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The model of Ramadan diurnal intermittent fasting: unraveling the health implications, volume III

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The model of Ramadan diurnal intermittent fasting: unraveling the health implications, volume III

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Editorial: The model of Ramadan diurnal intermittent fasting: unraveling the health implications, volume III

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Editorial on the Research Topic
The model of Ramadan diurnal intermittent fasting: unraveling the health implications, volume III

Introduction

Intermittent fasting (IF) has emerged as a widely studied dietary practice with substantial metabolic and physiological benefits, including improved insulin sensitivity, weight regulation, reduced inflammation, and enhanced circadian rhythm synchronization (1). Among various intermittent fasting (IF) regimens, Ramadan intermittent fasting (RIF) presents a unique model due to its structured, religiously mandated practice, which involves daily fasting from dawn to sunset for a month. Observed by nearly 1.5 billion Muslims globally, RIF provides an invaluable natural framework for studying the implications of diurnal fasting on human health (2). Despite extensive research on fasting, RIF offers distinct insights due to its periodic dry fasting nature, which differentiates it from water-based intermittent fasting protocols (3, 4).

This Research Topic, "*The model of Ramadan diurnal intermittent fasting: unraveling the health implications – volume III*," presents a collection of cutting-edge studies that explore the physiological, metabolic, behavioral, and clinical effects of RIF. By integrating findings from various populations and health conditions, this body of research contributes to the growing literature on fasting. It provides evidence to optimize fasting-based interventions for diverse health outcomes. The articles featured in this Research Topic collectively highlight the role of RIF in shaping metabolic health, disease prevention, and overall wellbeing.

The impact of Ramadan fasting on health

The growing interest in RIF has catalyzed research into its multifaceted impact on human physiology. One of the most significant findings is the role of RIF in enhancing metabolic health, characterized by favorable changes in glucose homeostasis, lipid metabolism, and body composition (5). Studies have demonstrated that fasting-induced metabolic shifts can be leveraged to manage conditions such as diabetes, obesity, and cardiovascular disease; however, individual variability necessitates further exploration (6–8).

Beyond metabolic regulation, RIF has shown promising effects on inflammatory and oxidative stress markers, reinforcing its potential as a preventive strategy against chronic diseases (9). Given that oxidative stress plays a critical role in aging and various pathologies, the observed improvements in antioxidant pathways during RIF underscore its broader health benefits. Moreover, emerging research on gene expression suggests that RIF modulates pathways associated with autophagy, circadian rhythm regulation, longevity, and stress response mechanisms, offering novel insights into its systemic effects (10–12).

Key themes in this Research Topic

This Research Topic encompasses a diverse range of studies examining various aspects of RIF, thereby further solidifying its importance in the health sciences. The included articles collectively enhance our understanding of the effects of fasting on various health parameters without being confined to metabolic benefits alone.

The 13 studies in this Research Topic provide comprehensive insights into the diverse health implications of RIF. Conducted by 82 authors from 15 countries, these studies examine the RIF's effects on metabolic health (validating risk assessment tools for diabetic patients (Alketbi et al.), assessing benefits for NAFLD (Lin et al.), metabolic profile, and blood pressure (Al-Jafar et al.), hormonal adaptations (in pre-and post-menopausal women) (Al Zunaidy et al.) and effect of calorie restriction and intermittent fasting, including RIF, on PCOS (Kalsekar et al.). Effect on physical performance [timing of resistance training (Triki et al.), impact on bioenergetic (Özbay et al.) pathways during exercise], molecular mechanisms (gene expression related to inflammation and oxidative stress, influence of haptoglobin polymorphism) (Madkour et al.), nutritional interventions (benefits of Ramadan-specific nutrition education) (Gul et al.), physiological adaptations (changes in body water compartments) (Najafi et al.), gut microbiome (Saglam et al.) alterations (shifts in bacterial composition and correlations with dietary components), as well as the changes in inflammatory markers and mental health (Ghashang et al.) parameters. Furthermore, the effect of combining exercise and fasting on animal models of osteoporosis (Albrahim et al.) was examined. Together, these studies highlight the potential of RIF to improve metabolic health, including inflammatory markers and body composition, while emphasizing that these benefits result from a combination of fasting and dietary and lifestyle habits during non-fasting hours.

One critical area explored is the role of RIF in managing chronic diseases. Research examining the application of RIF in diabetes risk assessment highlights the need for personalized medical guidance for individuals with type 2 diabetes who fast. Similarly, findings on cardiovascular risk markers highlight the potential of RIF in modulating lipid profiles and blood pressure, reinforcing its relevance in cardiometabolic health strategies (Madkour et al.).

Another emerging theme is the interaction between fasting and physical performance. Studies investigating resistance training and exercise timing during Ramadan provide valuable insights into optimizing muscle function and hormonal balance in athletes and active individuals. The debate over training in the fasting vs. fed state remains ongoing, and contributions to this Research Topic offer evidence-based recommendations for maintaining strength and performance during fasting periods [(13, 14); Triki et al.].

Furthermore, this volume expands on the relationship between fasting and the gut microbiome, an area of increasing scientific interest. The gut microbiota plays a pivotal role in maintaining metabolic health, regulating immune function, and controlling inflammation. This Research Topic suggests that RIF induces shifts in microbial composition, with potential implications for long-term gut health (Saglam et al.). These findings highlight the need for further investigations into dietary modifications during RIF to optimize gut microbiome adaptations and overall health benefits.

Future directions in Ramadan and health research

While the studies in this Research Topic offer significant advancements in understanding RIF, several key areas require further investigation. Future research should prioritize:

1. **Longitudinal studies on RIF**: most existing research focuses on the short-term effects of fasting. Long-term follow-up studies are needed to evaluate whether RIF-induced metabolic adaptations persist beyond Ramadan and contribute to long-term health benefits or risks.
2. **Personalized approaches to fasting**: given individual differences in metabolic responses, genetics, age, and health status, future research should explore personalized fasting protocols that maximize benefits while minimizing potential adverse effects.
3. **Molecular and systems biology investigations**: advanced omics technologies, including metabolomics, lipidomics, proteomics, and epigenetics, should be leveraged to elucidate the precise mechanisms through which RIF influences cellular function and disease prevention.
4. **Comparative studies with other IF models**: although RIF shares similarities with other IF protocols, it differs in duration, hydration status, and cultural context. Comparative studies can help delineate its unique effects and guide recommendations for those interested in adopting IF beyond Ramadan.
5. **Clinical applications and public health policies**: the integration of RIF into clinical and public health

recommendations requires robust evidence. Studies on fasting interventions in patient populations, particularly those with chronic illnesses, are crucial for informing guidelines and policy decisions.

Conclusion

Ramadan intermittent fasting serves as a valuable model for understanding the broader implications of intermittent fasting on human health. This Research Topic presents compelling evidence that RIF extends beyond religious observance and represents a structured dietary intervention with diverse physiological benefits. The insights gained from these studies pave the way for refining fasting strategies to optimize health outcomes in various populations. As interest in IF continues to grow, future research should strive to bridge existing knowledge gaps, ensuring that fasting recommendations are evidence-based, personalized, and accessible to those seeking to harness its health-promoting potential.

Author contributions

MF: Conceptualization, Funding acquisition, Investigation, Writing – original draft, Writing – review & editing. MK: Writing – original draft, Writing – review & editing. HC: Writing – original draft, Writing – review & editing. FK: Writing – original draft, Writing – review & editing. DA: Writing – original draft, Writing – review & editing. AB: Writing – original draft, Writing – review & editing.

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We extend our sincere appreciation to all authors who contributed to this Research Topic. Their dedication to advancing knowledge in fasting research has enriched the scientific discourse on RIF. We also acknowledge the reviewers for their critical insights, which have enhanced the rigor and impact of these studies. The collective expertise represented in this volume reflects the growing global interest in RIF and its health implications, reinforcing the importance of continued interdisciplinary collaboration in this field.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Alteration in body water compartments following intermittent fasting in Ramadan

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Concerning the health outcomes of intermittent fasting in Ramadan, loss of fat-free mass (FFM) and changes in the content of body water are of paramount importance. In this study, we aimed to assess the concomitant alterations in body water compartment and composition following Ramadan fasting in healthy individuals. We conducted an open-label cohort with longitudinal follow-up, involving 73 healthy medical staff who planned to fast for at least 20 consecutive days during Ramadan. The primary outcomes of the cohort were changes in parameters related to body composition and water content, which were measured using bioelectrical impedance analysis by InBody S10 (InBody, Seoul, South Korea). Based on the results, the participants' weight decreased significantly by approximately 1,030 g after the fasting period ($p < 0.001$). There was a significant reduction in the fat mass of an average 828 g ($p < 0.001$), which accounted for more than 80% of the weight loss. The decline in FFM was not significant (190 g; $p = 0.234$). The amount of total body water (TBW) and extracellular water (ECW) did not change, while intracellular water (ICW) decreased significantly by about 160 mL ($p = 0.027$). A strong correlation was observed between the reduction of phase angle and the increase in ECW/TBW ratio ($R = -0.71$, $p < 0.001$). Overall, our findings revealed a minimal amount of weight loss after Ramadan fasting, which was mainly due to the loss of fat mass. The parallel decrease in ICW and phase angle indicated impaired cell membrane integrity, with subsequent movement of water from the intracellular to the extracellular compartment.

KEYWORDS

body composition, Ramadan, intermittent fasting, body water, weight loss

Introduction

Intermittent fasting is described as an interventional strategy for weight loss, with alternating periods of fasting and feasting (1). This type of fasting can restore cellular homeostasis, increase antioxidant defense, and suppress inflammation; these alterations ultimately improve cardiovascular health and exert beneficial metabolic effects (2–4).

Although in recent years, the popularity of dietary regimens based on intermittent fasting has grown, intermittent fasting during the month of Ramadan has been practiced by many Muslims for many years. Each year, in the ninth month of the lunar calendar, Muslims abstain

from food and fluid intake during daylight hours for 29–30 consecutive days. The fasting window usually begins with a meal before sunrise ("Suhoor") and ends with a meal after sunset ("Iftar"). The duration of fasting varies from about 12 to more than 18 h, based on solar season and geographic location (5).

The effects of Ramadan fasting on various aspects of health, including metabolic parameters, blood pressure control, sleep quality, and physical performance, have been well investigated in previous researches (6–9). Most of these effects are attributed to the reduced calorie intake of individuals and the consequent alterations in body weight and composition due to fasting (10). Nonetheless, studies evaluating the alterations in body composition due to Ramadan fasting have yielded inconclusive results. While the majority of these studies have reported a significant decrease in weight and fat mass following Ramadan fasting, others have failed to show any significant changes (11). Such heterogeneity may be attributed to several factors, such as the amount of calorie intake in the feasting window, duration of fasting, and cultural rituals affecting the intake of macro-and micronutrients. In this regard, a meta-analysis by Jahrami et al. showed that intermittent fasting in Ramadan conferred a significant, albeit small, amount of weight loss, which was associated with the duration of fasting (12).

Although some minor fluctuations may occur in the amount of body water, it remains relatively constant during a normal life. Abstinence from water and other beverages during the fasting window in Ramadan may result in some degree of hypovolemia and cause subsequent symptoms, such as headache, impaired cognition, and decreased physical performance (13–15). Besides, the amount of fluid intake in the feasting window may be below the recommended level (16). Very few studies have directly investigated alterations in the content of body water following Ramadan fasting. In a study using deuterium oxide as a water tracer, Leiper et al. revealed that the total body water (TBW) content was conserved during Ramadan (17). Additionally, in a bioimpedance analysis, Alinezhad-Namaghi et al. documented a significant, but small amount of loss in the TBW content (<1%) after Ramadan fasting (18). Other studies have investigated indirect measures, such as urine and plasma osmolarity, urine flow rate, and hematocrit and serum creatinine levels to assess alterations in the body water content during Ramadan fasting (15, 19–21).

Concerning the health outcomes of Ramadan fasting, two main alterations need to be addressed; one is the reduction of fat-free mass (FFM), which reflects the depletion of body cell mass, and the other is the alteration of body water compartments, leading to hypovolemia and related complications. While changes in the body composition following Ramadan fasting have been extensively reported, alteration in the water content of body has not been well documented in previous studies. Furthermore, the relationship between the changes in body composition and water content is not yet clear. Thus, in this study, using the validated method of bioimpedance analysis, we aimed to assess the alterations in body water compartments and composition following Ramadan fasting in the healthy medical staff of our hospital.

Methods

Ethical considerations

The study protocol was designed according to the Declaration of Helsinki and approved by the local ethics committee of our institute (IR.TUMS.IKHC.REC.1399.324). All participants signed written informed consent forms before entering the study.

Study design and population

The study followed an open label longitudinal follow-up design with convenient sampling to compare anthropometric indices, body composition and water content at two points: baseline and after Ramadan fasting. The study population consisted of the healthy medical staff of Imam Khomeini Hospital Complex (Tehran, Iran). Participants were recruited via an e-mail from the secretary's office of the hospital. Adult individuals who planned to fast for at least 20 consecutive days in Ramadan were included if they provided written informed consent forms for participation in the study. The exclusion criteria were: being affected by any acute or chronic medical conditions, having a cardiac electrical device implanted, and recent use of any nutritional supplement.

The study was conducted during May–June 2018 (Ramadan 1439 AH) in Tehran, Iran. In this year, Ramadan started on May 17 and finished on June 14. The average length of fasting was more than 16 h each day. The mean ambient temperature was about 23°C and the maximum daytime temperature rose from 25°C at the start of Ramadan to 35°C by the end.

Sample size calculation

The sample size was calculated based on the primary outcome of weight loss. Assuming $\alpha = 0.05$ and $\beta = 0.8$, about 66 participants were required to detect a weight loss of 700 g (22). At the beginning of the study, a total of 86 individuals were enrolled. Of this sample, 73 individuals were included in the study, while 13 were excluded due to failure to fast during Ramadan ($n = 6$) or failure to return for the second evaluation ($n = 7$).

Anthropometric measurements

The anthropometric indices were measured for each participant during the week before Ramadan and then after 20 consecutive days of fasting during Ramadan. Body weight was measured using a calibrated electronic scale (Rasa, Tehran, Iran), wherein the subject wore light clothes with no shoes on, and measurements were recorded to the nearest 100 g. Moreover, height measurements were performed by a portable stadiometer (OTM, Tehran, Iran) while standing barefoot and rounded to the nearest 10 mm. To increase accuracy, the measurements were taken in the NPO state ("nothing by mouth") before Ramadan and after midday during Ramadan, at least 8 h after the last meal.

Measurement of body composition

The body composition was evaluated based on a bioelectrical impedance analysis by InBody S10 body water analyzer (InBody, Seoul, South Korea). This method was previously validated for the assessment of body composition in Iranian people (23). Generally, this

TABLE 1 Alterations in the body composition, body water compartments, and 50-kHz phase angle after Ramadan fasting in the study.

	Before fasting (mean ± SD)	After fasting (mean ± SD)	Amount of change	Percentage of change	*p* value
Weight	74.53 ± 1.7	73.52 ± 1.7	1.019	1.29	<0.001
BMI	27.11 ± 0.4	26.73 ± 0.4	0.37	1.36	<0.001
Fat mass	26.63 ± 0.9	25.8 ± 0.9	0.828	3.29	<0.001
Percent of body fat	35.78 ± 0.9	35.11 ± 0.9	0.66	1.84	0.001
Fat free mass	47.9 ± 11.8	47.71 ± 11.65	0.190	0.39	0.234
Skeletal muscle mass	26.36 ± 0.8	26.15 ± 0.8	0.211	0.66	0.028
Total body water (TBW)	35.19 ± 1	35.07 ± 1	0.121	0.21	0.28
Extracellular water (ECW)	13.44 ± 3.13	13.48 ± 3.13	−0.04	0.29	0.39
Intracellular water (ICW)	21.75 ± 0.6	21.59 ± 0.6	0.161	0.61	0.027
ICW/TBW	61.71 ± 0.67	61.47 ± 0.64	0.24	0.39	<0.001
ECW/TBW	38.3 ± 0.65	38.53 ± 0.61	0.23	0.6	<0.001
Protein mass	9.39 ± 2.37	9.32 ± 2.34	0.078	0.83	0.018
Body cell mass	31.15 ± 7.88	30.92 ± 7.77	0.235	0.75	0.025
50-kHz driven phase angle	5.62 ± 0.07	5.49 ± 0.07	0.13	2.33	<0.001

device applies a small alternating current to the body via tetrapolar eight-point tactile electrodes and separately measures the impedance of the trunk, arms, and legs at six different frequencies, including 1, 5, 50, 250, 500, and 1000 kHz. Impedance at high frequencies represents the conductivity of the TBW compartment, while impedance at low frequencies relies on the conductive properties of the extracellular water (ECW) compartment. Additionally, FFM was estimated based on the TBW content, and thereafter, the fat mass was calculated (24). Moreover, the phase angle, defined as the ratio of electric reactance to electric resistance at 50 kHz, was calculated according to the phase angle formula (25).

Statistical analysis

Statistical analyses were performed in SPSS Version 13 for Windows (SPSS Inc., IL, USA). Quantitative variables were reported by measuring the mean values and standard deviations, and qualitative variables were described by calculating frequency and percentage. A paired *t*-test was performed to compare numerical body composition variables before and during Ramadan fasting, if they were normally distributed. Moreover, Wilcoxon test was used for non-parametric analyses of data with a skewed distribution. To compare the mean values of variables between the two groups, an independent sample *t*-test or the Mann–Whitney *U* test was carried out if appropriate. Finally, relationships between continuous variables were evaluated using Pearson's correlation coefficient test. A *p*-value less than 0.05 was considered statistically significant.

Results

Body weight changes

The mean age of the participants was 41.78 years, and 26% of them ($n = 19$) were male. The fasters were observed for an average of 22.4 days. Nearly 75% of the participants ($n = 55$) lost weight after Ramadan fasting, while about 15% ($n = 11$) experienced weight gain. On the other hand, the weight of nearly 10% of the participants ($n = 7$) remained unchanged.

The subjects' weight decreased significantly by 1,030 g on average from the baseline ($p < 0.001$). The mean amount of weight loss was 1,380 and 0.890 g in male and female subjects, respectively, without any significant difference ($p = 0.16$). Similarly, no significant difference was observed between individuals older or younger than 40 years. Approximately two-thirds of the participants ($n = 46$) had an initial body mass index (BMI) of greater than 25 kg/m^2. These individuals experienced a significantly higher mean weight loss than those with a BMI of less than 25 kg/m2 (1243 vs. 588 g; $p = 0.046$). Additionally, a significant correlation was observed between the initial BMI and the amount of weight loss ($R = 0.239$, $p = 0.042$).

Body composition changes

Table 1 presents the weight and body composition of the study sample before and after Ramadan fasting, as well as the percentage of change in these variables. The participants significantly lost their fat mass following Ramadan fasting (828 g, 3.29%), while the amount of FFM reduction was not significant (190 g, 0.39%). According to the results, losses of skeletal muscle mass, protein mass, and body cell mass were all significant after the fasting period (Table 1).

Changes in body water compartments

The mean values of TBW and ECW contents did not significantly change after fasting, whereas the intracellular water (ICW) content decreased significantly by almost 160 mL (Table 1). Moreover, the ICW/TBW ratio decreased from 61.71 to 61.47% after Ramadan fasting ($p < 0.001$).

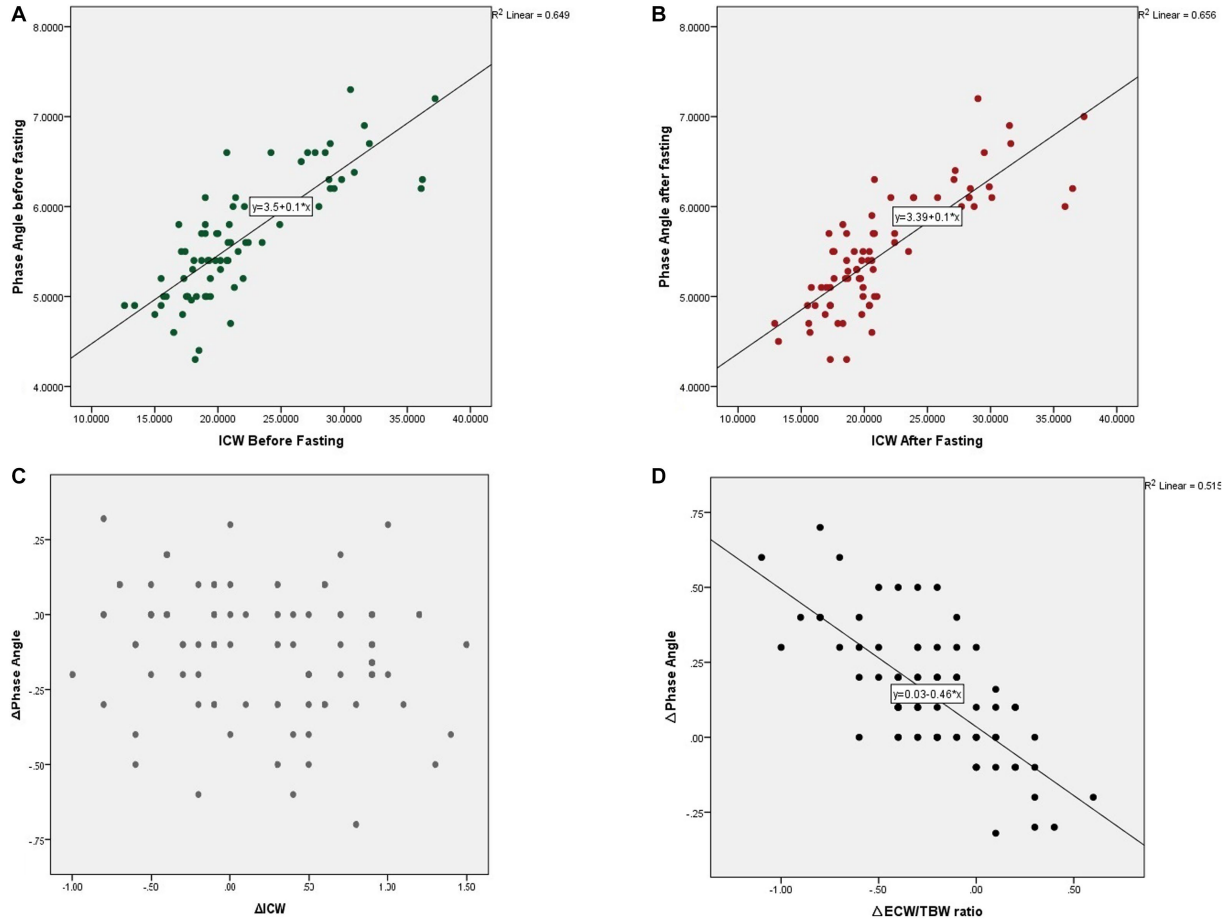

FIGURE 1
Correlation between 50-kHz phase angle and ICW before **(A)** and after Ramadan fasting **(B)**. The Δphase angle and ΔICW are not correlated **(C)**, but a strong correlation can be observed between the Δphase angle and ΔECW/TBW ratio **(D)** (ICW, Intracellular water; Δphase angle, Alteration in 50-kHz phase angle after fasting; ΔICW, Alteration in ICW after fasting; ΔECW/TBW, Alteration in the ratio of extracellular body water to total body water after fasting).

Changes in 50-kHz phase angle

The 50-kHz phase angle decreased significantly by about 0.13° (Table 1). The decrease in the phase angle was correlated with a reduction in the protein mass ($R = 0.25$, $p = 0.03$). Figure 1 shows the correlation between phase angle and ICW before and after Ramadan fasting, as well as the correlation between alterations in phase angle and ECW/TBW ratio following the fasting period. Although alterations in the phase angle and ICW were not correlated, a strong correlation was found between the amount of reduction in the phase angle and the increase in the ECW/TBW ratio ($R = -0.71$, $p < 0.001$).

Discussion

Fasting in the month of Ramadan, characterized by diurnal time-restricted feeding, is an obligatory practice for healthy adult Muslims. This form of intermittent fasting is unique, since practitioners abstain from both food and fluid in the fasting window. It is estimated that annually, 1 billion Muslims follow intermittent fasting in Ramadan. Thus, Ramadan fasting can provide valuable insights into the effects of intermittent fasting on metabolic parameters and body composition. In this study, we sought to explore the concomitant effects of Ramadan fasting on body composition and water content.

Highlights of principal findings

Following the participants for more than 22 days of fasting, we indicated a significant, albeit small, amount of weight loss. Utilizing a two-compartment model for the evaluation of body composition, our findings revealed that more than 80% of this weight loss was due to the reduction in fat mass. Conversely, the loss of FFM contributed to nearly 20% in body weight loss. These findings can explain the observed link between pre-fasting BMI and the amount of weight loss.

From a nephrologic point of view, ongoing water loss due to abstinence from fluid intake can result in some degree of volume depletion during Ramadan fasting. In this study, the month of Ramadan was in the late spring with a relatively high ambient

temperature. Despite such environmental conditions, our findings highlighted two important points; unchanged levels of TBW and a significant decrease in ICW content. These alterations ultimately led to an increased ECW/TBW ratio after the fasting period.

The phase angle, defined as the ratio of reactance to resistance, represents body cell mass, cell membrane integrity, and cell function (25). Despite negligible decrease in FFM, we found a significant reduction (2.33%) in the 50-kHz whole body phase angle, which was in parallel with the amount of loss in protein mass. Therefore, phase angle can serve as a more sensitive marker for nutritional assessment in Ramadan fasting.

Comparison with other studies

In terms of weight loss, our findings are consistent with the results of most previous studies, which showed a transient reduction in weight following Ramadan fasting. A meta-analysis by Jahrami et al., reported that diurnal intermittent fasting in Ramadan led to a weight loss of about 1,022 g (12). Consistent with our results, age and sex were not related to the amount of weight loss. Unlike the study by Norouzy et al., our population was heterogeneous in terms of weight loss, and even some cases experienced weight gain after the fasting period (26). Several factors may underlie this heterogeneity, including daily physical activities, amount of calorie intake in the non-fasting window, and different dietary habits that need to be evaluated in future studies. In line with our study, a meta-analysis by Fernando et al. indicated an association between the pre-Ramadan BMI and the amount of weight loss (27). Regarding weight loss, Ramadan fasting may be more effective in individuals with a higher pre-Ramadan BMI.

Although the proportional loss of fat mass to FFM may provide important clues about the safety of weight loss strategies, the amount of excessive loss in FFM and its safe level remain unclear. A study by Alinezhad-Naghami et al. showed that 76% of weight loss was attributed to the loss of fat mass (18). In another study by Syam et al., the reduced body fat had the greatest contribution to weight loss, while unlike our study, the protein mass remained unchanged (28). In a meta-analysis by Fernando et al., more than half of weight loss was related to a decrease in the fat mass (27). A systematic review by Chatson et al. on weight loss of more than 10 kg through diet and exercise showed that the loss of FFM accounted for 4.3–38.3% of the weight loss in an obesity intervention (29). Comparison of our results with previous research suggests that the effect of Ramadan fasting on the loss of FFM is at an acceptable and safe level.

Only few studies have directly investigated the impact of Ramadan fasting on the content of body water. A study by Alinezhad-Namaghi et al. showed that TBW had a significant, though small, reduction (0.75%) during Ramadan fasting (18). In the study by Leiper et al., by using a radiotracer technique, it was found that TBW was conserved in Ramadan despite a mild decrease in the daily water turnover (13). These findings may represent a new steady-state balance between fluid intake and water loss, leading to the relative preservation of TBW.

Considering the important role of phase angle in nutritional assessment, few studies have reported the impact of Ramadan fasting on phase angle. Similar to our study, Alinezhad-Namaghi et al., documented a significant reduction of about 1.7% in phase angle during Ramadan fasting (18). Further high-throughput studies are needed to evaluate the clinical importance and safe levels of alterations in the phase angle after Ramadan fasting.

Possible explanations of the findings

Similar to intermittent fasting in Ramadan, short-term fasting of obese individuals was also associated with a loss of ICW content (18, 30). Several mechanistic explanations were proposed for the reduced ICW following fasting. It is estimated that about 2.7–4 g of water is bound to each 1 g of glycogen (31). Therefore, the reduction of the glycogen content of skeletal muscle cells after fasting can result in the extracellular shift of water and reduction of ICW. In contrast to Ramadan fasting, a study by Shiose et al. revealed that the ICW content increased in the lower limbs via carbohydrate loading and increased muscle glycogen stores (32). Aside from glycogen depletion, proteolysis and decreased activity of Na-K-ATPase ionic pump due to insulin deficiency may also alter the hydroelectrolytic balance of the intracellular compartment, resulting in a shift of water to the extracellular space (33). Confirming the redistribution of water after Ramadan fasting, we found a strong relationship between the reduction of 50-kHz phase angle and the increased ECW/TBW ratio. This correlation indicated an impairment of cell membrane integrity and the subsequent shift of water from the intracellular to the extracellular compartment.

Study limitations

The present study had some limitations. First, a standardized method of bioimpedance analysis was applied in this study for the evaluation of body composition; however, we did not consider other variables related to the hydration status, such as urine output and osmolality, plasma osmolality, or serum creatinine. Second, data pertaining to physical activity, calorie intake, and dietary habits were not available to us; such data might explain the heterogeneity of our findings and account for the various metabolic effects of Ramadan fasting. Finally, we did not follow our participants after Ramadan; therefore, we cannot draw any definite conclusions about the reversibility of alterations induced by intermittent fasting during Ramadan.

Conclusion

In this study, we found a significant, albeit small, amount of weight loss in the study samples after 20 consecutive days of fasting in Ramadan, which depended on the pre-fasting BMI. The loss of fat mass was the main contributor to the weight loss, and the amount of FFM loss was negligible. We also observed a significant decrease in the ICW content and an increase in the ECW/TBW ratio. These alterations in the body water compartments were accompanied by a decrease in the 50-kHz phase angle, which might indicate a shift of water from the intracellular to the extracellular compartment due to impaired cell membrane integrity. Future studies are needed to clarify the clinical correlation and safety of such alterations in body water compartments and composition following Ramadan fasting.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.IKHC.REC.1399.324). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

MK, MA, and HM conceived and designed the study. MN, AS, AG, and MS collected the data. MS and AS analyzed the data. MS, MN, and HM wrote the first draft of manuscript. MK and MA revised the manuscript. All authors contributed, read, and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Effects of Ramadan intermittent fasting on gut microbiome: is the diet key?

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Much research has been conducted regarding the impact of diet on the gut microbiota. However, the effects of dietary habits such as intermittent fasting are unclear. This study aimed to investigate the effect of intermittent fasting during Ramadan on the gut microbiota. The study was conducted on 12 healthy adult individuals who practiced fasting 17 h per day for 29 consecutive days during the month of Ramadan. To determine the dietary intake of individuals, a 3-day dietary record was kept at the beginning and end of the study. Reads that passed quality filtering were clustered, and custom-prepared 16S rRNA gene regions of bacteria associated with the human microbiome were used as a reference. Consensus sequences were created, and genus-level taxonomic annotations were determined using a sequence identity threshold of 95%. The correlations between the dietary intake measurements of the participants and the respective relative abundance of bacterial genera were investigated. The results showed that Firmicutes were higher in abundance in the gut microbiota before fasting among participants, while they were significantly lower in abundance at the end of Ramadan fasting ($p < 0.05$). Proteobacteria were significantly higher in abundance at the end of the month of Ramadan ($p < 0.05$). Fasting was associated with a significant decrease in levels of seven genera: *Blautia*, *Coprococcus*, *Dorea*, *Faecalicatena*, *Fusicatenibacter*, *Lachnoclostridium*, and *Mediterraneibacter*. Conversely, the abundances of two bacterial genera were enhanced at the end of the fasting month: *Escherichia* and *Shigella*. The results of the dietary intake analysis showed that a negative correlation was detected for three comparisons: *Ihubacter* and protein (rho = −0.54, $p = 0.0068$), *Fusicatenibacter* and vegetables (rho = −0.54, $p = 0.0042$), and *Intestinibacter* and nuts (rho = −0.54, p-value = 0.0065). The results suggest that even when the fasting period during Ramadan is consistent, the types of food consumed by individuals can affect the gut microbiota.

KEYWORDS

Ramadan, diet, gut, microbiota, intermittent fasting

Introduction

The human intestinal microbiota is composed of trillions of microorganisms. Over 90% of the gut microbiota is comprised of Bacteroidetes and Firmicutes species. Proteobacteria, Verrucomicrobia, Fusobacteria, and Actinobacteria are the other prominent genera in the intestine. The rates of presence of these groups of bacteria in the intestine vary depending on various factors, such as age, genetics, dietary habits, and physical activity (Eckburg et al., 2005). The development of the gut microbiota during early life is influenced by various factors, including the microbiome of the mother, the delivery method, and breast

milk consumption. The gut microbiota remains relatively stable throughout adulthood. The stability of a healthy gut microbiome can be affected by various factors, such as body mass index (BMI) level, lifestyle factors, and cultural and dietary habits. These changes in the intestinal ecosystem can have both temporary and long-lasting effects (Rinninella et al., 2019). The determination of the optimal composition of intestinal microbiota remains uncertain. However, it is crucial to maintain the stability, diversity, and symbiotic interactions with the host to promote a robust immune and metabolic response (Rinninella et al., 2019).

Diet is a factor that impacts the gut microbiota (Rinninella et al., 2023). Studies evaluating different dietary models have established the impact of diet on the gut microbiota. The Western-style diet model leads to a decrease in the total number of bacteria and *Bifidobacterium* species. However, there is strong evidence that the Mediterranean diet beneficially modulates the gut microbiota by increasing the abundance of Bacteroidetes, *Clostridium cluster XIVa*, *Faecalibacterium prausnitzii*, *Lactobacilli*, and *Bifidobacteria* and decreasing the abundance of Firmicutes (Moszak et al., 2020). Dietary composition and behaviors also contribute to gut microbiota variations (Rinninella et al., 2019). Low microbiota-accessible carbohydrates are associated with certain types of bacterial depletion due to their content, decreasing microbial diversity (Moszak et al., 2020). Desai et al. found that the consumption of low-fiber diets triggered the spread of mucus-disrupting bacteria, including *Akkermansia muciniphila* and *Bacteroides caccae* (Desai et al., 2016). It has been demonstrated that a diet high in saturated fats significantly lowers *Lactobacillus* and increases *Oscillibacter*. These changes have been associated with significantly increased permeability in the proximal colon (Lam et al., 2012). In a study comparing the gut microbiota of Italian and African children, in which the effect of proteins on the gut microbiota was shown, it was determined that the gut microbiota of Italian children who consumed a lot of animal protein was rich in *Bacteroides* and *Alistipes* species (De Filippo et al., 2010). High intake of protein (more than 200 g/day) increases pathogens such as *Coliforms, Streptococcus,* and *Bacillus*, while low protein intake reduces the concentration of butyrate-containing bacteria such as *Lactobacilli, Bifidobacteria*, and saccharolytic bacteria (Zhao et al., 2019).

Fasting is another factor that has been investigated regarding the intestinal microbiota. Fasting is the voluntary deprivation of some or all foods and beverages for therapeutic, spiritual, or political reasons (Attinà et al., 2021). Ramadan intermittent fasting (RIF) is the most common form of time-restricted feeding in which food and liquid drinking are restricted from dawn to sunset during Ramadan, the ninth lunar month (Azizi, 2010). The duration of the daily fasting period depends on the geographical location and season, ranging from 11 to 22 h. During Ramadan, some changes in dietary habits may occur, such as reducing the frequency of meals and increasing the variety of foods consumed (Osman et al., 2020). In one study, Lebanese adults' intake of vegetables, dried fruit, Arabic sweets, cakes, pastries, and sugar-sweetened beverages was reported to be higher during Ramadan compared to the rest of the year ($p < 0.05$) (Shatila et al., 2021). According to Karaagaoglu et al., in Turkey, the sahur meal typically includes breakfast foods, whereas the iftar meal exhibits greater variability in food choices (Karaagaoglu and Yücecan, 2000). Despite the change in dietary habits, the results regarding the changes in individuals' energy and macronutrient intake are contradictory (Karaagaoglu and Yücecan, 2000; Nachvak et al., 2019; Osman et al., 2020; Shatila et al., 2021). It has been stated that these differences are due to the different dietary habits of individuals in various geographies (Osman et al., 2020).

Intermittent fasting has been found to have positive effects on cardiometabolic risk factors in healthy subjects (Jahrami et al., 2021). Furthermore, intermittent fasting may alter the gut microbiota composition (Zeb et al., 2023). Intermittent fasting has been reported to induce significant changes in the gut microbiota, increase the production of short-chain fatty acids (SCFAs), decrease the circulating lipopolysaccharides levels, and ameliorate obesity and metabolic risks (Karakan, 2019; Guo et al., 2021).

Few studies have been conducted regarding the connection between RIF and gut microbiota. In their study, Özkul et al. reported that while Bacteroidetes and *A. muciniphila* increased during RIF, the abundance of the Firmicutes *Butyricicoccus, Faecalibacterium,* and *Roseburia* also increased (Ozkul et al., 2020). Ikram et al. found that *Dorea*, *Klebsiella,* and *Faecalibacterium* were more common in the Muslim Chinese group after RIF, and *Sutterella, Parabacteroides,* and *Alistipes* were significantly enriched in the Pakistani group. In both groups, *Coprococcus, Clostridium_XlV*, and *Lachnospiraceae* decreased significantly after RIF. According to this study, the impact of RIF on the gut microbiota can vary based on cultural and dietary differences (Ali et al., 2021).

The cultural variations in dietary habits during RIF could be the reason for the changes in the gut microbiota. This study aimed to determine the effect of changing dietary habits during RIF on gut microbiota in the Turkish Muslim population.

Methods

Participants

This study was carried out with adults who were physically inactive, who did not receive medical drug therapy and diet therapy, and who were not participating in any weight loss programs. Individuals were reached through an announcement made by the Nutrition and Dietetics Department of Acibadem University on social media. At the beginning of the study, 16 participants were included. However, four people were excluded from the study: one because they could not give fecal samples, one due to antibiotic use, and two could not continue fasting. The study was therefore conducted with 12 healthy adults (7 women, aged 26.7 ± 6.7 years, and 5 men, aged 33.2 ± 9.2 years) who practiced fasting 15 h per day for 29 days. Participants were required to abstain from food and drink from dawn (05:30) until sunset (20:30) daily during the month of Ramadan (26.05.2017–23.06.2017). Participants who (1) self-reported chronic disease or gastrointestinal disease, (2) had undergone bariatric surgery, (3) were following an exercise or weight-reduction plan, (4) followed a special diet for any reason (such as gluten-free or vegetarian), (5) used medication such as antibiotics, proton pump inhibitors, metformin, or probiotic supplements in the last 3 months, (6) smoked and/or drank alcohol, and (7) were pregnant or lactating were excluded from the study.

At the beginning of the study, it was stated that individuals should continue their routine diets and avoid exercise during RIF.

All of the study procedures were approved in terms of medical ethics with the decision of the Ethics Committee of Acibadem University (ATADEK) (number 2017–17/8). Written informed consent was obtained from the participants.

Study design

Anthropometric measurements, dietary records, and fecal samples were collected the day before RIF (25.05.2017) and the last day of RIF in the morning (23.06.2023).

Anthropometric measurements

The anthropometric measurements included body weight and height, and the body composition analysis was performed by bioelectrical impedance (BIA) and waist circumference measurements. Body composition analysis using the BIA method was used to determine body fat mass (kg). In addition, percentage, lean body mass (kg), body water volume (L), basal metabolic rate (kcal), and BMI (kg/m^2) calculations were made. A Tanita MC 180 was used for BIA measurement. The BIA measurement conditions were met for each participant. These included not doing heavy physical activity 24 h before the test, not drinking alcohol for 24 h before the test, not having eaten for at least 2 h, not drinking water before the test, and not drinking tea, coffee, or cola 4 h before the test. Participants were asked to remove all metal objects (e.g., watches or jewelry) prior to measurement.

Standing height was measured with the help of a height meter, with the feet side by side and the head in the Frankfurt plane (the eye triangle and auricle are at the same level, parallel to the ground). BMI (kg/m^2) values were calculated using the equation body weight (kg)/height (m^2). Waist circumference was measured from the midpoint of the lowest rib with the lateral iliac prominences while standing (Ma et al., 2013).

Determining and monitoring the food intake

To determine the food intake before and during Ramadan, researchers took a 3-day dietary record at the beginning and end of the study. The dietary records of individuals were taken on 3 consecutive days (two on weekdays and one on weekends). The dietitian provided a brief education on "standard portion sizes and amounts of foods according to food groups for Turkey" so that individuals could record the amount of food consumed.

The study participants were educated on portion sizes using the "Food and Food Photo Catalog" (Rakicioglu Neslisah TNAAPG, 2015). The cookbook "Standard Food Recipes" was used to determine the ingredients in recipes where the contents were unknown (Kutluay Merdol Türkan, 2011). The daily energy and nutrients taken by the participants were evaluated using the "Nutrition Information System (BEBIS) 7.2" program. The obtained results were compared with the dietary reference intake. Anthropometric measurements and dietary records were compared at the end of the study to assess the changes in nutritional status.

Microbiota analysis

Fresh fecal samples were taken from each participant at the beginning and end of the study for the microbiota analyses. The dietitian verbally explained the conditions for taking fecal samples to the individuals and provided them with containers to collect them. The conditions were as follows: (1) The samples should be taken into sealed, sterile containers with red caps. (2) A small amount the size of a walnut is sufficient. (3) The name, surname, sex, and date of birth of the patient should be written on the sides of the containers. (4) The sampling day (0 or 30) must be specified. (5) The container must be closed and delivered to the laboratory within 4 h of taking the sample. Fresh fecal samples were stored at −80°C after collection.

Fecal DNA extraction, 16S amplicon sequencing, and bioinformatics analyses

All the steps, from sample processing to the end of taxonomic annotation and abundance table preparation, were carried out by Epigenetiks Inc. (Istanbul/Turkey). For DNA extraction, up to 200 mg of each fecal sample was processed using a ZymoBIOMICS DNA Miniprep Kit (Zymo Research, Cat. No. D4300). DNA concentrations were measured using a Qubit dsDNA HS Assay Kit (Invitrogen, Cat. No. Q32854). Library preparation was carried out using the 16S barcoding kit containing the complete 16S rRNA gene from V1 to V9 regions (Oxford Nanopore Technologies, Cat. No. SQK-RAB204), following the manufacturer's guidelines (v.RAB_9053_V1_REVR_14AUG2019). Prepared libraries were loaded onto R9.4.1 FLO-MIN106D flow cells, and sequencing was performed on a MinION sequencer (Oxford Nanopore Technologies).

Obtained raw FAST5 reads were converted to.fastq format using Guppy (ver. 6.0.5). The primer sequences were removed from the amplicon reads, and quality trimming was performed using BBTools v.38.94 (Bushnell et al., 2014). Reads passing quality filtering were clustered using Magicblast v.1.6.0 (Boratyn et al., 2019) by the Human Microbiome Project, using the custom-prepared reference of 16S rRNA gene regions of bacteria associated with human microbiome (16S NCBI reference sequences as of 12/08/2022). The consensus sequences were created, and.sam files were produced in Samtools (Danecek et al., 2021). Taxonomic annotations were determined through BLAST + v.2.12.0 (Boratyn et al., 2012) in the NCBI nr database (12/08/2022), using a sequence identity threshold of 95% for genus level. Finally, relative abundance percentages were calculated at phylum and genus levels.

Data analysis

All downstream data analysis steps were performed using R v.4.1.3). Alpha diversity analysis was performed with the "phyloseq" package (v.1.38.0) to investigate the within-sample community diversity (McMurdie and Holmes, 2013). For that purpose, the observed features, Shannon index, and Simpson's index were calculated at the phylogenic and genomic levels (10.1002/ece3.1155). The changes before and at the end of Ramadan fasting were tested via the paired Wilcoxon signed

rank test for each metric. A beta diversity analysis was carried out by calculating Bray–Curtis dissimilarities using phylum- and genus-level bacterial relative abundances to evaluate the differences between sample diversity among the members of time groups. PERMANOVA was carried out between two-time groups via the "adonis" function in the "vegan" package (v.2.5.7, Oksanen et al., 2022).

Before proceeding to bacterial taxa comparisons between groups, filtering was applied to capture the bacteria present in most samples. To this end, phyla with a relative abundance of 0.1% and above and genera with a relative abundance of 0.05% and above in at least 80% of overall samples were retained, while the rest were discarded. As a result, 4 phyla and 41 genera abundances for 24 samples were obtained. The paired Wilcoxon signed rank test was applied between two groups, and taxa with p-values of <0.05 were assigned as "significantly altered bacteria." Due to the low sample size, no *p*-value adjustment was applied, and raw *p*-values were used.

In addition to differential abundance analysis, linear discriminant effect size (LEfSe) analysis was used for biomarker discovery on phylum and genus abundance tables (Segata et al., 2011). The analysis was conducted using the web-based Galaxy tool (https://huttenhower.sph.harvard.edu/galaxy/) with the default settings.

For correlation analysis between bacteria and diet, either at phylum or genus level, the alpha diversity measures, relative abundance of bacteria, and amount of nutrients consumed by participants were used. Before correlation, taxa correlating with age were removed. Spearman's correlation coefficients and correlation test *p*-values were calculated. No *p*-value correction was carried out, and correlations with an absolute value (rho) higher than 0.5 and with a *p*-value smaller than 0.05 were considered significant. A correlation plot was drawn using the "corrplot" package (v.0.92, Wei et al., 2021). Relative abundance percentiles were submitted as medians of the groups.

All the scripts described above in the data analysis steps are available at https://github.com/eray-sahin/Saglam_2023_Fasting_Study.

Results

At the phylum level, decreased bacterial richness was observed in most samples, while the changes in genus level divided the subjects into two almost equal portions: 5 of the 12 subjects had a decreased number of genera after fasting, and the remaining 7 had an increased number of bacteria. However, none of those observations were determined to be significant ($p > 0.05$, Figures 1A, B). In contrast, the Shannon and Simpson indices, which take both richness and evenness in the community into account, revealed significantly enhanced diversity at the phylum level for all the subjects tested (Figures 1A, B). At the genus level, one-third of the subjects had decreased diversity at the end of Ramadan fasting, and the significance was a little higher than the threshold of 0.05 (Figures 1A, B). Similar to the alpha diversity, the beta diversity analysis showed clear and significant distinction of the communities at each time point at the phylum level (Figure 1C), based on observation of failed significance in comparing the community structure of two clusters at the genus level (Figure 1D). Overall, fasting was associated with a shift in gut microbiota composition at the phylum level, but the responses at the genus level were more heterogeneous among the subjects.

For the alpha diversity plots, the metrics calculated for each sample before and at the end of Ramadan fasting are represented by violin and boxplots (with a black middle line representing the median and boxes between interquartile ranges of the 25th and 75th percentiles). The changes between two-time points for each subject are shown by red, gray, or blue lines for increased, stationary, or decreased values, respectively. The Wilcoxon rank-sum test was performed to compare two-time groups, and respective *p*-values are presented on top of the figures. For beta diversity plots, ellipses correspond to 95% confidence intervals for each time group. *F*- and *p*-values obtained after PERMANOVA tests are shown at the bottom of each figure.

After filtering rare taxa at the phylum level, 4 of the 36 phyla survived, constituting a median value of 99.95% for the overall composition. Among the participants, Firmicutes (mean relative abundance) and Proteobacteria were the two dominant phyla. Together, they accounted for 98.36% and 97.73% of the median relative abundances for pre- and post-fasting communities, respectively (Figure 2A). There were consistent changes in the relative abundances of these two phyla in response to fasting: a decrease in Firmicutes (97.58% to 92.2%) and an increase in Proteobacteria (0.67% to 6.08%) (Figure 2B). In agreement, LEfSe analysis revealed higher Firmicutes enrichment in the samples collected before fasting and higher enrichment of Proteobacteria at the end of Ramadan fasting (Figure 2C). As another important indicator of the changes at the phylum level, the Bacteroidetes/Firmicutes ratio was also calculated and tested between two-time points. Even though an increase was observed at the end of Ramadan fasting, that change was not significant ($p = 0.055$, Supplementary material).

The differences in compositional changes were investigated at the genus level for samples from 12 participants (Figure 3A). Rare taxa filtration resulted in the survival of 41 genera representing 90.92% of the community composition at this taxonomic level. A comparison of the % relative abundances before and at the end of Ramadan fasting revealed significant alterations in nine genera levels (Figure 3B). Between the two-time points, before and at the end of the month of Ramadan, Ramadan fasting was associated with decreased levels of seven genera: *Blautia* (from 15.58% to 11.06%), *Coprococcus* (4.67% to 3.43%), *Dorea* (4.17% to 3.4%), *Faecalicatena* (0.5% to 0.41%), *Fusicatenibacter* (2.59% to 2%), *Lachnoclostridium* (0.16% to 0.1%), and *Mediterraneibacter* (0.75% to 0.23%). Conversely, the abundances of two bacterial genera were enhanced after fasting: *Escherichia* (0.24% to 2.94%) and *Shigella* (0.08% to 0.69%). LEfSe analysis did not reveal any significant species in the genus, possibly due to a stricter statistical test strategy of LEfSe, which results in the loss of significant changes in genus levels.

Several correlation patterns between alpha diversity and dietary intakes and between taxa abundance and dietary intakes were investigated, and all pairwise correlation results are submitted in the Supplementary material. At the phylum level, no significant correlation was detected between any of the three alpha diversity metrics and dietary measurements. By the relative abundance

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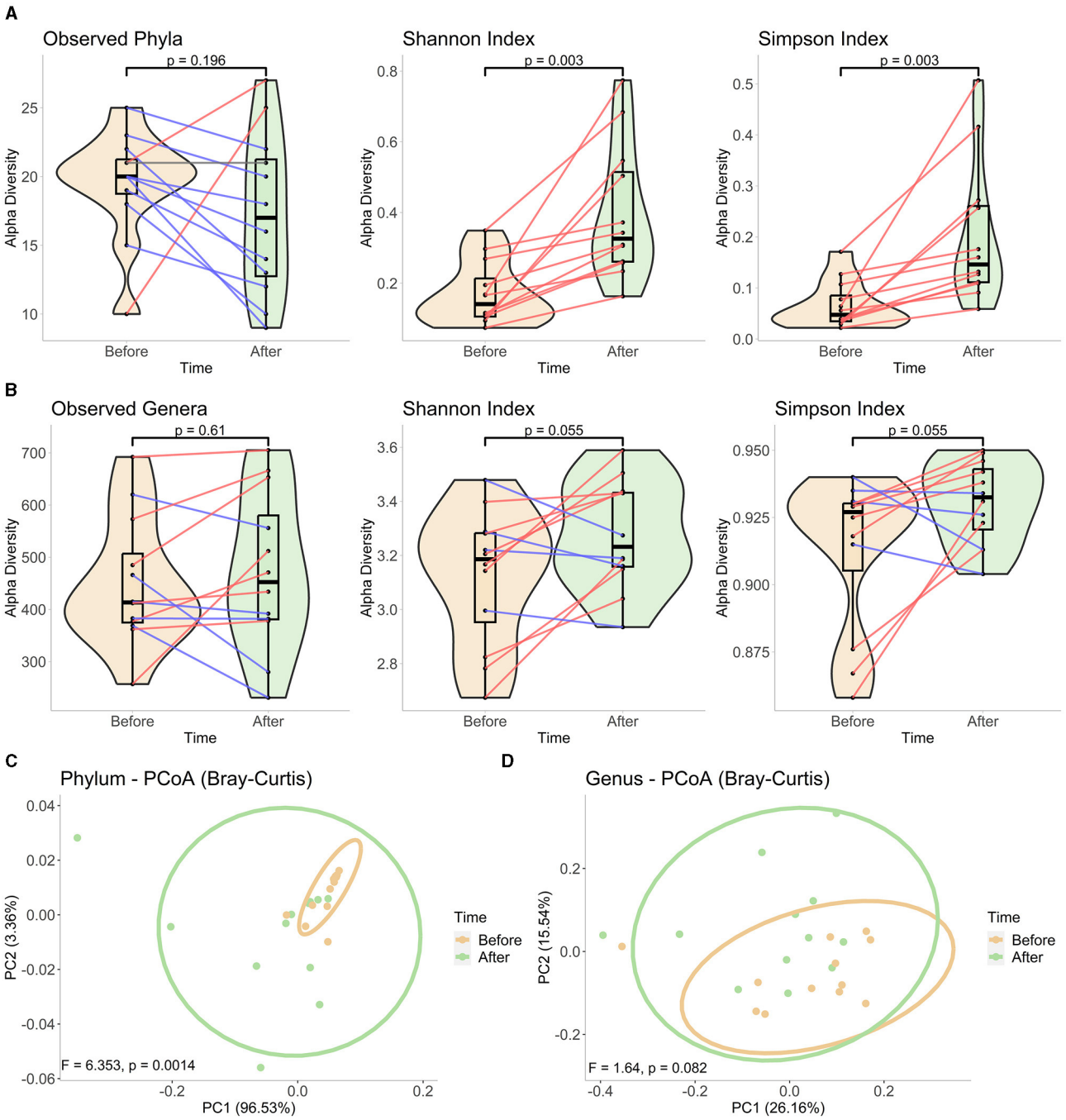

FIGURE 1
Diversity analyses in phylum and genus levels. Alpha diversity measurements using observed taxa, Shannon, and Simpson indices in **(A)** phylum and **(B)** genus levels. We constructed mixed violin and box plots to represent the sample groups before and at the end of Ramadan fasting. Changes between two-time points for each subject are shown by red or blue lines for increased or decreased values, respectively. The Wilcoxon rank-sum test was performed to compare two-time groups, and respective p-values are presented on top of the figures. The PCoA plots of beta diversity **(C)** in phylum- and **(D)** genus-level compositions at each time point, based on Bray–Curtis dissimilarity. The plots were drawn using the first two axes, and the percentage of variation explained by each axis is represented in parentheses. Each point represents one sample in each group, and ellipses correspond to a 95% confidence interval. The significance was tested by PERMANOVA, and obtained F-scores and *p*-values are submitted at the bottom of the plots.

of phyla, only one correlation calculated was determined to be significant: *Actinobacteria* and nut consumption showed a positive correlation (rho = 0.56, p-value = 0.005). Before proceeding with individual genera and diet relationship analysis, we inspected the effect of different nutrient groups on overall genus diversity. Accordingly, genus diversity was revealed to be inversely affected by fat or carbohydrate consumption, while increased carbohydrate consumption was associated with decreased genera diversity (rho = −0.62, *p*-value = 0.0013) and more diverse genus composition was observed in individuals who consumed a fat-rich diet (rho = 0.61,

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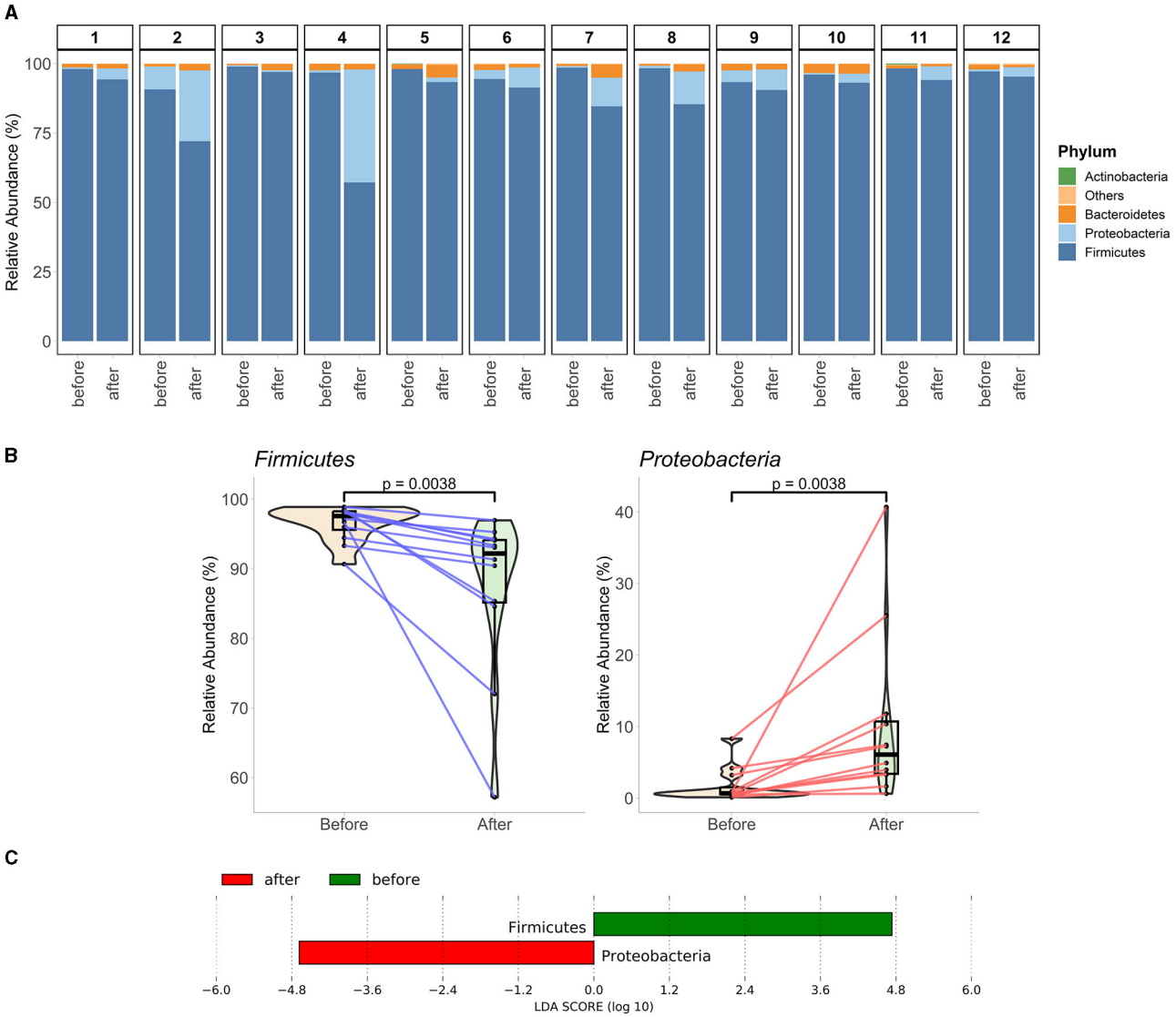

FIGURE 2
Phylum-level gut microbiota alterations at the end of fasting. **(A)** The composition of each paired sample from 12 participants is represented in the phylum level. The top three phyla of each sample are individually represented, while the rest are summed in "Others." **(B)** Phyla significantly changes at the end of Ramadan fasting. The mixed violin and box plots (with a black middle line representing the median, and boxes between interquartile ranges of the 25th and 75th percentiles) represent the sample groups before and at the end of Ramadan fasting. Changes between two-time points for each subject are shown by red or blue lines for increased or decreased values, respectively. Wilcoxon rank-sum test was performed to compare two-time groups, and respective p-values are presented on top of the figures. **(C)** Phyla obtained from LEfSe biomarker discovery analysis. A histogram shows two phyla with their respective LDA scores concerning their differential abundance in participants before and at the end of Ramadan fasting.

p-value = 0.0014). Similarly, the Shannon diversity measurements were higher for participants with a diet higher in polyunsaturated fat (g) (rho = 0.51, p-value = 0.011) and higher fat percentage (rho = 0.56, p-value = 0.0042).

