

## The effect of early time-restricted eating on muscle damage-related biomarkers and anxiety in older men

Maha Gasmi<sup>a</sup>, Antonella Muscella<sup>b,\*</sup>, Santo Marsigliante<sup>b</sup>, Kawther Missawi<sup>c</sup>, Riadh Khalifa<sup>a</sup>

<sup>a</sup> Higher Institute of Sport and Physical Education of Ksar Said, Tunis, Tunisia

<sup>b</sup> Department of Biological and Environmental Science and Technologies (DiSTeBA), University of Salento, 73100, Lecce, Italy

<sup>c</sup> Laboratory of Exercise Physiology and Pathophysiology: From Integrated To Molecular "Biology, Medicine and Health", UR12ES06, Faculty of Medicine Ibn Jazar Sousse, University of Sousse, 4002, Sousse, Tunisia

### ARTICLE INFO

Section Editor: Christiaan Leeuwenburgh

#### Keywords:

Early time-restricted eating  
Body composition  
Liver biomarkers  
Kidney function  
Muscle damage biomarkers  
Inflammation  
Cognitive anxiety  
Somatic anxiety  
Elderly men

### ABSTRACT

This study investigates the effects of early Time-Restricted Eating (eTRE) on body composition, biochemical markers of muscle damage, and anxiety levels in healthy elderly men. Thirty participants were randomly assigned to either an eTRE intervention group or a control group. The eTRE group consumed all daily caloric intake within an 8-h window (7:00 AM to 3:00 PM) for a period of two months, while the control group maintained their usual eating habits. Assessments were conducted at baseline (T0) and post-intervention (T1), including anthropometric measurements, blood biomarker analysis, and anxiety evaluation using the State-Trait Inventory for Cognitive and Somatic Anxiety (STICSA). Within-group analyses using the Wilcoxon signed-rank test and paired *t*-test revealed significant improvements in weight, body mass index (BMI), body fat, and fat-free mass in the eTRE group (all  $p < 0.01$ ). Significant decreases were also found in muscle damage and inflammation markers, including CPK ( $p = 0.0067$ ), AST ( $p < 0.0001$ ), CRP ( $p = 0.0011$ ), and bilirubin ( $p = 0.044$ ), indicating reduced physiological stress and improved liver function. Two-way repeated measures ANOVA revealed significant time by group interactions for BMI ( $F_{1,28} = 4.453$ ;  $p = 0.007$ ), body fat ( $F_{1,28} = 3.059$ ;  $p = 0.037$ ), fat-free mass ( $F_{1,28} = 3.908$ ;  $p = 0.014$ ), uric acid ( $F_{1,28} = 4.947$ ;  $p = 0.004$ ), CRP ( $F_{1,28} = 2.853$ ;  $p = 0.045$ ), AST ( $F_{1,28} = 14.332$ ;  $p < 0.001$ ), and STICSA scales ( $p < 0.05$ ). These interactions indicate that the observed changes were significantly more pronounced in the eTRE group compared to the control group. Mediation analyses revealed that biomarkers related to **muscle recovery** and **metabolic regulation** (such as **LDH**, **ALT**, and **AST**) significantly mediated the relationship between eTRE and **anxiety reduction**. These findings suggest that eTRE may be an effective dietary approach to improve body composition, reduce muscle stress and inflammation, and lower anxiety levels in elderly individuals. Future studies are needed to confirm these effects and explore their long-term sustainability.

### 1. Introduction

As men age, maintaining muscle health becomes increasingly challenging yet vital for overall well-being. Muscle stress, repair, and general function are key components that influence muscle health and recovery. Addressing these aspects can help mitigate the effects of aging, such as sarcopenia, and improve quality of life (Larsson et al., 2019). Muscle stress, induced by physical activity, especially resistance training, plays a critical role in maintaining and enhancing muscle mass and strength (Militello et al., 2024; Li et al., 2024).

However, as men age, their muscles undergo several physiological changes, including reduced satellite cell activity and decreased muscle

protein synthesis (MPS) rates, which make recovery more difficult (Kaczmarek et al., 2021). Older adults also experience increased oxidative stress and inflammation, exacerbating muscle damage (Chen et al., 2022). Maintaining general muscle function is critical for mobility, balance, and overall health in older men (Billot et al., 2020).

Regular physical activity, particularly resistance training, is essential to induce beneficial muscle stress, but the increased susceptibility to damage necessitates tailored exercise regimens and recovery strategies to minimize adverse effects (Cannataro et al., 2022).

Early time-restricted eating (eTRE) has emerged as a promising dietary intervention for improving health outcomes. This eating pattern involves consuming all daily calories within a specific window early in

\* Corresponding author at: Department of Biological and Environmental Science and Technologies (DiSTeBA), University of Salento, 73100, Lecce, Italy.

E-mail addresses: [antonella.muscella@unisalento.it](mailto:antonella.muscella@unisalento.it) (A. Muscella), [santo.marsigliante@unisalento.it](mailto:santo.marsigliante@unisalento.it) (S. Marsigliante).

<https://doi.org/10.1016/j.exger.2025.112896>

Received 18 April 2025; Received in revised form 8 September 2025; Accepted 12 September 2025

Available online 14 September 2025

0531-5565/© 2025 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

the day, typically within 6–8 h, and fasting for the remaining 16–18 h (Kim and Song, 2023). Recent studies suggest that eTRE may have significant benefits for older adult men, impacting metabolic health, body composition, and overall well-being (Mishra et al., 2023).

Body composition, including muscle mass and fat distribution, is another critical aspect of health in older men. eTRE may aid in reducing body fat while preserving lean muscle mass, a combination that is often challenging to achieve through traditional calorie restriction alone. A study by Anton et al. (2019) demonstrated that participants following an eTRE regimen experienced significant reductions in body weight and fat mass. This finding is particularly relevant for older men, who often struggle with maintaining muscle mass while trying to lose weight.

eTRE has been shown to positively influence also various metabolic parameters, which are crucial for older adult men who are at increased risk for metabolic disorders such as type 2 diabetes and cardiovascular diseases. One study found that eTRE improved insulin sensitivity and reduced insulin levels, which are key factors in managing and preventing diabetes (Sutton et al., 2018). By aligning food intake with the body's circadian rhythms, eTRE can enhance metabolic health and reduce the risk of metabolic syndrome (Manoogian and Panda, 2017).

Cardiovascular health is a major concern for older adults, and eTRE has shown potential benefits in this area as well. Research indicates that eTRE can lead to improvements in blood pressure, cholesterol levels, and other cardiovascular risk factors. Wilkinson et al. (2020) reported that participants adhering to an eTRE schedule had lower systolic blood pressure and improved lipid profiles. These changes contribute to a lower risk of heart disease, which is a leading cause of mortality in older men.

Although eTRE has gained popularity for its metabolic and cardiovascular benefits, only a limited number of interventional studies have investigated its impact on biomarkers potentially associated with muscle stress or injury and anxiety symptoms.

Some recent trials have explored the effects of TRE on markers of systemic inflammation and oxidative stress, which are closely related to muscle recovery processes (Wilkinson et al., 2020; Moro et al., 2016).

However, direct evidence linking TRE or eTRE to specific biomarkers often interpreted as related to muscle damage (e.g., creatine kinase [CK], aspartate aminotransferase [AST], lactate dehydrogenase [LDH]) remains scarce. It is important to note that elevated levels of CK and LDH have multiple interpretations and do not definitively confirm muscle damage without clinical symptoms.

A few pilot studies on younger populations have suggested that intermittent fasting protocols might reduce exercise-induced muscle damage and improve muscle recovery (Gasmí et al., 2018; Moro et al., 2016), yet these findings need validation in older adults who often have impaired muscle regeneration and slower recovery rates.

Although research on eTRE has shown various health benefits, particularly for older adults, few studies have investigated its effects on biomarkers commonly associated with muscle stress or injury and overall muscle health (Gasmí et al., 2018).

Thus, the objective of this study is to demonstrate the impact of eTRE on biomarkers that may reflect muscle stress or injury and anxiety levels in older men.

We have chosen to measure a panel of biomarkers (CPK, AST, total bilirubin, CRP, and LDH) critical for assessing muscle health and recovery due to their roles in indicating inflammation, systemic stress responses and potential muscle injury. Their combined analysis provides a comprehensive picture of the physiological changes associated with muscle injury and recovery. In particular creatine phosphokinase (also known as creatine kinase, CK) levels in the blood increase when muscle tissue is damaged or stressed, making it a widely used biomarker for muscle stress and injury, although it is not conclusive without clinical symptoms (Brancaccio et al., 2010). Aspartate aminotransferase (AST) is also released into the bloodstream following muscle damage. Thus, elevated AST levels, when considered alongside other muscle-specific biomarkers like CPK, can indicate muscle injury (Petterson et al.,

2008). Total bilirubin levels can be influenced by oxidative stress and inflammation, which are associated with muscle damage and recovery (Gonçalves et al., 2015). High levels of C-reactive protein (CRP) are associated with systemic inflammation. Monitoring CRP levels helps to understand the inflammatory response to muscle damage and the subsequent recovery process (Pedersen and Hoffman-Goetz, 2000). Lactate dehydrogenase (LDH) elevated levels in the blood can indicate muscle cell damage or lysis, but this enzyme is a non-specific marker and should be interpreted in conjunction with other muscle-specific biomarkers (Adibi et al., 2013).

Anxiety levels in older men have been the focus of various studies; common symptoms of anxiety in older adults include shakiness, difficulty breathing, dizziness, digestion problems, muscle tension, irritability, and obsessive thoughts (Penninx et al., 2021). These symptoms can significantly impair daily functioning and quality of life in older adults, leading to a decline in physical health, reduced ability to perform daily activities, and a lower overall sense of well-being (Kavelaars et al., 2023). Recent evidence suggests that chronic systemic inflammation plays a crucial role in the development and persistence of anxiety disorders. Elevated inflammatory markers such as interleukin-6 (IL-6) and C-reactive protein (CRP) have been consistently associated with higher anxiety symptomatology, potentially through neuroimmune mechanisms that affect brain function and mood regulation (Felger and Lotrich, 2013; Dowlati et al., 2010). This is particularly relevant in older adults, who often experience chronic low-grade inflammation (“inflammaging”) that may increase vulnerability to anxiety and other mood disorders (Miller and Raison, 2016).

The interplay between circadian rhythms, metabolic regulation, and inflammation is increasingly recognized as a key factor influencing mental health. Disruptions in circadian metabolism can exacerbate systemic inflammation, which in turn negatively affects brain function and mood regulation. Early time-restricted eating (eTRE), by aligning food intake with circadian biology, may help restore metabolic balance and reduce inflammatory burden, potentially improving anxiety symptoms and overall psychological well-being, especially in older adults.

Similarly, research on the psychological effects of TRE, including anxiety modulation, is emerging but still limited. Some clinical studies suggest that fasting regimens can influence stress hormone regulation and inflammatory pathways, thereby reducing anxiety symptoms (Lin et al., 2023; Moro et al., 2016). Intermittent fasting has been associated with decreased cortisol levels and improved mood states in both animal and human models (Moro et al., 2016). Nevertheless, evidence on the effects of eTRE specifically on anxiety symptoms in aging populations is insufficient and warrants further investigation.

Therefore, understanding the combined effects of eTRE on both physiological and psychological health markers in older adults is critical, as these factors collectively influence overall health, functional capacity, and quality of life in aging populations.

Thus, further well-controlled intervention trials are necessary to elucidate the effects of eTRE on inflammatory biomarkers linked to anxiety, as well as on muscle-related markers, in aging populations.

The objective of this study is to evaluate the impact of an eTRE protocol on a panel of muscle injury and inflammation markers and anxiety levels in older men.

We hypothesized that TRE, could have effects on mental health and mood improvements also in our study sample.

## 2. Materials and methods

### 2.1. Participant selection and inclusion

Eligible individuals provided written consent, which was approved by the Scientific Committee of the Higher Institute of Sport and Physical Education of XXXX. The study adhered to the ethical principles outlined in the 1964 Helsinki Declaration and its subsequent revisions. Sixty healthy men were enrolled in the investigation. The physical condition

of participants was evaluated using an adapted version of the Baecke questionnaire (Hertogh et al., 2008) Additionally, all participants underwent a clinical examination conducted by a physician to confirm their health status and ensure eligibility for the study.

