2

by Cek Turnitin

Submission date: 11-Sep-2025 01:56PM (UTC+0300)
Submission ID: 2747840444
File name: cek_plagiarism.docx (223.98K)
Word count: 5491
Character count: 33271

The Effects of Time-Restricted Eating and Alternate-Day Modified Fasting on Interferon-γ and Interleukin-10 Levels in Asian Young Women with Obesity: A Quasi-Experimental Study.

Abstract: Background/Objectives: Obesity induces chronic low-grade inflammation marked by elevated pro-inflammatory cytokines, such as interferon-gamma (IFN-γ), and reduced anti-inflammatory cytokines like interleukin-10 (IL-10), contributing to immune dysregulation. Intermittent fasting (IF) may restore immune balance through metabolic and circadian mechanisms. This study compared the effects of time-restricted eating (TRE) and alternate-day modified fasting (ADMF) on IFN-γ and IL-10 levels in young women with obesity. **Methods:** A 20-day quasi-experimental study with a pretest–posttest control group design included 23 non-diabetic women with obesity (aged 18–25 years; BMI ≥ 25 kg/m² according to the Asia-Pacific classification), randomized into control (n = 8), TRE 18:6 (n = 8), and ADMF (n = 7) groups. IFN-γ and IL-10 serum levels were measured pre- and post-intervention using ELISA kits. **Results:** TRE significantly reduced IFN-γ levels ($p = 0.025$), while no significant change was observed in the ADMF or control groups. No significant changes were found in IL-10 levels. **Conclusions:** TRE effectively reduced pro-inflammatory IFN-γ levels without significantly altering anti-inflammatory IL-10 levels, suggesting an anti-inflammatory effect primarily mediated through suppression of IFN-γ rather than IL-10 upregulation. The absence of significant IL-10 changes may reflect complex immunoregulatory dynamics in obesity. ADMF showed no significant immunomodulatory impact. These findings support TRE as a promising non-pharmacologic strategy to attenuate inflammation and improve immune balance in young women with obesity.

Keywords: IFN-γ; IL-10; obesity; intermittent fasting; immune regulation; young women

1. Introduction

Obesity is a complex and multifactorial condition characterized by the accumulation of excess fat in the body, which can disrupt physiological functions due to an imbalance between energy intake and energy expenditure over a long period [1]. Obesity has become a major global health concern, marked by a continuous increase in prevalence across the world. In 2020, 2.6 billion adults (39% of the global population) were overweight, including 13% classified as obese, a figure projected to surpass 4 billion by 2035, affecting 17% of the population [2]. Asia, particularly the Asia-Pacific region, is experiencing a rapid increase in obesity due to urbanization, sedentary lifestyles, and the adoption of Western dietary habits, including

higher consumption of high calorie processed foods and sweetened beverages [2–4]. Indonesia mirrors this trend, with national data indicating an increase in adult obesity from 21.8% in 2018 to 23.4% in 2021 [5]. This rising obesity trend threatens both individual health and national systems, with projected burdens on Indonesia's healthcare budget and economy by 2035 [2]. Globally, obesity prevalence is higher in women [6], particularly among urban women aged 18–25 years, who are disproportionately affected by modifiable lifestyle factors such as poor diet and physical inactivity [7,8]. In Indonesia, the proportion of women with obesity over 18 years rose sharply from 32.9% in 2013 to 44.4% in 2018 [9]. These data highlight the urgent need for early, targeted preventive strategies in young urban women to mitigate long-term health and economic impacts. Importantly, young women with obesity represent a metabolically vulnerable population, as early adulthood is a critical window when obesity-related immune dysregulation begins to emerge, and sex-specific differences in immune–hormonal responses may lead to distinct cytokine and inflammatory profiles compared to men.

Obesity is closely linked to chronic low-grade inflammation, which alters immune homeostasis and increases susceptibility to infections. This immune dysregulation is largely driven by adipocyte dysfunction and the gradual activation of macrophages within adipose tissue, especially in visceral fat depots [10,11]. Hypertrophic adipocytes release chemokines that recruit monocytes and promote their differentiation into pro-inflammatory M1 macrophages, shifting the adipose immune profile away from the regulatory M2 phenotype [12,13]. This alteration leads to immunological remodeling characterized by increased secretion of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interferon-gamma (IFN-γ), while the production of anti-inflammatory mediators like adiponectin and interleukin-10 (IL-10) is diminished [12,14]. This cytokine imbalance promotes immune cell infiltration, macrophage polarization, and activation of inflammatory signaling pathways, leading to systemic immune dysregulation [15]. In individuals with obesity, IFN-γ is produced primarily by activated Th1 cells and M1 macrophages. It exacerbates systemic inflammation by promoting insulin resistance via the activation of nuclear factor kappa B (NF-κB) and upregulation of suppressor of cytokine signaling 3 (SOCS3), thereby impairing metabolic homeostasis [16,17]. In contrast, IL-10 is an anti-inflammatory cytokine secreted by M2 macrophages and regulatory T cells (Tregs), playing a crucial role in dampening immune overactivation [17]. However, in obesity, the number and function of Tregs in visceral adipose tissue decline, contributing to IL-10 suppression and amplifying local and systemic inflammation [18]. Therefore, addressing obesity through targeted interventions that not only reduce adiposity but also restore immune balance is essential for mitigating the risk of obesity-related diseases and preventing long-term metabolic and inflammatory complications.