Lastly, significant correlations between the dietary intake measurements of participants and the relative abundance of bacteria at the genus level were investigated. Considering the small sample size, significant correlations (p-value of <0.05) with absolute Spearman's rho value of ≥0.5 were filtered out, and the surviving ones are represented in Figure 4. Among them, most of the correlations were positive, indicating that higher consumption of the respective nutrients results in the enrichment of the genus in the gut. However, a negative correlation was detected for three comparisons: between *Ihubacter* and protein (rho = −0.54, p-value = 0.0068), *Fusicatenibacter* and vegetables (rho = −0.54, p-value = 0.0042), and *Intestinibacter* and nut consumption (rho = −0.54, p-value = 0.0065). Among the 13 detected genera, the abundances of 6 genera were shown to be affected by nut consumption. However, it is worth noting that among the 12 participants, only half consumed nuts, while the amount was zero for the rest for both pre- and post-fasting.

Table 1 describes the sociodemographic characteristics of study participants The study was carried out with a total of 12 cases, 41.6 % (n = 5) male and 58.3% (n = 7) female individuals. The ages of

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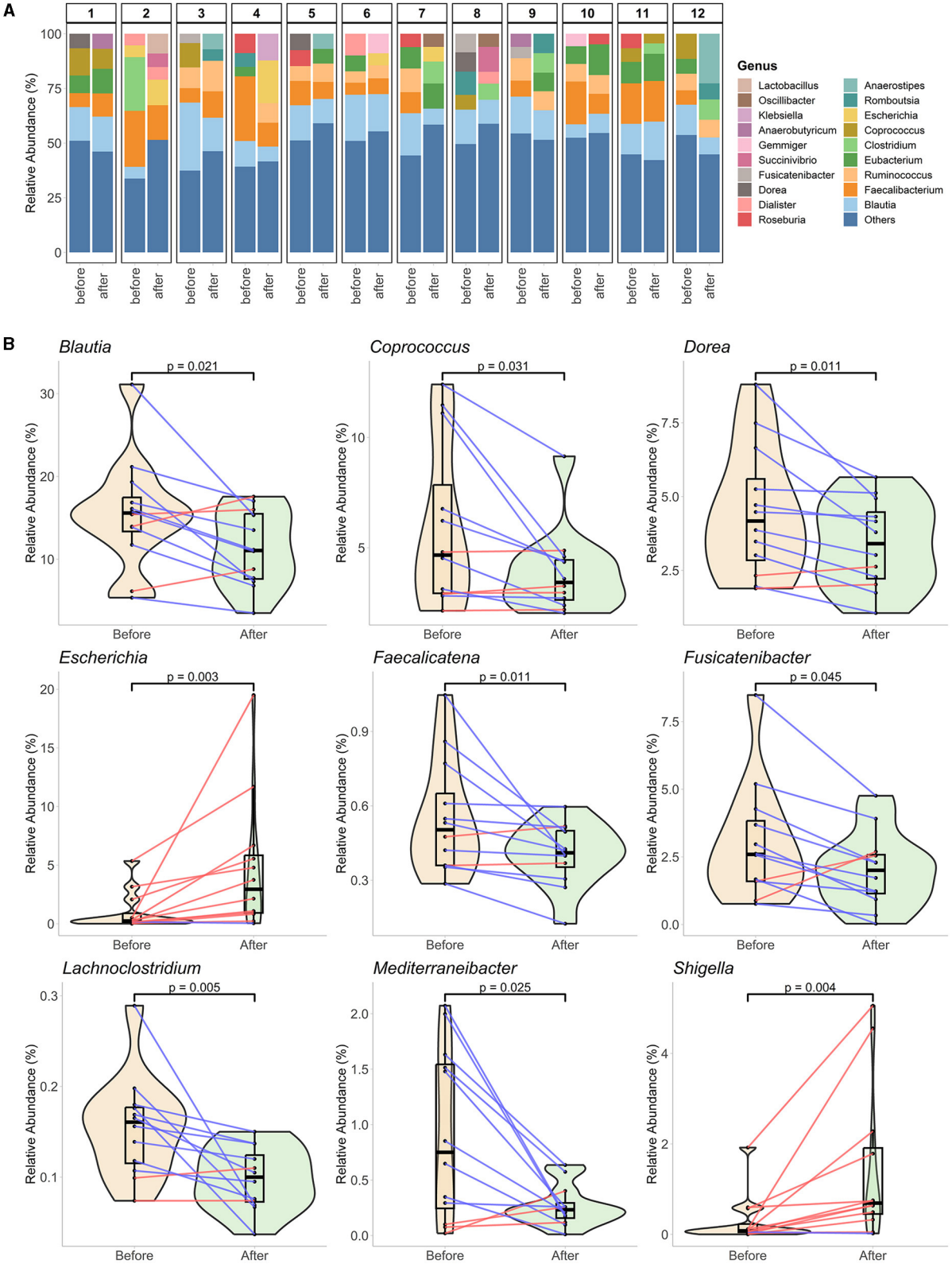

FIGURE 3
Genus-level gut microbiota alterations at the end of fasting. **(A)** The genus level represents the overall compositions of each paired sample from 12 participants. The top five genera of each sample are individually represented, while the rest are summed in "Others." **(B)** Nine genera significantly change at the end of Ramadan fasting. The mixed plots of violin and boxplots (with a black middle line representing the median and boxes between interquartile ranges of the 25th and 75th percentiles) represent the sample groups before and at the end of Ramadan fasting. The changes between two-time points for each subject are shown with red or blue lines for increased or decreased values, respectively. The Wilcoxon rank-sum test was performed to compare two-time groups, and respective *p*-values are presented on top of the figures.

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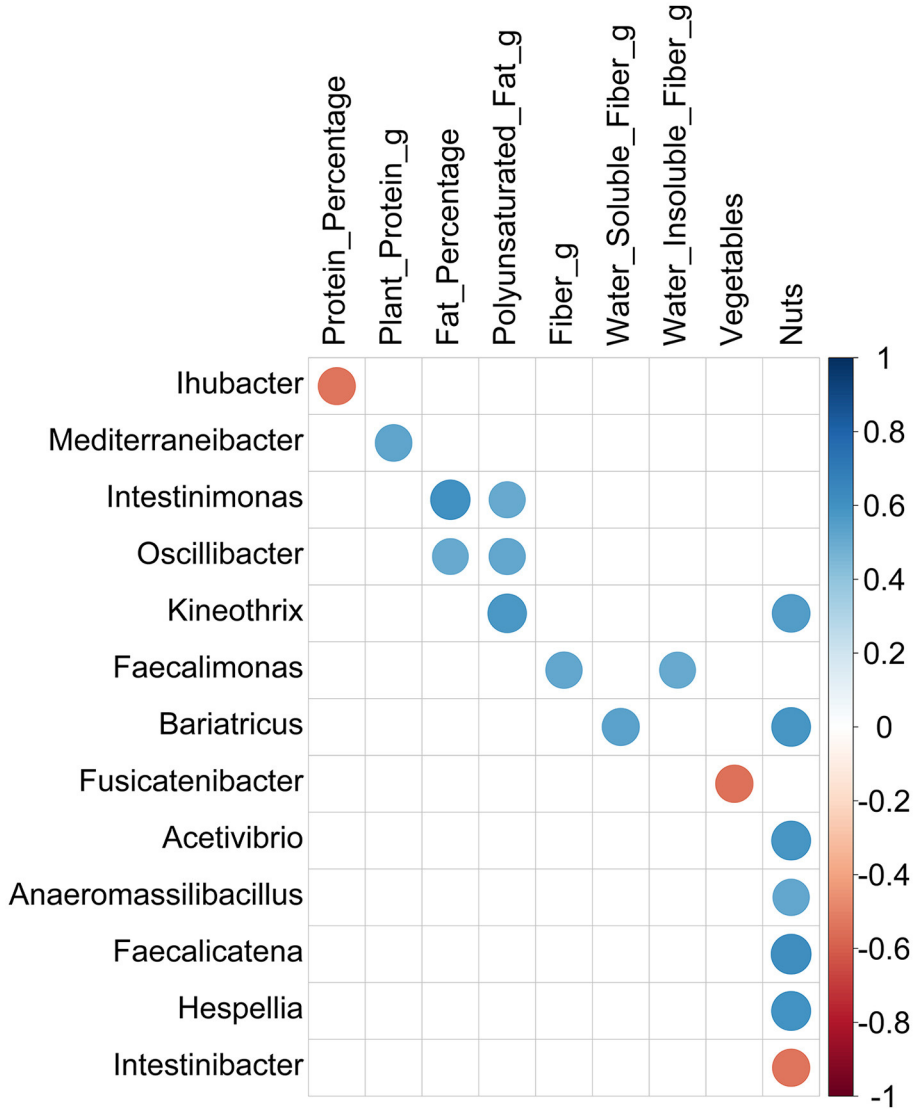

FIGURE 4
Correlations between genera and dietary intake measurements filtered by abs (Spearman's rho) ≥ 0.5. Circles represent the correlations passing a significance threshold of 0.05 after Spearman's rank correlation test. The size of the circles is proportional to the correlation coefficient value, and the color illustrates positive (blue) or negative (red) correlation between the two variables.

the cases ranged between 22 and 47, with an average of 29.1 ± 8.1 years. More than half (75%) were single, and the majority (91.6%) had a university degree.

Differences in the anthropometric measurements of the study participants before and at the end of RIF are shown in Table 2. The body weight of the individuals decreased by 0.661 kg compared to the baseline ($p = 0.034$; $p < 0.05$), which was evaluated to be statistically significant. The BMIs were 22.9 4 kg/m^2 at the beginning of the study and decreased by 0.23 ± 0.36 kg/m^2 at the end of 4 weeks ($p = 0.034$; $p < 0.05$). There was no significant change in waist circumference measurements (82.1 ± 16.9 cm vs. 81 ± 16.9 cm; $p > 0.05$). Differences in the average dietary intake of the study participants before and at the end of RIF are shown in Table 3. Changes in the amount of energy (kcal), energy percentage for protein (%), plant protein (g), saturated fat (g), polyunsaturated fat (g), monounsaturated fat (g), energy percentage for carbohydrate (%), and fiber (g) intake were not statistically significant ($p > 0.05$). In contrast, a 5.43% ± 9.07% increase in the fat percentage of energy and a 1.32 ± 1.87 g decrease in the amount of water-soluble fibers were statistically significant ($p = 0.046$, $p = 0.016$, respectively) (Table 3).

Differences in the average food/food group intakes of the study participants before and at the end of RIF are shown in Table 4. While the change in the consumption amounts of grain, egg, fruit, vegetable, nuts, cheese, dietary fat, added sugar, and meat group compared with the beginning was not statistically significant ($p > 0.05$), an average increase of 8.21 ± 14.22 g in fat intake was found to be statistically significant ($p = 0.018$; $p < 0.05$) (Table 4).

TABLE 1 Sociodemographic characteristics of the study population.

		Min-Max (Median)	Mean ± SD
Age (years)		22-47 (29.4)	29.1 ± 8.1
		n	**%**
Gender	Man	5	41.6
	Woman	7	58.3
Marital status	Married	3	25
	Single	9	75
Education level	Up to high school level	1	8.4
	University	11	91.6

TABLE 2 Differences in the anthropometric measurements of the study participants before and at the end of RIF.

		Baseline	Week 4	*P*
Body weight (kg)	Mean ± SD	66.8 ± 16.4	66.1 ± 16.7	[a]0.025*
	Median (min–max)	60.1 (49.7–101)	58 (49.1–101)	
BMI (kg/m^2)	Mean ± SD	22.9 ± 4	22.7 ± 4	[a]0.019*
	Median (min–max)	21.7 (18.6–24.8)	21.4 (18.1–30.2)	
Waist circumference (cm)	Mean ± SD	82.1 ± 16.9	81 ± 16.9	[a]0.057
	Median (min–max)	76 (63–120)	76 (62–120)	
Body fat (%)	Mean ± SD	21.5 ± 7.2	20.7 ± 7.6	[a]0.053
	Median (min–max)	22.9 (6.2–30)	23.5 (5.1–30)	
Lean body mass	Mean ± SD	49.7 ± 12.1	50 ± 12.2	[a]0.624
	Median (min–max)	42.6 (37.4–69.8)	43.3 (37.4–69.8)	

[a]Paired Wilcoxon signed rank test.
*$p < 0.05$.

Discussion

This study was conducted on 12 healthy participants who fasted during Ramadan to examine the effects of fasting on the gut microbiota. Intermittent fasting is associated with a richness and variety of gut microbiota (Khan et al., 2022). During RIF, changes in diet could be associated with changes in the intestinal microbiota (Ali et al., 2021). There are conflicting reports on the relationship between BMI and the diversity of intestinal microbiota bacteria (Lin, 2015), but it is widely reported that alterations in body weight can lead to changes in the composition and diversity of gut bacteria (Li et al., 2016). Furthermore, fibers and other non-digestible carbohydrates are known to have the highest effect on microbial diversity and metabolic profile (Garcia-Mantrana et al., 2018).

In this study, along with the decrease in BMI during the 29-day RIF period, a general decrease in the richness of the gut bacteria was detected. Meanwhile, a statistically significant increase in the evenness metric of bacterial diversity was observed according to the alpha diversity calculations at the phylum level. When compared with the changes in fundamental nutrients, phyla did not show a statistically significant diversity change in terms of their richness and evenness measures (Supplementary Table 1). Additionally, the beta diversity plot for phyla depicted a more comprehensive result, as the shift of ellipses drawn with 95% confidence showed a significant distinction between the prior and at the end of Ramadan fasting during the Ramadan month.

In our study, Firmicutes were higher in abundance in the prefasting stage than at the end of Ramadan fasting, while they were significantly decreased at the end of Ramadan fasting ($p < 0.05$). Ali et al. (2021) conducted a study on the impact of fasting for 29 days in two different ethnic groups. The results showed that at the phylum level, Firmicutes decreased only in the Pakistani group. However, Özkul et al. found no significant changes in Firmicutes levels after Ramadan (Ozkul et al., 2020). According to Mohammadzadeh et al., there was a significant increase in Firmicutes (13%) after Ramadan ($p < 0.05$) (Mohammadzadeh et al., 2021). Our study did not show any change in the Bacteriodetes levels, contrary to the results of these previous reports (Ozkul et al., 2020; Mohammadzadeh et al., 2021). Bacteroidetes and Proteobacteria, known to utilize host-derived energy substrates, simultaneously increased. Many previous studies have shown that dietary composition and behaviors are potent influences that can alter gut microbiota structure (David et al., 2014; Mesnage et al., 2019). Changes in dietary composition and behavior, such as prolonged fasting, can affect meal time and size, leading to rapid metabolic changes and altering the ratio of Firmicutes and Bacteroidetes (Jumpertz et al., 2011; Li et al., 2017, 2020). Our study

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TABLE 3 Differences in the average dietary intakes of the study participants before and at the end of RIF.

		Baseline	Week 4	*p*
Energy (kcal)	Mean± SD	1731 ± 443.8	1574 ± 450.4	[a]0.196
	Median (min–max)	1641 (1097–2586)	1524.7 (932.3–2409.2)	
Protein (%)	Mean± SD	17.4 ± 4.46	14.8 ± 4.4	[a]0.130
	Median (min–max)	16 (10–25)	14.5 (8–23)	
Plant protein (g)	Mean ± SD	25.7 ± 9.5	21.1 ± 6.3	[a]0.196
	Median (min–max)	25.2 (11.5–41.6)	20.3 (10.1–31.7)	
Animal protein (g)	Mean ± SD	46.2 ± 22.9	36.1 ± 20.3	[a]0.224
	Median (min–max)	47.5 (5–92.6)	31 (9–68.4)	
Fat (%)	Mean± SD	35.7 ± 10	42.3 ± 9.7	[a]0.014*
	Median (min–max)	37 (11–49)	44 (19–59)	
Saturated Fat (g)	Mean ± SD	23.1 ± 13.4	25.8 ± 12.9	[a]0.845
	Median (min–max)	20.1 (5.6–58.8)	21.4 (11.1–51.6)	
Polyunsaturated fat (g)	Mean ± SD	17.2 ± 9.7	16.6 ± 5.2	[a]0.666
	Median (min–max)	15.4 (6.5–38.9)	16.8 (4.4–23.4)	
Monounsaturated fat (g)	Mean ± SD	25.2 ± 13	26.9 ± 10.6	[a]1.000
	Median (min–max)	23.4 (5.8–48.9)	25.3 (7.5–43.5)	
Carbohydrate (%)	Mean ± SD	47 ± 12.2	43.3 ± 10.8	[a]0.126
	Median (min–max)	43.5 (35–79)	40.5 (31–72)	
Fibers (g)	Mean ± SD	19.8 ± 6.4	16.7 ± 6.1	[a]0.126
	Median (min–max)	19.5 (11.7–32.4)	17.4 (6–27.3)	
Water-soluble fibers (g)	Mean± SD	5.9 ± 1.8	4.7 ± 1.5	[a]0.045*
	Median (min–max)	5.7 (3.9–9.9)	4.6 (1.7–7.6)	
Water insoluble fibers (g)	Mean ± SD	12.9 ± 3.8	10.7 ± 3.9	[a]0.078
	Median (min–max)	13.3 (7.8–20.4)	11.17 (4–18.1)	

[a]Paired Wilcoxon signed rank test.
*$p < 0.05$.

also observed a significant increase in Proteobacteria at the end of Ramadan fasting ($p < 0.05$). Similarly, Ali et al. (2021) also found an increase in Proteobacteria. This can be explained, in part, by the enrichment of anaerobic fermenting taxa that can break down stubborn substrates and convert complex polysaccharides into simple sugars for ATP production (Dahiya et al., 2017). Research suggests that increased Proteobacteria in the gut may indicate dysbiosis and serve as a marker for developing diseases (Shin et al., 2015).

The genus alpha analysis in our study showed a mixed result, in which almost half of the samples showed an increase while the other half revealed a decreasing trend in all diversity measures. However, the correlation analysis showed a statistically significant positive association between the fat percentage of an individual's diet and both the richness and evenness of alpha diversity measures. In addition, the evenness measure of the gut bacterial community was higher in individuals with a higher amount of polyunsaturated fat in their diet. Conversely, the genera amount was lower in individuals with high carbohydrate levels.

At the genus level, RIF was associated with significant drops in the levels of seven genera at the end of Ramadan: *Blautia, Coprococcus, Dorea, Faecalicatena, Fusicatenibacter, Lachnoclostridium, and Mediterraneibacter*. *Blautia* produces acetic acid and significantly correlates with host physiological dysfunctions, such as obesity, diabetes, and various inflammatory diseases (Liu et al., 2021). An increase in *Blautia* abundance may inhibit insulin signaling and prevent fat accumulation in adipocytes (Kimura et al., 2013). In line with our findings, Ali et al. (2021) showed that *Blautia* was more abundant before fasting.

Coprococcus produce butyrate and may play an important role in host health by producing vitamins B and SCFA (Nogal et al., 2021). Ali et al. (2021) also found that the genera *Coprococcus* significantly decreased after RIF. Maifeld et al. found that the levels of SCFA producers such as *Coprococcus* experienced a decrease during starvation, but later on, they increased during the refeeding (Maifeld, 2021). Unlike our study, Ali et al. (2021) showed that *Dorea* was found in higher concentrations in the Chinese group at the end of Ramadan fasting. Companys et al. showed that *Dorea formicigenerans*? and *Dorea longicatena* had a positive association

TABLE 4 Differences in the average food/food group intakes of the study participants before and at the end of RIF.

		Baseline	Week 4	*p*
Grains intake (g)	Mean ± SD	250.7 ± 140.4	200.8 ± 94.4	[a]0.367
	Median (min–max)	186(85–512)	169.5 (79–375)	
Egg intake (g)	Mean± SD	38.1 ± 46.4	34.5 ± 31.4	[a]1.000
	Median (min–max)	12 (0–134)	34.5 (0–73)	
Fruit Intake (g)	Mean ± SD	265.8 ± 265.1	188.4 ± 199.8	[a]0.0504
	Median (min–max)	212.5 (0–845)	134.5 (0–645)	
Vegetable Intake (g)	Mean± SD	150.7 ± 129.9	168.8 ± 126.8	[a]0.624
	Median (min–max)	151.5 (0–469)	149.5 (0–398)	
Nuts intake (g)	Mean ± SD	21.3 ± 38.9	14.5 ± 18.6	[a]1.000
	Median (min–max)	0 (0–130)	7.5 (0–60)	
Cheese intake (g)	Mean ± SD	29.1 ± 31.9	48.1 ± 40.9	[a]0.310
	Median (min–max)	30 (0–90)	30 (0–143)	
Dietary Fat Intake (g)	Mean ± SD	10.8 ± 7.4	18.8 ± 13.6	[a]0.036*
	Median (min–max)	10.5 (0–21)	17 (1–51)	
Added Sugar Intake (g)	Mean ± SD	35.8 ± 42.9	40.2 ± 49.6	[a]0.964
	Median (min–max)	20 (0–100)	24 (0–133)	
Meat intake (g)	Mean ± SD	93.8 ± 87.6	66.3 ± 68.5	[a]0.178
	Median (min–max)	81.5 (0–261)	46.5 (0–225)	

[a]Paired Wilcoxon signed rank test.
*$p < 0.05$.

with BMI and body weight. Our study also showed that both the *Dorea* levels and BMI significantly decreased after Ramadan, showing a positive association.

The results of this study indicated that these species could be considered gut microbiota biomarkers of obesity (Companys et al., 2021). These findings are important because it has been observed that individuals with an overweight phenotype have increased levels of specific bacterial genera in the Firmicutes phylum, including *Blautia, Coprococcus,* and *Dorea* (Castaner et al., 2018; Companys et al., 2021). Our study showed that the relative abundance values of *Lachnoclostridium* decreased. *Lachnoclostridium* could affect cardiometabolic health by lowering acetate levels and producing harmful lipid compounds, including trimethylamine and CDP-diacylglycerol (Nogal et al., 2021). Additionally, increased *Lachnoclostridium* is linked to visceral adipose tissue (Wu et al., 2022). The changes at the genus level suggest that RIF can protect against obesity and cardiometabolic risk factors, even without restricting energy intake. Although the Enterobacteriaceae family comprises mostly beneficial bacteria in the digestive tract, there are a few potentially pathogenic bacteria within the same family, such as *Salmonella, Escherichia, Shigella*, and *Yersinia* (Gu et al., 2019). In our study, two bacteria were positively associated with fasting: *Escherichia* and *Shigella*. Mesnage et al. (2019) found that *Escherichia coli* were more abundant at the end of Ramadan fasting. Fasting-induced alterations in the gut microbiota may affect human energy metabolism since variations in taxonomic abundance were linked to changes in blood glucose and branched-chain amino acids in the feces (Mesnage et al., 2019). Maifeld et al. reported that following the refeeding period, a consistent decline in Enterobacteriaceae, one of its members, specifically *Escherichia coli*, was observed (Maifeld, 2021).

Based on our findings, there was no significant difference at the genus levels of *Akkermansia*, belonging to the Verrucomicrobia phylum, before and after RIF (*unpublished data*). Özkul et al. reported that *A. muciniphila* becomes more abundant after RIF (Özkul et al., 2019). *A. muciniphila* is a type of bacteria that breaks down mucin in the mucus layer. Interestingly, its presence in the body is linked to lower body weight (Santacruz et al., 2010; Everard et al., 2013). A previous clinical study showed that the abundance of *F. prausnitzii* and *A. muciniphila* increased in subjects subjected to a 1-week fasting program followed by probiotic administration (Remely et al., 2015). In another study (Dao et al., 2016), calorie restriction among obese individuals increased the quantity of *A. muciniphila*, significantly improving their metabolic wellbeing. Based on our analysis of individual *Akkermansia* levels of the participants of our study, it was observed that the relative abundance of *Akkermansia* increased in 2 out of 12 participants, decreased in 1, and remained relatively stable in the others. In another clinical study, no association was found between *Akkermansia* and the duration of overnight fasting (Kaczmarek et al., 2017). The controversial outcome could be because of variations in dietary routines during RIF.

It is well-known that weight loss is associated with reduced butyrate production by specific gut microbiota bacteria, such as the *Lactobacillus* and *Bifidobacterium* genera belonging to the Firmicutes phylum (Seganfredo et al., 2017).

Vazquez-Moreno et al. (2021) found that obesity was positively associated with *Fusicatenibacter* and *Romboutsia*, which are the genus members of the Firmicutes phylum, as well, in a study conducted in Mexico City. In two other studies, *Fusicatenibacter* and *Romboutsia* were positively correlated with an increase in BMI z-score in New Zealander children and BMI in Chinese adults (Zeng et al., 2019; Leong et al., 2020). Our study also found that there was a significant decrease in *Fusicatenibacter* levels following RIF. This can also explain the decrease in weight and body fat mass in the participants after Ramadan since no significant changes were observed in the diet's energy amount.

Nutrients can influence the prevalence of certain types of bacteria (Özdemir and Büyüktuncer Demirel, 2017). The Lachnospiraceae family (*Coprococcus, Blautia,* etc.) is known as a type of anaerobic, fermentative, and carbohydrate-metabolizing bacteria (Vacca et al., 2020). A study of two cohort data showed that microbiome diversity increased following RIF and was particularly associated with the upregulation of Lachnospiraceae. Similarly, Su et al. found that fasting promotes the growth of Lachnospiraceae (Su et al., 2021). Contrary to these studies, *Lachnoclostridium, Dorea, Blautia,* and *Coprococcus,* which are among the members of the Lachnospiraceae family, were found to be significantly reduced in our study after RIF. Coinciding with our findings, Mesnage et al. (2019) reported a decrease in Lachnospiraceae abundance after Buchinger fasting. Due to many conflicting claims in the literature, the exact relationship between Lachnospiraceae abundance and fasting cannot be made clear. The differences found in the results could be attributed to the diversity of the populations studied and the presence of limited research examining the relationship between food and nutrients and Lachnospiraceae levels.

Members of the Lachnospiraceae family can utilize various polysaccharides from their diet. However, there is significant variability in this capacity among different species and strains (Vacca et al., 2020). Thus, this could explain the difference observed in various studies.

Demirel (2019) found a negative relationship between daily dietary soluble fiber intake and *Lachnoclostridium* ($r = -0.656$, $p = 0.029$). However, in our study, although there was no statistically significant correlation between the *Lachnoclostridium* levels and the total fiber intake, it was observed that both the total fiber intake and the abundance of *Lachnoclostridium* were decreased. According to Wu et al., there is a positive correlation between the presence of *Lachnoclostridium* pathobiont species and animal protein consumption. At the same time, there is a negative correlation with the consumption of plant protein sources (Wu et al., 2022). Our study found no statistically significant difference between the consumption of plant and animal protein sources before and after Ramadan; however, a significant decrease in this *Lachnoclostridium* genus was determined. Huda et al. found that another member of the Lachnospiraceae family, *Faecalicatena* genus, increases with high fiber intake (Huda et al., 2022). This may be associated with decreased fiber intake during RIF.

A significant decrease in *Coprococcus* abundance was determined after Ramadan in this study. Ali et al. found that *Coprococcus* is positively associated with fat-derived energy (Ali et al., 2021). However, in our study, there was an increase in the energy obtained from the dietary fat at the end of Ramadan ($p = 0.014$), while the abundance of *Coprococcus* decreased. In another study, *Coprococcus* abundance was decreased in subjects who were fed a ketogenic diet (≤20g carbohydrates and ≥70% of daily energy from fat) for 6 weeks (Akansel, 2021). Similarly, Kohnert et al. found that a strict vegan diet led to an increase in *Coprococcus* levels, while a meat-rich diet (>150 g of meat per day) resulted in a decrease (Kohnert et al., 2021). The variations in the findings could be attributed to variations in the type of fat and dietary composition being researched. It has also been reported that a higher intake of polysaccharides and plant protein leads to a higher abundance of *Coprococcus* and other butyrate-producing bacteria (Garcia-Mantrana et al., 2018). Although the plant protein intake decreased in this study, it was not statistically significant.

Another finding of this study was the inverse relationship between *Fusicatenibacter* and vegetable consumption. According to one study, *Fusicatenibacter* has a strong correlation with higher levels of propionate in fecal samples. This correlation was linked to unhealthy dietary habits and obesity (Takada et al., 2013). There is currently no research in the literature that demonstrates a relationship between *Fusicatenibacter* and nutrient consumption.

It has been reported that consuming refined sugar can influence both the function and composition of the intestines. Ali et al. (2021) observed that sweet consumption was positively associated with the prevalence of *A. muciniphila.* They stated that further studies are necessary to confirm this effect. Our research did not detect any relationship between dietary patterns and *Akkermansia.* Consuming whole grains plays a role in gut microbiome modulation (Martínez, 2013) although no evidence of gut microbiota shift from grain consumption was found in our study. *Intestinibacter* of Firmicutes phylum was positively correlated with protein-derived energy. Our study found no significant correlation between protein intake and *Intestinibacter.* However, we did observe a negative correlation between *Intestinibacter* and the consumption of nuts.

In this study, in which we evaluated the effects of RIF during the standard fasting period, it was very difficult to evaluate the differences in interindividual diet patterns since they did not follow a standard dietary pattern. Using a standard dietary pattern in future studies will aid in effectively evaluating the results.

This research has several limitations. First, the sample size is limited because it was challenging to find individuals that met the inclusion criteria. The small sample size makes it essential to do more research on the impacts of dietary practices like fasting on a greater study cohort. Further comprehensive and prolonged research is necessary to evaluate the complete influence of RIF on gut microbiota modulation. Another limitation of this study is sleep and changes in dietary intake during RIF. Sleep restriction could alter gut microbiota composition because RIF is associated with sleep duration and nighttime sleepiness. Therefore, sleep may be a confounding factor in the alteration of gut microbiota. In this study, although there was no change in the amount of energy, carbohydrate, and protein taken in the diet, the dietary fat and water-soluble fibers were different. Therefore, differences in dietary intakes may also be a confounding factor in gut microbiota change.

In conclusion, diet and fasting have important effects on the gut microbiota. These effects differ between individuals. During Ramadan, although the duration of fasting is similar, there are

significant differences between the foods consumed by individuals. The effects of these consumed foods are the basis of the different changes in the gut microbiota. Therefore, future studies must evaluate the effects of people following similar dietary patterns during Ramadan.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI-PRJNA957131.

Ethics statement

The studies involving humans were approved by Ethical Committee of the Acibadem Mehmet Ali Aydinlar University (ATADEK) (decision number 2017-17/8). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

DS contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript. GAC contributed to the design and implementation of the research. ES designed and performed data analysis, prepared figures, and contributed to writing and reviewed the manuscript. BE and US have contributed to writing and reviewing of the manuscript. MB has contributed to design of the research, write, and review of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2023.1203205/full#supplementary-material

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Ramadan-specific nutrition education improves cardio-metabolic health and inflammation—a prospective nutrition intervention study from Pakistan

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There are recent reports that Ramadan fasting (RF) results in weight gain instead of weight loss. In addition, the data on the efficacy of brief nutrition education on healthy eating practices in Ramadan for better health are scarce. Therefore, a study was conducted to investigate the effects of brief nutrition education before the start of RF on healthy eating practices during RF. For this purpose, a prospective observational study focused on "Dietary Education and Awareness for Ramadan (DEAR)" as an intervention was carried out. The participants ($n = 74$) were recruited and divided into two groups, i.e., intervention and control groups ($n = 37$ each). As an intervention, nutrition education lessons were given before and during RF month. The control group did not attend these nutrition education lessons. Data on anthropometrics, dietary intake, and other parameters were collected at three time points: before, in the end, and 4 weeks after RF. Weight was measured in kg; height, waist circumference (WC), and hip circumference (HC) were measured in cm; and body mass index (BMI) was calculated. Waist-to-hip ratio (WHR) was calculated by dividing the waist value by the hip value. Body composition analysis was performed by the body composition analyzer (BF-907). Blood pressure (BP) was measured using a validated automated blood pressure. A 3–5 ml of venous blood was collected, and plasma and serum were separated. Serum and plasma samples were processed for general blood chemistry (blood lipid profile, glucose, and CRP) within 2 h. CRP was determined by the immunoturbidimetry method using an auto-analyzer. An enzyme-linked immunosorbent assay (ELISA) was used to determine cytokine/chemokines. Adherence to nutrition education (intervention) was assessed. The results show that nutrition education has positive effects on overall nutrition. Significant improvement in dietary adherence to dietary advice in the intervention group was noted. Significant BW loss (mean loss: 1.21 kg) in the intervention group was observed. The majority (63.3%) had lost BW ≥ 1.0 kg. Other changes observed as a result of the intervention included improvements in blood glucose, cholesterol, CRP levels, and systolic and diastolic BP. There was a notable shift in pro- and anti-inflammatory cytokine concentrations: IL-7, IL-4,

and TGF-α decreased, while IL-2, TNF-α and resistin, IL-1 RA, IL-17 A, and sCD40 increased. In conclusion, RF resulted in a loss in mean BW and an improvement in related blood chemistry and cytokine profiles. Furthermore, nutrition education before RF resulted in better nutrition practices during RF and a desirable healthy BW, blood lipid, and cytokine profiles.

KEYWORDS

DEAR, PDGN, inflammatory cytokine, CRP, Ramadan fasting, NEAT

Background

Ramadan is the ninth month of the Islamic Calendar. Muslims all over the world observe fasting during this month from dawn to sunset. During fasting hours, Muslims refrain from fluid and food intake (1, 2). The duration of fasting hours is variable and ranges from 12 to 18 h, depending on the season and geographical location (3). The prolonged daytime fasting during Ramadan makes it a unique model of weight management strategy. Furthermore, during this month, changes in a number of dietary habits occur. These changes include quality, quantity, timing of food intake, physiological, and biochemical factors (4, 5). These changes may be helpful in reducing the prevalence of certain chronic disorders, such as obesity, diabetes, and cardiovascular diseases.

Obesity, a chronic inflammation, is globally prevalent at alarming rates. Obesity is indisputably related to an increased prevalence of many preventable diseases, a significant log-linear increase in the risk of all-cause mortality, an increasing body mass index (BMI) value of >25, and enormous economic losses. While a number of strategies have been in place in an attempt to minimize the undesirable changes in metabolism and appetite, the benefits of an intermittent fasting regimen have begun to demonstrate strong efficacy in studies including ours (6) and others (7–9). A change in body weight is associated with other changes in the body, for example, changes in body composition, blood biochemistry, and a number of inflammatory biomarkers. Extended durable changes in the circadian clock of the human body may encourage numerous changes in cardio-metabolic profile; thus, fasting during Ramadan is well-established to have an overall impact on endocrine and cardio-metabolic health (7–9).

A number of classical prospective epidemiological studies have reported a constant relationship between high CRP levels and an increased possibility of cardiovascular episodes comprising myocardial infarction, stroke, and cardiovascular disease (CVD)-related mortality (10–14). Other markers that are related to obesity and cardiovascular health include cytokines (pro- and anti-inflammatory) and chemokines. Although the effects of short-term fasting on the profile of inflammatory markers have also been previously documented (15), the effect of long-lasting variations of food consumption in a model such as Ramadan fasting on the markers of inflammation has not been extensively studied. Nevertheless, the control diet intake has been recognized as an effective mechanism for managing obesity (16).

For most individuals, diet control may be persuaded by nutrition education; the education may, therefore, possibly aid the improvement of dietary interventions. Interestingly, controlling excessive food intake is a commendable act in Islamic teachings and health guidelines (17, 18). Faith-based interventions such as the dietary interventions adopted by Christians have also been reported to be fruitful in managing weight and its associated health risks (19, 20). Ramadan Iftar buffet, for example, is a common tradition now throughout the Muslim world (21), and the common belief among Muslims is that Ramadan is a month of 'giving charities' and 'generosity' (22). It is obvious that there is a gap between thoughtful knowledge and attention in rationalizing feasting and expenditure. The gap needs to be spanned by more nutrition/behavior sensitive education programs specially premeditated for the local public about the concept that Ramadan is also a month of modesty—modesty in food intake as well. There is a dire need for nutrition educationalists and researchers to be involved in augmenting the gap—a notion supported by many researchers. As Ramadan fasting is a natural way of observing dietary restrictions and neither exaggerate nor extremely relaxes dietary and lifestyle changes, any nutrition education is supposed to be very successful.

In addition, despite the role of Ramadan-specific nutrition education, it has been extensively studied in metabolic disorders patients; however, no studies, to the best of our knowledge, have been reported in healthy individuals. Having in mind that the observed variation in changes in body weight and body composition may be a result of Ramadan fasting, we hypothesize that fasting with some advice on healthy dietary intake may have an impact on overall health. We were of the view that any healthy dietary modification is deemed to be easy to take place as well as to be adhered to during the post-Ramadan period as well. We looked to create long-term dietary behavioral changes as proposed elsewhere (23, 24) and learned during the month of Ramadan through developing practical skills that help in healthy dietary intake.

We, therefore, conducted this study to investigate any long-term positive effects of Ramadan fasting through a follow-up post-Ramadan phase to observe adherence to healthy dietary and lifestyle modifications brought about during Ramadan. Furthermore, we wanted to observe the efficiency of nutrition education-based dietary interventions to maneuver post-Ramadan weight regain. We expected that the intervention would help in encouraging participants to keep track of their food intake at feasting times during Ramadan fasting. It was hypothesized that the control food intake and the detainment of Ramadan-induced weight loss would be more obvious in the nutrition intervention group than in the control group. In order to investigate the rationale of how fasting during Ramadan affects the body weight and body composition of practicing Muslims, it might lead to a better interpretation of the global practicing Muslim community being influenced by observing the religious obligations of Ramadan. Furthermore, it might be valuable to take into account the

epidemic of overweight and obesity, which has affected nations around the world, including Muslim-majority countries (25).

Materials and methods

The study used a pre- and post-test design with random assignments to the intervention group, who received special structured nutrition education through structured lectures, individual telephone counseling, and other educational tools. The control group did not receive any specially structured nutrition education lessons. Both groups completed pre- and post-test as reported elsewhere (6).

Recruitment of study participants

The participants of this study were either members of a registered organization, i.e., nutrition education, awareness, and training (NEAT: Social Welfare Department Khyber Pakhtunkhwa, Govt. of Pakistan) or persons known to members of the organizations. Potential participants were identified from the member registration record of NEAT. The registered members were encouraged to invite their other family members, relatives, and friends to join a pre-education session 1 week before the start of Ramadan fasting month in 2018.

On their arrival, the anthropometrics and body compositions of all participants were measured. Participants were encouraged to provide their willingness to participate as participants in the control group of the study or the nutrition intervention group. Initially, 37 male participants showed their interest in participating in the nutrition intervention group. These were enrolled as 'the intervention group'. A group of $n = 37$ perfectly matched those in the intervention group with respect to their baseline education, age, weight, and BMI was to act as the control group.

Inclusion and exclusion criteria

The detailed inclusion and exclusion criteria have been reported previously (6), but briefly, the study excluded bedridden and sick individuals, antenatal and breastfeeding females, and patients with conditions that necessitate dietary adjustments. Participants with pre-diabetes symptoms as monitored by a medical doctor were also not included. All participants were screened for their general health by a medical doctor before they entered the study. The research team had access to the personal health files of the selected participants as most of them were members of NEAT and were routinely checked for their health status from time to time. There were no refusals among the respondents who were chosen.

Intervention

The intervention was in the form of brief sessions of nutrition education. Nutrition education was primarily based on a set of dietary advice for Ramadan fasting, specifically developed for the purpose of the present study, i.e., Dietary Education and Awareness for Ramadan (DEAR; Supplementary File 1). These advices were prepared using information from Pakistan Dietary Guidelines for better nutrition (PDGN). The nutritional recommendations of the World Health Organization were also incorporated into these guidelines (26).

Data collection

All data were collected at three time points (T1, T2, and T3; repeated measures). Data at T1 were collected 2–3 days before the start of the fasting month of Ramadan. Data at T2 were collected in the last week of Ramadan fasting, preferably 2–3 days before the end of Ramadan fasting. Data at T3 were collected 1 month after Ramadan fasting. To ensure relatively huge data in a comparatively short time span, the help of trained field and clinical data collectors was sought.

Dietary data

The participant's dietary data were collected using a 3-day-24 HDR and a Food Frequency Questionnaire (FFQ; 27). The FFQ was specially developed for this research. Dietary recalls were conducted in the form of face-to-face interview. During the interviews, participants were asked to recall their food intake during the fasting period in a certain order, starting with breakfast in the morning (Sahar meal), Iftar meal, and ending with their last meal before sleep. Data on cooking methods (roasted, fried, boiled, steamed, etc.) and food sources (homemade vs. outsourced) were also gathered. Meanwhile, a family adult confirmed the nutritional report in interviews to reduce misinterpretations. Models of household utensils, such as bowls, spoons, and cups, were utilized to aid in the measurement of food consumption.

Amounts were reported based on how much food was consumed from each bowl, i.e., a half-filled small bowl. Respondents were asked to cup equals when they gave an unclear answer (e.g., "I used a little or a lot of milk in tea"). Food portion sizes were calculated using data from Pakistan's Dietary Guidelines for Better Nutrition (28).

The average amount of each food item consumed for 3 days was determined, and nutrients were calculated. Nutrient intake was calculated using an in-house nutrient calculator based on the data from Pakistan's food composition tables and previously published research (6, 29). Carbohydrates, lipids, protein, total energy, and a few vitamins and minerals were among the nutrients examined. There were additional percentages of energy from carbohydrates, lipids, and protein. Given the importance of energy distribution in solid vs. liquid foods (30), we also calculated the percentage of energy contribution by solid and liquid diets to assess the caloric intake in terms of 'solid calories' and 'liquid calories'. Participants were also requested to disclose all types of prescribed medicines and supplements consumed.

Anthropometric measurements

Weight was measured in kg, and height, waist circumference (WC), and hip circumference (HC) were measured in cm, as previously reported (6). In brief, body mass index (BMI) was calculated by dividing weight by squared height. The waist circumference was recorded at the height of the navel, and the hip circumference was measured at the crest of the buttocks by using a non-stretchable measuring tape. Waist-to-hip ratio (WHR) was

calculated. Participants were categorized as obese (OB), overweight (OW), and normal weight (NW) based on their BMI values according to WHO standards as previously reported (6).

Assessment of body composition

Body composition analysis of the study participants was performed using a body composition analyzer (BF-907) as reported previously (6). It is portable, non-invasive battery-operated equipment connected to the body through four electrodes.

Blood pressure measurement

The study participants' blood pressure (BP) was measured using a validated automated blood pressure monitor (Model M: 6, Omron Healthcare, Japan; 31). All participants were asked to relax for 5 min at room temperature before reporting their blood pressure results. Each person's blood pressure was measured three times. The measurements were taken on the right arm using a monitor with a proper cuff size and short intervals between readings. For analysis, the average of the three measurements was recorded (32). Both systolic and diastolic blood pressure readings were reported for all the participants at three time points of the study.

Blood sampling and analysis

Blood sampling was performed as reported previously (6). In brief, all blood samples at three different time points were collected in a way to ensure at least 8–10 h of fasting. In this way, pre-Ramadan (T1) and post-Ramadan (T3) fasting blood samples were collected in the morning, 7–8 a.m. before the morning breakfast. During Ramadan (T2), blood samples were collected at 11.00 a.m., approximately 8 h after the Sahar meal. Biochemical analyses were performed on blood samples following standard protocols at the Biology of Aging Laboratory, Singapore Immunology Network, Agency for Science Technology and Research, 8A Biomedical Grove, Singapore 138,648, Singapore. Consequently, blood lipid profile, blood glucose, hsCRP, and an extended panel of cytokines and chemokines were determined. Details of the procedures for the determination of these are provided in Supplementary File 2.

Nutrition education strategy

The nutrition education strategy was partially based on adult learning theory (33). It consisted of four steps: (1) assessing the needs of the participants, (2) setting educational objectives, (3) choosing/using a variety of methods, and (4) assessing that learning occurred (33). The unique learning styles of participants were identified in a pilot study (visual, auditory, collage, etc.). Objectives that focus on what the participants will do with the contents of the nutrition education strategy in order to learn it were set, and the intervention was designed accordingly. Participants in the intervention group attended a multi-component nutrition education entitled "Dietary Education and Awareness for Ramadan: Healthy Eating for a Healthy Living during Ramadan" using three different modes of instruction (lecture/talk, leaflet, and picture collage). During the study period throughout the 4 weeks of Ramadan fasting, participants were kept in contact through mobile phones, personal contact, or in groups in Iftar Buffet.

Procedure for nutrition education

The participants in the intervention group were gathered in three sessions for an average of 2 h. This was considered workable as previously prepared by Liu et al. (34). A mixture of Pashto and Urdu, as the local/national languages, was used during these instructions. All sessions were held in groups and were carried out by the investigator. The perception of the educational tools was pretested in a sample of 10 adults before the actual intervention.

The first session used a focal group technique. It was diagnostic in character. It was designed to identify limitations and/or barriers to healthy food consumption during Ramadan in the community. The second session was motivational in nature. It was formatted as a culinary workshop. The main purpose was to promote contact with different types of healthy foods for Ramadan fasting month, which therefore included the preparation and degustation of various recipes containing fruits and vegetables as the primary ingredients. This session also comprised a lecture guided by a poster and covered topics that addressed overall dietary quality, including: (a) identifying a preferable overall distribution of types of food in a diet using the plate model from the dietary guidelines for Pakistan (28) (Figure 1); (b) increasing the consumption of fresh fruits and vegetables, whole grains, and their products; (c) discouraging the consumption of fried foods (e.g., samosa, pakora, and kabab), foods high in fat and sugar (*jalaibee*, *sweets*, *puddings*, etc.), and (d) providing alternatives to low-nutrient snacks. This session also consisted of a brief summary of all topics discussed during the previous session and a picture collage was introduced where participants had to use pictures and place them in appropriate portions on a designed plate model. At the end of the second session, a leaflet consisting of topics discussed above together with a "sample menu" of iftar, dinner, sahar, and "alternatives to junky snacks (low nutrients snacks)" featuring mainly the local snack types. This was distributed to the participants as "take home lessons" to enhance their understanding of the whole session. The third and last session was essentially informative, mainly focusing on addressing the issues of nutritional recommendations for Ramadan, health benefits associated with fresh fruit and vegetable consumption in general, and during Ramadan in particular, ways to increase the consumption of such foods, and replacement of less healthy foods with fruit and vegetables. Question and answer sessions were conducted during the lecture to enable active participation.

In addition, participants in the intervention group with a normal baseline BMI (18.5–24.9) were encouraged to maintain their weight with no increase or decrease while still adjusting their diet to a healthier pattern and incorporating certain changes in their daily intake. The obese and overweight participants in the intervention group were encouraged to lose at least 5% of their weight at baseline by following a healthier pattern by incorporating certain changes in their daily intake. All participants in the intervention group were

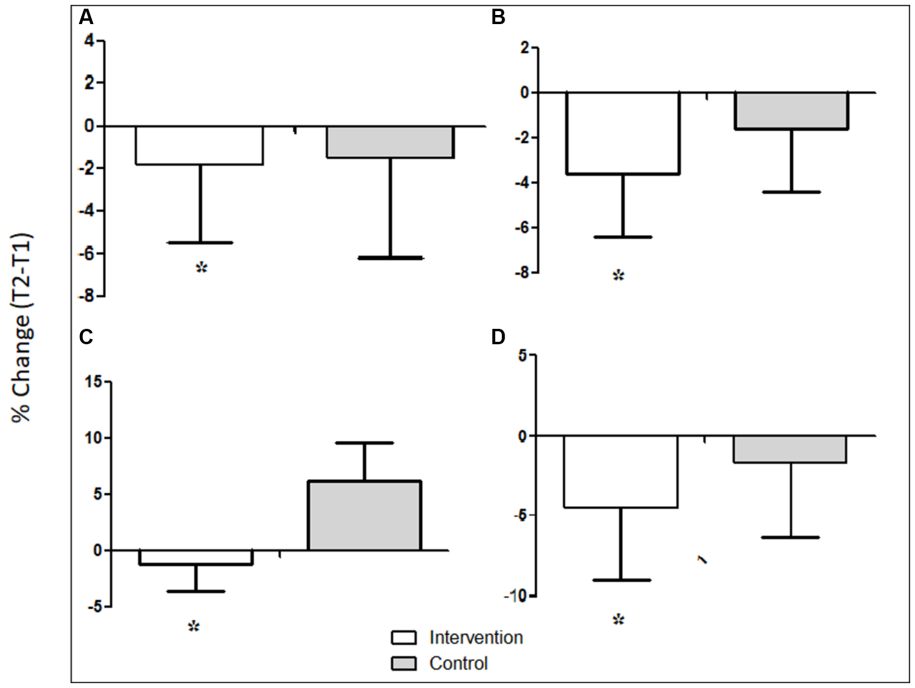

FIGURE 1
Mean percent reduction in anthropometrics. **(A)** Mean percent reduction in weight, **(B)** mean percent reduction in BMI, **(C)** mean percent reduction in WC, and **(D)** mean percent reduction in %BF. White bars represent the 'intervention group'. Gray bar represent the 'control group'.

advised in a personalized manner. Targets in terms of weight and diet were defined and explained on an individual basis.

Assessment of adherence to nutrition education

Adherence to nutrition education was assessed twice—1 day before the commencement of Ramadan fasting, just before the educational sessions, and a second time on the last day of Ramadan fasting. The purpose of assessing adherence to nutrition education at two time points was to know whether there was any difference in nutritional behavior between the two time points of the study (T1 and T2) in order to assess the overall impact of nutrition education on nutrition behavior and dietary intake.

The questionnaire used to assess nutrition education was adapted from the one developed by Permatasari et al. (35; Supplementary File 3). The questionnaire consisted of 13 questions, with answers graded using a Likert scale. Favorable questions (1–5) were scored as follows: 1 = strongly disagree, 2 = disagree, 3 = agree, and 4 = strongly agree. For unfavorable questions (6–13), this scoring was reversed as follows: 1 = strongly agree, 2 = agree, 3 = disagree, and 4 = strongly disagree. The questionnaire was completed before the intervention (baseline T1) and at the end of the intervention (i.e., at the end of Ramadan fasting, T2).

Nutrition education impact assessment

It was necessary to assess how effective nutrition education was in modifying dietary behavior. This was done by estimating how much the participants in the intervention group demonstrated adherence to the Pakistan Dietary Guidelines for Better Nutrition (28). According to the PDGN, there must be 2–3 servings each of meat and pulses, vegetables, milk and milk products, fruits, and 4–5 times cereals and grains. The adherence to dietray advice given in the PDGN was calculated as reported previously (36). The ratings of the consumption of each food group (from 0 to 5 or the reverse) were adapted from Panagiotakos et al. (37) and are given in Table 1. The dietary adherence score included non-refined cereals and bread (whole bread, rice, pasta, and other grains), fruit, vegetables, legumes, fish, olive oil, meat and meat products, poultry, full-fat dairy products, sweets, and oils. For the intake of food items assumed to be close to the PDGN or higher (non-refined cereals, fruits, and vegetables), we allocated a score of 0 when the individual stated no consumption, a score of 1 when they stated the consumption of 1–4 servings/month, a score of 2 for 5–8 servings/month, a score of 3 for 9–12 servings/month, a score of 4 for 13–18 servings/month, and a score of 5 for more than 18 servings/month. Moreover, we included legumes, fish, and olive oil in this group after separating them from the meat and oil groups. In contrast, for the intake of food items assumed to be limited in PDGN (i.e., rare or monthly intake; meat and meat products, poultry, and full-fat dairy products), we allocated the scores on a reverse scale (i.e., 5, when individuals stated no intake, to 0, when they stated almost daily intake). Hence, the scores ranged from 0 to 60. Higher scores show better adherence to the Pakistan Dietary Guidelines for better nutrition.

Ethical consideration

The ethics approval for the study was granted by the Human Research Ethics Committee of the Department of Human Nutrition, The University of Agriculture, Peshawar (Ref: HN-HREC/2017-022).

TABLE 1 Scoring system for adherence to dietary PDGN score.

No.	Food groups	Frequency of consumption (Servings/Month)					
		Never	1–4	5–8	9–12	13–18	>18
1	Non-refined cereals and bread[a]	0	1	2	3	4	5
2	Fruit[b]	0	1	2	3	4	5
3	Vegetable	0	1	2	3	4	5
4	Legumes	0	1	2	3	4	5
5	Fish	0	1	2	3	4	5
6	Olive oil	0	1	2	3	4	5
7	Meat and meat products	5	4	3	2	1	0
8	Poultry	5	4	3	2	1	0
19	Full-fat dairy products	5	4	3	2	1	0
10	Sweets	5	4	3	2	1	0
11	Oils	5	4	3	2	1	0

[a]Whole-grain bread, rice, pasta etc.
[b]Fresh (e.g., apple, oranges, banana, grapes, etc.) and dried fruit, including dates.

Statistical analysis

Data obtained from the questionnaires were processed using Microsoft Excel and analyzed using SPSS v22 software. Participants' baseline demographics were expressed as categorical data, with frequencies and percentages, or continuous data, with mean and standard deviation. Confidence intervals (CIs) were calculated and presented in separate tables in the supporting files (Supplementary File 4). Continuous data were analyzed using an independent sample t-test and categorical data by applying Fisher's exact test. Adherence to PDGN was scored and displayed as continuous data. The differences in scores at the start and end of the study were measured using the Wilcoxon signed-rank test. Baseline differences between these variables were tested using the Mann–Whitney U-test. A value of p of <0.05 was considered significant.

Results

Selected socio-demographic characteristics of the participants are presented in Table 2. The two groups were matched in age, employment status, education, monthly income, and nutritional status. The mean (SD) age of the case and control groups was 47.1 ± 6.7 years for the intervention group and 49.9 ± 7.5 years for the control group.

Adherence to nutrition education

All participants in both the intervention group and control group completed the follow-up assessment at the two time points (T1 and T2) of the study. The mean start score for adherence to nutrition education was 16.0 (8.1; range: 9–24) for the control group and 16.6 (4.8; range: 9–27) for the intervention group ($p = 0.406$). The end scores were 17.1 (9.9; range: 10–25) and 42.5 (13.2; range: 30–48) in the control and intervention groups, respectively. There was a significant improvement in the intervention group's adherence to nutrition education test scores, with an absolute increase in a mean score of 25.6 ($p < 0.0001$). No significant difference was observed in the control group test scores (absolute difference 1.3; $p = 0.224$).

Effect of nutrition education

Primary outcome

Dietary adherence to the PDGN score

Table 3 shows mean (SD) dietary adherence scores to PDGN at three time points for intervention and control groups. In general, the mean (SD) dietary adherence to PDGN scores at T2 for all parameters for the intervention group was significantly higher as compared to T1. These scores remained stable to some extent even after the 4-week post-Ramadan period (T3). The total dietary adherence to PDGN score at T2 was significantly higher as compared to T1 for the intervention group (36.7 vs. 14.2; $p < 0.05$). The total dietary adherence to the PDGN score for the control group between T1 and T2 differed non-significantly (14.0 vs. 11.8; $p > 0.05$).

Secondary outcomes

Changes in anthropometrics

As shown in Table 4, at baseline, the intervention and control groups had the same anthropometrics and body composition parameters (p, for all trends>0.05). Details of the ANOVA associated with Table 4 are given in Supplementary File 4. Overall, there was a general trend of a decrease in body weight, BMI, WC, and % BF at T2 and then a slight increase by T3. However, most of these variables were still lower at T3, despite an increase after T2 during the post-Ramadan period. The time x group interactions for BMI, WC, and % BF were as follows: significant for BMI [F (2, 116) =10; $p < 0.0001$], WC [$F_{(2, 115)} = 10.5$; $p < 0.0001$], and non-significant for % BF [$F(2, 116) = 1.5$; $p = 0.221$].

TABLE 2 Baseline demographics of the study participants.

Characteristics	Intervention group ($n = 37$)	Control group ($n = 37$)	t/χ^2	p-value
Age [years; mean (SD)]	30.1 (12.2)	31.3 (13.5)	$t = 0.65$	0.511
Employment status, n (%)			$\chi^2 = 1.412$	0.265
Unemployed	20 (66.7)	27 (90.0)		
Employed	10 (33.3)	23 (76.7)		
Education, n (%)				
Primary school	12 (40)	18 (60.0)	$\chi^2 = 4.52$	0.386
Secondary school	10 (33.3)	12 (40.0)		
High School	4 (13.3)	4 (13.3)		
Undergraduate/Bachelor	4 (13.3)	4 (13.3)		
Monthly income*, n (%)			$\chi^2 = 0.889$	0.345
Below regional wage	16 (53.3)	18 (60.0)		
Above regional wage	14 (46.7)	12 (40.0)		
BMI, n (%)				
Normal Weight (18.5–24.9)	17 (56.7)	27 (90.0)	$\chi^2 = 0.290$	0.861
Overweight (25–30)	8 (26.7)	16 (53.3)		
Obese (>30)	5 (16.7)	7 (23.3)		

Results significant at $p < 0.05$; χ^2 = Chi square test; BMI=Body mass index. *The Khyber Pakhtunkhwa Minimum Wages Act, 2013.

TABLE 3 Mean (SD) adherence to dietary PDGN scores.