The study employed specific inclusion and exclusion criteria to ensure the selection of eligible participants. Inclusion criteria were as follows: i) adherence to a non-vegetarian or vegan diet; ii) no use of ergogenic supplements in the previous six months; iii) no current or prior use of medications known to affect immune or metabolic function; iv) not participating in structured physical activity programs or behavioral interventions targeting sedentary behavior; v) no involvement in calorie-restricted diets or other nutritional interventions; vi) generally good health; vii) no exposure to radioactive procedures in the past 12 months; and viii) regular sleep patterns and not working night shifts. Exclusion criteria included: i) a history of chronic diseases such as cardiovascular conditions, diabetes mellitus, hypertension, metabolic syndrome, or chronic gastrointestinal disorders (e.g., inflammatory bowel disease); ii) current or recent use (within the last 6 months) of medications that could significantly influence metabolism, immune function, or muscle activity, such as corticosteroids, certain antidepressants, or endocrine-disrupting drugs; iii) prior diagnosis of eating disorders, including anorexia nervosa, bulimia nervosa, or binge eating disorder; iv) history of significant musculoskeletal injuries or surgeries within the past year; v) current pregnancy or breastfeeding; vi) heavy smoking or substance/alcohol abuse; vii) presence of severe psychiatric conditions or poorly controlled mental health disorders that could impact anxiety or compliance with the protocol; and viii) participation in other clinical trials or dietary/behavioral studies that could introduce confounding variables or interfere with adherence to the early time-restricted eating (eTRE) protocol.

Participants meeting these criteria were recruited for the study. After recruitment and familiarization with the procedures, participants were randomized into an experimental group and a control group (Fig. 1).

The required sample size was determined a priori using G\*Power

version 3.1.9.4 (Düsseldorf, Germany). A power of 80 % ( $1 - \beta$ ) and a significance level of 0.05 ( $\alpha$ ) were used for the calculation, assuming a medium effect size ( $f = 0.25$ ). Based on these parameters, the minimum total sample size was calculated to be 28 participants (14 per group), allowing for adequate power to detect significant differences between groups. The effect size was derived from previous studies on time-restricted eating interventions and their effects on body composition and metabolic parameters (e.g., Moro et al., 2016; Tinsley and La Bounty, 2015). To account for potential dropouts, 30 participants (15 per group) were ultimately enrolled.

## 2.2. Randomization and blinding

Participants were randomly assigned to either the early TRE intervention group or the control group in a 1:1 ratio using a computer-generated randomization list (created in Microsoft Excel). The randomization list was prepared by an independent researcher not involved in recruitment or assessments. To reduce potential measurement bias, the assessors who conducted anthropometric measurements, biochemical analyses, and psychological evaluations were blinded to group assignments throughout the study.

Baseline demographic and anthropometric characteristics, including age, height, weight, BMI, and body composition, are summarized in Fig. 1 (Study flow chart). Statistical analyses confirmed no significant differences between the early TRE and control groups at baseline (all  $p > 0.05$ , using Mann–Whitney  $U$  tests or independent  $t$ -tests where appropriate), indicating a homogeneous distribution of these variables.

Demographic information, including ethnicity and education level (mainly secondary and higher education), was also collected for all participants. No significant differences were found between the groups, supporting comparability at baseline.

Given this, these factors were not included as covariates in the main analyses. However, in studies with baseline imbalances, adjusting for such covariates would be warranted.

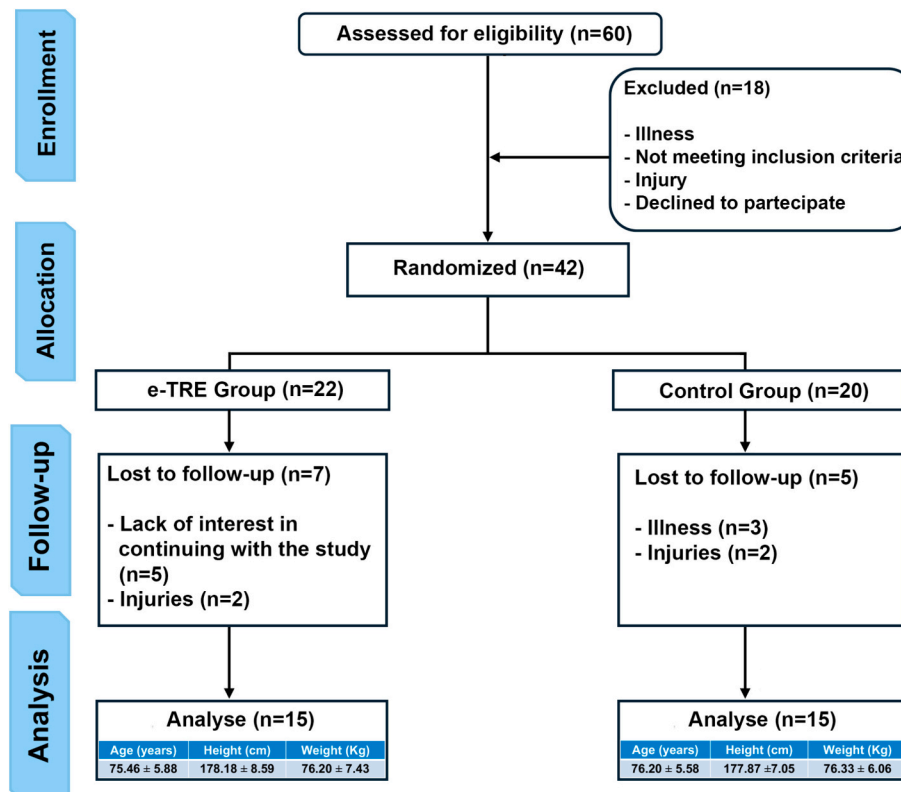


Fig. 1. Study flow chart.

### 2.3. eTRE diet program

The early TRE intervention group followed a time-restricted eating protocol, where they were instructed to consume all their daily calories within a specific time window, typically from 7:00 AM to 3:00 PM, for a duration of two months. This time window was chosen based on previous research suggesting optimal metabolic benefits and alignment with natural circadian rhythms. During the fasting period outside of this window, participants were allowed to consume water, herbal tea, or black coffee without any caloric additives.

In contrast, the control group maintained their regular dietary habits without any time restrictions.

All participants, regardless of group assignment, received personalized dietary guidelines and meal plans tailored to their caloric needs by a registered dietitian. These dietary plans aimed to ensure adequate nutrient intake and adherence to any specific dietary preferences or restrictions. Participants were encouraged to follow their assigned dietary protocol diligently throughout the two-month intervention period.

Additionally, participants in the experimental group received comprehensive education and counseling sessions on the principles and benefits of early time-restricted eating, as well as strategies for meal planning, portion control, and managing hunger during fasting periods. Regular follow-up sessions were conducted to monitor adherence to the dietary protocols, address any questions or concerns, and provide ongoing support throughout the intervention period.

Adherence was verified through the review of 24-h dietary recalls and 4-day food records, as well as weekly structured telephone interviews conducted by a registered dietitian to confirm compliance with the assigned eating window and dietary recommendations. In addition, participants maintained a daily paper food diary in which they recorded the exact start and end times of all food and beverage consumption. These diary entries were reviewed by the research staff during follow-up visits to assess compliance with the prescribed eating and fasting windows. No electronic devices or apps were used for monitoring. Importantly, the researchers conducting the follow-up assessments were blinded to the group assignments to reduce bias.

Participants were instructed to maintain their habitual physical activity levels, which primarily consisted of light activities such as daily walking or household chores, throughout the intervention period. Adherence was rigorously monitored through weekly detailed physical activity logs combined with structured, standardized interviews conducted by trained research staff to ensure accurate reporting and detect any deviations. This multi-method monitoring approach was implemented to minimize potential confounding effects of physical activity on muscle biomarkers.

#### 2.3.1. Dietary survey

Participants were advised by a registered nutritionist to avoid foods with high glycemic loads, unhealthy fats, caffeine, alcohol, recreational drugs, dietary supplements, and low-fiber intake. To assess their habitual dietary intake and eating patterns, all participants completed a validated “Diet History Questionnaire” (Lührmann et al., 1999) (see Table 2). A structured dietary interview was conducted by the dietitian to monitor the timing and composition of meals. Compliance with the dietary protocol was regularly monitored through weekly follow-up phone calls. Based on the collected data, the dietitian made individualized adjustments to optimize adherence. Additionally, dietary intake was evaluated using both a 24-h recall and a 4-day food record, allowing for detailed tracking of eating habits and nutrient intake. This approach helped identify any significant differences in energy or macronutrient consumption between groups. Nutritional analysis was performed using the ESHA Food Processor SQL Nutrition and Fitness Software version 10.5, which includes a database of over 35,000 food items and provides information on more than 160 nutrients (ESHA Research).

To characterize the dietary habits of the study population, a dietary survey conducted by a certified nutritionist advised participants to avoid

high glycemic loads, unhealthy fats, caffeine, alcohol, drugs, supplements, and low-fiber diets. Participants completed a “Diet History Questionnaire” to assess habitual diet and dietary patterns (Guallar-Lührmann et al., 1999) (Table 1). The dietitian controlled all participants by performing a structured interview to control meal timing and composition. Adherence to diet protocol was verified by contacting the participants (via phone call) every week. The dietitian utilizes the results to calibrate the diet. The dietitian utilizes the 24-h recall and a 4-day food record to pursue every participant's habit and dietary consumption and verify any difference in energy and macronutrient composition between the groups. ESHA Food Processor SQL Nutrition and Fitness Software 10.5 was used in the present study.

Although participants in both groups received personalized diet plans, caloric and macronutrient intakes were not strictly matched across groups. The experimental group showed a spontaneous reduction in energy and fat intake, which is consistent with previous reports on eTRE interventions (Sutton et al., 2018; Gabel et al., 2018). Thus, the effects observed in our study may reflect a combination of meal timing and changes in dietary intake.

#### 2.4. Blood collection and analysis

Upon arrival, participants had a heparinized catheter (Insyte-W, 1.1 mm o.d. × 30 mm) inserted into an ante-cubital vein after 20 min of sitting. In our study, blood samples were collected in the morning within a standardized time window (08:00–10:00 a.m.) after an overnight fast of at least 10–12 h. The room temperature was maintained at a controlled level of approximately 22–24 °C to minimize potential confounding effects.

Venous blood samples were drawn at rest (after 20 min of sitting), with 15 mL collected in tubes containing ethylene di-amine tetra-acetic acid (EDTA) to analyze blood count and muscle injury biomarkers. Samples were immediately centrifuged (at 3000 rpm for 15 min at 4 °C) and divided into appropriate aliquots.

Resting blood samples were collected at two time points: before (T0) and after (T1) the 8-week eTRE diet period. Serum glucose and uric acid levels were determined using enzymatic colorimetric methods following the Trinder enzymatic process. Aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) levels were measured using an enzymatic rate method with a reagent kit. Plasma creatinine was analyzed using both the kinetic colorimetric Jaffes method and enzymatic colorimetric method, while C-reactive protein (CRP) was measured using an enhanced immuno-turbidimetric assay. Creatine phosphokinase (CPK) was measured using an enzymatic method (CPK assay kits, Sigma-Aldrich).

#### 2.5. Anxiety measurement

The State-Trait Inventory for Cognitive and Somatic Anxiety (STICSA) test was administered to all participants twice: once the day before other measurement activities began, and once after the early Time-Restricted Eating (eTRE) diet. The STICSA (Barros et al., 2022) is an instrument that measures both state and trait anxiety, with each scale

**Table 1**  
Summary of Dietary Intake and Adherence During the Intervention Period.

		eTRE group	p	Control group	p
Energy (kcal/day)	T0	2352.5 ± 87.1	0.0001	2250.6 ± 85.6	0.94
	T1	2131.3 ± 35.3		2240.1 ± 108.5	
Protein (g/day)	T0	78.1 ± 2.5	0.1	77.9 ± 2.1	0.044
	T1	80.1 ± 3.1		79.3 ± 1.0	
Carbohydrate (g/day)	T0	211.6 ± 10.7	0.031	327.8 ± 12.1	0.38
	T1	208.7 ± 7.9		330.3 ± 26.8	
Fat (g/day)	T0	90.5 ± 2.8	0.005	90.1 ± 1.5	0.49
	T1	89.6 ± 4.4		91.1 ± 2.5	

p-values calculated using the Wilcoxon signed-rank test

comprising 21 items rated on a 4-point Likert scale (Gros et al., 2010). Both the STICSA State and Trait scales were divided into two forms evaluating cognitive (consisting of 10 items) and somatic (comprising 11 items) components of anxiety (Barros et al., 2022).

