. Changes in body composition, blood pressure, glucose metabolism, lipid metabolism, and liver markers end points in the time–restricted eating groups compared to each other after the 12–week intervention.

Men			
End point	**Early vs. Late TRE**	**Early vs. Self-selected TRE**	**Late vs. Self–selected TRE**
	Mean difference (95% CI)	Mean difference (95% CI)	Mean difference (95% CI)
Body composition			
Weight (kg)	1.4 (-1.2, 4.1)	-0.9 (-3.7, 1.8)	-2.4 (-5.2, 0.4)
Weight (%)	1.3 (-1.2, 3.8)	-1.2 (-3.8, 1.5)	-2.5 (-5.1, 0.1)
Fat–free mass (kg)	0.9 (-1.3, 3.2)	-0.2 (-2.5, 2.2)	-1.1 (-3.5, 1.2)
Appendi lean mass (kg)	0.7 (-0.8, 2.2)	0.0 (-1.6, 1.6)	-0.7 (-2.3, 0.8)
Fat mass (kg)	0.4 (-2.0, 2.8)	-0.2 (-2.8, 2.3)	-0.6 (-3.1, 1.8)
Fat mass (%)	-0.1 (-2.1, 1.9)	-0.3 (-2.4, 1.7)	-0.2 (-2.3, 1.8)
VAT mass (g)	-26.2 (-139.4, 86.9)	-68.5 (-186.7, 49.7)	-42.2 (-159.3, 74.8)
Blood pressure			
Systolic BP (mmHg)	5.2 (-2.7, 13.2)	1.9 (-6.4, 10.2)	-3.3 (-11.5, 4.8)
Diastolic BP (mmHg)	2.5 (-2.9, 8.0)	0.6 (-5.1, 6.2)	-2.0 (-7.5, 3.6)
Glucose metabolism			
Fasting glucose (mg/dL)	8.4 (1.5, 15.3)*	4.8 (-2.4, 12.0)	-3.6 (-10.7, 3.4)
Insulin (mU/L)	0.4 (-3.1, 4.0)	-1.7 (-5.4, 2.0)	-2.2 (-5.8, 1.5)
HOMA–IR	0.3 (-0.6, 1.2)	-0.3 (-1.2, 0.6)	-0.6 (-1.5, 0.3)
HbA1c (%)	0.0 (-0.3, 0.2)	-0.1 (-0.4, 0.1)	-0.1 (-0.3, 0.2)
Lipids metabolism			
Total cholesterol (mg/dL)	2.5 (-21.0, 26.0)	-4.3 (-28.8, 20.3)	-6.7 (-30.8, 17.3)
HDL–C (mg/dL)	0.8 (-5.1, 6.8)	-1.2 (-7.4, 5.0)	-2.0 (-8.1, 4.1)
LDL–C (mg/dL)	-2.8 (-20.9, 15.3)	-4.8 (-23.7, 14.1)	-2.0 (-20.5, 16.5)
Triglycerides (mg/dL)	25.4 (-21.8, 72.7)	1.6 (-47.7, 51.0)	-23.8 (-72.3, 24.6)
APOA1 (mg/dL)	-3.4 (-25.5, 18.6)	-10.8 (-33.9, 12.3)	-7.4 (-30.0, 15.3)
APOB (mg/dL)	0.5 (-13.2, 14.2)	-2.7 (-17, 11.6)	-3.2 (-17.2, 10.9)
Liver markers			
ALT (U/L)	-0.2 (-11.6, 11.1)	-1.6 (-13.4, 10.2)	-1.3 (-13.0, 10.3)
GGT (U/L)	0.2 (-20.9, 21.3)	-1.8 (-23.9, 20.2)	-2.1 (-23.7, 19.5)
ALP (U/L)	-4.6 (-11.8, 2.7)	-5.9 (-13.5, 1.7)	-1.3 (-8.8, 6.1)
Women			
End point	**Early vs. Late TRE**	**Early vs. Self-selected TRE**	**Late vs. Self–selected TRE**
	Mean difference (95% CI)	Mean difference (95% CI)	Mean difference (95% CI)
Body composition			
Weight (kg)	-0.5 (-2.6, 1.6)	0.7 (-1.4, 2.8)	1.2 (-1.0, 3.3)
Weight (%)	-0.7 (-2.8, 1.5)	1.0 (-1.1, 3.2)	1.7 (-0.5, 3.9)
Fat–free mass (kg)	0.2 (-1.4, 1.7)	0.0 (-1.6, 1.6)	-0.2 (-1.8, 1.5)
Appendi lean mass (kg)	-0.3 (-1.6, 1.0)	0.5 (-0.8, 1.8)	0.8 (-0.5, 2.1)
Fat mass (kg)	-0.9 (-2.7, 1.0)	0.1 (-1.8, 2.0)	0.9 (-1, 2.9)
Fat mass (%)	-0.7 (-2.4, 0.9)	0.0 (-1.7, 1.7)	0.7 (-1, 2.4)
VAT mass (g)	-39.5 (-131.8, 52.9)	-0.1 (-93.5, 93.4)	39.4 (-55.9, 134.6)
Blood pressure			
Systolic BP (mmHg)	1.1 (-6.1, 8.4)	3.8 (-3.6, 11.1)	2.7 (-4.7, 10.0)
Diastolic BP (mmHg)	1.0 (-4.4, 6.5)	2.4 (-3.1, 7.9)	1.4 (-4.2, 7.0)
Glucose metabolism			

Fasting glucose (mg/dL)	7.4 (0.6, 14.2)*	5.4 (-1.5, 12.2)	-2.1 (-9, 4.9)
Insulin (mU/L)	-0.8 (-4, 2.5)	-0.7 (-4, 2.7)	0.1 (-3.2, 3.5)
HOMA–IR	0.0 (-0.7, 0.8)	0.0 (-0.8, 0.8)	-0.1 (-0.9, 0.8)
HbA1c (%)	0.0 (-0.2, 0.1)	-0.1 (-0.3, 0.1)	0.0 (-0.2, 0.1)
Lipids metabolism			
Total cholesterol (mg/dL)	9.3 (-11.1, 29.6)	10.2 (-10.4, 30.7)	0.9 (-19.8, 21.6)
HDL–C (mg/dL)	0.6 (-3.8, 5.0)	3.0 (-1.4, 7.4)	2.4 (-2.1, 6.9)
LDL–C (mg/dL)	8.6 (-8.2, 25.4)	10.2 (-6.8, 27.2)	1.6 (-15.5, 18.7)
Triglycerides (mg/dL)	-12.4 (-49.6, 24.8)	-8.1 (-45.6, 29.5)	4.3 (-33.5, 42.1)
APOA1 (mg/dL)	-4.6 (-26.3, 17.1)	-3.4 (-25.3, 18.5)	1.2 (-20.8, 23.2)
APOB (mg/dL)	5.9 (-8.0, 19.9)	4.2 (-9.9, 18.3)	-1.7 (-16.0, 12.5)
Liver markers			
ALT (U/L)	-3.3 (-11.0, 4.3)	-2.5 (-10.2, 5.3)	0.9 (-6.9, 8.7)
GGT (U/L)	-2.1 (-10.7, 6.6)	0.0 (-8.8, 8.7)	2.0 (-6.8, 10.9)
ALP (U/L)	-3.8 (-11.1, 3.4)	0.8 (-6.5, 8.1)	4.6 (-2.8, 12.1)

Sample size: early time–restricted eating (TRE) = 25 men and 24 women; late TRE = 27 men and 25 women; self-selected TRE = 22 men and 25 women. ALP, alkaline phosphatase; ALT, alanine aminotransferase; APOA1, apolipoprotein A; APOB, apolipoprotein B; Appendi, appendicular; BP, blood pressure; CI, confident interval; GGT, gamma-glutamyltransferase; HbA1c, glycated haemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; MG, mean glucose; VAT, visceral adipose tissue. *Represent significant differences between groups as determined by post hoc Tukey analysis ($P<0.05$).

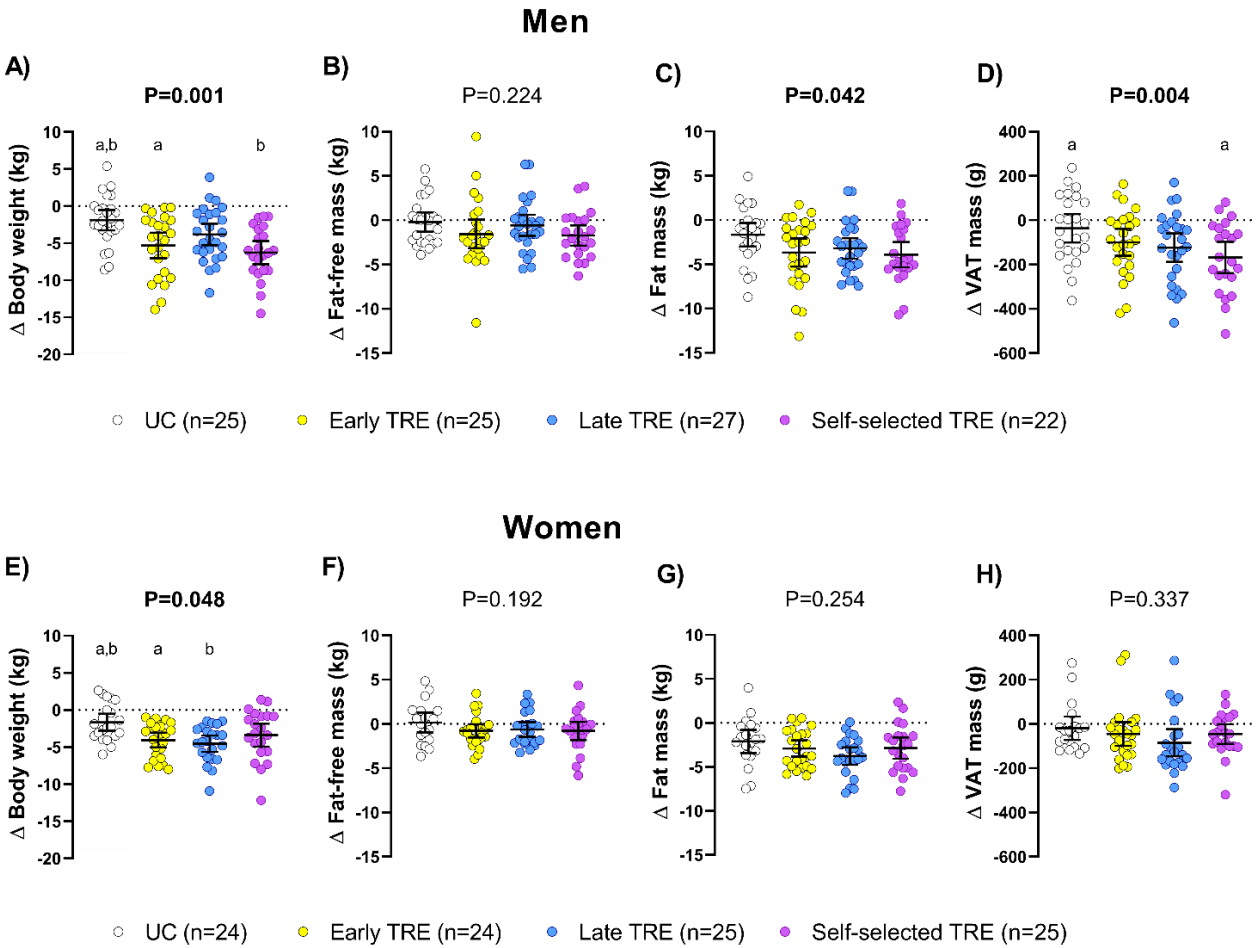

Figure 2. Changes in body weight (A and E), fat–free mass (B and F), fat mass (C and G), and visceral adipose tissue mass (VAT; D and H) in men and women among the usual–care (UC), early time–restricted eating (TRE), late TRE, and self–selected TRE groups after the 12–week intervention. Dara are raw means with 95% confident interval. P–value from group × time interaction term from a linear mixed-effects model that included study group, time (baseline and 12 weeks), and study group × time interaction term as fixed effects and participant as random effect. Similar letters represent significant differences between groups as determined by post hoc Tukey analysis (P<0.05).