Intermittent fasting (IF), a dietary strategy involving periodic abstention from caloric intake, has emerged not only as a metabolic intervention but also

as a modulator of immune function. Recent evidence suggests that IF influences both innate and adaptive immunity by altering immune cell metabolism and cytokine production [19,20]. IF has been shown to influence the composition and function of immune cells, including Tregs, which play a critical role in maintaining immune tolerance and homeostasis by secreting anti-inflammatory cytokines such as IL-10 [20]. During fasting, energy depletion at the cellular level increases the AMP/ATP ratio, activating AMP-activated protein kinase (AMPK), a central regulator of energy sensing and metabolic homeostasis [21]. AMPK activation inhibits the differentiation of pro-inflammatory T cells and suppresses the production of inflammatory cytokines, including IFN-γ [21]. Among various IF protocols, time-restricted eating (TRE) and alternate-day modified fasting (ADMF) have gained prominence for their potential immunometabolic benefits. TRE, characterized by daily fasting–feeding cycles aligned with circadian rhythms (e.g., 18:6 or 16:8 patterns), has been shown in rodent models to enhance immune rhythmicity, reduce systemic inflammation, and suppress IFN-γ expression while promoting IL-10 signaling [22,23]. These effects are largely attributed to the synchronization of feeding windows with endogenous clock gene expression (e.g., *Bmal1* and *Clock*), which regulates T cell function and cytokine transcription [22]. Additionally, TRE also contributes to reducing adipose fat accumulation by promoting metabolic switching through activation AMPK and inhibition of the mechanistic target of rapamycin (mTOR) [24]. ADMF represents an adaptation of the classical alternate-day fasting (ADF) protocol [25,26]. In the classical ADF regimen, individuals undergo complete fasting for 24 hours alternating with days of unrestricted eating, which often results in poor long-term adherence due to the difficulty of sustaining zero-calorie days [27]. In contrast, ADMF permits about 20–25% of daily energy intake on fasting days, with *ad libitum* intake on non-fasting days. This modification was designed to improve feasibility and adherence while retaining the metabolic benefits of IF [28,29]. ADMF promotes metabolic health by inducing cellular adaptation to fluctuating energy availability. Through alternating energy restriction and *ad libitum* intake, ADMF triggers metabolic stress that activates energy-sensing pathways and enhances the use of stored substrates, fostering metabolic flexibility and resilience [30]. ADMF also upregulates sirtuins, particularly *Sirtuin-1 (SIRT1)* and *Sirtuin-3 (SIRT3)*, via increased NAD^+ levels, supporting fatty acid oxidation, stress resistance, and cellular longevity [31]. However, evidence from human studies on the immunological impacts of different IF protocols, particularly regarding IFN-γ and IL-10 regulation, remain limited. Given the high prevalence of immune dysregulation in individuals with obesity, it is crucial to clarify how IF influences cytokine dynamics in this population.

Considering the previously outlined challenges, further investigation is needed to elucidate the immunomodulatory potential of various IF regimens, particularly among women with obesity. In this study, we specifically recruited metabolically healthy young women with obesity and examined the impact of two IF protocols, TRE and ADMF, on immune function by

assessing serum concentrations of the pro-inflammatory cytokine IFN-γ and the anti-inflammatory cytokine IL-10. The selection of TRE and ADMF is grounded in prior evidence demonstrating that both regimens are associated with relatively high adherence and notable efficacy in supporting weight loss and metabolic improvement [32,33]. TRE, particularly popular for its simplicity and minimal behavioral demand, enables individuals to lose weight without calorie counting by restricting the daily eating window to 4–10 hours, which naturally leads to a spontaneous reduction in energy intake by 200–550 kcal/day [34]. Meanwhile, ADMF has been reported to reduce body weight by approximately 3–7% within a 2–3 month period, even when modest caloric intake is allowed on fasting days [35]. This study hypothesizes that both TRE and ADMF interventions may reduce the pro-inflammatory cytokine IFN-γ and increase the anti-inflammatory cytokine IL-10, thereby enhancing immune homeostasis in young women with obesity. Furthermore, TRE is expected to have a more pronounced effect due to its alignment with circadian rhythms, promoting more consistent metabolic and immunological adaptations compared to ADMF. The findings are expected to support the development of evidence-based nutritional strategies aimed at improving immune function and mitigating inflammation-related complications associated with obesity.

2. Materials and Methods

2.1. Study Design

This study employed a quasi-experimental design, incorporating a pretest–posttest control group design to explore the physiological effects of fasting interventions in individuals with obesity. The sample selection followed inclusion and exclusion criteria, resulting in a total of 24 participants. The required minimum sample size was estimated a priori using GPower software to ensure adequate statistical power. Subsequently, a randomization method was applied to allocate participants into three groups: Control (n = 8), TRE (n = 8), and ADMF (n = 8). Randomization was performed using the random number generator function in IBM SPSS Statistics version 27. The fasting interventions were implemented over 20 days, participants assigned to the TRE and ADMF groups followed specific IF regimens with defined fasting hours and dietary allowances, whereas participants in the Control group were advised to maintain their habitual dietary patterns.

Ethical approval for this study was granted by the Ethics Committee of the Faculty of Medicine, Universitas Airlangga (Ref: 36/EC/KEPK/FKUA/2024). Written informed consent was obtained from all participants prior to their participation in this study.