## 2.6. Statistical analysis

Analyses were performed using the commercial software Statistical Package for the Social Sciences (SPSS) version 23.0 for Windows (SPSS, Inc., Chicago, IL, USA). Statistical power calculations were performed a priori using G\*Power version 3.1.9.4 to ensure an adequate sample size for detecting meaningful effects with 80 % power at  $\alpha = 0.05$ .

Effect sizes (partial eta squared for ANOVA, Cohen's *d* for *t*-tests, and *r* for non-parametric tests) and 95 % confidence intervals were calculated for all key analyses to provide estimates of practical significance. Cohen's *d* values were interpreted as follows: values around 0.2 indicate a small effect, around 0.5 a medium effect, around 0.8 a large effect, and values  $\geq 1.0$  indicate a very large effect, suggesting a highly relevant change.

Data normality was assessed using the Shapiro-Wilk test, and the equality of variances was assessed using Levene's test. The non-parametric Wilcoxon signed-rank test was employed to assess differences in muscle damage-related biomarkers and anxiety levels before and after early Time-Restricted Eating (eTRE), as these parameters were not normally distributed. In contrast, to compare anthropometric parameters before the intervention between the experimental and control groups, the Student's paired *t*-test was used, as these parameters were normally distributed.

Additionally, to evaluate the effects of eTRE on body composition, biochemical markers of muscle damage, and anxiety levels, a two-way repeated measures ANOVA was performed with Group (eTRE vs. Control) as the between-subjects factor and Time (baseline [T0] vs. post-intervention [T1]) as the within-subjects factor.

To control for inflated Type I error risk due to multiple comparisons, Bonferroni correction was used. When multiple pairwise comparisons were conducted after significant ANOVA results, Bonferroni correction was applied to adjust for multiple testing and reduce the risk of Type I error.

A significance threshold of  $p < 0.05$  was adopted for all statistical tests.

To examine the role of biomarkers as mediators in the relationship between eTRE and anxiety scores, mediation analyses were conducted using the bootstrap method with 5000 resamples. The mediated effect was calculated as the product of the effect of treatment on biomarkers and the effect of biomarkers on anxiety. The 95 % bootstrap confidence intervals for the mediated effects were determined using the percentile method. The analysis was performed separately for each muscle biomarker with respect to anxiety scores in the STICSA State and STICSA Trait domains. Significant effects were defined when the bootstrap confidence interval did not include zero.

## 3. Results

### 3.1. Dietary survey

In the experimental group, energy intake significantly decreased at T1 ( $p = 0.0001$ , Cohen's *d* = 1.35, 95 % CI [0.85, 1.85], indicating a large effect), whereas in the control group, no significant variation was found ( $p = 0.94$ , Cohen's *d* = 0.05, 95 % CI [-0.30, 0.40], indicating a trivial effect).

Regarding protein intake, control group showed a significant increase in protein intake ( $p = 0.044$ , Cohen's *d* = 0.95, 95 % CI [0.45, 1.45], indicating a large effect), while no significant changes were observed in the experimental group (Cohen's *d* = 0.85, 95 % CI [0.52, 1.18], indicating a large effect).

For carbohydrate intake, no significant differences were observed in eTRE group ( $p = 0.38$ ,  $r = 0.12$ , 95 % CI [-0.15, 0.39], indicating a

small effect).

Finally, fat intake significantly decreased in experimental group ( $p = 0.005$  Cohen's *d* = 0.85, 95 % CI [0.35, 1.35], indicating a large effect), while no significant differences were observed in eTRE group ( $p = 0.49$  Cohen's *d* = 0.08, 95 % CI [-0.27, 0.43], indicating a trivial effect). (Table 1).

A two-way ANOVA examining the interaction effects between time and group confirmed no significant interaction for protein intake (partial  $\eta^2 = 0.03$ ), indicating similar consumption across groups. Significant main effects of time were detected for energy intake (partial  $\eta^2 = 0.24$ ) and carbohydrate intake (partial  $\eta^2 = 0.12$ ).

### 3.2. Effect of eTRE on body composition

Weight, BMI, body fat, and fat-free mass before the intervention were not significantly different ( $p > 0.05$  for all parameters) between the control and experimental groups (Fig. 2).

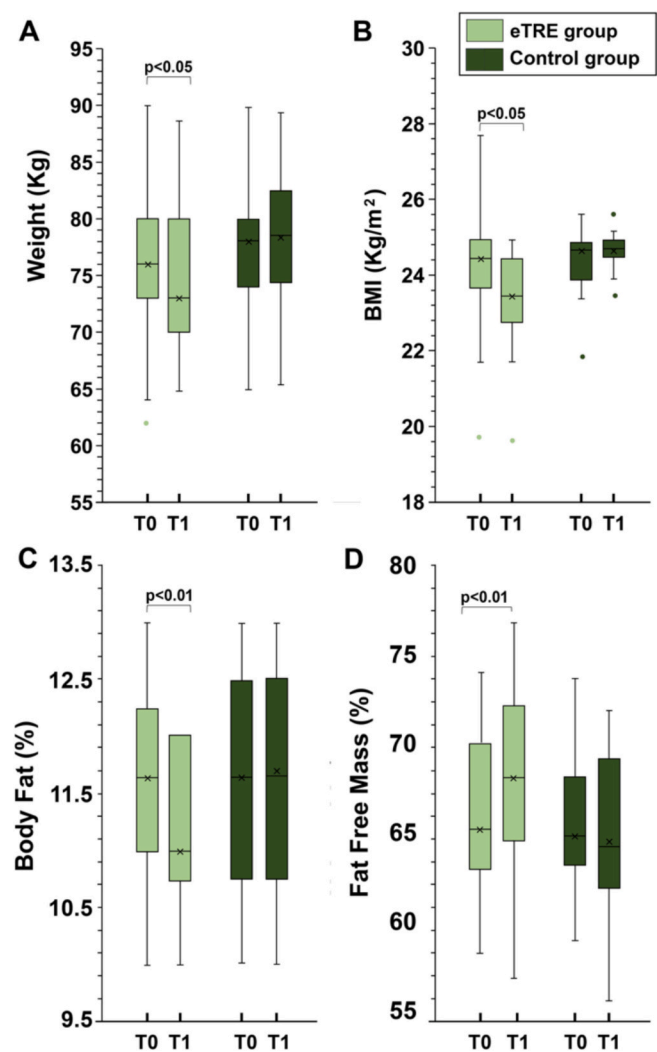
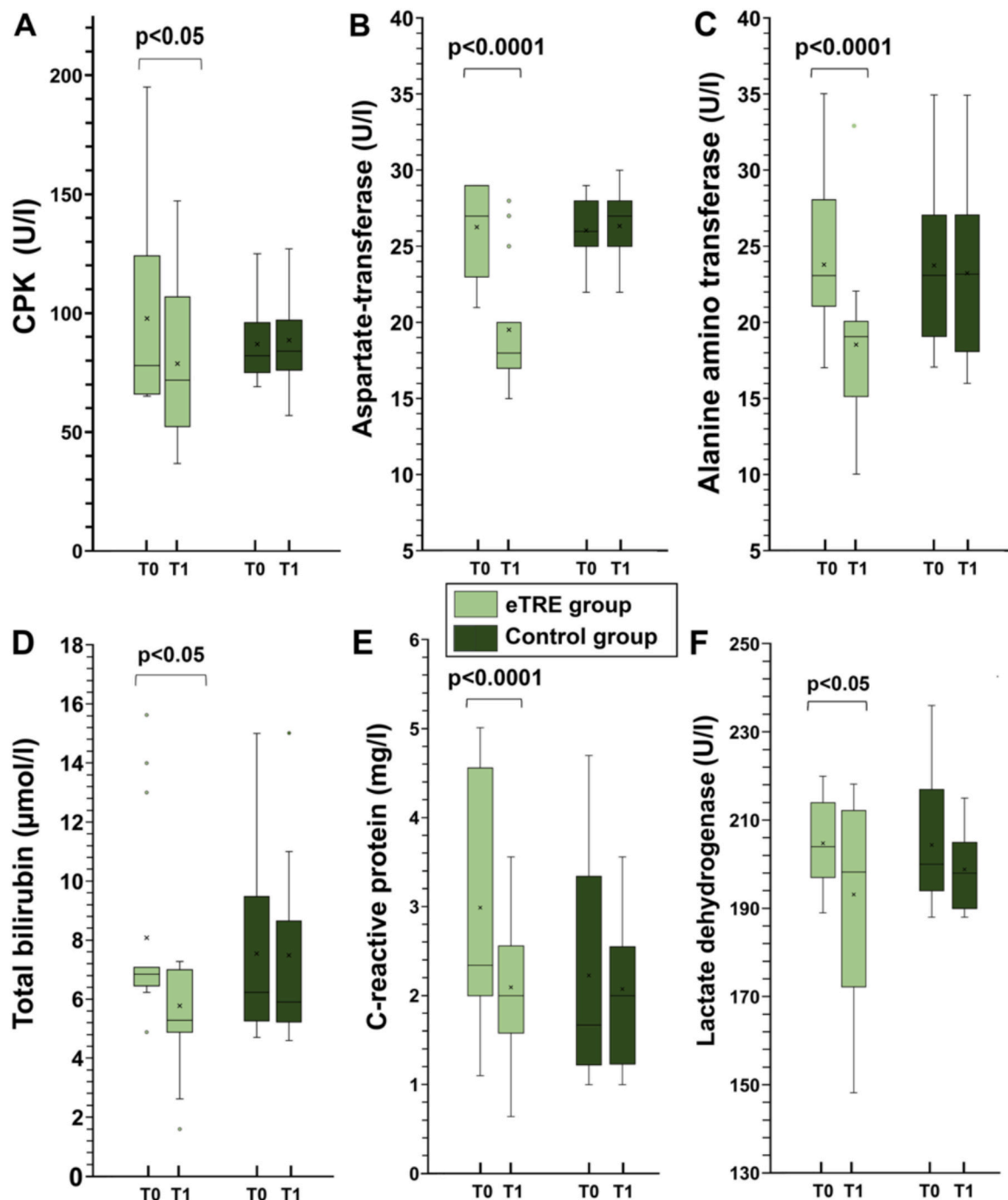


Fig. 2. The effects of early Time-Restricted Eating (eTRE) diet on BMI (A) Body fat (B), and Fat Free Mass (C). Box and whiskers representation of markers evaluated first (T0) and after the 8-week eTRE diet period (T1) and in control group (dark green). In this representation, the central box covers the middle 50 % of the data values, between the upper and lower quartiles. The bars extend out to the extremes, while the central line is at the median. *p*-values for within-group differences between T0 and T1 were calculated using paired Student's *t*-tests and are displayed directly on the graphs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

At T1, significant reductions in weight ( $p = 0.038$ , Cohen's  $d = 0.52$ , 95 % CI [0.03, 1.01], indicating a medium effect), BMI ( $p = 0.003$ , Cohen's  $d = 0.58$ , 95 % CI [0.10, 1.05], indicating a medium effect), and body fat ( $p = 0.039$ , Cohen's  $d = 0.47$ , 95 % CI [0.02, 0.92], indicating a medium effect) were observed in the TRE group compared to the control group (Fig. 2). ANOVA analysis revealed a significant main effect of time for BMI ( $p = 0.029$ , partial  $\eta^2 = 0.13$ ) and fat-free mass ( $p = 0.022$ ,

partial  $\eta^2 = 0.14$ ) for the eTRE group. Additionally, the intervention led to a significant increase in fat-free mass ( $p = 0.005$ , Cohen's  $d = 0.63$ , 95 % CI [0.15, 1.10], Fig. 2D) at post-intervention (T1) compared to pre-intervention (T0).