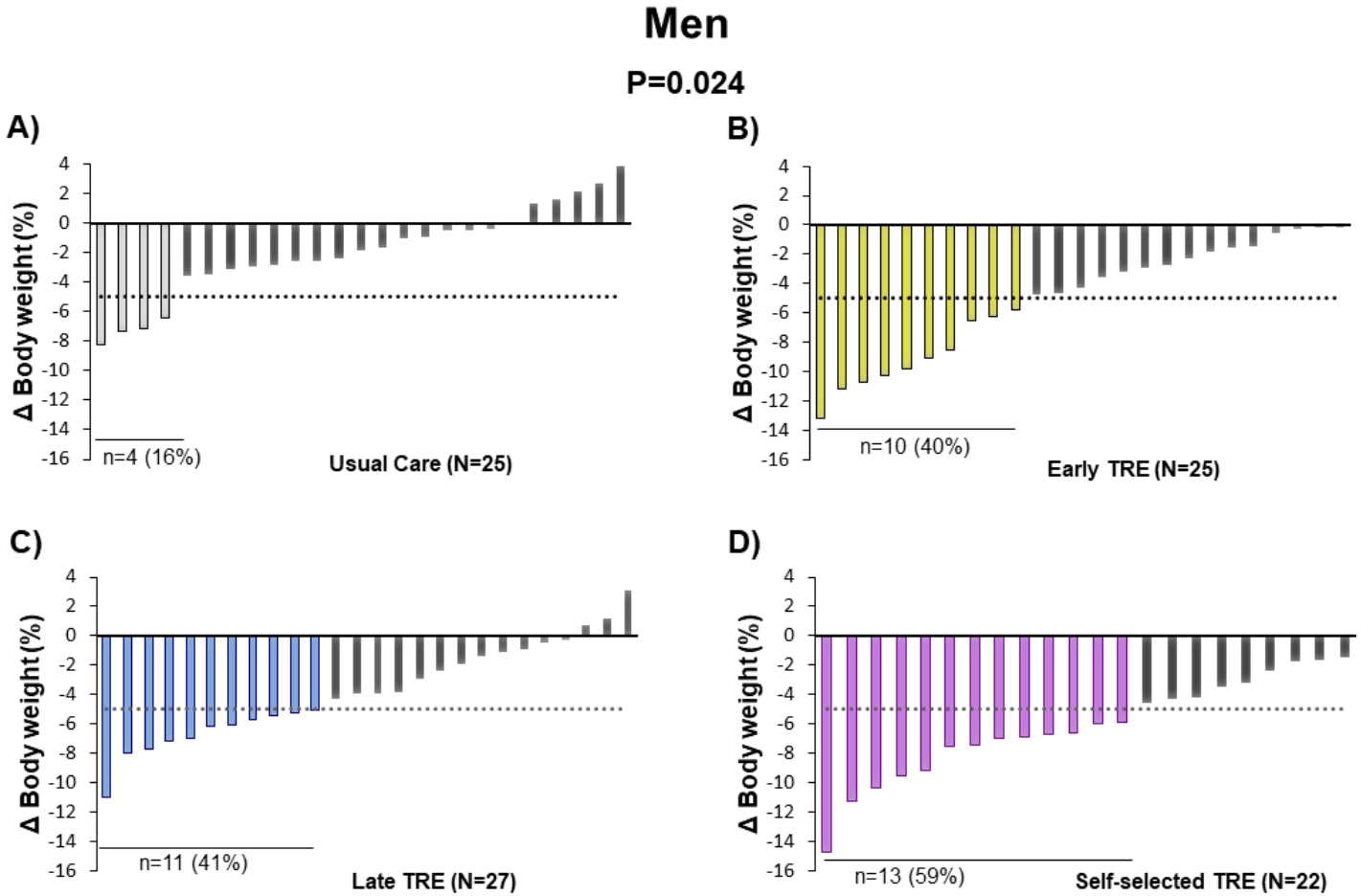

Figure 3. Changes in body weight percentage in men among the usual–care (UC; A), early time–restricted eating (TRE; B), late TRE (C), and self–selected TRE (D) groups after the 12–week intervention. The columns represent the raw data of individual study participants. The dotted line represents a clinically meaningful weight loss (≥5%). P-value from Pearson's chi-square.

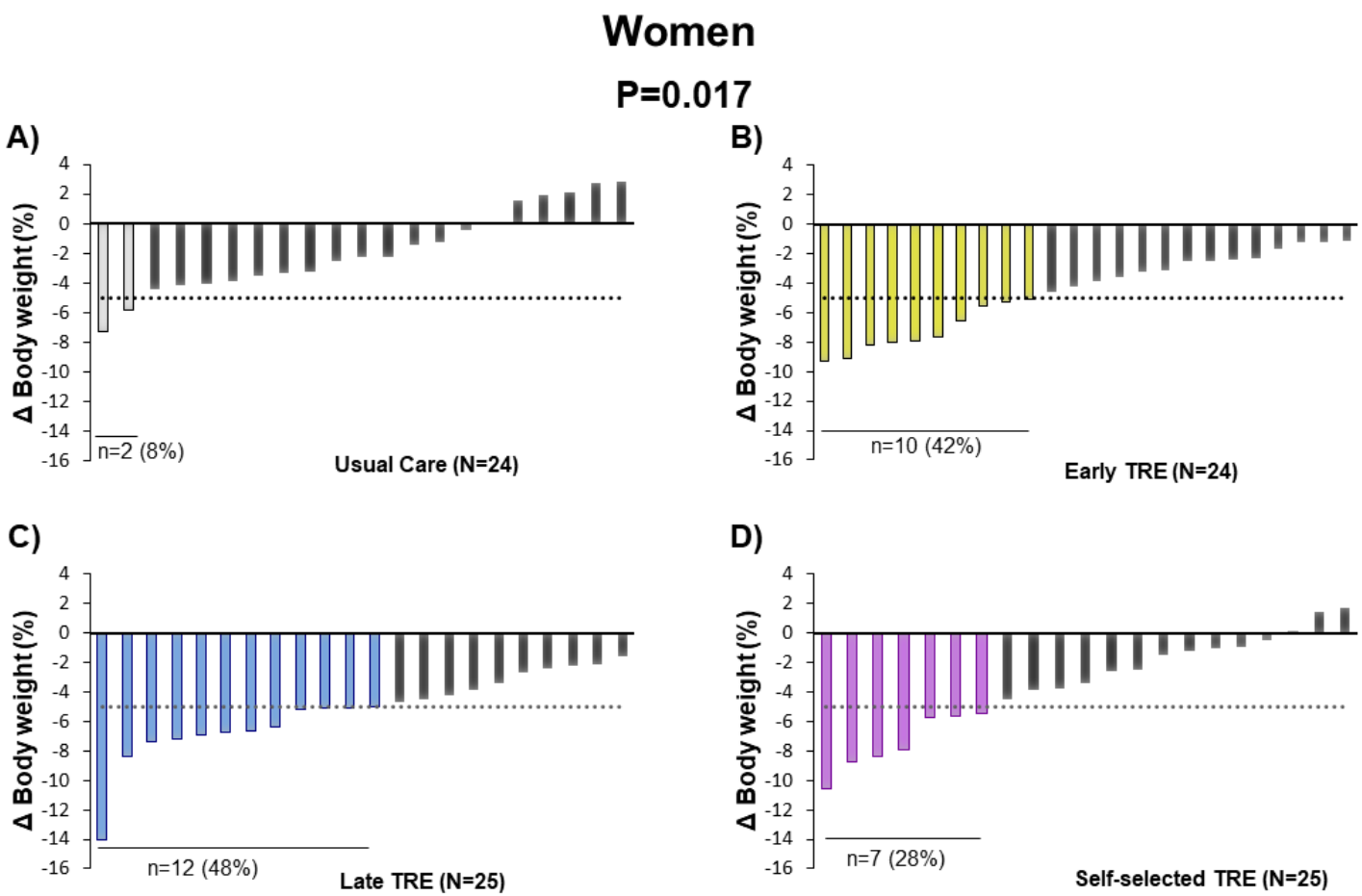

Figure 4. Changes in body weight percentage in women among the usual–care (UC; A), early time–restricted eating (TRE; B), late TRE (C), and self–selected TRE (D) groups after the 12–week intervention. The columns represent the raw data of individual study participants. The dotted line represents a clinically meaningful weight loss (≥5%). P-value from Pearson's chi-square.

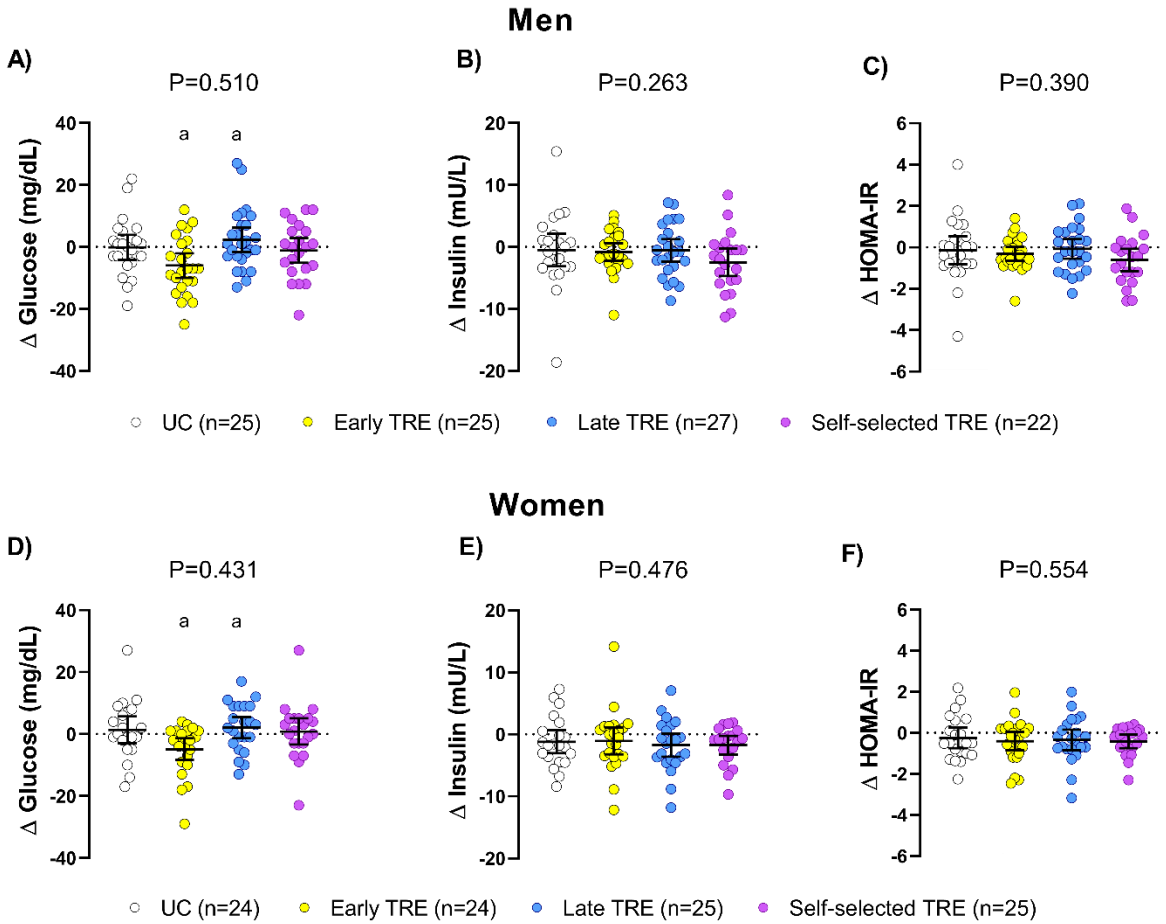

Figure 5. Changes in fasting glucose (A and D), insulin (B and E), and homeostasis model assessment of insulin resistance (HOMA-IR; C and F) in men and women among the usual–care (UC), early time–restricted eating (TRE), late TRE, and self–selected TRE groups after the 12–week intervention. Dara are raw means with 95% confident interval. P–value from group × time interaction term from a linear mixed-effects model that included study group, time (baseline and 12 weeks), and study group × time interaction term as fixed effects and participant as random effect. Similar letters represent significant differences between groups as determined by post hoc Tukey analysis (P<0.05).

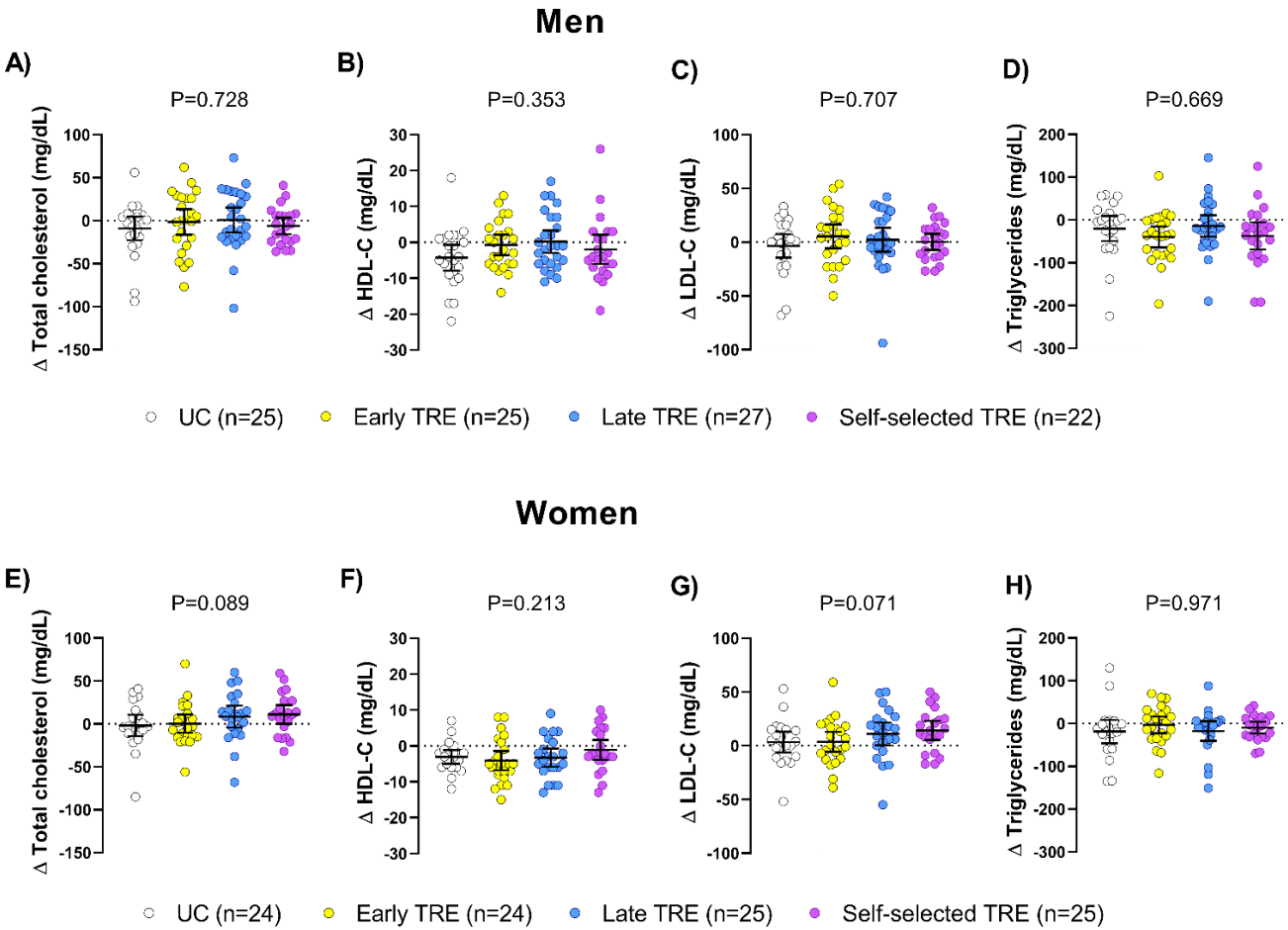

Figure 6. Changes in fasting total cholesterol (A and E), high–density lipoprotein cholesterol (HDL-C; B and F), low–density lipoprotein cholesterol (LDL-C; C and G), and triglycerides (D and H) in men and women among the usual–care (UC), early time–restricted eating (TRE), late TRE, and self–selected TRE groups after the 12–week intervention. Dara are raw means with 95% confident interval. P–value from group × time interaction term from a linear mixed-effects model that included study group, time (baseline and 12 weeks), and study group × time interaction term as fixed effects and participant as random effect.