Anthropometric data and blood specimens were obtained at baseline (pre-test) and immediately following the intervention (post-test). Baseline data were collected before the fasting intervention commenced, while post-intervention measurements were taken one day after the final fasting period. To ensure data reliability, all procedures were conducted directly by the research team. Participants underwent an 8–10 hour fasting period prior to

each measurement, with their final meal taken at 10:00 PM the night before, and measurements carried out between 06:00 and 08:00 AM. Blood specimens were collected from the median cubital vein and processed for serum preparation, which was stored at –80 °C until analysis of IFN-γ and IL-10 using Enzyme-Linked Immunosorbent Assay (ELISA) kits. Sample collection and processing were conducted at the Integrated Medical Laboratory Unit, Faculty of Medicine, Universitas Airlangga.

2.2. *Inclusion and Exclusion Criteria*

This study enrolled young women with obesity aged 18 to 25 years from Surabaya, East Java, Indonesia, based on predefined inclusion and exclusion criteria. Participants were classified as obese class I or II according to the Asia-Pacific BMI classification (BMI ≥25 kg/m²). To strengthen the diagnosis of obesity, body fat percentage was also assessed, and only individuals with values exceeding 30% were included, ensuring a consistent high adiposity profile across participants. Eligible individuals also demonstrated normal hemoglobin (Hb) and fasting blood glucose (FBG) levels and had no history of diabetes mellitus.

Exclusion criteria encompassed a history of chronic gastrointestinal conditions (e.g., chronic gastritis), cardiovascular diseases (e.g., hypertension or atherosclerosis), or malignancies. Individuals who reported alcohol use, smoking, or regular consumption of weight-loss pills, anti-inflammatory drugs, or other medications affecting immune function were excluded. In addition, participants with a history of bariatric surgery were not eligible for enrollment, as these factors could influence inflammatory markers. Furthermore, participants who were absent during screening or data collection or who failed to adhere to at least 80% of the fasting regimen due to illness were not included in the final analysis. Those who initially met eligibility requirements underwent further screening to confirm health status and suitability for participation.

2.3. *Intermittent Fasting Protocol*

Two intermittent fasting protocols, TRE and ADMF were implemented in this study. TRE followed an 18:6 protocol, where subjects fasted for 18 hours consecutively and consumed all their calories within a 6-hour eating window [36]. The fasting period began at 8:00 PM and lasted until 2:00 PM the next day, with the feeding timeframe from 2:00 PM to 8:00 PM. During fasting, participants were allowed water only without any flavorings, sweeteners, or other calorie-free beverages, in order to minimize potential confounding effects of caffeine or bioactive compounds on inflammatory markers and immune responses [37,38]. During the eating window, participants could eat *ad libitum* without calorie restrictions. The ADMF protocol involved a 24-hour fasting period during which participants received 25% of their calculated daily energy needs [26], as determined by a nutritionist using the Harris-Benedict formula [39]. Meals were prepared and supplied by the research team through a specialized catering service. On non-

fasting days, subjects ate *ad libitum* without restrictions. This protocol was maintained for 20 continuous days. Throughout the intervention phase, subjects were instructed to maintain daily food records using an approximate food diary method, following standardized guidance provided before the intervention. In addition, daily physical activity was monitored using a standardized activity log that participants were required to complete and report to the research team. While physical activity was monitored, it was not strictly controlled to allow participants to maintain their habitual lifestyle patterns.

2.4. Outcomes Measurement

2.4.1. Screening Measurements and Tools

Hemoglobin levels, FBG, heart rate, blood pressure, and body composition were evaluated during the initial screening phase. Hemoglobin concentrations were measured via finger-prick test using a hemoglobinometer (Accu-Chek Performa; Roche Diabetes Care, Mannheim, Germany), while FBG levels were assessed utilizing a glucometer (Easy Touch GCU ET322; Zhejiang Easy-Touch Medical Instruments Co., Ltd., Zhejiang, China). Heart rate and blood pressure were measured using the digital sphygmomanometer (Omron HEM-8712; Omron Healthcare Co., Ltd., Kyoto, Japan). Body composition was evaluated through bioelectrical impedance analysis (BIA) employing the Omron HBF-375 Karada Scan Body Fat Composition Analyzer (Omron Healthcare Co., Ltd., Japan) according to established protocols [40].

2.4.2. Blood Sampling and Immunoassay Protocol

Blood samples were drawn from the median cubital vein between 06:00 and 08:00 AM using a 10 mL Terumo syringe with a 20 G needle, and subsequently transferred into BD Vacutainer SST II Advance Plus collection tubes. Samples were centrifuged at 3000 rpm for 10 min at 23 °C within 20 minutes of collection to separate serum from red blood cells, and the serum aliquots were stored at −80 °C until analysis. The serum samples were used for immunological analysis, particularly to measure IFN-γ and IL-10 levels, key cytokines indicative of immune response modulation. Serum concentrations of IFN-γ and IL-10 were analyzed using commercially available ELISA kits validated for high sensitivity and accuracy to ensure precise immunological profiling. This methodology adhered to manufacturer instructions and previous validated studies for cytokine measurement in the context of intermittent fasting [41]. IFN-γ levels were measured using the Human IFN-γ ELISA Kit (catalogue No. E0105Hu, BT Lab Bioassay Technology Laboratory, Shanghai, China). This kit demonstrates a sensitivity of 0.49 ng/mL and a detection range of 1–400 ng/mL [42,43]. IL-10 levels were measured using the Human IL-10 ELISA Kit (catalogue No. E0102Hu, BT Lab, Bioassay Technology Laboratory, Shanghai, China), which provides a sensitivity of 2.59 pg/mL and a broad detection range of 5–1500 pg/mL [44,45].