Two-way ANOVA revealed significant time by group interactions for BMI ( $F_{1,28} = 4.453$ ;  $p = 0.014$ , partial  $\eta^2 = 0.14$ ), body fat ( $F_{1,28} = 3.059$ ;  $p = 0.0037$ , partial  $\eta^2 = 0.10$ ), and fat-free mass ( $F_{1,28} = 3.908$ ;  $p =$



**Fig. 3.** The effects of early Time-Restricted Eating (eTRE) diet on Creatine phosphokinase (CPK) (A) aspartate-transferase (B), total bilirubin (C), C-reactive protein (D) and lactate dehydrogenase (E) concentration in elderly men. Data are presented using box-and-whisker plots illustrating individual variability and distribution. Each panel shows marker concentrations at baseline (T0) and after the 8-week eTRE intervention period (T1) in the intervention group (light green) and in the control group (dark green). In this representation, the central box covers the middle 50 % of the data values, between the upper and lower quartiles. The bars extend out to the extremes, while the central line is at the median. Statistical differences within groups between T0 and T1 were assessed using the Wilcoxon signed-rank test, and p-values are reported for significant changes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

0.014, partial  $\eta^2 = 0.12$ ), indicating that the changes over time differed significantly between experimental and control groups. Specifically, the TRE group showed reductions in BMI and body fat and an increase in fat-free mass, whereas no significant changes were observed in the control group.

### 3.3. Effect of eTRE on systemic inflammation related biomarkers

In the experimental group, a significant decrease in creatine phosphokinase (CPK) levels was observed post-intervention ( $p = 0.024$ , Cohen's  $d = 0.56$ , 95 % CI [0.09, 1.02], indicating a medium effect), suggesting a reduction in muscle damage or physiological stress (Fig. 3A). Significant reductions were also found in aspartate aminotransferase (AST) ( $p = 0.00086$ , Cohen's  $d = 1.97$ , 95 % CI [1.29, 2.62], indicating a very large effect) and alanine aminotransferase (ALT) levels ( $p = 0.00064$ , Cohen's  $d = 1.04$ , 95 % CI [0.52, 1.55], indicating a very large effect), indicating a possible improvement in liver function and/or reduced muscle damage (Fig. 3B).

Additionally, total bilirubin levels significantly decreased following the intervention ( $p = 0.0013$ , Cohen's  $d = 0.84$ , 95 % CI [0.34, 1.33], indicating a very large effect; Fig. 3C). A similar trend was observed for C-reactive protein (CRP), a marker of systemic inflammation, which significantly declined after the intervention ( $p = 0.0007$ , Cohen's  $d = 1.17$ , 95 % CI [0.63, 1.70], indicating a very large effect; Fig. 3D). Lactate dehydrogenase (LDH) levels also showed a significant post-intervention reduction ( $p = 0.015$ , Cohen's  $d = 1.00$ , 95 % CI [0.48, 1.51], indicating a very large effect; Fig. 3E).

Two-way ANOVA revealed significant time by group interactions for several biochemical markers, including uric acid ( $F_{1,28} = 4.947$ ;  $p = 0.004$ , partial  $\eta^2 = 0.15$ ), aspartate aminotransferase (AST) ( $F_{1,28} = 14.332$ ;  $p < 0.001$ , partial  $\eta^2 = 0.34$ ), alanine aminotransferase (ALT) ( $F_{1,28} = 3.44$ ;  $p = 0.023$  partial  $\eta^2 = 0.11$ ), C-reactive protein (CRP) ( $F_{1,28} = 2.853$ ;  $p = 0.045$ , partial  $\eta^2 = 0.09$ ), and lactate dehydrogenase (LDH) ( $F_{1,28} = 4.56$ ;  $p = 0.006$ , partial  $\eta^2 = 0.14$ ). These results suggest that the intervention had a beneficial effect on biochemical markers associated with muscle damage, liver function, and systemic inflammation.

In the control group, we did not find significant differences in any measured parameter. Thus, the experimental group showed improved markers of muscle stress and inflammation compared to the control group.

These positive outcomes suggest that the intervention may support muscle recovery and promote overall muscle health. However, within-group comparisons using the Wilcoxon signed-rank test revealed no significant changes in creatinine ( $p = 0.27$ , Cohen's  $d = 0.22$ , 95 % CI [-0.49, 0.53], indicating a small effect) and uric acid ( $p = 0.13$ , Cohen's  $d = 0.20$ , 95 % CI [-0.31, 0.71], indicating a small effect), levels between pre- and post-intervention (T0 vs. T1) in the experimental group (Fig. 4).

Despite the absence of significant within-group changes, two-way ANOVA showed a significant time  $\times$  group interaction for both creatinine ( $F_{1,28} = 3.321$ ;  $p = 0.026$ , partial  $\eta^2 = 0.11$ ) and uric acid ( $F_{1,28} = 4.947$ ;  $p = 0.004$ , partial  $\eta^2 = 0.15$ ), suggesting that these parameters evolved differently over time between the intervention and control groups.

These findings highlight that, although within-group changes were not significant, the overall interaction effects suggest that the intervention had a differential impact on renal function markers over time, potentially reflecting improved metabolic regulation or reduced physiological stress in the experimental group.

Within-group differences between T0 and T1 were analyzed using the Wilcoxon signed-rank test, and  $p$ -values are reported to highlight statistically significant changes.

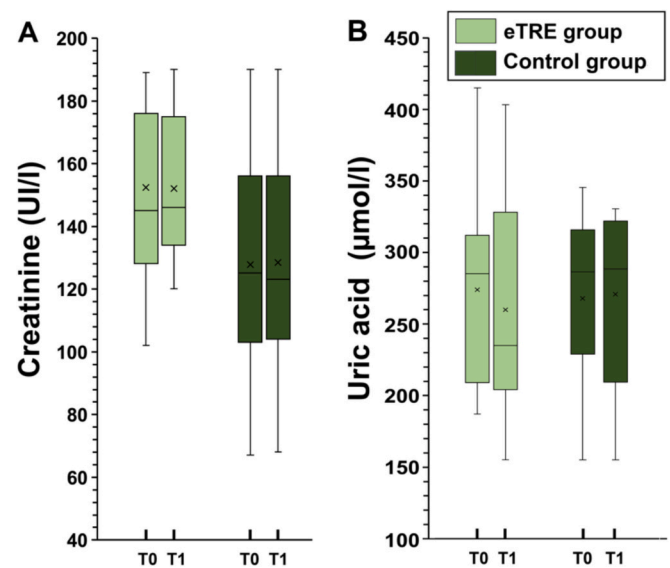


Fig. 4. The effects of early Time-Restricted Eating (eTRE) diet on creatine (A) Creatine phosphokinase and Uric acid (B) concentration in elderly men. Box and whiskers representation of markers evaluated first (T0) and after the 8-week eTRE diet period (T1) and in control group (dark green). In this representation, the central box covers the middle 50 % of the data values, between the upper and lower quartiles. Out to the extremes, while the central line is at the median. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

### 3.4. Effect of eTRE on anxiety state

The State-Trait Inventory for Cognitive and Somatic Anxiety (STICSA) is a self-assessment tool designed to comprehensively evaluate anxiety across four dimensions: cognitive trait, somatic trait, cognitive state, and somatic state. Data collected before and after the eTRE intervention showed a significant overall decrease in anxiety levels.

For instance, the average state anxiety scores declined noticeably following the intervention ( $p < 0.001$ , Cohen's  $d = 0.87$ , 95 % CI [0.47, 1.28], indicating a large effect size; Fig. 5A). Similarly, state somatic anxiety scores also decreased post-TRE (from  $18.27 \pm 2.61$  to  $16.79 \pm 2.46$ ;  $p < 0.0001$ , Cohen's  $d = 0.70$ , 95 % CI [0.33, 1.07], medium-to-large effect; Fig. 5B). Additionally, state cognitive anxiety scores, which reflect anxiety related to cognitive processes and thoughts, exhibited a significant reduction (from  $22.22 \pm 3.26$  to  $19.77 \pm 3.09$ ;  $p < 0.0001$ , Cohen's  $d = 0.87$ , 95 % CI [0.46, 1.27], large effect; Fig. 5C). This suggests that eTRE could help reduce intrusive or distressing thoughts associated with anxiety.

Regarding trait anxiety, which measures the general tendency to experience anxiety across different situations, the results indicate that the eTRE diet has a positive impact on reducing trait anxiety ( $p < 0.0001$ , Cohen's  $d = 1.35$ , 95 % CI [0.85, 1.85], very large effect; Fig. 5D), including both cognitive and somatic aspects. Specifically, trait somatic and trait cognitive anxiety scores showed significant reductions: trait somatic anxiety decreased from  $18.93 \pm 2.14$  to  $16.67 \pm 2.45$  ( $p < 0.0001$ , Cohen's  $d = 0.74$ , 95 % CI [0.35, 1.12], medium-to-large effect; Fig. 5E), and trait cognitive anxiety decreased from  $25.53 \pm 3.07$  to  $21.53 \pm 2.81$  ( $p < 0.0001$ , Cohen's  $d = 0.81$ , 95 % CI [0.39, 1.22], large effect; Fig. 5F).

Two-way ANOVA revealed a significant time by group interaction for STICSA State ( $F_{1,28} = 3.752$ ;  $p = 0.016$ , partial  $\eta^2 = 0.09$ ), STICSA Trait ( $F_{1,28} = 3.316$ ;  $p = 0.026$ , partial  $\eta^2 = 0.09$ ), and STICSA Trait Somatic ( $F_{1,28} = 6.103$ ;  $p = 0.001$ , partial  $\eta^2 = 0.09$ ), indicating a differential reduction in anxiety symptoms over time between the experimental and control groups.

Box and whiskers representation of state anxiety (A-C) and trait

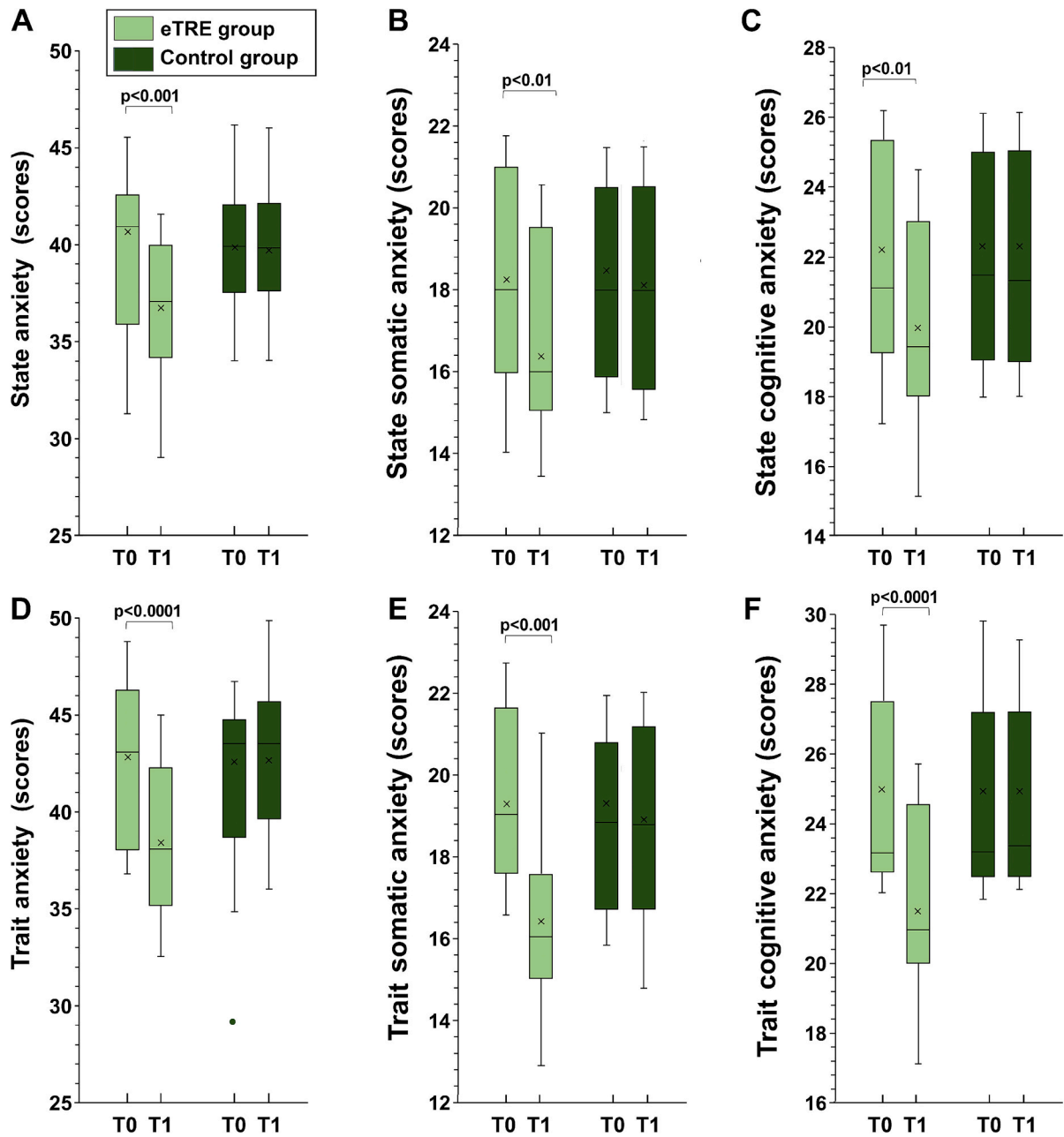


Fig. 5. The effects of early Time-Restricted Eating (eTRE) diet on state and trait anxiety levels in elderly men.

anxiety (D–F) scores evaluated at baseline (T0) and after the 8-week eTRE diet period (T1), and in the control group (dark green). In this representation, the central box covers the middle 50 % of the data values, between the upper and lower quartiles, while the whiskers extend to the most extreme data points not considered outliers. The central line within each box indicates the median value. Within-group differences between T0 and T1 were analyzed using the Wilcoxon signed-rank test, and *p*-values are reported to highlight statistically significant changes.