	Intervention[1] ($n = 37$)			Control[1] ($n = 37$)			p-value*
	T1	T2	T3	T1	T2	T3	
Non-refined cereals and bread	1.2 (1.1)[a]	3.1 (1.4)[b]	1.5 (1.1)[a]	1.3 (0.9)[a]	1.3 (1.2)[a]	1.4 (1.1)[a]	<0.0001
Fruit	1.9 (1.7)[a]	4.4 (2.1)[b]	2.5 (1.2)[b]	1.8 (0.1)[a]	1.9 (1.1)[a]	1.5 (1.2)[a]	<0.0001
Vegetable	3.9 (2.1)[a]	4.5 (2.7)[b]	4.5 (1.5)[a]	3.9 (2.1)[a]	2.9 (1.1)[b]	3.7 (1.1)[a]	0.05
Legumes	1.6 (1.2)[a]	3.6 (2.3)[b]	2.6 (2.1)[b]	1.6 (1.2)[a]	1.8 (1.2)[a]	1.7 (1.2)[a]	<0.0001
Fish	1.8 (1.3)[a]	3.6 (2.5)[b]	2.7 (1.3)[b]	1.6 (1.2)[a]	1.3 (1.1)[a]	1.5 (0.4)[a]	<0.0001
Vegetable/Olive oil	0.7 (1.2)[a]	2.1 (1.2)[b]	1.2 (1.5)[b]	0.8 (0.4)[a]	0.9 (0.4)[a]	0.2 (0.1)[b]	0.01
Red Meat and meat products	0.5 (1.1)[a]	1.4 (1.1)[b]	0.5 (0.2)[a]	0.4 (0.1)[a]	0.5 (0.4)[a]	0.5 (0.2)[a]	<0.0001
Poultry	0.5 (1.0)[a]	2.6 (1.3)[b]	1.2 (0.6)[b]	0.5 (0.2)[a]	0.8 (0.4)[a]	0.6 (0.3)[a]	<0.0001
Full-fat dairy products	1.1 (1.1)[a]	3.6 (2.1)[b]	1.1 (0.1) [a]	1.3 (0.4)[a]	1.2 (1.2)[a]	1.2 (0.4)[a]	<0.0001
Sweets	0.5 (0.4)[a]	4.1 (2.7)[b]	1.4 (0.5) [b]	0.2 (0.1)[a]	0.7 (0.2)[a]	0.5 (0.4)[a]	<0.0001
Ghee	0.5 (0.3)[a]	3.7 (2.1)[b]	1.7 (1.1)[b]	0.8 (0.4)[a]	0.7 (0.2)[a]	0.5 (0.4)[a]	<0.0001
Total adherence to PDGN	14.2 (9.2)[a]	36.7 (12.7)[b]	20.9 (11.9)[b]	14.2 (6.3)[a]	14 (6.3)[a]	11.8 (5.9)[a]	<0.0001

*p-value, based on mean differences between T1 and T2 for intervention and control groups.
[1]Means followed by the same letter do not differ significantly. T1 = baseline; T2 = at the end of Ramadan fasting month; T3 = 1 month post-Ramadan; Two Way ANOVA, with $p < 0.05$.

Figure 1 shows that compared to the control group, participants in the intervention group had a greater percent reduction in weight (−1.8 vs. −1.5%.), BMI (−3.6 vs. −1.6%), WC (−1.2 vs. 6.2%), and % BF (−4.4 vs. −1.1%; p, for all trends < 0.05).

Table 5 shows the mean (SD) intake of energy, macronutrients, and fiber. In general, mean energy intake significantly differed at three time points of the study in both the intervention and control groups. Details of these analyses associated with Table 5 are given in Supplementary File 4.

Figure 2A shows the %age of energy distribution in carbohydrates, protein, and fats in the intervention and control groups. The percent energy contribution by carbohydrates in the intervention and control groups slightly increased at T2 compared to T1 and then decreased at T3. The percent energy contribution by protein slightly decreased at T2 as compared to T1 in the intervention group but increased in the control group. The percent energy contribution by fats also slightly decreased at T2 as compared to T1 in the intervention and control groups.

Figure 2B shows the %age change in the intake of saturated and unsaturated fats between T1 and T2. In the intervention group, saturated fat intake decreased by 20.9%, while unsaturated fat intake increased by 147.8%. In the control group, the saturated fat intake

TABLE 4 Mean (SD) of Anthropometrics of Intervention and Control Groups.

Variables	Intervention ($n = 30$)			Control ($n = 30$)			Two-way analysis of variance (p-value)*		
	T1	T2	T3	T1	T2	T3	Group	Time	Group × Time
Weight	74.2 (10.8)[a]	72.9 (10.68)[b]	73.8 (10.73)[a]	71.5 (10.54)[a]	70.4 (10.39)[a]	71.7 (10.59)[a]	0.925	<0.0001	<0.0001
BMI	25.1 (3.95)[a]	24.6 (3.8)[b]	24.9 (3.9)[c]	24.5 (3.62)[a]	24.1 (3.60)[a]	24.5 (3.60)[a]	0.901	<0.0001	<0.0001
WC	90.0 (10.8)[a]	88.9 (10.49)[b]	89.9 (10.47)[a]	90.0 (8.9)[a]	95.6 (8.77)[a]	88.2 (8.93)[a]	0.116	<0.0001	<0.0001
%BF	29.1 (6.68)[a]	24.8 (5.12)[b]	27.2 (5.84)[c]	31.1 (6.81)[a]	29.4 (5.88)[a]	29.0 (6.08)[c]	0.294	<0.0001	0.217

[1]Means followed by the same letter do not differ significantly. One-way ANOVA, with $p < 0.05$; T1 = baseline; T2 = at the end of Ramadan fasting month; T3 = 1 month post-Ramadan; Two Way ANOVA, with $p < 0.05$. *p-value, Two Way ANOVA, $p < 0.05$.

TABLE 5 Mean (SD) of nutrients intake of Intervention and Control Groups.

Variables	Intervention ($n = 30$)			Control ($n = 30$)			p-value*		
	T1	T2	T3	T1	T2	T3	Group	Time	Group × Time
Energy (Kcal)	2052	2,191	2,322	2,302	2,193	2,389	<0.0001	0.0003	0.0012
Carbohydrates (g)	301.9	332.5	347.9	353.2	345.3		<0.0001	<0.0001	<0.0001
Total Protein (g)	71.2	70.4	72.1	71.3	72.5	72.3	0.542	0.636	0.856
Fats (g)	62.2	64.4	71.3	67.1	58.0	71.9	0.854	<0.0001	0.021
Fiber (g)	11.1	10.7	10.5	10.9	1.0	10.2	0.654	0.340	0.759

[1]Means followed by the same letter do not differ significantly. One-way ANOVA, with $p < 0.05$; T1 = baseline; T2 = at the end of Ramadan fasting month; T3 = 1 month post-Ramadan; Two Way ANOVA, with $p < 0.05$. *p-value, Two Way ANOVA, $p < 0.05$.

decreased by 8.2%, and the intake of unsaturated fats decreased by 78.4%.

Changes in biochemical profiles

Table 6 shows the mean (SD) of various blood biochemicals at three time points of the study. Compared to T1, mean (SD) values of blood glucose, cholesterol, sodium, CRP, and diastolic and systolic blood pressure were lower at T2 for the intervention group. These differences were less evident for the control group. Details of the ANOVA associated with Table 6 are given in Supplementary File 4.

Changes in cytokines/chemokines levels

Table 7 shows the mean (SD) concentrations of cytokines/chemokines stratified by the intervention and control groups and by the three time points of the study. The table also gives the results of the ANOVA. For the intervention group, mean (SD) values of pro-inflammatory cytokines/chemokines (IL-2, IL-7, TNF-α, and Resistin) were higher at T2 as compared to T1 (p, for all trends<0.05). For these cytokines/chemokines, the control group followed almost the same trend. In case of pro-inflammatory cytokines, for the intervention group, the levels of these cytokines at T2 were also higher as compared to T1. For the control group, with the exception of IL-1 and TGF-α, the mean (SD) values of these cytokines were lower at T2 as compared to T1.

Changes in the cytokines/chemokines levels through the three time points of the study for both the intervention and control groups are also depicted in Figure 3. As evident, in most of the cases, the blood concentration of cytokines/chemokines elevated during Ramadan fasting and then either further went up or came back closer to the baseline level at the post-Ramadan period.

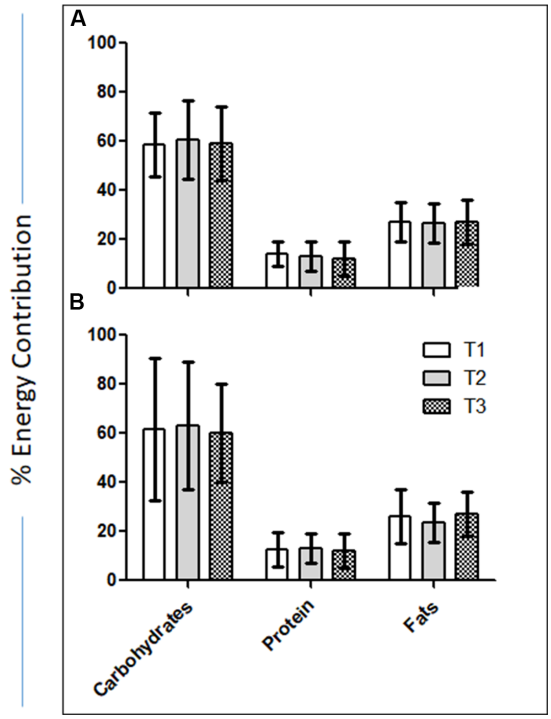

FIGURE 2
(A) Percent change in the intake of saturated and unsaturated fats between T1 and T2. **(B)** Percentage of energy distribution in carbohydrates, protein, and fats in the intervention and control groups.

TABLE 6 Mean (SD) of selected bio-chemicals and diastolic and systolic blood pressure.

Variables	Intervention ($n = 30$)			Control ($n = 30$)			Two-way analysis of variance (p-value)		
	T1	T2	T3	T1	T2	T3	Group	Time	Group × Time
Glucose (mg/dl)	123.4 (14.93)[a]	98.65 (14.7)[b]	105 (13.2)[c]	120.9 (21.4)[a]	101.3 (13.59)[b]	100.03 (27.85)[c]	0.698	<0.0001	0.397
Cholesterol (mg/dl)	149.5 (65.3)[a]	84.48 (74.2)[b]	150.1 (112.4)[a]	133.5 (82.1)[a]	107.9 (76.12)[a]	96.3 (78.81)[c]	0.340	0.0503	0.0330
HDL (mg/dl)	35.9 (9.11)[a]	34.4 (12.22)[a]	33.4 (11.88)[c]	30.9 (9.2)[a]	32.4 (8.4)[a]	30.4 (11.2)[a]	<0.0001	<0.0001	<0.0001
LDL (mg/dl)	112.6 (74.2)[a]	50.1 (32.21)[b]	116.7 (57.31)[a]	85.6 (23.11)[a]	75.5 (34.34)[b]	76.9 (31.11)[c]	<0.0001	<0.0001	<0.0001
Sodium (mg)	3.2 (0.32)[a]	3.0 (0.56)[a]	3.2 (0.81)[a]	2.9 (0.21)[a]	2.7 (0.24)[a]	3.0 (0.33)[a]	<0.0001	<0.0001	<0.0001
CRP (pg/ml)	3.4 (1.11)[a]	2.9 (1.25)[b]	5.47 (2.11)[c]	4.1 (2.08)[a]	3.6 (1.80)[b]	3.3 (2.18)[c]	<0.0001	0.601	0.521
Diastolic BP (mmHg)	80.5 (1.52)[a]	78.3 (2.39)[a]	79.8 (4.11)[a]	80.8 (2.12)[a]	79.2 (2.11)[a]	80.4 (1.37)[a]	<0.0001	<0.0001	<0.0001
Systolic BP (mmHg)	123 (7.51)[a]	116.2 (6.11)[b]	112.0 (6.10)[c]	123.2 (7.40)[a]	114.8 (6.35)[b]	122.6 (4.77)[a]	<0.0001	<0.0001	<0.0001

*p-value, Two Way ANOVA, $p < 0.05$. [1]Means followed by the same letter do not differ significantly. One-way ANOVA, with $p < 0.05$.

TABLE 7 Mean (SD) of cytokines/chemokines.

Variables	Intervention ($n = 30$)			Control ($n = 30$)			p-value*		
	T1	T2	T3	T1	T2	T3	Group	Time	Group × Time
IL-2 (pg/ml)	1.37 (0.50)[a]	1.99 (1.05)[b]	1.65 (0.79)[c]	1.6 (0.98)[a]	1.84 (1.09)[a]	1.62 (0.86)[a]	0.393	<0.0001	0.497
IL-7 (pg/ml)	5.2 (4.3)[a]	5.4 (2.1)[a]	5.8 (2.1)[a]	5.1 (4.2)[a]	4.9 (2.3)[a]	5.0 (5.2)[a]	0.299	0.853	0.722
TNF-α (pg/ml)	36.45 (32.42)[a]	75.23 (61.18)[b]	52.1 (41.63)[c]	44.1 (3.7)[a]	56.1 (17.1)[b]	54.9 (13.01)[c]	0.792	0.008	0.219
Resistin (pg/ml)	3,655 (1777)[a]	3,976 (3083)[b]	3,786 (5446)[c]	3,655 (2015)[a]	3,986 (1482)[b]	3,716 (1633)[a]	<0.0001	0.662	0.700
IL-1 RA (pg/ml)	81.0 (87.01)[a]	140.4 (127.2)[b]	92.14 (68.25)[a]	111 (97.69)[a]	117.1 (77.61)[b]	126 (68.26)[c]	0.383	0.077	0.0871
IL-4 (pg/ml)	46.4 (28.52)[a]	54.33 (33.74)[b]	52.64 (34.36)[c]	44.3 (32.37)[a]	56.1 (29.89)[b]	54.9 (31.91)[b]	0.744	0.771	0.281
IL-17 A (pg/ml)	5.2 (3.15)[a]	5.45 (3.39)[a]	5.89 (3.29)[c]	5.32 (2.94)[a]	4.65 (4.02)[b]	5.83 (2.47)[a]	<0.0001	0.667	0.553
sCD40 (pg/ml)	20.47 (8.01)[a]	20.35 (10.19)[a]	13.51 (11.78)[c]	44.05 (8.7)[a]	56.02 (11.4)[b]	54.61 (9.56)[b]	0.001	0.622	0.392
TGF-α (pg/ml)	6.82 (3.3)[a]	7.91 (5.52)[a]	7.83 (3.98)[a]	7.08 (4.78)[a]	6.84 (3.05)[a]	6.93 (3.69)[a]	<0.0001	0.621	0.504

[1]Means followed by the same letter do not differ significantly. One-way ANOVA, with $p < 0.05$. *p-value, Two Way ANOVA, $p < 0.05$.

Discussion

Major findings

1. Assessment of nutrition education showed a significant improvement in the intervention group adherence test scores, while no significant difference was observed in the control group test scores. This shows that nutrition education may be a successful strategy in bringing positive and healthy dietary changes in Ramadan fasting.
2. Nutrition education was successful in improving dietary adherence to PDGN scores in the intervention group. The total dietary adherence to the PDGN score significantly improved at T2 compared to T1 for the intervention group (mean scores: 36.7 vs. 14.2; $p < 0.05$). The total dietary adherence to PDGN score for the control group between T1 and T2 differed non-significantly (14.0 vs. 11.8; $p > 0.05$).
3. Compared to control, there was significant weight loss in the intervention group at T2 ($p > 0.05$).
1. Overall, there was a general trend of a decrease in body weight, BMI, WC, and % BF at T2 and then a slight increase by T3. However, most of these variables were still lower at T3 despite an increase after T2 during the post-Ramadan period.
2. Compared to the control group, participants in the intervention group had a greater percent reduction in weight (−1.8 vs. −1.5%.), BMI (−3.6 vs. −1.6%), WC (−1.2 vs. 6.2%), and % BF (−4.4 vs. −1.1%).
3. Compared to T1, mean (SD) values of blood glucose, cholesterol, sodium, CRP, and diastolic and systolic blood pressure were lower at T2 for the intervention group. These differences were less evident for the control group.
4. Concentration of most of the cytokines/chemokines elevated during Ramadan fasting and then either further went up or came back closer to the baseline level at the post-Ramadan period in both the intervention and control groups.
5. In the intervention group, saturated fat intake decreased by 20.9%, while unsaturated fat intake increased by 147.8%. In the control group, the saturated fat intake decreased by 8.2% and the intake of unsaturated fats decreased by 78.4%.

Ramadan fasting brings numerous changes in dietary intake. These changes may have beneficial or adverse impacts on overall health, as fasting for 29–30 days is a relatively longer period. Ramadan is now of a 'feasting' than 'fasting' occasion. This may modify dietary patterns in an unhealthy way. The likelihood of unhealthy dietary practices in Ramadan is higher. Previous studies have recommended a dire need for nutrition education for Ramadan fasting. As part of this study, we investigated the effect of formal nutrition education before the start of Ramadan on dietary adherence to the PDGN score (as our primary outcome), weight, nutritional intake, and selected biochemicals and cytokines (as our secondary outcomes) in a group of 30 adult male participants compared

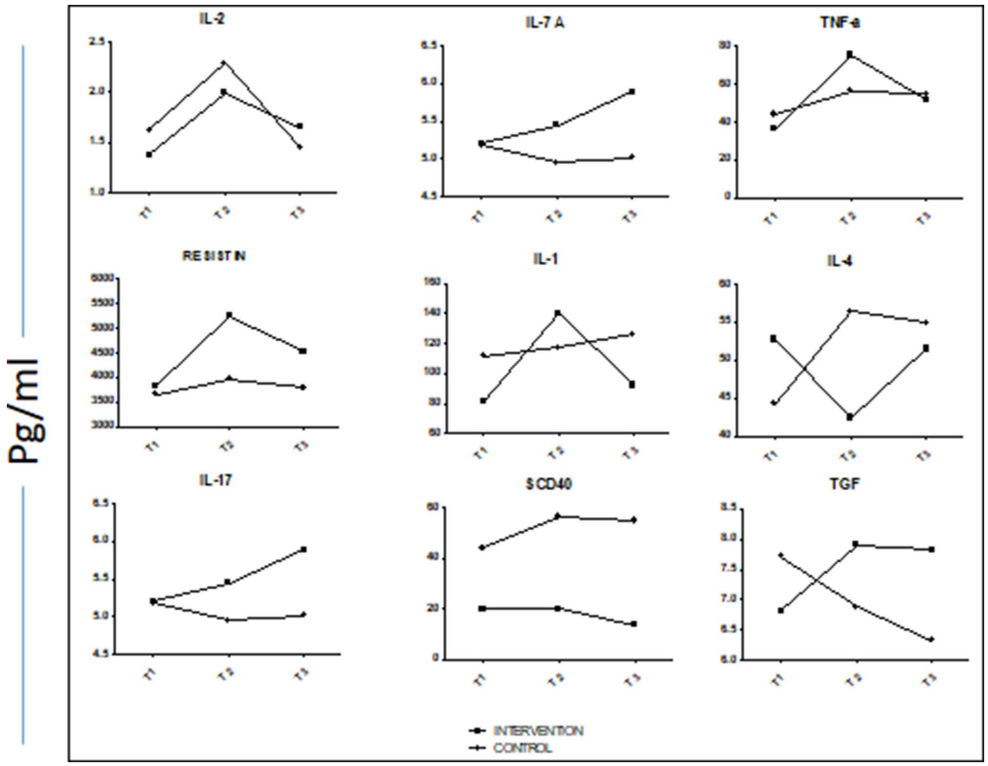

FIGURE 3
Trend lines of mean concentrations of selected cytokines/chemokines at the three time points both of control and intervention groups.

to weight and BMI-matched 30 participants in the control group. Adherence rate and adherence to nutrition education were very high (Table 3), and hence the non-adherence rate was very, i.e., 18.9% (7 out of 37), which was very low compared to 50 and 80% of non-adherence rate for chronic disease management, including medication and lifestyle changes (38). Nutrition education improved adherence to dietary advice as there was a significant improvement in the intervention group test scores, with an absolute increase in the mean score of 25.6, while the control group, who received no nutrition education, failed to improve their adherence to PDGN score (Figure 4). The concept of 'adherence' recognizes the patient's right to choose whether or not to follow advice and implies an individual's active participation in the treatment regimen [Cohen (39)]. Thus, poor adherence can be a serious threat to the overall health and wellbeing of an individual (40) and also carry an economic burden (41). Adherence is particularly important in the context of chronic diseases that require long-term therapy and a number of permanent rather than temporary changes in lifestyle behaviors, including diet (38). The extent to which risk-reduction interventions proved to be as effective in research settings as in individuals' real-life settings depends on the individual's adherence to treatment advice. In that regard, results from a randomized clinical trial that wanted to assess the adherence to and effectiveness of various types of diets revealed that the level of adherence to dietary advice, rather than the type of diet, was the key determinant of effective weight loss (42).

As a primary outcome of interest, dietary adherence to the PDGN score improved in the intervention group, who received nutrition education, as compared to the control group, who did not get any

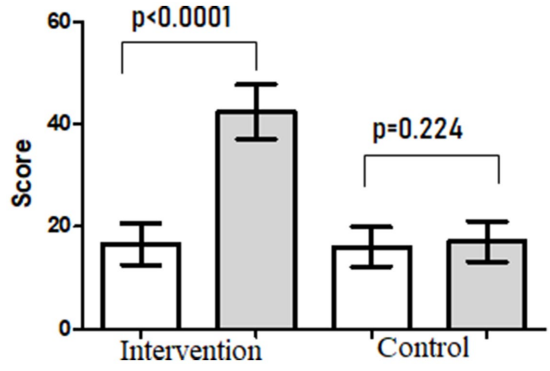

FIGURE 4
Dietary adherence at the start and end of the study.

nutrition education but followed their usual dietary intake patterns throughout the Ramadan fasting month. Dietary adherence to PDGN scores for all parameters in the intervention group significantly improved after they received nutrition education. The total dietary adherence to the PDGN score assessed at the end of Ramadan fasting was significantly higher as compared to the baseline score (36.7 vs. 14.2; $p < 0.05$). On the other hand, the total dietary adherence to PDGN score for the control group at the end of Ramadan did not differ much from the dietary adherence to PDGN score at baseline (14.0 vs. 11.8; $p > 0.05$; Table 2). These data from the present study show that nutrition education at the start of Ramadan brings positive

and healthy changes in the dietary patterns of adult fasting individuals. To the best of our knowledge, there has been no study conducted on otherwise normal adult subjects investigating the impact of nutrition education on dietary modification, and we are therefore unable to compare our results with previous studies during Ramadan fasting. Nevertheless, nutrition education studies done on diabetic patients have shown the overall efficacy of such strategies (43). Participation in structured education programs has shown improvement in their body weight, BMI, and dietary fiber intake in addition to self-empowerment, knowledge of diabetes, and self-management skills (44, 45). In a systematic review of randomized controlled trials of the effectiveness of self-management training in people with type 2 diabetes, educational interventions that involved patient collaboration were more effective than didactic interventions in improving short-term glycemic control (< 6 months), weight, and lipid profiles (46).

As a secondary outcome, the present study found significant mean weight loss in the intervention group who received nutrition education. The loss was much more pronounced compared to that in the control group, which might indicate an effect of nutrition education on healthy weight management. Other anthropometric variables of interest (e.g., BMI, WC, and %BF) also changed during Ramadan fasting as expected. Participants in the intervention group had a greater percent reduction in weight (−1.8 vs. −1.5%.), BMI (−3.6 vs. −1.6%), WC (−1.2 vs. 6.2%), and % BF (−4.4 vs. −1.1%; Figure 1). In addition, the results of the present study also showed improvements in the quality of fat intake. In the intervention group, saturated fat intake decreased by 20.9%, while unsaturated fat intake increased by 147.8%. In the control group, the saturated fat intake decreased by 8.2% and the intake of unsaturated fats decreased by 78.4% (Figure 2B). These positive changes in the nutrient intake, particularly the quality of fat intake, in the intervention group, might have been reflected in a much-pronounced improvement in blood, glucose, cholesterol, sodium, CRP, and diastolic and systolic blood pressure in the intervention groups compared to the control group (Table 6). One of the reasons for these positive effects may be that the participants in the intervention group with improvements in their dietary adherence to the PDGN score had a much more balanced diet with increased servings of grains, vegetables, and fruits and a lower amount of red meat, dairy products, and hydrogenated cooking oil (ghee). Recently, much emphasis has been placed on certain dietary patterns to prevent chronic diseases, for example, the Mediterranean diet, the healthy American diet, and the vegetarian diet (47). All of these dietary patterns incorporate traditional advice to eat more fresh fruits, vegetables, and whole-grain cereals. Therapeutic diets such as the Dietary Approaches to Stop Hypertension (DASH) (48) and the dietary portfolio recommended in the Canadian Cardiovascular Society guidelines (49) to lower cholesterol also emphasize these principles and consistently result in large reductions in blood pressure and lipids when taken under metabolically controlled conditions (50–52). However, despite many efforts to encourage the general public to increase plant food consumption, the response has been slow (48). In this context, the results of the present study show that Ramadan fasting combined with nutrition education has pronounced positive effects on dietary behavior.

Conclusion

Nutrition education before Ramadan fasting is an effective strategy to modify dietary behavior in such a way that it improves adherence to dietary guidelines, hence affecting weight management goals.

Limitations

Measurement of adherence to prescribed dietary advice typically involves the following: (1) the assessment of what the client eats through self-reported methods (e.g., 24-h recall, food records, food frequency questionnaires, and diet history) and (2) the determination of the degree to which the diet approximates the recommended dietary plan (e.g., the difference between clients' recommended macronutrient goals and their self-reported intake). Although sparsely used, more objective measures of adherence to diets also exist (e.g., 24-h urinary sodium excretion to assess adherence to a low sodium diet) (53). However, there is no gold standard for the accurate determination of dietary intake. Self-report of energy intake is a characteristic inherent to nutrition-related topics and is found to be underestimated compared to objective measures such as resting energy expenditure assessed by indirect calorimetry (54). Underreporting energy intake is observed more frequently in female participants as compared to male participants (55), in older individuals as compared to young people (56), and in obese individuals as compared to normal-weight individuals (57). Although self-reported measures are often regarded as more susceptible to bias (e.g., over-reliance on memory; daily dietary variability; report error related to portion size and meal composition; and social desirability), they are a direct, simple, and inexpensive method and are readily available for use in practice. Self-reported measures can be improved and validated by using multiple measures of adherence and controlling statistically for bias or by using constructs, for example, body weight, blood pressure, or plasma cholesterol concentrations (58–60).

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the ethical approval for the study was granted by the Human Research Ethics Committee of the Department of Human Nutrition, The University of Agriculture, Peshawar (Ref: HN-HREC/2017-022). The patients/participants provided their written informed consent to participate in this study.

Author contributions

RG, IK and IA have substantial contribution to the concept and design of the study, data collection, analysis and interpretation. IH and MH assisted in data collection and data entry. RG, IK, AMA, and IA drafted the article and revised it critically for important intellectual content. NS, HC, SS and AA helped in data entry, data interpretation,

reviewing the revised draft. All authors contributed to the final revision of the article and all approved the final submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1204883/full#supplementary-material

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The impacts of Ramadan fasting for patients with non-alcoholic fatty liver disease (NAFLD): a systematic review

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Background: Numerous studies have explored the impacts of Ramadan fasting on Non-alcoholic fatty liver disease (NAFLD). Therefore, the objective of this systematic review was to analyze and summarize all clinical studies regarding the impacts of Ramadan fasting for patients with NAFLD.

Methods: We performed a comprehensive search of the Embase, Cochrane, and PubMed databases from inception to September 1, 2023. All clinical studies concerning the impacts of Ramadan fasting on patients with NAFLD were included.

Results: In total, six studies with 397 NAFLD patients comprising five prospective studies and one retrospective study were included in the systematic review. All six studies were assessed as high-quality. Ramadan fasting may offer potential benefits for patients with NAFLD, including improvements in body weight, body composition, cardiometabolic risk factors, glucose profiles, liver parameters, and inflammation markers.

Conclusion: Ramadan fasting might be an effective dietary intervention for NAFLD. However, the number of studies examining the impacts of Ramadan fasting for patients with NAFLD is relatively limited. Therefore, more high-quality research is needed to further our understanding of the benefits of Ramadan fasting for NAFLD.

Systematic review registration: https://inplasy.com, identifier 202390102.

KEYWORDS

Ramadan fasting, non-alcoholic fatty liver disease (NAFLD), impacts, liver parameters, a systematic review

Introduction

Non-alcoholic fatty liver disease (NAFLD) encompasses a spectrum of liver conditions, ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), and can progress to more severe forms such as cirrhosis and hepatocellular carcinoma (HCC), which is defined by the presence of fat in more than 5% of hepatocytes, and not attributed to the causes such as viral hepatitis, excessive alcohol consumption, steatogenic medications, or other causes of fatty liver disease (1, 2). Its development is influenced by an interplay of genetic

predisposition and environmental factors. A recent study found that the global incidence of NAFLD is 4,613 per 100,000 person-years, with a higher incidence among overweight or obese individuals, and men (3). It is estimated that the global prevalence of NAFLD is 25%. NAFLD poses a significant public health challenge worldwide. So far, there is no approved drug therapies for NAFLD. The complex pathophysiology and substantial heterogeneity in its phenotypes suggest that combination treatments might be necessary. For the prevention and treatment of NAFLD, a healthy lifestyle is of vital importance.

A balanced diet is the main component for those with NAFLD and is endorsed by current guidelines. Numerous studies have shown that specific dietary interventions can positively affect liver and metabolic parameters in patients with NAFLD. These interventions include the Mediterranean diet (4), time-restricted eating (TRE) (5, 6), low-carb diets (7), alternate day fasting (ADF) (8), the 5:2 diet (9), and Ramadan fasting. Among them, Ramadan fasting is a unique form of obligatory intermittent fasting observed annually during the month of Ramadan by approximately 1.5 billion Muslims worldwide. This fasting regimen offers various health benefits such as improved body weight and composition, reduced complications from metabolic syndrome, enhanced lipid profile, and other cardiometabolic advantages. Furthermore, Ramadan fasting fosters better glucose homeostasis, reduces inflammatory and oxidative stress markers, and may influence gene expression associated with anti-inflammatory and antioxidant defenses. Previous studies have investigated the effects of Ramadan fasting for healthy adults (10–17), patients with prediabetes and diabetes (18–29), and individuals with NAFLD (30–34). Among them, many studies have delved into the relationship between Ramadan fasting and NAFLD. For example, Badran et al. (30) investigated the effects of Ramadan fasting on 98 patients with NAFLD. They found that Ramadan fasting led to significant improvements in anthropometric biochemical, and ultrasonographic parameters for NAFLD patients, especially in the early phases and among prediabetics. Mari et al. (32) explored the effects of Ramadan fasting on patients with NASH and found that fasting could improve NASH severity, insulin sensitivity, and inflammatory markers. However, no systematic review has been performed to compile these clinical trials. Therefore, the aim of our study is to conduct a systematic review to summarize and analyze all clinical studies regarding the impacts of Ramadan fasting on patients with NAFLD.

Methods

Search strategy

We conducted this systematic review in accordance with the Cochrane Collaboration guidelines. Our review protocol was pre-defined and registered on the INPLASY website (ID:202390102), and we have reported the results in line with the PRISMA checklist (35). We undertook a comprehensive search of the Embase, PubMed, and Cochrane databases from their inception up to September 1, 2023, and without language restrictions. We included the following terms: ("Non-alcoholic Fatty Liver Disease" OR "Fatty Liver, Non-alcoholic" OR "Livers, Non-alcoholic Fatty" OR "Non-alcoholic Fatty Liver" OR "Liver, Non-alcoholic Fatty" OR NAFLD OR "Non-alcoholic Fatty Liver Disease" OR "Non-alcoholic Fatty Livers" OR "Steatohepatitis, Non-alcoholic") AND ("Ramadan fasting" OR Ramadan OR "Recurrent circadian fasting" OR "Islamic fasting" OR "Ramadan diurnal fasting" OR "intermittent fasting" OR "Ramadan intermittent fasting" OR "Ramadan model of intermittent fasting" OR "Ramadan intermittent fasting" OR Ramadan OR RF OR intermittent OR "Intermittent prolonged fasting during Ramadan" OR "Ramadan fast"). We also manually searched the references of relevant reviews and articles. Two authors (Lin and Wu) conducted the search and in cases of uncertainty, they consulted with a third author (Huang).

Inclusion criteria

Using the PICOS framework, we set the following inclusion criteria: (P) Populations: individuals with NALFD. (I) Interventions: Ramadan fasting. (C) Control: without Ramadan fasting. (O) Outcomes: Effects of Ramadan fasting on liver parameters, body weight, body composition, cardiovascular markers, glucose profiles, and inflammatory markers. (S) Study Types: Clinical studies, including cross-sectional studies, case-control studies, cohort studies, and randomized controlled trials (RCTs). We excluded editorials, duplicates, commentaries, conference abstracts, supplements, and case reports.

Quality appraisal and data extraction

Various tools were utilized to evaluate study quality, depending on the specific study design. For randomized controlled trials (RCTs), we used the RoB2 tool. For non-RCTs, the ROBINS-I tool designed specifically for these types of studies was employed (36). The Newcastle-Ottawa Scale (NOS) was used to assess the quality of case-control and cohort studies (37). This scale comprises eight key questions addressing aspects such as participant selection, the comparability of study groups, and verification of exposure. Two authors (Lin and Wang) independently carried out the data extraction process. They collated the information into two pre-prepared tables, detailing the characteristics and main findings of the studies, including study year, design, mean age, mean BMI, study duration, main measured parameters, and body weight and composition, lipid profile, glucose and insulin metabolism, liver parameters, other outcomes.

Results

Literature search

Our initial database search yielded a total of 778 records. After removing duplicates, 598 records remained for screening by titles, abstracts, and full texts. Ultimately, we included six studies in the systematic review of five were prospective studies and one retrospective study (30–34, 38). The progression of the search and selection process is illustrated in **Figure 1**.

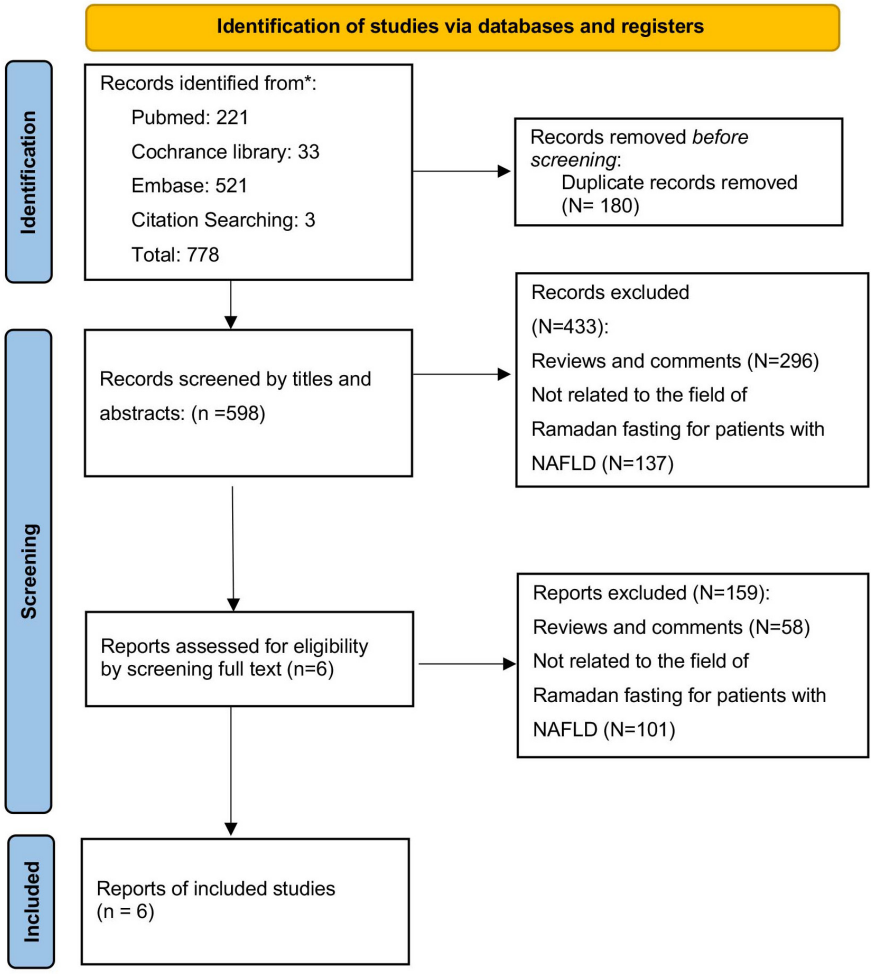

FIGURE 1
PRISMA 2020 flow diagram for new systematic reviews which included searches of databases and registers only.

Study and patient characteristics and the assessment of quality

A total of 397 participants were encompassed in the studies. Individual study sample sizes varied, with a range from 16 to 155 participants. The mean age spanned from 30.9 ± 2.42 to 51.8 ± 20.9 years, while the mean BMI ranged from 29.46 ± 4.52 to 37.03 ± 6.56. Each study had a duration of 4 weeks. Key measured parameters included body weight, body composition, cardiometabolic risk factors, glucose profiles, liver parameters, and inflammation markers. In the quality assessment, three studies (32, 33, 38) received a score of 9 and other three studies (30, 31, 34) were a score of 8 on the NOS scale and were thus classified as high quality. A detailed summary of the study characteristics and their quality assessments can be found in **Tables 1**, **2**.

The impact of Ramadan fasting for NAFLD

All six studies we reviewed investigated the effects of Ramadan fasting on body weight and composition (30–34, 38). While the majority showed that Ramadan fasting led to a decrease in body weight or an improvement in body composition, one study found no significant changes in these factors. Cardiometabolic risk factors were measured in four studies (30, 31, 33, 34), including lipid profiles, such as reductions in the levels of triglycerides, LDL, and total cholesterol, and an increase in HDL level. Three studies (30, 31, 33) found that Ramadan fasting had benefits for cardiometabolic risk while one not (32). Out of the five studies that assessed glucose parameters (30–34), four studies (30, 31, 33, 34) reported improvements in insulin resistance and sensitivity and a decrease in fasting blood sugar levels after Ramadan fasting. However, one study (32) noted an increase in fasting blood sugar levels post-fasting. Increased lipid and FBS levels may result from eating more high-carb and sugar foods and exercising less in the Ramadan period in this study. Liver parameters were a common focus across all studies. Of the five that investigated changes in ALT and AST post-fasting (30–32, 34, 38), four (30–32, 34) observed a decrease in these levels, but one study (38) found an increase in ALT levels, it may be due to individuals consume large amounts of food high in fat and sugar before dawn and sunset, which causes increased stress on liver parameters including ALT in this study. Mari et al. (32) reported significant decreases in NAFLD Fibrosis

TABLE 1 The study characteristics.

References	Design	*N*	Mean age	Mean BMI	Study duration	Main measured parameters
Aliasghari (33)	The prospective study	102	37.59 ± 7.06	30.09 ± 4.49	4 weeks	Body weight, BMI, WC, Waist/Hip ratio, Fat mass, FBG, Insulin, HOMA-IR, IL-6, Hs-CRP
Arabi (34)	The prospective study	50	40.52 ± 10.90	31.38 ± 4.9	4 weeks	BMI, WC, Fat mass, Free fatty mass, SBP, DBP, ALT, AST, FBG, Insulin, TG, HDL, LDL
Badran (30)	The prospective study	16	46.03 ± 11.72	37.03 ± 6.56	4 weeks	BMI, FIB-4, cholesterol, triglycerides, HDL, LDL, cholesterol/HDL risk ratio
Mari (32)	The retrospective study	155	51.8 ± 20.9	36.7 ± 7.1	4 weeks	BMI, ALT, AST, Insulin, HOMA-IR, hs-CRP
Rahimi (38)	The prospective study	34	46.03 ± 11.72	29.46 ± 4.52	4 weeks	Body weight, BMI, ALT
Gad (31)	The prospective study	40	30.9 ± 2.42	30.9 ± 2.42	4 weeks	BMI, WC, Fat mass, FBG, Insulin, HOMA-IR, FIB-4, CAP, and LSM

TABLE 2 The quality assessments.

Items	Aliasghari (33)	Arabi (34)	Badran (30)	MARI (32)	Rahimi (38)	Gad (31)
Selection (0–4 stars):	4 stars	3 stars	3 stars	4 stars	4 stars	3 stars
Representativeness of the exposed cohort (1 star)	☆	☆	☆	☆	☆	☆
Selection of the non-exposed cohort (1 star)	☆	NA	NA	☆	☆	NA
Ascertainment of exposure (1 star)	☆	☆	☆	☆	☆	☆
Demonstration that the outcome of interest was not present at the start of the study (1 star)	☆	☆	☆	☆	☆	☆
Comparability (0–2 stars):	2 stars	2 stars	2 stars	2 stars	2 stars	2 stars
Comparability of cohorts on the basis of the design or analysis, controlled for the most important factor (1 star)	☆	☆	☆	☆	☆	☆
Control for any additional factor (a maximum of 1 star)	☆	☆	☆	☆	☆	☆
Outcome (0–3 stars):	3 stars	3 stars	3 stars	3 stars	3 stars	3 stars
Assessment of outcome (1 star)	☆	☆	☆	☆	☆	☆
Was the follow-up long enough for outcomes to occur (1 star)	☆	☆	☆	☆	☆	☆
Adequacy of follow-up of cohorts (1 star)	☆	☆	☆	☆	☆	☆
Total scores	**9**	**8**	**8**	**9**	**9**	**8**

Total scores (0–9 stars). Total scores are the sum total of the scores of selection, comparability, and outcome.

Score (NFS) (from 0.45 ± 0.25 to 0.23 ± 0.21, $P < 0.05$), fibrosis-4 (FIB4) scores (from 1.93 ± 0.76 to 1.34 ± 0.871, $P < 0.05$), and BARD scores (from 2.3 ± 0.98 to 1.6 ± 1.01, $P < 0.05$). Badran's study (30) highlighted significant improvements in fibrosis markers, namely AST to platelet ratio index (APRI) and FIB-4 ($p \leq 0.05$). Gad et al. (31) presented significant clinical improvements in FIB-4 (1.31 ± 0.26 to 1.24 ± 0.25, $p < 0.05$), controlled attenuation parameter (CAP) (from 318.52 ± 34.59 to 294.0 ± 20.34, $p < 0.05$), and liver stiffness measurement (LSM) (from 6.95 ± 1.62 to 6.59 ± 1.49, $p < 0.05$) after fasting. Inflammatory markers were evaluated in three studies, with each noting a decrease in C-reactive protein (CRP) levels post-Ramadan. Aliasghari's study also identified a decrease in interleukin 6 (IL-6) levels after fasting. The primary findings of each study are summarized in Table 3.

Discussion

To the best of our knowledge, this study represents the first comprehensive systematic review that summarizes and analyzes all clinical studies examining the effects of Ramadan fasting on

TABLE 3 The primary findings of each study.

References	Body weight and composition	Lipid profile	Glucose and insulin metabolism	liver parameters	Other outcomes
Aliasghari (33)	Decreased BW	Decreased cholesterol level	decreased fasting blood glucose and plasma insulin	Decrease the level of steatosis	Decreased IL-6 and hs-CRP
Arabi (34)	No change in BMI, fat mass, fat-free	Increased HDL-C, TC, and triglycerides, Decreased Systolic blood pressure	Increased fasting blood glucose	Decrease ALT	NA
Badran (30)	Decreased BW and BMI	Decreased cholesterol level	decreased fasting blood glucose	Decreased Fib4 score and APRI score Decrease ALT	Decreased CRP
MARI (32)	Decrease BMI	NA	Decreased HOMA-IR	Decreased NFS score and BARD score, decrease ALT	Decreased CRP
Rahimi (38)	Decrease in weight gain	NA	NA	Increased ALT	NA
Gad (31)	Decrease BMI	Decreased cholesterol level, triglycerides, LDL cholesterol, total cholesterol, improve HDL cholesterol	Decreased fasting glucose level and HbA1c	Decreased liver steatosis and liver stiffness, Decrease ALT, FIB-4, CAP, and LSM	Decreased serum albumin and total protein

patients with NAFLD. In our systematic review, we included six studies, all of which were assessed as high quality. Our findings suggest that Ramadan fasting could serve as an effective dietary intervention for NAFLD patients, leading to improvements in body weight, liver parameters, lipid profiles, and inflammatory markers.

Ramadan fasting, an annual religious observance practiced by a significant portion of the global Muslim population, involves extended periods of abstention from food and fluid intake from dawn to sunset. Ramadan presents a significant departure from regular eating patterns and offers a distinctive, real-world context to investigate the effects of fasting and altered energy distribution on patients with NAFLD (39). This unique form of intermittent fasting has elicited considerable scientific interest in its potential therapeutic implications for metabolic disorders, notably NAFLD. Emerging research suggests that such protracted fasting intervals can induce beneficial metabolic shifts conducive to improved liver health. Specifically, Ramadan fasting has been associated with reductions in liver enzymes, signifying diminished hepatic stress and inflammation. Additionally, the fasting period can enhance insulin sensitivity, a paramount consideration given the integral role of insulin resistance in NAFLD pathogenesis. The potential for modest weight loss during Ramadan, coupled with shifts in lipid profiles toward a more cardioprotective phenotype, further underscores the prospective benefits for individuals with NAFLD. In total, the potential beneficial mechanisms of Ramadan fasting for patients with NAFLD include: Ramadan fasting is usually linked with weight loss, a decrease in total fat mass, and enhancements in cardiometabolic risk factors, including improvements in lipid profiles, blood pressure, and glycemic parameters; Ramadan fasting was shown to improve HOMA-IR and fasting glucose; Ramadan fasting may reduce the levels of inflammatory cytokines including CRP and IL-6, and oxidative stress; Ramadan fasting has significant improvements in alkaline phosphatase, AST, and bilirubin. In our systematic review, six studies with 397 patients with NAFLD were included. Collectively, these findings suggest that Ramadan fasting could serve as a promising non-pharmacological approach, complementing existing therapeutic strategies for NAFLD. However, the potential benefits may be offset by the customary, and sometimes excessive, unhealthy dietary intake during Iftar—the meal to break the fast. This could potentially worsen NAFLD pathophysiology. For example, in Rahimi' study, they found that an increase in ALT after Ramadan, and in Arabi's study, they found the increased lipid and FBS levels, since participants following a period of fasting is to eat large meals that are high in fats and sugars at the beginning and end of the day. This eating pattern can place significant stress on the liver as it works to metabolize the nutrients that have been ingested. The complex relationship between Ramadan fasting and NAFLD warrants further investigation to ensure that religious practices harmoniously align with optimal liver health. Our results are consistent with a recent study which evaluate the impact of IF on older patients with NAFLD based on Clinicaltrials.gov Registry. Ramadan fasting, as well as IF, may have potential benefits for patients with NAFLD (40).

Our systematic review has several limitations. Firstly, only six studies have investigated the effects of Ramadan fasting on patients with NAFLD. Secondly, there are no randomized controlled trials (RCTs) examining Ramadan fasting in the context of NAFLD. Thirdly, the sample sizes in the existing studies are relatively small, ranging from 16 to 155 subjects. In addition, geographic dietary habits during Ramadan may impact outcomes and weight reductions and may explain some of the contradictory results reported in this review. Investigating the effects of Ramadan fasting on the discussed parameters with a larger sample would provide more comprehensive insights. Future research would benefit from higher-quality studies featuring rigorous designs, longer-term interventions, and extended follow-up periods.

In conclusion, Ramadan fasting has potential as an effective dietary intervention for NAFLD. However, the existing body of research on the effects of Ramadan fasting for NAFLD patients

is limited. To solidify our understanding of its benefits, we need further high-quality studies in this field.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

XL: Data curation, Formal analysis, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review and editing. GW: Data curation, Formal analysis, Methodology, Project administration, Supervision, Validation, Writing – original draft. JH: Conceptualization, Funding acquisition, Investigation, Resources, Software, Visualization, Writing – original draft, Writing – review and editing.

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Alterations in anthropometric, inflammatory and mental health parameters during Ramadan intermittent fasting in a group of healthy people: a prospective cohort study

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Fasting has been practiced with different time span in different areas of the world and for various reasons. One of the types of fasting regimens is Ramadan intermittent fasting (RIF), which is described as intermittent dry fasting and known as the most commonly practiced form of religious fasting. Different studies have shown its effects on body composition parameters and mental health, fatigue and quality of life (QoL). Elucidating the relationship of RIF on biological parameters would also be of importance to show its mechanism. Therefore, we evaluated several biological mediators related to mental health, such as ß-nerve growth factor (ß-NGF), brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), and insulin-like growth factor-1 (IGF-1), interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), and matrix-metalloproteinase-9 (MMP-9). This study consisted of fasting (FG; $n = 25$) and non-fasting group (NFG; $n = 25$). Four different time points were assessed for FG: one week before (T1), mid (T2), last days (T3), and one week after (T4) RIF. T1 and T3 were the assessment time points for NFG. Biological mediators were determined from serum samples by using Human Magnetic Luminex and enzyme-linked immunosorbent assay. Furthermore, we then performed correlation analyses between biological mediators and our previously published clinical parameters including body composition and mental health parameters at all time points. Significant alterations were shown in FG for ß-NGF (T2vsT3, $p < 0.05$; T2vsT4, $p < 0.05$), GDNF (T1vsT4, $p < 0.05$; T2vsT4, $p < 0.05$), IL-8 (T2vsT3, $p < 0.05$; T3vsT4, $p < 0.05$), TNF-α (T1vsT3, $p < 0.05$; T1vsT4, $p < 0.001$; T2vsT4, $p < 0.001$), and MMP-9 (T1vsT4, $p < 0.01$). There were no statistically significant differences between FG and NFG in all biological mediators at T1 and T3. Correlation analysis showed that MMP-9 levels had negative correlation with body mass index (BMI) at T3. At T3 BDNF levels had negative correlation with Epworth Sleepiness Scale (ESS) as one of measured QoL parameters. ß-NGF, GDNF, TNF-α, and MMP-9 had positive correlation with some of body composition and mental health parameters. Findings demonstrate that RIF

altered different biological mediators could give benefit to health. Its benefit is mediated by the alteration of biological mediators.

KEYWORDS

Ramadan intermittent fasting, neurotrophic factors (NTFs), Interleukin-8 (IL-8), TNF-α – tumor necrosis factor alpha, anthropometric, matrix metalloprotein-9 (MMP-9), mental health parameters and quality of life (QoL) parameters

Introduction

Intermittent fasting (IF) has become a lifestyle of eating during specific period of time and fasting for the rest of the day. IF has shown many of the health benefits. It is not only reducing free-radical production and weight loss, but also the adaptive cellular responses between and within organs that restores glucose metabolism, increases stress resistance and suppresses inflammation (1). Previous studies showed the positive effect of IF in animal models on chronic disorders such as obesity, diabetes, cardiovascular disease, cancer and neurological disorders (2, 3). Ramadan intermittent fasting (RIF) is one type of IF that performed by about 1.5 billions of Muslims worldwide annually from dawn to sunset for one month. The time periods of RIF vary from 9 to 22 h per day due to geographical differences and seasonal variation (4). Food, water, smoking and sexual activities are forbidden during the fasting daytime hours of Ramadan month. Our previous studies demonstrated that not only RIF can improve anthropometric parameters, but also can improve mental health, fatigue and quality of life (QoL) (5). However, the physiological mechanism behind these beneficial effects need to be elucidated.

Depression is related to irregularities in hypothalamic–pituitary–adrenal (HPA) axis, altered insulin resistance, plasma glucose, pro-inflammatory cytokines, leptin resistance, and disturbance of neurotransmitters, neuropeptides and neurotrophic factors (6, 7). Interestingly, several studies demonstrated that IF has positive effects on the neuroendocrine system and on depression. IF might induce an antidepressant effect on depression, providing potential new treatment options. It included neurotrophic effects and orexin signaling activation that can release endorphin and ketone via the increased of cAMP response element-binding (CREB) phosphorylation (8). Hence, it is of interest to study the effect of IF on biological mediators related to depression, particularly on neurotrophic factors.

Nerve growth factor (NGF) was the first member described among the neurotrophic factors (9). NGF has been known to involve in the regulation of neurotransmitters and neuropeptides synthesis of nerve cells. It is important for growth, maintenance, proliferation and survival of nerve cells (10). Various clinical studies in human and animals showed an association of low NGF levels with depression compared with healthy controls (11). Brain-derived neurotrophic factor (BDNF), another member of neurotrophic factors, was described as being involved in synaptic plasticity (12), depression (8), and increasing survival of dopaminergic neurons of the developing substantia nigra (13). BDNF has been also studied in different neurological-related diseases, such as major depressive disorders, fibromyalgia syndromes and chronic low back pain (14). Glial cell-derived neurotrophic factor (GDNF) is a small protein and member of neurotrophic factors that are responsible for the development, maintenance and survival of different neurons (15). In mice model, induced GDNF-expression showed protective effects to obesity. In addition, several studies showed that GDNF is known to the mechanism underlying depressive disorders (16).

Other biological mediators related to depression and body composition parameters such as insulin growth factor-1 (IGF-1), tumor necrosis factor alpha (TNF-α), interleukin-8 (IL-8), and matrix metalloproteinase-9 (MMP-9) were also determined (17–19). One of the biological mechanisms for depression is related to inflammation. People with major depressive disorder (MDD) and obesity have been shown to have higher inflammatory markers as compared to healthy control (20–22). IGF-1 has been shown to have anti-inflammatory action by inhibiting the expression of proinflammatory cytokines including IFN-γ, IL-1β and TNF-α and enhancing the production of anti-inflammatory cytokines including IL-4 and IL-10 (23). The abnormalities of IGF-1 in MDD patients have been suggested as a marker and predictive role of the neurotrophin for depression and treatment effectiveness (24). Meanwhile, individuals who had increased level of both pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-α) and interleukin 8 (IL-8) have been reported to develop psychiatric disorders such as major depression, bipolar disorder, schizophrenia and sleep disorder (25, 26). Other study showed that depression condition in young patient, levels of serum MMP-9 were increased which might be used as biomarker for bipolar disorder (27). Another study also showed that patients in acute phase of ischemic stroke condition, serum MMP-9 levels were elevated (28). In addition, animal were treated with MMP-9 inhibitor showed improve specific neurobehavioral deficits associated with Alzheimer's disease (29).

As mentioned before, neurotrophic factors play a role not only related to depressive disorders, but also to body composition parameters. Therefore, this study was an explorative study that aimed at elucidating the changes in neurotrophic factors upon the observation of RIF. Additionally, we aimed to measure the inflammatory cytokine TNF-α, the chemokine IL-8 and MMP-9. Furthermore, in our previous study showed that RIF improved body composition and mental health parameters, fatigue and QoL (5). For the body composition parameters, we measured body weight (BW), body mass index (BMI), skeletal muscle mass (SMM), body fat mass (BFM), fat free mass (FFM), body fat percentage (BFP), body water mass (BWM) and wait and hip ratio (WHR). For mental health parameters, we determined the effects on fatigue [Fatigue Severity Scale (FSS) and visual analogue scale (VAS)], depression (The Hospital Anxiety and Depression Score (HADS) and Beck's Depression Inventory (BDI)-II) and QoL [Epworth Sleepiness Scale (ESS)] (5). In this study, we aimed to analyze the correlation between measured biological mediators with our previously published clinical parameters including body composition and mental health parameters at all time points.

Materials and methods

Local Ethic Committee of Hannover Medical School approved this study (Ethics No. 6899) in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. This study registered at German Registry of Clinical Trial (DRKS-ID: DRKS00008181). Written informed consent was obtained from all subjects and/or their legal guardian(s).

Participants

This study was a sub analysis from previous report (5). Healthy male participants which mostly students of Hannover Medical School were recruited. We divided them into two groups fasting group (FG) and non-fasting group (NFG). The health status of participants were assessed by experienced doctors at Department of Rehabilitation and Sport Medicine, Hannover Medical School. Study inclusion for FG required a minimum age of 18 years old, intended to fast the whole month of Ramadan, have fasted during Ramadan at least once before, understood German or English language. Study inclusion for NFG required a minimum age of 18 years old and did not fast during the study period.

Study design

In this current study, the fasting period was about 18–19 h per day. In the FG, subjects were assessed at four time points (Figure 1): one week before the beginning of Ramadan/baseline (T1); middle of Ramadan (T2: day 14th or 15th or 16th); the last days of Ramadan (T3: day 28th or 29th or 30th); and one week after the Ramadan fasting finished (T4: day 6th, 7th, 8th from the end of Ramadan).

In the NFG, subjects were assessed only at 2 time points: before Ramadan (T1) and during the last days of Ramadan (T3).

Serum preparation

Serum was prepared by collecting blood samples from peripheral venous by using Monovette serum tube (Monovette®, Sarstedt, Germany). It was done in the morning time between 08:00 and 10:00 for all time points. Centrifugation was performed after allowing blood clotting at 1500 g for 15 min (Universal 320R, Hettich, Tuttlingen, Germany). These serum samples were stored at −80°C until determination.

ß-NGF, BDNF, GDNF, TNF-α, IL8 and MMP-9 serum level were determined using customized Human Magnetic Luminex Assay (R&D Systems) according to the manufacturer's instructions. IGF-1 serum levels were determined using Quantikine ELISA Human IGF-1 Immunoassay (R&D Systems Inc.) according to the manufacturer's instructions.

Body composition and mental health parameters

As discussed above, body composition and mental health parameters have been published in our previous publication (5). We determined the body composition parameters including body weight (BW), body mass index (BMI), skeletal muscle mass (SMM), body fat mass (BFM), fat free mass (FFM), body fat percentage (BFP), body water mass (BWM) and wait and hip ratio (WHR) by using InBody 230 (Model MW160, InBody Co., Ltd., Seoul, Korea). Mental health parameters, fatigue and QoL were assessed by using self-administered questionnaires. The Hospital Depression and Anxiety Score (HADS) and Beck's Depression Inventory (BDI)-II were used to assess the intensity of anxiety and depression or only depression, respectively. Fatigue was measured by the visual analogue scale (VAS) and fatigue severity scale (FSS). Day sleepiness as QoL parameter was measured by using the Epworth Sleepiness Scale (ESS). In this study, we only showed mental health parameters that have correlation with measured biological mediators (ESS, HADSA, HADSD, BDI-II).

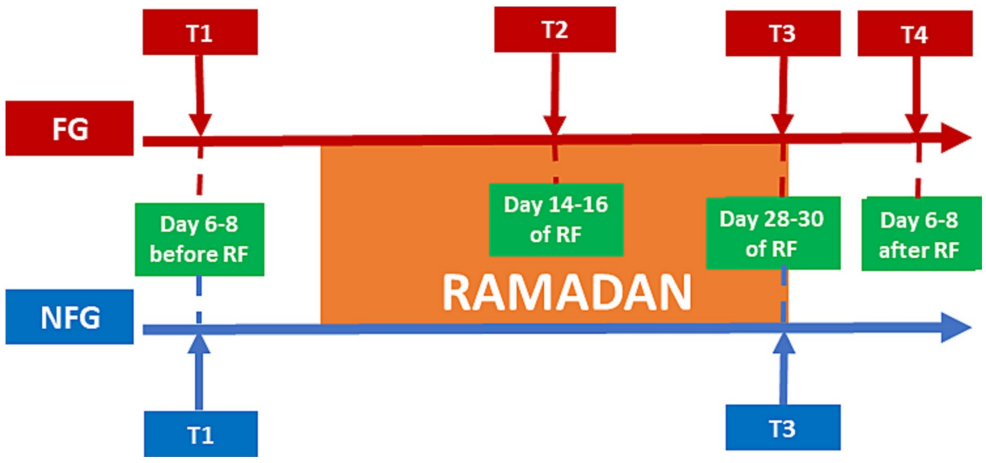

FIGURE 1
Study design.