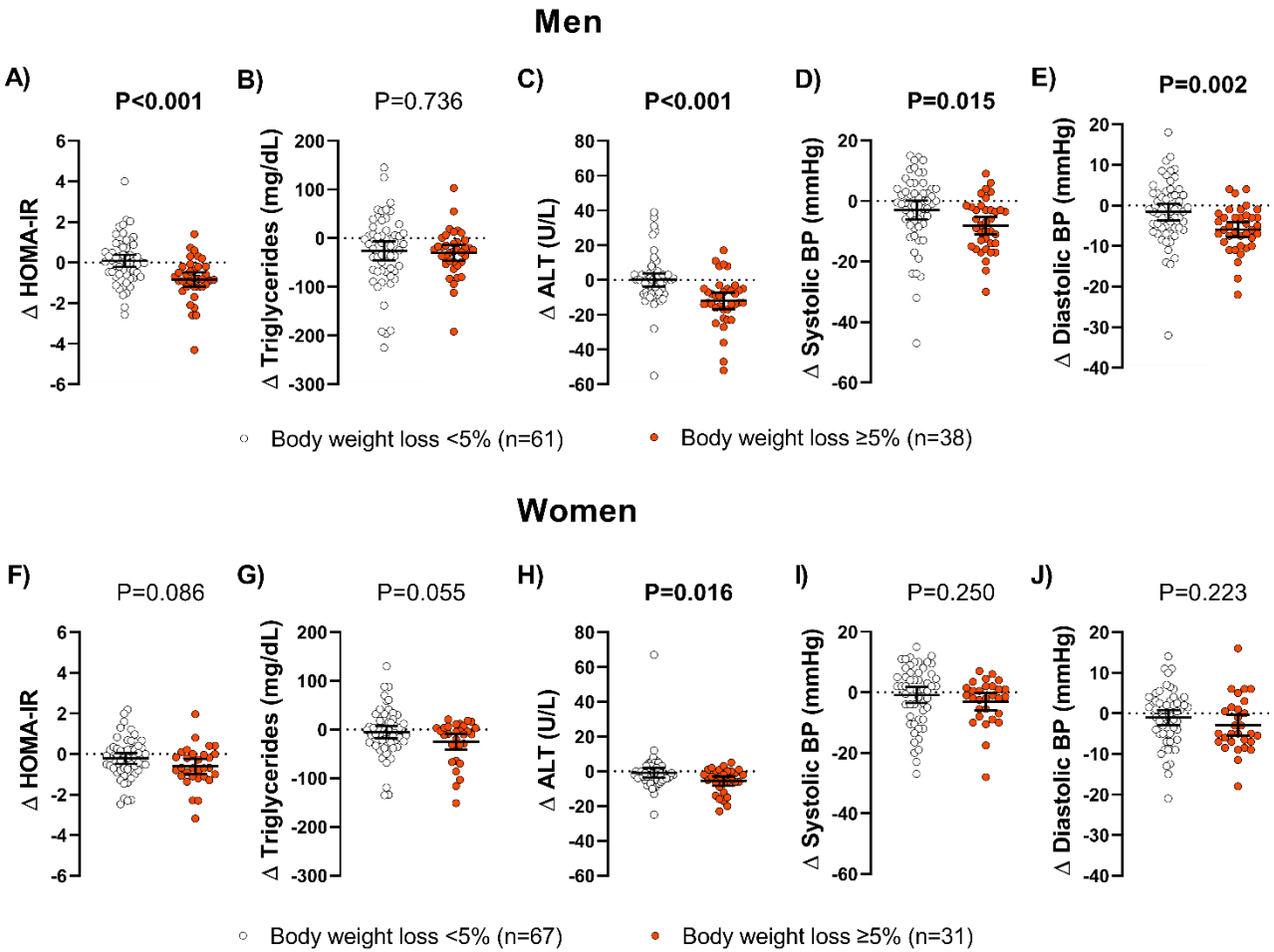

Figure 7. Changes in homeostasis model assessment of insulin resistance (HOMA-IR; A and F), triglycerides (B and G), alanine aminotransferase (ALT; C and H), systolic blood pressure (BP; D and I), and diastolic blood pressure (E and J) in men and women achieving clinically significant weight loss compared to those who did not. Dara are raw means with 95% confident interval. P–value from t-test.

Discussion

This multicenter randomized controlled trial demonstrated that self-selected TRE was more effective than a UC intervention in reducing VAT mass in men after a 12-week intervention. However, no significant differences in VAT mass reduction were observed among the intervention groups in women. In terms of body weight loss, men in the early and self-selected TRE groups achieved an additional reduction of 3.4 kg and 4.4 kg, respectively, compared to the UC group. Similarly, women in the early and late TRE groups achieved an additional reduction of 2.4 kg and 2.8 kg, respectively, compared to the UC group. Nonetheless, no significant differences were found in the change of fat-free mass, fat mass, or cardiometabolic risk factors among the intervention groups in both men and women. These findings suggest that self-selected TRE could be considered as a targeted strategy for reducing VAT in male individuals. Furthermore, our results indicate that implementing an 8-hour TRE, regardless of the timing of the eating window, within a UC intervention led to significantly greater body weight loss in both sexes without accompanying improvements in body composition or cardiometabolic risk factors.

The effect of TRE on VAT, a significant risk factor for cardiometabolic morbidity mortality[11], remains poorly understood. Only two studies have assessed the impact of TRE on VAT measured by computed tomography. Both studies reported that an 8-hour early TRE combined with CR reduced VAT in adults with obesity. However, the difference was not statistically significant when compared to CR alone after 6 and 12 months of intervention[9,10]. It's worth noting that both studies also found similar changes in body weight and fat mass between the groups. In another investigation by Jamshed et al.,[28] VAT measured by DXA decreased in adults with obesity after 14 weeks of 8-hour early TRE combined with CR, but there were no significant differences compared to CR alone. The early TRE group achieved an additional 2.3 kg of body weight loss; however, fat mass loss was similar between the groups, which may explain the lack of additional VAT reduction. Only two studies have examined the impact of early versus late TRE on VAT assessed by bioelectrical bioimpedance[18,19]. He et al.,[19] reported that participants with overweight/obesity who chose either an 8-hour early or late TRE experienced greater reductions in body weight, fat mass, and VAT compared to a low carbohydrate diet after a 12-week intervention period. Additionally, Zhang et al.,[18] found that both 6-hour early and late TRE led to greater reductions in body weight, fat mass, and VAT in adults with obesity

compared to an *ad libitum* control group after 8 weeks. These studies collectively suggest that CR and fat mass loss are the primary drivers of VAT reduction, while fasting and meal timing may have a less significant role[11]. Our findings align with these studies, as VAT remained similar in the UC group, where body weight and fat mass loss were small, while decreasing in the TRE groups with greater body weight and fat mass loss (i.e., early, and self-selected TRE in men, and late TRE in women). The only significant difference observed in comparison to the UC group was found in the self-selected TRE group among men. We believe this difference is attributed to the group's substantial absolute and relative reductions in body weight and fat mass, rather than sex differences in VAT reduction. Previous research has demonstrated that when changes in VAT are expressed relative to baseline values (e.g., percentage reduction), the reduction in VAT following lifestyle interventions is similar between men and women[11]. Our results suggest that incorporating an 8-hour TRE into a UC intervention without specific CR could be considered as a targeted strategy to create a greater energy deficit and thereby reduce VAT.

TRE has been shown to inadvertently reduce energy intake by 10-30% (approximately 300-500 kcal/day) leading to modest weight loss (<5%) in the short term (i.e., ≤3 months) among individuals with overweight/obesity compared to controls without energy and meal timing restrictions [6-8]. Our findings align with these previous TRE studies, as we observed a mean body weight loss of 3-6% in both men and women in the TRE groups. However, it is worth noting that less than 20% of individuals in the UC groups achieved clinically significant weight loss (≥5%), whereas it ranged from 30-60% in the TRE groups. This suggests that TRE may be an effective nutritional strategy when compared to *ad libitum* eating or when implemented alongside a UC intervention without prescribed energy restriction. Nevertheless, the 12-month studies by Liu et al.,[9] and Wei et al.,[10] found similar changes in body weight, fat mass, and fat-free mass between 8-hour early TRE plus CR and CR alone. Although Jamshed et al.,[28] observed an additional 2.3 kg of body weight loss in the 8-hour early TRE plus CR group compared to the CR alone group after 14 weeks of intervention, there were no differences in fat mass, fat-free mass, and appendicular lean mass changes between the two groups. These studies suggest that when the energy deficit is similar between TRE and CR, they result in comparable body weight and fat mass loss. Notably, our study found that men in both the early and self-selected TRE groups, as well as women in the late TRE group, achieved comparable body weight loss (5.3%, 6.3%, and 5.3% respectively) to the early TRE

group (5.7%) in the study by Jamshed et al.,[28]. Remarkably, these weight reductions were accomplished without prescribed CR and within a shorter intervention duration (12 weeks vs. 14 weeks). These findings highlight the efficacy of TRE in facilitating significant weight loss, even without enforced energy restrictions. Regarding sex differences, we observed that the weight loss efficacy of TRE does not vary according to sex, which is consistent with previous evidence showing that men and women achieve similar percentage body weight loss in response to lifestyle interventions[29,30]. Improvements in adherence to the Mediterranean diet were associated with body weight loss in the UC group among men and in the late TRE group among women, with a tendency observed in the self-selected TRE group among men.

The evidence on the effects of the timing of the eating window during TRE is limited. To the best of our knowledge, only four studies have directly compared early versus late TRE[16-19]. However, these studies have important limitations, including small sample sizes[17], durations shorter than 8 weeks[16-18], and participants being free to choose their preferred timing rather than being randomly assigned to an early or late TRE TRE[19]. Furthermore, a recent meta-analysis has examined the impact of the timing of the eating window during TRE on body weight loss in adults with overweight/obesity[15]. This meta-analysis included studies that compared at least early or late TRE to a non-TRE group. Among these preliminary studies comparing early versus late TRE, none of them have reported significant differences in body weight, fat mass, or fat-free mass loss[15-19]. Our findings are consistent with previous evidence as we did not observe any differences in body weight and composition among the early, late, and self-selected TRE groups. Our study provides additional valuable insights by extending the intervention duration to 12 weeks, considering the potential effects of sex, and including a self-selected TRE group, which may have improved adherence, acceptability, and overall efficacy. Another recent meta-analysis of 9 randomized controlled trials found that consuming a larger proportion of total energy intake early in the day during CR was associated with approximately 1.2 kg additional body weight loss compared to a late eating distribution[31]. This slight difference in weight loss could be attributed to the higher thermic effect of food observed in the morning compared to the evening[32], leading to increased energy expenditure during wake time, and reduced hunger levels[33], despite the same energy intake. However, these findings do not seem to extend to studies on TRE, as both previous research[15-19] and our study have reported that early and late TRE lead to similar changes in

body weight and composition. This suggests that regardless of the timing of the eating window, TRE induces a comparable energy deficit.

The effects of TRE on blood pressure are highly variable with some studies showing reductions in systolic and diastolic blood pressure while others show no effect[6-8,34]. In 12-month studies comparing early TRE plus CR to CR alone, similar reductions in systolic and diastolic blood pressure were observed[9,10]. However, Jamshed et al.,[28] found that early TRE plus CR was more effective than CR alone in reducing diastolic blood pressure. In our study, we observed decreases in both systolic and diastolic blood pressure in all intervention groups in men except for late TRE, but without significant differences between groups. A meta-analysis also reported that early, but not late TRE, decreased diastolic blood pressure compared to non-TRE[15]. During a 5-week crossover study, Sutton et al.,[14] found that eucaloric (i.e., energy intake equals energy needs, and thus in absence of weight loss) early TRE decreased systolic and diastolic blood pressure in men with prediabetes compared to a 12-hour eating window condition. It is possible that late TRE may affect natriuresis, as it could lead to increased salt intake later in the day when sodium excretion is downregulated by the circadian system[7]. Among women, there were no significant changes in systolic or diastolic blood pressure observed within or between the intervention groups. However, in a secondary analysis of women who achieved a clinically meaningful weight loss (≥5%), significant reductions in both systolic and diastolic blood pressure were observed. This suggests that women may require greater weight loss to see improvements in blood pressure, possibly due to the influence of sex hormones on blood pressure regulation[35].