### 3.5. Effects of eTRE on muscle biomarkers and anxiety scores

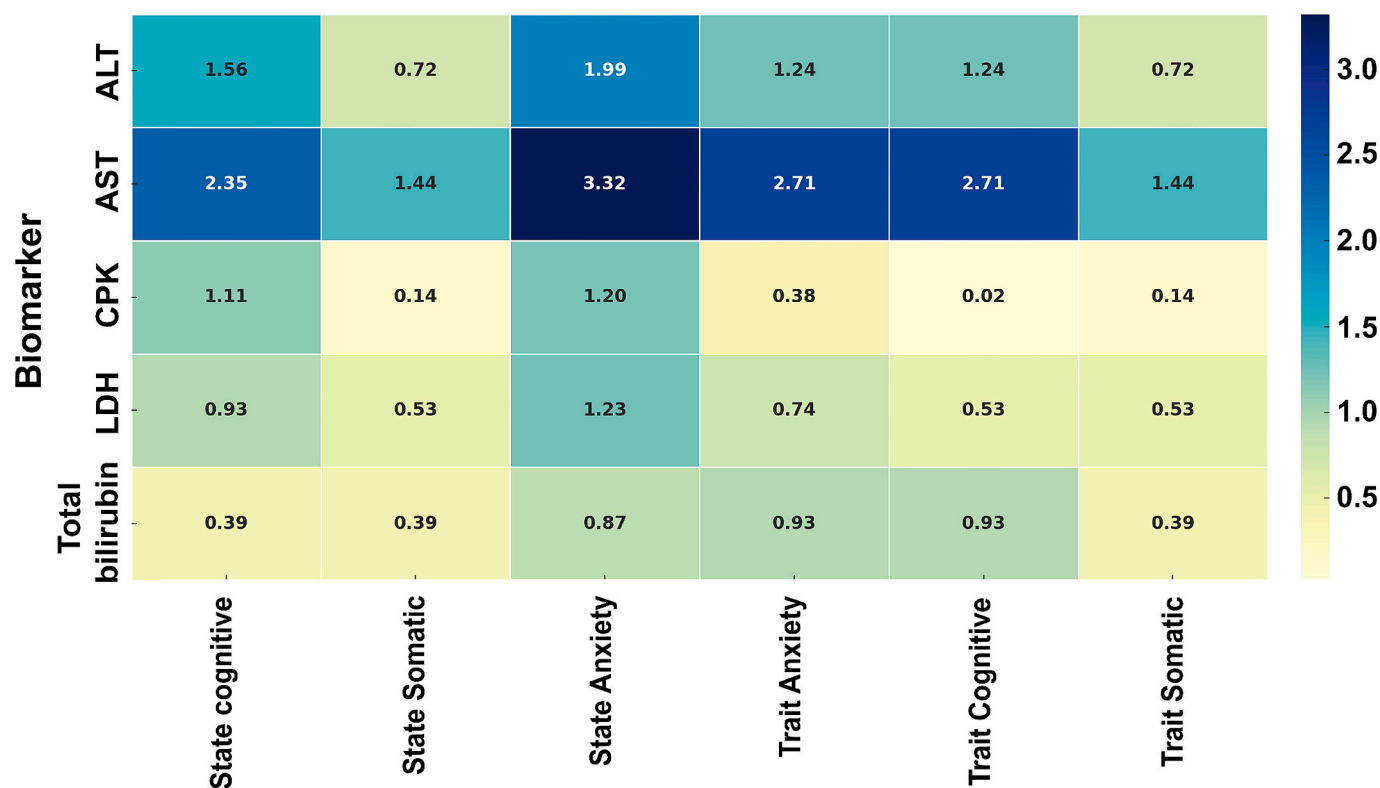
After conducting the mediation analyses, we observed that several muscle biomarkers significantly mediated the relationship between early Time-Restricted Eating (eTRE) and anxiety levels, particularly across the STICSA State and STICSA Trait domains. The heatmap (Fig. 6) illustrates these results, highlighting the biomarkers that played

a significant role in mediating the relationship between the intervention and anxiety scores.

Notably, biomarkers such as CPK, LDH, ALAT, and ASAT demonstrated significant mediation effects. These biomarkers showed varying degrees of influence across different anxiety domains, indicating their important role in the mediation process. On the other hand, CRP and Uric Acid, which did not show significant mediation effects, were excluded from the final analysis. This indicates that while certain biomarkers are key mediators, others do not contribute significantly to the relationship between eTRE and anxiety outcomes (Fig. 6).

## 4. Discussion

The study examined the effects of early Time-Restricted Eating (eTRE) on various health parameters in elderly men. The results indicate significant positive changes in body composition for those in the experimental group. Specifically, there was a significant reduction in



**Fig. 6.** Heatmap of the Indirect Effects of Biomarkers in Mediating the Relationship Between eTRE and Anxiety. The heatmap illustrates the indirect effects of various biomarkers on the relationship between **Group** (eTRE vs. control) and **anxiety scores** across six domains: **State Anxiety, Trait Anxiety, Cognitive Anxiety, Somatic Anxiety, Trait Cognitive Anxiety, and Trait Somatic Anxiety**, measured using the **State-Trait Anxiety Inventory (STICSA)**. The numbers inside each cell represent the **magnitude of the indirect effect ( $\beta_1 \times \beta_2$ )**, where **larger values indicate stronger mediation**. The **colour gradient** ranges from light yellow (weaker mediation) to dark blue (stronger mediation). Biomarkers that did not show significant mediation effects have been excluded from the heatmap.

weight, BMI, and body fat, while fat-free mass significantly increased. Additionally, we explored the impact of eTRE on muscle damage-related biomarkers. There was a significant decrease in creatine phosphokinase (CPK), aspartate aminotransferase (AST), total bilirubin, C-reactive protein (CRP), and lactate dehydrogenase (LDH) levels, suggesting a reduction in systemic inflammation and a potential decrease in muscle damage.

The observed significant reductions in inflammatory markers not only suggest a decrease in systemic inflammation and muscle damage but may also be mechanistically linked to the improvement in anxiety symptoms observed in the eTRE group.

Chronic systemic inflammation has been widely implicated as a contributing factor in the pathophysiology of anxiety disorders, particularly in older adults, where low-grade inflammation (“inflammaging”) can exacerbate neuroimmune dysfunction and mood regulation (Felger and Lotrich, 2013; Miller and Raison, 2016). Therefore, the modulation of these inflammatory biomarkers through eTRE may underlie, at least in part, the psychological benefits, highlighting the importance of targeting inflammation in interventions aimed at improving mental health in aging populations.

The study also evaluated the effects of eTRE on anxiety using the State-Trait Inventory for Cognitive and Somatic Anxiety (STICSA), a comprehensive self-assessment tool. This assessment aimed to expand the psychometric understanding of STICSA by evaluating its dimensionality, reliability, measurement invariance, and nomological validity specifically in older men following the eTRE diet. This part of the study aimed to provide deeper insights into the psychological effects of the eTRE intervention on older adults.

Recent studies indicate that eTRE may significantly benefit older adult men by improving metabolic health, body composition, and

overall well-being (Mishra et al., 2023). Notably, the observed increase in fat-free mass alongside reductions in fat mass suggests that eTRE may help preserve or even enhance muscle mass—an outcome of particular importance given the common age-related loss of lean tissue.

This observation is consistent with previous studies that reported significant reductions in body weight, BMI, and body fat with eTRE protocols lasting from 4 to 14 weeks, and with eating windows ranging from 8 to 16 h, across various populations including healthy young adults and individuals with obesity (Ameur et al., 2024; Jamshed et al., 2022).

However, other research has reported negligible or insignificant effects of eTRE on body weight, particularly in men with prediabetes and healthy men (Jones et al., 2020; Sutton et al., 2018). Such discrepancies might arise from differences in protocol design, including the duration of eating windows, timing relative to circadian rhythms, participants' baseline health, and adherence levels. For instance, some studies utilized shorter intervention periods or different fasting schedules, which may not elicit comparable physiological adaptations. Furthermore, the timing of fasting onset relative to seasonality could affect energy expenditure and hormonal responses, as suggested by recent chronobiology research (Kim and Song, 2023). Such heterogeneity in study designs and participant characteristics complicates the ability to draw firm conclusions on the efficacy of eTRE across populations.

Regarding biomarkers associated with muscle and liver stress and systemic inflammation, our study revealed significant decreases in CPK, AST, ALT, LDH, total bilirubin, and CRP levels. These changes suggest possible improvements in muscle metabolic status, hepatic function, and a reduction in systemic inflammatory state. However, it is important to note that elevations in CK and LDH are not specific to muscle damage and may also reflect adaptive metabolic responses or changes in physical

activity levels. These findings are particularly relevant given the scarcity of robust clinical trials investigating these biomarkers within the context of eTRE, especially in older populations.

Research has indicated significant improvements in metabolic profiles, including glucose levels and markers of the circadian clock, which are linked to better muscle function and repair (Anton et al., 2019; Sundaram and Yan, 2018). These metabolic adaptations may indirectly support muscle health by enhancing insulin sensitivity, reducing oxidative stress, and modulating anabolic-catabolic balance.

While research on eTRE has highlighted numerous health benefits, especially for older adults, there remains a paucity of studies specifically investigating its impact on biomarkers associated with muscle stress and recovery and overall muscle health, thereby limiting the strength of current conclusions (Gasmı et al., 2018; Mulas et al., 2023).

Maintaining general muscle function is critical for mobility, balance, and overall health in older men. For older men, a multifaceted approach is necessary to optimize muscle health and recovery.

The significant decrease in muscle damage-related biomarkers levels observed in the study suggests a notable reduction in muscle damage and systemic inflammation.

Specifically, lower creatine kinase (CK) levels may suggest reduced muscle membrane disruption and a lower degree of muscle stress or inflammation, although CK is a non-specific marker and elevations can also occur in various physiological and metabolic contexts (Brancaccio et al., 2007). The observed reduction in CK levels might also reflect an overall decrease in systemic inflammatory responses, which is relevant for the prevention of chronic diseases (Ridker et al., 2000).

Similarly, decreased aspartate aminotransferase (AST) and total bilirubin levels could indicate improved liver function and a reduction in tissue stress or muscle turnover (Wang et al., 2016). However, it is important to interpret these biomarkers cautiously, as they are influenced by multiple physiological processes and do not provide a definitive diagnosis of muscle or liver damage in the absence of clinical symptoms.

Bilirubin, a byproduct of heme metabolism, can be influenced by various factors, including liver function and oxidative stress (Nitti et al., 2020). While IF has been shown to improve metabolic health and reduce inflammation (Diab et al., 2024), its impact on bilirubin levels specifically remains unclear.