2.5. Statistical Analysis

The sample size for this study was determined using G*Power software version 3.1.9.7. A one-tailed independent-samples t-test was selected to assess differences between two groups. The calculation was based on an effect size (Cohen's d) of 2.34, which was derived from previous IFN-γ mean values ± SD of 4.51 ± 1.03 versus 2.74 ± 0.28 [46]. With a significance level of 0.05, 95% statistical power, and a 1:1 group allocation, the analysis determined that at least five participants per group were needed. The final sample size was recalculated using the Higgins and Kleinbaum formula, accounting for participant dropout rates reported in previous research [46] was found to be n = 7 in each group, resulting in a minimum total of 21 subjects across the 3 groups.

Statistical analysis was performed using IBM SPSS Statistics version 27. Data normality was tested with the Shapiro–Wilk test. For normally distributed variables, paired t-tests and one-way ANOVA with Tukey's HSD post hoc test were applied. Non-normally distributed data were analyzed using the Wilcoxon signed-rank and Kruskal–Wallis tests, followed by Games–Howell post hoc analysis. Data are expressed as mean ± standard deviation (SD) for normally distributed data or median (interquartile range, IQR1-IQR3) for non-normally distributed data. All statistical data analyses used a level of significance at $p < 0.05$.

3. Results

3.1. Baseline Participant Characteristics

One participant from the ADMF group was excluded from the final analysis due to being identified as an outlier. ELISA results for both IFN-γ and IL-10 in this subject exceeded the upper limit of the standard curve and were statistically flagged using SPSS outlier diagnostics. Consequently, 23 participants were included in the final statistical analysis. All participants were young women with obesity aged 18–25 years, with an average age of 21.4 ± 1.8 years. Based on the Asia-Pacific classification of obese (BMI > 25 kg/m²), the average BMI at baseline was 30.18 ± 3.65 kg/m², and the mean body fat percentage was 37.07 ± 3.13%, exceeding the 30% clinical cutoff for adiposity. Baseline characteristics such as age, BMI, systolic and diastolic blood pressure, heart rate, FBG, fat mass, visceral fat, and resting metabolic rate did not differ significantly between the control, TRE, and ADMF groups (all p-values > 0.05), indicating successful randomization and group comparability. Full baseline data are presented in Table 1. Notably, participant adherence to the assigned fasting protocols was 100% in all groups, as confirmed by daily self-reporting logs and supervised compliance checks. This high level of compliance strengthens the internal validity of the intervention outcomes.

Table 1. Participants' baseline characteristics.

Parameter	Control (n = 8)	TRE (n = 8)	ADMF (n = 7)	*p*-Value
Age, years [a]	21.00 ± 1.69	22.00 ± 1.85	21.14 ± 1.95	0.513
Blood pressure, mmHg				
Systolic [a]	111.25 ± 9.37	117.50 ± 13.33	110.42 ± 7.16	0.362
Diastolic [a]	81.12 ± 6.64	80.62 ± 10.44	78.42 ± 4.03	0.775
FBG, mg/dL [a]	99.75 ± 8.03	100.12 ± 14.87	101.85 ± 13.74	0.943
Hemoglobin, mg/dL [a]	13.01 ± 1.52	12.71 ± 1.63	12.75 ± 2.12	0.936
Weight, kg [a]	71.92 ± 11.69	78.59 ± 8.88	74.95 ± 9.94	0.443
Height, cm [a]	157.93 ± 5.51	159.37 ± 4.54	155.50 ± 5.67	0.375
BMI, kg/m² [b]	27.60 (25.75-29.90)	31.35 (28.07-32.57)	31.40 (28.00-33.20)	0.184
Fat Mass, % [b]	36.40 (33.30-38.17)	38.05 (34.85-39.42)	38.90 (36.70-39.60)	0.457
Visceral Fat, level [a]	10.43 ± 3.74	10.87 ± 2.91	11.64 ± 2.74	0.765
Resting Metabolic Rate, Kcal [a]	1433.75 ± 163.59	1525.12 ± 126.74	1484.14 ± 128.23	0.446
IFN-γ, ng/ml [b]	82.89 (76.31-92.62)	106.46 (86.57-137.17)	91.53 (79.50-116.37)	0.181
IL-10, pg/ml [b]	386.77 (338.52-530.18)	357.27 (316.77-502.64)	347.07 (298.04-563.12)	0.841

Description: Data are presented as mean ± standard deviation (SD) or median (interquartile range [IQR1–IQR3]). p-values indicate comparisons of baseline characteristics among the three groups.
[a] p-values were calculated using One-way ANOVA. [b] p-values were calculated using Kruskal–Wallis. TRE, Time-Restricted Eating; ADMF, Alternate-Day Modified Fasting; IFN-γ, Interferon-Gamma; IL-10, Interleukin-10; BMI, Body Mass Index; FBG, Fasting Blood Glucose.

3.2. Dietary Intake and Physical Activity Compliance

All participants completed daily food and physical activity records throughout the intervention. In the control group, no notable changes in dietary intake were reported compared with baseline. In the ADMF group, participants consumed only meals provided by the research team on fasting days and ate ad libitum on non-fasting days. In the TRE group, participants ate *ad libitum* within the 6-hour eating window period. Physical activity was monitored using standardized daily activity logs. While participants were required to report their activities, these were not strictly controlled, and most participants maintained their habitual patterns during the study period.

3.3 Effects of Intermittent Fasting on Anthropometric Profile

Table 2 presents the within-group comparisons of anthropometric parameters before and after the 20-day intervention. No significant changes were observed in BMI, fat mass, or visceral fat percentage across all groups following the intervention ($p > 0.05$).