TRE has consistently shown improvements in glucose levels and HOMA-IR when compared to *ad libitum* eating control groups[6,8]. However, when directly compared to CR, TRE appears to be equally effective in improving fasting glucose, insulin, and HOMA-IR[6,9,10,17,28]. The evidence regarding the timing of the eating window during TRE is mixed. While three out of the four published studies did not find significant differences in fasting glucose, insulin, and HOMA-IR between early and late TRE[17-19], Xie et al.,[16] reported a greater reduction in HOMA-IR, but not in fasting glucose, with early TRE compared to late TRE in non-obese adults after 5 weeks of intervention. A meta-analysis also indicated a small yet significant difference in improving HOMA-IR between early and late TRE, but not for fasting glucose[15]. Another meta-analysis reported that consuming a larger proportion of total energy intake early in the day during

CR was associated with a greater decrease in fasting glucose and HOMA-IR compared to a late eating distribution[31]. Additionally, Sutton et al.,[14] demonstrated that eucaloric (i.e., without weight loss) early TRE improved insulin sensitivity and β cell responsiveness in men with prediabetes in comparison to a condition involving a 12-hour eating window. These findings partially align with our study, as we observed a decline in fasting glucose levels only in the early TRE group, with significant differences compared to the late TRE group in both men and women. However, this decline in fasting glucose levels in the early TRE group was not accompanied by a similar reduction in HOMA-IR, possibly due to a potential limitation in statistical power. When analyzing the combined data from both men and women, the early TRE group showed a significant reduction in HOMA-IR after the 12-week intervention, without observing any significant differences compared to the other intervention groups (data not shown). Additionally, in the combined analysis of men and women, the early TRE group demonstrated a significant decrease in fasting glucose levels (5-7 mg/dL) compared to the UC, late TRE, and self-selected TRE groups (data not shown). It is well-established that glucose tolerance is higher in the biological morning, which is likely influenced by diurnal variations in β-cell responsiveness, peripheral insulin sensitivity, insulin clearance, and glucose effectiveness[36]. Only men in the self-selected TRE group exhibited decreases in insulin and HOMA-IR. This group demonstrated the greatest reductions in body weight, fat mass, and VAT mass. Furthermore, a secondary analysis of participants who achieved clinically meaningful weight loss (≥5%) revealed significant reductions in insulin and HOMA-IR, highlighting the role of energy deficit in improving insulin sensitivity. However, it remains challenging to determine whether early and late TRE have distinct clinical effects on glucose metabolism, independent of changes in body weight and fat mass. Future studies that utilize more precise methods to assess insulin sensitivity, such as the hyperinsulinemic-euglycemic clamp technique, and involve individuals with prediabetes or diabetes are still necessary.

TRE, regardless of the timing of the eating window, does not appear to significantly affect blood lipid metabolism compared to *ad libitum* eating control groups[6,8,15]. Our findings are consistent with previous research, as we did not observe any differences in lipid metabolism between the intervention groups in both men and women. However, it is noteworthy that in the late TRE group among women, we observed a decrease in HDL-C and an increase in LDL-C and apolipoprotein B, despite weight loss. Further investigations are required to

determine if late TRE adversely affects lipid profiles in women. The effects of TRE on liver enzymes remain uncertain, and our study did not reveal a consistent pattern. In men, we observed a decrease in alanine aminotransferase levels in the late and self-selected TRE groups, while alkaline phosphatase levels increased in the early TRE group. Previous studies have suggested that combining TRE with CR, which leads to greater weight loss, may be necessary to enhance lipid metabolism[9,10] and liver function markers[10]. Indeed, in a secondary analysis, we observed significant improvements in lipid metabolism and liver markers among both men and women who achieved a clinically meaningful weight loss (≥5%).

Limitations

The study has several limitations that should be acknowledged. Firstly, while VAT and other ectopic fat depots were measured using magnetic resonance imaging, they were not included in the analysis conducted for this study. The reason for their exclusion is that the data pertaining to VAT and ectopic fat depots were not ready in time for the thesis defense. Therefore, the focus of the analysis and findings in this study primarily revolve around VAT assessed by DXA, which was readily available and suitable for analysis at the time. Secondly, the duration of the study was relatively modest, which may limit the ability to observe long-term effects of the interventions. Additionally, important data on glycemic control, adherence rates, physical activity, and dietary patterns were not included in the analysis. These factors could have provided further insights into the mechanisms underlying the observed outcomes. Lastly, a 12-month follow-up was not included in this study, which would have allowed for the assessment of the sustainability of the observed effects over a longer period.

Conclusions

In this 12-week multicenter randomized controlled trial involving men and women with overweight/obesity and slight metabolic impairments, self-selected TRE was more effective to reduce VAT mass than a UC intervention in men. Additionally, our results demonstrate that an 8-hour TRE, irrespective of the timing of the eating window, led to significantly greater body weight loss compared to the UC intervention in both men and women. Lastly, all intervention groups exhibited similar effects on body composition and cardiometabolic risk factors.

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Supplementary material

Table S1. Body composition, blood pressure, glucose metabolism, lipid metabolism, and liver markers end points at baseline and after the 12–week intervention in men.

End point	UC (n=25)	Early TRE (n=25)	Late TRE (n=27)	Self–selected TRE (n=22)
	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)
Body composition				
Weight (kg)				
Preintervention	101.8 (96.2, 107.3)	106.3 (100.7, 111.9)	101.6 (96.3, 107.0)	99.8 (93.9, 105.8)
Postintervention	99.9 (94.3, 105.4)	101.0 (95.4, 106.6)	97.8 (92.4, 103.2)	93.6 (87.6, 99.5)
Change	-1.9 (-3.3, -0.4)*	-5.3 (-6.8, -3.9)*	-3.9 (-5.3, -2.4)*	-6.3 (-7.8, -4.7)*
Weight (%)				
Preintervention				
Postintervention	98.1 (97.1, 99.0)	95.0 (94.0, 95.9)	96.3 (95.3, 97.2)	93.8 (92.8, 94.8)
Change	-1.9 (-3.3, -0.6)*	-5.0 (-6.4, -3.7)*	-3.7 (-5.1, -2.4)*	-6.2 (-7.7, -4.7)*
Fat–free mass (kg)				
Preintervention	63.2 (60.3, 66.1)	65.8 (62.9, 68.8)	63.2 (60.4, 66)	63.3 (60.1, 66.4)
Postintervention	63.0 (60.1, 65.9)	64.3 (61.4, 67.2)	62.6 (59.8, 65.4)	61.5 (58.4, 64.7)
Change	-0.2 (-1.4, -1.1)	-1.5 (-2.8, -0.3)*	-0.6 (-1.8, 0.6)	-1.7 (-3.0, -0.4)*
Appendi lean mass (kg)				
Preintervention	25.7 (24.3, 27.1)	26.9 (25.5, 28.3)	25.4 (24.1, 26.8)	26.0 (24.5, 27.5)
Postintervention	25.2 (23.8, 26.6)	25.4 (23.9, 26.8)	24.6 (23.2, 26.0)	24.4 (22.9, 25.9)
Change	-0.5 (-1.3, 0.4)	-1.5 (-2.4, -0.7)*	-0.8 (-1.6, 0.0)*	-1.6 (-2.4, -0.7)*
Fat mass (kg)				
Preintervention	37.4 (34.2, 40.7)	39.5 (36.2, 42.7)	37.6 (34.5, 40.7)	35.3 (31.8, 38.7)
Postintervention	35.7 (32.5, 39.0)	35.8 (32.5, 39.0)	34.3 (31.2, 37.4)	31.4 (27.9, 34.8)
Change	-1.7 (-3.0, -0.4)*	-3.7 (-5.0, -2.4)*	-3.3 (-4.5, -2.0)*	-3.9 (-5.3, -2.5)*
Fat mass (%)				
Preintervention	37.0 (35.2, 38.8)	37.3 (35.5, 39.1)	36.8 (35.1, 38.5)	35.6 (33.7, 37.4)
Postintervention	35.8 (34.0, 37.6)	35.5 (33.7, 37.3)	35.0 (33.3, 36.7)	33.4 (31.6, 35.3)
Change	-1.2 (-2.3, -0.1)*	-1.8 (-2.8, -0.7)*	-1.9 (-2.9, -0.8)*	-2.1 (-3.3, -1.0)*
VAT mass (g)				
Preintervention	1039.6 (944.5, 1134.7)	1042.4 (947.4, 1137.5)	1041.6 (950.1, 1133.1)	986 (884.6, 1087.3)
Postintervention	1001.4 (905.6, 1097.2)	943.0 (847.9, 1038.1)	915.9 (823.7, 1008)	818.0 (716.7, 919.4)
Change	-38.2 (-100.8, 24.3)	-99.5 (-160.9, -38.1)*	-125.7 (-185.8, -65.6)*	-167.9 (-233.4, -102.5)*
Blood pressure				
Systolic BP (mmHg)				
Preintervention	130.3 (125.4, 135.3)	131.6 (126.7, 136.5)	124.7 (119.9, 129.4)	125.7 (120.5, 130.9)
Postintervention	121.8 (116.8, 126.9)	125.1 (120.1, 130.1)	123.4 (118.6, 128.2)	121.1 (115.9, 126.3)
Change	-8.5 (-13.0, -4.1)*	-6.5 (-10.9, -2.2)*	-1.3 (-5.4, 2.9)	-4.6 (-9.2, -0.1)*
Diastolic BP (mmHg)				
Preintervention	84.8 (81, 88.5)	84.3 (80.6, 88)	81.4 (77.8, 85)	81.9 (77.9, 85.8)
Postintervention	81.6 (77.8, 85.4)	79.9 (76.2, 83.7)	79.6 (76, 83.2)	78 (74.1, 82.0)
Change	-3.2 (-6.2, -0.1)*	-4.4 (-7.3, -1.4)*	-1.8 (-4.7, 1.0)	-3.8 (-6.9, -0.7)*
Glucose metabolism				
Fasting glucose (mg/dL)				
Preintervention	94.4 (89.7, 99.1)	96.5 (91.8, 101.2)	99.4 (94.9, 103.9)	95.9 (90.9, 100.9)
Postintervention	94.3 (89.5, 99.1)	90.6 (85.8, 95.3)	101.9 (97.3, 106.5)	94.7 (89.7, 99.7)
Change	-0.1 (-4.0, 3.7)	-5.9 (-9.7, -2.1)*	2.5 (-1.1, 6.1)	-1.1 (-5.1, 2.8)
Insulin (mU/L)				
Preintervention	12.8 (10.8, 14.8)	11.2 (9.2, 13.2)	11.6 (9.6, 13.5)	11.5 (9.3, 13.7)

Postintervention	12.3 (10.2, 14.3)	10.5 (8.5, 12.5)	11.3 (9.3, 13.2)	9.1 (6.9, 11.2)
Change	-0.5 (-2.5, 1.4)	-0.7 (-2.6, 1.2)	-0.3 (-2.2, 1.6)	-2.4 (-4.5, -0.4)*
HOMA–IR				
Preintervention	3.0 (2.5, 3.5)	2.7 (2.1, 3.2)	2.9 (2.4, 3.4)	2.8 (2.2, 3.4)
Postintervention	2.9 (2.3, 3.4)	2.4 (1.8, 2.9)	2.9 (2.4, 3.4)	2.2 (1.6, 2.8)
Change	-0.1 (-0.6, 0.4)	-0.3 (-0.8, 0.2)	0.0 (-0.5, 0.5)	-0.6 (-1.1, -0.1)*
HbA1c (%)				
Preintervention	5.4 (5.2, 5.6)	5.4 (5.2, 5.6)	5.4 (5.2, 5.6)	5.3 (5.1, 5.5)
Postintervention	5.5 (5.3, 5.6)	5.4 (5.2, 5.6)	5.4 (5.2, 5.6)	5.2 (5, 5.4)
Change	0.0 (-0.1, 0.2)	0.0 (-0.1, 0.1)	0.0 (-0.1, 0.1)	-0.1 (-0.2, 0.0)
Lipids metabolism				
Total cholesterol (mg/dL)				
Preintervention	222.7 (208.6, 236.8)	206.5 (192.4, 220.6)	218.1 (204.5, 231.6)	206.2 (191.1, 221.3)
Postintervention	214.3 (199.9, 228.8)	204.4 (190.2, 218.7)	218.6 (204.9, 232.3)	200.0 (184.9, 215.2)
Change	-8.4 (-21.5, 4.7)	-2.1 (-14.9, 10.8)	0.5 (-11.8, 12.9)	-6.1 (-19.6, 7.3)
HDL–C (mg/dL)				
Preintervention	54.5 (50.3, 58.7)	48.7 (44.5, 52.9)	50.8 (46.7, 54.8)	50.0 (45.5, 54.4)
Postintervention	50.6 (46.3, 54.9)	47.9 (43.7, 52.2)	50.9 (46.8, 55)	48.0 (43.5, 52.5)
Change	-3.9 (-7.2, -0.6)	-0.7 (-4.0, 2.5)	0.1 (-3.0, 3.2)	-2.0 (-5.4, 1.5)
LDL–C (mg/dL)				
Preintervention	143.6 (132.0, 155.3)	132.2 (120.6, 143.9)	140.8 (129.6, 152.0)	129.4 (117.0, 141.8)
Postintervention	140.2 (128.3, 152.1)	137.3 (125.5, 149.1)	143.0 (131.7, 154.4)	129.7 (117.3, 142.1)
Change	-3.5 (-13.5, 6.6)	5.0 (-4.8, 14.9)	2.3 (-7.2, 11.8)	0.3 (-10.1, 10.6)
Triglycerides (mg/dL)				
Preintervention	138.7 (108.8, 168.5)	150.9 (121.1, 180.8)	143.8 (115.1, 172.5)	150.4 (118.6, 182.2)
Postintervention	119.7 (88.6, 150.7)	112.0 (81.8, 142.2)	130.3 (101.3, 159.4)	113.1 (81.3, 144.9)
Change	-19.0 (-45.8, 7.8)	-38.9 (-64.8, -13.1)*	-13.5 (-38.3, 11.4)	-37.3 (-64.4, -10.2)*
APOA1 (mg/dL)				
Preintervention	161.9 (150.5, 173.2)	143.8 (132.5, 155.1)	149.7 (138.8, 160.7)	156.7 (144.6, 168.8)
Postintervention	161.7 (149.9, 173.4)	150.3 (138.7, 161.8)	152.8 (141.7, 163.9)	152.4 (140.3, 164.5)
Change	-0.2 (-12.5, 12.0)	6.5 (-5.6, 18.5)	3.0 (-8.6, 14.7)	-4.3 (-17.0, 8.4)
APOB (mg/dL)				
Preintervention	111.8 (102.7, 120.9)	103.5 (94.3, 112.6)	111.5 (102.7, 120.3)	100.8 (91.0, 110.5)
Postintervention	107.3 (97.9, 116.6)	103.9 (94.7, 113.2)	112.4 (103.6, 121.3)	98.5 (88.8, 108.3)
Change	-4.5 (-12.2, 3.1)	0.5 (-7.0, 8.0)	0.9 (-6.3, 8.2)	-2.2 (-10.1, 5.6)
Liver markers				
ALT (U/L)				
Preintervention	35.2 (28.0, 42.5)	37.5 (30.2, 44.7)	35.1 (28.1, 42.1)	34.5 (26.8, 42.2)
Postintervention	35.9 (28.5, 43.4)	31.7 (24.3, 39.0)	29.0 (22.0, 36.1)	27.1 (19.4, 34.8)
Change	0.7 (-5.6, 7.0)	-5.8 (-12.0, 0.4)	-6.1 (-12.0, -0.1)*	-7.4 (-13.9, -0.9)*
GGT (U/L)				
Preintervention	70.6 (45.4, 95.9)	34.8 (9.5, 60)	48.9 (24.6, 73.2)	37.0 (10.1, 63.9)
Postintervention	64.6 (39.1, 90.0)	30.4 (5.0, 55.7)	44.7 (20.3, 69.1)	30.8 (3.9, 57.7)
Change	-6.1 (-17.8, 5.7)	-4.4 (-15.9, 7.2)	-4.2 (-15.3, 6.9)	-6.2 (-18.3, 5.8)
ALP (U/L)				
Preintervention	66.1 (59.6, 72.6)	61.5 (55.0, 68.0)	68.2 (62.0, 74.5)	62.5 (55.6, 69.5)
Postintervention	67.6 (61, 74.2)	67.3 (60.7, 73.8)	69.4 (63.1, 75.7)	62.4 (55.5, 69.4)
Change	1.5 (-2.6, 5.6)	5.8 (1.8, 9.7)*	1.2 (-2.6, 5)	-0.1 (-4.3, 4.0)