Contradictory findings in the literature regarding bilirubin necessitate cautious interpretation of its changes, highlighting the need for further targeted investigations (Kalhor et al., 2020). While some investigations have shown an increase in bilirubin during IF (Kalhor et al., 2020), the overall evidence is sparse, and further research is needed to clarify the relationship between IF and bilirubin levels.

Aspartate aminotransferase and alanine aminotransferase are enzymes commonly used to assess liver health. Elevated levels of AST and ALT can indicate liver damage or inflammation. Previous research has shown that caloric restriction and IF, including eTRE, can improve liver function markers by reducing oxidative stress and inflammation, which are common in metabolic disorders and aging (Kord-Varkaneh et al., 2023; Lange et al., 2023).

These findings corroborate the present study's results and further suggest a mechanistic link between eTRE and improved hepatic resilience in aging.

Lastly, since LDH is a multi-organ damage marker that affects more than only liver and heart function (Tegeler et al., 2016), lower LDH levels, an enzyme associated with tissue damage, further corroborate the beneficial effects of the intervention on tissue health (Kalhor et al., 2020).

High levels of C-reactive protein (CRP) are commonly observed in older adults being associated with the state of chronic low-grade inflammation that characterizes the aging process (Tegeler et al., 2016) and contributes to the development of age-related diseases (Kushner, 2001; Wyczalkowska-Tomasik et al., 2016; Løfblad et al., 2021).

During chronic conditions such as cardiovascular disease, diabetes, and metabolic syndrome, CRP may be a useful marker for evaluating inflammatory status (Libby, 2012; Ridker et al., 2000). In addition, elevated CRP levels also reflect ongoing neuroinflammation and neurodegenerative diseases such as Alzheimer's and Parkinson's disease and are associated with cognitive decline (Qiu et al., 2019; Luan and Yao, 2018).

Therefore, tracking CRP in the context of dietary and lifestyle modifications can help assess their impact on systemic inflammation, potentially serving as a surrogate marker for intervention efficacy in older adults. Because potentially reducing age-related inflammatory responses, improves overall health in older populations.

Recent studies have shown that some nutritional regimens, such as the Mediterranean diet, rich in antioxidants and phenolic compounds characterized by anti-inflammatory properties, are associated with a lower concentration of CRP in older men (Salas-Salvadó et al., 2008). In agreement with our findings, Ramadan fasting (Maaloul et al., 2023) and eTRE reduced CRP levels (James et al., 2024) or on the contrary remained unchanged (Mulas et al., 2023). These mixed results highlight the complex and multifactorial nature of inflammation modulation by dietary interventions, emphasizing the relevance of our study's contribution to this field.

Therefore, the observed CRP reduction in the elderly experimental group following eTRE likely reflects beneficial changes in metabolic health and inflammation.

Regarding biomarkers of renal function, some studies have reported unaltered levels of uric acid (Ashkbari et al., 2024), while others showed an increase (Mohamed et al., 2023). In addition, studies inconsistently showed no change (Bragazzi, 2015; Zouhal et al., 2020) or an increase (Maughan et al., 2008) in creatinine levels. In the present study, there were no significant changes concerning these two parameters, aligning with the findings of studies that reported stable biomarker levels despite dietary interventions.

This suggests that the eTRE regimen employed may not significantly affect these renal function markers, or that any potential changes might be subtle and not detectable within the study's timeframe. However, longer interventions may reveal different outcomes, which future research should explore.

These findings collectively underscore the potential of eTRE as a valuable strategy for enhancing muscle and liver health and reducing inflammation in older adults (Moro et al., 2016; Gasmı et al., 2018).

By improving metabolic and inflammatory profiles, eTRE can support better muscle recovery and overall function, which are critical for older adults (Moro et al., 2016; Gasmı et al., 2018).

In summary, eTRE shows promise in improving muscle health and recovery in older adult men by modulating metabolic and inflammatory responses, thereby aiding in muscle stress management and repair.

Our results indicate that the **eTRE diet** has a positive impact on reducing both **state** and **trait anxiety**, including cognitive and somatic aspects, among elderly men. These findings align with previous research suggesting that dietary patterns and metabolic health can significantly influence mental well-being (Wheaton et al., 2020; Gabel et al., 2018; Bowers et al., 2019).

Although direct studies specifically linking TRE with improved anxiety in older adults are relatively limited, emerging studies are beginning to explore related concepts and mechanisms.

For example, eTRE can improve several health markers that might indirectly affect anxiety levels, such as oxidative stress and insulin sensitivity, which are connected to mental health (Sutton et al., 2018; Manoogian and Panda, 2017; Moro et al., 2016; Lin et al., 2023).

Another important aspect is the regulation of circadian rhythms. eTRE aligns eating patterns with the body's natural circadian rhythms, which can improve overall metabolic health and stabilize mood. By eating within a specific time window, usually during daylight hours, eTRE helps reset and maintain these rhythms, leading to better mental health outcomes (Sharifi et al., 2024; Longo and Panda, 2016).

Improved sleep quality also plays a significant role. As people age, sleep quality often declines, and poor sleep is a major risk factor for anxiety. eTRE can improve sleep patterns by regulating melatonin production and reducing late-night eating, which can interfere with sleep. Better sleep quality has a direct positive impact on anxiety levels (Lowe et al., 2019).

Furthermore, eTRE helps in the regulation of neurotransmitters. Eating at regular intervals can stabilize blood sugar levels, which in turn helps regulate the production and function of neurotransmitters like serotonin and dopamine. These neurotransmitters are crucial for mood regulation, and stable levels can reduce anxiety symptoms (Chaix et al., 2014).

In summary, while more research is needed to fully understand the direct link between reduced muscle damage and improved anxiety, the evidence suggests that minimizing muscle damage and the associated inflammatory response may alleviate some of the physiological contributors to anxiety.

Evidence suggesting that a decrease in markers of muscle damage may be linked to improvements in anxiety, although this connection is not fully understood and is an area of active research. Muscle damage often results in inflammation, which is a key factor in the development of anxiety. When muscle damage occurs, inflammatory cytokines are released as part of the body's repair process. These cytokines can cross the blood-brain barrier and affect brain function, potentially leading to mood disorders such as anxiety (Dantzer et al., 2008).

Reducing muscle damage, lead to reduction in systemic inflammation that can, in turn, have a positive impact on mental health by lowering anxiety levels (Gleeson et al., 2011).

However, the specific connection between systemic inflammation and muscle damage in relation to anxiety following eTRE has not been fully explored. Therefore, to examine the role of various biomarkers as mediators in the relationship between eTRE and anxiety scores, mediation analyses were conducted to investigate how these biomarkers, systematically assessed during eTRE, mediate the effects of the intervention on anxiety levels.

The results show that certain biomarkers, such as CPK, LDH, ALT, and AST, significantly mediated the effects of eTRE on anxiety levels across these domains. These findings suggest that metabolic and biochemical processes are involved in the physiological mechanisms by which eTRE influences anxiety. Interestingly, biomarkers related to inflammation and metabolic regulation, such as CRP and Uric Acid, did not show significant mediation effects, suggesting that the impact of eTRE on anxiety might be less related to these factors within the scope of this study.

The distinction between State and Trait Anxiety is crucial for understanding how biomarkers mediate the relationship between eTRE and anxiety. State Anxiety refers to temporary anxiety triggered by specific events (e.g., exams), while Trait Anxiety is a general predisposition to feel anxious.

State anxiety is often linked to acute stress responses, while Trait anxiety relates to chronic stress and neurobiological imbalances (Daviu et al., 2019). Our findings suggest that biomarkers like CPK, LDH, ALT, and AST are more strongly associated with changes in State anxiety, consistent with their roles in metabolic responses and muscle recovery after short-term stress. The lack of significant mediation by biomarkers such as CRP and Uric Acid indicates that inflammation and metabolic regulation may not be key factors in mediating State anxiety in the short term. This aligns with studies suggesting that inflammatory cytokines are more relevant to chronic anxiety (Rohleder, 2014).

In contrast, Trait anxiety may involve biomarkers linked to long-term stress, such as cortisol and neurotransmitters (Noushad et al., 2021).

On the other hand, Trait Anxiety, a more enduring predisposition to anxiety, may involve biomarkers linked to long-term stress, such as cortisol and neurotransmitters (Noushad et al., 2021), and was mediated by biomarkers related to longer-term metabolic regulation and muscle health, specifically Total Bilirubin, ALT, and AST, which were found to

be significant in this study. These findings suggest that eTRE helps reduce both acute and chronic anxiety by influencing biochemical pathways that regulate muscle recovery, metabolic health, and inflammation.

The distinction between Cognitive Anxiety and Somatic Anxiety is also critical in understanding how biomarkers mediate eTRE's effects on anxiety.

Somatic Anxiety, which is linked to autonomic nervous system (ANS) activity, could be influenced by biomarkers such as Heart Rate Variability (HRV), blood pressure, and adrenaline (McEwen, 2006). In line with this, biomarkers related to HRV, and the sympathetic nervous system are likely more strongly associated with somatic symptoms of anxiety, such as heart racing and muscle tension. However, biomarkers like LDH, ALT, and AST were found to mediate Somatic Anxiety, highlighting their role in muscle recovery and metabolic regulation. These biomarkers also had meaningful effects on Trait Anxiety, suggesting their broader influence on both physical and emotional responses to stress.

Cognitive Anxiety, which is associated with worry, rumination, and anticipation of negative outcomes (Chaix et al., 2014), could be influenced by biomarkers related to muscle recovery, such as Total Bilirubin, LDH, ALT, AST, and CPK, which mediate the cognitive symptoms of anxiety, further emphasizing the role of metabolic biomarkers in emotional regulation and the interplay between physiological and psychological responses to stress, consistent with previous research suggesting that muscle recovery biomarkers influence cognitive aspects of anxiety through biological feedback loops (Vints et al., 2024; Kim et al., 2007).

In conclusion, these results provide evidence that biomarkers associated with muscle recovery and metabolic regulation mediate the relationship between eTRE and anxiety. These findings contribute to our understanding of how biological mechanisms, such as inflammation, muscle repair, and neurotransmitter regulation, underpin the effects of eTRE on mental health. By integrating biomarker research into interventions like eTRE, we can potentially improve both physical and emotional well-being, offering a promising approach to managing anxiety, particularly among elderly adults.

Our study is not without limitations. Firstly, the sample size was relatively small, which may limit the generalizability of the findings. However, this sample size met the minimum calculated a priori to ensure adequate statistical power (80 %) based on an assumed medium effect size, and no dropouts occurred during the study, thus preserving the planned power. To enhance the applicability of the results, future studies should include larger and more diverse populations. Additionally, the intervention period was short, restricting the ability to observe the long-term effects and sustainability of early Time-Restricted Eating. Longitudinal studies are needed to assess the prolonged impact of eTRE on health outcomes. Furthermore, while eTRE focuses on meal timing, this study did not closely monitor dietary composition and caloric intake, which could influence the observed effects. Future studies should include detailed dietary tracking to isolate the effects of meal timing from those of diet quality.

In conclusion, eTRE offers a range of potential benefits for older adult men, including improvements in body composition, liver function markers, muscle damage biomarkers and inflammation. Additionally, eTRE diet positively reduces state and trait anxiety, encompassing cognitive and somatic aspects, among elderly men. While more research is needed to fully understand the long-term effects and optimal implementation of eTRE, the current evidence suggests that it could be a valuable dietary strategy for enhancing health and well-being in older men. The mediation analysis further highlights the role of biomarkers in the relationship between eTRE and anxiety reduction, suggesting that metabolic and muscle recovery pathways are integral to both physical and emotional well-being. These findings pave the way for future research to explore the long-term impact of eTRE on mental health and its potential to be integrated into health promotion strategies for aging

populations.

## 5. Conclusion

In older men, muscle stress, repair, and general function are key components that influence muscle health and recovery. Addressing these aspects can help mitigate the effects of aging, such as sarcopenia, and improve quality of life.

Maintaining muscle health and facilitating effective recovery in older men requires a comprehensive approach that addresses muscle stress, repair, and general function.

eTRE shows promise in improving muscle health and recovery in older adult men by modulating metabolic and inflammatory responses, thereby aiding in muscle stress management and repair. Intermittent fasting that confines eating to the early part of the day, improves anxiety in elderly men by reducing inflammation. These eTRE combined effects contribute to better overall health and reduced anxiety symptoms.