Table 2. Comparison of anthropometric profile between pre-test and post-test for each group.

Parameters	Group	Pre-Test	Post-Test	*p*-Value
BMI, kg/m² [b]	Control (n = 8)	27.60 (25.75–29.90)	28.05 (26.05–29.70)	0.622
	TRE (n = 8)	31.35 (28.07–32.57)	31.40 (28.35–33.05)	0.833
	ADMF (n = 7)	31.40 (28.00–33.20)	31.30 (27.70–32.50)	0.445

Fat Mass, % [b]	Control (n = 8)	36.40 (33.30–38.17)	35.65 (33.00–39.10)	0.735
	TRE (n = 8)	38.05 (34.85–39.42)	38.65 (35.70–39.07)	0.140
	ADMF (n = 7)	38.90 (36.70–39.60)	37.40 (35.00–39.20)	0.735
Visceral Fat, level [a]	Control (n = 8)	10.43 ± 3.74	10.50 ± 3.67	0.763
	TRE (n = 8)	10.87 ± 2.91	10.81 ± 2.91	0.785
	ADMF (n = 7)	11.64 ± 2.74	10.78 ± 3.40	0.111

Description: Data are presented as mean ± standard deviation (SD) or median (interquartile range [IQR1–IQR3]). p-values indicate within-group comparisons between pre-test and post-test values.
[a] p-values were calculated using Paired sample t-test. [b] p-values were calculated using Wilcoxon sum rank test. TRE, Time-Restricted Eating; ADMF, Alternate-Day Modified Fasting; BMI, Body Mass Index.

3.4. Effects of Intermittent Fasting on IFN-γ levels and IL-10 levels

Table 3 presents within-group comparisons of IFN-γ and IL-10 levels between baseline and post-intervention. No significant changes in IFN-γ levels were observed in the control (p = 0.123) and ADMF groups (p = 0.866), while a significant reduce was observed in the TRE group (p = 0.025). Regarding IL-10, no significant changes were observed in any of the groups (p > 0.05). Between-group analysis showed a significant difference in IFN-γ changes (p = 0.009; Figure 1A), whereas IL-10 levels remained stable across groups (p > 0.05; Figure 1B). Further post-hoc testing using Tukey's HSD indicated that TRE induced a significantly greater reduction in IFN-γ compared to the control group (p = 0.041). However, no significant differences were detected between the control and ADMF groups or between ADMF and TRE groups.

Table 3. Comparison of IFN-γ and IL-10 levels between pre-test and post-test for each group.

Variable	Group	Pre-Test	Post-Test	P Value
IFN-γ levels, ng/ml	Control (n = 8)	82.89 (76.31–92.62)	88,80 (80.08–99.07)	0.123
	TRE (n = 8)	106.46 (86.57–137.17)	87.89 (67.44–109.25)	0.025*
	ADMF (n = 7)	91.53 (79.50–116.37)	94.58 (78.52–117.74)	0.866
IL-10 levels, pg/ml	Control (n = 8)	386.77 (338.52–530.18)	306.03 (262.58–380.25)	0.123
	TRE (n = 8)	357.27 (316.77–502.64)	321.13 (250.29–434.04)	0.069
	ADMF (n = 7)	347.07 (298.04–563.12)	289.37 (265.28–776.45)	1.000

Description: Data are presented as median (interquartile range [IQR1–IQR3]). p-values indicate within-group comparisons between pre-test and post-test values and calculated using Wilcoxon sum rank test. * Significant difference between pre-test and post-test values ($p < 0.05$). TRE, Time-Restricted Eating; ADMF, Alternate-Day Modified Fasting; IFN-γ, Interferon-Gamma; IL-10, Interleukin-10.

The primary differences in biomarker levels are depicted graphically. Figure 1 illustrates the post hoc analysis results using Tukey's HSD test for IFN-γ levels (Figure 1A) and IL-10 levels (Figure 1B) among the groups.

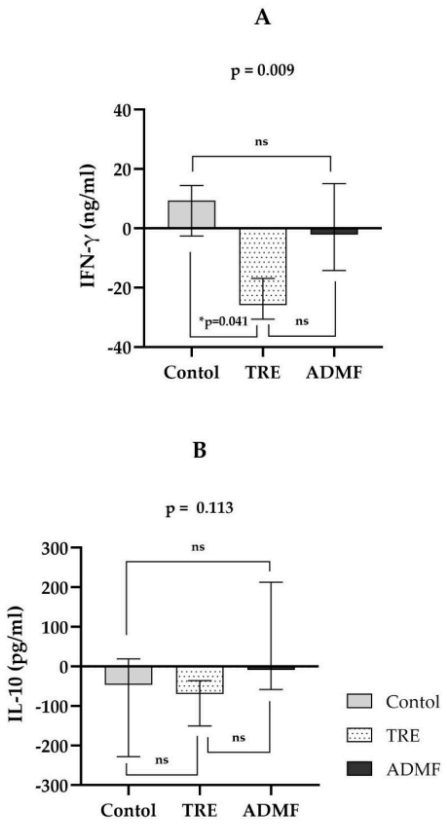

Figure 1. Comparison of changes (Δ values: post-test minus pre-test) in serum IFN-γ (A) and IL-10 (B) levels among the control, TRE, and ADMF groups after the 20-day intervention. Data are presented as median (interquartile range [IQR1–IQR3]). Statistical analysis was performed using the Kruskal–Wallis test to compare Δ values among the groups, followed by Tukey's HSD post hoc test for pairwise comparisons. * Significant reduction in IFN-γ was observed in the TRE group compared with the control group ($p < 0.05$). TRE, Time-Restricted Eating; ADMF, Alternate-Day Modified Fasting.