ALP, alkaline phosphatase; ALT, alanine aminotransferase; APOA1, apolipoprotein A; APOB, apolipoprotein B; Appendi, appendicular; BP, blood pressure; CI, confident interval; GGT, gamma-glutamyltransferase; HbA1c, glycated haemoglobin; HDL-

C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; MG, mean glucose; VAT, visceral adipose tissue. *Represent significant differences within groups as determined by pairwise comparison with Tukey adjustment (P<0.05).

Table S2. Body composition, blood pressure, glucose metabolism, lipid metabolism, and liver markers end points at baseline and after the 12–week intervention in women.

End point	UC (n=24)	Early TRE (n=24)	Late TRE (n=25)	Self–selected TRE (n=25)
	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)
Body composition				
Weight (kg)				
Preintervention	90.3 (85.6, 94.9)	88.9 (84.2, 93.5)	85.2 (80.6, 89.7)	88.2 (83.6, 92.8)
Postintervention	88.6 (83.9, 93.3)	84.8 (80.2, 89.5)	80.6 (76, 85.2)	84.8 (80.3, 89.4)
Change	-1.7 (-2.8, -0.5)*	-4.1 (-5.1, -3.0)*	-4.5 (-5.7, -3.4)*	-3.4 (-4.5, -2.2)*
Weight (%)				
Preintervention				
Postintervention	98.2 (97.3, 99.0)	95.4 (94.6, 96.2)	94.7 (93.9, 95.6)	96.4 (95.6, 97.3)
Change	-1.8 (-3, -0.7)*	-4.6 (-5.8, -3.5)*	-5.3 (-6.4, -4.1)*	-3.6 (-4.8, -2.4)*
Fat–free mass (kg)				
Preintervention	47.5 (45.2, 49.8)	46.4 (44.1, 48.7)	44.1 (41.8, 46.3)	45.9 (43.6, 48.1)
Postintervention	47.7 (45.4, 50.1)	45.6 (43.3, 48)	43.4 (41.2, 45.7)	45.1 (42.8, 47.4)
Change	0.2 (-0.7, 1.1)	-0.8 (-1.6, 0.0)	-0.6 (-1.5, 0.2)	-0.8 (-1.7, 0.1)
Appendi lean mass (kg)				
Preintervention	18.1 (17.0, 19.2)	17.7 (16.7, 18.8)	17.1 (16.0, 18.1)	17.4 (16.3, 18.4)
Postintervention	17.7 (16.6, 18.8)	16.7 (15.6, 17.7)	15.7 (14.6, 16.8)	16.8 (15.7, 17.9)
Change	-0.4 (-1.1, 0.4)	-1.1 (-1.8, -0.4)*	-1.4 (-2.1, -0.7)*	-0.6 (-1.3, 0.1)
Fat mass (kg)				
Preintervention	42 (38.9, 45.1)	41.4 (38.4, 44.5)	40.5 (37.5, 43.5)	41.7 (38.7, 44.7)
Postintervention	39.8 (36.7, 42.9)	38.5 (35.4, 41.6)	36.7 (33.7, 39.8)	38.8 (35.8, 41.9)
Change	-2.2 (-3.3, -1.1)*	-2.9 (-3.9, -1.9)*	-3.8 (-4.8, -2.7)*	-2.8 (-3.9, -1.8)*
Fat mass (%)				
Preintervention	46.8 (45.0, 48.6)	46.9 (45.1, 48.7)	47.7 (45.9, 49.4)	47.3 (45.6, 49.1)
Postintervention	45.1 (43.3, 47.0)	45.6 (43.8, 47.4)	45.6 (43.8, 47.4)	46.0 (44.2, 47.8)
Change	-1.6 (-2.6, -0.7)*	-1.3 (-2.2, -0.5)*	-2.1 (-3.0, -1.2)*	-1.4 (-2.3, -0.4)*
VAT mass (g)				
Preintervention	842 (744.3, 939.7)	866.2 (768.5, 963.9)	911.2 (815.5, 1007)	873 (777.3, 968.8)
Postintervention	822.3 (721.8, 922.8)	820.9 (723.2, 918.6)	826.5 (729.3, 923.6)	827.6 (729.9, 925.4)
Change	-19.7 (-73.9, 34.6)	-45.3 (-93.9, 3.2)	-84.8 (-135.3, -34.2)*	-45.4 (-97.0, 6.3)
Blood pressure				
Systolic BP (mmHg)				
Preintervention	120.3 (114.5, 126.1)	119.9 (114.1, 125.7)	117.8 (112, 123.5)	120 (114.3, 125.8)
Postintervention	117.2 (111.3, 123.2)	116.9 (111, 122.8)	115.9 (110.1, 121.7)	120.8 (114.9, 126.7)
Change	-3.1 (-7.0, 0.9)	-3.0 (-6.9, 0.8)	-1.9 (-5.8, 2.0)	0.8 (-3.2, 4.8)
Diastolic BP (mmHg)				
Preintervention	77.3 (72.9, 81.7)	79.5 (75, 83.9)	77.1 (72.7, 81.4)	77.7 (73.4, 82.1)
Postintervention	75.3 (70.7, 79.8)	76.8 (72.3, 81.3)	75.4 (70.9, 79.8)	77.5 (73, 81.9)
Change	-2.0 (-5.0, 1.0)	-2.7 (-5.6, 0.2)	-1.7 (-4.6, 1.3)	-0.3 (-3.3, 2.7)
Glucose metabolism				
Fasting glucose (mg/dL)				
Preintervention	94.3 (90.7, 97.9)	91.5 (87.9, 95.1)	93.4 (89.9, 97)	91.0 (87.5, 94.5)

Postintervention	95.1 (91.3, 98.9)	86.6 (82.9, 90.2)	95.9 (92.2, 99.6)	91.4 (87.7, 95.2)
Change	0.8 (-3, 4.6)	-4.9 (-8.6, -1.3)*	2.5 (-1.2, 6.1)	0.4 (-3.3, 4.2)
Insulin (mU/L)				
Preintervention	12.4 (10.4, 14.4)	10.7 (8.7, 12.7)	12 (10, 13.9)	10.5 (8.6, 12.5)
Postintervention	11.4 (9.3, 13.4)	9.6 (7.6, 11.7)	10.1 (8.1, 12.1)	8.8 (6.7, 10.9)
Change	-1 (-2.8, 0.8)	-1.1 (-2.8, 0.6)	-1.9 (-3.6, -0.1)*	-1.7 (-3.6, 0.1)
HOMA–IR				
Preintervention	2.9 (2.4, 3.4)	2.4 (2, 2.9)	2.8 (2.3, 3.2)	2.4 (1.9, 2.9)
Postintervention	2.7 (2.2, 3.2)	2.0 (1.5, 2.5)	2.4 (1.9, 2.9)	2.0 (1.5, 2.5)
Change	-0.2 (-0.6, 0.2)	-0.4 (-0.8, 0.0)	-0.4 (-0.8, 0.1)	-0.4 (-0.9, 0.0)
HbA1c (%)				
Preintervention	5.4 (5.2, 5.5)	5.4 (5.2, 5.5)	5.4 (5.2, 5.5)	5.3 (5.2, 5.4)
Postintervention	5.4 (5.3, 5.6)	5.4 (5.3, 5.6)	5.4 (5.2, 5.5)	5.3 (5.2, 5.4)
Change	0.1 (0.0, 0.2)	0.1 (0.0, 0.2)	0.0 (-0.1, 0.1)	0.0 (-0.1, 0.1)
Lipids metabolism				
Total cholesterol (mg/dL)				
Preintervention	197.3 (184.2, 210.4)	210.0 (196.9, 223.0)	212.9 (200.1, 225.7)	211.8 (198.9, 224.6)
Postintervention	196.2 (182.6, 209.8)	209.8 (196.5, 223.0)	222.0 (208.7, 235.3)	221.7 (208.3, 235.2)
Change	-1.1 (-12.4, 10.2)	-0.2 (-11, 10.6)	9.1 (-1.9, 20.1)	10.0 (-1.2, 21.2)
HDL–C (mg/dL)				
Preintervention	57.3 (52.8, 61.7)	55.5 (51.0, 60.0)	58.5 (54.1, 62.9)	55.0 (50.6, 59.4)
Postintervention	54.2 (49.6, 58.8)	51.5 (47.0, 56.0)	55.1 (50.6, 59.6)	54.0 (49.5, 58.5)
Change	-3.1 (-5.5, -0.6)*	-4.0 (-6.4, -1.7)*	-3.4 (-5.8, -1.0)*	-1.0 (-3.4, 1.4)
LDL–C (mg/dL)				
Preintervention	116.6 (105.0, 128.2)	131.7 (120.1, 143.3)	133.9 (122.5, 145.2)	135.0 (123.7, 146.4)
Postintervention	120.4 (108.4, 132.4)	134.8 (123.1, 146.6)	145.6 (133.9, 157.3)	148.4 (136.5, 160.2)
Change	3.8 (-5.5, 13.1)	3.1 (-5.8, 12.1)	11.7 (2.6, 20.8)*	13.3 (4.0, 22.6)*
Triglycerides (mg/dL)				
Preintervention	126.3 (105.4, 147.2)	124.0 (103.1, 144.8)	121.6 (101.2, 142.1)	108 (87.5, 128.5)
Postintervention	110.7 (88.8, 132.6)	120.3 (99.1, 141.5)	105.6 (84.2, 127)	96.3 (74.5, 118.0)
Change	-15.6 (-36.2, 5.0)	-3.6 (-23.5, 16.2)	-16.0 (-36.1, 4.1)	-11.7 (-32.2, 8.8)
APOA1 (mg/dL)				
Preintervention	167.3 (155.9, 178.6)	160 (148.6, 171.4)	165.9 (154.8, 177.1)	158.4 (147.3, 169.6)
Postintervention	163.0 (151.0, 174.9)	159.9 (148.3, 171.4)	161.2 (149.5, 172.9)	154.9 (143, 166.9)
Change	-4.3 (-16.3, 7.7)	-0.1 (-11.7, 11.4)	-4.7 (-16.4, 7.0)	-3.5 (-15.4, 8.4)
APOB (mg/dL)				
Preintervention	92.2 (82.6, 101.8)	100.4 (90.8, 110.0)	101.6 (92.2, 111.0)	101.6 (92.1, 111.0)
Postintervention	91.3 (81.4, 101.3)	103.3 (93.6, 113.0)	110.4 (100.7, 120.2)	108.7 (98.8, 118.5)
Change	-0.8 (-8.5, 6.9)	2.9 (-4.5, 10.4)	8.8 (1.3, 16.4)*	7.1 (-0.6, 14.8)
Liver markers				
ALT (U/L)				
Preintervention	24.4 (19.6, 29.2)	19.3 (14.5, 24.1)	21.7 (17.0, 26.4)	20.2 (15.5, 24.9)
Postintervention	20.1 (15.1, 25.1)	19.3 (14.5, 24.2)	18.4 (13.5, 23.3)	17.7 (12.8, 22.7)
Change	-4.3 (-8.5, 0.0)*	0.0 (-4.1, 4.1)	-3.3 (-7.5, 0.8)	-2.4 (-6.7, 1.8)
GGT (U/L)				
Preintervention	25.8 (20.8, 30.9)	21.7 (16.6, 26.8)	20.6 (15.6, 25.5)	21.2 (16.2, 26.2)
Postintervention	23.2 (17.9, 28.5)	19.4 (14.3, 24.5)	16.2 (11.0, 21.4)	18.9 (13.6, 24.1)
Change	-2.6 (-7.4, 2.1)	-2.3 (-6.9, 2.3)	-4.4 (-9.1, 0.3)	-2.3 (-7.1, 2.4)
ALP (U/L)				
Preintervention	64.9 (57.9, 71.9)	69.1 (62.1, 76.1)	68.7 (61.8, 75.5)	68.8 (62.0, 75.7)

Postintervention	64.2 (57.1, 71.3)	69.3 (62.2, 76.3)	65 (58.1, 72.0)	69.8 (62.8, 76.8)
Change	-0.7 (-4.7, 3.4)	0.2 (-3.7, 4.0)	-3.7 (-7.6, 0.3)	1.0 (-3.0, 5.0)

ALP, alkaline phosphatase; ALT, alanine aminotransferase; APOA1, apolipoprotein A; APOB, apolipoprotein B; Appendi, appendicular; BP, blood pressure; CI, confident interval; GGT, gamma-glutamyltransferase; HbA1c, glycated haemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; MG, mean glucose; VAT, visceral adipose tissue. *Represent significant differences within groups as determined by pairwise comparison with Tukey adjustment ($P<0.05$).