While the benefits of eTRE are promising, practical considerations and challenges must be addressed, especially for older adults. Adhering to a strict eating window can be difficult, and individual preferences, lifestyles, and health conditions should be considered when recommending eTRE. Ensuring adequate nutrient intake within the restricted eating window is essential to prevent deficiencies and support overall health.

In conclusion, early time-restricted eating offers a range of potential benefits for older adult men, including improvements in metabolic health, body composition, cardiovascular health, and inflammation. While more research is needed to fully understand the long-term effects and optimal implementation of eTRE, the current evidence suggests that it could be a valuable dietary strategy for enhancing health and well-being in older men.

## CRedit authorship contribution statement

**Maha Gasmí:** Writing – review & editing, Validation, Investigation, Data curation, Conceptualization. **Antonella Muscella:** Writing – original draft, Visualization, Validation, Supervision, Formal analysis, Conceptualization. **Santo Marsigliante:** Writing – review & editing, Validation, Funding acquisition, Formal analysis. **Kawther Missawi:** Writing – review & editing, Validation, Data curation. **Riadh Khalifa:** Writing – review & editing, Validation, Supervision.

## Research ethics

We further confirm that any aspect of the work covered in this manuscript that has involved human participants has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

IRB approval was obtained.

## Funding

No funding was received for this work.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## References

- Adbi, A., Shahmohammadi, S., Yazdani, F., Hashemi, H., 2013. Serum enzymes of muscle damage: comparison of two methods of resistance training. *J. Exerc. Physiol. Online* 16 (5), 25–34.
- Ameur, R., Maaoulou, R., Tagougui, S., Neffati, F., Hadj Kacem, F., Najjar, M.F., Ammar, A., Hammouda, O., 2024. Unlocking the power of synergy: High-intensity functional training and early time-restricted eating for transformative changes in body composition and cardiometabolic health in inactive women with obesity. *PLoS One* 19 (5). <https://doi.org/10.1371/journal.pone.0301369>.
- Anton, S.D., Lee, S.A., Donahoo, W.T., McLaren, C., Manini, T., Leeuwenburgh, C., Martin, B., 2019. The effects of time restricted feeding on overweight, older adults: a pilot study. *Nutrition and Healthy Aging* 5 (1), 23–24.
- Ashkbari, A., Nikbakht, H.A., Amirhanlou, S., Elahi, G., Salahi, M., Ebrahimi, S., Golfroozi, S., Hosseini, S.A., Ghelichi-Ghojogh, M., 2024. Jun. Impact of Ramadan fasting on lipid profile, uric acid, and HbA1c in CKD: a systematic review and meta-analysis. *Prim. Care Diabetes* 18 (3), 277–283. <https://doi.org/10.1016/j.pcd.2024.03.007>.
- Barros, F., Figueiredo, C., Brás, S., Carvalho, J.M., Soares, S.C., 2022. Multidimensional assessment of anxiety through the State-Trait Inventory for Cognitive and Somatic Anxiety (STICSA): From dimensionality to response prediction across emotional contexts. *PLoS One* 17 (1), e0262960. <https://doi.org/10.1371/journal.pone.0262960>. Jan 25.
- Billot, M., Calvani, R., Urtamo, A., Sánchez-Sánchez, J.L., Ciccolari-Micaldi, C., Chang, M., Roller-Wirnsberger, R., Wirnsberger, G., Sinclair, A., Vaquero-Pinto, N., Jyväkorpi, S., Öhman, H., Strandberg, T., Schols, J.M.G.A., Schols, A.M.W.J., Smeets, N., Topinkova, E., Michalkova, H., Bonfigli, A.R., Lattanzio, F., Rodríguez-Mañas, L., Coelho-Júnior, H., Broccatelli, M., D'Elia, M.E., Biscotti, D., Marzetti, E., Freiberg, E., 2020. Preserving mobility in older adults with physical frailty and sarcopenia: opportunities, challenges, and recommendations for physical activity interventions. *Clin. Interv. Aging* 16 (15), 1675–1690. <https://doi.org/10.2147/CIA.S253535>. Sep.
- Bowers, A.W., Khosla, P., Bhatti, J.S., 2019. Impact of time-restricted eating on cognitive function and mental health in older adults. *J. Clin. Psychol.* 75 (6), 1127–1136. <https://doi.org/10.1002/jclp.22740>.
- Bragazzi, N.L., 2015. Ramadan fasting and chronic kidney disease: does estimated glomerular filtration rate change after and before Ramadan? Insights from a mini meta-analysis. *Int. J. Nephrol. Renov. Dis.* 8 (Jun 1), 53–57. <https://doi.org/10.2147/IJNRD.S61718>.
- Brancaccio, P., Lippi, G., & Maffulli, N. (2010). Biochemical markers of muscular damage. *Clinical Chemistry and Laboratory Medicine (CCLM)*, 48(6), 757–767. cclm.
- Brancaccio, P., Maffulli, N., Limongelli, F.M., 2007. Creatine kinase monitoring in sport medicine. *Br. Med. Bull.* 81-82 (1), 209–230. <https://doi.org/10.1093/bmb/ldm014>.
- Cannataro R, Cione E, Bonilla DA, Cerullo G, Angelini F, D'Antona G. Strength training in elderly: a useful tool against sarcopenia. *Front Sports Act Living.* 2022Jul 18;4: 950949. doi: <https://doi.org/10.3389/fspor.2022.950949>.
- Chaix, A., Zarrinpar, A., Miu, P., Panda, S., 2014. Time-restricted feeding is a preventative and therapeutic intervention against diverse nutritional challenges. *Cell Metab.* 20 (6), 991–1005. <https://doi.org/10.1016/j.cmet.2014.11.001>.
- Chen M, Wang Y, Deng S, Lian Z, Yu K. Skeletal muscle oxidative stress and inflammation in aging: focus on antioxidant and anti-inflammatory therapy. *Front. Cell Dev. Biol.* 2022Aug 30;10:964130. doi: <https://doi.org/10.3389/fcell.2022.964130>.
- Dantzer, R., O'Connor, J.C., Freund, G.G., Johnson, R.W., Kelley, K.W., 2008. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat. Rev. Neurosci.* 9 (1), 46–56.
- Daviu, N., Bruchas, M.R., Moghaddam, B., Sandi, C., Beyeler, A. Neurobiological links between stress and anxiety. *Neurobiol Stress.* 2019Aug 13;11:100191. doi: <https://doi.org/10.1016/j.ynstr.2019.100191>.
- Diab, R., Dimachkie, L., Zein, O., Dakroub, A., Eid, A.H., 2024. Intermittent fasting regulates metabolic homeostasis and improves cardiovascular health. *Cell Biochem. Biophys.* <https://doi.org/10.1007/s12013-024-01314-9>.
- Dowlati, Y., Herrmann, N., Swardfager, W., Liu, H., Sham, L., Reim, E.K., Lancot, K.L., 2010. A meta-analysis of cytokines in major depression. *Biol. Psychiatry* 67, 446–457.
- Felger, J.C., Lotrich, F.E., 2013. Inflammatory cytokines in depression: neurobiological mechanisms and therapeutic implications. *Neuroscience* 246, 199–229. <https://doi.org/10.1016/j.neuroscience.2013.04.060>. Aug 29.
- Gabel, K., Kalani, M., Varady, K.A., 2018. Effect of intermittent fasting and time-restricted eating on metabolic health, and the potential mechanisms of action. *Nutr. Rev.* 76 (1), 1–19. <https://doi.org/10.1093/nutrit/nux069>.
- Gasmí M, Sellami M, Denham J, Padulo J, Kuvacic G, Selmi W, Khalifa R. Time-restricted feeding influences immune responses without compromising muscle performance in older men. *Nutrition* 2018Jul-Aug;51-52:29–37. doi: <https://doi.org/10.1016/j.nut.2017.12.014>.
- Gleeson, M., Bishop, N.C., Stensel, D.J., Lindley, M.R., Mastana, S.S., Nimmo, M.A., 2011. The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. *Nat. Rev. Immunol.* 11 (9), 607–615.
- Gonçalves, R.L., Quinlan, C.L., Perevoshchikova, I.V., Hey-Mogensen, M., Brand, M.D., 2015. Sites of superoxide and hydrogen peroxide production by muscle mitochondria assessed ex vivo under conditions mimicking rest and exercise. *J. Biol. Chem.* 290 (1), 209–227.
- Gros, D.F., Simms, L.J., Antony, M.M., 2010. Psychometric properties of the state-trait inventory for cognitive and somatic anxiety (STICSA) in friendship dyads. *Behav. Ther.* 41 (3), 277–284. <https://doi.org/10.1016/j.beth.2009.07.001>.