4. Discussion

This study investigated the immunomodulatory effects of two distinct IF regimens, TRE and ADMF, on the pro-inflammatory cytokine IFN-γ and the anti-inflammatory cytokine IL-10 in young women with obesity. The findings demonstrate that TRE significantly reduced IFN-γ levels without significantly affecting IL-10 levels, whereas ADMF produced no significant changes in either cytokine. These results suggest that TRE may exert an anti-inflammatory effect by selectively attenuating pro-inflammatory pathways rather than augmenting anti-inflammatory responses.

The 20-day duration of this intervention was chosen to capture early immunometabolic responses to intermittent fasting. Evidence indicates that meaningful adaptations can occur within short timeframes; for instance, two weeks of early time-restricted feeding in healthy men significantly enhanced insulin sensitivity and skeletal muscle glucose and BCAA uptake, independent of weight loss [47] . Likewise, laboratory-controlled fasting studies demonstrated reductions in IL-1β and IL-6 after only one week of daytime fasting, with effects persisting or re-emerging after two weeks of Ramadan fasting [48]. Together, these findings suggest that the short-term design employed here was sufficient to detect initial changes in IFN-γ and IL-10, while longer interventions may be necessary to establish the durability of such effects.

The observed reduction in IFN-γ levels in the TRE group support the initial hypothesis that IF interventions can attenuate systemic inflammation. Similar findings have been reported in Ramadan fasting, where fasting was associated with significant reductions in pro-inflammatory cytokines and improvements in immune balance [49,50]. Although studies investigating the impact of IF on IFN-γ remain limited, emerging evidence from preclinical models indicates its potential in modulating pro-inflammatory cytokines. In particular, studies utilizing rodent models have shown that IF protocols, including the fasting-mimicking diet and alternate-day fasting, significantly reduce levels of IFN-γ and other inflammatory mediators such as TNF-α and IL-17, suggesting a strong anti-inflammatory potential [51,52]. This study is among the first to demonstrate that a 20-day TRE (18:6) intervention significantly reduced serum IFN-γ levels in young women with obesity, supporting its potential as an immunometabolic intervention to reduce obesity-related inflammation.

TRE appears to reduce IFN-γ levels through integrated immunometabolic mechanisms, including suppression of cytokine-producing immune cells, enhanced metabolic switching, and circadian alignment [20,23,53–55]. IFN-γ is predominantly secreted by $CD4^+$ T helper 1 (Th1) cells, $CD8^+$ cytotoxic T cells, and NK cells [56,57]. Previous research in older men have shown that TRE reduces circulating lymphocytes and NK cells, implying decreased IFN-γ synthesis and attenuated systemic inflammation [58]. Thus, the reduction in lymphocyte and NK cell populations may play a key role in decreasing IFN-γ production, thereby supporting immune homeostasis and alleviating chronic inflammation linked to obesity. Moreover, TRE consistently induces metabolic switching—

a shift from glucose metabolism to fatty acid oxidation and ketogenesis, typically activated 12 hours after food intake cessation [59], making TRE—with its consistent daily 18-hour fasting window, an effective trigger of this metabolic state. This daily engagement may offer more sustained anti-inflammatory signaling than ADMF, which includes caloric intake and occurs on alternate days. TRE also modulates key metabolic regulators: activating AMPK and SIRT1, while inhibiting mTOR, pathways associated with reduced T cell activation and cytokine production [55,60]. These changes promote autophagy, mitochondrial function, and immune quiescence [55]. Compared to ADMF, TRE's consistent fasting–feeding cycles may more effectively reprogram the immune environment toward reduced IFN-γ expression and improved immune homeostasis.

Circadian realignment and gut microbiota modulation plays a fundamental role in regulating immune responses, and its alignment through TRE has been linked to reduced inflammation and suppressed pro-inflammatory cytokines expression [61,62]. Study in IBD relevant mouse model, TRE synchronizes feeding–fasting cycles with circadian genes such as Clock and Bmal1, the latter of which downregulates IFN-γ transcription by inhibiting pro-inflammatory signaling pathways [23]. In this study, the TRE protocol exhibited significantly lower IFN-γ levels compared to other groups, likely due to its consistent daily fasting pattern (18:6), which enhances circadian entrainment and immune regulation [61]. By contrast, ADMF's alternating schedule and irregular feeding windows may impair circadian alignment, reducing its immunomodulatory potential [63,64]. In addition, accumulating evidence in young healthy male adults suggests that IF may reshape gut microbiota composition and function, particularly increasing the abundance of *Prevotellaceae* and *Bacteroidaceae* species [61]. Similarly, study in a mouse model of chronic inflammation that time-restricted feeding restored rhythmic expression of *Bmal1* and *Per2* toward normal circadian patterns These microbial shifts have been associated with reductions in pro-inflammatory cytokines and improvements in systemic low-grade inflammation [23]. These findings suggest that TRE may exert anti-inflammatory effects by synchronizing circadian oscillations and reshaping gut microbiota composition.

Despite IL-10's role as a key anti-inflammatory cytokine, this study found no significant changes in IL-10 levels following either ADMF or TRE. In contrast to previous studies that reported IL-10 elevation after diurnal fasting during Ramadan [65]. Several factors may explain the lack of IL-10 responsiveness in our study. The absence of significant anthropometric changes, as adipose tissue reduction is often necessary to elicit anti-inflammatory responses. Additionally, chronic inflammation and metabolic dysfunction in obesity may impair IL-10 production by regulatory immune cells [14,66], and IL-10 exerts dual immunoregulatory roles, functioning both as an anti-inflammatory cytokine and in modulating immune cell responses depending on the physiological context [67].