Statistics analysis

The aim of this study is to assess the level of ß-NGF, BDNF, GDNF, IGF-1, IL-8, TNF-α, and MMP-9 before (T1), during (T2 and T3) and after (T4) RIF. Kruskal-Wallis or two-way Friedman ranked test were used to analyze the data, after we tested the data distribution using the Kolmogorov–Smirnov test. We performed the *post hoc* tests, and Bonferroni correction were used to adjust the significances. We used Spearman's correlation tests to determine correlation between clinical parameters and biological mediators. Missing data were replaced by using a mean. Statistical analysis was performed by using SPSS version 22 (IBM, New York, United States).

Results

Baseline characteristics of FG and NFG

Table 1 shows the baseline data of FG and NFG regarding anthropometric measurements and mental health parameters of both groups that were measured from our previous publication (5). This data demonstrated that there were no statistically significant differences at baseline between FG and NFG. Table 2 shows the baseline data of FG and NFG regarding biological mediators measured in this study. There were no statistically significant differences at baseline between FG and NFG.

Effect of RIF on neurotrophic factors

In this study, both ß-NGF and GDNF showed significant changes during RIF in FG only (Figures 2A,C). ß-NGF levels showed significant increased from the middle of Ramadan (T2) to the last days of Ramadan (T3) and one week after the RIF finished (T4). Whereas GDNF levels showed significant decreased from one week before the beginning of Ramadan/baseline (T1) to T4, but also significant decreased from T2 to T4. Furthermore, BDNF and IGF-1 levels showed no significant changes in FG during the study period (Figures 2B,D). In addition, we observed no statistically significant differences between FG and NFG in ß-NGF, BDNF, GDNF and IGF-1 levels from 1 week before RIF (T1) to the last week of RIF (T3) (Figures 3A–D).

Effect of RIF on IL-8, TNF-a, and MMP-9

In this study, we measured TNF-α and IL-8 levels during IF in healthy male participants in order to determine the effect of RIF on cytokines and chemokines levels. TNF-α levels were significantly decreased from T1 to T3 and T4. TNF-α levels were also decreased from T2 to T4 (Figure 4A). We also observed decreased levels of IL-8 from T3 to T4, although IL-8 levels were increased from T2 to T3 (Figure 4B).

Furthermore, we measured MMP-9 levels that play an important role in obesity-mediated adipose tissue remodeling. Interestingly, we found that MMP-9 was significant decreased from T1 to T4 in FG (Figure 4C). In addition, we compared the levels of IL-8, TNF-α, and MMP-9 levels of both FG and NFG. No

TABLE 1 Baseline data of fasting and non-fasting group (Body composition and mental health parameters).

	FG (N = 25)	NFG (N = 25)	p
	Mean ± SEM	Mean ± SEM	
Age	26.12 ± 0.98	26.20 ± 0.98	0.977
Ethnicity			
White/Asian	21/4	16/9	0.196[§]
Body composition			
BW (kg)	77.82 ± 2.46	76.16 ± 4.29	0.739
BMI (kg/m^2)	24.78 ± 0.73	24.56 ± 0.78	0.84
SMM (kg)	34.66 ± 1.02	34.60 ± 1.05	0.972
BFM (kg)	16.65 ± 1.48	17.44 ± 1.68	0.726
FFM (kg)	61.18 ± 1.72	61.10 ± 0.176	0.98
BFP (%)	20.92 ± 1.41	21.54 ± 1.41	0.797
BWM (kg)	44.88 ± 1.25	44.77 ± 1.80	0.96
WHR	0.88 ± 0.04	0.92 ± 0.02	0.293
Mental health			
Anxiety (HADSA)	4.92 ± 3.82	4.26 ± 3.38	0.521
Depression (HADSD)	4.36 ± 3.88	3.06 ± 3.47	0.218
Depression (BDI-II)	8.36 ± 8.21	6.48 ± 5.97	0.359
Fatigue			
Fatigue (VAS)	3.01 ± 1.83	3.08 ± 1.96	0.893
Fatigue Severity Scale (FSS)	26.92 ± 8.65	26.44 ± 10.38	0.86
Epworth Sleepiness Scale (ESS)	7.96 ± 3.81	7.16 ± 3.73	0.457

All data are presented in Mean ± SEM; body weight (BW), body mass index (BMI), skeletal muscle mass (SMM), body fat mass (BFM), fat-free mass (FFM), body fat percentage (BFP), body water mass (BWM), waist-hip ratio (WHR), Hospital Anxiety and Depression Scale Anxiety (HADSA), Hospital Anxiety and Depression Scale Depression (HADSD), Beck's Depression Inventory (BDI)-II, Visual analogue scale (VAS), Fatigue Severity Scale (FSS), Epworth Sleepiness Scale (ESS). Student's T-test. [§]Fischer's Exact test. Data has been presented in previous publication (5).

TABLE 2 Baseline data of fasting and non-fasting group (biological mediators).

	FG (N = 25)	NFG (N = 25)	p
	[Median (IQR)]	[Median (IQR)]	
ß-NGF	2.1 (1.7–2.2)	2.1 (1.7–2.4)	0.992
BDNF	591.4 (291.2–860.5)	702.9 (369.7–1001.5)	0.410
GDNF	2.6 (1.8–3.5)	2.6 (1.8–3.3)	0.671
IGF-1	2548.6 (847.2–5583.4)	1760.8 (437.3–3651.3)	0.264
TNF-α	4.5 (3.4–5.4)	4.5 (3.6–5.4)	0.731
IL-8	3.0 (2.0–4.4)	3.7 (2.7–4.5)	0.307
MMP-9	4654.8 (3710.0–5827.2)	3544.0 (2652.1–6347.8)	0.123

All data are presented in median + interquartile range (IQR). ß-nerve growth factor (ß-NGF), brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF), tumor necrosis factor alpha (TNF-α), interleukin-8 (IL-8) and matrix metalloproteinase-9 (MMP-9), IQR: Interquartile range. Student's T-test.

statistically significant differences in IL-8, TNF-α, and MMP-9 were observed at T1 and T3 between FG and NFG (Figures 5A–C). Surprisingly, the TNF-α levels were significantly decreased at T1 and T3 of NFG (Figure 5B).

Correlations between biological mediators and clinical parameters during RIF

It is of interest to analyze the correlation between clinical parameters and biological mediators (Table 3). In the last days of Ramadan (T3), MMP-9 levels showed a negative correlation with body mass index (BMI). BDNF levels showed negative correlation with Epworth Sleepiness Scale (ESS). Interestingly, TNF-α levels showed positive correlation with Hospital Anxiety and Depression Scale-Anxiety (HADSA) and Hospital Anxiety and Depression Scale–Depression (HADSD). Meanwhile, GDNF and MMP-9 levels showed positive correlation with HADSD.

We observed positive correlations of TNF-α to body weight (BW) and body fat mass (BFM) at one week after the RIF finished (T4). GDNF levels had positive correlation with BFM and waist-hip-Ratio (WHR). Interestingly, Beck's depression inventory (BDI)-II, as depression parameter, showed negative correlation with ß-NGF levels.

Discussion

The most understanding by common people, the effect of IF is related to the alteration of anthropometric parameters, which include BMI, body weight, and fat loss (5, 30–32). Interestingly, our previous studies, RIF has a positive influence to mental health, fatigue and QoL (5, 30). This effect might be mediated by different biological mediators, particularly cortisol and BDNF (33). Another study also observed that RIF improved body composition and did not exacerbate depression in MDD patients (34). In this current study, we wanted to elucidate the effects of RIF at different time points on neurotrophic factors such as ß-NGF, GDNF, BDNF and IGF-1. Additionally, we also measured other biological mediators such as TNF-α, IL-8 and MMP-9.

In our current study, ß-NGF showed significant increase from the middle of Ramadan (T2) to the last days of Ramadan (T3) and one week after the RIF finished (T4). A comparable study measuring NGF during RIF in woman only participants also showed significant increase of NGF during mid and end of RIF (35). It is known that NGF has an important role in the regulation of neurotransmitters and neuropeptides synthesis of nerve cells (10), but there is no previous evidence on the effect of fasting on NGF levels and it effects to improve brain health through the induction of the NGF levels. Interestingly, Beck's depression inventory (BDI)-II showed negative correlation with ß-NGF levels. A meta-analysis and systematic review observed that NGF levels were significantly lower in MDD than in healthy control (36).

Furthermore, we observed significantly decreased levels of GDNF from one week before the beginning of Ramadan/baseline (T1) to T4, and significantly decreased levels from T2 to T4. Interestingly, GDNF levels has a positive correlation with HADSD at T3 and BFM and WHR at T4. GDNF levels were shown to be significantly decreased in depression (37) involved in the dopamine system and has been linked to a potential treatment in drug abuse (38). Concerning special psychiatric conditions and its correlation to metabolic levels, a study by Skibinska et al. (39) showed that in psychiatric disorders, such as depression, GDNF and metabolic features such as cholesterol, showed significant correlation between GDNF and cholesterol

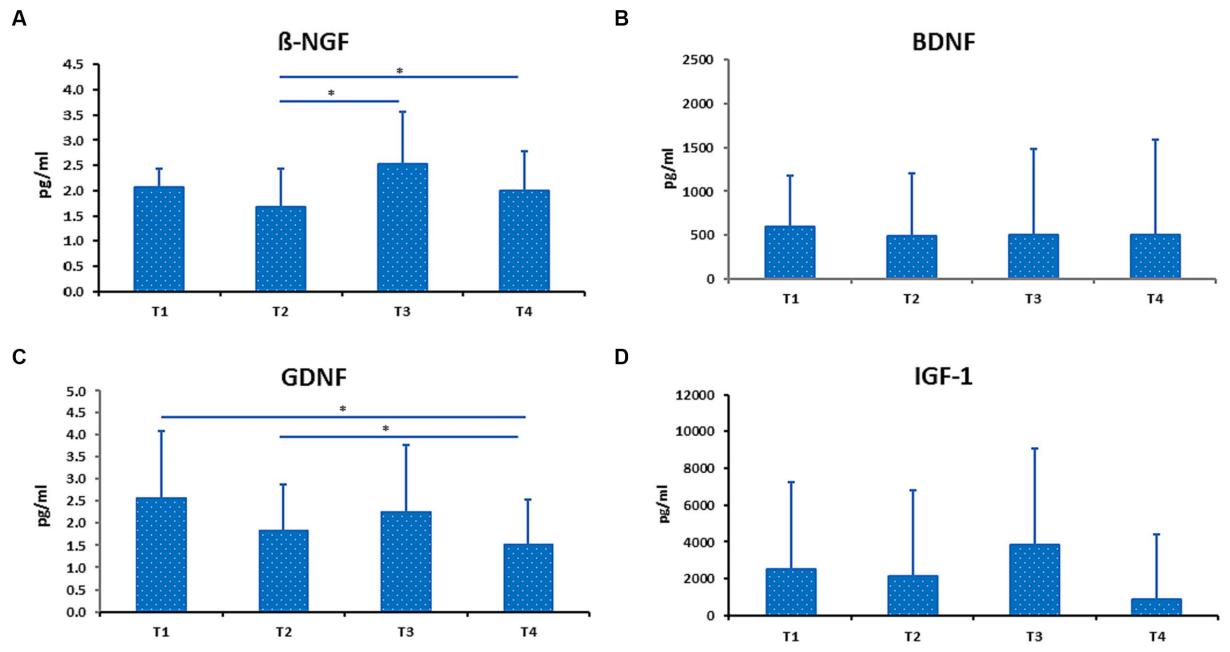

FIGURE 2
Level of ß-NGF **(A)**, BDNF **(B)**, GDNF **(C)**, and IGF-1 **(D)** in fasting group (FG). Data presented as median + interquartile range (IQR). * $p < 0.05$.

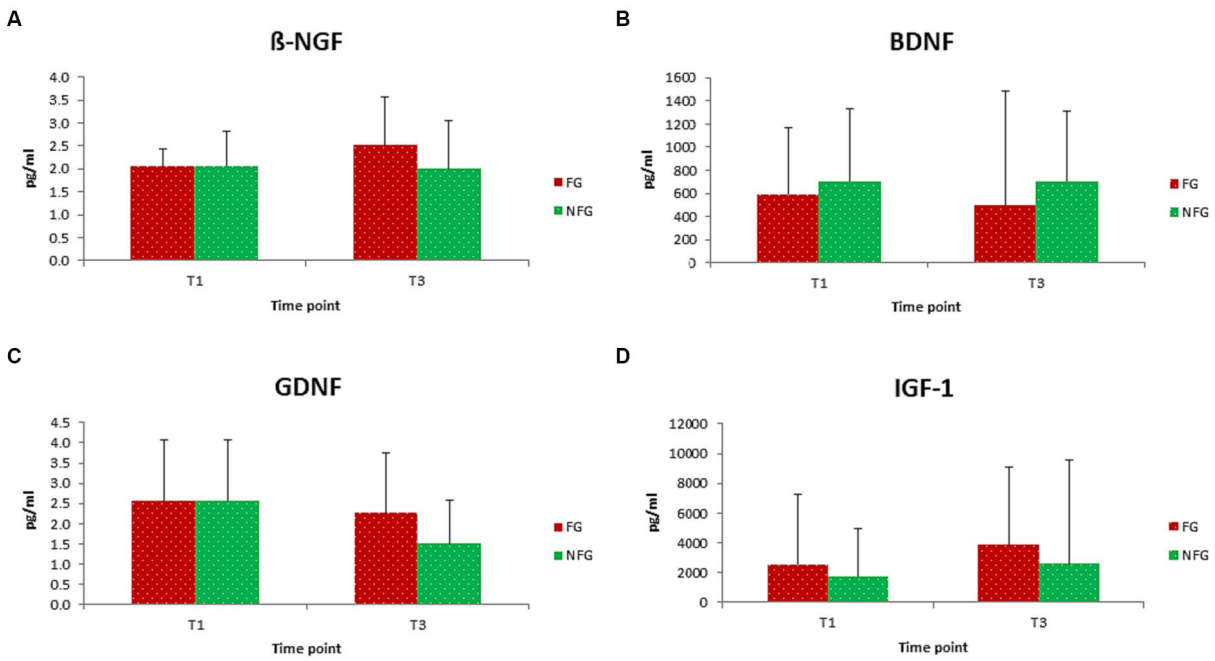

FIGURE 3
Level of ß-NGF **(A)**, BDNF **(B)**, GDNF **(C)**, and IGF-1 **(D)** in fasting (FG) and non-fasting group (NFG) at T1 and T3. Data presented as median + interquartile range (IQR).

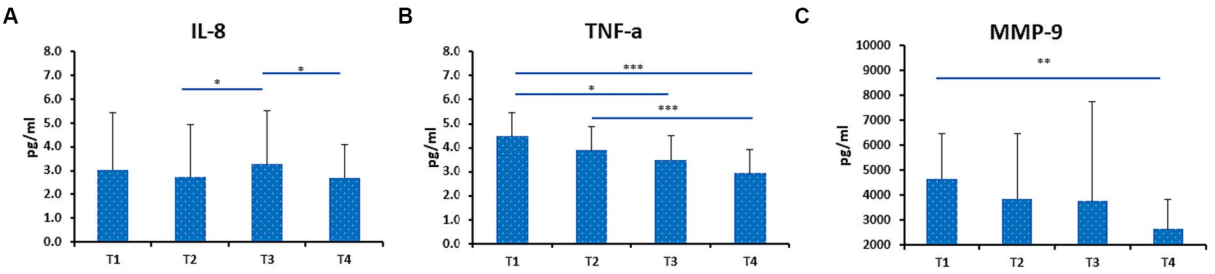

FIGURE 4
Level of IL-8 **(A)**, TNF-a **(B)** and MMP-9 **(C)** in fasting group (FG). Data presented as median + interquartile range (IQR). Data presented as median + interquartile range (IQR). * $p < 0.05$; ** $p < 0.01$; ***$p < 0.001$.

GDNF. Transgenic mice showed higher brown fat mass, energy expenditure and even increased expression in skeletal muscle. Nevertheless, GDNF does not appear as a predictor for treatment remission in other psychiatric disorders, such as general anxiety disorder (40). However, the findings towards GDNF and its (patho) physiological role remain controversial and further studies need to elucidate the role of GDNF. Best to our knowledge, this study is the first study to analyze the effect of fasting on GDNF.

RIF associated with the decrease of BDNF levels in schizophrenia patients (41). Furthermore, an increased BDNF level was linked to type II diabetes mellitus (T2DM) and obesity (42). A study using rats suggested that IF could potentially protect from T2DM by increasing the levels of BDNF and neurotrophin (NT)3 (43). Interestingly, contradictory results were obtained regarding BDNF levels during RIF in healthy participants. Some studies showed a significant increase of BDNF levels during RIF (35). Whereas another study showed a decrease of BDNF levels during RIF (44). These conflicting reports regarding how IF influence BDNF levels might be because of the different time periods of RIF due to geographical differences and seasonal variations. Different time point of collecting the samples also might resulting the differences. Meanwhile, our previous study showed significantly decreased (33), it could be that the latter study include both male and female participants.

Concerning to insignificant changes of IGF-1-levels during RIF, our results were in line with another study conducted on well trained men during RIF (45), although we cannot compare directly with subjects of this study. In contrast to these findings another study showed significant reduction of IGF-1 levels during RIF by adaptation mechanisms. As shown in mice model, it was hypothesized that short time starvation leads to survival mode and reduction of the growth hormone IGF-1 (46). Reduced IGF-1 levels were linked to extended life span in mice (47).

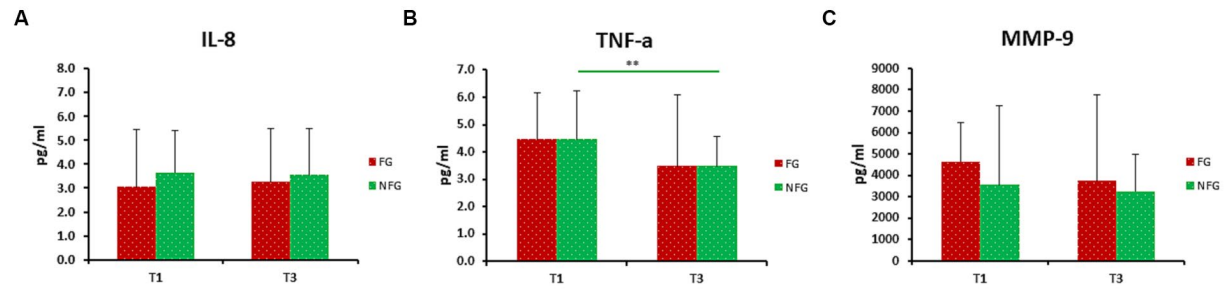

FIGURE 5
Level of IL-8 **(A)**, TNF-α **(B)**, and MMP-9 **(C)** in fasting (FG) and non-fasting group (NFG) at T1 and T3. Data presented as median+interquartile range (IQR). ** $p < 0.01$.

TABLE 3 Correlation between clinical parameters and biological mediators during RIF in fasting group ($N = 25$).

Time point	Clinical parameter data Mean ± SEM		Biological mediator data Mean ± SEM		*R* and *p*
T3	BMI	24.22 ± 0.73	MMP-9	4477.17 ± 552.28	*R*: −0.446; *p*: 0.025
	ESS	6.68 ± 0.88	BDNF	757.38 ± 126.89	*R*: −0.414; *p*: 0.040
	HADSA	4.16 ± 0.73	TNF-α	3.67 ± 0.31	*R*: 0.398; *p*: 0.049
	HADSD	4.16 ± 0.5	TNF-α	3.67 ± 0.31	*R*: 0.434; *p*: 0.030
			GDNF	2.21 ± 0.22	*R*: 0.526; *p*: 0.007
			MM*p*-9	4477.17 ± 552.28	*R*: 0.419; *p*: 0.037
T4	BW	76.54 ± 2.51	TNF-α	2.18 ± 0.13	*R*: 0.408; *p*: 0.043
	BFM	15.59 ± 1.38	TNF-α	2.18 ± 0.13	*R*: 0.621; *p*: 0.001
			GDNF	1.73 ± 0.13	*R*: 0.668; *p*: 0.000
	WHR	0.89 ± 0.07	GDNF	1.73 ± 0.13	*R*: 0.490; *p*: 0.013
	BDI-II	4.92 ± 1.03	ß-NGF	2.18 ± 0.13	*R*: −0.399; *p*: 0.048

All data are presented in Mean ± SEM. This table shows only the significant correlation data between clinical parameters and biological mediators. Data of clinical parameters of this table has been presented in previous publication (5).

Limitation

This study only evaluated the effect of RIF on healthy young males. Therefore, the results could be different in different sex, range of ages (younger/older) and health conditions. We did not include female participants, because they might not fast for the whole of Ramadan due to menstruation. During menstruation periods, females are not obligated to do fasting. It could alter the results. However, we included female participants in other fasting study, although we did not measure similar markers (33). Many biological parameters are quite sensitive to dietary consumption (48–51). Controlling food intake in all participants could lead to better understanding physiological mechanism. However, it could not be done as participants had different cultures, habits and preferences. Otherwise, recording food intake during the study should be done in the future. Assessment of NFG were performed only at T1 and T3, as we hypothesized that NFG would not show any changes during T2 and T4. Main focus of this study, among others, is the difference of FG between before fasting (T1) and the last days of the fasting period (T3). However, we agree that it should be done at all time points for future study.

In summary, RIF as a model of IF could give benefit to health in particular related to mental health and anthropometric parameters. Its benefit is mediated by the alteration of biological parameters, especially neurotrophic factors and cytokines.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethic Committee of Hannover Medical School (ethics no. 6899; registration code of trial: DRKS00008181). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

SG: Data curation, Investigation, Project administration, Writing – original draft, Writing – review & editing. AS: Data curation, Investigation, Methodology, Software, Validation, Writing – original draft, Writing – review & editing. MB: Data curation, Investigation, Methodology, Software, Writing – review & editing. IH: Data curation, Investigation, Project administration, Writing – review & editing. GG: Funding acquisition, Resources, Writing – review & editing. CG: Funding acquisition, Resources, Writing – review & editing. BN: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

BN and CG are employed by Hannover Rehabilitation Services and Science Consulting.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Effects of Ramadan intermittent fasting on performance, physiological responses, and bioenergetic pathway contributions during repeated sprint exercise

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Introduction: This investigation aims to elucidate the impact of Ramadan intermittent fasting on performance, physiological responses, and bioenergetic pathway contributions during repeated sprints.

Methods: Fourteen active male Muslim athletes (age = 22.4 ± 1.8 years, body weight = 69.5 ± 3.8 kg, height = 176 ± 5.1 cm) executed a repeated sprint protocol, consisting of ten 20-meter sprints with 15-s passive recovery intervals, during both fasting and non-fasting conditions. The fasting session was conducted after a 12–14 h fast following Sahur (the pre-dawn meal during Ramadan). In contrast, the non-fasting session occurred before the Ramadan fasting period began, during the same hours of the day, at a time when fasting was not yet required for the athletes. Bioenergetic pathway contributions during repeated sprints were quantified using the PCr-LA-O_2 method.

Results: The mean sprint time during fasting sessions was 3.4 ± 0.3 s compared to 3.3 ± 0.2 s in non-fasting sessions, indicating a trend approaching the threshold of significance for slower times in the fasted state ($p = 0.052$, effect size (ES) = 0.34). In terms of bioenergetic contributions, the total metabolic energy expenditure (TEE) was slightly lower during fasting sessions (236.5 ± 22 kJ) compared to non-fasting sessions (245.2 ± 21.7 kJ), but this difference was not statistically significant ($p = 0.102$, ES = 0.40). Similarly, metabolic energy expenditure per sprint was 23.7 ± 2.2 kJ in fasting conditions compared to 24.5 ± 2.2 kJ in non-fasting conditions ($p = 0.106$, ES = 0.35). The oxidative energy contribution did not differ significantly between fasting (34.2 ± 4.1 kJ) and non-fasting conditions (34.2 ± 4.1 vs. 35.5 ± 5.2 kJ; $p = 0.238$, ES = 0.28). Similarly, lactic (60.4 ± 7.6 vs. 59.2 ± 8.3 kJ; $p = 0.484$, ES = 0.15); and alactic (149.3 ± 19.9 vs. 143 ± 21.5 kJ; $p = 0.137$, ES = 0.30) energy contributions showed no significant differences between the fasting and non-fasting sessions. The percentage of performance decrement (Pdec) and the percentage contributions of oxidative, lactic, and alactic pathways to the total energy expenditure did not differ significantly

between the fasting and non-fasting conditions, indicating a similar bioenergetic profile across both conditions.

Conclusion: The present findings indicate no significant differences in performance metrics and metabolic outcomes between fasted and non-fasted states. Future assessments with longer duration and higher intensity protocols may provide further insights.

KEYWORDS

bioenergetics, Ramadan, fasting, repeated sprints, intermittent exercise

1 Introduction

As commonly known, for religious beliefs, Muslim athletes must observe fasting from dawn to dusk during the month of Ramadan as per the Islamic lunar calendar (1, 2). During this time, both food and fluid intake are restricted during daylight hours leading to impaired muscle function, diminished metabolic energy consumption, and compromised hydration status (3–5). The daily fast during the Ramadan month is variable, dependent on the latitude and seasonal variation, but commonly lasts 10 to 20 h daily across ~30 consecutive days (6). This religious observance often conflicts with athletes' training schedules and competitive events, as mainstream sporting calendars do not accommodate religious practices (1, 7). Ramadan intermittent fasting (RIF) has been shown to induce alterations in energy metabolism, hormonal fluctuations, and sleep pattern disruptions, which all could potentially influence athletic performances (8, 9). Although current evidence predominantly suggests that RIF may impair aerobic rather than anaerobic capacity, there is a conspicuous absence of research examining its effects on repeated sprint performance — a form of exercise that relies on both anaerobic and aerobic metabolic pathways (10, 11).

Although it is reported that maintaining adequate nutrition, sleep, and training can mitigate performance decrements during fasting (12), prolonged fasting is also associated with an inevitable decline in performance, highlighting the complex interplay between fasting duration and athletic outcomes (4). Notably, a significant gap in existing research is the insufficient focus on energy metabolism markers and other variables that could influence this process (13–15). Surveys among football players revealed that 39% of them abstain from fasting on match days and 81.5% believe that fasting could impair performance (7). These findings indicate that athletes' decisions to fast during training or competition are complex and multifaceted, deeply rooted in personal, cultural, and religious beliefs, and not solely based on empirical evidence. This complexity underscores the importance of considering both the physiological and psychosocial dimensions of fasting in sports settings.

A critical lacuna in the existing literature is the lack of comprehensive studies examining the specific effects of Ramadan intermittent fasting (RIF) on repeated sprint activities (RSA), particularly in relation to bioenergetic pathways activation. While RSA is recognized as a key performance metric in many sports, especially in team-based games like soccer (14, 15), its interaction with the physiological and bioenergetic challenges posed by RIF remains underexplored. Given the demanding nature of RSA (16–18), involving high-intensity bursts of energy and rapid recovery, understanding how RIF influences these aspects is crucial. The lactate system, which reflects anaerobic metabolism, is particularly relevant for sports requiring high-intensity efforts, such as repeated sprints in soccer (10, 17, 19). During periods of intense physical exertion, the production of blood lactate increases significantly, serving as a marker for anaerobic metabolism and a predictor for athletic endurance and performance (20, 21). In the context of Ramadan, the fasting practice significantly alters metabolic responses and energy utilization patterns (1, 3). Fasting can lead to changes in glycogen stores, impacting lactate production and clearance (5, 22). Understanding the bioenergetic alterations during Ramadan, particularly in terms of lactate dynamics, is crucial for comprehending how fasting affects athletes' performance during repeated sprints (23). However, the impact of fasting, particularly during Ramadan, on these high-intensity activities is not well-documented, leaving a gap in strategies for athlete preparation and training during fasting periods.

Previous research has often attributed the decline in physical performance during Ramadan to factors such as caloric restriction, altered macronutrient composition, and sleep deprivation (2, 12, 24). These elements are undoubtedly significant, yet there remains a critical gap in understanding the specific impacts of these factors on athletes' bioenergetic pathways during Ramadan. Our investigation, therefore, takes a novel approach by controlling for these variables and focusing on shifts in energy metabolism. By isolating the influence of energy metabolism, we aim to provide a deeper understanding of how Ramadan fasting specifically affects athletic performance. This approach allows us to distinguish the effects of altered nutritional and sleep patterns from the intrinsic metabolic changes induced by fasting. Our study is not just about observing performance decrements during Ramadan but about comprehensively understanding the underlying bioenergetic mechanisms. This focus on bioenergetics is crucial as it addresses a largely unexplored aspect of sports performance during Ramadan. We hypothesize that RIF could potentially impair RSA performance by altering bioenergetic pathway contributions, thereby affecting the overall energy availability and utilization during high-intensity efforts. By investigating these dynamics, we aim to contribute valuable insights into the planning and execution of training and competitive strategies for athletes who observe Ramadan fasting. Our study's findings could also inform approaches to optimize performance and energy management in sports disciplines where RSA is critical, extending beyond soccer to other team sports.

2 Materials and methods

2.1 Study design

This study utilized a quasi-experimental, crossover design with repeated measures to investigate the effects of RIF on RSA in active male Muslim athletes. The design involved assessing the same group of athletes under two different conditions: during Ramadan (fasting) and outside of Ramadan (non-fasting). The primary objective was to assess the impact of RIF on performance, physiological responses, and bioenergetic pathway contributions during RSA. This approach allowed for a direct comparison of each athlete's performance under fasting and non-fasting conditions, thereby providing insights into the specific effects of Ramadan fasting on RSA performance.

2.2 Participants

A priori power analysis was calculated using the G*Power software (Version 3.1.9.4, University of Kiel, Kiel, Germany) to determine the required sample size with α set at 0.05 and power (1-β) set at 0.80. The analysis revealed that a total sample size of 13 subjects would be sufficient to find significant differences with an actual power of 0.82. Fourteen active male Muslim athletes (mean ± SD: age = 22.4 ± 1.8 years, body mass = 69.5 ± 3.8 kg, height = 176 ± 5.1 cm) voluntarily enrolled in this study. All participants were briefed on the potential risks and benefits and provided informed written consent. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and received approval from the Ethics Committee of Atatürk University's Faculty of Sport Sciences. The athletes were classified as "Trained/Developmental" based on the Participant Classification Framework by McKay et al. (25). This categorization was determined by their training frequency (3–5 times per week) and affiliation with amateur soccer teams. These athletes typically represent a performance level that includes structured training but not at the elite or professional tiers. Inclusion criteria mandated that participants be in good health, free from acute or chronic medical conditions and not undergoing any medical treatment. Exclusion criteria encompassed contraindications to maximal effort testing, existing orthopedic injuries, use of dietary supplements and ongoing medical treatment. Participants were advised to obtain a minimum of 8 h of sleep prior to each testing session and to refrain from strenuous physical activity during the study period. Dietary intake was standardized across both test days, albeit distributed across three meals during the control session and two meals during the fasting session. Caloric intake and macronutrient distribution were set at 40 kcal/kg/day, comprising 20% protein, 30% fat and 50% carbohydrates (2, 8).

2.3 Procedures

Participants visited the Atatürk University Sports Sciences Implementation and Research Centre on three separate occasions: a familiarization session and two experimental sessions (Figure 1). The familiarization session was scheduled before the onset of Ramadan, during which participants were not fasting. During this session, participants were acclimated to exercising with a respiratory gas mask and a sample lactate measurement was obtained. Subsequently, maximal 20-meter sprint times were recorded twice (with a 3-min interval) and the best (shorter) time was used as the criterion score. Participants were required to achieve at least 95% of this criterion score during the initial sprints of both tests. Failure to do so resulted in test termination and a 5-min rest before reattempting.

The control session (non-fasting) was conducted within the last 3 days before the start of Ramadan. The experimental session (fasting) took place during the first 3 days of Ramadan, ensuring that each participant had a 48–72-h gap between their control and experimental sessions. All tests were administered between 16:00 and 18:00, corresponding to a fasting duration of 12–14 h for the experimental session. Participants executed a repeated sprint protocol consisting of ten 20-meter sprints with 15-s passive recovery intervals. Light-sensitive photocell gates were employed, and athletes were instructed to initiate their sprints following a 3-s countdown indicated by the lighting sequence. Sprints began 50 cm behind the photocell gate. Repeated-sprint performance metrics, including total time and percentage of speed decrement, were calculated based on formulas proposed by Glaister et al. (26).

$$\text{Percentage of speed decrement} = \left[100 \times \left(\frac{\text{total sprint time}}{\text{ideal sprint time}} \div\right)\right] - 100$$

$$\text{Total sprint time} = \text{sum of sprint times from all sprints}$$

$$\text{Ideal sprint time} = \text{the number of sprints} \times \text{fastest sprint time}$$

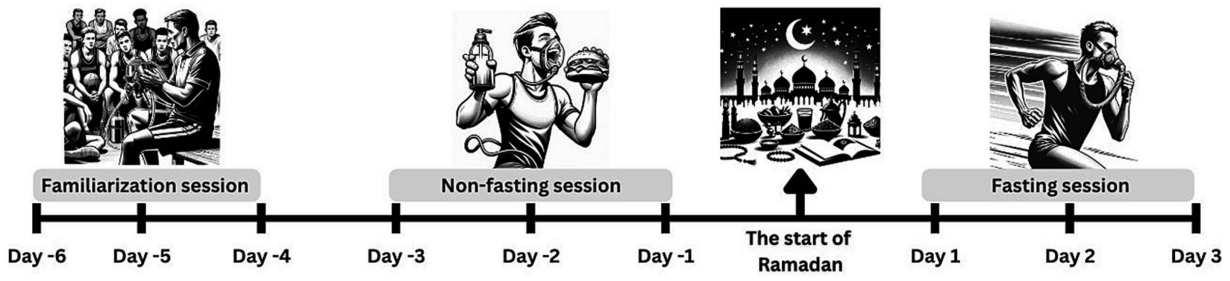

FIGURE 1
Flowchart of the study design process.

A standardized warm-up protocol was performed before each testing session, consisting of 10 min of jogging and stretching and followed by three 5-meter acceleration drills. Efforts were made to minimize long-duration and high-intensity actions to prevent blood lactate accumulation and fatigue. Testing started 3 min post-warm-up. Anthropometric assessments were conducted using standardized techniques on the initial test day. Height was measured using a portable stadiometer (Holtain Ltd., Crosswell, Crymych, Dyfed, United Kingdom), whereas body mass and fat were evaluated via air displacement plethysmography (Bod Pod, Cosmed, Chicago, IL, United States) calibrated as per manufacturer guidelines. Oxygen consumption (VO_2) was measured using a portable metabolic gas analyzer (K5, Cosmed, Rome, Italy) during and 15 min post-exercise. Blood lactate concentrations were assessed pre-exercise and at the 1st, 3rd, 5th, 7th and 10th minutes post-exercise, using capillary blood samples from the left-hand fingertip and analyzed with a portable hand analyzer (Lactate Scout +, SensLab GmbH, Germany). Laboratory conditions were maintained at approximately 22°C and 45% humidity throughout all testing sessions.

2.4 Calculation of the energy pathway contributions

The contributions of the oxidative, lactic and alactic metabolic pathways were quantified using the PCr-LA-O2 method (27, 28). Oxygen uptake was assessed during exercise and a 15-min post-exercise period using a portable gas exchange system operating in breath-by-breath mode. Prior to each test, the portable metabolic gas analyzer was calibrated in accordance with the manufacturer's guidelines. Blood lactate concentrations were ascertained from capillary blood samples obtained from the left-hand fingertip using a portable hand analyzer. Blood samples (20 µL) were collected at baseline and at subsequent intervals during the recovery phase (1st, 3rd, 5th, 7th, and 10th minutes) to determine peak lactate levels [BLa_{peak}]. The delta lactate concentration [BLa_{delta}] was calculated as the difference between BLa_{peak} and baseline values. The oxidative pathway contribution was derived from VO_2 levels exceeding the standing baseline (4.5 mL·kg^{-1}·min^{-1}) during the tests. The area under the curve of the actual VO_2 minus the standing VO_2 was used to quantify this contribution (29).

$$\text{Oxidative contribution}\,(\text{kj}) = \left[\begin{matrix}\text{actual } VO_2\,(\text{ml}) - \\ \text{standing } VO_2\,(\text{ml})\end{matrix}\right] / 1000 \times 20.9 \quad (1)$$

The lactic system contribution was calculated from the BLadelta with the di Prampero equivalent (29).

$$BLa_{\text{delta}}\,(\text{mmol}) = \text{Peak BLa}\,(\text{mmol}) - \text{Resting BLa}\,(\text{mmol}) \quad (2)$$

$$\begin{aligned}\text{Lactic contribution}\,(\text{kj}) = {} & BLa_{\text{delta}}\,(\text{mmol}) \times \\ & \text{body mass}\,(\text{kg}) \times \\ & 3\,(\text{ml})] / 1000 \times 20.9\end{aligned} \quad (3)$$

The alactic pathway contribution was determined using bi-exponential curves generated to examine the fast and slow components of excess post-exercise oxygen consumption (EPOC) kinetics utilizing OriginPro software (version 2019b, OriginLab, Northampton, MA, United States). The fast phase of EPOC (actual VO_2 − slow phase VO_2) was utilized as the representative of the alactic contribution since ATP-PCr resynthesis occurs in this phase. Additionally, the energy contribution during the breaks was attributed to the alactic pathway considering that rest intervals are predominantly devoted to the repayment of PCr stores. Nonetheless, it should be noted that our calculations operate under the assumption that the post-exercise replenishment of phosphocreatine (PCr) stores is solely mediated by the oxidative pathway. This model does not account for potential minor contributions from the glycolytic system to PCr resynthesis. Additionally, we assume that VO_2 observed during the fast phase of EPOC is entirely dedicated to the replenishment of PCr stores, thereby neglecting any VO_2 that may be utilized for the re-binding of myoglobin (29).

$$VO_{2(t)} = VO_{2\text{baseline}} + A_1 \times \left[e^{-(t-td)/\tau 1}\right] + A_2 \times \left[e^{-(t-td)/\tau 2}\right] \quad (4)$$

$$EPOC_{\text{fast}}\ VO_2 = A_1 \times \tau 1 \quad (5)$$

$$\begin{aligned}\text{ATP} - \text{PCr contribution}\,(\text{kj}) = {} & EPOC_{\text{fast}}\ VO_2 + \text{number of} \\ & \text{breaks} \times \text{integral of} \\ & VO_{2(\text{EPOC\_15\_sec})} \times 20.9\end{aligned} \quad (6)$$

$EPOC_{fast}$ is alactic pathway contribution calculated from the oxygen kinetic during the fast component of EPOC, $VO_{2(t)}$ is the oxygen uptake at time *t*, $VO_{2baseline}$ is the asymptotic y-value of the curve, A is the amplitude, td is the time delay, τ is the time constant, $VO_{2(EPOC\_15\_sec)}$ is the integral value of the curve for the first 15 s (recovery period between the sprints) and 1 and 2 denote the fast and slow components, respectively.

2.5 Statistical analysis

Data-processing procedures were conducted using SPSS 21.0 (IBM Corp, Armonk, NY, United States) and OriginPro 2019b software (OriginLab Corp., Northampton, United States). The measures are reported as means and standard deviations. Normality of distribution was verified using the Shapiro–Wilk test. Differences between variables were calculated using the paired sample t-test. In addition, effect sizes were calculated using Cohen's *d* (30) and were classified according to Hopkins (31). The Hopkins' classification for interpreting effect sizes is as follows: Effect sizes less than 0.2 are considered trivial, effect sizes between 0.2 and 0.6 are categorized as small, effect sizes between 0.6 and 1.2 are considered moderate, effect sizes between 1.2 and 2.0 are classified as large, and effect sizes greater than 2.0 are considered very large. For comparisons of sprint times within a protocol, a one-way analysis of variance with repeated measurements was used. A two-way analysis of variance (ANOVA) with repeated measures was used to determine the effects of the state (control and fasting), sprints (1, 13, 22, 24, 27, 28, 32–35) and interaction (state×sprints),

TABLE 1 Metabolic outputs of repeated sprints performed during fasting and control sessions.

	$RS_{control}$	$RS_{fasting}$	t	p	ES (d)
Mean time (s)	3.3 ± 0.2	3.4 ± 0.3	2.144	0.052	0.34
Worst time (s)	3.4 ± 0.3	3.6 ± 0.5	2.180	0.048	0.39
P_{dec} (%)	6.4 ± 2.7	6.6 ± 3.3	0.373	0.715	0.07
Oxidative (kJ)	35.5 ± 5.2	34.2 ± 4.1	1.236	0.238	0.28
Lactic (kJ)	60.4 ± 7.6	59.2 ± 8.3	0.720	0.484	0.15
Alactic (kJ)	149.3 ± 19.9	143 ± 21.5	1.586	0.137	0.30
TEE (kJ)	245.2 ± 21.7	236.5 ± 22	1.759	0.102	0.40
EE per sprint	24.5 ± 2.2	23.7 ± 2.2	1.757	0.106	0.35
Oxidative (%)	14.4 ± 1.3	14.5 ± 1.5	0.798	0.846	0.07
Lactic (%)	24.9 ± 4.3	25.3 ± 4.6	0.551	0.591	0.09
Alactic (%)	60.7 ± 3.8	60.2 ± 4.5	0.596	0.561	0.12

TEE, total metabolic energy expenditure; EE, metabolic energy expenditure; P_{dec}, Percentage of performance decrement.

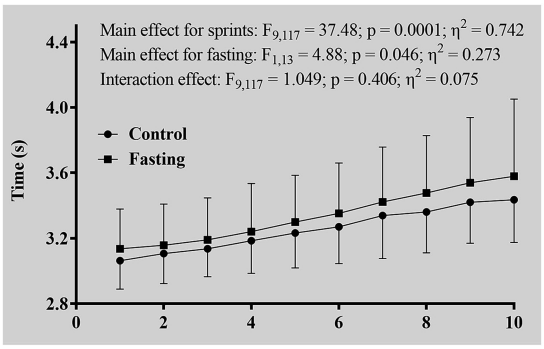

FIGURE 2
Sprint times of repeated sprints performed during fasting and control sessions.

followed by multiple comparisons. The assumptions of sphericity were assessed by Mauchly's test. Whenever an assumption was violated, Greenhouse–Geisser correction if epsilon (ε) value was <0.75 and Huynh-Feldt correction if ε was >0.75 were applied to the degree of freedom. Statistical tests were deemed to be significant at $p < 0.05$.

3 Results

The worst sprint time during the fasting repeated sprints ($RS_{fasting}$) was significantly higher than that during the non-fasting repeated sprints ($RS_{control}$). However, no differences were recorded in the mean sprint time or the percentage of performance decrement (Table 1). Subsequent analysis two-way ANOVA revealed the main effects for both sprint sequence and nutritional states. Specifically, later sprints exhibited slower times compared to earlier ones and the fasting state was associated with slower times relative to the control state. Importantly, no interaction effect was detected between the nutritional state and the sprint sequence in terms of absolute performance metrics (Figure 2). With respect to metabolic contributions, both in relative and absolute terms, no significant differences were identified in the contributions from the oxidative, lactic and alactic pathways (Table 1; Figures 3, 4).

4 Discussion

The decline in physical performance during Ramadan has commonly been attributed to factors such as caloric restriction, alterations in macronutrient composition and sleep deprivation. However, the present investigation controlled for these variables and concentrated on shifts in energy metabolism. Our data revealed no statistically significant variations in the contributions of alactic, lactic and oxidative metabolic pathways during RSA in both fasting and non-fasting conditions contrary to our hypothesis. It should be noted, however, that whereas the volume of fluid intake remained constant over the two different conditions, the timing of fluid consumption was markedly different due to the restrictions imposed by the fasting period.

In this study involving athletes positioned in the middle of the training and performance caliber scale, we have identified that bioenergetic activities are similar in both fasting and non-fasting conditions. However, the findings indicate that there may be a tendency for performance outcomes to decline during more demanding exercise tasks while fasting. A recent systematic review by Abaïdia et al. (1) highlighted a critical methodological consideration in interpreting the outcomes of studies on this subject. The review concluded that elite athletes did not experience a significant decline in performance, whereas sub-elite athletes with less frequent training sessions exhibited a more pronounced performance decrement. Given that our study population consisted of trained/developmental-level football players (25), our findings align with this observation suggesting that fasting had a negligible impact on bioenergetic contributions and performance metrics. Nonetheless, the data imply that these effects could become more pronounced under more strenuous and extended exercise protocols. Therefore, it is imperative to consider multiple variables including the athletes' training level, exercise intensity and duration, fatigue etiology and overall energy metabolism, when evaluating the effects of RIF on physical performance.

This study demonstrates that similar bioenergetic contributions can be achieved during repeated sprints, which predominantly activate the lower body muscle groups, while fasting. It has been established that approximately half of an athlete's body weight comprises muscle mass, predominantly localized in the lower extremities (36, 37). This translates to an estimated 250–300 grams of glycogen storage in the muscles of athletes engaged in sprint activities. Given that this storage is sufficient to meet the metabolic energy demands of typical sprinting protocols, performance decrements during RIF are likely attributable to factors extrinsic to muscle cell activity, such as dehydration or hypoglycemia. In a standard feeding regimen, elevated blood glucose levels are expected within a few hours post-suhoor (i.e., the meal eaten before dawn during Ramadan). The rate of post-absorptive blood glucose utilization, primarily for hepatic glycogen synthesis and glycolysis, is approximately 2 mg/kg/min, although glucose levels remain comparatively low until the iftar meal (i.e., the meal eaten at sundown during Ramadan; (22, 38)).

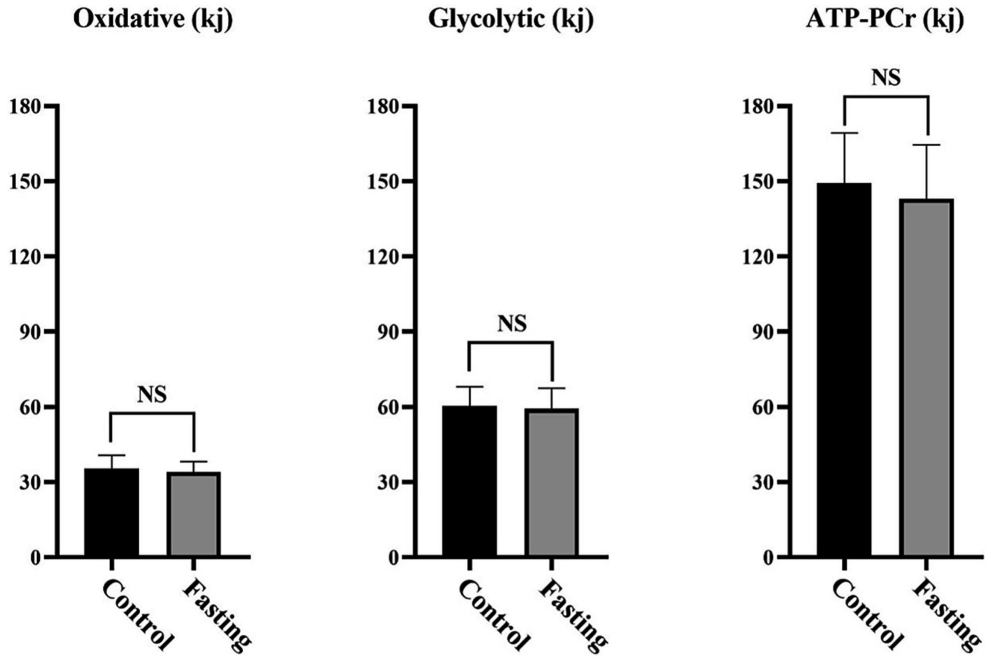

FIGURE 3
Absolute metabolic energy contribution of the bioenergetic pathways (in kilojoules) during repeated sprints for fasting and control sessions (NS, non-significant).

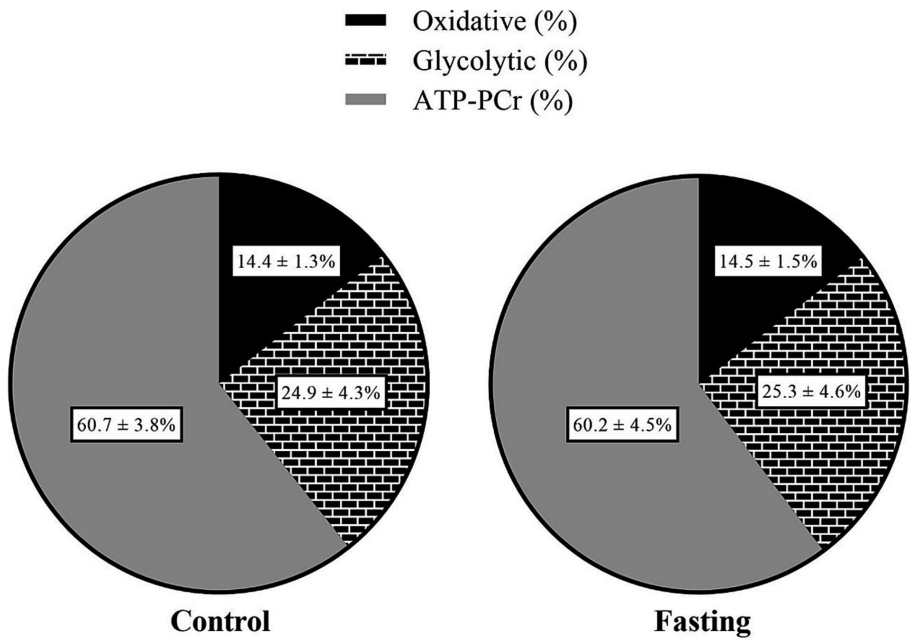

FIGURE 4
Relative metabolic energy contribution of the respective metabolic pathways (in kilojoules) for fasting and control states.

During the afternoon phase of RIF, skeletal muscles exhibit metabolic plasticity, transitioning from carbohydrates to lipids as the primary fuel source to conserve hepatic glycogen and maintain blood glucose levels (39, 40). However, high-intensity exercises like sprinting predominantly rely on muscle glycogen. Theoretically, the preservation of muscle glycogen stores until training implies that the fuel requirements during sprints remain consistent with non-fasting conditions. During RSA conducted shortly before the iftar meal, working muscles require high-energy phosphates (ATP and CP) and glucose. Given that blood glucose equilibrium is primarily regulated

by the insulin/glucagon balance, hepatic activation and ketone metabolism, it can be inferred that intramuscular glycogen stores are adequate for high-intensity muscle activation. Nonetheless, cognitive impairments associated with low blood glucose levels suggest that even when muscle cell metabolism is regular, disruptions may occur in the neural transmission pathways to the muscle cells.

Alterations in sleep and nutritional patterns during Ramadan induce endocrinological changes (9, 34, 35). Monoamines, particularly serotonin, modulate both sleep and metabolism (35). Prolonged carbohydrate deprivation may prevent tryptophan-dependent drowsiness, as dietary tryptophan is the biochemical precursor of serotonin and plasma tryptophan levels increase following carbohydrate ingestion (35). Additionally, melatonin induces drowsiness through its thermoregulatory effects and direct action on the central nervous system (33, 34). These endocrinological changes are closely related to the mechanisms for maintaining blood glucose and appear to support the use of muscle glycogen as an energy source during sprinting.

Cortisol and testosterone levels typically peak in the early morning (24, 33, 35), optimizing performance in activities requiring maximal effort or significant motor coordination. However, a shift in cortisol secretion patterns during Ramadan has been observed, characterized by reduced morning and increased evening secretion (12, 41). Consistent with these findings, no substantial alterations in respiratory variables were observed during Ramadan (42). Although some studies reported a metabolic slowdown characterized by lower resting afternoon VO_2 (1, 12, 32), our study suggests that metabolism can adequately respond to increased energy demands during repeated sprints.

In this study, we employed a design that focused on bioenergetic activity during repeated sprints, where both anaerobic and aerobic metabolism are active, while controlling for other variables. Our findings challenge the common assumption that fasting negatively impacts energy metabolism pathways during high-intensity exercise. Notably, the results related to performance were close to the threshold of statistical significance, suggesting that outcomes could vary in more challenging exercise tasks. We implemented a protocol of ten 20-meter repeated sprints with 15-s passive recovery intervals. Such designs have dynamic variables, including increasing the number of sprints, extending sprint distance, or reducing rest time, indicating that results could differ in more demanding exercise tasks. Furthermore, the athlete group in our study, when assessed in terms of training and performance caliber, is situated at the third level of a six-tier scale (2, 12, 24). It's conceivable that athletes at higher levels could withstand more strenuous exercise tasks even while fasting. Thus, it's important to remember that many factors need to be considered in such studies. In conclusion, our study highlights the complex nature of bioenergetic performance in athletes during Ramadan fasting and the potential impacts of various factors on this performance. It is clear that more research is needed to fully understand these effects and their implications for athletes' performance.

5 Conclusion

To the best of our knowledge, the present study represents the inaugural investigation of the effects of RIF on repeated sprint performance, with a specific focus on associated bioenergetic pathways. The worst sprint time during the fasting repeated sprints was significantly higher than that during the non-fasting repeated sprints. However, no differences were recorded in the mean sprint time or the percentage of performance decrement. In the context of bioenergetic contributions and total energy expenditure, values were not different between the two conditions. Consequently, while our experimental protocol did not yield unequivocal disparities between the fasting and non-fasting states in terms of performance metrics and metabolic parameters, it intimates that such differences could become more salient in protocols of extended duration and elevated intensity, favoring the non-fasting state.

6 Future studies

The study controls for key variables like calorie intake, macronutrient proportions and sleep duration, thereby isolating the effects of fasting on energy metabolism. However, the timing of fluid intake, which is significantly altered during Ramadan, could be a variable worth exploring in future research. Our findings lay the groundwork for future studies to delve deeper into the metabolic markers and energy production mechanisms associated with varying exercise intensities and durations during Ramadan. Understanding these nuances is crucial for athletes and coaches aiming to optimize training and performance during this period. Moreover, the study opens avenues for investigating how religious and cultural practices intersect with sports science, offering a multidisciplinary lens that could enrich both fields. In summary, while our study does not provide definitive answers, it raises pertinent questions and provides a robust framework for future research in exercise physiology, particularly in the context of religious fasting.

7 Strengths and limitations

The present study uniquely investigates the effects of fasting on athletic performance from the perspective of bioenergetic contributions. We utilized a comprehensive methodology that allowed us to discern the contributions from all three energy systems—anaerobic alactic, anaerobic lactic, and aerobic—providing a holistic understanding of how fasting impacts different aspects of energy utilization during repeated sprint activities. Furthermore, the use of repeated sprints as the performance metric aligns with the demands of many team sports, enhancing the ecological validity of our findings. Additionally, this study design carefully controlled the timing of data collection to ensure that both fasting and non-fasting sessions occurred within the same week, minimizing potential confounding factors due to time variations. However, it's important to note some limitations. First, our study had a relatively small sample size of intermediate-level football players, which may limit the generalizability of our findings to elite or sub-elite athletes. Second, the study design focused on the initial days of Ramadan, and different results might be observed in the later stages of fasting. Finally, while we controlled for several variables, there may still be unaccounted factors that could influence the outcomes. Future research with larger and more diverse populations and extended observation periods could provide further insights into the effects of fasting on athletic performance.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Ethics Committee of Atatürk University's Faculty of Sport Sciences. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

SÖ: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. SU: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. CG: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. IO: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. FÖ: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. HY: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. NK: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. FK: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. SA: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. İU: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. LPA: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Effect of calorie restriction and intermittent fasting on glucose homeostasis, lipid profile, inflammatory, and hormonal markers in patients with polycystic ovary syndrome: a systematic review

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Background and objective: Polycystic ovary syndrome (PCOS) is a complex hormonal disorder that leads to ovarian cysts, irregular ovulation, and hormonal swings in women. It is a complex and heterogeneous condition that affects 4 to 20% of women of reproductive age worldwide and relates to reproductive, metabolic, and psychosocial dysfunction. Dietary and lifestyle modifications have been proposed to play a central role in the management of PCOS. This study aimed to provide a comprehensive systemic overview of the existing literature on the effects of intermittent fasting (IF) and calorie restriction (CR) regimens on disease markers of PCOS.

Designs and methods: Several databases, such as CINAHL, Cochrane, EBSCOhost, EMBASE, Google Scholar, ProQuest Medical, PubMed/MEDLINE, ScienceDirect, Scopus, and Web of Science databases were searched for clinical trials and observational studies examined the effects of IF regimens such as time-restricted eating and Ramadan model of IF (RIF) on glucose homeostasis, lipid profile, inflammatory and hormonal markers in patients with PCOS.

Results: This systematic review solicited three articles, comprising a collective sample size of 75 females diagnosed with PCOS. The studies were published between 2015 to 2023 and were undertaken in three countries: China, Turkey, and Iran. The research articles examined the effects of intervention with IF and CR on PCOS-related parameters such as anthropometric measures and biochemical tests which included enzymes, glycemic control, lipid profile, hormonal, and oxidative stress, and inflammatory markers. The articles yielded mixed results, with two of them showing significant changes across all tested parameters. One of the three studies did not exhibit any significant changes.

Conclusion: Very limited studies examined the relationship between IR and CR with markers of PCOS. Further well-controlled studies need to be undertaken the combined results from the limited studies illustrate the intricate and diverse

nature of IF, including the RIF, and its influence on measurements of body composition and biochemical markers related to PCOS.

KEYWORDS

time-restricted eating, alternate-day fasting, Ramadan fasting, diurnal fasting, gynecology, infertility

Introduction

Polycystic ovary syndrome (PCOS) is a prevalent endocrine condition distinguished by persistent anovulation, biochemical and/or clinical hyperandrogenism, and the presence of polycystic ovary morphology (1). The World Health Organization (WHO) estimates that PCOS affects more than 116 million women worldwide (1). This disease has significant clinical implications and can lead to health issues related to the accumulation of adipose tissue, including obesity, insulin resistance (IR), metabolic syndrome (MetS), and type 2 diabetes mellitus (T2DM) (2). Insulin resistance can be elucidated by the necessity of elevated insulin levels to support metabolic processes, as well as its involvement in mitogenic and reproductive functions (3). Previous studies have indicated that a substantial proportion of women with PCOS experience impaired glucose tolerance and IR and are at increased risk for developing T2DM (3). In general, the presence of abdominal obesity or visceral adiposity in individuals with PCOS may contribute to IR, potentially triggered by subclinical, systemic low-grade inflammation. Recent research has determined that obesity is the primary risk factor for IR in persons diagnosed with PCOS (3). Another significant issue related to PCOS is hyperandrogenism, a condition that is also associated with IR. The premature secretions of androgen during early stages are commonly regarded as a characteristic feature of PCOS and are believed to contribute to the development of IR in preceding stages (3).