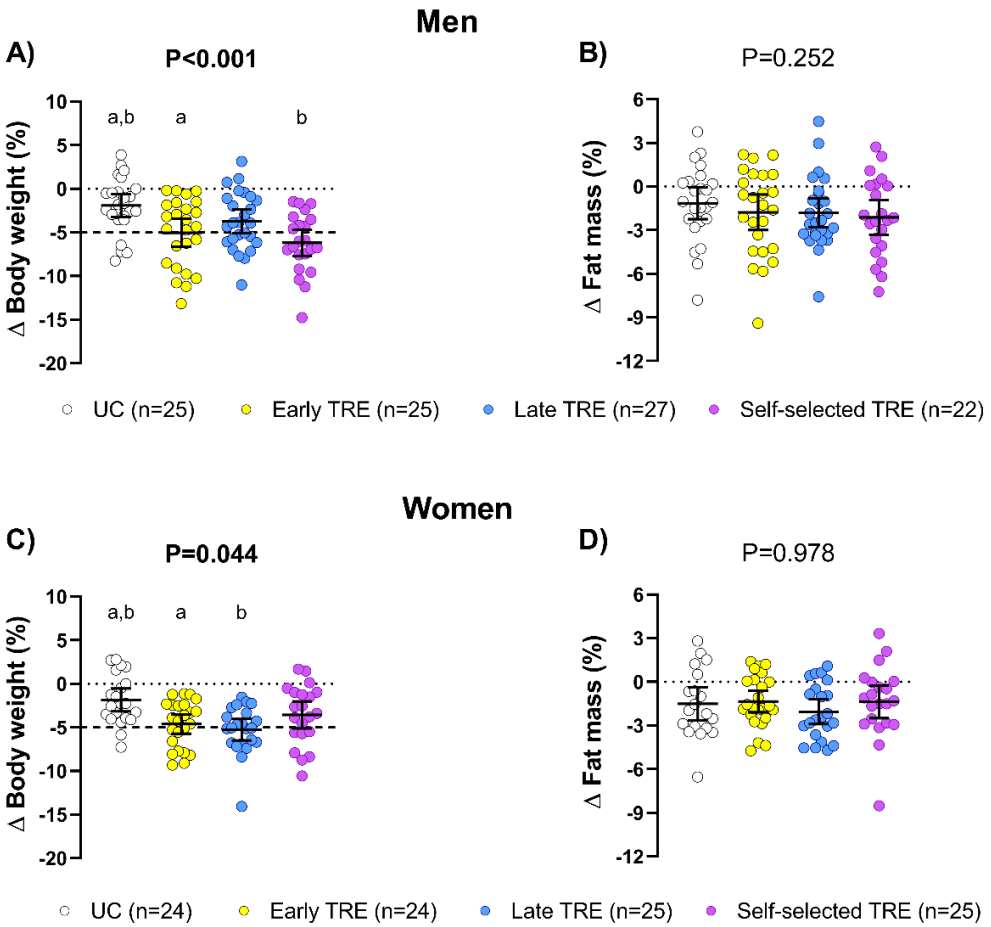

Figure S1. Changes in body weight percentage (A and C) and fat mass percentage (B and D) in men and women among the usual–care (UC), early time–restricted eating (TRE), late TRE, and self–selected TRE groups after the 12–week intervention. Dara are raw means with 95% confident interval. P–value from group × time interaction term from a linear mixed-effects model that included study group, time (baseline and 12 weeks), and study group × time interaction term as fixed effects and participant as random effect. Similar letters represent significant differences between groups as determined by post hoc Tukey analysis (P<0.05)

Table S3. CONSORT 2010 checklist of information to include when reporting a randomised trial

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	127
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	127-128
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	129-130
	2b	Specific objectives or hypotheses	129-130
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	130-131
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	-
Participants	4a	Eligibility criteria for participants	130
	4b	Settings and locations where the data were collected	130-131
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	132
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	131-133
	6b	Any changes to trial outcomes after the trial commenced, with reasons	-
Sample size	7a	How sample size was determined	132-133
	7b	When applicable, explanation of any interim analyses and stopping guidelines	-
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	130-131
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	130-131
Allocation Concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	130-131
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	130-131
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	130-131
	11b	If relevant, description of the similarity of interventions	132
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	132-133
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	132-133
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	133-134
	13b	For each group, losses and exclusions after randomisation, together with reasons	133-134
Recruitment	14a	Dates defining the periods of recruitment and follow-up	133-134

	14b	Why the trial ended or was stopped	130-131
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	135-136
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	130-131
Outcomes and	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	136-151
estimation	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	-
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	141
Harms	19	All-important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	-
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	157
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	157-158
Interpretation	22	Interpretation consistent with results, balancing benefits, and harms, and considering other relevant evidence	152-158
Other information			
Registration	23	Registration number and name of trial registry	130
Protocol	24	Where the full trial protocol can be accessed, if available	130
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	-


Eat
Fast
16 hrs

GENERAL DISCUSSION

Chapter 6. An integrative discussion of the International Doctoral Thesis

General discussion

Obesity is a pressing global health issue[1], it is linked to chronic conditions such as diabetes, cardiovascular disease, and cancer[1,2], placing a substantial burden on healthcare systems[1,3]. New strategies are needed to address the challenge of obesity. In recent years, meal timing and IF have emerged as promising dietary interventions for obesity treatment[4-6]. These interventions may offer potential advantages compared to continuous daily CR, including improved adherence for some individuals and independent effects beyond simply reducing calories[4-9]. The timing of our meals plays a crucial role in relation to our circadian system[10,11]. It affects peripheral clocks found in almost all cells and tissues[10,11]. In mice, fasting can amplify the life-extending effects of CR, particularly when eating is timed appropriately[7,8]. Furthermore, early TRE under eucaloric conditions (i.e., maintaining energy intake equal to energy needs, and hence without weight loss) demonstrated improvements in appetite regulation, blood pressure, oxidative stress, insulin sensitivity, and cellular responsiveness in men with prediabetes compared to a 12-hour eucaloric regimen[9]. Therefore, it is necessary to summarize the effects of different types of IF on body composition and cardiometabolic health in humans, which was the first aim of this International Doctoral Thesis (**Chapter 1**).

Overall, IF regimens have demonstrated improvements in body composition, ectopic fat levels, and classical cardiometabolic risk factors compared to *ad libitum eating* control groups. However, it appears that IF does not provide additional benefits compared to continuous daily caloric CR. This suggests that achieving a net energy deficit is the primary driver of the observed cardiometabolic health benefits. Therefore, it is crucial to match and carefully control energy balance to accurately compare the effects of meal timing, fasting and energy restriction on cardiometabolic health. However, it is worth noting that these comparisons have been limited to only two studies[9,12]. The mechanisms underlying the improvement in cardiometabolic health during fasting are believed to involve metabolic switch from glycogenolysis to gluconeogenesis, increased fat oxidation, and ketogenesis[13].

Whether the decreases in the different components of energy expenditure after

body weight loss are comparable between IF regimens and continuous daily CR is still unknown[13]. Although TRE does not seem to impact 24-hour energy expenditure when compared to larger eating windows (>12 hours), the few studies performed using room indirect calorimetry suggest that IF regimens may affect substrate oxidation, increasing protein and fat oxidation[13]. Long-term and well-powered trials are needed to ascertain whether IF regimens increase fat oxidation and if this ultimately leads to better weight management and cardiometabolic health than continuous daily CR.

The cardiometabolic health benefits of IF regimens can be optimized and their undesirable effects mitigated by combining them with exercise. Adding moderate- to vigorous -intensity exercise for ≥60 minutes performed in the fasted state may advance and strengthen the metabolic switch[13]. Additionally, incorporating structured endurance exercise can help prevent the reduction in low- to moderate-intensity activity energy expenditure observed during energy deficit[13]. Another concern with IF is the potential loss of fat-free mass, which may be more significant than in continuous daily CR due to increased proteolysis for gluconeogenesis during fasting periods. Therefore, resistance exercise can help counteract this effect[13].

The research on IF in humans is still in its early stages, making it challenging to reach conclusive findings. Limited knowledge exists regarding the impact of IF regimens on ectopic fat accumulation and postprandial metabolism, both of which play crucial roles in cardiometabolic health. Additionally, the effects of sex and the timing of the eating window during TRE remain poorly understood. Therefore, we first aimed to examine the relationship between meal timing and body composition, as well as cardiometabolic risk factors, in young men and women (**Chapter 4**).

We derived several meal timing parameters based on existing literature, including the eating window, which represents the duration between the first and last caloric intake, and the caloric midpoint, defined as the time when at least 50% of daily calories are consumed and expressed in local time. Additionally, we calculated eating jetlag to measure the variability of the eating midpoint between non-working days and working days. Furthermore, we determined the time from the midsleep point to the first food intake and the time from the last food intake to the midsleep point, providing insights into the timing of the initial and final food intake relative to the circadian time (midsleep point).

Our hypothesis posited that a shorter daily eating window, an earlier food intake schedule, and reduced variability in meal timing between non-working days and working days would correlate with improved body composition and cardiometabolic risk factors. However, our study did not yield any significant associations between meal timing and anthropometry/body composition parameters in both men and women. Similarly, no associations were found between caloric midpoint, eating jetlag, and the time from last food intake to the midsleep point with cardiometabolic risk factors. Nevertheless, we observed that a longer daily eating window and a shorter time from the midsleep point to the first food intake (i.e., an earlier first food intake within a 24-hour cycle) were linked to a healthier cardiometabolic profile, specifically lower HOMA-IR and cardiometabolic risk score, in young men.

Interpreting the cross-sectional relationship between meal timing and health status can be complex due to various influencing factors. One potential confounding factor is breakfast consumption, as epidemiological data consistently associate it with lower BMI and adiposity[14-16]. This association may be explained by factors such as higher energy intake, lower diet quality, lower physical activity levels, and disrupted circadian rhythms[14-16]. Therefore, breakfast consumption, which is indicative of a longer daily eating window, may be linked to healthier habits and better body composition and cardiometabolic health outcomes. In our cohort of young women, we observed that breakfast skippers had lower adherence to the Mediterranean diet and lower Mediterranean diet quality compared to breakfast consumers. When we performed a secondary analysis focusing only on women who consumed breakfast, we observed that a longer daily eating window was associated with poorer body composition, as indicated by higher BMI and VAT mass. These findings support our initial hypothesis and are consistent with similar studies conducted on middle-aged women[17] and older adults[18]. In our cohort of men, those who skipped breakfast had a more inflammatory diet and tended to be less physically active. These factors may contribute to the observed association between a longer daily eating window and lower HOMA-IR and cardiometabolic risk score in young men. This finding aligns with previous research conducted in middle-aged adults[19].

We observed that a shorter time from the midsleep point to the first food intake, indicating an earlier first food intake within a 24-hour cycle, was associated with lower HOMA-IR and cardiometabolic risk score in young men. These findings

support previous evidence suggesting that eating early in alignment with circadian rhythms may have a positive impact on cardiometabolic health. Circadian misalignment, which refers to a disruption between the central clock regulated by external light and the peripheral clocks influenced by factors such as eating, physical activity, and sleep, could potentially explain these results. Glucose tolerance is higher in the biological morning, which appear to be driven by diurnal variations in β-cell responsiveness, peripheral insulin sensitivity, insulin clearance, and glucose effectiveness[10,11]. Moreover, skeletal muscle fatty acid oxidation and the thermic effect of food are higher in the biological morning or around noon, suggesting that earlier in the daytime is optimal for eating, while nighttime is better suited for fasting and sleep. Lastly, it is worth noting that both our study and previous research[17] did not find a significant relationship between meal timing and cardiometabolic risk factors in women. Factors such as hormonal fluctuations, body composition, and dietary habits may contribute to the varying results observed in men and women.

After assessing the cross-sectional relationship between meal timing, body composition, and cardiometabolic risk factors, we concluded that conducting a randomized controlled trial was necessary to gain a deeper understanding of the effects of IF. To focus our investigation, we specifically chose TRE as the IF regimen for two key reasons: Firstly, TRE places a strong emphasis on the timing of food intake, which has the potential to boost the effects of fasting[7-9]; Secondly, TRE is widely recognized as a feasible and widely accepted approach to IF, making it suitable for a broader population. We identified three key research questions based on our previous review: 1) What is the impact of TRE on ectopic fat accumulation, particularly VAT? 2) Are there any sex differences in the effects of TRE? 3) Does the timing of the eating window during TRE influence its efficacy? Therefore, the last aim of this International Doctoral Thesis was to determine the efficacy of three different 8-hour TRE schedules (i.e., early, late, self-selected vs. UC) on VAT, body composition and cardiometabolic health in men and women with overweight/obesity (**Chapter 5**). Based on previous evidence, we hypothesized that an earlier 8-hour eating window during TRE would result in greater improvements in body composition and cardiometabolic health than UC treatment, late or self-selected 8-hour eating window, following a 12-week intervention.