In this study, 20-day ADMF and TRE interventions did not significantly reduce BMI, fat mass, or visceral fat, suggesting that short-term IF without caloric restriction may be insufficient to improve body composition in young women with obesity. In contrast, 4 weeks of Ramadan diurnal fasting in overweight and obese adults led to significant reductions in visceral adiposity and body weight, while paradoxically increasing circulating IL-10 levels [68]. The unrestricted food intake in this study's eating windows, combined with individual variability in metabolic rate, physical activity, and hormonal status, may have limited the observed effects. The absence of significant fat mass reduction may also explain the limited changes observed in IL-10 levels, given the role of adipose tissue in regulating anti-inflammatory cytokines. Moreover, immune dysfunction associated with obesity may limit IL-10 responses, as chronic inflammation and excess adiposity impair IL-10 secreting lymphocytes, reducing the anti-inflammatory capacity even during IF [69]. Consistent with this, studies in overweight women also report stable IL-10 levels following IF or caloric restriction. Interestingly, IF may elicit tissue-specific immune adaptations, such as increased M2 macrophages in skeletal muscle, without altering systemic IL-10, suggesting that alternative anti-inflammatory mechanisms may play a more prominent role in obesity [70]. In summary, systemic IL-10 responses to IF in obesity appear to be shaped by multiple factors beyond adiposity alone, and future studies are required to clarify the interplay between adipose tissue, immune dysfunction, and tissue-specific adaptations.

IL-10 is widely recognized for suppressing pro-inflammatory cytokines and maintaining immune homeostasis [71]. However, recent findings also emphasize its paradoxical role as a pro-inflammatory by enhancing cytolytic $CD8^+$ T cell activity and promoting IFN-γ production under certain conditions [67]. The dual immunoregulatory role of IL-10 may explain the unchanged IL-10 levels observed in this study, despite a significant reduction in IFN-γ. The absence of IL-10 upregulation could reflect a diminished need for compensatory anti-inflammatory signaling following reduced inflammatory burden. These findings highlight the complex immunoregulatory role of IL-10 during intermittent fasting and suggest that inflammation may be attenuated without requiring IL-10 mediated modulation.

A key strength of this study is the homogeneity of baseline characteristics across groups, indicating successful randomization and minimizing potential confounders, thereby strengthening causal inferences regarding the observed effects of the fasting interventions. This study acknowledges several limitations that may influence the generalizability and interpretation of the findings. Firstly, the study population was restricted to young adult women aged 18–25 years, thereby limiting the applicability of the results to other age groups. Future research should consider a broader age range to enhance external validity. Additionally, individual variability in dietary patterns, physical activity, and baseline health status may have

introduced confounding effects that were not fully controlled. While both ADMF and TRE were implemented as IF interventions, participants were allowed to consume food *ad libitum* during eating windows without restrictions on dietary composition. This lack of dietary and physical activity control may have influenced cytokine responses independently of the fasting protocols. Another limitation of this study is the use of the Harris–Benedict (HB) equation to estimate resting metabolic rate. Although widely applied, this equation carries inherent limitations, as it may not fully account for individual variability in body composition, ethnicity, and metabolic adaptations, potentially leading to under- or overestimation of energy requirements in individuals with obesity. Therefore, these limitations should be carefully considered when interpreting the results and in designing future studies aimed at refining the immunometabolic effects of intermittent fasting.

5. Conclusions

This study highlights the potential of TRE to attenuate systemic inflammation in young women with obesity by significantly reducing the pro-inflammatory cytokine IFN-γ, without concurrently altering IL-10 levels. The absence of significant changes with ADMF may be explained by its alternating schedule, which may have diminished its potential immunomodulatory effects. These findings support TRE as a promising non-pharmacological approach to immunometabolic regulation in obesity. Future research should focus on elucidating the molecular and cellular pathways through which different IF regimens modulate cytokine networks, particularly in young women with obesity, to better inform precision-targeted lifestyle interventions for immune balance restoration.

ORIGINALITY REPORT

22%	18%	19%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.ncbi.nlm.nih.gov Internet Source	6%
2	Sheeny Priska Purnomo, Purwo Sri Rejeki, Raden Argarini, Shariff Halim et al. "Regulation of Metabolic Aging Through Adenosine Mono Phosphate-Activated Protein Kinase and Mammalian Target of Rapamycin: A Comparative Study of Intermittent Fasting Variations in Obese Young Women", Nutrients, 2025 Publication	3%
3	www.mdpi.com Internet Source	2%
4	www.frontiersin.org Internet Source	1%
5	www.sportedu.org.ua Internet Source	<1%
6	e-sciencecentral.org Internet Source	<1%
7	api.mountainscholar.org Internet Source	<1%
8	Dominik Rachoń, Guillermo Rimoldi, Wolfgang Wuttke. " Effects of Benzophenone-2 and Octyl-Methoxycinnamate on the Production of Interferon-γ and Interleukin-10 by Murine Splenocytes ", Immunopharmacology and Immunotoxicology, 2008 Publication	<1%