Sarahian et al. (4) propose that PCOS can arise from a combination of lifestyle, genetic, and prenatal influences, hence giving rise to a range of effects with differing magnitudes. The risk of PCOS is heightened by an unhealthy lifestyle and dietary choices, or exposure to infectious agents (5, 6). Environmental factors, such as physical exercise, dietary and lifestyle behaviors, and food choices exhibit significant variability among different populations.

Frequently, PCOS treatment focuses on the management of underlying symptoms, typically involving progestin therapy and a combination of birth control medications (7). Furthermore, the implementation of lifestyle modifications, specifically the adoption of healthful dietary practices and nutritious food choices, is highlighted as a viable approach for effectively managing the condition (5). The overall condition is improved by the combination of pharmacological treatments and dietary and lifestyle modifications. According to Xu and Qiao (3), lifestyle therapies, including exercise, weight loss, and nutrition therapy, have demonstrated favorable results in individuals with PCOS.

The implementation of nutrition therapy for weight reduction in women diagnosed with PCOS has been found to have a substantial influence on metabolic conditions. Previous research has demonstrated that patients with obesity who lose between 5 and 10 percent of their body weight (BW) experience significant health benefits (8). In recent decades, fasting regimens, especially what is commonly known as intermittent fasting (IF), have emerged as a non-pharmaceutical lifestyle approach in integrative medicine and a means to reduce and control weight to enhance health (9). The implementation of IF has been shown to have the potential to reduce adiposity and improve IR through decreased calorie intake and metabolic reprogramming. It can result in several positive health outcomes, such as enhanced metabolic efficiency, enhanced cognitive acuity, and an extended life span (10, 11).

Ramadan intermittent fasting (RIF), is another type of IF regimen. During Ramadan, adult Muslims are mandated to refrain from food and drink for 12–22 h during the day, depending on the season and geographical location. This fasting pattern is followed consistently for 29–30 days. Multiple studies, including original research, systematic reviews, and meta-analyses, have provided evidence that RIF is linked to decreased BW, body fat mass especially visceral fat, serum lipids, and other cardiometabolic risk factors, inflammatory and oxidative stress markers, with slight improvements in glucometabolic regulation and liver function tests (12–19). Most, if not all, of these aforementioned factors, improved upon RIF have been implicated in the etiopathogenesis of PCOS in variable degrees and dimensions (20–22).

Based on the above literature, and previous research examining the impact of different models of IF on various metabolic, hormonal, and inflammatory markers and health indicators, as well as their reported protective effect, it is hypothesized that CR and IF will improve metabolic parameters in PCOS patients. Therefore, this review aims to provide a comprehensive summary of the existing literature on the effects of Ramadan and non-Ramadan IF and CR regimens on PCOS markers.

Methods

This systematic review followed Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) to guide the reporting of the findings (23).

Review question

The PICO model was employed to expand the return from the review and applicability of the data collected (Table 1).

Database searches

An electronic search of the databases was conducted of the Google Scholar, PubMed/MEDLINE, EBSCO-host, CINAHL, ScienceDirect,

TABLE 1 PICO Model.

Population	Females above the age of 18 years diagnosed with PCOS using Rotterdam criteria
Intervention	Any form of intermittent fasting regimen such as time-restricted eating (TRE), alone or associated with pharmacological therapy, exercise, and weight loss. To include 16:8 method, 18:6 method, 5:2 diet, alternate day fasting, intermittent fasting, Ramadan model of intermittent fasting, or other suitable religious intermittent fasting regimens
Comparator	Comparison to the usual *ad libitum* diet or no dietary or non-fasting intervention, standard treatment-as-usual, and pharmacological therapy
Outcomes	Any PCOS metabolic, hormonal, inflammatory, or anthropometric markers including insulin levels, C-peptide concentrations, glucagon, insulin-like growth factor 1 (IGF-1), glycated hemoglobin (HbA1c), homeostatic model assessment for insulin resistance (HOMA-IR), fasting blood glucose, oral glucose tolerance test (OGTT), lipid profile such as and serum lipid profile parameters, low-density lipoprotein (LDL), high-density lipoprotein (HDL).; hormonal markers such as sex hormone binding globulin (SHBG), follicular stimulating hormone (FSH), luteinizing hormone (LH)

Cochrane, ProQuest Medical, Web of Science, and Scopus databases for targeted studies published from 1970 to the end of November 2023. The keywords were taken from a bibliometric analysis paper by Obaideen et al. (24). Accordingly, the following search criteria were applied: TITLE-ABS-KEY ("intermittent fasting") OR ("Ramadan") OR (Ramadhan) OR (Ramazan) OR ("Islamic fasting") OR ("diurnal fasting") OR ("Ramadan intermittent fasting") OR ("Ramadan diurnal intermittent fasting") OR ("consecutive 30 days of fasting") OR ("religious fasting") AND ("PCOS" OR "polycystic ovary syndrome" or "polycystic ovarian syndrome").

Inclusion criteria

Both observational and experimental studies investigating the impact of CR, IF including RIF on markers related to PCOS were included. The inclusion criteria for research articles were as follows: (1) experimental and observational studies; (2) adult female participants (>18 years) diagnosed with PCOS; (3) endpoints that included changes in at least one PCOS diagnostic biomarker before and after following the CR or IF regimen.

Exclusion criteria

Specific exclusion criteria were applied to eliminate any potential quality or methodological issues: (1) studies involving patients with any other disease apart from PCOS; (2) lacking full-text; (3) non-English language; (4) lack of clear and pre and post data; (5) editorials, abstracts case reports, and review articles; and (6) non-peer-reviewed and unpublished data. The steps for study selection are summarized in the PRISMA flow diagram (Figure 1).

Main outcomes and measures

The main outcome was to report the effect of practicing CR and IF regimens including RIF-induced changes in PCOS-related parameters, namely anthropometric, glycemic control, lipid profile, hormonal, oxidative, and inflammatory parameters. The first step of screening was examining all titles and abstracts to exclude irrelevant publications.

Two authors (AK and DA) independently screened the titles and abstracts of identified studies and assessed them for eligibility against the inclusion criteria. Discrepancies during screening were resolved by the principal investigator (MF). All titles and abstracts were screened to exclude irrelevant publications. To standardize data collection, the researchers reported and tabulated the study characteristics, including the main author's name, study country, year of publication, sample size, type of fasting, study design, and main findings for the examined outcomes.

Results

Three studies with a total of 75 participants were included in this systematic review. Details of the study authors, year of publication, country, design and type of IF regimen, sample size, age, and biomarkers tested, and the effects of CR and IF regimens are shown in Table 2. The included studies were conducted in 3 different countries, i.e., China, Turkey, and Iran. The parameters included in these studies were as follows: anthropometrics and biochemical tests including enzymes, glycemic control, lipid profile, hormonal parameters, oxidative stress, and inflammatory markers.

Anthropometric measures

In terms of the results obtained for the anthropometric parameters, Li et al. (25) in their study, reported a significant decrease in all anthropometric parameters, i.e., BMI, BW, BFM, BF%, and VFA ($p \leq 0.001$), with the exception of WHR and SMM, which showed no significant association with IF. Feyzioglu et al. (26) reported significant reductions in anthropometric parameters, namely BMI and WHR. Asemi et al. (27) did not find any significant effect of RIF on anthropometric parameters.

Liver enzymes and glucometabolic markers

In terms of enzymes, Li et al. (25) reported no significant effect of IF on enzymes such as UA and AST, while there was a significant decrease in ALT ($p = 0.027$). In relation to the glycemic control parameters, Li et al. (25) reported significant decreases in all glycemic control parameters, i.e., FINS, HOMA-IR, and HbA1c, with the exception of FBG. Similarly, Feyzioglu et al. (26) reported significant decreases in all glycemic control parameters, i.e., FINS, FBG, HOMA-IR, and HbA1c. However, Asemi et al. (27) did not find any significant effect of RIF on glycemic control parameters.

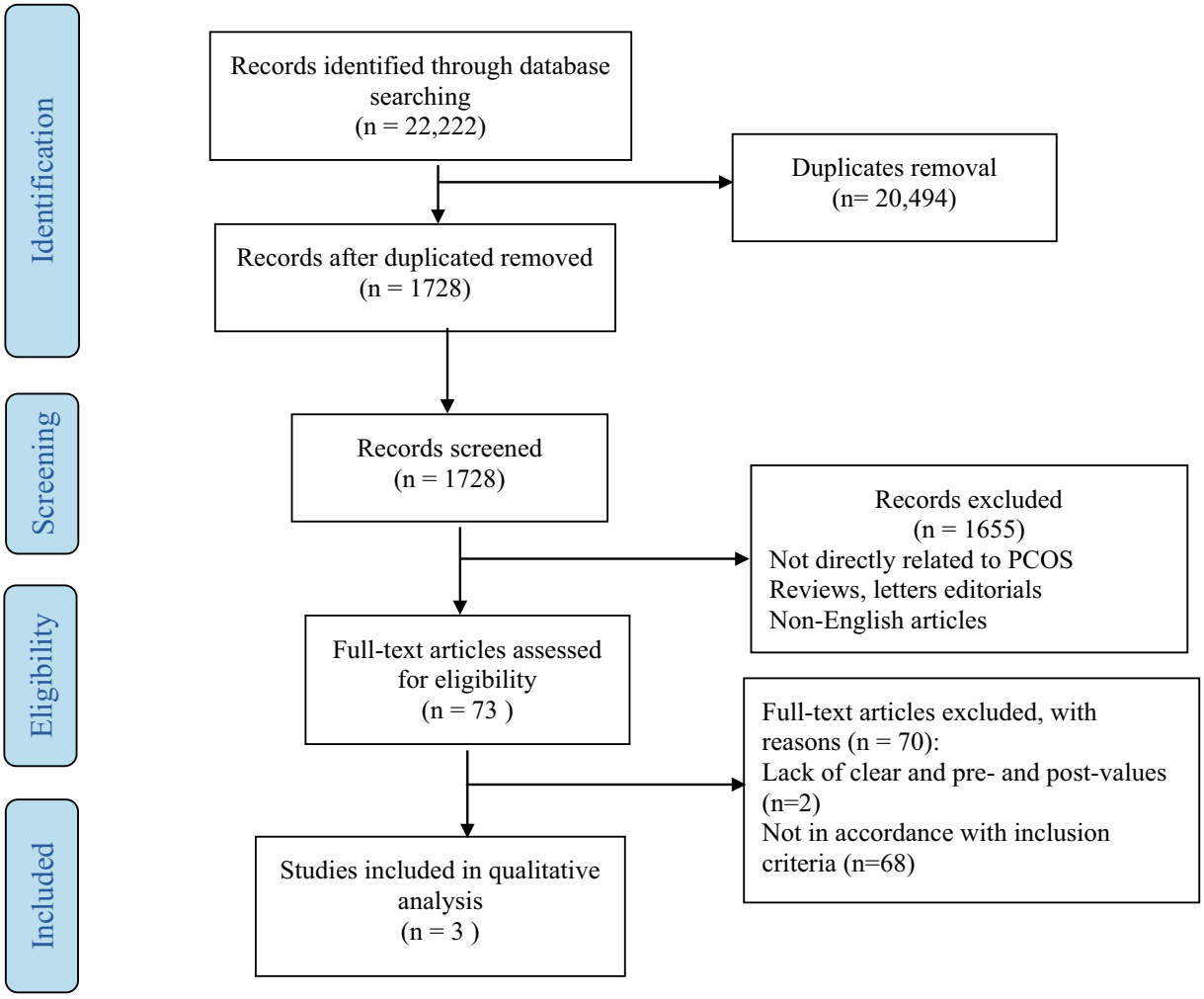

FIGURE 1
PRISMA flowchart for systematic review search.

Lipid markers

Additionally, all the 3 included studies reported on the effect of IF and RIF on the lipid profile. In their study, Li et al. (25) did not find any significant effect of IF on the serum lipids. Similarly, Asemi et al. (27), in their study, did not find any significant effect of RIF on the lipid profile parameters. Conversely, Feyzioglu et al. (26) reported a positive effect of IF on serum lipids, with significant decreases in LDL and TG and a significant increase in HDL.

Hormonal markers

Li et al. (25) reported no significant effects of IF on hormonal parameters such as LH and FSH, while a significant increase in IGF-1 and SHBG was observed after IF. Alternatively, they found significant reductions in TT and FAI. Feyzioglu et al. (26) reported significant effects of IF on all hormonal parameters, with decreases reported in FSH, LH, E2, Prolactin, TT, FT, FAI, DHEAS, and AMH, while reported significant increases in TSH and SHBG. The study by Asemi et al. (27) did not report any hormonal markers in their study.

Oxidative stress and inflammatory markers

Li et al. (25) found a significant decrease in inflammatory markers, *hs*-CRP. Feyzioglu et al. (26) found a significant decrease in the oxidative stress marker, calprotectin. Asemi et al. (27) found no significant effect of RIF on inflammatory and oxidative stress markers such as TAC, MDA, and *hs*-CRP. On the other hand, the authors reported significant increases in NO and GSH.

Discussion

The current study tried to systematically elaborate on the effect of following CR and IF regimens on the disease parameters of PCOS. Only three studies were selected after applying the inclusion/exclusion criteria, a matter that denotes the relatively emerging topic we are currently addressing and the mass need for further studies to be conducted in this regard. The scarcity of studies reviewed is consistent with the finding of Floyd et al. (28), who examined the effect of practicing TRE on insulin levels and insulin sensitivity in patients diagnosed with PCOS. After screening 2,662 studies and

TABLE 2 Characteristics and major findings of the included studies on the effect of intermittent fasting on patients with PCOS.

Author, publication year	Country	Sample Size (*n*)	Age range (average)	Type of fasting	Study Design	PCOS Markers						
						Anthropometric measurements	Glycemic control	Lipid Profile	Hormones	Liver enzymes	Oxidative stress and inflammatory markers	Major findings
Li et al. (25)	China	18	18–31	IF	Clinical trial	BW (kg) BMI (kg/m^2) WHR SMM (kg) BFM (kg) BF% VFA (cm^2)	FG (mmol/L) FINS (μU/mL) AUCIns (mU/L*min) AUCGlu (mmol/L*min) AUCIns/AUCGlu HOMA-IR IGF-1 (ng/mL)	TG (mmol/L) TC (mmol/L) LDL-C (mmol/L)	TT (ng/mL) SHBG (nmol/L) FAI (%) LH (mIU/mL) FSH (mIU/mL) LH/FSH	AST (U/L) ALT (U/L)	hs-CRP (mg/L)	BW ↓, BMI ↓, BFM ↓, BF% ↓ VFA ↓ ALT ↓ FINS ↓, AUCIns ↓ AUCIns/AUCGlu ↓, HOMA-IR ↓ IGF-1 ↑, TT ↓, SHBG ↑, FAI ↓ CRP ↓
Feyzioglu et al. (26)	Turkey	30	21–33	IF	Clinical trial	BMI (kg/m^2) WHR	FINS (μU/mL) FG (mg/dL) HOMA-IR IR (>2.4) HbA1c	HDL-C (mg/dL) LDL-C (mg/dL) TG (mg/dL)	FSH (mIU/mL) LH (mIU/mL) E2 (mIU/mL) TSH mIU/mL Prolactin (ng/mL) TT (ng/dL) Free testosterone (pg/mL) SHBG (nmol/L) FAI (%) Hyperandrogenism (≥8) DHEAS (μg/dL) AMH (ng/mL)		Calprotectin (μg/g)	BMI ↓, WHR↓ FBG ↓, FINS ↓, HOMA-IR ↓, IR ↓ HbA1c ↓ TG ↓, LDL ↓, HDL ↑, TT ↓ SHBG ↑, FAI ↓, LH ↓, FSH ↓, E2 ↓ TSH ↑, Prolactin ↓, Free Testosterone ↓, DHEAS ↓, AMH ↓, Hyperandrogenism ↓ Calprotectin ↓
Asemi et al. (27)	Iran	27	18–40	RIF	Cross-section study	Height (cm) Weight (kg) BMI (kg/m^2)	FG (mg/dL) FINS (IU/mL) HOMA-IR HOMAB QUICKI	TG (mg/dL) VLDL-C (mg/dL) TC (mg/dL) LDL-C (mg/dL) HDL-C (mg/dL)			NO (mol/L) TAC (mmol/L) GSH (mmol/L) MDA (mmol/L) hs-CRP (ng/mL)	NO ↑, GSH ↑

↓ Significantly decreased; ↑ Significantly increased. AMH, anti-mullerian hormone; AUC GIu., area under the curve (AUC) for glucose; AUC Ins., area under the curve (AUC) for insulin; BF%, body fat percent; BFM, body fat mass; BMI, body-mass index; BW, body weight; DHEAS, dehydroepiandrosterone sulfate; E2, estradiol; FAI, free androgen index; FG, fasting glucose; FINS, fasting insulin; FSH, follicle stimulating hormone; GSH, glutathione; HbA1c, glycated hemoglobin A1c; HDL-C, high-density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance; hs-CRP, high-sensitivity C-reactive protein; IR, insulin resistance; LDL-C, low-density lipoprotein-cholesterol; LH, luteinizing hormone; MDA, malondialdehyde; NO, nitric oxide; SHBG, sex hormone binding globulin; SMM, skeletal muscle mass; TAC, total antioxidant capacity; TC, total cholesterol; TG, triglycerides; TSH, thyroid stimulating hormone; TT, total testosterone; VFA, visceral fat adiposity; VLDL-C, very low-density lipoprotein-cholesterol; WHR, waist-hip ratio.

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assessing 37 eligible studies, only one study was found by Floyd and colleagues.

Anthropometric markers

Across different studies, the effect of IF on anthropometric measurements has been considered. For instance, Li et al. (25) and Feyzioglu et al. (26) reported remarkable decreases in various anthropometric markers. Whilst, the study by Asemi et al. (27) which studied RIF in particular, revealed no significant relationship between RIF and these anthropometric markers. However, previous studies by Faris et al. (16) and Hooshiar et al. (29) state that RIF lowers visceral adiposity hence it can reduce BW and other parameters in individuals with PCOS. Additionally, Madkour et al. (30), in their study found that RIF resulted in weight loss, and reduced BMI, BF%, and waist circumference. In their study, the beneficial effects of RIF were observed across all subgroups in the study, regardless of age, sex, and fasting duration. Another meta-analysis was conducted by Jahrami et al. (17) to investigate the impact of RIF on BW. It was found that RIF can cause variable changes in BW, body composition, and fat mass. The reduction in BW and visceral adiposity could be attributed to the metabolic shift to ketogenesis and fatty acid oxidation during fasting (15). However, weight changes induced by RIF were found to be mostly reversed post-Ramadan, indicating that weight loss during this period is transient. This difference in findings represents a split landscape; some studies show that IF positively influences body composition, while others fail to back it up.

Glucometabolic markers and liver enzymes

In terms of enzymes, the included studies reported minimal effect on the liver enzymes. Regarding the glycemic control parameters, two of the included reported a significant reduction in all glycemic control parameters (23, 26). However, Asemi et al. (27) did not find any significant effect of RIF on the glycemic control parameters. Previous studies have also reported such contradictory findings. A systematic review and meta-analysis by Faris et al. (14) examined the effects of RIF on glucometabolic markers in healthy people. The study found that RIF had a minimal impact on these markers and highlighted the influence of various factors such as sex, age, fasting duration, and country on glucometabolic changes. Conversely, Faris et al. (14) reported studies conducted on rodents, which showed that IF improved insulin sensitivity and glucose tolerance, and preserved β-cell mass in obesity-induced diabetes. Likewise, in a study by Carter et al. (31), the researchers conducted a large trial to evaluate the impact of IF versus a continuous energy-restricted diet on glycemic control in individuals with type T2DM. After a year of intervention, both groups showed comparable decreases in HbA1c levels. However, in a study by Faris et al. (16) despite a significant drop in plasma adiponectin levels, no significant change was found in insulin sensitivity or glucose homeostasis markers. Additionally, the study also found a significant increase in apelin levels, which could be responsible for the lack of significant change in insulin resistance, despite an increase in total sugar intake during Ramadan. The study also noted that the level of IGF-1 significantly decreased by the end of RIF, which may be another reason for the non-significant changes in markers of glucose homeostasis.

Lipid markers

Regarding the lipid markers, 2 of the included studies did not find any significant effect of IF on the lipid profile parameters (23, 27) Conversely, Feyzioglu et al. (26) reported a positive effect of IF on serum lipids, with a significant reduction in LDL and TG and an increase in HDL. Similar findings have been reported by Jahrami et al. (18) in their meta-analysis, they reported that RIF improved lipid profile and coagulation parameters, and these improvements persisted for 4 weeks after fasting. The effects of RIF on the lipid markers are consistent with the impacts of other forms of IF and energy-restricted diets. Additionally, the study also found that IF has cardioprotective effects, possibly due to increased cellular stress resistance, reduced oxidative damage, and changes in the brain-derived neurotrophic factor signaling in the brain.

Hormonal markers

In terms of the hormonal marker, following IF, Li et al. (25) observed no significant effects of IF on hormonal parameters such as LH and FSH, while significant increases in IGF-1 and SHBG. Alternatively, they found significant reductions in TT and FAI. Likewise, Feyzioglu et al. (26) reported significant effects of IF on all hormonal parameters, with decreases reported in FSH, LH, E2, Prolactin, TT, FT, FAI, DHEAS, and AMH, while reported significant increases in TSH and SHBG. Similar findings have been reported by Cienfuegos et al. (32) in their study which suggested that IF can potentially lower androgen levels, specifically TT and FAI, and increase SHBG in obese premenopausal women, offering a potential treatment for hyperandrogenic conditions like PCOS. Additionally, Han et al. (33) in their study investigated the effects of time-restricted feeding (TRF) on a mouse model of PCOS. The study observed that TRF treatment significantly lowered plasma androgen levels and the LH/FSH ratio in PCOS mice, consistent with other dietary interventions. This suggests that TRF may regulate gonadotropin-releasing hormone secretion, influencing the synthesis of steroid hormones. During fasting, certain gut microbes can use host substrates to produce beneficial metabolites like butyrate, acetate, and mucin stimulants. This suggests that IF's influence on reproductive hormones may be mediated by alterations in the gut microbiome (32).

Oxidative stress and inflammatory markers

Among the three included studies, following IF, Li et al. (25) and Feyzioglu et al. (26) found a significant decrease in the inflammatory and oxidative stress markers, *hs*-CRP and calprotectin, respectively. Similar findings have been reported in previous studies. For instance, Faris et al. (16) in their review, reported the effect of RIF on proinflammatory cytokines and oxidative stress markers in both obese and non-obese individuals. The studies included in the review reported a slight decrease in these markers after Ramadan, suggesting short-term protection against low-grade systemic inflammation and

oxidative stress. This reduction could be due to weight loss during Ramadan or the lowering of the IGF-1 which is associated with inflammation and oxidative stress. Further, RIF has been linked with reduced serum glucose, insulin, and IR levels in obese individuals with metabolic syndrome. Additionally, RIF may also increase the expression of certain antioxidant genes, providing another potential mechanism for its health benefits (15). A study conducted by Madkour et al. (30) provides the first evidence of a link between RIF and fat mass and obesity-associated (*FTO*) gene expression in overweight/obese individuals. The authors found an association between reduced *FTO* expression and favorable effects such as suppression of pro-inflammatory markers and improved lipid profile. *FTO* is broadly distributed in many organs, and its expression may be influenced by dietary conditions. The study also found a reduction in pro-inflammatory cytokines such as interleukin (IL)-6, tumor necrosis factor- α (TNF-α), and an increase in IL-10, an anti-inflammatory cytokine, during RIF. These findings are consistent with previous research showing significant reductions in pro-inflammatory cytokines and improvements in cardiometabolic risk factors during RIF (12, 34). The study by Madkour et al. (30) found no correlation between *FTO* expression and high-energy intake, waist circumference, or obesity, suggesting that RIF's beneficial effects occur independently of dietary and anthropometric factors. The study also found that RIF upregulates several key regulatory proteins involved in tumor suppression, DNA repair, insulin signaling, glucose and lipid metabolism, circadian clock regulation, immune system, and cognitive function. Similarly, another study by Madkour et al. (35) investigated the impact of RIF on the genetic expression of metabolic and cellular regulator genes (*SIRT1* and *SIRT3*) and antioxidant defense enzyme system genes (*TFAM, SOD2,* and *Nrf2*). The research revealed that the expression of SIRT1 shows a minor reduction at the end of RIF, while SIRT3 shows a significant reduction, which could be due to the lack of significant changes in total energy and fat intake during Ramadan. The study further revealed that the expression of antioxidant defense genes (*SOD2, TFAM*, and *Nrf2*) increases, suggesting their role in counteracting the increased oxidative stress during fasting. The research also notes that the significant reduction of IGF-1 reflects the significant activation of antioxidative stress genes, providing a positive transient protective impact against oxidative stress and subsequent pathological conditions.

Association between PCOS, gut microbiota, and bile acid metabolism

Studies on the gut microbiota may be useful in interpreting the impact of IF, which may be mediated by alterations brought about by IF on the gut microbiota, and in explaining the plausible effect of IF on PCOS. Recent research by Dong and Rees (36) suggests a link between the gut microbiome and the development of PCOS, wherein they reported that women with PCOS exhibit higher levels of *Bacteroides vulgatus* (*B. vulgatus*) and lower levels of certain bile acids in their intestines. Experiments with mice showed that introducing these gut bacteria from PCOS patients or pure *B. vulgatus* led to insulin resistance, altered bile acid metabolism, and disrupted ovarian function. However, administration of IL-22 or glycodeoxycholic acid improved these symptoms, suggesting potential therapeutic strategies. Furthermore, genome-wide association studies have identified various susceptibility loci for PCOS, particularly in metabolic and neuroendocrine pathways. These include loci near genes such as the insulin receptor, follicle-stimulating hormone receptor, and others (36). Additionally, Cienfuegos et al. (32), in their study reported that IF has been found to positively affect the gut microflora's composition and diversity, and reduce gut permeability, thereby diminishing obesity-linked postprandial endotoxemia and systemic inflammation.

The strength of our review is that is the first comprehensive systematic review that tackles the effect of different forms of IF and CR regimens on the different aspects related to PCOS, including anthropometrics and biochemical parameters including enzymes, glycemic control, lipid profile, hormonal parameters, oxidative stress, and inflammatory markers. However, the study also had some limitations that should be considered when interpreting the findings. Limited research and scarcity of works render the generalizability of the results unattainable. Additionally, the inclusion of an observational study as part of the reviewed articles is another weakness of the study as causality cannot be inferred in such a study design.

Conclusion

The following review thus presents IF and CR as a subject of much interest and promise in modulating different physiological responses in individuals with PCOS. A scarce of studies have examined the effect of IF and CR on markers of PCOS. Even so, such limited findings demonstrate the complicated and multifaceted nature of the IF regimen and its impact on body composition, metabolic, hormonal, and other biochemical markers related to PCOS. This diversity in outcomes, however, highlights the complex interaction between IF and responses at an individual level. Therefore, the current findings must be interpreted with caution due to these inconsistencies and different outcomes in various studies, alongside the very limited number. Like many developing fields of study, the research on IF highlights the need for more rigorous testing presented in terms of more well-controlled clinical trials. In the future, such efforts should focus on developing comprehensive and well-standardized research protocols; multifaceted populations of women diagnosed with PCOS must be selected for assessment. Longer-term assessments are needed, with more mechanisms revealed. Intermittent fasting may be a modulatory tool for many health parameters, but we must understand this more holistically and nuancedly.

Data availability statement

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

Author contributions

AK: Data curation, Investigation, Software, Writing – original draft, Writing – review & editing. DA: Data curation, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. MF: Conceptualization, Data curation, Investigation, Methodology, Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing.

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The dietary changes during Ramadan and their impact on anthropometry, blood pressure, and metabolic profile

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Background: The effect of Ramadan intermittent fasting (RIF) on the metabolic profile, anthropometry and blood pressure has been investigated in multiple studies. However, it is still unknown to what extent changes in nutrient intakes contribute to these changes.

Methods: This observational study was conducted in London (UK) in 2019. The study collected diverse data from a community-based sample in London before and during/after Ramadan. Collected data included a 3-day food diary (before and during Ramadan), as well as blood samples, anthropometric measurements and blood pressure (before and after Ramadan). The food diary was translated into nutritional data using nutrition software "Nutritics." The changes in nutrient intakes were investigated using a mixed-effects regression model. The impact of adjusting for nutrient intake change was investigated on the absolute difference of metabolites (Nightingale platform), systolic/diastolic blood pressure and anthropometric measures.

Results: The study collected data on food intake before and during Ramadan from 56 participants; the mean age was 44.7 ± 17.3, and 51.8% ($n = 29$) were females. We found a change in the intake of 11 nutritional factors, glucose, fructose, betaine, sugars, sugars as monosaccharide equivalents, lutein/zeaxanthin, starch, starch as monosaccharide equivalents, proline, glutamic acid and lycopene. No changes in quantities or proportions of macronutrients, carbohydrates, protein and fat. Mainly, the changes in diet during Ramadan are characterized by more consumption of sugars (62%, $p < 0.001$) and a lower intake of starch (−21%, $p = 0.012$). The changes in 14 metabolite levels (two glycolysis-related metabolites, one amino acid, two ketone bodies, two triglyceride, six lipoprotein subclasses, and an inflammation marker) after Ramadan were partially associated with some changes in nutrient intakes during Ramadan, especially betaine, fructose, glucose, starches and sugars. The lutein/zeaxanthin intake change explained inversely 14% of systolic blood pressure changes. Moreover, BMI and weight changes were partially explained by changes in intake of fat (7%; 9%), monounsaturated fat (6%; 7%), starch (8%; 9%), and starch as monosaccharide equivalents (8%; 9%) intakes in a direct relationship.

Conclusion: Diet changes during Ramadan were associated partially with the observed changes in the metabolic profile, blood pressure and anthropometry.

This confirms the changes associated with RIF in the metabolic profile, blood pressure and anthropometry are not an absolute physiological response to the diet transition occurring during Ramadan.

KEYWORDS

food intake, nutrients, metabolomics, intermittent fasting, dietary transition

1 Introduction

Ramadan intermittent fasting (RIF) is associated with a sudden drastic change in the dietary behavior of observers. Although observing RIF does not dictate restriction on calorie intake or type of food, previous studies showed that it incurs significant changes in eating habits, patterns, duration, frequency of meals and intakes of energy and macronutrients (1–4). Observers feast after sunset (Iftar) and eat another lighter meal before dusk (Suhur) (5) and more often in groups of family and friends (6). These changes, along with changes in sleep duration and quality (7), circadian rhythm (8) and physical activity (9), affect cardiometabolic risk markers (10) and inflammatory and oxidative stress markers (11).

Understanding these dietary changes during Ramadan is crucial for developing evidence-based guidelines for the public and healthcare professionals. This is particularly important for individuals with metabolic disorders such as diabetes or hypertension who want to practice this ritual safely.

Previous studies have reported inconsistent results with regards to energy intake ranging from an increase (12, 13), to no change (14, 15) or even a reduction (16–18) during Ramadan. Likewise, reported studies are inconsistent on the intake of macronutrients, including fat (3, 14, 19), carbohydrates (13, 17, 19), cholesterol (13, 14), and protein (3, 19). Recently, Abdelrahim et al. conducted a meta-analysis and reported a reduction in total energy intake, carbohydrates, protein and water intakes and no change in fat or protein intakes during Ramadan (4). The previous observational studies often focused on specific ethnic groups or nationalities, potentially skewing the results. Therefore, investigating these changes in a multi-ethnic sample is essential to minimize cultural bias.

Moreover, most previous studies used 24-h dietary recalls (14, 20) and food frequency questionnaires (17, 18) to measure diet for their ease of use, which are subject to recall bias. Food diary is a cheap and convenient real-time recording method that provides a more accurate measure of diet (21).

In addition, former studies have not investigated to what extent the changes in dietary intake during Ramadan explain the subsequent changes in metabolites, systolic/diastolic blood pressure and anthropometric indices. Investigating this will allow more structured nutritional advice to Ramadan observers, especially individuals with chronic diseases. We hypothesize that diet changes during Ramadan are associated with the changes observed after Ramadan in blood pressure, anthropometry and metabolic profile.

This study aims to explore changes in energy, macronutrient and nutritional factors intakes during Ramadan in a multi-ethnic population. It will investigate whether these dietary changes explain the changes in the metabolic profile, blood pressure and anthropometric indices after Ramadan. The findings could contribute to a better understanding of the health impacts of Ramadan fasting.

2 Methods

LORANS is an observational study conducted to observe the effect of RIF on cardiometabolic health in 2019. The study was conducted in five large mosques in London, UK; details of the study are described elsewhere (22). In LORANS, participants were given a 3-day food diary to monitor the change in their dietary habits during Ramadan. The food diary had an introduction illustrating how to describe types/amounts of food and two sections to record food intake 3 days before and 3 days during RIF. An example of 1 day's food intake was given to show the participant how to fill in food intake accurately. The 3 days consisted of one weekend day and two weekdays to ensure we captured a holistic picture of dietary habits. Each food diary was labeled with a unique barcode to identify the participant (Supplementary material 1). In LORANS, we collected diverse data from 146 participants who attended the first visit before Ramadan to participate in the study. Of those, 56 participants (response rate = 38.5%) agreed to fill out the food diary and returned it completed on the second visit after Ramadan.

We used "Nutritics," a nutrition software, to translate the food diaries into nutritional data (23). The platform output provided the intakes of macronutrients and nutritional factors. However, the energy value from each macronutrient was not provided, so some extra calculations were needed. Our estimation of the macronutrients' energy values was based on the fact that 1 g of protein, fat and carbs are 4 kcal, 9 kcal and 4 kcal, respectively (24).

As described previously (22, 25, 26), we measured systolic blood pressure (SBP), diastolic blood pressure (DBP), weight, waist circumference (WC), hip circumference (HC), body mass index (BMI), fat mass (FM), fat percentage (F%), fat-free mass (FFM), and total body water (TBW). Also, we collected two blood samples from LORANS participants a few days before and 8–12 days after Ramadan. Metabolomic profiling was performed using nuclear magnetic resonance (NMR) spectroscopy to assess metabolite changes.

2.1 Statistical analysis

Considering fat as the primary outcome, we calculated that based on a significant level of 0.05 and 80% power, we need to collect before and after Ramadan food intake data from 25 subjects to detect a significant difference of 5%. Using the "lme4" package in R (version 4.1.0), we constructed mixed-effects models adjusted for fixed (age

and sex) and random variables (mosque) to estimate the transition in diet during Ramadan. In addition, we applied a False Discovery Rate (FDR) method to correct for multiple testing. The same test was applied to metabolites for men and women separately to investigate whether the effect of RIF on metabolites varies between men and women and impacts the metabolomic changes upon the observance of RIF. This analysis was performed using the "lmer" package in R. We presented all variables in the form of mean ± SD before and during Ramadan and all results as a mean difference with 95% CI. Results with an FDR-adjusted *p*-value of less than 0.05 were considered statistically significant.

To explore to what extent the changes in diet explain the changes in metabolites, we calculated the absolute differences in nutrients (intake during minus intake before Ramadan) and metabolites (before and after Ramadan) that changed significantly. Afterwards, each metabolite's absolute difference was added to a basic linear regression model as a dependent variable with age, sex, number of fasting days and mosque as independent variables, and the R-squared value was reported. Then, a nutrient was added to the same model to report the change in the R-squared value (compared to the original R-squared value in the basic model), which was interpreted as the proportion of the metabolite's change explained by the change in the nutrient intakes. The same process was repeated for SBP, DBP, weight, WC, HC, BMI, FM, F%, FFM, and TBW as dependent variables that changed after RIF (22, 25).

TABLE 1 Baseline characteristics of LORANS's participants who filled out and returned the food diary ($n = 56$).

Variable	Sub-groups	Value
Age (mean ± SD)	Total	44.7 ± 17.3
	18–40 years (%)	37.5%
	40–60 years (%)	42.8%
	60–80 years (%)	17.9%
	> 80 years (%)	1.8%
Sex (number of females (%))	*n* = 29 (51.8%)	
Ethnic background (%)	South Asian	59%
	Arab	22%
	Other	14%
	Unknown	5%
Marital status (%)	Single	23.2%
	Married/living with a partner	67.9%
	Divorced/separated	3.5%
	Unknown	5.4%
With chronic diseases (%)	Diabetes	8.9%
	Hypertension	19.6%
	Cardiovascular diseases	1.8%
Education (%)	No formal qualification	8.9%
	Secondary school or equivalent	26.8%
	Higher education: College/HNC/HND	17.9%
	Bachelor's degree	26.8%
	Postgraduate degree	14.3%
	Unknown	5.3%
Smoking (%)	Never	83.9%
	Stopped	11.6%
	Occasionally	0.9%
	Yes, most or all days	3.6%

3 Results

The mean ± SD age of the 56 participants who completed the food diaries before and during Ramadan was 44.7 ± 17.3, and 29 (51.8%) of them were females. Table 1 shows the baseline characteristics of this group.

During Ramadan, there were significant changes in the intake of 11 nutritional factors. Table 2 indicates that RIF is mainly characterized by the consumption of more sugars and a lower intake of starch. However, there was no change in the quantities or proportions of macronutrients (Table 3).

We reported significant changes in 78 metabolites in women, while only one metabolite changed in men (Supplementary materials 2, 3). When investigating metabolite changes based on having a chronic disease (with/without a chronic disease), no changes were observed in the two groups' metabolites (Supplementary materials 4, 5).

The key nutrients that were associated with the metabolite changes were lutein/zeaxanthin, lycopene, fiber, fructose, glucose, starch, starch as monosaccharide equivalents, sugars and sugars as monosaccharide equivalents. Notably, the most substantial changes in metabolites, as explained by alterations in nutrient intake, were observed in large high-density lipoprotein (HDL) with 25% explained by alteration in glucose intake followed by.

Starch as monosaccharide (25%), starch (24%), fructose (24%) and betaine (24%). Similarly, 25% of pyruvate change was explained by fructose intake alteration followed by glucose (21%), and sugar (21%). Also, fructose and sugar intake alterations were deemed to explain changes in glutamine by 24 and 24%, respectively (Figure 1).

SBP, DBP, weight, WC, HC, BMI, FM, F%, and TBW were less affected by changes in nutrient intakes. The largest influence was reported for SBP, where 14% of the change was explained by the change in Lutein/Zeaxanthin intake. Additionally, changes in the intake of carbohydrates (and their monosaccharide equivalents), fat, monounsaturated fat, and starch (and their monosaccharide equivalent) were associated with changes in weight and BMI (association range from 5 to 9%) (Figure 2).

4 Discussion

4.1 Principal findings

In a general population from different ethnic backgrounds, RIF was associated with a diet transition in which the intake of 11 dietary variables changed significantly. The main changes were increased sugar consumption and reduced consumption of starch. Despite an observed change of up to 10% in the intake of macronutrients, none of these changes were statistically significant. Observing changes in some nutritional factors and no changes in macronutrients indicates changes in food choices during Ramadan.

TABLE 2 Before and after Ramadan intakes of nutritional factors ($n = 11$), whose intakes significantly changed during Ramadan, besides some essential nutrients whose intakes did not change ($n = 6$).

Nutrient	Intake before Ramadan mean (SD)	Intake during Ramadan mean (SD)	Mean difference (95% CI)	Percentage of change	FDR-adjusted *p*-value
Glucose (g)	8.6 (10)	27.2 (17)	18.6 (14.2 to 23)	216%	<0.001
Fructose (g)	10.2 (11)	28.1 (17)	18 (13.5 to 22.4)	175%	<0.001
Betaine (mg)	0 (0)	0.2 (0)	0.2 (0.15 to 0.26)	NA	<0.001
Sugars (g)	58.7 (29)	95.2 (47)	36.5 (25.7 to 47.3)	62%	<0.001
Sugars as monosaccharide equivalents (g)	57.1 (30)	92 (48)	34.9 (23.4 to 46.4)	61%	<0.001
Lutein/Zeaxanthin (mg)	2.2 (9)	12.9 (11)	−10.7 (−14.2 to −7.2)	486%	<0.001
Starch (g)	116.1 (41)	91.7 (41)	−24.4 (−37.1 to −11.7)	−21%	0.012
Starch as monosaccharide equivalents (g)	121.9 (44)	95 (46)	−26.9 (−41 to −12.8)	−22%	0.012
Proline (mg)	2,740 (1688)	1986 (1098)	−754 (−1,176 to −332)	−28%	0.022
Glutamic acid (mg)	8,379 (5653)	5,890 (3388)	−2,489 (−3951.9 to −1026.3)	−30%	0.032
Lycopene (mg)	830 (2339)	3,886 (7089)	3,056 (1,241 to 4,871)	368%	0.033
Potassium (mg)	2104.4 (725)	2343.5 (820)	239 (64 to 414)	11%	0.087
Sodium (mg)	1,431 (572)	1291.4 (684)	−139 (−330 to 51)	−10%	0.344
Magnesium (mg)	200.1 (71)	210.2 (75)	10.2 (−7.8 to 28.2)	5%	0.488
Cholesterol (mg)	204.5 (165)	181.9 (126)	−22.6 (−63 to 17.4)	−11%	0.488
Fibre (g)	15.9 (6)	16.6 (6)	0.7 (−0.6 to 2)	4%	0.521
Calcium (mg)	529.2 (221)	523.2 (261)	−6 (−75 to 63)	−1%	0.999

TABLE 3 Changes in energy and macronutrients during Ramadan compared to regular diet before Ramadan.

Nutrient	Intake before Ramadan mean (SD)	Intake during Ramadan mean (SD)	Mean difference (95% CI)	Percentage of change	FDR-adjusted *p*-value
Energy (kcal)	1526.7 (487)	1504.2 (577)	4.2 (−115 to 123)	0%	0.973
Carbohydrates					
(g)	176.4 (58)	192 (72)	16.9 (0.3 to 34)	10%	0.214
% of energy	47.1 (9)	51.9 (9)	4.4 (1.3 to 7.5)	9%	0.079
Protein					
(g)	70.8 (34)	62.1 (25)	−7.4 (−16.1 to 1.3)	−10%	0.292
% of energy	18.5 (6)	16.8 (4)	−1.7 (−3.2 to −0.1)	−9%	0.196
Fat					
(g)	59.7 (25)	54.3 (29)	−3.8 (−10.4 to 3)	−6%	0.506
% of energy	34.1 (7)	31.2 (8)	−2.6 (−5 to −0.1)	−8%	0.208
Saturated fat (g)	20.1 (9)	18 (11)	−1.7 (−4.5 to 1.1)	−8%	0.473
Monounsaturated fat (g)	18.5 (9)	16.5 (9)	−1.3 (−3.6 to 0.9)	−7%	0.473
Polyunsaturated fat (g)	8.5 (5)	7.8 (4)	−0.4 (−1.6 to 0.9)	−4%	0.715

The diet adopted during Ramadan could explain a limited part of the changes in the metabolic profile following RIF. Consequently, a greater proportion of the changes in the metabolic profile is assumed to be due to the underlying mechanisms of fasting and other factors such as gut microbiome or lifestyle related changes (e.g., sleep, physical activity). This assumption is in agreement with the reported results in previous studies when adjusting for energy intake did not influence changes in blood pressure or body composition (22, 25). Moreover, the nutrient intakes changes did not explain much of the changes in SBP, DBP, weight, BMI, WC, HC, FM, F% and TBW.

4.2 Changes in metabolites, anthropometry and blood pressure

In LORANS, we investigated earlier changes in metabolites, anthropometry and blood pressure after RIF. We reported significant

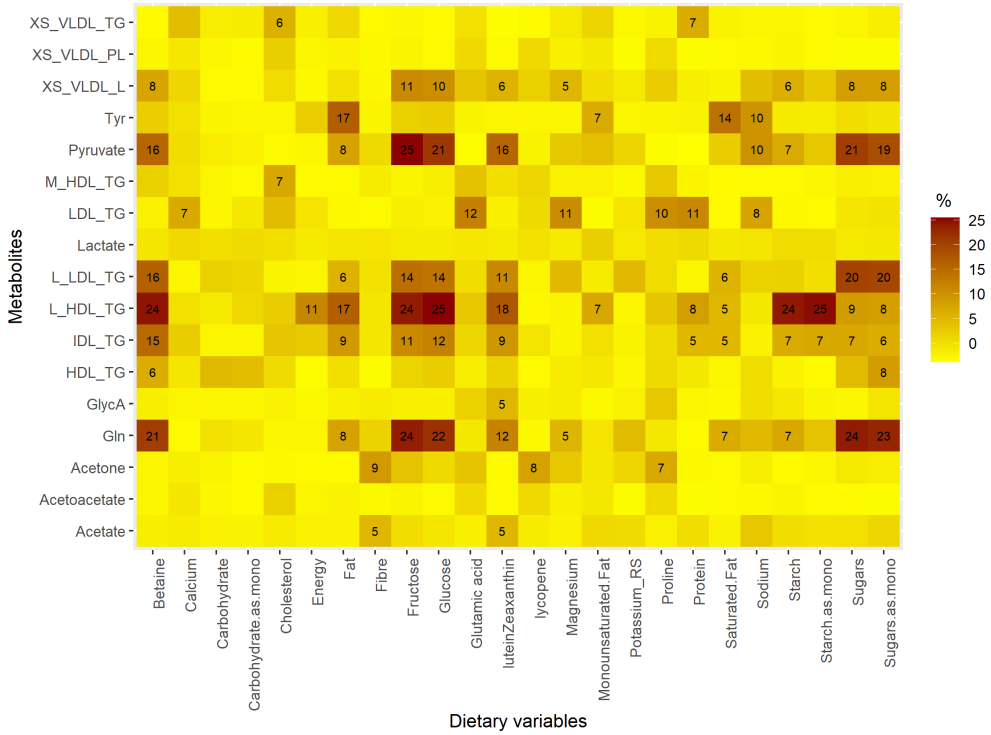

FIGURE 1
The proportions of metabolites explained by changes in intakes of dietary variables. Gln, Glutamine; GlycA, Glycoprotein acetyls; L_HDL_TG, Triglycerides in large HDL; IDL_TG, Triglycerides in IDL; M_HDL_TG, Triglycerides in medium HDL; L_LDL_TG, Triglycerides in large LDL; HDL_TG, Triglycerides in HDL; Tyr, Tyrosine; LDL_TG, Triglycerides in LDL; XS_VLDL_PL, Phospholipids in very small VLDL; XS_VLDL_TG, Triglycerides in very small VLDL. The metabolites are those that changed significantly after Ramadan. The dietary variables are those whose intake changed significantly during Ramadan, macronutrients, energy, potassium, sodium, magnesium, cholesterol, fiber and calcium. Mono, monosaccharide equivalents.

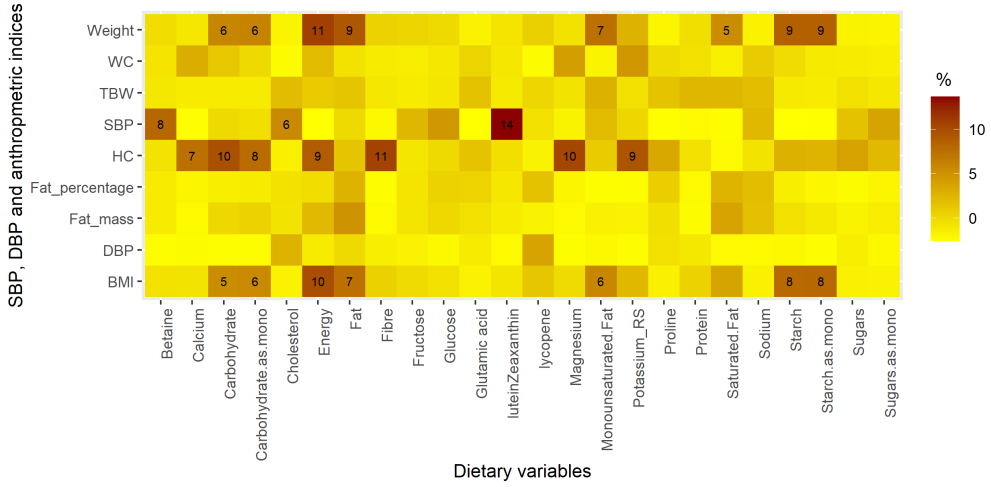

FIGURE 2
The proportions of blood pressure and anthropometric parameters explained by changes in intakes of dietary variables. The dietary variables are those whose intake changed significantly during Ramadan, macronutrients, energy, potassium, sodium, magnesium, cholesterol, fiber and calcium.

changes in 14 metabolite levels (normalized between 0 and 1) after RIF (26), namely lactate, acetate, glycoprotein acetyls, triglycerides in large high-density lipoprotein (HDL), triglycerides in intermediate-density lipoprotein (IDL), triglycerides in medium HDL, triglycerides in large low-density lipoprotein (LDL), triglycerides in HDL, tyrosine, pyruvate, acetone, triglycerides in LDL, phospholipids in very small very-low-density lipoprotein (VLDL), triglycerides in very small VLDL ($\beta = -0.31$, $P = <0.001$; −0.22, <0.001; −0.07, 0.006; −0.06, 0.006; −0.06, 0.007; −0.08, 0.013; −0.05, 0.014; −0.07, 0.019; −0.1, 0.019; −0.09, 0.019; 0.10, 0.019; −0.05, 0.035; −0.05, 0.041; −0.05, 0.041). However, the current study found that changes in metabolites after RIF are sex-dependent. Moreover, in terms of SBP, DBP and

anthropometric measurements, we observed a reduction (22, 25) after RIF in SBP (−7.3, <0.001), DBP (−3.4, <0.001), weight (−1.6, <0.001), body mass index (−0.60, <0.001), waist circumference (−1.95, 0.011), hip circumference (−2.86, <0.001), fat mass (−1.24, <0.001), fat percentage (−1.05, <0.001) and total body water (−1.45, <0.001).

4.3 Comparison to previous studies

Although LORANS is the first study to report the changes in diet during Ramadan in a multi-ethnic group, its results agreed with studies that recruited samples from one ethnic background. In LORANS, the caloric intake was the same before and during Ramadan. This finding is consistent with most previous studies (3, 14, 19, 20, 27). Similarly, the intake and percentage contribution of the three macronutrients (protein, fat and carbohydrates) during Ramadan was the same as before. Likewise, numerous studies observed no change in the protein (1, 14, 17, 19, 20, 27, 28), fat (1, 13, 14, 19, 28) and carbohydrates (3, 19, 20) intake. Still, some studies reported a significant increase or decrease in some of these macronutrients (3, 15, 18, 20). The observed inconsistency could potentially stem from the fact that these studies were done in various countries, given that diet is tightly linked to culture. Nevertheless, two of the former studies were conducted in the same country (Iran) and yet reported inconsistent changes in macronutrients.

In LORANS, the intakes of nutritional factors such as cholesterol, sugars and starch were investigated. In agreement with a former study by Lamri-Senhadji et al. (13), we did not observe changes in cholesterol intake. The increase in sugar consumption by more than 50% in LORANS is in accordance with all former studies that observed higher sugar intake (3, 14, 19). None of the previous studies investigated the change in starch consumption, so we could not directly compare the observed decrease in our research to other studies. However, some foods rich in starch were found to be consumed less during Ramadan in some former studies (14, 18).

Although some former studies (29, 30) had assessed the changes in metabolites and lipids after RIF, the direct comparison of observed metabolic changes in LORANS to previous studies was not possible due to the use of different metabolic profiling methods.

4.4 Explaining changes in the metabolic profile

It is well-established that sugar intake is negatively associated with HDL and positively with total triglycerides (31); however, the increased sugar consumption in this study was associated with a non-significant reduction in HDL and no change in total triglycerides. Also, this cannot be explained by the non-significant drop in total fat intake and the different types of fat (saturated fat, monounsaturated and polyunsaturated). This might be due to the limited statistical power of our study and the effect of change in sugar intake in a month might be smaller than what our study could have detected. Moreover, the change in ketone bodies was almost independent of the changes in daily food intake. This was expected as the increased abundance of ketone bodies is due to compensating for the absence of glucose intake for more than 8 h, regardless of quantity of glucose intake before starting fasting, through converting ketogenic amino acids and fatty acids into ketones (32).

4.5 Strengths and limitations

This study has several strengths. Usin a 3-day food diary, including one weekend day, is a rigorous method to observe the day-to-day variation in food intake across the different days of the week compared to other methods (33). Also, the food diary reduced the risk of recall bias compared to the 24-h dietary recalls, dietary history and food frequency questionnaires. Translating the food diaries into nutrient data using "Nutritics" may have reduced human error in calculating the nutrients. In addition, LORANS is the only study investigating the dietary change during Ramadan in a multi-ethnic sample, giving an overall picture of the changes rather than specific changes in a certain culture. Moreover, the study was community-based, which makes it generalizable. Furthermore, utilizing the collected metabolomics data alongside the dietary data emphasized the magnitude of fasting effect on the metabolic profile apart from the diet adopted during Ramadan.

Also, this study presents some limitations. Although using the 3-day food diary was optimal for this study, it may have discouraged some participants from providing the dietary data due to the time and effort required and led to a low response rate (38.5%). Still, the number of participants who completed the food diary makes it among the largest studies on this topic, especially studies in which a food diary was used to measure diet.

Having a period of 8–12 days between the end of Ramadan and the second visit may have diluted the effect on the metabolic profile. In turn, it may have weakened the explanation of the change in the metabolic profile by diet change. However, reporting a significant difference in 14 metabolites after Ramadan indicates that the effect is still detectable.

5 Concluding comments

RIF is associated with a quick transition in dietary habits. The main changes are the higher consumption of sugars and the lower consumption of starch. However, the changes in the metabolic profile, blood pressure, and anthropometry after RIF are not merely due to the diet adopted during Ramadan.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Imperial College Research Ethics Committee. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

RA-J: Data curation, Formal analysis, Visualization, Writing – original draft. YW: Writing – original draft, Writing – review & editing. PE: Writing – review & editing. KT: Conceptualization,

Supervision, Writing – review & editing. AD: Conceptualization, Methodology, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1394673/full#supplementary-material

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Changes in haptoglobin genotype-based gene expressions upon the observance of dawn-to-dusk intermittent fasting: a prospective cohort study on overweight and obese individuals

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Introduction: Intermittent fasting (IF) has been reported to be involved in ameliorating oxidative stress and lessening the systemic-low grade inflammation that predisposes to chronic diseases. Gene polymorphism is currently a main determining factor for the metabolic responses to different dietary and lifestyle modifications.

Methods: The current study was designed to explore the effect of observing four-week, dawn to dusk IF by participants with obesity on gene expression of the anti-inflammatory *CD163*, oxidative stress, and bioenergetics enzymes (*SOD2*, *Nrf2*, and *TFAM*), as well as metabolic and cellular regulatory genes (*SIRT1* and *SIRT3*). Further, the study aimed to find out how haptoglobin (Hp) polymorphism modulates gene expression of the aforementioned genes and to determine changes in relative gene expressions of the aforementioned six genes based on Hp polymorphism in response to IF. Haptoglobin genotype was determined for the study subjects, and gene expressions were determined using qPCR. Gene expressions were assessed before and at the end of four consecutive weeks, dawn to sunset IF.

Results: The expressions of *CD163*, *SOD*, *NfF2*, and *TFAM* genes have significantly increased at the end of IF. At the same time, *SIRT3* significantly decreased, implying that observing four consecutive weeks of dawn-to-dusk IF may enhance antioxidative stress response and reduce systemic inflammation.

Conclusion: Participants with genotypes Hp2-1 and Hp2-2 revealed upregulation of the antioxidant genes in response to the metabolic stress induced by IF compared with Hp1-1, implying that Hp polymorphism plays a key role in shaping the body's response to dietary modifications such as fasting.

KEYWORDS

calorie restriction, time-restricted eating, oxidative stress, inflammation, nutritional genomics, Gene polymorphisim

Introduction

Obesity is a significant global health burden that adversely affects human health due to its numerous disease complications (1). Many of these complications are driven by obesity-induced oxidative stress (OS) and low-grade systemic inflammation, including insulin resistance, diabetes, infertility, asthma, and liver disease (2).

Intermittent fasting (IF) has grasped significant attention in recent years as a potential strategy for weight management and metabolic health improvement. However, numerous studies have explored the effects of IF on metabolic parameters, which include reducing the risk of obesity-related cardiometabolic diseases, lowering oxidative stress, improving lipid profiles, inhibiting atherosclerosis, and mitigating inflammation (3, 4). However, limited research has investigated the genetic and molecular mechanisms underlying these effects, particularly in the context of specific genetic variations.

Among the various forms of IF, Ramadan intermittent fasting (RIF) involves abstaining from food and drink from dawn to sunset. Observed by about 1.5 billion Muslims out of more than two billion, RIF mandates complete abstention during this period (5, 6). This unique form of fasting has provided researchers with a natural model to study the physiological and molecular impacts of IF on human health. Numerous studies have examined the effects of observing RIF on different aspects related to obesity and metabolism, including body weight (7) and body fatness (8), with particular attention toward visceral adiposity (9), OS and inflammation markers (10–12), glucometabolic regulation (13), liver disease, metabolic syndrome and cardiometabolic risk factors (7, 14), circadian rhythm and eating regulation hormones (15), and sleep quality (16).

Haptoglobin (Hp) is a plasma protein primarily known for its role in binding free hemoglobin (Hb) and preventing oxidative damage. The Hp gene exhibits genetic polymorphism, resulting in three primary genotypes: Hp1-1, Hp2-1, and Hp2-2 (17). These genotypes differ in their biochemical properties and functional capacities, which may influence individual responses to metabolic stress and oxidative challenges (17). The variation in the Hp genotype has been implicated in various health conditions, including cardiovascular diseases, diabetes, and inflammatory disorders (18–20). Compared to Hp2-1 and Hp2-2, which have a lower Hp-Hb complex stability, the Hp2-2 genotype is associated with an increased OS and proinflammatory response, making it a risk factor for inflammation (18, 19, 21). People with different Hp genotypes have been shown to respond differently to dietary modifications such as IF, where Hp2-2 individuals had a higher anti-inflammatory response than Hp2-1 and Hp1-1 individuals upon the observance of RIF (22). Thoroughly in our previous study, we reported that 4 weeks of dawn-to-dusk-RIF was associated with significantly altered anthropometric, metabolic, and inflammatory markers, with variable responses observed based on Hp polymorphisms. According to our findings, RIF is associated with reduced low-grade systemic inflammation, enhanced anti-inflammatory markers, and ameliorated obesity-related health conditions (22).