- Hertogh, E.M., Monninkhof, E.M., Schouten, E.G., Peeters, P.H., Schuit, A.J., 2008. Validity of the modified Baecke questionnaire: comparison with energy expenditure according to the doubly labeled water method. *Int. J. Behav. Nutr. Phys. Act.* 27 (5), 30. <https://doi.org/10.1186/1479-5868-5-30>. May.
- James DL, Hawley NA, Mohr AE, Hermer J, Ofori E, Yu F, Sears DD. Impact of intermittent fasting and/or caloric restriction on aging-related outcomes in adults: a scoping review of randomized controlled trials. *Nutrients* 2024, Jan 20;16(2):316. doi: <https://doi.org/10.3390/nu16020316>.
- Jamshed, H., Steger, F.L., Bryan, D.R., Richman, J.S., Warriner, A.H., Hanick, C.J., Martin, C.K., Salvy, S.J., Peterson, C.M., 2022. Effectiveness of Early time-restricted eating for weight loss, fat loss, and Cardiometabolic health in adults with obesity: a randomized clinical trial. *JAMA Intern. Med.* 182 (9), 953–962. <https://doi.org/10.1001/jamainternmed.2022.3050>. Sep 1.
- Jones, R., Pabla, P., Mallinson, J., Nixon, A., Taylor, T., Bennett, A., Tsintzas, K., 2020. Two weeks of early time-restricted feeding (eTRF) improves skeletal muscle insulin and anabolic sensitivity in healthy men. *Am. J. Clin. Nutr.* 112 (4), 1015–1028. <https://doi.org/10.1093/ajcn/nqaa192>. Oct 1.
- Kaczmarek A, Kaczmarek M, Ciałowicz M, Clemente FM, Wolański P, Badicu G, Murawska-Giałowicz E. The role of satellite cells in skeletal muscle regeneration—the effect of exercise and age. *Biology (Basel)*. 2021Oct 18;10(10):1056. doi: <https://doi.org/10.3390/biology10101056>.
- Kalhor, M., Malekpour, K., Pazouki, A., et al., 2020. The effects of Islamic fasting on blood biochemical parameters in morbidly obese patients undergone bariatric surgery. *Indian J. Surg.* <https://doi.org/10.1007/s12262-020-02231-w>.
- Kavelaars, R., Ward, H., Mackie, D.S., Modi, K.M., Mohandas, A., 2023. The burden of anxiety among a nationally representative US adult population. *J. Affect. Disord.* 336, 81–91. <https://doi.org/10.1016/j.jad.2023.04.069>. Sep 1.
- Kim, H.J., Lee, Y.H., Kim, C.K., 2007. Biomarkers of muscle and cartilage damage and inflammation during a 200 km run. *Eur. J. Appl. Physiol.* 99 (4), 443–447. <https://doi.org/10.1007/s00421-006-0362-y>. Mar.
- Kim, J., Song, Y., 2023. Early time-restricted eating reduces weight and improves glycemic response in young adults: a pre-post single-arm intervention study. *Obes. Facts* 16 (1), 69–81. <https://doi.org/10.1159/000527838>.
- Kord-Varkaneh H, Salehi-Sahlabadi A, Tinsley GM, Santos HO, Hekmatdoost A. Effects of time-restricted feeding (16/8) combined with a low-sugar diet on the management of non-alcoholic fatty liver disease: a randomized controlled trial. *Nutrition* 2023, 105:111847. doi: <https://doi.org/10.1016/j.nut.2022.111847>.
- Kushner, I., 2001. C-reactive protein elevation can be caused by conditions other than inflammation and may reflect biologic aging. *Cleve. Clin. J. Med.* 68 (6), 535–537. <https://doi.org/10.3949/cjcm.68.6.535>.
- Lange M, Nadkarni D, Martin L, Newberry C, Kumar S, Kushner T. Intermittent fasting improves hepatic end points in nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Hepatal. Commun.* 2023, Aug 3;7(8):e0212. doi: <https://doi.org/10.1097/HCG.0000000000000212>.
- Larsson, L., Degens, H., Li, M., Salvati, L., Lee, Y.I., Thompson, W., Kirkland, J.L., Sandri, M., 2019. Sarcopenia: aging-related loss of muscle mass and function. *Physiol. Rev.* 99 (1), 427–511. <https://doi.org/10.1152/physrev.00061.2017>. Jan 1.
- Li, D.C.W., Rudloff, S., Langer, H.T., Norman, K., Herpich, C., 2024. Age-associated differences in recovery from exercise-induced muscle damage. *Cells* 13 (3), 255. <https://doi.org/10.3390/cells13030255>. Jan 30.
- Libby, P., 2012. Inflammation in atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* 32 (11), 2045–2051. <https://doi.org/10.1161/ATVBAHA.108.179705>.
- Lin S, Cienfuegos S, Ezpeleta M, Pavlou V, Chakos K, McStay M, Runcney MC, Alexandria SJ, Varady KA. Effect of time-restricted eating versus daily calorie restriction on mood and quality of life in adults with obesity. *Nutrients* 2023Oct 10;15(20):4313. doi: <https://doi.org/10.3390/nu15204313>.
- Lofblad, L., Hov, G.G., Åsberg, A., et al., 2021. Inflammatory markers and risk of cardiovascular mortality in relation to diabetes status in the HUNT study. *Sci. Rep.* 11, 15644. <https://doi.org/10.1038/s41598-021-94995-8>.
- Longo, V.D., Panda, S., 2016. Fasting, circadian rhythms, and time-restricted feeding in healthy lifespan. *Cell Metab.* 23 (6), 1048–1059.
- Lowe, D.A., et al., 2019. Time-restricted eating in women prevents adverse metabolic effects of a high-fat diet despite weight loss. *Obesity* 27 (3), 377–384.
- Luan, Y.Y.; Yao, Y.M. The clinical significance and potential role of C-reactive protein in chronic inflammatory and neurodegenerative diseases. *Front. Immunol.* 2018, Jun 7; 9:1302. doi: <https://doi.org/10.3389/fimmu.2018.01302>.
- Lührmann, P.M., Herbert, B.M., Gaster, C., Neuhauser-Berthold, M., 1999. Validation of a self-administered 3-day estimated dietary record for use in the elderly. *Eur. J. Nutr.* 38 (5), 235–240. <https://doi.org/10.1007/s003940050066>. Oct.
- Maaloul, R., Marzougui, H., Ben Dhia, I., Ghroubi, S., Tagougui, S., Kallel, C., Driss, T., Elleuch, M.H., Ayadi, F., Turki, M., Hammouda, O., 2023, Mar. Effectiveness of Ramadan diurnal intermittent fasting and concurrent training in the management of obesity: is the combination worth the weight? *Nutr. Metab. Cardiovasc. Dis.* 33 (3), 659–666. <https://doi.org/10.1016/j.numecd.2022.12.004>.
- Manoogian, E.N.C., Panda, S., 2017. Circadian rhythms, time-restricted feeding, and healthy aging. *Ageing Res. Rev.* 39, 59–67.
- Maughan, R.J., Leiper, J.B., Bartagi, Z., Zrifi, R., Zerguini, Y., Dvorak, J., 2008. Effect of Ramadan fasting on some biochemical and haematological parameters in Tunisian youth soccer players undertaking their usual training and competition schedule. *J. Sports Sci.* 26 (Suppl. 3), S39–S46. <https://doi.org/10.1080/02640410802491368>.
- McEwen, B.S., 2006. Protective and damaging effects of stress mediators: central role of the brain. *Dialogues Clin. Neurosci.* 8 (4), 367–381. <https://doi.org/10.31887/DCNS.2006.8.4/bmcewen>.
- Militello, R.; Luti, S.; Gamberi, T.; Pellegrino, A.; Modesti, A.; Modesti, P.A. Physical activity and oxidative stress in aging. *Antioxidants* 2024, 13, 557. <https://doi.org/10.3390/antiox13050557>.
- Miller, A.H., Raison, C.L., 2016. The role of inflammation in depression: from evolutionary imperative to modern treatment target. *Nat. Rev. Immunol.* 16 (1), 22–34. <https://doi.org/10.1038/nri.2015.5>. Jan.
- Mishra S, Persons PA, Lorenzo AM, Chaliki SS, Bersoux S. Time-restricted eating and its metabolic benefits. *J. Clin. Med.* 2023Nov 9;12(22):7007. doi: <https://doi.org/10.3390/jcm12227007>.
- Mohamed, A.I.; Mohamed, J.; Abdilahi, M.M.; Abdek, B.A.; Abate, K.H. Effect of Ramadan intermittent fasting on renal and liver function markers among healthy overweight and obese males: a prospective cohort study. *Human Nutrition & Metabolism* 2023, 34, 200227. <https://doi.org/10.1016/j.hnm.2023.200227>.
- Moro, T., Tinsley, G., Bianco, A., Marcolin, G., Pacelli, Q.F., Battaglia, G., Palma, A., Gentil, P., Neri, M., Paoli, A., 2016. Effects of eight weeks of time-restricted feeding (16/8) on basal metabolism, maximal strength, body composition, inflammation, and cardiovascular risk factors in resistance-trained males. *J. Transl. Med.* 14 (1), 290. <https://doi.org/10.1186/s12967-016-1044-0>. Oct 13.
- Mulas, A.; Cienfuegos, S.; Ezpeleta, M.; Lin, S.; Pavlou, V.; Varady, K.A. Effect of intermittent fasting on circulating inflammatory markers in obesity: a review of human trials. *Front. Nutr.* 2023, 10, 1146924. doi: <https://doi.org/10.3389/fnut.2023.1146924>.
- Nitti, M.; Furfaro, A.L.; Mann, G.E. Heme oxygenase dependent bilirubin generation in vascular cells: a role in preventing endothelial dysfunction in local tissue microenvironment? *Front. Physiol.* 2020, Jan 29;11:23. doi: <https://doi.org/10.3389/fphys.2020.00023>.
- Noushad, S., Ahmed, S., Ansari, B., Mustafa, U.H., Saleem, Y., Hazrat, H., 2021. Physiological biomarkers of chronic stress: a systematic review. *Int J Health Sci (Qassim)*. 15 (5), 46–59. Sep-Oct.
- Pedersen, B.K., Hoffman-Goetz, L., 2000. Exercise and the immune system: regulation, integration, and adaptation. *Physiol. Rev.* 80 (3), 1055–1081.
- Penninx BW, Pine DS, Holmes EA, Reif A. Anxiety disorders. *Lancet* 2021 Mar 6;397 (10277):914–927. doi: [https://doi.org/10.1016/S0140-6736\(21\)00359-7](https://doi.org/10.1016/S0140-6736(21)00359-7). (Epub 2021 Feb 11).
- Pettersson, J., Hindorf, U., Persson, P., Bengtsson, T., Malmqvist, U., Werkström, V., & Ekelund, M. (2008). Muscle injury and exercise: effects on serum levels of the muscle enzymes CK, AST, ALT and LDH. *Clin. Chim. Acta*, 376(1–2), 126–132.
- Qiu, X.; Xiao, Y.; Wu, J.; Gan, L.; Huang, Y.; Wang, J. C-reactive protein and risk of Parkinson's disease: a systematic review and meta-analysis. *Front. Neurol.* 2019, Apr 17;10:384. doi: <https://doi.org/10.3389/fneur.2019.00384>.
- Ridker, P.M., Cushman, M., Stampfer, M.J., Tracy, R.P., Hennekens, C.H., 2000. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N. Engl. J. Med.* 336 (14), 973–979. <https://doi.org/10.1056/NEJM199704033361401>. Apr 6.
- Rohleder, N., 2014. Stimulation of systemic low-grade inflammation by psychosocial stress. *Psychosom. Med.* 76 (3), 181–189. <https://doi.org/10.1097/PSY.0000000000000049>. Apr.
- Salas-Salvadó, J., García-Arellano, A., Estruch, R., Marquez-Sandoval, F., Corella, D., Fiol, M., Gómez-Gracia, E., Viñoles, E., Aros, F., Herrera, C., Lahoz, C., Lapetra, J., Perona, J.S., Muñoz-Aguado, D., Martínez-González, M.A., Ros, E., 2008. PREMIDED investigators. Components of the Mediterranean-type food pattern and serum inflammatory markers among patients at high risk for cardiovascular disease. *Eur. J. Clin. Nutr.* 62 (5), 651–659. <https://doi.org/10.1038/sj.ejcn.1602762>.
- Sharifi S, Rostami F, Babaei Khorzoughi K, Rahmati M. Effect of time-restricted eating and intermittent fasting on cognitive function and mental health in older adults: a systematic review. *Prev. Med. Rep.* 2024May 9;42:102757. doi: <https://doi.org/10.1016/j.pmedr.2024.102757>.
- Sundaram, S., Yan, L., 2018. Time-restricted feeding mitigates high-fat diet-enhanced mammary tumorigenesis in MMTV-PyMT mice. *Nutr. Res.* 59, 72–79.
- Sutton, E.F., Beyl, R., Early, K.S., Cefalu, W.T., Ravussin, E., Peterson, C.M., 2018. Early time-restricted feeding improves insulin sensitivity, blood pressure, and oxidative stress even without weight loss in men with prediabetes. *Cell Metab.* 27 (6), 1212–1221.e3. <https://doi.org/10.1016/j.cmet.2018.04.010>.
- Tegeler, C., O'Sullivan, J.L., Bucholtz, N., Goldeck, D., Pawelec, G., Steinhagen-Thiessen, E., Demuth, I., 2016. The inflammatory markers CRP, IL-6, and IL-10 are associated with cognitive function: data from the Berlin aging study II. *Neurobiol. Aging* 38, 112–117. <https://doi.org/10.1016/j.neurobiolaging.2015.10.039>.
- Tinsley, G.M., La Bounty, P.M., 2015. Effects of intermittent fasting on body composition and clinical health markers in humans. *Nutr. Rev.* 73 (10), 661–674. <https://doi.org/10.1093/nutrit/nuv041>.
- Vints, W.A.J., Gökcü, E., Šeikinaite, J., Kusleikienė, S., Česnaitenė, V.J., Verbunt, J., Levin, O., Masiulis, N., 2024. Resistance training's impact on blood biomarkers and cognitive function in older adults with low and high risk of mild cognitive impairment: a randomized controlled trial. *Eur. Rev. Aging Phys. Act.* 21 (1), 9. <https://doi.org/10.1186/s11556-024-00344-9>. Apr 10.
- Wang, Y., Tuomilehto, J., Jousilahti, P., Antikainen, R., Männistö, S., 2016. Serum total bilirubin levels and the risk of cardiovascular disease in a Finnish population. *Atherosclerosis* 246, 217–223. <https://doi.org/10.1016/j.atherosclerosis.2016.01.002>.
- Wheaton, A.G., McKnight, K., Roberge, J., 2020. Effects of time-restricted eating on anxiety and mental health in older adults: a pilot study. *J. Nutr. Aging* 24 (4), 345–357. <https://doi.org/10.1007/s12603-020-1402-2>.

- Wilkinson, M.J., Manoogian, E.N.C., Zadourian, A., Lo, H., Fakhouri, S., Shoghi, A., Panda, S., 2020. Ten-hour time-restricted eating reduces weight, blood pressure, and atherogenic lipids in patients with metabolic syndrome. *Cell Metab.* 31 (1), 92–10.
- Wyczalkowska-Tomasik, A., Czarkowska-Paczek, B., Zielenkiewicz, M., Paczek, L., 2016. Inflammatory markers change with age, but do not fall beyond reported normal ranges. *Arch. Immunol. Ther. Exp.* 64 (3), 249–254. <https://doi.org/10.1007/s00005-015-0357-7>.
- Zouhal, H.; Bagheri, R.; Ashtary-Larky, D.; Wong, A.; Triki, R.; Hackney, A.C.; Laher, I.; Abderrahman, A.B. Effects of Ramadan intermittent fasting on inflammatory and biochemical biomarkers in males with obesity. *Physiol. Behav.* 2020Oct 15;225: 113090. doi: <https://doi.org/10.1016/j.physbeh.2020.113090>.