In our multicenter randomized controlled trial, self-selected TRE was more effective in reducing VAT in men compared to the UC intervention, while no

significant difference was observed in women. Previous research on TRE has emphasized the role of fat mass loss in VAT reduction[20-22], which could explain the results seen in men in the self-selected TRE, as they experienced the greater fat mass loss. Notably, both the early TRE group in men and the late TRE group in women, with the second highest fat mass loss, showed reductions in VAT compared to their baseline values, although these differences were not statistically significant when compared to the UC group. These findings suggest that meal timing and sex may not have a significant impact on the efficacy of TRE in VAT reduction, with fat loss playing a major role. Future studies should aim to match fat mass loss between continuous daily CR and TRE to isolate the specific effects of fasting and explore whether fasting preferentially leads to subcutaneous or ectopic fat loss.

Our findings indicate that regardless of the timing of the eating window, an 8-hour TRE resulted in significantly greater body weight loss compared to a UC intervention without prescribed energy restriction, in both men and women. For instance, men in the early and self-selected TRE groups achieved an additional reduction of 3.4 kg and 4.4 kg, respectively, compared to the UC group. Similarly, women in the early and late TRE groups achieved an additional reduction of 2.4 kg and 2.8 kg, respectively, compared to the UC group. However, we did not observe significant differences in changes in fat-free or fat mass among the intervention groups in either men (mean differences in fat mass: UC vs. Early TRE: -2.0 kg, 95% CI -4.4 to 0.5; UC vs. Late TRE: -1.6 kg, 95% CI -4.0 to 0.9; UC vs. Self-selected TRE: -2.2 kg, 95% CI -4.8 to 0.3) or women (mean differences in fat mass: UC vs. Early TRE: -0.7 kg, 95% CI -2.7 to 1.2; UC vs. Late TRE: -1.6 kg, 95% CI -3.6 to 0.4; UC vs. Self-selected TRE: -0.7 kg, 95% CI -2.7 to 1.3). This may be due to the need for greater statistical power to detect differences in fat-free or fat mass compared to body weight. To further explore the effects of TRE, future studies should aim to match the energy deficit between continuous daily CR and TRE. This would allow for a more comprehensive examination of whether fasting preferentially leads to fat or fat-free mass loss, potentially driven by increased proteolysis for gluconeogenesis during fasting periods.

We did not observe any significant differences in cardiometabolic risk factors between the intervention groups. However, we found that an 8-hour TRE regimen, regardless of meal timing, resulted in clinically meaningful weight loss (≥5%) in a higher proportion of participants (30-60%) compared to the UC groups (less than 20%). Among those participants who achieved a clinically meaningful

weight loss, both men and women showed improvements in glucose and lipid metabolism, liver markers, and blood pressure compared to their baseline values. These improvements were particularly significant in men when compared to participants who did not achieve a weight loss greater than 5%. Based on our findings, an 8-hour TRE can be considered as a simple strategy for clinicians to teach their patients during routine care. It is also intuitive for patients to implement and maintain in their daily lives, leading to weight loss exceeding 5% in some individuals, which is known to have positive effects on cardiometabolic health[23]. However, it is crucial to address the barriers that prevent certain participants from achieving a weight loss greater than 5% with TRE. These obstacles may include feelings of hunger and sluggishness, social situations that discourage adherence to TRE, busy or irregular schedules, and inadequate diet quality during the eating window[24]. Future studies should aim to investigate whether tailoring TRE to individuals' diverse behavioral patterns and preferences would result in greater improvements. Furthermore, it is important to examine whether alternative forms of IF or continuous daily CR are more effective for individuals who face challenges with TRE.

In **Chapter 4**, we observed an association between meal timing and cardiometabolic risk factors in men, but not in women. Furthermore, in **Chapter 5**, men demonstrated greater improvements in cardiometabolic risk factors compared to women, despite losing a similar percentage of body weight. These intriguing findings emphasize the importance of gaining a deeper understanding of potential sex-specific differences in the effects of meal timing and fasting on cardiometabolic health. Further research is needed to elucidate these differences and their implications.

Limitations

The present International Doctoral Thesis has several limitations that should be acknowledged. In **Chapter 4**, the cross-sectional design limits the ability to establish causal relationships. The measurement of food intake timing relied on three non-consecutive 24-hour recalls, which may not capture the broader variability over a longer period. The study population consisted of young and healthy adults, which may limit the generalizability of the findings to older or metabolically compromised individuals. The statistical power of the study may have been insufficient to thoroughly explore potential sex differences in the relationship between meal timing, body composition, and cardiometabolic risk

factors.

In **Chapter 5**, while magnetic resonance imaging was used to measure VAT and other ectopic fat depots, these data were not included in the analysis due to their unavailability at the time of the thesis defense. Therefore, the focus of the analysis and findings primarily revolved around VAT assessed by dual-energy X-ray absorptiometry. The duration of the study was relatively short, which may restrict the ability to observe long-term effects of the interventions. Additionally, important data on glycemic control, adherence rates, physical activity, and dietary patterns were not included in the analysis, which could have provided further insights into the underlying mechanisms. Lastly, a 12-month follow-up was not included, preventing an assessment of the sustainability of the observed effects over a longer timeframe.

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Eat
Fast
16 hrs

CONCLUSIONS

Chapter 7. Conclusions of the International Doctoral Thesis

Conclusions

This International Doctoral Thesis provides two specific conclusions that add new insights into the understanding of the role of meal timing and IF in body composition and cardiometabolic health in humans.

- Specific conclusion I (**Chapter 4):** Meal timing is not cross-sectionally associated with either anthropometry or body composition parameters in young adults. Similarly, caloric midpoint, eating jetlag and the time from last food intake to midsleep point are not associated with cardiometabolic risk factors. Nonetheless, a longer daily eating window and a shorter time from midsleep point to first food intake (i.e., earlier first food intake in a 24-hour cycle) are associated with better cardiometabolic health (i.e., lower HOMA-IR and cardiometabolic risk score) in young men.

- Specific conclusion II (**Chapter 5**): In a 12-week multicenter randomized controlled trial involving men and women with overweight/obesity and slight metabolic impairments, self-selected TRE was more effective to reduce VAT mass than a UC intervention in men. Additionally, an 8-hour TRE, irrespective of the timing of the eating window, led to significantly greater body weight loss compared to the UC intervention in both men and women. Lastly, all intervention groups exhibited similar effects on body composition and cardiometabolic risk factors.

Conclusiones

Esta Tesis Doctoral Internacional proporciona dos conclusiones específicas que aportan nuevas perspectivas sobre el conocimiento del rol del horario de las comidas y del ayuno intermitente en la composición corporal y la salud cardiometabólica en humanos.

- Conclusión específica I (**Capítulo 4**): El horario de las comidas no se asocia de manera transversal con los parámetros de antropometría ni de composición corporal en adultos jóvenes. De manera similar, el punto medio calórico, el desfase alimentario y el tiempo transcurrido desde la última ingesta de alimentos hasta el punto medio del sueño no se asocian con los factores de riesgo cardiometabólico. Sin embargo, una ventana de alimentación diaria más prolongada y un tiempo más corto desde el punto medio del sueño hasta la primera ingesta de alimentos (es decir, una primera ingesta de alimentos más temprana en un ciclo de 24 horas) se asocian con una mejor salud cardiometabólica (es decir, menor HOMA-IR y menor puntuación de riesgo cardiometabólico) en hombres jóvenes.

- Conclusión específica II (**Capítulo 5**): En un ensayo controlado aleatorizado multicéntrico de 12 semanas que incluyó a hombres y mujeres con sobrepeso/obesidad y leves alteraciones metabólicas, la restricción temporal de la ingesta nutricional autoseleccionada resultó ser más efectivo para reducir la masa de grasa visceral que una intervención estándar en hombres. Además, una restricción temporal de 8 horas de la ingesta nutricional, independientemente del momento de la ventana de ingesta, produjo una pérdida significativamente mayor de peso corporal en comparación con la intervención estándar tanto en hombres como en mujeres. Por último, todos los grupos de intervención mostraron efectos similares en la composición corporal y los factores de riesgo cardiometabólico.


Eat
Fast
16 hrs

FUTURE PERSPECTIVES

Chapter 8. Future perspectives

Future perspectives

Despite significant advancements in the study of meal timing and IF, there are still many unanswered questions that require further investigation. Future research should focus on the following areas:

- Matching energy deficit: It is important to compare the effects of continuous daily CR and IF regimens by ensuring a similar energy deficit. This will help determine if fasting preferentially leads to fat or fat-free mass loss, potentially due to increased proteolysis during fasting periods.

- Fat oxidation and weight management: Investigate whether IF regimens enhance fat oxidation and if this translates into better weight management and improved cardiometabolic health compared to continuous daily CR.

- Matching fat loss: Match fat mass loss between continuous daily CR and IF regimens to isolate the specific effects of fasting and examine whether it leads to preferential subcutaneous or ectopic fat loss.

- Timing of eating window: Compare different schedules of TRE with matched energy deficits to explore the impact of the timing of the eating window on cardiometabolic health.

- Postprandial metabolism: Investigate the effects of IF regimens during the postprandial period, particularly on glucose metabolism.

- Barriers to adherence: Identify and address barriers that hinder adherence to IF regimens to promote successful implementation and compliance.

- Sex-specific differences: Gain a deeper understanding of potential sex-specific differences in the effects of IF regimens on body composition and cardiometabolic health.

- Long-term adaptations: Examine whether the acute metabolic response

to fasting changes over time after prolonged adaptation to IF, and how it may influence body composition and cardiometabolic health.

- Aerobic exercise in the fasted state: Investigate whether moderate- to high-intensity exercise performed in the fasted state can enhance the metabolic switch from glycogenolysis to gluconeogenesis, increase fat oxidation, and promote ketogenesis.

- Resistance exercise in the fed state: Explore whether resistance exercise performed during the fed state of an IF regimen can help preserve lean mass and mitigate the loss typically associated with an energy deficit.

By addressing these research areas, we can gain a deeper understanding of the effects of IF and meal timing on various aspects of health and metabolism, leading to more effective and personalized strategies for weight management and cardiometabolic health.


Eat
Fast
16 hrs

ANNEXES

Manuscripts derived from the present International Doctoral Thesis

- **Dote-Montero M**, Sanchez-Delgado G, Ravussin E. Effects of Intermittent Fasting on Cardiometabolic Health: An Energy Metabolism Perspective. *Nutrients*. 2022, 14(3), 489. doi: 10.3390/nu14030489. PMID: 35276847; PMCID: PMC8839160.

- **Dote-Montero M**, Acosta FM, Sanchez-Delgado G, Merchan-Ramirez E, Amaro-Gahete FJ, Labayen I, Ruiz JR. Association of meal timing with body composition and cardiometabolic risk factors in young adults. *European Journal of Nutrition*. 2023, 1-13. doi: 10.1007/s00394-023-03141-9. PMID: 37100891.

- **Dote-Montero M,** Merchan-Ramirez E, Oses M, Echarte J, Clavero-Jimeno A, Alcantara JMA, Camacho-Cardenosa A, Cupeiro R, Rodríguez-Miranda MR, López-Vázquez A, Amaro-Gahete FJ, González Cejudo MT, Martin-Olmedo JJ, García Pérez PV, Contreras-Bolívar V, Muñoz-Garach A, Andreo-López MC, Carneiro-Barrera A, Miranda-Ferrúa E, Zugasti, A Petrina E, Álvarez de Eulate N, Goñi E, Ribelles MJ, Armendáriz Brugos C, Izquierdo C, Fernández-Puggioni V, Galbete A, Villanueva A, Medrano M, Alfaro-Magallanes VM, Muñoz-Torres M, Martín-Rodríguez JL, Idoate F, Cabeza R, Ruiz JR, Labayen I. Efficacy of different 8h time-restricted eating schedules on visceral adipose tissue and cardiometabolic health: A study protocol. Revised Version Under Review (*Nutrition, Metabolism and Cardiovascular Diseases*).

- **Dote-Montero M,** Merchan-Ramirez E, Oses M, Echarte J, Clavero-Jimeno A, Alcantara JMA, Camacho-Cardenosa A, Cupeiro R, Rodríguez-Miranda MR, López-Vázquez A, Amaro-Gahete FJ, González Cejudo MT, Carneiro-Barrera A, Galbete A, Muñoz-Torres M, Martín-Rodríguez JL, Idoate F, Cabeza R, Ruiz JR, Labayen I. Effects of three 8-hour time-restricted eating schedules on visceral adipose tissue, body composition and cardiometabolic health in men and women with overweight/obesity: A multicenter randomized controlled trial. In preparation.

Short Curriculum Vitae

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CV SUMMARY

Manuel Dote Montero (MDM) holds a bachelor's degree in Sport and Exercise Sciences (2017) and a master's degree in Research in Sport and Physical Activity (2018). During his master's degree, he received a research initiation grant. Later on, MDM was awarded the highly coveted pre-doctoral grant in Spain to do a PhD Thesis (FPU18/03357 grant 2018-2023). MDM has actively contributed to numerous research projects (see specific section).

As a doctoral candidate, he has led the EXTREME study as project manager, which encompasses a team of more than 30 members across two research centers (the University of Granada and the University of Navarra, Spain). The overarching aim of the EXTREME study is to explore the feasibility and efficacy of three different 8-hour time-restricted eating schedules (i.e., early, late, and self-selected) compared to a usual-care intervention over a 12-week duration on visceral adipose tissue, body composition, and cardiometabolic health in adults with overweight/obesity (ClinicalTrials.gov: https://bit.ly/3HUyhf9).