With the rising interest in the effects of IF on weight regulation and overall health and the integration between dietary modifications and gene expressions as part of the study of nutritional genomics, it is crucial to examine how individuals with different Hp genotypes respond to dietary modifications like IF. Additionally, it is important to explore how IF influences the expression of genes responsible for inflammation, OS, and cellular regulation and metabolism. Therefore, the primary objective of this study is to examine how gene expressions change upon the observance of a four-week, dawn-to-sunset RIF among overweight/obese participants with different Hp genotypes. Specifically, the study investigates how Hp modulates the expression of genes related to inflammation and OS, such as *CD163*, *SOD2*, *Nrf2*, and *TFAM*, and metabolic and cellular regulatory genes like *SIRT1* and *SIRT3*. We hypothesize that overweight/obese participants with different Hp genotypes will respond differently to RIF and that the six gene expressions (*CD163*, *SOD2*, *Nrf2*, *TFAM*, *SIRT1*, and *SIRT3*) will vary accordingly.

Our previous work examined general changes in the expression of five genes (*SOD2*, *Nrf2*, *TFAM*, *SIRT1*, and *SIRT3*) during RIF, regardless of Hp gene polymorphism (22). Hence, the novelty of this current study lies in analyzing the expression of these six genes based on Hp gene polymorphism, thereby demonstrating how gene expressions respond differently to dietary modifications like IF among overweight/obese individuals according to their Hp genetic profile.

In a large scope, the study focuses on examining the interplay between genetic predisposition and environmental factors, such as dietary patterns and fasting, in influencing metabolic health outcomes. The investigation of Hp genotype-based gene expressions during IF can provide insights into the molecular mechanisms driving the metabolic benefits of this dietary practice. By examining gene expression profiles across different Hp genotypes, this study aims to elucidate the genotype-specific molecular pathways that mediate the beneficial effects of IF. This research contributes to the development of our understanding of gene-nutrient interactions in the context of the nutritional genomics field of study.

Methods

Study design

A prospective cohort design was employed in this study to examine the variations in OS, cell regulatory, and metabolic gene expression resulting from Ramadan intermittent fasting (RIF) among participants with overweight/obesity and three distinct haptoglobin (Hp) genotypes. The study was conducted over two Ramadan fasting months in two consecutive years, specifically during May and June of 2017 and 2018. Data collection occurred at two different time points: baseline, which took place 2 to 7 days before the start of RIF, and at the

end of the fourth week of the Ramadan month, following 28 to 30 consecutive days of dawn-to-sunset RIF. Throughout the fasting period, which lasted approximately 15 h per day, participants refrained from oral intake, including both food and water, from dawn until sunset. No specific dietary or physical activity guidelines were given to the participants at any stage of the study. In accordance with Islamic fasting laws, menstruating women were exempted from Ramadan fasting during their menstrual period, resulting in a shorter fasting period for female participants (23–25 days) compared to male participants (28–30 days).

Participant selection

A convenience sampling method was applied in this study. Upon announcing the research through social media, institutional emails, and personal communications, individuals who expressed their interest in observing Ramadan fasting and visited the University Hospital Sharjah (UHS)/UAE for screening were recruited. The study adhered to the principles of the Declaration of Helsinki and received approval from the UHS Research Ethics Committee (Reference No. REC-16-05-11-01). Each participant was given an information sheet detailing the research plan, objectives, and requirements for involvement, and they provided signed informed consent before participating. To be eligible for the study, male or female participants had to be overweight/obese (body mass index, BMI >25 kg/m^2), willing to fast during Ramadan, and willing to take part in the research. Data collection involved a self-report questionnaire covering the medical history and demographic details, which were gathered through face-to-face interviews conducted by trained research assistants. Exclusion criteria encompassed a history of metabolic syndrome, diabetes, or cardiovascular disease; neuro-psychiatric patients on regular medications; individuals following a weight-reducing diet; those who had undergone bariatric surgery within the last 9 months before starting RIF; and pregnant or premenopausal women.

As an observational, non-interventional, or experimental study, it is acceptable to use a self-control, pre-post design to demonstrate the effects of dietary or lifestyle modifications on specific health outcomes. In the case of the Ramadan fasting model, having a non-fasting control group from the same ethnic population during this short period of the Holy month of Ramadan is very challenging. Being a religious ritual that is strictly observed and spiritually significant for the Muslim community, it is very challenging to secure non-fasting individuals. Even non-religious people often adhere to fasting during Ramadan out of empathy and solidarity with the fasting community. This challenge is further emphasized in gene testing studies, where ethnic background plays a key role in determining gene expression levels (23).

Blood samples collection

Blood samples were obtained from the participants after an 8–10 h fasting period at both time intervals. A total of 10 mL of blood was collected during each of the two-time intervals. To maintain consistency in the duration of fasting and eliminate the impact of timing and dietary intake on measured biochemical parameters, the blood samples were collected between 11 am and 1 pm on both occasions. After collection, the blood samples were split into two aliquots. One aliquot was subjected to centrifugation at 2,500 rpm for 15 min within 1 h of collection. The resulting serum was then divided into coded aliquots and stored at −80°C until it was ready for use in biochemical analyses. The second aliquot was reserved for RNA extraction.

Haptoglobin genotype determination

Hp genotype distribution was determined using 8% vertical polyacrylamide gel electrophoresis (24). Briefly, the Hp-hemoglobin (Hp-Hb) complex solution was prepared by adding 5 mL of 10% Hb A to 40 mL of the sample buffer (50% glycerol), followed by 10 mL of the sample serum. Electrophoresis was then run at room temperature for 4 h at 130 volts. The gel was removed from the apparatus and immersed in benzidine solution for 30 min to visualize the Hp bands. The benzidine solution was prepared by dissolving 0.2 g of benzidine in 250 mL boiling water. Just before staining, 1.5 mL glacial acetic acid and 0.6 mL H_2O_2 were added to the benzidine solution (25).

Quantitative real-time PCR

First, blood RNA extraction was performed using the Norgen Biotek Total RNA Purification kit as per manufacturer instructions. The total RNA yield was assessed spectrophotometrically using (Nanodrop 2000, Thermo Scientific) by measuring the A260/A280 ratio to check the purity, where only the samples with a 1.6 ratio and above were chosen. Norgen's TruScript™ First Strand cDNA Synthesis Kit has been used for our sample to get a cDNA complementary strand, as per the manufacturer's instructions. qPCR reaction was performed at the volume of 20 µL, including 100 ng of cDNA with QuantiTect SYBR Green PCR mixture (Qiagen, Germany). The cycling conditions included initial activation of the polymerase for 15 min at 95°C, followed by 45 cycles of 15 s denaturation at 94°C, annealed at 55°C for 30 s, followed by extension at 72°C for 30 s. The primers used are listed in Table 1. For each sample, the expression of each gene was normalized to the housekeeping (GAPDH) at the same time point. The data were compared with a pool of six healthy controls with normal BMI (18.5–24.9 kg/m^2) at each time point. The relative expression was shown as fold change according to Livak and Schmittgen (26) and presented as mean and standard deviation (SD), as described elsewhere (27).

Statistical analysis

Statistical analyses were performed using SPSS 29 (IBM, Armonk, NY, United States). The mean and standard deviation (SD) were calculated for continuous variables and the percentage (%) for categorical variables. The normality distribution of the data was tested using Kolmogorov Smirnov. The change was calculated as [the endpoint value − baseline value], and the % change was calculated as [(endpoint value − baseline value) /baseline value] × 100%. The *p*-value for the trend was analyzed using a linear trend test. The Wilcoxon signed-rank test for paired samples was used to compare changes within groups over

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TABLE 1 Forward and reverse primers for the main six genes tested in the study.

Gene	Primer	References
CD163	Forward: 5′-CCAGTCCCAAACACTGTCCT-3′	(46)
	Reverse: 5′-ATGCCAGTGAGCTTCCCGTTCAGC-3′	
Nrf2	Forward: 5′-ATGGATTTGATTGACATACTTT	(47)
	Reverse: 5′-ACTGAGCCTGATTAGTAGCAAT	
SOD2	Forward: 5′-GCTCCGGTTTTGGGGTATCTG	(48)
	Reverse: 5′-GCGTTGATGTGAGGTTCCAG	
TFAM	Forward: 5′-ATGGCGTTTCTCCGAAGCAT	(49)
	Reverse: 5′-CAGATGAAAACCACCTCGGTAA	
SIRT1	Forward: 5′GCCTCACATGCAAGCTCTAGTGAC	(50)
	Reverse: 5′-TTCGAGGATCTGTGCCAATCATAA	
SIRT3	Forward: 5′-ACCCAGTGGCATTCCAGAC	(51)
	Reverse: 5′-GGCTTGGGGTTGTGAAAGAAG	
GAPDH	Forward: 5′-CCAGGTGGTCTCCTCTGACTTC	(52)
	Reverse: 5′-TCATACCAGGAAATGAGCTTGACA	

the time course of the study. Changes in variables within groups are presented as adjusted *p*-values derived from a general linear model after adjusting for the baseline age, sex, waist circumference, and total caloric intake. The significance was considered at *p*-values <0.05.

Results

Basic demographic, anthropometric, and inflammatory characteristics

A total of 114 overweight and obese participants (75 males and 39 females, 38.7 ± 11.7 years) with a mean BMI of 30.4 ± 5.09 kg/m^2 were recruited in this observational study. Changes in anthropometric, metabolic, and inflammatory markers before and at the end of RIF are shown in our previously published work of Madkour et al. (22). According to the data in that work, the patients experienced a significant average weight loss of 1.6% of their baseline body weight. Additionally, there were significant decreases in other anthropometric measurements, including body mass index, body fat percent, fat mass, fat-free mass, muscle mass, visceral fat surface area, waist circumference, hip circumference, and waist: hip ratio at the end of the fasting period compared to their levels before fasting. Furthermore, there were notable decreases in serum triglycerides, total cholesterol, and LDL levels. At the same time, there was a significant increase in serum HDL levels at the conclusion of the fasting month for the entire population. At the end of the month of fasting, the study individuals showed a notable decrease in the levels of serum Hp, IL-6, and TNF-α compared to their levels before fasting. At the end of Ramadan, the study participants exhibited a considerable increase in serum CD163 and IL-10 levels. These results also revealed that Hp2-2 was the most frequently abundant genotype among the study participants, representing 48.24% ($n = 55$), followed by Hp 2-1 (46.5%, $n = 53$), and by Hp 1-1 (5.26%, $n = 6$).

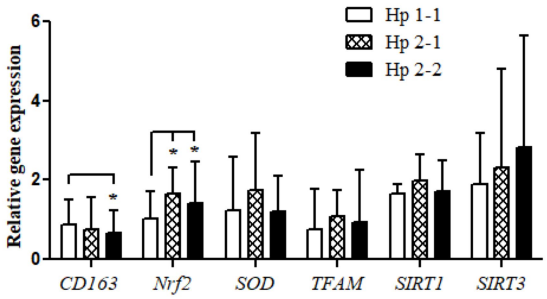

FIGURE 1
Relative gene expressions for the *CD163*, antioxidant (*Nrf2*, *SOD2*, and *TFAM*), and metabolic and cellular regulatory (*SIRT1* and *SIRT3*) genes according to the Hp genotype for the study subjects. *$p < 0.05$.

Relative gene expression according to the haptoglobin polymorphism

Relative gene expression of CD163, antioxidant enzymes (*Nrf2*, *SOD2*, and *TFAM*), and metabolic and cellular regulatory (*SIRT1* and *SIRT3*) genes in overweight and obese participants in comparison to counterpart gene expressions for controls based on their Hp genotype distribution as depicted in Figure 1. The gene expression of CD163 represents a trend of value decrements from Hp1-1 to Hp2-1 to Hp2-2 with a significant difference between Hp2-2 compared to Hp1-1. Also, at the same pattern, the results show a higher significance of *Nrf2* gene expression in Hp2-1 and Hp2-2 in comparison to Hp1-1 individuals with overweight/obesity. In contrast, no significant differences were observed in gene expressions between Hp1-1, Hp2-1, and Hp2-2 in the antioxidant enzymes (*SOD2* and *TFAM*) and metabolic and cellular regulatory genes (*SIRT1* and *SIRT3*).

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Changes in relative gene expressions before and after IF

Relative gene expressions in participants with overweight and obese for *CD163*, antioxidant (*SOD2*, *Nrf2*, and *TFAM*), and metabolic and cellular regulatory (*SIRT1* and *SIRT3*) genes assessed for study participants are shown in Figure 2. The results of the expressions showed a highly significant increase in the *CD163*, *Nrf2*, *SOD2*, and *TFAM* genes in the study participants at the end of Ramadan compared with the pre-fasting levels. In contrast, the relative gene expression of the metabolic and cellular regulatory *SIRT3* gene showed a significant reduction in the study participants at the end of RIF in comparison with the levels before RIF. Moreover, *SIRT1* gene expression represented a clear decremental trend at the end of RIF in comparison to the expressions before RIF, but without a statistically significant value (Figure 2).

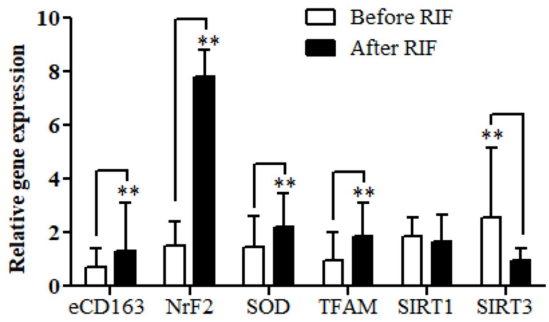

FIGURE 2
Changes in relative gene expressions for *CD163*, antioxidant (*SOD2*, *Nrf2*, and *TFAM*), and metabolic and cellular regulatory (*SIRT1* and *SIRT3*) genes before and after observing RIF month. $^{**}p < 0.001$.

Changes in relative gene expression before and after RIF according to Hp genotype

Relative gene expression in obese participants of *CD163*, antioxidant (*SOD2*, *Nrf2*, and *TFAM*), and metabolic and cellular regulatory (*SIRT1* and *SIRT3*) genes in response to RIF-based Hp genotype are resented in Table 2. A highly significant increase in *CD163* gene expression for those participants with Hp2-2, with no such significant differences was observed in participants with Hp1-1 and Hp2-1 polymorphism genotypes at the end of Ramadan fasting in comparison with the pre-fasting expression levels (Figure 3). Besides, the mean difference in the expression of the *CD163* gene showed a significant increase between Hp polymorphism Hp2-2 in comparison with Hp1-1 groups. Likewise, *Nrf2* gene expression showed significant increases in participants with Hp1-1, Hp2-1, and Hp2-2 genotypes at the end of RIF in contrast with the pre-fasting levels (Figure 4). Also, the mean of difference for *Nrf2* gene expression between the Hp polymorphism genotype revealed a trend of increment associated with the expression through Hp1-1, Hp 2-1, and Hp 2-2, respectively, but without significant changes.

TABLE 2 Changes in relative gene expressions before and after RIF based on Hp genotype.

Gene	Hp1-1 (n = 6)		Hp2-1 (n = 53)		Hp2-2 (n = 55)	
	Before RIF	End of RIF	Before RIF	End of RIF	Before RIF	End of RIF
CD163	0.88 ± 0.66	0.60 ± 0.41	0.77 ± 0.80	0.99 ± 0.92	0.67 ± 0.57	1.75 ± 2.36*
Nrf2	1.04 ± 0.68	5.46 ± 3.31*	1.67 ± 0.68	5.52 ± 5.88*	1.44 ± 1.04	9.92 ± 13.56*
SOD2	1.23 ± 1.39	0.71 ± 0.01	1.74 ± 1.43	2.04 ± 1.68	1.21 ± 0.92	2.49 ± 2.24*
TFAM	0.76 ± 1.04	1.57 ± 1.55	1.07 ± 0.67	1.73 ± 1.05*	0.93 ± 1.33	2.01 ± 1.47*
SIRT1	1.66 ± 0.26	1.53 ± 0.16	1.99 ± 0.66	1.67 ± 0.90	1.73 ± 0.77	1.64 ± 1.25
SIRT3	1.91 ± 1.29	0.87 ± 0.56	2.32 ± 2.51	1.01 ± 0.44*	2.85 ± 2.80	0.97 ± 0.44*

CD163, Cluster of Differentiation-163 gene; *Nrf2*, nuclear factor Erythroid 2–related factor 2; *SOD2*, superoxide dismutase 2; *TFAM*, Mitochondrial transcription factor A; *SIRT1*, silent information regulator sirtuin 1; *SIRT3*, NAD-dependent deacetylase sirtuin-3. *Indicates significance.

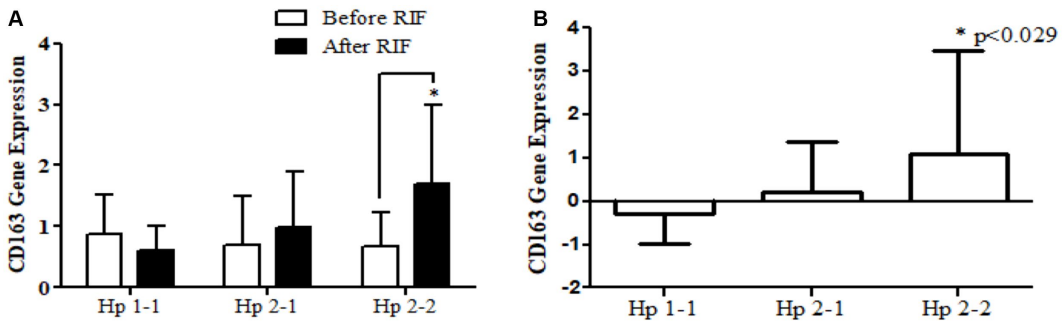

FIGURE 3
(A) Changes in CD163 gene expression before and after RIF based on Hp genotype. **(B)** Mean of differences in CD163 gene expressions before and after RIF based on Hp genotype. $^{*}p < 0.05$.

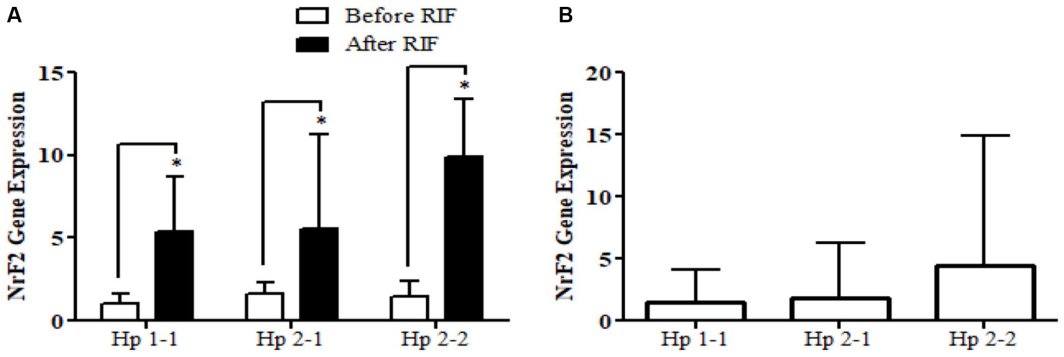

FIGURE 4
(A) Changes in *Nrf2* gene expression before and after RIF based on Hp genotype. **(B)** Mean of differences in *Nrf2* gene expressions before and after RIF based on Hp genotype.

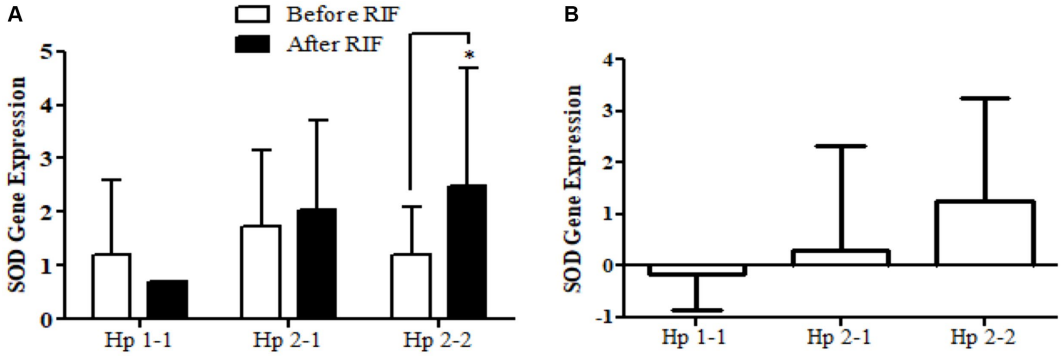

FIGURE 5
(A) Changes in *SOD2* gene expression before and after RIF based on Hp genotype. **(B)** Mean of differences in *SOD2* gene expressions before and after RIF based on Hp genotype.

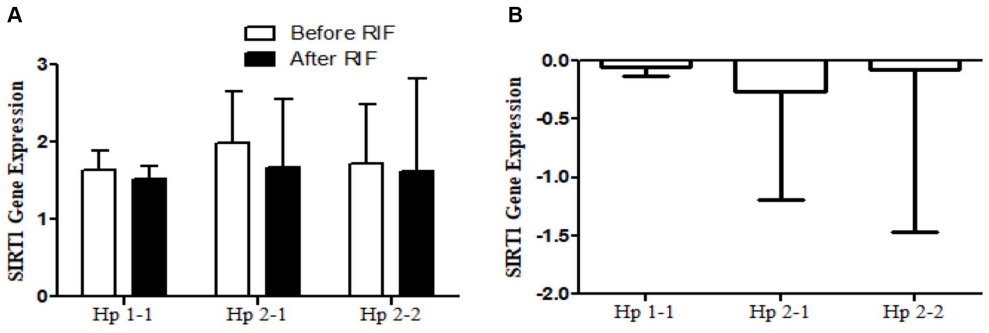

FIGURE 6
(A) Changes in *SIRT1* gene expressions before and after RIF based on Hp genotype. **(B)** Mean of differences in *SIRT1* gene expressions before and after RIF based on Hp genotype.

Similarly, the *SOD2* gene expression revealed a significant increase in participants with the Hp2-2 polymorphism genotype, not the other two genotypes (Figure 5). Further, the means of differences for *SOD2* gene expressions for the three polymorphism genotypes Hp showed a trend of increase in Hp1-1, Hp2-1, and Hp2-2 but without significant changes. Regarding *TFAM* gene expressions, Figure 6 depicts significant increases for Hp2-1 and Hp2-2 genotypes at the end of RIF in comparison with the levels of expression before. Alongside, the mean of difference for the expression of the *TFAM* gene showed a clear trend of increment associated with the values of the expression through Hp1-1, Hp2-1, and Hp2-2, respectively, but without significant changes (Figure 7).

The results show no significance for *SIRT1* gene expression values in Hp1-1, Hp2-1, and Hp2-2 genotypes as individual groups after RIF

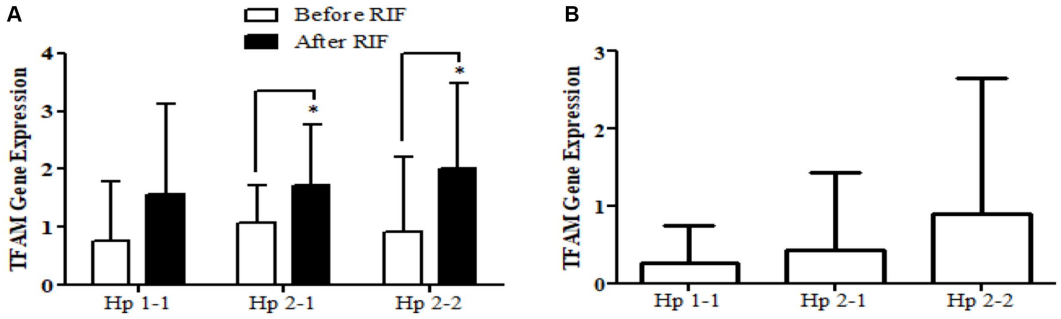

FIGURE 7
(A) Changes in *TFAM* gene expressions before and after RIF based on Hp genotype. **(B)** Mean of differences in *TFAM* gene expressions before and after RIF based on Hp genotype.

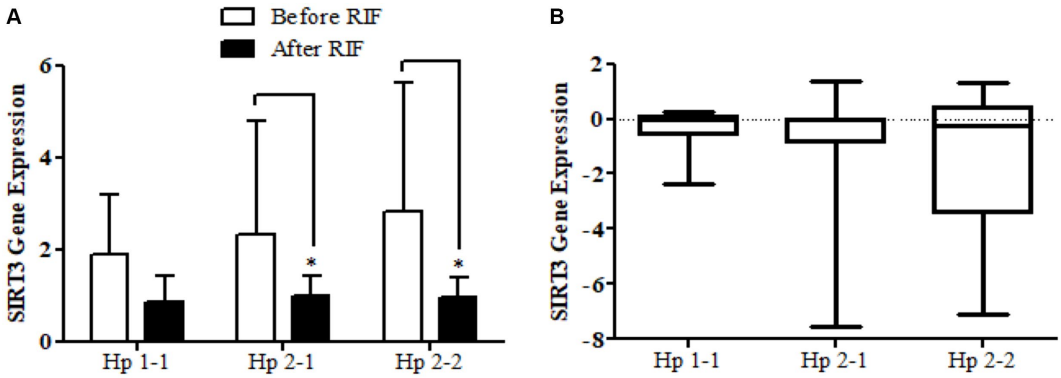

FIGURE 8
(A) Changes in *SIRT3* gene expressions before and after RIF based on Hp genotype. **(B)** Mean of differences in *SIRT3* gene expressions before and after RIF based on Hp genotype.

in comparison to values before RIF (Figure 6). Also, with no significant values for *SIRT1* gene expression in the mean of the difference between Hp polymorphism genotypes. Unlike, the result expressed significantly lower values in *SIRT3* gene expression for Hp 2-1 and Hp2-2 genotypes and no significant differences in Hp1-1 individual groups after RIF in corresponding to values before RIF (Figure 8). Also, the *SIRT3* gene expression means of difference values between Hp polymorphism genotypes showed no significant differences (Figure 8).

Discussion

This groundbreaking research investigates for the first time the changes in genetic expressions implicated in the regulation of inflammation and antioxidant defense enzyme system (*CD163*, *TFAM*, *SOD2*, and *Nrf2*), as well as metabolic and cellular regulation (*SIRT1* and *SIRT3*). Notably, our study showed that *CD163* gene expression levels were significantly upregulated after RIF, which supports our hypothesis that RIF had a positive effect in modulating pro-and anti-inflammatory markers. However, there may be other unexamined factors that have an impact on these factors during Ramadan, such as changes in circadian rhythm and physical activity, which may be markers, as reported in non-fasting research (28). Moreover, we report that key antioxidant defense system-controlling genes (*SOD2*, *TFAM*, and *Nrf2*) exhibited significantly elevated expression, highlighting their role in mitigating OS during fasting-induced physiological stress. This gene expression pattern aligns with findings from a meta-analysis showing decreased inflammatory and OS markers upon the observance of RIF (10). The significance of *SOD2* in counteracting the damaging effect of reactive oxygen species generated by mitochondria upon cellular respiration was confirmed in the present study. Moreover, no significant correlations were found between the genetic expressions of any of the five tested genes and the independent variables (sex, waist circumference, and BMI) except for *SOD2* and caloric intake. The *SOD2* expression was significant ($p < 0.05$) and directly associated with increased caloric intake (>2,000 kcal/day vs. <2,000 kcal per day) among fasting participants during Ramadan. The upregulation of the antioxidant defense enzyme genes (*TFAM*, *SOD2*, and *Nrf2*) augmented the anti-inflammatory status of fasting individuals.

Nrf2 has emerged nowadays as a pivotal link between managing antioxidant gene expression during stress response and cell survival. It regulates the expression of genes encoding antioxidant enzymes, detoxification enzymes, and other cytoprotective proteins. *Nrf2* activity is influenced by various factors, including fasting and dietary components. Intermittent fasting can activate *Nrf2*, leading to

increased expression of antioxidant enzymes like SOD2, which helps mitigate oxidative damage (29, 30). Therefore, it is reasonable that *SOD2* is overexpressed in parallel with the higher expression of the *Nrf2* gene following RIF, which may be explained by the direct effect of *Nrf2* on *SOD2* expression and activity.

While the overexpression of antioxidative stress genes during RIF might raise concerns about increased ROS production, Ristow and Schmeisser (31) defended this argument by reporting that several longevity-promoting modifications, such as IF and caloric restriction, may converge by causing activation of mitochondrial oxygen consumption to promote the increased formation of ROS. These dietary interventions may serve as molecular signals to exert downstream impacts that ultimately trigger endogenous defense mechanisms, which culminate in improved stress resistance and longevity, an adaptive response called mitohormesis or mitochondrial hormesis. Moreover, RIF increases *SOD2* expression, enhancing the cell's ability to cope with OS. *SIRT1* and *SIRT3*, as part of the Sirtuins family of NAD^+-dependent protein deacetylases, play key roles in cellular metabolism, stress response, and longevity.

We found that *SIRT1* gene expression decreased slightly after RIF, while *SIRT3* gene expression significantly decreased following RIF. The expression of Sirtuins is enhanced upon acute/chronic inflammation. Our findings may explain the lower expression of *SIRT1* and *SIRT3* upon RIF due to the modulation of inflammatory markers, as anti-inflammatory markers were improved, as reported in our previous study. In the context of this study, *SIRT3*, the primary mitochondrial deacetylase enzyme, orchestrates fatty acid breakdown during prolonged fasting. While *SIRT1* expression remained unchanged, *SIRT3* showed a significant decrease post-RIF. These results could be explained by the lack of significant differences in fat intake and total energy between baseline and post-Ramadan, alongside excessive simple sugar intake throughout feeding night hours of Ramadan month, as shown in the dietary intake of the present study (data not shown). Several studies have reported that *SIRT1* and *SIRT3* were overexpressed following excessive caloric restriction and prolonged fasting (32, 33), which is unlike in RIF where it involves 12–17 fasting hours/day without any intake (including water), with the remainder of the night hours available for eating without restriction (5).

SIRT3 gene expression is acutely responsive to a cell's primary nutrient availability. Caloric restriction, exercise, and fasting enhance SIRT3 expression in diverse tissues (34–36). Conversely, another study indicated reduced SIRT3 during skeletal muscle fasting (37), contrasting with reports of decreased SIRT3 in high-fat-fed rodents and humans with metabolic syndrome (38–40). Our study notes increased carbohydrate, total sugar, and total fat consumption during Ramadan night hours, potentially explaining the lowered SIRT3 gene expression during Ramadan fasting.

To our knowledge, this is the first study to investigate the role of the Hp genotype (Hp1-1, Hp2-1, and Hp2-2) in *CD163* gene expression, antioxidants enzymes including (*Nrf2*, *SOD2*, and *TFAM*), and cellular regulatory genes (*SIRT1* and *SIRT3*) in response to RIF. Our results showed a significant enhancement of CD163 gene expression among Hp2-2 individuals compared with Hp2-1 and Hp1-1 individuals. These results are confirmed by using the mean of difference statistical methods, which shows a significant response of Hp2-2 to RIF compared to Hp1-1 and Hp2-1. This may explain the marked improvement in action and response to RIF among individuals with obesity, where Hp2-2 individuals had a stronger reaction and secreted more anti-inflammatory markers in our previous study (22). Additionally, we previously showed that IL-10 has significantly increased after RIF. This could be correlated with another study, which reported that CD163 expression is controlled and upregulated considerably by IL-10. They also noted that CD163 has an anti-inflammatory effect by inhibiting phorbol ester-induced human T-lymphocyte activation, resulting in the weakening of the immune response to the inflammatory mediator (41). Another explanation for how high levels of CD163 modulate the anti-inflammatory effect is by directly inducing intracellular signaling that leads to the secretion of anti-inflammatory cytokines. Second and perhaps even more important, the CD163-mediated delivery of hemoglobin to the macrophage may trigger an anti-inflammatory response because heme metabolites have potent anti-inflammatory effects (42). These findings demonstrated the benefits of RIF in modulating and improving the systemic inflammation of individuals with obesity, especially those with the Hp2-2 genotype.

Similarly, the antioxidant enzyme *Nrf2* gene expression demonstrated a significant increment in individuals with obesity in the Hp1-1, Hp2-1, and Hp2-2 groups after RIF compared with before RIF; the values were more than five-fold in Hp1-1 and Hp2-1 and more than eight-fold in Hp2-2. In addition, the mean difference for *Nrf2* gene expression between Hp genotypes revealed an insignificant trend of increment associated with Hp1-1, Hp2-1, and Hp2-2. Similarly, the antioxidant enzyme *SOD2* gene expression revealed significant enhancement in the Hp2-1 and Hp2-2 groups following RIF. For *Nrf2*, the mean differences in *SOD2* and *TFAM* between Hp genotype groups showed an important trend of increment in Hp1-1, Hp2-1, and Hp2-2, in that order. These results support the hypothesis of a previous study that suggested that the genotype of Hp has a crucial role in modulating the oxidative-antioxidative status in both obesity and diabetes (43) and reveal the positive impact/response of RIF based on the inflammatory status, which significantly elevated in the higher proinflammatory genotype Hp2-2 in order to modulate the inflammatory response. In another large-scale research, where they studied the levels of many antioxidant enzymes, including SOD, in 165 diabetic patients, they reported that diabetic patients with Hp2-2 genotype have lower levels of SOD compared to Hp2-1 and Hp1-1 (25). We suggest that fasting could reverse this and rather lead to increased levels of antioxidants like SOD enzyme in diabetic patients. In contrast, we observed significantly lower values in *SIRT3* gene expression for the Hp2-1 and Hp2-2 genotypes and no significant differences for Hp1-1 after RIF compared with before RIF. No significant difference was observed for *SIRT1* in any Hp genotype in response to RIF.

We can claim that this is the first study that examined the changes in the gene expressions of *Nrf2*, *SOD2*, *TFAM*, *SIRT1*, and *SIRT3* in the context of IF research and based on the Hp genotype. This uniqueness arises from the fact that RIF is the only dry and diurnal fasting model among the different IF models. While IF is known to trigger various cellular pathways that can impact health and longevity, these mechanisms are regulated partly by key players at the molecular and cellular pathways, which are *Nrf2*, *TFAM*, *SOD2*, *SIRT1*, and *SIRT3*. These bioactive molecules, in summary, are key players in cellular responses to IF. Their interplay helps regulate OS, mitochondrial function, and cellular metabolism, contributing to the health-promoting effects of IF.

A few limitations should be considered when interpreting the findings of the current study. First, causality cannot be inferred due to the observational prospective design and the lack of a control group. Undetected confounding factors could contribute to the downregulation

or upregulation of the tested genes during RIF. Further, changes in circadian rhythm and sleep patterns, known to affect gene expression, are among the potential factors influencing these changes (44). Although physical exercise levels did not significantly change during Ramadan compared to the pre-fasting stage, physical activity could still have a substantial impact, and future studies should incorporate objective measurements of exercise levels. Further, seasonal variations should be considered in future research, as the month of Ramadan spans different solar seasons, which are known to influence gene expressions (45). Lastly, the findings from the current work cannot be generalized to other ethnicities or the general population. Ethnic variations should be considered and examined in the context of the effects of dawn-to-dusk intermittent fasting on various health outcomes.

Conclusion

Our findings suggest that the observance of 4 weeks of dawn-to-dusk IF during Ramadan could significantly alter the expression of genes associated with inflammation and OS regulation in individuals with obesity. Specifically, the Hp2-1 and Hp2-2 genotypes of the Hp polymorphism demonstrated a more pronounced antioxidative and anti-inflammatory response to the RIF model compared to the Hp1-1 genotype. Consequently, a four-week period of dawn-to-dusk IF during Ramadan may enhance health outcomes in obese individuals by modulating and improving their oxidative and inflammatory mechanisms. These results underscore the potential health benefits of RIF and its role in managing obesity-related inflammation and oxidative stress.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by University of Sharjah Research Ethics Committee. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

MM: Conceptualization, Investigation, Data curation, Methodology, Writing – original draft, Formal analysis, Software. RH: Conceptualization, Project administration, Supervision, Writing – review & editing. NS: Writing – review & editing. SA: Conceptualization, Writing – review & editing. NF: Data curation, Methodology, Writing – review & editing. DA: Data curation, Formal analysis, Software, Writing – review & editing. FA: Data curation, Writing – review & editing. JT: Writing – review & editing. MF: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Software, Supervision, Validation, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Ramadan fasting and exercise combination therapy: A novel approach for osteoporosis prevention in ovariectomized rats

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Background: Osteoporosis is a chronic bone metabolic disease that affects millions of people worldwide, particularly the elderly and postmenopausal women. It is characterized by weakened bones, increasing the risk of fractures and leading to significant morbidity and mortality. The goal of the current study is to examine the reported osteo-preservative effects of exercise and/or fasting in the Ramadan fasting model (RFM) in ovariectomized (OVX) rats.

Methods: The experimental intervention started 1 month following the ovariectomy procedure and consisted of five 15-min exercise sessions per week at 18–25 m/min and/or an approximately 13-h fast from sunrise to sunset (6:00 AM–19:00 PM). Serum bone metabolism biomarker levels were measured, and mineral concentrations in femoral ashed bones and digested serum were determined. Additionally, serum bone alkaline phosphatase (b-ALP), parathyroid hormone, osteocalcin, calcitonin, and vitamin D3 concentrations were measured using the competitive enzyme immunoassay technique.

Results: Calcium, magnesium, and phosphorus showed a notable decrease in mineral concentration among OVX rat femurs compared with the combination group (OVX + RFM + E) and control groups. In addition, homeostasis of serum concentrations of calcium, magnesium, and phosphorus was observed to increase in the OVX + RFM + E group rather than in the OVX group without intervention when compared with a control group. Furthermore, fasting and exercise, either alone or concurrently with ovariectomy, induced a non-significant elevation in osteocalcin, parathyroid hormone, and vitamin D3, whereas b-ALP and calcitonin increased significantly compared with those in control rats.

Conclusion: The combination of the Ramadan fasting model and moderate intensity exercises among OVX rats manifested advantageous effects in bone biomarkers compared with OVX rats without intervention. This could be

recommended as a lifestyle modification that is protective against osteoporosis, especially in the context of depleted estrogen hormone after menopause.

KEYWORDS

ovariectomy, Ramadan fasting, exercise, bone metabolism, estrogen deficiency

Introduction

Osteoporosis, a chronic bone metabolic disease characterized by weakened bones, which increases the risk of fractures, is a serious public health problem affecting millions of people, especially the elderly and postmenopausal women (Al-Ansari et al., 2022; Jaul and Barron, 2017). A bone fracture seriously affects the daily activities and quality of life of individuals and can result in complete paralysis or death (Al-Ansari et al., 2022). Worldwide, osteoporotic fracture occurs every 3 seconds, with over 8.9 million fractures occurring yearly (Jaul and Barron, 2017). The possible outcomes of vertebral compression fractures include pain, deformity, disability, and increased mortality (Al-Saleh et al., 2023). Furthermore, those with hip fractures experienced a significant increase in morbidity and medical expenses (Al-Saleh et al., 2023). Osteoporosis has many risk factors, such as disruptive endocrine changes like menopause, obesity, and vitamin D deficiency (Al-Ansari et al., 2022). Estrogen hormone deficiency may also lead to bone and muscle loss and impaired physical function, which leads to obesity, hyperleptinemia, and leptin resistance (Ezzat-Zadeh et al., 2017; Colleluori et al., 2022).

The prevalence of osteoporosis is noteworthy, even in regions like Saudi Arabia, where an epidemiologic analysis revealed that 34% of healthy women and 30.7% of men aged 50–79 years are osteoporotic (Ezzat-Zadeh et al., 2017). Given these statistics, screening recommendations suggest that women aged 65 years and older, or those between 60 and 64 years with additional risk factors, should undergo osteoporosis screening (Al-Ansari et al., 2022; Al-Saleh et al., 2023). Hence, early detection and intervention are crucial in mitigating the personal and societal costs associated with this condition.

A change in a lifestyle, such as quitting smoking, exercising, and following an appropriate diet, is one way to prevent osteoporosis (Alwahhabi, 2015). Exercise is considered to positively impact osteoporosis and bone mineral density (BMD) (Zhang et al., 2022). In this context, different types of physical exercise, including aerobic exercise, which speeds up the heart rate and breathing, strength exercise, balance exercise, high-impact exercise, resistance training, and plyometric exercise, which utilizes the stretch-shortening cycle by using a lengthening movement (eccentric) which is quickly followed by a shortening movement (concentric), are used in clinical practice to maintain or increase BMD (Li et al., 2021). A study has reported that exercise can increase bone strength and functional performance in postmenopausal women (Watson et al., 2018). Recently, a study demonstrated an indirect effect of exercise on bone tissue, by reducing leptin levels and improving insulin sensitivity (Khalafi et al., 2023). Therefore, scientists began to study alternative diets such as fasting, which are thought to decrease the incidence of osteoporosis.

Intermittent fasting can increase bone mass by decreasing parathyroid hormones and osteoclastogenesis and enhancing the osteoblast mechanism in a rat model (Alrowaili et al., 2021). Ramadan fasting is a special type of fasting, where during fasting hours, eating, drinking, and smoking are not allowed, but individuals are allowed to eat or drink from sundown until sunset (Wang and Wu, 2022). Ramadan fasting hours range from 13 to 18 depending on the geographical area, as the fasting hours are more than intermittent fasting (Correia et al., 2020). The Ramadan model (RM) protects the body from pro-inflammatory factors including TNF-α and IL-6 (Correia et al., 2020). Moreover, a study found a significant reduction of lowering blood sugar and LDL after 1 month of RM (Jahrami et al., 2022). Several studies have suggested that there is an association between inflammatory factors and insulin resistance with increased bone resorption and, thus, osteoporosis (Sanguineti et al., 2014; Xia et al., 2012).

To date, the mechanisms behind how Ramadan fasting and exercise individually and in combination affect bone health in ovariectomized rats are not fully understood. Most previous studies have focused on either Ramadan fasting or exercise alone, and there is a lack of research examining the combined effects of Ramadan fasting and exercise on bone health. The primary objective of the current study is to observe the preventive approach of an exercise program combined with RFM on osteoporosis in ovariectomized rats. The aim of the study was to examine the preventive approach of Ramadan fasting model and exercise, individually and in combination, to determine the bone health on ovariectomy-induced osteoporosis in a rat model.

Materials and methods

Animal preparation

Fifty female Sprague Dawley rats aged 8 weeks (weighing approximately 300 ± 20 g) were housed in the experimental surgery and animal lab, faculty of medicine, King Saud University. All animals were kept under controlled environmental temperature (22°C ± 1°C), humidity (50%–55%), and 12 day/night cycle. All the experimental procedures followed the guidelines of the Institutional Animal Care and Use Committee (IACUC) of King Saud University.

Ethical considerations

All the experimental procedures followed the guidelines of the Institutional Animal Care and Use Committee (IACUC) of Princess Nourah bint Abdulrahman University (approval no. HAP-01-R-059; IRB log number: 22-1141; category of approval: EXEMPT).

Experiment design and grouping

All rats were randomly divided into five groups (ten rats in each group) as follows: 1) Control group, 2) OVX group, 3) OVX with

RFM, 4) OVX with Exercise, and 5) OVX with both RFM and Exercise. The rats had free access to AIN 93G diet and filtered water. Nutritional markers, body weight (in grams), food intake (in grams), and water consumption (in ml) were measured weekly throughout the experiment period.

Bilateral ovariectomy procedure

All animals were deeply anesthetized using flows (8% sevoflurane inhalation anesthesia and 3% oxygen) in a rat chamber. Rats were placed in the chamber until the deep anesthesia. The rat was placed on a surgical table (temperature was fixed at 37°C). After that, a sterilized fine surgical blade was used to cut a 1-cm opening at the center of the pelvis region skin, and the muscle was opened carefully. Then, both ovaries were pushed out gently using locking straight forceps fixed on the tube and the ligament. Then, the ovary was removed using a surgical cautery machine to avoid bleeding. Then, the muscle was closed with two sutures, and the same was done for the skin.

All the rats were placed in the recovery unit after the surgery under veterinary care until they healed. After that, all the animals were returned to their cages.

Ramadan fasting model (RFM)

Postmenopausal female rats in the chronic phase after 1 month of bilateral ovariectomy were fasted from sunrise to sunset (6: 00 AM–19:00 PM) according to Riyadh time, which estimates around 13 h. The fasting was forced by removing the food and water at dawn and returning them at sunset for 4 weeks. A schematic representation of the timing of different interventions is illustrated in Figure 1.

Exercise session

After 2 weeks of bilateral ovariectomy, post-menopausal female rats in the chronic phase were trained on the treadmill for eight sessions over 2 weeks to adapt to the exercise. Then, animals were exposed to exercise five times a week on a 0% slope and for 15 min at 18–25 m/min. For 4 weeks, the running session lasted 30 min. A schematic representation of the timing of different interventions is illustrated in Figure 1.

Sampling and sacrificing

All animals were anesthetized with ketamine/xylazine at a dose of (9.1/91 mg/kg); blood was withdrawn by using direct heart puncture and then transferred to a gel separation tube (yellow cap) and incubated at room temperature for 30 min before centrifuging at 3,500 RPM for 10 min, and the serum was kept at −80°C in a freezer until use.

The removal of muscle and tissue from the femoral bone started with washing the femoral bone. Next, the bone femoral was submerged in a solution of water and a gentle detergent. After soaking, the bone was removed from the solution and rinsed thoroughly with clean water. A sharp instrument was used to carefully scrape away any remaining tissue or mucus on the surface of the bone. Then, the bone was rinsed thoroughly with clean water once more to remove any remaining residue. The femoral bone was dried and stored in a cool, dry place until it was ready for use. After that, both sides of the femur were kept at the −80°C in a freezer until use.

Additionally, the kidney and liver were immediately removed and washed with isotonic saline. After the specimens were dissected, one half was promptly frozen at −80°C in order to harvest RNA. The second portion of the tissue was homogenized to a 10% (w/v) homogenate in 10-mM phosphate buffer (pH 7.4) that was ice-cold for biochemical investigations.

Biomarkers

Measurement of mineral concentration in femoral bone and serum

Dried femoral bones were weighed and placed in a heat-resistant pottery bowl at 700°C in a furnace for 16 h using muffle furnace (Lenton Thermal design, Hope Valley, United Kingdom). Then, the bone ash was collected and weighed to calculate the ash/bone ratio; moreover, 100 mg of bone ash was added to 3 µm of nitric acid and 0.5 µm of hydrochloric acid (HCl), mixed, and made to wait for approximately 10 min before closing the vessel for microwave digestion for approximately 20 min. For the serum, 0.5 mL of each sample was directly digested with 2 mL of nitric acid. The concentration of magnesium (Mg^{+2}), phosphorus (P^{+}), and calcium (Ca^{+2}) were measured using inductively coupled plasma mass spectroscopy (ICP-MS) after being diluted with 45 mL of distal water to reach 70% of dilution and filtrated. The concentrations of minerals to ash in bones are reported as mg/100 bone ash, whereas mmol/L is used to express the concentrations in serum.

Measurement of serum biochemical levels

The levels of serum bone alkaline phosphatase (b-ALP), parathyroid hormone (PTH), osteocalcin, calcitonin, and 25-hydroxyvitamin D3 (Vit D3) were then measured using rat-specific ELISA kits. ELISA kits with the following numbers were utilized: E-EL-R1109, E-PP-1730, E-EL-R0243, E-EL-R0047, and E-EL-0014, all from Elabscience in Wuhan, China.

Assessment of the oxidative stress index

To determine the level of lipid peroxidation, malondialdehyde (MDA) was quantified using the thiobarbituric acid method developed by Ohkawa et al. (1979). As previously mentioned (Green et al., 1982), the amount of nitric oxide (NO) in the cerebral cortex was measured by measuring dye generation at 540 nm following the injection of Griess reagent. Ellman's reagent was used to quantify the GSH levels, and the yellow chromogen was measured at 412 nm, as was previously described (Ellman, 1959). Furthermore, the manufacturer's instructions were followed while using the Abcam (catalog number: ab65329; Cambridge, United Kingdom) colorimetric assay kit to measure the total antioxidant capacity (TAC).

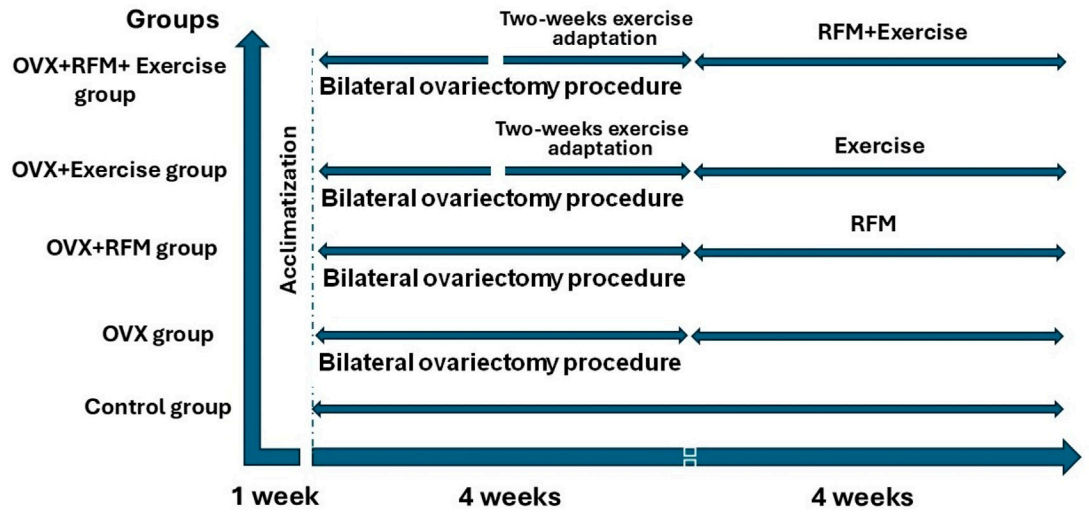

FIGURE 1
Diagram of schematic representation of the study protocol of the rat groups in weeks.

Additionally, using the procedures described by Paglia and Valentine (1967), the activity of glutathione peroxidase (GPx) was assessed. The catalase (CAT) enzyme activity in the homogenates was determined using the method described by Aebi (1984). Superoxide dismutase (TSOD) activity was measured at 480 nm using the protocol described by Misra and Fridovich (1972).

Measuring the inflammatory cytokines

The manufacturer's instructions were followed while using the cyclooxygenase-2 (Cox-2), interleukin 1 beta (IL-1β), and tumor necrosis factor alpha (TNF-α) ELISA kits (MyBioSource, San Diego, CA, United States) to measure the levels of inflammatory cytokines. MBS725633, MBS2023030, and MBS175904, in that order, are the catalog numbers of the measured inflammatory markers.

Real-time quantitative polymerase chain reaction

The harvested tissues' total cellular RNA was separated with the aid of the TRIzol reagent (Invitrogen, Life Technologies Corporation, Carlsbad, CA, United States). We already mentioned the Q-PCR experiment and the reverse transcription reaction solution in our work (Albrahim et al., 2023). Using the $2^{-\Delta\Delta Ct}$ technique, the relative fold change was determined. The primers were synthesized by Sigma-Aldrich (St. Louis, MO, United States) and listed in Table 1.

Statistical analysis

All data will be statistically analyzed using GraphPad Prism software version 9. The one-way ANOVA will assess the significant difference in minerals concentration between different groups, followed by Tukey's *post hoc* test. Before using an analysis of variance (ANOVA), all parameters with continuous data were put through Levene's test to ensure that the variance was homogeneous. A Mann–Whitney U test was used to determine the significance. The Shapiro–Wilk test was used to first determine whether the data were normal. The obtained data were displayed as the mean ± S.D. A p-value of equal or less than 0.05 is considered significant.

Results

Effects of exercise and fasting on body weight

Figure 2 displays the effects of exercise and fasting on weekly weight gain. At baseline, the body weights of all animals were

TABLE 1 Gene primer sequences examined by real-time PCR analysis.

Name	Accession number	Forward primer (5′---3′)	Reverse primer (5′---3′)
GAPDH	NM_017008.4	CTCTCTGCTCCTCCCTGTTC	TACGGCCAAATCCGTTCACA
SOD2	NM_017051.2	CGGGGGCCATATCAATCACA	GCCTCCAGCAACTCTCCTTT
GPx1	NM_030826.4	CCTGGTATCTGGGCTTGGTG	TTAGGCGTAAAGGCATCGGG

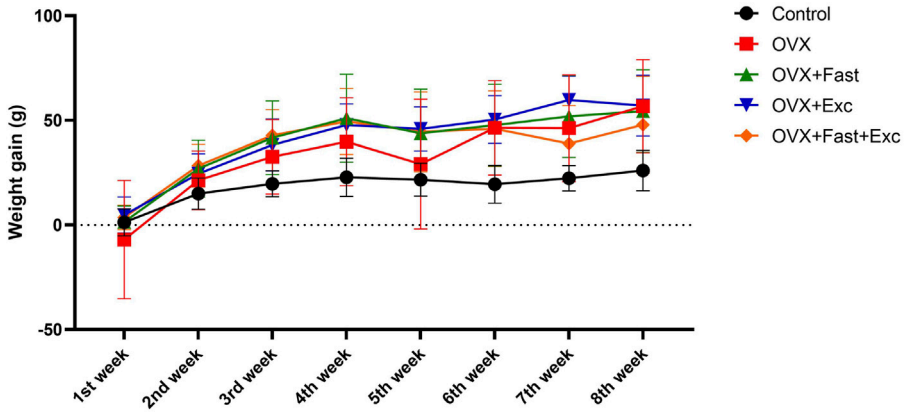

FIGURE 2
Effects of exercise and/or fasting on weekly weight gain.

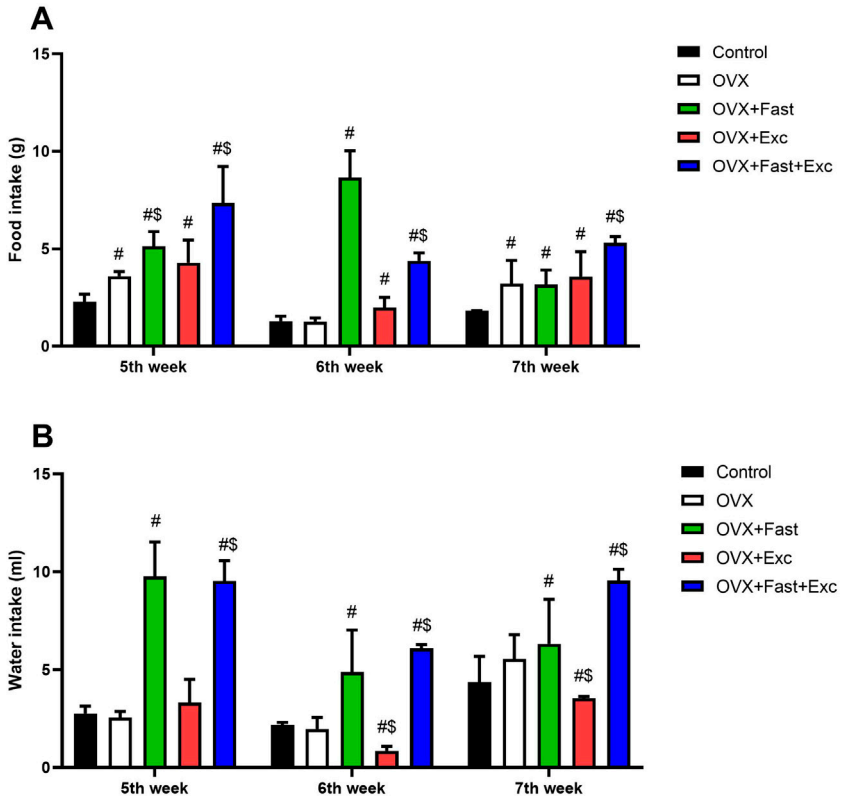

FIGURE 3
Change in **(A)** food consumption and **(B)** water consumption of all rats before intervention, after intervention, and at the end of the experiment. The numerical parameter is presented as the mean ± S.D. of each group (n = 6/group). Data were statistically evaluated using one-way ANOVA followed by the Tukey multiple comparison *post hoc* test. $^{\#}p < 0.05$ compared to the control group; $^{\$}p < 0.05$ compared to the OVX group.

within the same range (280–320 g). However, after 2 weeks of ovariectomy procedures, the gain in body weight was increased, as expected. There was no effect in the OVX, RFM, exercise, or combination of RFM and exercise groups to decrease the increment in body weight gain compared with the control group ($p < 0.05$).

The numerical parameters are presented as the mean ± S.D. of each group (n = 6/group).

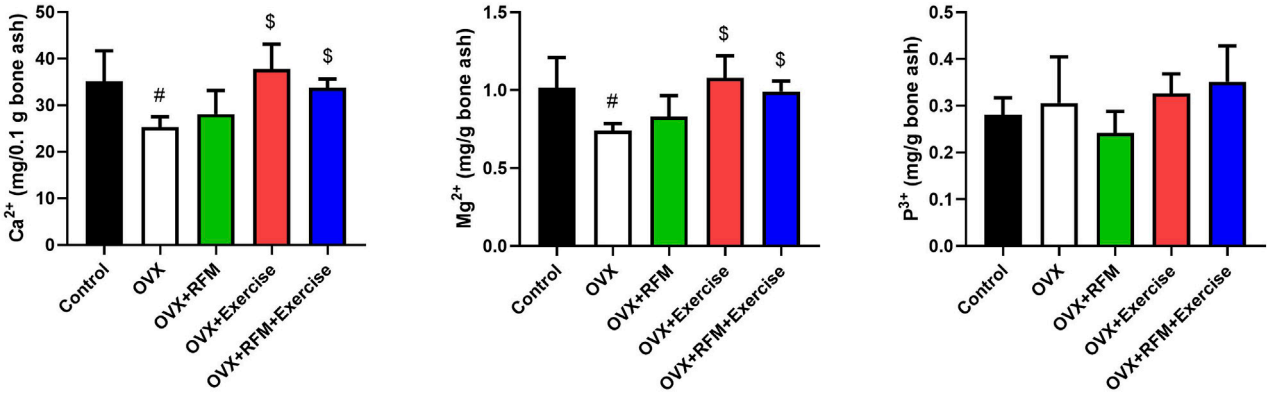

FIGURE 4
Effect of fasting and/or exercise on bone mineral concentration of femur bone in rats. The numerical parameter is presented as the mean ± S.D. of each group (n = 6/group). Data were statistically evaluated using one-way ANOVA followed by the Tukey multiple comparison *post hoc* test. $^{\#}p < 0.05$ compared to the control group; $^{\$}p < 0.05$ compared to the OVX group.