9	worldwidescience.org Internet Source	<1 %
10	Dilip Ghosh, Benny Antony. "Ashwagandha - Potential Drug Candidate from Ancient Ayurvedic Remedy", CRC Press, 2025 Publication	<1 %
11	Miaoqing Tan, Yuxi Liang, Wenting Lv, Hao Ren, Qijun Cai. "The effects of inspiratory muscle training on swimming performance: A study on the cohort of swimming specialization students", Physiology & Behavior, 2023 Publication	<1 %
12	journal.razavihospital.ir Internet Source	<1 %
13	Neeraj Mishra, Sumel Ashique, Anoop Kumar. "Gastrointestinal Inflammations and Gut Microbiota - Pathophysiology, Management, and Emerging Therapies", Routledge, 2025 Publication	<1 %
14	Robert Jones, Pardeep Pabla, Joanne Mallinson, Aline Nixon, Tariq Taylor, Andrew Bennett, Kostas Tsintzas. "Two weeks of early time-restricted feeding (eTRF) improves skeletal muscle insulin and anabolic sensitivity in healthy men", The American Journal of Clinical Nutrition, 2020 Publication	<1 %
15	Rahmani Mohammad Malyar, Quanwei Wei, Linsong Hou, Shoura Hytham Elsaid et al. "Fermented Bamboo Powder Activates Gut Odorant Receptors, and Promotes Intestinal Health and Growth Performance of Dwarf Yellow-Feathered Broiler Chickens", Poultry Science, 2024 Publication	<1 %

16	Rizwan Akbar Ali, Sandeeka Mannakkara. "Phased Post-disaster Recovery Challenges: 2016-2017 Floods and Landslides in Colombo and Kalutara, Sri Lanka", International Journal of Disaster Risk Reduction, 2024 Publication	<1 %
17	Victor R. Preedy. "Plant and Animal Proteins in Health and Disease Prevention", CRC Press, 2025 Publication	<1 %
18	bmchealthservres.biomedcentral.com Internet Source	<1 %
19	clinicalproteomicsjournal.biomedcentral.com Internet Source	<1 %
20	www.spandidos-publications.com Internet Source	<1 %
21	mgend.med.kyoto-u.ac.jp Internet Source	<1 %
22	pmc.ncbi.nlm.nih.gov Internet Source	<1 %
23	surface.syr.edu Internet Source	<1 %
24	Amanpreet Kaur Kalsi, Ashutosh Halder, Manish Jain, P K Chaturvedi, Mary Mathew, J B Sharma. "Association of raised levels of IL-4 and anti-TPO with hyperprolactinemia", American Journal of Reproductive Immunology, 2019 Publication	<1 %
25	discovery.researcher.life Internet Source	<1 %
26	www.labroots.com Internet Source	<1 %

27	www.researchgate.net Internet Source	<1 %
28	cardiab.biomedcentral.com Internet Source	<1 %
29	synapse.koreamed.org Internet Source	<1 %
30	www.i-scholar.in Internet Source	<1 %
31	Submitted to Trinity College Dublin Student Paper	<1 %
32	assets-eu.researchsquare.com Internet Source	<1 %
33	ir.ymlib.yonsei.ac.kr Internet Source	<1 %
34	www.preprints.org Internet Source	<1 %
35	Yimei Xiao, Longcheng Chen, Yuewen Xu, Xiaolin He, Shangquan Gan, Fuquan Yin. "The Effects of Tea Polyphenols in Feed on the Immunity, Antioxidant Capacity, and Gut Microbiota of Weaned Goat Kids", Animals, 2025 Publication	<1 %
36	www.aging-us.com Internet Source	<1 %
37	João Victor Laurindo dos Santos, Dafiny Rodrigues Silva, Samyra Araujo Monteiro de Carvalho, Débora Cavalcante Ferro et al. "Association between the consumption of ultra-processed foods and generalized anxiety disorder in adults with obesity seeking weight loss", Springer Science and Business Media LLC, 2025 Publication	<1 %

38	arxiv.org Internet Source	<1 %
39	biologydirect.biomedcentral.com Internet Source	<1 %
40	link.springer.com Internet Source	<1 %
41	mspace.lib.umanitoba.ca Internet Source	<1 %
42	www.researchsquare.com Internet Source	<1 %
43	5dok.org Internet Source	<1 %
44	Hong Liu. "Interleukin-27-Mediated Suppression of Human Th17 Cells Is Associated with Activation of STAT1 and Suppressor of Cytokine Signaling Protein 1", Journal of Interferon & Cytokine Research, 01/15/2011 Publication	<1 %
45	Jing Wu, Xin-Yue Li, Xia Fang, Fang-Yuan Teng, Yong Xu. "Decreased Serum Maresin 1 Concentration Is Associated With Postmenopausal Osteoporosis: A Cross-Sectional Study", Frontiers in Medicine Internet Source	<1 %
46	Mozaniel Santana de Oliveira, Leo M.L. Nollet. "Bioactive Compounds - Identification and Characterization of their Food and Pharmacological Potential", CRC Press, 2025 Publication	<1 %
47	article.sciencepg.net Internet Source	<1 %
48	mdpi-res.com Internet Source	<1 %

49	openmicrobiologyjournal.com Internet Source	<1 %
50	scie.online Internet Source	<1 %
51	www.grafiati.com Internet Source	<1 %
52	Celeste M. Lavallee, Andreina Bruno, Christopher Ma, Maitreyi Raman. "The Role of Intermittent Fasting in the Management of Nonalcoholic Fatty Liver Disease: A Narrative Review", Nutrients, 2022 Publication	<1 %
53	George Bray, Claude Bouchard. "Handbook of Obesity - Volume 2 - Clinical Applications, Fourth Edition", CRC Press, 2019 Publication	<1 %

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	On		