MDM also underwent an extended research stay at the prestigious Pennington Biomedical Research Center, (Baton Rouge, Louisiana, USA) under the mentorship of Prof. Eric Ravussin (January-August 2021), supported by the highly competitive Fulbright scholarship. During this research stay, MDM received comprehensive training in cutting-edge techniques such as indirect calorimetry in metabolic chambers, hyperinsulinemic-euglycemic clamps for insulin sensitivity evaluation, and magnetic resonance spectroscopy to measure ectopic fat (1H-MRS) or ATP turnover (31P-MRS). MDM also undertook a research stay at the IMDEA Food Institute (Madrid, Spain) from May to August 2023, under the guidance of Prof. Rafael de Cabo and Prof. Alberto Díaz-Ruiz. This research stay provided him with training in fundamental cellular and

molecular biology techniques to examine the effects of intermittent fasting on molecular mechanisms of ectopic fat deposition and insulin sensitivity in preclinical models.

MDM has contributed to 16 research articles, with nine as the first and corresponding author, with 50% appearing in Q1. He has also submitted 15 meeting abstracts, with nine as the first author, and has been invited to speak thrice at national and international conferences. Currently, MDM maintains an active and productive collaboration with Prof. Eric Ravussin at Pennington Biomedical Research Center (Baton Rouge, Louisiana, USA) and Prof. Jose E Galgani at the Pontifical University of Santiago de Chile (Santiago de Chile, Chile). MDM overarching career goal is to advance our understanding of the intricate regulation of energy balance, with the ultimate aim of identifying innovative and effective interventions for the treatment of obesity.

EDUCATION

- **2018-2023** PhD Student in Biomedicine, University of Granada, Spain.
- **2017-2018** Master's degree in Research in Sport and Physical Activity (Grade: 9.2/10) Faculty of Sport Sciences, University of Granada, Spain.
- **2013-2017** Bachelor's degree in Sport and Exercise Sciences (Grade: 8.6/10), Faculty of Sport Sciences, University of Granada, Spain.

NATIONAL FELLOWSHIPS

- **2019-2023** FPU pre-doctoral research fellowship (FPU18/03357) for PhD studies, funded by the Spanish Ministry of Universities: 56,000€.
- **2017** Research initiation fellow for official master's students, funded by the University of Granada: 1,800€.

NATIONAL AND INTERNATIONAL INTERNSHIPS

- **2023** Department of Sport Science, University of Innsbruck, Innsbruck, Austria. Prof: Justin Stevan Lawley. Duration: **5 days**. Funded by the European Union (Erasmus+): 800€.
- **2023** Nutritional Interventions Group, IMDEA Food Institute, Madrid, Spain. Prof: Alberto Díaz-Ruiz. Duration: **3 months.**
- **2021** AdventHealth Translational Research Institute, Orlando, FL, USA. Prof: Lauren M. Sparks. Duration: **5 days**.

- **2021** Section of Clinical Science, Pennington Biomedical Research Center, Baton Rouge, LA, USA. Prof: Eric Ravussin. Duration**: 6 months**. Funded by Fulbright Spain and Regional Government of Andalusia: 20,900USD.
- **2016-2017** Department of Health and Human Performance, University of Montana, Missoula, MT, USA. Duration: **9 months**. Funded by the University of Granada: 1,200€.
- **2015-2016** Università degli Studi di Cassino e del Lazio Meridionale, Cassino, LZ, Italy. Erasmus programme. Duration: 9 months. Funded by the Spanish Service for the Internationalization of Education and the Regional Government of Andalusia: 5,400€.

PUBLICATIONS

Only the 10 most relevant publications are included. For more information see PubMed: https://bit.ly/3RPfwOS and ResearchGate: https://bit.ly/3YjVzC6

1. **Dote-Montero M**, Acosta FM, Sanchez-Delgado G, Merchan-Ramirez E, Amaro-Gahete FJ, Labayen I, Ruiz JR. Association of meal timing with body composition and cardiometabolic risk factors in young adults. Eur J Nutr. 2023. PMID: 37100891. **IF: 4.86; Rank: 35/90 (Q2)** Nutrition & Dietetics.
2. **Dote-Montero M**, Sanchez-Delgado G, Ravussin E. Effects of intermittent fasting on cardiometabolic health: an energy metabolism perspective. Nutrients. 2022. PMID: 35276847. **IF: 6.70; Rank: 15/90 (Q1)** Nutrition & Dietetics.
3. **Dote-Montero M**, Carneiro-Barrera A, Martinez-Vizcaino V, Ruiz JR, Amaro-Gahete FJ. Acute effect of HIIT on testosterone and cortisol levels in healthy individuals: A systematic review and meta-analysis. Scand J Med & Sci Sports. 2022. PMID: 34022085. **IF: 4.64; Rank: 14/88 (Q1**) Sport Sciences.
4. **Dote-Montero M**, Pelayo-Tejo I, Molina-Garcia P, Carle-Calo A, García-Ramos A, Chirosa-Ríos LJ, Chirosa-Ríos IJ, Amaro-Gahete FJ. Effects of post-tetanic potentiation induced by whole-body electrostimulation and post-activation potentiation on maximum isometric strength. Biol Sport. 2022. PMID: 35309538. **IF: 4.60; Rank: 16/88 (Q1)** Sport Sciences.
5. Perez-De-Arrilucea-Le-Floc'h UA*, **Dote-Montero M***, Carle-Calo A, Sánchez-Delgado G, Ruiz JR, Amaro-Gahete FJ. Acute effects of whole-body electromyostimulation on energy expenditure at resting and

during uphill walking in healthy young men. Metabolites. 2022. PMID: 36144186. IF**: 5.58; Rank: 90/297 (Q2)** Biochemistry & Molecular Biology. *Equally contributed.

6. **Dote-Montero M**, De-la-O A, Jurado-Fasoli L, Ruiz JR, Castillo MJ, Amaro-Gahete FJ. The effects of three types of exercise training on steroid hormones in physically inactive middle-aged adults: a randomized controlled trial. Eur J Appl Physiol. 2021. PMID: 33890158. IF: 3.34; **Rank: 32/81 (Q2)** Physiology.
7. **Dote-Montero M**, Amaro-Gahete FJ. Methodological issues related to exercise interventions during fasted or fed state. PMID: Med Sci Sports Exerc. 2020.PMID: 33103865. IF: 5.41; **Rank: 8/88 (D1)** Sport Sciences.
8. **Dote-Montero M**, De-la-O A, Castillo MJ, Amaro-Gahete FJ. Predictors of sexual desire and sexual function in sedentary middle-aged adults: the role of lean mass index and s-klotho plasma levels. The FIT-AGEING Study. J Sex Med. 2020. PMID: 32089483. **IF: 3.93; Rank: 30/90 (Q2)** Urology & Nephrology.
9. **Dote-Montero M**, Amaro-Gahete FJ, De-la-O A, Jurado-Fasoli L, Gutierrez A, Castillo MJ. Study of the association of DHEAS, testosterone and cortisol with s-klotho plasma levels in healthy sedentary middle-aged adults. Exp Gerontol. 2019. PMID: 30928678. **IF: 4.25; Rank: 26/67 (Q2)** Geriatrics & Gerontology.
10. Alcantara JMA, Galgani JE, Jurado-Fasoli L, **Dote-Montero M**, Merchan-Ramirez E, Ravussin E, Ruiz JR, Sanchez-Delgado G. Validity of four commercially available metabolic carts for assessing resting metabolic rate and respiratory exchange ratio in non-ventilated humans. Clin Nutr. 2022. PMID: 35180452. **IF: 7.64; Rank: 13/90 (Q1)** Nutrition & Dietetics.

Publications by authorship position and WoS categories journal rank.

	Authorship position				
	1st	2nd	3rd	Others	**Total**
D1	1				**1**
Q1 (not D1)	3		2	3	**8**
Q2	5	1			**6**
Q3				1	**1**
Total	**9**	**1**	**2**	**4**	**16**

D, Decile; Q, Quartile; WoS, Web of Science

CONFERENCE ABSTRACTS

Only the conference abstracts as first author from the last two years are included:

1. **2022** Dote-Montero M et al. Accuracy and precision of four metabolic carts in comparison to Deltatrac II. *Recent Advances & Controversies in the Measurement of Energy Metabolism (RACMEM)*. Québec, Canadá.
2. **2022** Dote-Montero M et al. Efficacy and feasibility of three different 8h time-restricted eating schedules on body composition and cardiometabolic health in adults with overweight/obesity: a randomized controlled trial. *Danish Diabetes Association Postdoc Summit*. Rønne, Danmark.
3. **2021** Dote-Montero M et al. Sex differences in metabolic adaptation after weight loss: a secondary analysis of CALERIE studies. *New Trends in Sex and Gender Medicine*. Online, USA.
4. **2021** Dote-Montero M et al. Effects of three different 8h time-restricted eating schedules over 4 weeks on body weight and waist/hip circumferences in adults with overweight/obesity: a pilot randomized trial. *Virtual 81st Scientific Sessions American Diabetes Association.* Online, USA.
5. **2021** Dote-Montero M et al. Feasibility of Three Different 8h Time-Restricted Eating Schedules Over 4 Weeks in Spanish Adults With Overweight/Obesity: A Pilot Randomized Controlled Trial. *NUTRITION 2021 LIVE ONLINE.* Online, USA.
6. **2021** Dote-Montero M et al. Association of meal timing with body fat and cardiometabolic health in young healthy adults. *Precision Nutrition: Research Gaps and Opportunities Workshop*. Online, USA.

INVITED SPEAKER

1. **2019** Simpósio Internacional de Investigação sobre Sono, Exercício & Saúde. Setúbal, Portugal.
2. **2019** V Biomedical Research Student Congress (CEIBS). Granada, Spain.
3. **2018** IV Biomedical Research Student Congress (CEIBS). Granada, Spain.

PARTICIPATION IN RESEARCH PROJECTS

Manuel Dote-Montero participated in 8 funded research projects:

1. EXTREME: Efficacy and feasibility of a time-restricted eating intervention on visceral adipose tissue and cardiometabolic health in adults with overweight/obesity. Funded by the Regional Government of

Andalusia. Budget: 30,000€. Principal investigator (PI): Jonatan R. Ruiz. Duration: 2021-2023. Role: Project manager.

2. Congruent validity and inter-day reliability of four recently released indirect calorimeter metabolic carts. Funded by Cosmed Inc, CareFusion Inc, Mastritch Instrument Inc, and Medical Graphics Inc. Budget: 60,000€. PI: Jonatan R. Ruiz. Duration: 2019-2021. Role: Researcher.
3. Impact of Whole Body Electromyostimulation on energy metabolism. Funded by Wiemspro S.L. Budget: 30,000€. PI: Francisco J. Amaro Gahete. Duration: 2018-2023. Role: Researcher.
4. ACTIFOX: Effects of the acute ingestion of dihydrocapsiate during aerobic exercise on energy metabolism in adults with overweigth/obesity. Funded by the Regional Government of Andalusia. Budget: 21,000€. PI: Jonatan R. Ruiz. Duration: 2018-2020. Role: Researcher.
5. SmartMove: Exercise in the prevention and treatment of obesity and insulin resistance: Smart analysis-smart interventions. Funded by the Spanish Ministry of Economy and competitiveness. Budget: 100,000€. PI: Jonatan R. Ruiz and Francisco B. Ortega. Duration: 2016-2020. Role: Researcher.
6. BEER-HIIT: Beer effects on the physical adaptation to intensive training. Funded by the Centro de Información Cerveza y Salud (CICS). Budget: 40,000€. PI: Manuel J. Castillo. Duration: 2018-2019. Role: Researcher.
7. FIT-AGEING: Role of physical exercise on the S-Klotho protein regulation and other ageing biomarkers in healthy adults. Funded by the Regional Government of Andalusia. Budget: 65,000€. PI: Manuel J. Castillo. Duration: 2015-2018. Role: Researcher.
8. ACTIBATE: Activating brown adipose tissue through exercise. effects of an exercise intervention on activity and quantity of brown adipose tissue. Funded by the Spanish Ministry of Economy and competitiveness among others. Budget: 600,000€. PI: Jonatan R. Ruiz. Duration: 2014-2018. Role: Researcher.

UNIVERSITY TEACHING

- **2021-2023** Functional Anatomy of the Locomotor System (90 hours of teaching, 9 ECTS [European Credit Transfer and Accumulation System] credit). Degree/Bachelor: Sport and Exercise Sciences, University of Granada (Spain).

- **2020** Fundamentals of Sport 3: Artistic Gymnastics (30 hours of teaching, 3 ECTS credit). Degree/Bachelor: Sport and Exercise Sciences, University of Granada (Spain).
- **2019** Motor Control and Learning (60 hours of teaching, 6 ECTS credit). Degree/Bachelor: Sport and Exercise Sciences, University of Granada (Spain).
- **2021-2023** Master's dissertation supervisor of two students from the master's degree in Research in Sport and Physical Activity, University of Granada, Spain.
- **2022** Master's dissertation supervisor of two students from the master's degree in Human Nutrition, University of Granada, Spain.
- **2022** Supervisor of an undergraduate dissertation for a student pursuing a bachelor's degree in Human Nutrition and Dietetics at the University of Granada, Spain.
- **2020** Master's dissertation supervisor of two students from the master's degree in Food and Sports for Health (Food & Fit), University of Granada, Spain.

PRIZES AND AWARDS

- **2023** The paper "Acute effect of HIIT on testosterone and cortisol levels in healthy individuals: A systematic review and meta-analysis" was one of the most downloaded during its first 12 months of publication in Scandinavian Journal of Medicine & Science in Sports.
- **2018** The best master's dissertation in the master's degree in Research in Sport and Physical Activity at the University of Granada for the academic year 2017-2018.
- **2018** Award for excellence in international mobility for the stay at the University of Montana, funded by the University of Granada: 1,000€.
- **2017** Certificate of accomplishment awarded by the University of Montana.

LANGUAGES

- Certificate in Advanced English (CAE) by Cambridge. Council of Europe level C1.