Weekly change in food and water consumption

Ovariectomized rats showed a significant increase in food intake before intervention and after ovariectomy procedures compared with the control rats ($p < 0.05$). However, at the end of the experiment, the combination of RFM and exercise significantly increased food consumption compared with that of control and ovariectomized rats ($p < 0.05$) (Figure 3A). Moreover, ovariectomy procedures in rats did not affect water intake ($p > 0.05$). After 1 week of intervention, RFM and combination of RFM and exercise significantly increased water intake compared with that in control and ovariectomized rats ($p < 0.05$). In addition, at the end of the experiment, there was a significant increase ($p < 0.05$) in water intake in the combination group (OVX + RFM + Exercise) compared with that in other groups (Control, OVX, OVX + Exercise, and OVX + RFM) (Figure 3B).

Effects of exercise and fasting on bone minerals concentrations

After 1 month of intervention, there was no significant difference in bone mineral concentration within the groups ($p > 0.05$). Otherwise, the OVX combination group (OVX + RFM + Exercise) and the OVX + Exercise group show approximately similar concentration in bone Ca^{2+} as the control group. In addition, non-intervention OVX groups show low bone Ca^{2+} compared to the OVX + Exercise group (Figure 4A). Moreover, the combination group (OVX + RFM + Exercise) had higher bone Mg^{2+} concentration than the control group (Figure 4B). Furthermore, the group that exercised over 1 month had more Mg^{2+} bone concentration than the OVX group without intervention. Interestingly, the combination group (OVX + RFM + Exercise) had more bone K^{+} concentrations than the control group. Otherwise, the OVX group had lower concentrations in bone K^{+} than the OVX + Exercise group (Figure 4C).

Effects of exercise and fasting on serum minerals concentrations

The effects of exercise and fasting either alone or in combination on OVX rats are shown in Figure 5. At the end of the experiment, there is no significant difference in serum Ca^{2+} concentration within the groups ($p > 0.05$). However, the serum Ca^{2+} concentration in the OVX combination group (OVX + RFM + Exercise) is similar to that in the control group (Figure 5A). In addition, slightly positive effects on Ca^{2+} serum concentration were observed in OVX + RFM and OVX + Exercise groups compared with the OVX groups without intervention. Moreover, significant increase in serum Mg^{2+} concentrations was observed in the group that exercised along with the fasting combination intervention compared with that in the control group ($p < 0.05$) (Figure 5B). In addition, a significant increase ($p < 0.05$) was found in serum P^{+} concentrations in the ovariectomized rats groups (OVX, OVX + RFM, and OVX + Exercise) compared with the control group, and there was no notable change in serum P^{+} concentrations in the combination group (OVX + RFM + Exercise) when contrasted with the control group ($p > 0.05$) (Figure 5C).

Effects of exercise and/or fasting on serum biochemical parameters

Ovariectomy in rat induced a non-significant decline in serum Vit. D3 compared to that in control rats. However, fasting and exercise, each one alone or concurrently with ovariectomy, induced a non-significant elevation in Vit. D3 compared with that in control rats. This increment in fasting and exercise, each one alone, was non-significant when compared with OVX rats, but the increment was significant during combined fasting and exercise with ovariectomy compared with that in OVX rats (Figure 6A). Furthermore, parathyroid hormone and osteocalcin levels were minimally decreased non-significantly in ovariectomy rats compared with that in control rats and increased non-significantly in fasting and exercise group rats, each one alone or

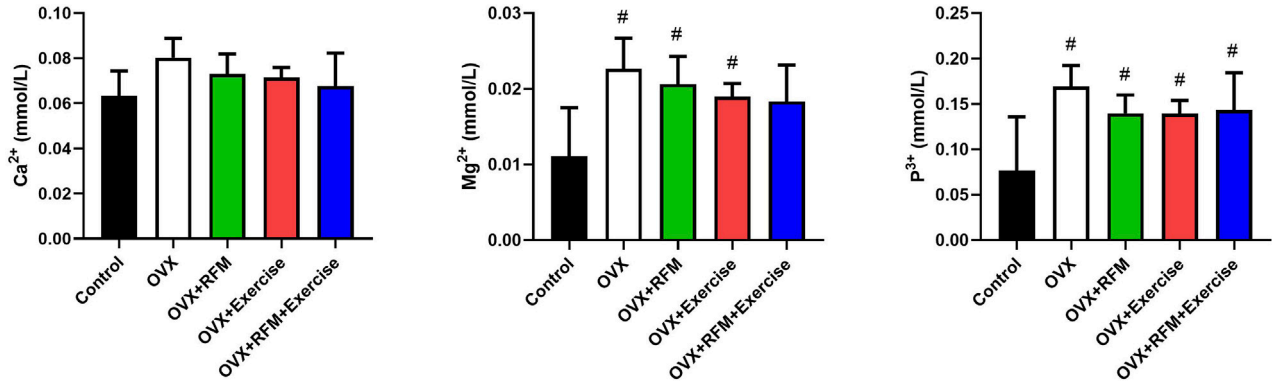

FIGURE 5
Effect of fasting and/or exercise on serum minerals concentration in rats. The numerical parameter is presented as the mean ± S.D. of each group (n = 6/group). Data were statistically evaluated using one-way ANOVA followed by the Tukey multiple comparison *post hoc* test. $^{\#}p < 0.05$ compared to control the group; $^{\$}p < 0.05$ compared to the OVX group.

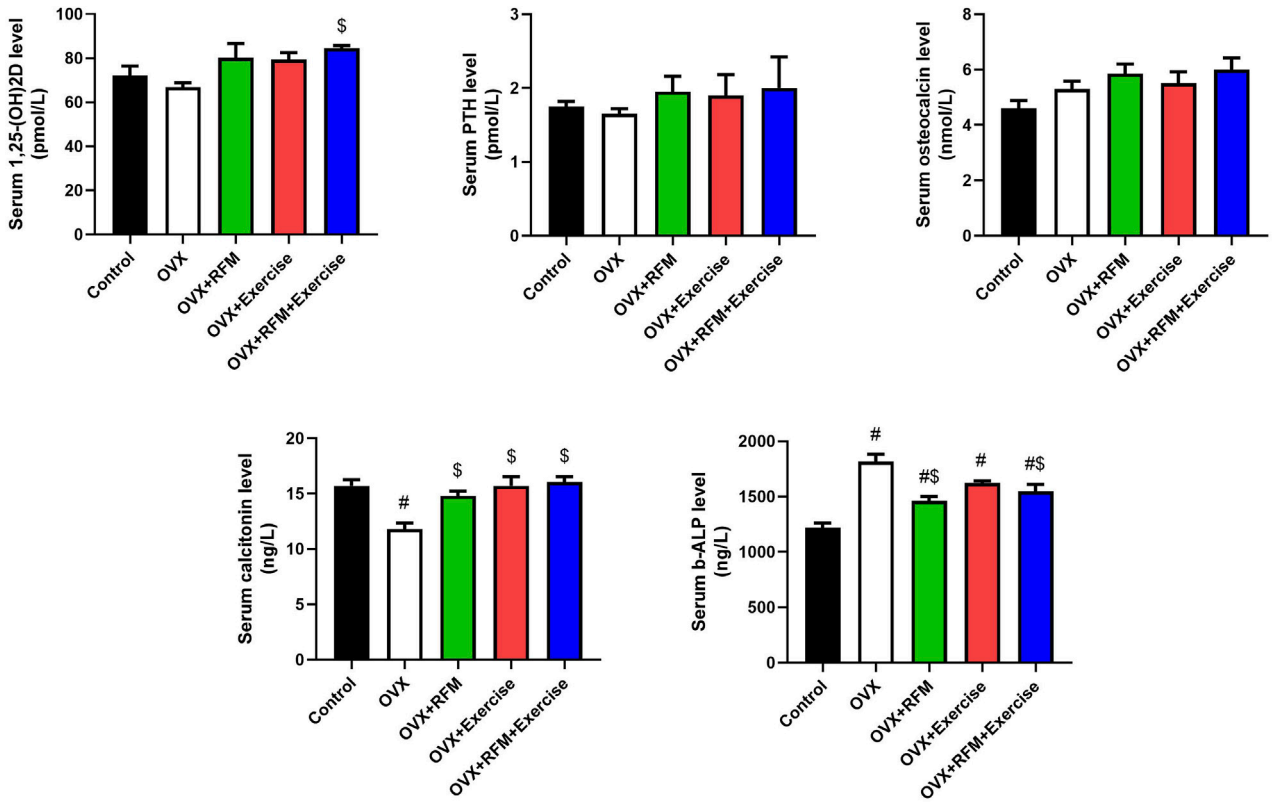

FIGURE 6
Effects of exercise and/or fasting on serum vitamin D3, osteocalcin, calcitonin, parathyroid hormone, and bone-alkaline phosphatase concentrations. The numerical parameter is presented as the mean ± S.D. of each group (n = 6/group). Data were statistically evaluated using one-way ANOVA followed by the Tukey multiple comparison *post hoc* test. $^{\#}p < 0.05$ compared to the control group; $^{\$}p < 0.05$ compared to the OVX group.

concurrently with ovariectomy, compared to that in control and OVX rats (Figures 6B, C). However, the serum calcitonin level decreased significantly in ovariectomy rats compared with that in control rats. However, the decrease in calcitonin was non-significant in fasting and exercise group rats, each one alone or concurrently with ovariectomy, compared to that in control rats. Fasting and exercise, each one alone or concurrently with ovariectomy, induced significant increase in serum calcitonin compared to that in ovariectomized rats (Figure 6D). Additionally, the serum b-ALP level was increased significantly in ovariectomized rats compared to

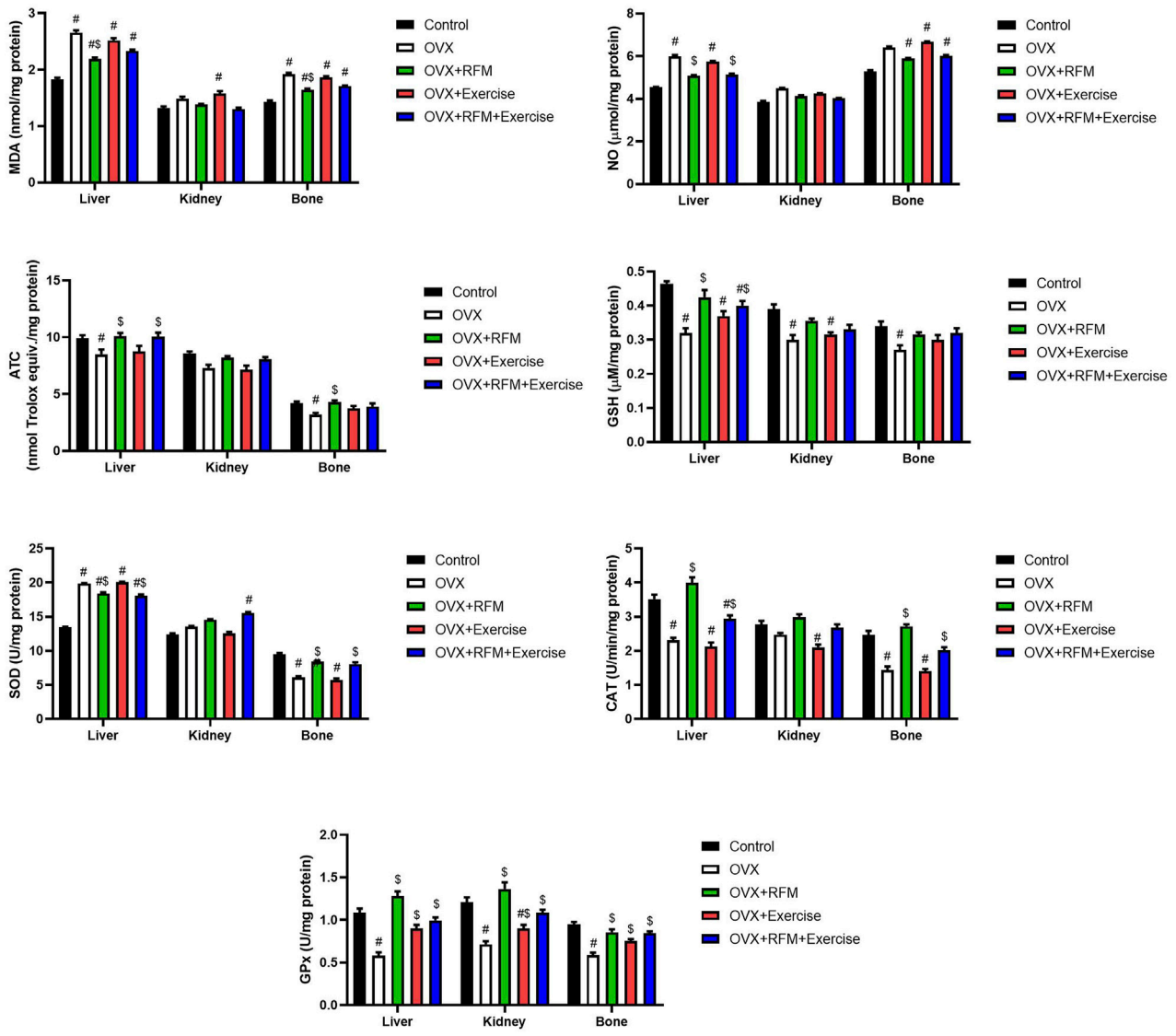

FIGURE 7
Effects of exercise and/or fasting on oxidative stress markers concentrations and mRNA expression of related genes in the liver, kidney, and serum. The numerical parameter is presented as the mean ± S.D. of each group (n = 6/group). Data were statistically evaluated using one-way ANOVA followed by the Tukey multiple comparison *post hoc* test. $^{\#}p < 0.05$ compared to the control group; $^{\$}p < 0.05$ compared to the OVX group.

that in control rats. Moreover, exercise alone with ovariectomy failed to reduce the increment in b-ALP (Figure 6E). However, fasting alone or concurrently with exercise in ovariectomy rats significantly decreased the level of b-ALP compared to that in OVX rats, but the level was still significantly higher than that in control rats.

Effects of exercise and/or fasting on tissue oxidative stress biomarkers

Ovariectomy in rats induced oxidative stress, evidenced by a significant increase in MDA (Figure 7A) and NO (Figure 7B) concurrently with a marked decrease in GSH (Figure 7C), associated with a significant inhibition in antioxidant enzymes, namely, SOD (Figure 7D), CAT (Figure 7E), and GPx (Figure 7F). Oxidants/antioxidants imbalance in ovariectomized rats' liver, kidney, and bones was confirmed by the reduction in the total antioxidant capacity (TAS) level (Figure 7G). However, the obtained results revealed that ovariectomy is associated with a significant stimulation in SOD activity in the liver of rats. Interestingly, fasting of ovariectomized rats significantly attenuated oxidative stress induction. Exercise was not effective in the restoration of oxidants/antioxidants balance in ovariectomized rats. Furthermore, the combination of fasting and exercise also failed to restore the redox status in ovariectomized rats. mRNA expression of SOD2 (Figure 8A) and GPx1 (Figure 8B) confirmed the biochemical results, as indicated by significant downregulation in GPx1 and upregulation in SOD2 in the liver in ovariectomized rats, and fasting successfully attenuated this downregulation.

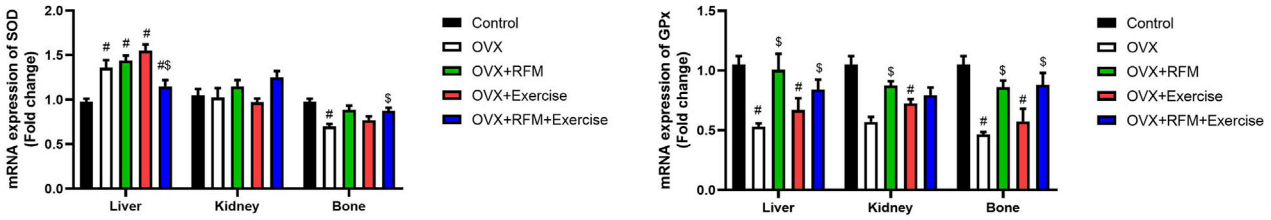

FIGURE 8
Effects of exercise and/or fasting on oxidative stress mRNA expression of related genes in the liver, kidney, and serum. The numerical parameter is presented as the mean ± S.D. of each group (n = 6/group). Data were statistically evaluated using one-way ANOVA followed by the Tukey multiple comparison *post hoc* test. $^{\#}p < 0.05$ compared to the control group; $^{\$}p < 0.05$ compared to the OVX group.

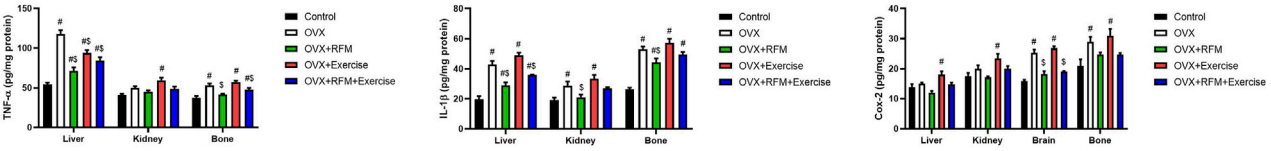

FIGURE 9
Effects of exercise and/or fasting on pro-inflammatory cytokines concentrations in the liver, kidney, and serum. The numerical parameter is presented as the mean ± S.D. of each group (n = 6/group). Data were statistically evaluated using one-way ANOVA followed by the Tukey multiple comparison *post hoc* test. $^{\#}p < 0.05$ compared to the control group; $^{\$}p < 0.05$ compared to the OVX group.

Effects of exercise and/or fasting on tissue pro-inflammatory markers

Ovariectomy significantly increased the pro-inflammatory cytokines/mediators compared to that in the control rats ($p < 0.05$). Exercise unfortunately augmented the inflammatory response in ovariectomized rats. However, fasting of the ovariectomized rats reduced the inflammatory response, evidenced by the significant reduction in TNF-α (Figure 9A), IL-1β (Figure 9B), and Cox-2 (Figure 9C). Moreover, the combination of fasting and exercise successfully restrained pro-inflammatory cytokines (TNF-α and IL-1β) and mediator (Cox-2) elevations.

Discussion

Osteoporosis is a worldwide common disease that leads to many complications and increases morbidity and mortality, which causes a burden on health-care systems (Al-Ansari et al., 2022; Jaul and Barron, 2017). Lifestyle modification is the first line of treatment, and few studies investigated the effect of lifestyle on preventing osteoporosis. The ovariectomized rat model is one of the approved animal models to mimic postmenopausal, induced osteoporosis (Li et al., 2014; Yousefzadeh et al., 2020). In this sense, the current study investigated the protective effect of exercises and RFM and their combination against osteoporosis on the OVX rat model. Our results showed significantly increased body weight among the OVX group compared with the control group. These findings are explained by an observational cohort study; osteoporosis in postmenopausal women may be due to their altered hormonal status, including estrogen and follicle-stimulating hormone (FSH), which are noted to be associated with obesity occurring in women (Ebong et al., 2022). Apparently, the drop in circulating estradiol and the increase in FSH went along with the shift in visceral adiposity. Furthermore, menopause is linked to an increase in visceral and total body fat due to estrogen's effects on regulating the expansion and metabolism of adipocytes, including lipolysis and lipoprotein lipase activity, as well as the correlated relationship between FSH and lipid production (Ebong et al., 2022; Curtis et al., 2018).

According to Burch et al. (2021), ovariectomized rats experience estrogen level depletion, which consequently leads to a rapid and significant increase in body weight, whereas intact rats gain weight slowly and more moderately. Moreover, the OVX group shows a significant increase in free fatty acids (FFAS) and triglyceride levels from baseline to complete 8 weeks. At the end of the experiment period, it was observed that the OVX group gained more weight than the control group (Curtis et al., 2018). Another experimental study carried out by Damirchi et al. (2010) made an interesting observation that ovariectomy in rats leads to a dramatic increase in body weight, as shown in the study results by the eighth week. Furthermore, there was a higher visceral fat level in the OVX group than in the control rats. These results of the previous studies are consistent with the findings of the present study. As was foreseeable, ovariectomy was followed by a significant gain in body weight after 2 weeks of the experiment. At the seventh week, there was a significant difference in body weight gain between the OVX and control groups. Furthermore, the weight gain continued in the OVX group until the end of the trial at the eighth week. A study demonstrated the effectiveness of exercise in postmenopausal women by reducing 6%–7% of body weight, leading to positive consequences on estradiol, free estradiol, androstenedione, and free testosterone as well as improved physical fitness (Moro et al., 2016). An additional study discovered that exercise dramatically decreased

visceral fat in the OVX, with the exercise group contrasted to the OVX group; however, the body weight and BMI remained unchanged (van Gemert et al., 2015). In addition to exercise, fasting improves the metabolic state and weight loss and changes in body composition. A study conducted for 8 weeks demonstrated the effectiveness of fasting for 16 h/8 h feeding concluded that there was a drop in fat mass (Agostini et al., 2018).

Consistent with these studies, our findings observed that there was a positive effect of fasting and exercise on body weight change over the study duration, as observed when comparing the OVX–RFM group and the OVX–Exercise group with the OVX group. While previous studies' findings are instructive in the context of exercise and fasting effects on body weight, the current study provides the effect of exercise and fasting combined on the body weight. There was a huge difference in body weight gain in the OVX, OVX + Exercise, and OVX + RFM groups with the control groups at the seventh week. However, by the eighth week of the experiment, it was noted that the control group alongside the combination group (OVX + RFM + Exercise) had the lowest body weight gains in contrast with the OVX, OVX + Exercise, and OVX + RFM groups. Overall, post-menopause leads to hormonal chaining linked to obesity in women. Moreover, fasting and exercise help promote losing body weight. Thereby, the combination of fasting with exercise shows a positive effect in terms of losing weight in the OVX rats. Our results support the hypothesis in accordance with the previous study, and this study shows significant increase in body weight and significant decrease in bone markers among ovariectomized rats without intervention. Previous studies illustrated a relation between sarcopenia and osteoporosis due to the biomechanical and biochemical relationship between muscles and long bones' tissues (Barengolts et al., 1993). Skeletal muscle produces many proteins and peptides that can impact bone health; therefore, exercise is beneficial for osteoporosis prevention and treatment (Barengolts et al., 1993).

A study suggests that in the early stages of estrogen deficiency, endurance exercise can postpone the initial stage of bone mineral loss (Bahijri et al., 2015) and promote the development of muscle mass, endurance, and strength. Physical activity demands additional strain, so the formation of bone occurs, whereas lower strains encourage bone resorption; in addition, exercise can increase the myokines and osteokines, which are secreted by the muscle and bone, respectively, and has anabolic effects on the muscle and the osteoblast lineage through improving bone-forming cell differentiation and activity (Barengolts et al., 1993). However, it has been demonstrated that resistance exercise has a higher impact than endurance exercise on bone mass maintenance and improvement because of the nature of the mechanical stimulus. In addition, if we perform an endurance exercise without the right caloric intake, we can have the opposite effect with the induced protein catabolism. Amato et al. (2022) demonstrated the efficacy of resistance training in the elderly population on bone remodeling.

Furthermore, through changes in bone structure and/or localized adaptation in bone distribution in the regions subjected to the greatest strain, exercise training may improve bone strength independent of changes in BMD (Allison et al., 2015; Warden et al., 2007). A bone's resistance to bending increases during exercise as a result of increased cortical thickness brought on by load-induced periosteal apposition and, to a lesser extent, decreased endocortical resorption (Warden et al., 2014). In this regard, Krogh et al. (2024) recently found that resistance training led to significantly increased levels of fasting N-terminal propeptide of type-I procollagen (P1NP, a bone formation marker) in both pediatric hematopoietic stem cell transplantation patients and controls with no significant changes in fasting C-terminal telopeptide of type-I collagen (CTX, a bone resorption marker) levels.

Dietary adjustments related to fasting during Ramadan regulated PTH secretion in a way that would be advantageous to bone health (Adawi et al., 2017). Fasting may have a positive impact on bone turnover while also lowering pro-inflammatory markers. A shift in eating habits during RFM influences PTH secretion by raising the calcium content in the evening and reducing bone resorption at night (Adawi et al., 2017; Hisatomi and Kugino, 2019). Furthermore, the current study discovered that intermittent fasting for 16–18 h in rats led to a large rise in serum levels of osteoprotegerin and a significant drop in serum levels of RANK, indicating that RFM can suppress osteoclast activity and stimulate osteoblast activity (Park and Shin, 2022). Similarly, Alrowaili et al. (2021) found that in rats with glucocorticoid-induced osteoporosis that were subjected to intermittent fasting for 16–18 h per day for 90 days, the serum levels of the bone formation biomarkers, namely, osteoprotegerin, ALP, and osteocalcin, were significantly increased, and those of the bone resorption markers, namely, tartrate-resistant acid phosphatase (TRAP)-5b, amino-terminal cross-linking telopeptide of type-I collagen (NTX-1), and deoxypyridinoline (DPD), were significantly decreased. This suggests that intermittent fasting slows the progression of glucocorticoid-induced osteoporosis by inhibiting osteoclast activity and promoting osteoblast osteogenesis. In a similar vein, it was discovered that intermittent fasting counteracted both the rise in the serum bone resorption marker TRAP and the BMD reduction brought on by a ketogenic diet (Xu et al., 2019). Controversy around animal studies shows that fasting shows no significant differences in lumbar vertebral body height and cortical bone thickness compared to the non-fasting group; however, the duration of fasting in the study was only 4 days (Masanova et al., 2022).

Supporting the beneficial effect of fasting, in a 6-month randomized controlled trial, the effects of alternate-day fasting regimens and caloric restriction on bone metabolic markers in overweight and obese individuals were investigated in relation to religious Ramadan fasting. The results showed good weight reduction in both the caloric restriction and alternate-day fasting groups, and no significant effects on bone mineral content (BMC), bone mineral density (BMD), or the markers related to bone metabolism, type-I collagen carboxy-terminal peptide (CTX-1), and OPG (Barnosky et al., 2017) were observed. Comparable findings were reported by Clayton et al. (2020), who discovered no impact of a 24-h fast on serum levels of PTH, procollagen type-I N-terminal propeptide (PINP), or CTX-1. In addition, Martens et al. (2020) demonstrated that neither the total BMD nor the regional BMD of middle-aged and elderly non-obese individuals differed from a control group after 6 weeks of time-restricted feeding, nor did these individuals' bone mass decrease.

In clinical studies, Ramadan fasting could relieve the symptoms of the patients with RA or SpA by regulating inflammatory cytokines (decreasing CRP, IL1, or 6), lipid profiles, antropometric features, or intestinal microbial compositions (Ben Nessib et al., 2021;

Rodopaios et al., 2024). As RIF has been found to have a beneficial effect on the secretion of PTH, it was hypothesized that it could positively affect the bone metabolism. RIF has also a positive impact on the activation of dipeptidyl peptidase 4 (DPP-4) inhibitors as the DPP-4 gene has been identified as an important genetic factor contributing to the progression of osteoporosis (Ben Nessib et al., 2021; Bahijri et al., 2015). However, there are no clinical investigations and basic research that indicates that RIF and exercise can ameliorate osteoporosis.

Overall, there is a significant reduction in bone density among ovariectomized rats, especially in the concentrations of calcium and magnesium in the bones, and this is mainly due to the depletion in the estrogen level (Lei et al., 2009; Vaananen and Harkonen, 1996). Estrogen deficiency affects bone density by increasing PTH, IL-1β, and IL-6, which are cytokines that increase the process of osteoclasts formation (Stern et al., 1988; Masanova et al., 2022).

The current study results found that the femur bone calcium concentration and serum calcium concentrations were higher in the OVX + Exercise group than in the OVX group without interventions. Recently, in some studies, the relationship between calcium homeostasis and exercise had been investigated. Some experimental animal studies found that there is an increase in the absorption of calcium from the intestine due to the stimulation of exercise to increase bone density, which leads to an increase in vitamin D3 absorption, resulting in the inhibition of PTH secretion (Yeh and Aloia, 1990; Ashizawa et al., 1997). Furthermore, exercise can help balance calcium levels in the bones and blood (Tella and Gallagher, 2014). Calcium homeostasis is important because a chronical decrease in calcium serum concentrations is associated with increase in PTH levels, which leads to the replacement of calcium from the bones to the blood (Peterson and Heffernan, 2008).

The results of the current study also showed that there was a slight unremarkable elevation and hemostasis in the OVX + RFM group compared to the OVX group in the serum samples. To explain our results, a study by Park and Shin (2022) found that intermittent fasting protects bone mass by reducing TNF-α levels in the serum. TNF-α can affect calcium absorption by decreasing the level of calcitriol in the blood (Attarzadeh Hosseini et al., 2013). In contrast, previous studies that examined the effect of Ramadan fasting on blood calcium levels have shown that blood calcium levels may be normal or slightly low due to the increased excretion of calcium in the urine and due to PTH abnormalities after Ramadan fasting (Bahijri et al., 2015; Castiglioni et al., 2013).

The main results of the current study investigated the effect of exercise and fasting on OVX rats compared with the control group. There is an identical result of calcium concentration in the bones and serum of the OVX + RFM + Exercise group compared to that in the control group that had not undergone an ovariectomy surgery, and this may be due to the combined benefits of fasting and exercise in regulating PTH and RANKL levels.

In addition, magnesium deficiency affects the absorption of vitamin D and PTH, which affects the absorption of calcium and bone density (Vazquez-Lorente et al., 2020; Marcus, 2018). Furthermore Vazquez-Lorente et al. (2020) investigated the mechanism effect of magnesium in bones, and they found that hypomagnesemia was associated with a increase in cytokines that accelerate the process of osteoclast, resulting in an increase in bone stiffness and osteoclast's function. In OVX rats, they were exposed to magnesium deficiency, as depletion of the estrogen level was associated with low magnesium and BMD due to an increase in pro-inflammatory factors (Toba et al., 2000). In the experimental animal study, OVX rats fed with magnesium supplements had shown an increase in osteocalcin, which is a protein matrix that is considered as a marker for bone building. Our findings demonstrated higher Mg^{2+} bone concentration in the OVX + Exercise group than in the OVX group. Previous experimental animal studies have shown that there is a strong relationship between aerobic exercise such as treadmill and swimming and an increase in BMD by stimulating bone formation processes (Kang et al., 2017; Oh et al., 2016), which includes magnesium concentrations in the bones.

In our main finding, there was a notable increase in the bone magnesium level and serum magnesium level in comparison between the combination groups (OVX + RFM + Exercise) versus the control group, which supports the effectiveness of intervention in homeostasis magnesium concentration.

Our experimental results showed that ovariectomy in rats leads to a decrease the in bone potassium concentration level compared with that in the control group, potentially contributing to the development of osteoporosis. This finding is consistent with previous experimental animal studies conducted by Lei et al. (2009) and Ha et al. (2020), which reported a significant decrease in bone potassium concentrations levels in ovariectomized rats. Moreover, the present study found that OVX + Exercise group and OVX + RFM group interventions have differential effects on bone potassium concentrations in ovariectomized rats compared with the OVX group without intervention. In addition, the combination group (OVX + RFM + Exercise) had a higher bone potassium concentration level than the control group, which indicated an increase in BMD and a protective approach against osteoporosis (Ji et al., 2007).

Our findings also show a significant increase in serum potassium concentrations in the ovariectomized groups compared to that in the control group, consistent with a previous experimental study conduct by Kaastad et al. (2001), which claimed that the decrease in potassium concentrations levels in OVX rats is due to the deficiency of estrogen hormone, which causes the deficiency of the aldosterone hormone level.

Furthermore, an experimental animal study found that ovariectomy led to changes in metabolic function, specifically an increase in blood glucose levels and a decrease in insulin sensitivity. These findings suggest that the observed increase in potassium levels in the current study may be due to changes in metabolic function caused by ovariectomy (Shaban et al., 2017). Another possible explanation by Ciarambino et al. (2022) is that the increase in potassium levels in the ovariectomy group may be related to changes in renal function. Potassium levels are primarily regulated by the kidneys, and ovariectomy has been shown to alter renal function in rats, specifically a decrease in renal blood flow and the glomerular filtration rate.

In our findings, there was no significant increase or decrease in serum potassium levels in the combination group (OVX + RFM + Exercise) compared with that in the control group, suggesting that the combination of exercises and fasting may have improved effect in potassium homeostasis.

Vitamin D affects bone density, especially in the femur bones, and this effect increases when estrogen and vitamin D deficiency

occur in cooperation, which leads to the high risk of fractures (Lips and van Schoor, 2011; Ali et al., 2014). This is the reason how vitamin D supplementation can prevent osteoporosis in postmenopausal women (Elwakeel et al., 2020). Vitamin D deficiency can cause imbalance in BMD levels, resulting in potassium and calcium deficiency (Lips and van Schoor, 2011). Furthermore, vitamin D suppresses the parathyroid hormone, which leads to increased calcium absorption in the intestine and deposition in the bones (Lips and van Schoor, 2011; Elwakeel et al., 2020). Our results reflect a decrease in serum levels of vitamin D in OVX rats. The relationship between OVX rats and depletion of the vitamin D level is explainable by the study of Sundell and Björnsson, who found a significant increase in the level of vitamin D in the serum of OVX rats after treatment with estrogen supplements (Sundell and Bjornsson, 1990). In another experimental animal study, there was a positive correlation between estrogen and vitamin in rats, as vitamin D increases estrogen and *vice versa* (Nashold et al., 2009). This process is due to the enhancement of the estrogen hormone of the vitamin D receptors in the central nervous system, which increases the absorption of vitamin D in the blood (Dzik et al., 2022).

Our study suggested that exercise intervention in the OVX + Exercise group can increase the level of vitamin D serum when compared with that in the OVX group. In agreement with our results, a randomized control trail found a significant increase in the vitamin D level in the participant after aerobic exercise (Dzik et al., 2022). In another experimental animal study conducted by Aly et al. (2016), they found a positive effect of swimming exercise on vitamin D serum among albino rats. This action may be due to the ability of exercise to increase the anabolic reaction of vitamin D in various tissues, especially in the muscle (Aly et al., 2016).

A recent study by Karras et al. (2023) investigated the effectiveness of intermittent fasting on vitamin D concentration among overweight participants. Interestingly, free 25-hydroxy vitamin D [25(OH)D] in the fasting group showed an increase that was considerable for the full study duration. In contrast to the control group, the fasting group demonstrated increased free 25(OH)D concentrations at the seventh week. Moreover, it was observed that body fat has an inverse relationship with vitamin D (Karras et al., 2023). This finding supports the present study results, which show an increase in serum vitamin D levels among the fasting group compared with the control group.

In the present study, it was found that the combination effect of RFM and exercise interventions in OVX rats eliminate the negative effects of ovariectomy and estrogen deficiency on decreasing the serum vitamin D level when compared with that in the control group.

However, in the present study, one possible explanation for the lack of effect of ovariectomy on calcium and vitamin D levels could be related to the timing of the measurements. It is possible that changes in calcium and vitamin D levels as a result of ovariectomy take longer than 1 month to manifest (Lei et al., 2009). Additionally, it is important to consider additional measures of bone health in conjunction with calcium and vitamin D levels, such as bone mineral density or bone microarchitecture. These measures may provide a more comprehensive understanding of the effects of ovariectomy on bone health in rats.

Overall, our results support the hypothesis accordant with the previous study, and this study shows significant increase in body weight and decrease in bone markers among ovariectomized rats without intervention.

Study limitations

The study by Alrowaili et al. (2021) used an 18-h IF protocol for 3 months, whereas the current work only covered 8 weeks. This shorter duration may be insufficient to observe the full effects of IF on bone metabolism and osteoporosis management. In addition, our work may not fully account for the potential impact of nutritional changes during IF, particularly protein intake, which can significantly affect bone metabolism. Moreover, further study is needed for more detailed cellular investigations of bone cells to better understand the mechanisms mediating osteoporosis in this context. The current study may not provide sufficient insight into the cellular processes involved.

Conclusion

In conclusion, this study demonstrated that a combination of exercise and Ramadan fasting intervention can regulate calcium, magnesium, and potassium homeostasis in femoral bone and serum concentration, in addition to maintaining bone metabolism, oxidative stress, and inflammatory status levels in the liver, kidney, and bone, which have a positive effect on bone health. The combination of the Ramadan fasting model and moderate intensity exercises could be recommended as a lifestyle modification that is protective against osteoporosis, especially in the context of depleted estrogen hormone after menopause. However, further investigation is needed to confirm the results of this study.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by King Saud University's Institutional Animal Care and Use Committee (IACUC) regulations. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

TA: conceptualization, data curation, funding acquisition, investigation, methodology, project administration, resources, supervision, writing–original draft, and writing–review and editing. RAy: formal analysis and writing–review and editing. RAi: formal analysis and writing–review and editing. LA: formal analysis and writing–review and editing. SA: formal analysis and writing–review and editing. HA: conceptualization, data curation,

formal analysis, and writing–review and editing. MhA: conceptualization, data curation, formal analysis, and writing–review and editing. MnA: conceptualization, data curation, formal analysis, and writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Effects of time-of-day resistance training on muscle strength, hormonal adaptations, and sleep quality during Ramadan fasting

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Objectives: We investigated the timing of resistance training (RT) during Ramadan fasting (RF) on muscle strength, hormonal adaptations, and sleep quality.

Methods: Forty healthy and physically active male Muslims (age = 25.7 ± 5.6 years, body mass = 85.1 ± 17.5 kg, height = 175 ± 9 cm, BMI = 28.3 ± 5.7 kg/m^2) were enrolled in this study and 37 completed pre and post-tests. Subjects were randomly allocated into two experimental groups. Group 1 (FAST, $n = 20$) completed an 8-week whole-body RT in the late afternoon (between 16 h and 18 h) while fasting. Group 2 (FED, $n = 20$) completed the similar RT protocol compared with FAST at night (between 20 h and 22 h). The following parameters were analyzed at various time-points: 2 weeks before the start of RF (T0), on the 15th day of Ramadan (T1), on the 29th day of Ramadan (T2), and 21 days after the last day of RF (T3) where both groups were in a fed state. One-repetition maximum tests (1-RM) were conducted for the squats (1-RM$_{SQ}$), the deadlift (1-RM$_{DL}$) and the bench press (1-RM$_{BP}$). Sleep quality was assessed using the full Pittsburgh Sleep Quality Index (PSQI). Blood samples were taken to determine cortisol, testosterone and IGF-1 levels. Additionally, acute hormonal responses were evaluated before (BF), immediately after (AF), and 30 min after a RT session (AF-30min) at T0, T1, T2, and T3.

Results: Significant group-by-time interactions were identified for 1-RM$_{SQ}$ ($p = 0.001$; effect size [ES] = 0.43) and 1-RM$_{DL}$ ($p = 0.001$; ES = 0.36). *Post-hoc* tests indicated significant 1-RM$_{SQ}$ ($p = 0.03$; ES = 0.12) and 1-RM$_{DL}$ ($p = 0.04$; ES = 0.21) improvements from T0-T2 for FED. Additionally, significant group-by-time interactions were observed for the chronic effects on cortisol ($p = 0.03$; ES = 0.27) and testosterone levels ($p = 0.01$; ES = 0.32). *Post-hoc* tests indicated significant increases of cortisol levels among FAST at T1 and T2 compared to T0 ($p = 0.05$; ES = 0.41, $p = 0.03$; ES = 0.34) and a significant increase in cortisol levels in FED at T1 ($p = 0.05$; ES = 0.29) and T2 ($p = 0.04$; ES = 0.25). However, the observed increase was lower compared to FAST. *Post-hoc* tests also indicated significant increases of testosterone only among FED at T2 ($p = 0.04$; ES = 0.31). A

significant group-by-time interaction was found for the acute effect of exercise on cortisol level ($p = 0.04$; ES = 0.34). The cortisol level immediately after RT was higher in FAST only at T1 ($p = 0.03$; ES = 0.39) and T2 ($p = 0.05$; ES = 0.22) compared with T0. No significant group-by-time interactions were identified for sleep quality ($p = 0.07$; ES = 0.43).

Conclusion: Muslims can safely practice RT during RF. However, training in a fed state during Ramadan might be more effective than during fasted state for the enhancement of maximal strength with better hormonal responses observed.

KEYWORDS

fasting, muscle performance, training, hormonal adaptation, sleep quality

Introduction

More than 1.9 billion healthy Muslims worldwide fast intermittently during the Ramadan month (RF) as one of the fundamental obligations of the Muslim faith according to the Pew Research Center (1). During this religious month, Muslims abstain from any type of food and fluid intake, engaging in sexual activities, and smoking from dawn *(suhoor)* to sunset *(iftar)* (2, 3). Thus, consuming food and drinks during RF is restricted to the hours of darkness (4). Additionally, the timing of meals and caloric restriction have previously been associated with changes in physiological, chronobiological, and social behaviors (5–8). Hormonal secretions (catecholamines, steroids, growth, and gut hormones) and sleep–wakefulness patterns are modified by circadian changes in food intake and social habits during RF (3, 9, 10).

The effect of engaging in sports during RF was underlined during the London Olympic Games 2012 and also the FIFA Soccer World Cup 2014, as they staged during the RF (11, 12). Some studies reported that training during RF is an effective strategy to improve physical fitness and overall health despite the dietary challenges (13, 14). Regular physical activity during fasting can improve cardiovascular health, reduce the risk of chronic diseases, and improve insulin sensitivity (15). Additionally, integrating sports and physical activity into the life style routine during RF helps to increase spiritual awareness and mindfulness. Many Muslims find that training during RF helpful to connect with their bodies and their faith (16).

Some researchers observed that low energy intake alters the availability and utilization of energetic substrates that can have adverse effects on metabolic, immune, and inflammatory responses and may thus lead to impaired physical fitness (17–20). In addition, insufficient sleep can affect physical fitness by increasing fatigue perception and decreasing mental alertness in response to the same exercise load during RF (8, 21).

It is therefore important to implement effective strategies such as a balanced diet during the non-fasting period, scheduling sports activities, and exercise outside fasting hours, and reducing the intensity and duration of exercise in order to maintain physical performance and adapt to the physiological changes associated with fasting (22).

The maintenance of physical exercise during RF is also challenging for athletes and physically active people who continue resistance training (RT). While there is a plethora of research on the effects of RT applied during RF on measures of body composition and muscular fitness such as muscle power, strength, and local muscular endurance (23, 24), there is limited data on the effects of RT during RF on sleep patterns and hormonal adaptations. Accordingly, we examined the timing of RT during RF when RT is performed either after breaking fast during a fed state to minimize fasting-induced performance degradation (25, 26), or during a fasting state to prevent sleep and hormonal perturbation after night training that could negatively affect muscle strength (27, 28). Additionally, researchers from previous studies also investigated the acute effects of exercise during fasting on hormonal secretion of growth hormones (GH), testosterone, and cortisol levels, which are higher after exercise and remain elevated 60 min after exercise during caloric restriction (29, 30).

Therefore, it is important to choose the right time of day for RT training during RF to minimize the effects of changes in mealtimes and the disruption of hormonal responses and sleep quality during this month, thus offering better physical performance and muscle maintenance. This study aimed to investigate the effects of performing RT in fasted state (FAST, i.e., training before breaking fast) or fed state (FED, i.e., training after breaking fast) on measures of muscle strength, sleep quality, and chronic hormonal responses in healthy and physically active young male adults. A secondary aim was to verify the effects of RT during FED or FAST on acute hormonal responses. With reference to the literature (23, 24), it was hypothesized that RT could be safely practiced during RF with both schedules; and that training after breaking fast may increase muscle strength while preserving normal hormonal responses.

Methods

Experimental approach to the problem

This longitudinal study was conducted during the Ramadan of the lunar year 1442 *Hijri* (from March 12th to April 13th, 2021), with 15 ± 1 h of fasting every day. The study was conducted in Tunisia, where daytime temperatures during the study period were ~ 23 ± 4°C with a relative humidity of 65 ± 5%.

Data on maximal strength, sleep quality, and hormonal adaptations (cortisol, testosterone, and IGF-1) were collected at four different time points: 2 weeks before RF (T0), on the 15th day of (T1), on the 29th day of (T2), and 21 days after the end of Ramadan (T3). All tests were conducted in the same order and time of day during pre and post-tests (see Figure 1).

Participants

The sample size of our study was determined using the power analysis program G*Power (version 3.1.9.3, University of Kiel, Kiel, Germany). The *a priori* power analysis was calculated using the F-test

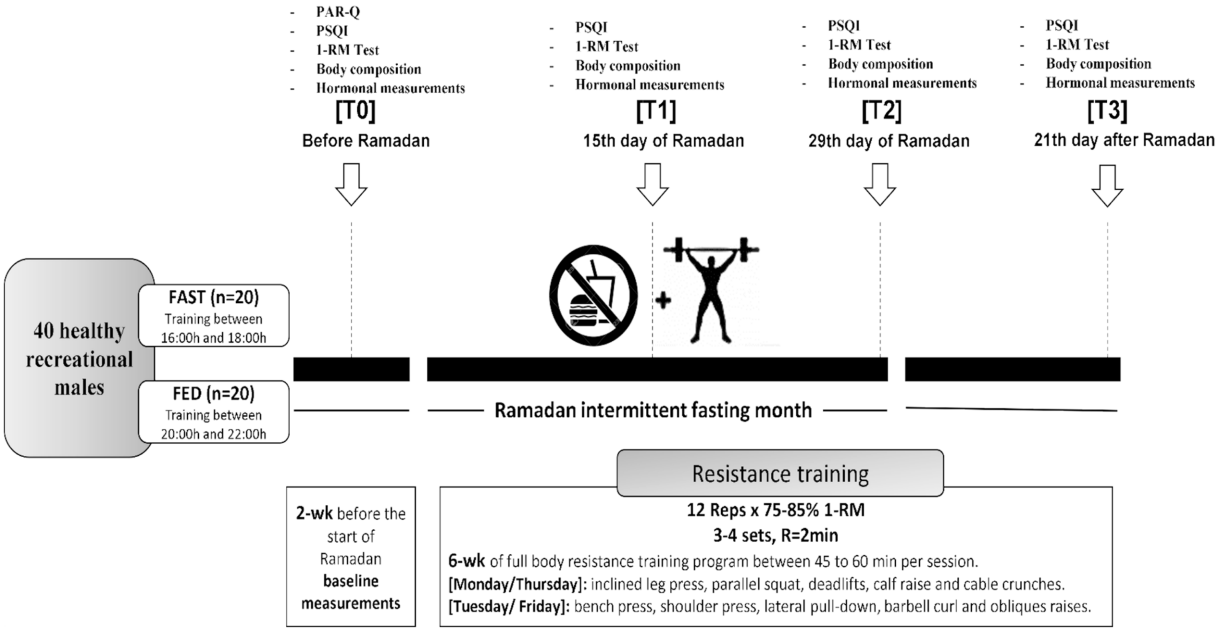

FIGURE 1
Study design. PSQI, Pittsburgh Sleep Quality Index; PAR-Q, physical activity readiness questionnaire; 1-RM, one repetition maximum; R, rest; wk, week; reps, repetitions.

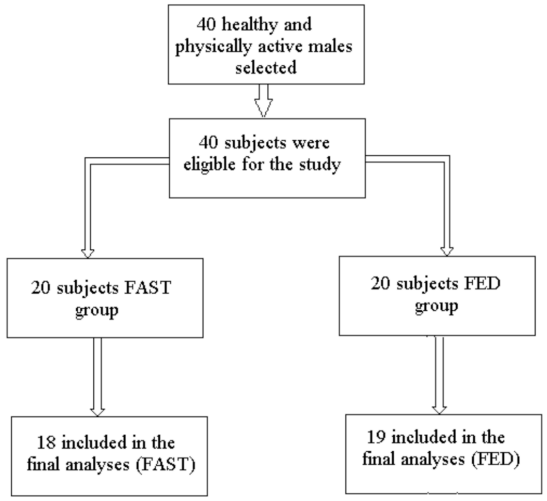

FIGURE 2
Flowchart of the sampling process.

family (i.e., ANOVA repeated measures within-between interaction; power = 0.8, alpha = 0.05, effect size Cohen's $f = 0.3$), and a related study that examined the effects of RF intermittent fasting on 1-RM performance (31). Outcomes from the power analysis indicated that a total sample size of 24 subjects to be sufficient (31). The sample size was increased to 40 to allow for potential dropouts of study participants (Figure 2).

Forty healthy and physically active males aged 25.7 ± 5.6 years (body mass = 85.1 ± 17.5 kg, height = 175 ± 9 cm, BMI = 28.3 ± 5.7 kg/m^2) volunteered to participate in this study. Subjects were randomly assigned into two groups: FAST ($n = 20$) practiced RT in the afternoon before breaking fast (4–6 pm); FED ($n = 20$) practiced RT in the evening 1–2 h after breaking fast (8–10 pm) during RF. We added a control period of 21 days after the end of RF. During this time, both groups continued with the same training program at the same time of day under a normal nutritional regime (fed state). The study goal was to investigate whether the time-of-day training has an effect under the same nutritional conditions in either of the study groups.

Subjects were eligible for the study if they were healthy males aged between 18 and 35 years old, had at least 2 years of RT experience, lacked a history of cardiometabolic diseases or muscle injuries that could have precluded participation in the RT program, did not use medications, ergogenic aids, anabolic steroids or dietary supplements. Individuals had to refrain from other physical exercise during the study duration. All subjects had a medical examination and were fully informed about all experimental procedures, possible discomforts, risks, and benefits of the participation. Thereafter, they were kindly asked to sign the written informed consent form prior to the start of the study. The applied study procedures were in accordance with the principles outlined in the latest version of the Declaration of Helsinki. The study was approved by the local University Ethics Committee on Human Research of the University of Manouba, Tunisia (Ethics Code: Tn, UM2019-73).

Subjects visited the laboratory to become acquainted with all procedures before the start of data collection. The first day of testing was devoted to signing the informed consent form, answering the physical activity readiness questionnaire (PAR-Q), and the Pittsburgh Sleep Quality Index (PSQI), undergoing anthropometric measurements, and completing the one repetition maximum (1-RM) test. On the second test day, the 1-RM tests were repeated to determine the loads that were used during the RT programs and to assess test–retest reliability of the applied tests. During the third test day, blood samples were collected to analyze testosterone, cortisol, and the insulin-like growth factor-1 (IGF-1). The test sessions were separated by 48 h.

Exercise program

A whole-body RT program was performed on 4 days per week (Monday, Tuesday, Thursday, and Friday) for 8 weeks (1 week before Ramadan, 4 weeks during Ramadan, and 3 weeks after the end of Ramadan). Each workout included five exercises that were repeated twice. The applied exercises on Monday and Thursday involved the inclined leg press, the parallel squat exercise, deadlifts, calf raises, and cable crunches. On Tuesday and Friday, subjects performed the bench press, the shoulder press, the lateral pull-down, the barbell curl, and obliques raises.

Each exercise session lasted 45–60 min and started with a general warm-up (10 min) and included a cool-down period (5–10 min) of low-intensity aerobic and dynamic stretching exercises. Subjects performed 4 sets × 12 repetitions at 75–85% of the 1-RM for every exercise with 2 min of passive recovery between sets and exercises. Exercise intensities were gradually increased during the 8-week training period, from 75% of the 1-RM at the start of the program to 85% of the 1-RM at week 3, until the end of the intervention. Each Subject was individually supervised by an experienced strength and conditioning specialist (RT) who managed load progression to ensure that each exercise was performed with adequate exercise technique during the concentric and eccentric phases of movement. Time under tension amounted to 2 s during the concentric phase and 2 s during the eccentric phase. The person responsible for exercise supervision monitored compliance and individual workout data for each exercise session (e.g., number of repetitions and sets, rest, movement velocity, and intensity).

Anthropometric tests

Body composition was measured in the morning (10:00 a.m.) at T0, T1, T2, and T3 following an overnight fast (10:00 h) and after more than 36 h following the last exercise bout.

Height was measured using a stadiometer. Body mass was assessed using an electronic scale with a resolution of 50 g (Medisana®, Neuss, Germany). The percentage of body fat was determined with a Harpenden caliper (Baty international. RH15 9LR. England) in four skinfolds (biceps, triceps, subscapular, and suprailiac) (32). Lean body mass (LBM) was calculated as body mass minus body fat mass. All measurements were performed by the same investigator following standardized procedures (33).

Assessment of sleep quality

Sleep quality was assessed directly after taking the anthropometric and body composition measurements at T0, T1, T2, and T3 using the full Pittsburgh Sleep Quality Index (PSQI) validated for the Arabic language (34). The global PSQI score is composed of seven components: subjective sleep quality (individual's sleep quality perception), sleep latency (time between lying in bed and starting sleeping), sleep duration (period sleeping reported by the individual), habitual sleep efficiency (ratio between the time sleeping and the time in bed), sleep disturbance (problems to sleep expressed by subjects), sleeping medication (use of medication to sleep), and daytime dysfunction (intensity of diurnal somnolence) (35). The seven domains were separately scored from 0 (no difficulty) to 3 (very difficult), with a total or global score ranging from 0 to 21 to reflect sleep quality and disturbances over a 1-month period. Better sleep quality is indicated through have a lower global PSQI score, while higher scores indicate poor sleep quality.

Maximal strength tests

The 1-RM tests for the squat, bench press, and deadlift exercises were determined for each subject at T0, T1, T2, and T3 to evaluate the development of maximal strength during the study. Additionally, 1-RM tests were re-assessed every 2 weeks to maintain the targeted exercise intensity (75–85% 1-RM). The 1-RM testing and the start of the training sessions were separated by 10 min of rest to allow for an appropriate recovery.

Subjects were asked to perform 10 repetitions with the estimated 10-RM as a warm-up before the start of the test for each exercise using previously described procedures (36, 37). The 1-RM was determined with a maximum of six attempts, with a 5-min rest period between each attempt (37). In addition, a 3-min rest was allowed between the tests. Pilot data were obtained on 2 test days from 40 subjects to determine test–retest reliability. Our data revealed the following ICCs 1-RM parallel squat (0.929; 95%CI lower 0.929–upper 0.996), 1-RM bench press (0.779; 95%CI lower 0.581–upper 0.883), 1-RM deadlift (0.886; 95%CI lower 0.783–upper 0.940).

Blood samples

Four venous blood samples were taken at T0–T3. A heparinized catheter (Insyte-W, 1.1 mmo.d. × 30 mm, Biopol, Tunis, Tunisia) was inserted into an antecubital vein by using a 20-gauge needle and Vacutainer tubes before, at 0 min (immediately after), and 30 min after the training sessions. The blood was collected in test tubes containing EDTA and centrifuged at 1,500 × *g* for 10 min at 4°C, resultant serum was then removed and stored at −80°C until subsequently testosterone (nmol/L), cortisol (nmol/L), and IGF-1(ng/mL) were analyzed using radioimmunoassay commercial kits (Beckman Coulter and Diagnostic Systems Laboratories, France) according to the manufacturer's procedures. The limits of sensitivity were 0.5 nmol/L for testosterone, 5.79 nmol/L for cortisol, and 3 ng/mL for IGF-1. The intra-assay coefficients of variation were between 0.9 and 4.6% for testosterone, between 2.24 and 6.01% for cortisol, and between 6.3 and 6.8% for IGF-1. All samples were assayed in duplicate and were decoded only after the analyses were completed (i.e., blinded analysis procedure). All samples were analyzed on the same assay for each analyte to eliminate inter-assay variance.

Dietary habits

Participants were instructed to record the estimated quantities of all food and beverages consumed for at least 3 days per week (2 week-day and 1 day on the weekend), to offer a reliable estimate of food intake for the entire week. Total water intake was defined as the fluid volume of consumed beverages plus the water content of consumed foods.

TABLE 1 Estimated daily dietary intake (mean ± SD) recorded at T0, T1, T2, and T3 in FAST and FED groups ($N = 37$).

	Group	Phases				*p* values (ES)		
		T0	T1	T2	T3	Time	Group	Group × Time
Protein (% kcal)	FAST	18 ± 7	18 ± 9	18 ± 3	18 ± 8	0.07 (0.43)	0.07 (0.56)	0.1 (0.57)
	FED	18 ± 3	18 ± 7	18 ± 9	18 ± 7			
Carbohydrate (% kcal)	FAST	48 ± 2	47 ± 8	47 ± 3	47 ± 2	0.1 (0.35)	0.06 (0.54)	0.08 (0.45)
	FED	48 ± 3	46 ± 9	46 ± 9	46 ± 7			
Fat (% kcal)	FAST	36 ± 8	36 ± 5	36 ± 3	35 ± 7	0.09 (0.52)	0.1 (0.38)	0.08 (0.43)
	FED	36 ± 9	36 ± 3	37 ± 5	36 ± 5			
Energy (kcal/day)	FAST	3,456 ± 265	3,449 ± 270	3,435 ± 291	3,456 ± 275	0.2 (0.43)	0.08 (0.55)	0.08 (0.46)
	FED	3,481 ± 268	3,431 ± 237	3,471 ± 218	3,472 ± 232			
Total water intake (L/day)	FAST	4.3 ± 0.8	4.2 ± 0.4	4.3 ± 0.5	4.2 ± 0.7	0.06 (0.57)	0.08 (0.35)	0.1 (0.36)
	FED	4.2 ± 0.9	4.1 ± 0.7	4.3 ± 0.2	4.1 ± 0.9			

A nutritional tracking application[1] and the food composition tables of the National Institute of Statistics of Tunis (1978) were used to determine the distribution of macronutrients and the calories ingested. Each food item was individually entered into the application to provide data on total energy consumption, and the amounts of energy derived from proteins, fats, and carbohydrates for each period analyzed. The mean of nutrients consumed during the intervention was calculated using the method described by McCance and Widdowson (38).

Protein intake was supervised by a specialist to ensure that dietary protein needs were met. Subjects were instructed to consume dietary supplements on the training days containing 24 g of protein and 1 g of carbohydrate before sleeping (Iso100 Hydrolyzed Whey Protein Isolate; Dymatize Nutrition, Dallas, TX). There were no differences in the estimated nutrients consumed during the study between the experimental groups (Table 1).

Statistical analyses

Data were analyzed using SPSS software (v. 16.0, SPSS Inc., Chicago, IL, United States) and were expressed as means ± standard deviations (SD). The normality of data distribution was confirmed using the Shapiro–Wilk test. The effects of time of training during RF were evaluated using a 2 (groups: FAST, FED) × 4 (time: T0, T1, T2, T3) mixed model ANOVA. Additionally, hormonal data were assessed using a 2 (groups: FAST, FED) × 3 (time: BF, AF, AF-30 min) mixed model ANOVA. Where significant group-by-time interactions occurred, *post hoc* tests were computed using the Bonferroni adjustments to identify group-specific changes over time.

Effect sizes (ES) were calculated using ANOVA output by converting partial eta-squared η_p^2 to Cohen's *d* values. In addition, within-group ES were computed using the following equation: ES = (mean post—mean pre)/SD. ES were considered trivial (<0.2), small (0.2–0.6), moderate (0.6–1.2), large (1.2–2, 0), and very large (2.0–4.0) (39). The level of statistical significance was set at $p < 0.05$. Means, standard deviations, and 95% confidence intervals (CI) were presented for all data.

Results

Three subjects (one from FED and two from FAST) were not included in the final analyses due to low compliance (<90%). The remaining subjects ($N = 37$) met the 100% adherence requirement for continued participation in the study. There were no between-group differences for any anthropometric measures, strength parameters, and diurnal variation of hormonal secretions at baseline between the two groups. ES magnitudes ranged from ignored, small to moderate for all measurements.

Anthropometric tests

Means, standard deviations, and 95% CI of anthropometric parameters and body composition characteristics are presented in Table 2 for the study sample. No significant group × time interactions were found for all anthropometric measurements. However, a significant main time effect was reported for both FAST and FED for body mass ($p = 0.001$; ES = 0.37), BMI ($p = 0.001$; ES = 0.32), and body fat ($p = 0.001$; ES = 0.41) during Ramadan, with no significant main time effect for lean body mass ($p = 0.57$; ES = 0.42).

Maximal strength tests

Table 3 contains the primary findings with regards to maximal strength performance. There were significant group × time interactions for 1-RM_{SQ} ($p = 0.001$; ES = 0.43) and 1-RM_{DL} ($p = 0.001$; ES = 0.36). *Post-hoc* tests indicated significant increases for 1-RM_{SQ} ($p = 0.03$; ES = 0.13) and 1-RM_{DL} ($p = 0.04$; ES = 0.21) from T0-T2 among the FED group. No significant group × time effect was found for 1-RM_{BP} ($p = 0.68$; ES = 0.21). At T3, significant improvements were observed for 1-RM_{SQ} ($p = 0.04$; ES = 0.24), 1-RM_{BP} ($p = 0.04$; ES = 0.20), and 1-RM_{DL} ($p = 0.02$; ES = 0.18) in the FED group and for 1-RM_{SQ}

1 http://www.myfitnesspal.com

TABLE 2 Anthropometric and body composition characteristics (means ± SD) at T0, T1, T2 and T3 in FAST and FED groups (N=37).

Variables	Group	Phases				p values (ES)		
		T0	T1	T2	T3	Time	Group	Group x Time
Body mass (kg)	FAST	78.88±3.65	75.79±3.05*	74.01±3.14*#	77.10±4.68$	**0.001 (0.37)**	**0.06 (0.24)**	**0.79 (0.12)**
	FED	80.00±3.53	78.81±3.18*	76.08±2.99*	78.61±3.54$			
BMI (kg/m^2)	FAST	24.16±1.24	23.66±1.29*	22.35±1.31*#	23.66±1.15$	**0.001 (0.32)**	**0.28 (0.29)**	**0.58 (0.20)**
	FED	24.69±1.48	23.94±1.13	23.26±1.28*	24.02±1.25$			
Body fat (%)	FAST	16.61±1.14	15.38±1.52*	13.84±1.45*#	14.90±1.29*$	**0.001 (0.41)**	**0.98 (0.12)**	**0.4 (0.17)**
	FED	16.34±0.53	15.38±1.52*	13.95±1.56*#	15.01±1.34*$			
LBM (kg)	FAST	66.29±2.40	65.15±3.00	64.64±3.09	65.31±3.54	**0.57 (0.42)**	**0.06 (0.21)**	**0.84 (0.17)**
	FED	67.02±2.08	66.61±2.61	66.56±2.72	67.22±3.03			

BMI: body mass index; **LBM:** lean body mass; *: p< 0.05 from the corresponding time point T0; **#:** p< 0.05 from the corresponding time point T1; **$:** p<0.05 from the corresponding time point T2.

TABLE 3 One-repetition maximum (1-RM) changes (means ± SDs) at T0, T1, T2 and T3 in FAST and FED groups (N=37).

Variables	Group	Phases				p values (ES)		
		T0	T1	T2	T3	Time	Group	Group x Time
1-RM$_{SQ}$ (kg)	FAST	169.41±14.67	170.29±13.13	167.24±12.60	172.53±11.79*#	**0.001 (0.35)**	**0.39 (0.12)**	**0.001 (0.43)**
	FED	170.47±11.61	173.21±11.75	175.56±11.35*	178.00±12.35*#			
1-RM$_{BP}$ (kg)	FAST	106.47±10.21	110.94±11.70	110.29±10.87	120.12±11.27*	**0.001 (0.48)**	**0.66 (0.13)**	**0.68 (0.21)**
	FED	105.29±10.97	108.06±10.88	110.59±11.14	117.06±10.76*			
1-RM$_{DL}$ (%)	FAST	187.06±8.89	187.06±9.83	187.65±9.86	188.65±8.17*#	**0.001 (0.43)**	**0.04 (0.21)**	**0.001 (0.36)**
	FED	187.94±7.96	191.88±7.88	197.24±8.22*	199.59±8.67*#			

1-RM: one-repetition maximum; **SQ:** squat; **BP:** bench press; **DL:** deadlift; **QC:** quadriceps; **BB:** biceps brachii; *: p< 0.05 from the corresponding time point T0; **#:** p< 0.05 from the corresponding time point T1; **$:** p<0.05 from the corresponding time point T2.

($p = 0.02$; ES = 0.18), 1-RM$_{BP}$ ($p = 0.02$; ES = 0.23), and 1-RM$_{DL}$ ($p = 0.04$; ES = 0.19) in the FAST group in comparison with T0.

Sleep quality

Means, standard deviations, and 95% confidence intervals (CI) of the Pittsburgh Sleep Quality Index at T0, T1, T2, and T3 among FAST or FED groups are reported in Table 4. No significant group × time interaction was found for the total score of the PSQI ($p = 0.07$; ES = 0.43). There was a main effect of time for the total score of PSQI. A higher global PSQI score was observed RF in both groups at T1 ($p = 0.02$; ES = 0.36 for FAST and $p = 0.04$; ES = 0.26 for FED) and T2 ($p = 0.001$; ES = 0.46 for FAST and $p = 0.02$; ES = 0.35 for FED) compared to T0. Total PSQI scores were lower 21 days after RF (T3) compared to T1 ($p = 0.01$; ES = 0.32 for FED and $p = 0.01$; ES = 0.27 for FAST) and T2 ($p = 0.001$; ES = 0.16 for FED and $p = 0.03$; ES = 0.18 for FAST).

Blood samples

Means, standard deviations, and 95% confidence intervals (CI) of the acute and chronic effects of exercise (FED, FAST) during RF are illustrated in Figure 3.

Significant group × time interactions were identified for the chronic RT effects on measures of cortisol ($p = 0.03$; ES = 0.27). *Post-hoc* tests indicated significant increases in cortisol levels in the FAST group at T1 and T2 compared to T0 ($p = 0.05$; ES = 0.41 and $p = 0.03$; ES = 0.34). Significant increases in cortisol levels also occurred in the FED group at T1 ($p = 0.05$; ES = 0.29) and T2 ($p = 0.04$; ES = 0.25) compared to T0. However, the increases were significantly lower compared to the FAST group. Cortisol levels decreased after the Ramadan, and no significant differences were found for cortisol level at T3 compared with T0 among both groups ($p > 0.05$).

Significant group × time interactions occurred with regards to the chronic RT effects for testosterone levels ($p = 0.01$; ES = 0.32). *Post-hoc* tests indicated significant increases of testosterone among FED at T2 and T3 compared to T0 ($p = 0.04$; ES = 0.31 and $p = 0.03$; ES = 0.42). Additionally, testosterone levels remained significantly elevated after the end of RF at T3 vs. T0 ($p = 0.05$; ES = 0.28) and T3 vs. T1 ($p = 0.03$; ES = 0.25).

A significant group × time interaction occurred for cortisol levels ($p = 0.04$; ES = 0.34) for the acute effects of exercise. The level of cortisol immediately after RT was higher at T1 ($p = 0.03$; ES = 0.39) and T2 ($p < 0.05$; ES = 0.22) compared with T0 among the FAST group. However, there were no significant differences in the cortisol levels between T0 and T3 (i.e., for the acute effects of exercise) in the FAST group, immediately after RT.

No significant group × time interactions were detected for IGF-1 ($p = 0.07$; ES = 0.27).

Discussion

This study was conducted with healthy young and RT experienced males. In this study, we aimed to evaluate the effects of

TABLE 4 Sleep quality (mean ± SD) recorded at T0, T1, T2, and T3 in the FAST and FED groups ($N = 37$).

	Group	Phases				*p* values (ES)		
		T0	T1	T2	T3	Time	Group	Group x Time
Sleep quality	FAST	1.2 ± 0.6	2.3 ± 0.8*	2.3 ± 0.7*	1.3 ± 0.7#$	**0.006 (0.58)**	**0.08 (0.36)**	**0.09 (0.42)**
	FED	1.1 ± 0.7	2.4 ± 0.4*	2.4 ± 0.5*	1.2 ± 0.6#$			
Sleep latency	FAST	0.7 ± 0.4	0.7 ± 0.2	0.8 ± 0.4	0.7 ± 0.4	**0.07 (0.37)**	**0.1 (0.47)**	**0.1 (0.46)**
	FED	0.8 ± 0.1	0.7 ± 0.4	0.7 ± 0.2	0.8 ± 0.2			
Sleep duration	FAST	1.1 ± 0.5	1.3 ± 0.2*	1.3 ± 0.9*#	1.2 ± 0.5#$	**0.001 (0.45)**	**0.2 (0.34)**	**0.07 (0.65)**
	FED	1.1 ± 0.7	1.2 ± 0.3*	1.3 ± 0.7*#	1.1 ± 0.2#$			
Sleep efficiency	FAST	0.8 ± 0.2	0.8 ± 0.6*	0.8 ± 0.5*	0.7 ± 0.4#$	**0.004 (0.55)**	**0.07 (0.24)**	**0.08 (0.67)**
	FED	0.7 ± 0.5	0.8 ± 0.7*	0.8 ± 0.8*	0.7 ± 0.2#$			
Sleep disturbance	FAST	0.7 ± 0.5	0.7 ± 0.4	0.7 ± 0.5	0.6 ± 0.8	**0.08 (0.35)**	**0.08 (0.62)**	**0.1 (0.55)**
	FED	0.6 ± 0.9	0.7 ± 0.3	0.6 ± 0.7	0.6 ± 0.7			
Daytime dysfunction	FAST	0.4 ± 0.7	0.4 ± 0.4	0.4 ± 0.8	0.5 ± 0.1	**0.07 (0.45)**	**0.07 (0.38)**	**0.08 (0.56)**
	FED	0.5 ± 0.3	0.4 ± 0.7	0.5 ± 0.8	0.4 ± 0.3			
Total score of PSQI	FAST	3.7 ± 2.1	7.1 ± 2.3*	7.2 ± 2.1*	3.8 ± 2.2#$	**0.002 (0.42)**	**0.07 (0.56)**	**0.07 (0.43)**
	FED	3.7 ± 2.3	7.3 ± 2.1*	7.2 ± 2.6*	4.1 ± 1.1#$			

PSQI, Pittsburgh Sleep Quality Index.
*$p < 0.05$ from the corresponding time point T0; #$p < 0.05$ from the corresponding time point T1; $$p < 0.05$ from the corresponding time point T2.

RT applied during RF on muscular strength, sleep quality, and selected blood hormonal concentrations (chronic and acute effects) in a FED state vs. in a FAST state. To the best of our knowledge, this is the first study that investigates the importance of choosing the adequate day time RT training for a better muscle performance, taking into consideration the effects on sleep quality and hormonal secretions.

The key findings of our study include: (1) significant improvements for 1-RM_{DL} and 1-RM_{SQ} only in FED during RF (T1), however, while after the end of RF (T2) 1-RM_{DL}, 1-RM_{SQ}, and 1-RM_{BP} improved in both groups (2) significant chronic effects on hormonal concentrations was detected in both groups, cortisol level increased among FAST at the second day of RF (T1) and lasted till its end (T2), while testosterone increased in FED at the last week of the RF (T1) and remained to increase even after the end of RF (T3); (3) significant increases in cortisol levels were reported only among FAST, increased cortisol levels immediately after RT sessions during RF (T1) were higher than before the start of RF, while they returned to the normal level after the end of RF (T2). No significant between group differences were observed for sleep quality during the experiment.

The observed significant deadlift and squat improvements were found in the FED group only and might be due to adequate pre-exercise energy and fluid intake levels. The RT sessions of FED occurred at 21:00 h under favorable conditions as the athletes were able to refuel and rehydrate for ~90 min before the exercise sessions, while the FAST group trained after at least 13 h of fasting. Previous studies reported that the athletes' pre-exercise blood glucose concentration levels and fluid balance were within the normal range during the evenings, while training at 18:00 h was the least favorable time of day for training as the athletes abstained from food and fluid for more than 10 h (40).

The effects of RT applied during RF on measures of muscle strength were also reported by others who obtained similar results (41, 42). Authors from previous studies indicated that the optimal time of day to perform high-intensity exercises during RF is in the evening, after breaking the fast. Training-induced adaptations might be diminished during the fasting state of RF (25, 40).

Hormonal changes were also reported in our study, and showed significant increases in cortisol levels in FAST likely due to a chronic effect of training during fasting. Increases in cortisol levels were also higher after breaking fast (FED) compared to before RF levels, but remained within ranges. The modification of the time difference between the food and water intakes and training sessions could underly increases in cortisol levels in FAST compared with pre-RF levels and to the FED state group. The higher level of cortisol concentration during fasting is a response to a catabolic process to restore glucose needs by stimulating gluconeogenesis, proteolytic activity and increasing skeletal protein degradation after 12 h of fasting (44). Increases in cortisol levels during RF were also reported previously (45–47). However, some studies observed decreases in cortisol levels in judo athletes after a higher training load during RF (12). One reason for this discrepancy could be the timing of blood sampling collection, which occurred between 8:00 h and 10:00 h in the later study (12), and in the afternoon as in our study.

The cortisol levels immediately after a single bout of RT in FAST were also higher compared to pre-RF levels. Differences in the magnitude of acute RT-induced cortisol responses may be due to the effects of a new physiological stress (fasting) that could lead to increased catabolic processes to mobilize fatty acids from fat reserves, and activation of protein breakdown from muscle tissue to raise blood glucose concentrations during food restriction during exercise in the FAST state (48). Similar study results were reported previously. The reported increases in cortisol levels after exercise in a fasting state (caloric restriction) were greater than in fed state (30, 49).

Significant increases of testosterone levels were only found in the FED state group during RF, which may favor physiological responses to modulate the balance between hormone-mediated anabolic and catabolic activities after the slight increase of cortisol concentrations during RF. Thus, the observed increase in muscle strength in FED can be explained by favoring anabolic functions of testosterone in skeletal

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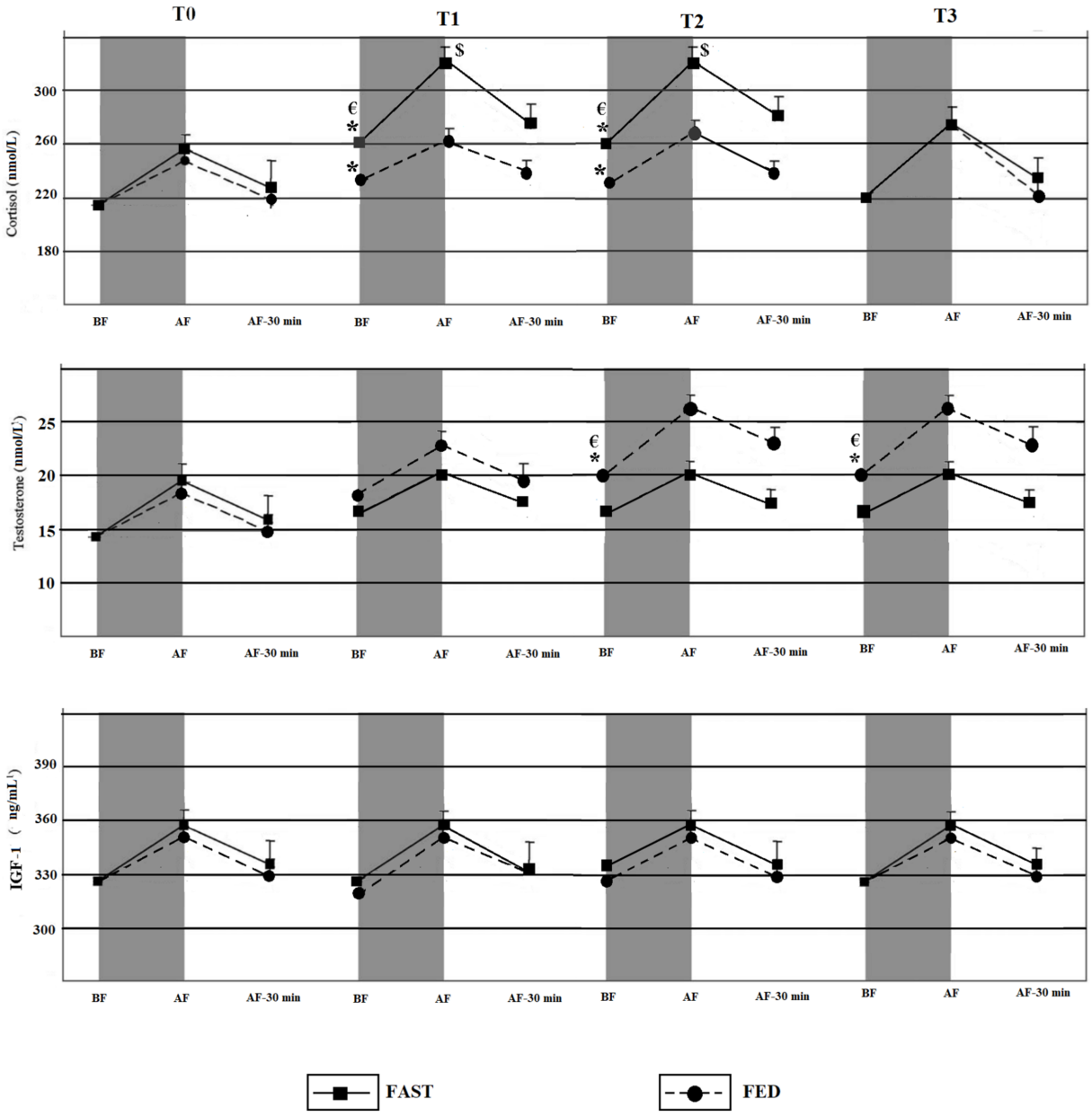

FIGURE 3
Acute and chronic effects of training during RF among FED and FAST groups. BF, Before exercise; AF, Immediately after exercise; AF-30 min, 30 min after exercise. *Significant time effect compared to T0 ($p < 0.05$); €Significant group × time chronic effect of training ($p < 0.05$); $significant group × time acute effect of exercise ($p < 0.05$).

muscle and neuronal tissue (50, 51). Similar findings were reported by Mira et al. (52) who observed significant increases in testosterone during RF in the evening hours and decreases in the morning. These findings were explained by the effects of RF on changes in the circadian rhythm and metabolic regulation during RF (52). The effects of fasting and training were also investigated in other studies, where testosterone levels were unchanged when measured before breaking the fast during RF (17, 53). To the best of our knowledge, no study investigated the effects of training times on testosterone secretion during RF.

Additionally, there were continued increases in testosterone levels only in the FED group even after the end of Ramadan (T3) in comparison with T0 and T1. Researchers from previous studies reported elevations in testosterone levels after long-term resistance training (8 weeks of training) in a non-fasting state while there were no increases in the FAST group (T3) undergoing only 3 weeks of training in a fed state (45, 46).

There were non-significant changes in IGF-1 levels in the two study groups. IGF-1 levels can be affected by food restriction and dehydration during RF or by changes in physical activity levels (54, 55). This was not the case in our study where there were no differences in total energy and water intake, or in total training volume between the two study groups. However, this non-significant change (tendency to decrease) may be due to increased cortisol levels which can decrease the skeletal IGF-I synthesis (44).

Sleep is an essential component of improving performance by promoting post-training recovery (56). Previous studies indicated that training at night causes sleep disturbances due to high pre-sleep arousal levels, especially if it occurs during 3 h before going to bed

(57). Researchers from other studies reported that RF affects the circadian rhythm and produces modifications in the sleep quality with significant reductions in total sleep time (58, 59).

Our results showed a non-significant group × time effect for the factor day time training on sleep quality. However, significant main time effects were found for sleep quality during RF fasting. In fact, the Ramadan month affords substantial lifestyle adjustments and traditions involving longer nightly prayers, sleep disturbances due to the timing of *suhur* meal with poor sleep experiences.

Study limitations

This study was conducted during the COVID partial lock down period, and training sessions for the FED group occurred between 20:00 h and 22:00 h before gym closing times (as mandated by the Tunisian Government), making it important to study late-night RT and its effects on sleep quality and hormonal secretion.

Additionally, it could be useful to add a third day-time point to investigate the effects of RT after a short time after starting fasting. In fact, a morning training in a FAST state, after a short time of taking *suhur* meal could provide important insights.

Practical applications

Our study examined the effects of RT during RF on body composition, strength performance, and hormonal secretion. The results of our study suggest that RT during RF induces favorable changes in body composition (loss of body fat, maintaining lean tissue). Moreover, practicing RT after breaking fast (Fed state) improved muscle strength, probably due to a more favorable anabolic and hormonal status compared with the FAST. Thus, the results of this study can guide coaching strategies for strength and conditioning specialists. Choosing the appropriate time of day to practice RT training during RF is an important consideration for recreational weightlifters, and also for athletes from other (strength-dominated) sports to avoid physiological and psychological challenges that occur during this month of daily fasting.

Conclusion

The aim of this study was to investigate the appropriate time of day for recreational athletes who continue to train during RF to maintain and improve muscle performance in order to minimize the effects of meal timing changes on muscle performance. Our study demonstrated that RT applied after breaking fast in a FED state improved energy intake and testosterone secretion, thus enhancing muscle strength. Additionally, practicing RT in a FAST state had no adverse effects on measures of muscle strength when maintaining the same training volume and energy intake.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The applied study procedures were in accordance with the principles outlined in the latest version of the Declaration of Helsinki. The study was approved by the local University Ethics Committee on Human Research of the University of Manouba, Tunisia (Ethics Code: Tn, UM2019-73). The study was conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

RT: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. AAb: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Writing – original draft, Writing – review & editing. IS: Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – review & editing. FR: Data curation, Investigation, Methodology, Software, Validation, Visualization, Writing – review & editing. AS: Data curation, Formal analysis, Validation, Visualization, Writing – review & editing. AAl: Data curation, Formal analysis, Funding acquisition, Resources, Software, Validation, Writing – review & editing. AH: Data curation, Formal analysis, Validation, Writing – review & editing. IL: Data curation, Formal analysis, Validation, Writing – review & editing. UG: Data curation, Formal analysis, Resources, Validation, Writing – review & editing. HZ: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer KI declared a past co-authorship with the authors UG and HZ to the handling editor.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

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The effect of Ramadan intermittent fasting on anthropometric, hormonal, metabolic, inflammatory, and oxidative stress markers in pre-and post-menopausal women: a prospective cohort of Saudi women

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Background: The menopausal transition significantly affects cardiometabolic health, primarily due to changes in reproductive hormones, particularly decreased estrogen levels and relative androgen excess. Adult Muslim women, both pre-and post-menopausal, are mandated to observe Ramadan intermittent fasting (RIF) every year. Therefore, the current study was designed to investigate RIF's effects on pre-menopausal (PRE-M) and post-menopausal (POST-M) healthy women's cardiometabolic health markers. This study further evaluated the relationship between tested markers and the participant's basic variables, such as BMI and body fatness. Due to differences in physiological and metabolic biomarkers between groups, RIF is likely to impact PRE-M and POST-M women differently.

Methods: This study included 62 healthy women (31 PRE-M, aged 21–42 years, and 31 POST-M, aged 43–68 years) who observed RIF. Anthropometrics, sex hormones, lipid profile, pro-inflammatory (TNF-α), anti-inflammatory (IL-10) cytokines, the oxidative stress markers malondialdehyde (MDA), total antioxidant capacity (TAC), superoxide dismutase (SOD), glutathione peroxidase (GPx), and aging biomarker insulin-like growth factor-1 (IGF-1); all were tested 1 week before and at the fourth week of Ramadan.

Results: Body weight, BMI, waist circumference, body fat percentage (BFP), fat mass, fat mass index, triglycerides, and diastolic blood pressure significantly ($p < 0.05$) decreased at the end of Ramadan in both groups in comparison to the pre-fasting period. Contrarily, HDL, SOD, GPx, and IL-10 significantly ($p < 0.05$) increased in both groups. Estrogen levels significantly ($p < 0.05$) decreased in PRE-M women, whereas significantly ($p < 0.05$) increased in POST-M women. The progesterone levels, TAC, MDA, and IGF-1 remained unchanged in both groups. TNF-α significantly decreased in both groups, but the magnitude of

reduction was higher in PRE-M women. Sex hormones and some metabolic biomarkers, especially in POST-M women, variably exhibited positive or negative relationships to BMI and BFP. RIF may influence the levels of estrogen, TNF-α, and IL-10 through improvements in metabolic health, reductions in body fat, activation of autophagy, modulation of immune responses, and changes in hormonal regulation.

Conclusion: The RIF was generally associated with improved anthropometric, metabolic, inflammatory, and oxidative stress markers in both PRE-M and POST-M healthy women. Adhering to healthy dietary and lifestyle guidelines by pre-and post-menopausal women during Ramadan may foster the health benefits gained.

KEYWORDS

intermittent fasting, diet, anthropometric, cytokines, oxidative stress, antioxidant, menopausal

Introduction

The transition from pre-menopausal (PRE-M) to post-menopausal (POST-M) phase is associated with drastic changes in many hormonal and metabolic markers. After menopause, women exhibit a sustained increase in hemodynamic load and altered sympathetic nervous system activity that may contribute to pathological functional and structural modifications in the blood vessels and heart (1). Modifications in estradiol and progesterone levels may cause changes during the PRE-M phase (2). Some studies (3, 4) revealed inconsistent results regarding the effects of IF and RIF on reproductive hormones in healthy women. According to Ko and Kim (5), due to hormonal changes during the menopausal transition period, such as decreased estrogen levels and increased levels of circulating androgens, a variety of lipid metabolic disorders emerge. These disorders may cause metabolic syndromes, including cardiovascular diseases and type 2 diabetes. They also stated that lipid metabolism abnormalities have an impact on body fat mass, fat-free mass, fatty acid metabolism, and many elements of energy metabolism, such as basal metabolic ratio, adiposity, and obesity. Menopause is also connected with changes in the amounts of various lipids in the blood, which causes lipid peroxidation and the development of insulin resistance, abdominal obesity, and dyslipidemia (5). According to El Khoudary et al. (6), the menopause transition has a substantial impact on cardiovascular health and results in a variety of lifestyle and dietary modifications. Furthermore, they discovered different patterns of sex hormone alterations and negative changes in body composition, lipoproteins, lipids, and vascular health during menopause. These modifications raise the likelihood of acquiring POST-M illnesses.

Various physiological effects and positive findings have been reported for caloric restriction and intermittent fasting (IF) in men and rodents on several disease conditions, including prolonged lifespan, decreased aging and its neurological diseases, cardiovascular diseases, and cancer-related mortalities, enhanced insulin sensitivity, and reduced inflammation, and oxidative stress (7–11). IF can affect energy signaling pathways that are regulated by cAMP-responsive element binding protein (CREB) and AMP-activated protein kinase (AMPK), as well as the pro-growth mTOR pathways and the expression of circadian clock genes. The fasting-sensitive AMPK is activated by elevated AMP levels, which can subsequently influence the circadian clock by modulating essential circadian regulators (12).

Ramadan fasting is a holy month for Muslims during which all adult healthy Muslims are mandated to abstain from food, drink (including water), and sexual activities from dawn to sunset. Thus, the month of Ramadan is associated with drastic lifestyle and dietary modifications. The main aspects of modification include changing food consumption patterns (13, 14), reduced physical activities (15), and night sleep periods with increased sleep intervals during the day (16). Considering that people alternate between fasting and feasting within 24 h, Ramadan fasting is a typical form of IF, and it is regarded as the most commonly and globally observed religious form of IF (17).

Epidemiological studies have established the beneficial impacts of observing Ramadan intermittent fasting (RIF), including body weight reduction (18), reduced body fat (19, 20), especially visceral fat (19), normalizing glucometabolic regulation (20), cardiometabolic (21), and liver functioning (22), accompanied by metabolomic (23), lipidomic (24), and microbiomic alterations (25). Improvements in metabolic health (21), reductions in body fat (26), activation of autophagy (27), modulation of immune responses (28), and changes in hormonal regulation (29, 30) are among the proposed mechanisms underpinning the reported health effects of observing RIF.

Sex, presented in terms of the body's sex hormones, directly impacts metabolic reactions in both males and females, and determines the magnitude of changes in anthropometric, metabolic, and physiological aspects, as well as dietary changes upon the observance of RIF in both healthy and disease conditions. This emphasizes the role of sex as one of the determinant factors that affect the health outcomes of observing RIF (31, 32) and necessitates examining RIF impacts on the two sexes separately.

Observing RIF presents unique health implications for both pre-and post-menopausal women. For pre-menopausal women, hormonal fluctuations may be affected by IF, potentially leading to altered menstrual patterns (33). During Ramadan, weight management can become a challenge, as fasting may result in weight loss or gain depending on dietary choices during non-fasting hours (13, 18). Improved insulin sensitivity and improved lipid profiles are possible benefits of observing RIF (20, 21, 34); however, there is a risk of hypoglycemia for those with metabolic conditions (35). For menopausal women, the effects of fasting can vary significantly due to decreased estrogen levels, which can lead to symptoms like hot flashes

(36). Additionally, menopausal women need to consider bone health, as adequate calcium and vitamin D intake is crucial during Ramadan to counter osteoporosis risk (37, 38). Fasting, including RIF, can also improve cardiovascular health and reduce inflammation, benefiting menopausal women who may suffer from related conditions (21, 39, 40).

Considering the vast metabolic and hormonal effects of practicing fasting in general, and RIF in particular, it becomes rationalized and necessary to examine the effect of the observance of RIF by pre-and post-menopausal Saudi women and to elaborate how different health markers change before and during the fasting days of Ramadan. Therefore, this study aimed to assess the effects of RIF on PRE-M and POST-M women's anthropometric indices, lipid profiles, and metabolic biomarkers. This study further evaluated the relationship between the tested biomarkers and participant variables, including age, body fat, and BMI.

Materials and methods

Study design and subjects

The current study used an open label, longitudinal, follow-up design with convenience sampling and pre-and post-test data collection, as described elsewhere (41, 42). This study was conducted during Ramadan, from the first of April to the first of May 2022. An information sheet was distributed to the people who met the eligibility requirements. This study's enrollment was advertised on social media, in hospital bulletins, and personal correspondence.

The data was gathered twice: once a week before Ramadan (R1) and again at the end of Ramadan's third week, after 21–29 days of fasting (R2). The participants in Ramadan observed fasting from dawn to sunset (about 14 h), with an average fasting hour of 350 h for the two groups. The participants in this study were not given any special dietary guidelines and were instructed to eat and exercise as normal before Ramadan. The size of the sampling was set based on the *A priori* power analysis, which was conducted using G*Power 3.1. software (43). The analysis indicated that a sample of 30 for each group, a total of 60 participants, would allow 80% power to detect a medium effect size ($f = 0.50$, $a = 0.05$), and the participants were selected using inclusion and exclusion criteria. Through social media, we conveniently recruited 62 women aged 21–68 from Saudi Arabia's Qassim (Unaiza area). We contacted all those interested in this study and requested that they attend an official meeting at King Saud Hospital, Unaiza. In this meeting, the participants were briefed about the study objectives and protocol, their eligibility and medical status were assessed, and they were given the opportunity to sign an informed consent form. The bodily measurements and blood samples were taken between 11 a.m. and 1 p.m. during both visits of the two time points.

Before the study started, all subjects provided written informed consent. According to the questionnaire and clinical data, those who responded were divided into two groups: PRE-M women (21–42 years old) and POST-M women (43–68 years old). Healthy women with regular menstrual cycles were clinically defined as PRE-M, while those who had stopped menstruating at least a year before sample collection were classified as POST-M; both categories were then considered. Those with a record of smoking, CVD, pregnancy, cancer, hypertension, breastfeeding, diabetes, and even non-diabetics but taking medicines for sugar control, as well as those taking medications that cause changes in metabolism such as antiretrovirals, corticosteroids, antiseizure, psychotropic, insulin, sulfonylurea, and thiazolidinediones, or experiencing a weight change of more than 3 kg right before the study period, were excluded. Inclusion and exclusion criteria applied are depicted in Figure 1.

Anthropometric measurement

Anthropometric parameters were measured in two visits (triplicate in each visit) using the body composition analyzer ACCUNIQ BC360 (SELVAS Healthcare Inc., Daejeon, Korea). The reproducibility and validity for body composition measuring BIA device in adult women is reported elsewhere (44). Electrodes were placed on the respondent's hands and feet in the standing position. The device was connected to an Ultrasonic Height meter, which automatically measured the body weight, body mass index (BMI), hip circumference (HC), waist circumference (WC), waist-to-height ratio (WHtR), and waist-to-hip ratio (WHR), fat mass (FM), and fat-free mass (FFM). The participants fasted for 4 h before testing, and their palms and soles were cleaned after each measurement. Fat mass index (FMI, kg/m^2) was calculated by dividing the fat mass (FM, kg) by height square (m^2), whereas the fat-free mass index (FFMI, kg/m^2) was calculated by dividing the fat-free mass (FFM, kg) by height square (m^2).

Blood sample collection and analysis

Blood samples were collected before and after Ramadan at the two time points. A venous blood sample (10 mL) was collected from each subject after at least 8 h of fasting. To eliminate the impact of timing and dietary intake on the measured biochemical parameters and to guarantee consistent fasting duration at both time points, the samples were taken between 11 a.m. and 1 p.m. during both visits. The blood was centrifuged for 15 min at 3000 rpm within an hour of collection. The serum was coded and kept at-80°C until utilized for biochemical analysis. The blood samples were analyzed in the laboratory of KSH, Unaizah (Qassim district), and the laboratory of Qassim University. ELISA kits were used to compare the required measurements to a specific standard for each parameter. Initially, the standard of each parameter was tested, and related regression and trend lines were plotted. Then, the relevant parameters from the blood samples were calculated accordingly. The kits were precisely handled by following the instructions to perform multiple replicates for each parameter.

Assessment of blood pressure

Following each interview (from baseline to end of research), blood pressure was recorded in the left arm (mmHg) using a standard mercury sphygmomanometer with an appropriate cuff size. Participants with an empty bladder were seated after resting for at least 10 min in a quiet area.

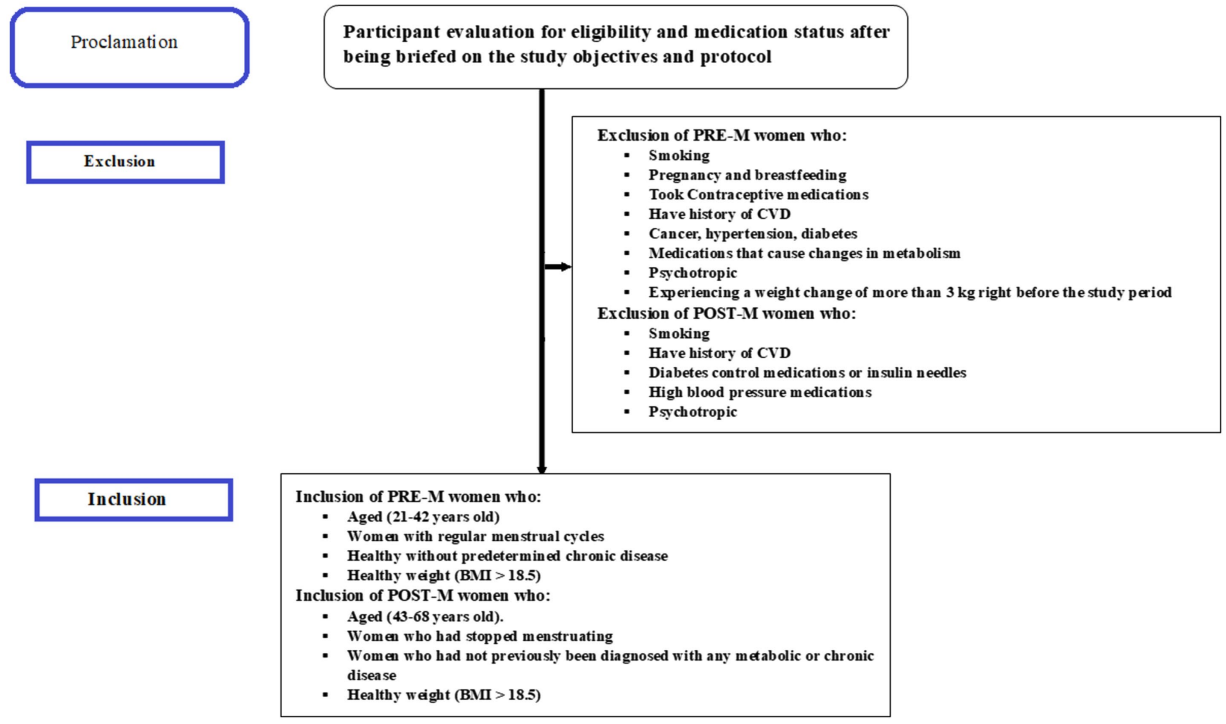

FIGURE 1
Participant recruitment flow chart.

Cytokines assay

ELISA-based techniques were adopted to analyze the cytokines quantitatively. Tumor necrosis factor-α (TNF-α) (Cat. No E0082Hu ELISA Kit, Shanghai, China) was measured as a pro-inflammatory biomarker. A standard curve was constructed using the standard solution in the kit. Interleukin (IL)-10 (Cat. No. E0102Hu, IL10 ELISA Kit, Shanghai, China) was measured as an anti-inflammatory biomarker and a standard curve was created using the standard solution.

Determination of oxidative stress biomarkers

ELISA kit (Cat. No. E2199Hu) was used to assess the Total antioxidant capacity (TAC) in samples. The absorbance of the final solution was measured at 450 nm, and a standard solution was used to generate a standard curve. A colorimetric assay kit (Cat. No E1371Hu, Shanghai, China) was used to calculate the lipid peroxidation as malondialdehyde (MDA) by measuring thiobarbituric acid reactive substance (TBARS). The standard curve was constructed using the standard solution in the kit. Superoxide dismutase (SOD) activity was determined using a SOD assay kit (Cat. No E0918Hu, Shanghai, China), whereas ELISA kits (Cat. No E3696Hu) were employed to assess the Glutathione peroxidase (GPx) activity. The standard curve was created using the standard solution. ELISA kit (Cat. No. E0103Hu) was used to measure the insulin-like growth factor-1 (IGF-1), and a standard curve was generated from the standard solution, all done in the laboratory of Qassim University.

C-reactive protein detection

The C-reactive protein (CRP) was tested in the laboratory of King Saud Hospital (KSH) using a qualitative CRP analyzing kit (BioTime, Xiamen, Fujian, China).

Blood glucose, lipid profile, and sex hormone determination

Blood glucose levels and lipid profile (total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG)) were determined using colorimetric kits and a Dimension Xpand Plus Chemistry Analyzer. Progesterone and estrogen were measured using XN1000-Cobas E411 (Sysmex-Rauch-Hitachi, Tokyo, Japan) in KSH.

Statistical analysis

Statistical analyses were carried out using SPSS software (version 25) (SPSS Inc., Chicago, IL, USA). Repeated measures of ANOVA were applied to study the correlation within and between experimental groups' data (anthropometric, blood glucose, lipid profile, blood pressure, and sex hormones levels as well as circulating levels of oxidative stress, aging, and inflammatory biomarkers) along with the time (before and at the end of Ramadan) of the measurements (time point 1, time point 2, etc.). Similar to an ANOVA, time is treated as a categorical variable rather than a continuous variable in repeated measures ANOVA. Spearman correlation coefficients and simple

regression analysis were used to determine the relationship between anthropometric indices and age as independent variables and sex hormones, circulating levels of oxidative stress, aging, and inflammatory biomarkers as dependent variables for PRE-M and POST-M healthy women.

Results

Changes in anthropometric indices, lipid profile, and sex hormone levels

Table 1 presents the anthropometric, basic hematological, and hormone levels in PRE-M and POST-M women before and at the end of RIF's period. The parameters, including weight, BMI, waist circumferences, the ratio of waist-to-height, PBF, FM, FMI, and TG, significantly decreased in PRE-M and POST-M women at the end of the fasting period (main effect of time, $p < 0.001$, or $p = 0.002$) without significant differences between the two groups. Contrarily, HDL-C levels significantly increased in PRE-M (1.62 to 4.75 mmol/L) and POST-M (1.40 to 2.56 mmol/L) women (main effect of time, $p = 0.027$); however, the difference between R1 and R2 was significant. The women belonging to PRE-M experienced greater increases in HDL-C levels compared to the POST-M group of women (group × time interaction, $p = 0.008$). Diastolic blood pressure was significantly increased in the PRE-M group, whereas it decreased in the POST-M group (main effect of time, $p < 0.001$); however, the difference between R1 and R2 in the POST-M was highly significant. The effect was higher in the POST-M women group as compared to the PRE-M women group (group × time interaction, $p = 0.021$). Estrogen also significantly decreased in the PRE-M group and increased in the POST-M group (main effect of time, $p = 0.046$); however, the difference between R1 and R2 in terms of estrogen in the PRE-M was highly significant. The change was greater in the PRE-M group than in the POST-M group of women (group × time interaction, $p = 0.01$). However, the parameters such as hip circumference, waist-to-hip ratio, total body water, total cholesterol, progesterone level, FFM, LDL-C, systolic blood pressure, and fasting glucose did not exhibit significant changes between and within groups.

Changes in cytokines, oxidative stress biomarkers, and C-reactive protein (CRP)

Table 2 demonstrates the circulating levels of oxidative stress, and inflammatory and anti-inflammatory markers. TAC, MDA, and IGF-1 remained unchanged between and within the two groups during fasting and non-fasting periods. There was a highly significant difference between R1 and R2 with regard to GPx, IL-10, and TNF-α in the PRE-M group than in the POST-M group of women. SOD activity significantly increased in both PRE-M and POST-M women groups (main effect of time, $p = 0.016$), and the two groups did not vary significantly. GPx (main effect of time, $p = 0.05$) and IL-10 (main effect of time, $p = 0.025$) increased dramatically in both PRE-M and POST-M women. The rise in GPx (group × time interaction, $p = 0.045$) and IL-10 (group × time interaction, $p = 0.029$) was significantly higher in PRE-M women compared to the POST-M women. Contrarily, TNF-α significantly decreased in both groups (main effect of time, $p = 0.021$). The reduction was found to be more significant in PRE-M women (group × time interaction, $p = 0.048$). Table 3 shows the presence or the absence of CRP in PRE-M and POST-M women. The results demonstrated the presence of inflammation in only two (6.45%) women in the PRE-M group, and the number decreased to one by the end of Ramadan. These findings suggest that fasting during the day in Ramadan could counter-elevate inflammatory and oxidative stress markers to some extent. It could further reduce low-grade systemic inflammation, oxidative stress, and related negative health effects in healthy individuals. At the end of Ramadan, however, the number of women in the POST-M group with oxidative stress and inflammation increased from 5 (16.13%) to 10 (32.26%). This may be temporary due to sudden hormonal changes during the month of Ramadan.

Association of BMI, PBF, and age with cytokines, aging, and oxidative stress biomarkers

Table 4 summarizes the relationship between BMI, percent body fat, and metabolic biomarkers. The findings revealed that the BMI of women in the PRE-M group was not associated with any of the biomarkers at both the testing periods (before and at the end of Ramadan). However, for the same group, BMI had a substantial positive connection with estrogen levels before and after fasting, as well as TNF-α before fasting. POST-M women's BMI had a significant negative correlation with the progesterone levels before and at the end of the fasting period. Similarly, a significant positive correlation of PBF was observed with the estrogen level in POST-M women, whereas it remained negatively correlated with the progesterone level in both groups (PRE-M and POST-M). A significant positive association was noted between PBF and TNF-α in both groups before fasting. The PBF of POST-M women also exhibited a significant positive association with MDA before fasting. A significant negative association was noted between BMI or PBF and IGF-1in both groups at the end of the fasting period. Table 5 presents the association between age and metabolic biomarkers. PRE-M women's age was significantly ($p \leq 0.01$) and negatively associated with progesterone level before the fasting period. Moreover, lipid MDA was significantly and negatively correlated with PRE-M age before and at the end of the fasting period. Before Ramadan, there was a positive relationship between IL-10 and age in both groups. However, age was negatively correlated with the IGF-1 marker before and after the fasting period in POST-M women. Other biomarkers were either positively or negatively related to the age of the respondents, but only at a non-significant level.

Discussion

The current study was designed to explore the effect of observing RIF on both pre-and post-menopausal healthy women in Saudi Arabia. This study unravels major differences between the two

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TABLE 1 Changes in anthropometric, blood glucose, lipid profile, blood pressure, and sex hormone levels before (R1) and at the end of Ramadan (R2) for PRE-M and POST-M healthy women (n = 62).

Parameter	PRE-M (n = 31)		Difference*	POST-M (n = 31)		Difference*	p-value time	p-value group × Time
	R1	R2		R1	R2			
	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD			
Weight (kg)	61.33 ± 11.73	59.42 ± 11.38	−1.91	69.08 ± 11.26	66.97 ± 11.28	−2.11	< 0.001	0.628
Body mass index (BMI) (kg/m^2)	24.45 ± 4.56	23.71 ± 4.49	−0.74	28.57 ± 3.83	27.43 ± 4.00	−1.14	< 0.001	0.129
Waist circumference (cm)	72.74 ± 9.17	68.90 ± 9.13	−3.84	86.63 ± 9.42	81.00 ± 8.50	−5.63	< 0.001	0.085
Hip circumference (cm)	99.71 ± 11.74	97.87 ± 7.94	−1.84	102.19 ± 18.99	98.69 ± 12.89	−3.5	0.168	0.664
Waist-to-height ratio (WHtR) (cm)	0.46 ± 0.06	0.44 ± 0.06	−0.02	0.56 ± 0.07	0.52 ± 0.06	−0.04	< 0.001	0.068
Waist-to-hip ratio (WHR) (cm)	0.73 ± 0.06	0.70 ± 0.05	−0.03	1.05 ± 1.30	0.84 ± 0.16	−0.21	0.618	0.187
Total body water (kg)	31.29 ± 3.13	30.89 ± 2.84	−0.40	30.68 ± 2.87	30.89 ± 3.30	0.21	0.679	0.135
Percent body fat (%)	29.62 ± 8.72	28.72 ± 8.47	−0.90	38.56 ± 5.43	37.18 ± 5.52	−1.38	< 0.001	0.293
Fat mass (FM)(kg)	19.08 ± 8.61	18.17 ± 8.27	−0.91	27.16 ± 7.82	25.76 ± 7.41	−1.40	< 0.001	0.191
Fat-free mass (FFM) kg	42.30 ± 3.94	42.16 ± 3.87	−0.14	41.93 ± 3.94	42.20 ± 4.52	0.27	0.661	0.222
FMI (kg/m^2)	7.56 ± 3.38	6.88 ± 3.28	−0.68	11.08 ± 2.95	10.52 ± 2.85	−0.56	< 0.001	0.148
FFMI (kg/m^2)	16.89 ± 1.59	16.83 ± 1.56	−0.06	17.23 ± 1.58	17.32 ± 1.54	0.09	0.796	0.277
LDL-C (mmol/L)	2.49 ± 0.99	2.67 ± 0.90	0.18	3.43 ± 0.89	3.51 ± 0.85	0.08	0.326	0.701
Total cholesterol (mmol/L)	4.49 ± 1.08	4.75 ± 0.69	0.26	5.47 ± 0.86	5.14 ± 1.56	−0.33	0.792	0.086
HDL-C (mmol/L)	1.62 ± 0.28^{bc}	4.75 ± 0.35^{a}	3.13	1.40 ± 0.43^{bc}	2.56 ± 0.39^{b}	1.16	0.027	0.008
Fasting glucose (mmol/L)	4.64 ± 1.85	4.06 ± 1.84	−0.58	5.82 ± 2.62	5.45 ± 2.40	−0.37	0.186	0.776
Triglycerides (TG) (mmol/L)	0.81 ± 0.32	0.72 ± 0.50	−0.09	1.16 ± 0.44	0.90 ± 0.51	−0.26	0.002	0.113
Systolic blood pressure (SBP) (mmHg)	110.81 ± 13.50	113.78 ± 8.82	2.97	127.47 ± 19.88	125.91 ± 23.98	−1.56	0.653	0.156
Diastolic blood pressure (DBP) (mmHg)	77.00 ± 12.20^{ab}	77.39 ± 7.99^{ab}	0.39	81.41 ± 13.24^{a}	71.00 ± 10.31^{c}	−10.41	< 0.001	0.021
Estrogen (pmol/L)	649.58 ± 95.03^{a}	417.13 ± 17.75^{b}	−232.45	42.51 ± 6.39^{cd}	49.44 ± 3.18^{c}	6.93	0.046	0.010
Progesterone (pmol/L)	9.74 ± 1.75	9.81 ± 2.05	0.07	1.27 ± 0.18	0.39 ± 0.07	−0.88	0.878	0.858

Values are expressed as means ± SD. p-value: Repeated measures ANOVA with groups (pre-menopausal women, post-menopausal women) as the between-subject factor and time before (R1) and at the end of Ramadan (R2) as the within-subject factor. *Difference = R2 – R1. Means not sharing a common letter are significantly different (group × time interaction).
The values with different superscript letters (a,b,c,d) in a column are significantly different ($p < 0.05$).

groups in terms of their hormonal and inflammatory responses to RIF. Interestingly, observing RIF was associated with improvements in some anthropometric, metabolic, inflammatory, and oxidative stress markers in both PRE-M and POST-M healthy women, with distinct differences in hormonal changes between the pre-and post-menopausal women.

Anthropometric measures

The reported effect of fasting during Ramadan on body fat, weight, and BMI is consistent with the significant reduction in waist circumference observed in both women PRE-M and POST-M groups. Fat levels considerably decreased at the end of Ramadan, paralleling a

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TABLE 2 Changes in circulating levels of oxidative stress, aging, and inflammatory biomarkers before (R1) and at the end of Ramadan (R2) for PRE-M and POST-M healthy women (n = 62).

Parameter	PRE-M (n = 31)			POST-M (n = 31)			p-value time	p-value group × time
	R1	R2	Difference*	R1	R2	Difference*		
	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD			
TAC (U/ml)	21.18 ± 5.08	24.40 ± 7.39	3.22	15.66 ± 6.01	18.01 ± 8.50	2.35	0.482	0.922
MDA (nmol/ml)	2.37 ± 9.48	2.31 ± 4.36	−0.06	2.14 ± 6.09	1.61 ± 2.89	−0.53	0.147	0.636
SOD (U/ml)	11.87 ± 1.72	15.96 ± 1.30	4.09	11.13 ± 1.43	13.10 ± 2.86	1.97	0.016	0.334
GPx (g/ml)	136.44 ± 10.67^{b}	143.86 ± 12.67^{a}	7.42	125.09 ± 5.90^{c}	126.62 ± 8.51^{c}	1.53	0.050	0.045
IGF-1 (ng/ml)	119.24 ± 4.01	119.62 ± 4.38	0.38	100.31 ± 3.84	100.69 ± 4.34	0.38	0.406	0.989
IL-10 (pg/ml)	30.31 ± 3.62 ^{b}	39.73 ± 2.19^{a}	9.42	21.46 ± 2.98^{c}	21.56 ± 1.95^{c}	0.10	0.025	0.029
TNF-α (ng/ml)	194.45 ± 8.02^{a}	183.30 ± 9.65^{b}	−11.15	123.43 ± 9.71^{c}	117.19 ± 2.13^{c}	−6.24	0.021	0.048

Values are expressed as means ± SD. p-value: Repeated measures ANOVA with groups (pre-menopausal women, post-menopausal women) as the between-subject factor and time before (R1) and at the end of Ramadan (R2) as the within-subject factor.
*Difference = R2 – R1. Means not sharing a common letter are significantly different (group × time interaction). TAC, Total antioxidant capacity; MDA, Lipid peroxidation as malondialdehyde; SOD, Super oxide dismutase; GPx, Glutathione peroxidase enzyme; IGF-1, Insulin-like growth factor-1; IL-10, Interleukin-10; TNF-α, Tumor necrosis factor-α.
The values with different superscript letters (a,b,c,d) in a column are significantly different ($p < 0.05$).

TABLE 3 Changes in qualitative C-reactive protein (CRP) before (R1) and at the end of Ramadan (R2) for PRE-M and POST-M healthy women (n = 62).

Results	PRE-M (n = 31)					POST-M (n = 31)				
	R1	%	R2	%	p-value	R1	%	R2	%	p-value
Positive	2	6.45	1	3.23		5	16.13	10	32.26	
Negative	29	93.55	30	96.77	0.071	26	83.87	21	67.74	0.015
Total	31	100.0	31	100.0		31	100.0	31	100.0	

* $p \leq 0.05$; ** $p \leq 0.01$ according to Chi-square test.

significant reduction in waist circumference (34). This could result in a shift in the pattern of body fat redistribution after Ramadan. The reported significant decreases in FM and FMI in both groups at the end of the RIF period came despite the notion that FFM was unaffected by the RIF. In contrast, RIF-induced weight loss associated with decreased FFM has already been observed in other studies (19). However, a significant effect of RIF on FFM was not observed during this study. This is a crucial physiological finding because FFM plays a key role in blood glucose homeostasis, functional capacity, and resting energy expenditure (45). RIF could facilitate losing body weight and FM in some individuals. The adaptations acquired during fasting are transient and generally reversible within a short period (46). Thus, developing long-term body composition maintenance strategies at the end of Ramadan is important. Because glucose and fluids are less readily available to the body, stored body fat acts as a key substrate for energy production, resulting in lower fat content and body weight (19, 26). A study linked Ramadan fasting-related weight loss to a decrease in total calorie intake, as energy balance is critical in regulating changes in body weight (26). The current findings on body weight and anthropometric changes are consistent with the broad literature and are reported in more than one systematic review and meta-analysis on Ramadan fasting in healthy people (18, 34, 47).

Glucose homeostasis, lipid profile, and blood pressure

Regarding the lipid profile, HDL-C significantly increased in both groups, especially in PRE-M women at the end of the RF period. TG significantly reduced in both groups, but the difference between the groups was not significant, whereas cholesterol and LDL-C remained unchanged, according to Kul et al. (47). In a study involving a healthy population, only women experienced a substantial increase in HDL-C levels. Factors including dietary habits, physical characteristics, type of fat saturation, fat percentage, simple sugars percentage in diet, and weight loss could affect the lipid profile. Furthermore, Kul et al. (47) found that participants' metabolic markers and body weight were altered during Ramadan fasting compared to pre-fasting values. These findings are compatible with the current study's findings since Ramadan fasting is different from other fasting regimens in that it entirely prohibits the ingestion of solid meals, liquids and water, which could impact blood sample concentration. Ramadan also significantly affects people's behavioral habits and lifestyles, such as shortened sleeping hours and patience. As a result, Ramadan fasting is distinct from alternate-day fasting. According to Osman et al. (48), fasting during Ramadan has minor benefits for body composition by reducing body mass in both healthy and obese individuals, and the consequences are generally temporary and varied. However, there is a more consistent improvement in blood lipid profile during Ramadan fasting, and this often lasts beyond Ramadan time.

The altered sleep–wake cycle influences food and fluid consumption, which regulates energy intake and expenditure throughout the Ramadan fasting period (49). As a result, at the end of RIF, glucose levels in both groups were stable in this study. Moreover, during Ramadan, altered sleep patterns and psychological/social activities may change the cyclical pattern of numerous hormonal variables related to energy intake regulation and energy metabolism (49). Both groups' systolic blood pressure remained stable; however,

TABLE 4 Association between body mass index, body fatness, and metabolic biomarkers before (R1) and at the end of Ramadan (R2) for PRE-M and POST-M healthy women (n = 62).

Independent variable/ dependent variable	PRE-M (n = 31)						POST-M (n = 31)					
	R1			R2			R1			R2		
	r	(β, r^2)	p-value	r	(β, r^2)	p-value	r	(β, r^2)	p-value	r	(β, r^2)	p-value
Body mass index (BMI)												
Estrogen	0.132	(−0.001, 0.017)	0.478	0.111	(0.028, 0.012)	0.551	0.286*	(0.017*, 0.082)	0.012	0.351*	(0.441*, 0.123)	0.049
Progesterone	−0.216	(−0.003, 0.047)	0.243	−0.111	(−0.028, 0.012)	0.551	−0.152**	(0.006**, 0.023)	0.007	−0.412*	(−0.800*, 0.169)	0.019
TAC	0.040	(0.006, 0.002)	0.829	0.092	(0.007, 0.001)	0.624	−0.026	(−0.008, 0.003)	0.889	−0.047	(−0.010, 0.002)	0.800
MDA	−0.032	(−0.002, 0.001)	0.866	0.125	(0.014, 0.016)	0.502	−0.178	(−0.011, 0.032)	0.330	−0.085	(−0.029, 0.169)	0.644
SOD	0.087	(0.003, 0.008)	0.642	−0.028	(−0.001, 0.001)	0.880	−0.055	(−0.002, 0.003)	0.764	−0.001	(−0.601, 0.0002)	0.994
GPx	0.054	(0.005, 0.003)	0.773	−0.204	(−0.015, 0.042)	0.271	−0.093	(−0.009, 0.009)	0.614	−0.019	(−0.003, 0.0002)	0.918
IL-10	0.059	(0.001, 0.004)	0.751	0.031	(0.001, 0.004)	0.867	−0.058	(−0.001, 0.003)	0.751	−0.047	(−0.001, 0.002)	0.800
TNF-α	0.122	(0.003, 0.015)	0.215	0.022	(0.015, 0.008)	0.906	0.075*	(0.012*,0.006)	0.043	0.040	(0.001, 0.002)	0.828
IGF-1	−0.054	(−0.018, 0.003)	0.773	−0.047*	(−0.015*, 0.002)	0.048	−0.093	(−0.040, 0.009)	0.614	−0.034**	(−0.071**, 0.005)	0.001
Percent body fat (PBF)												
Estrogen	−0.107	(−0.001, 0.011)	0.567	0.077	(0.031, 0.023)	0.679	0.239**	(0.120**, 0.057)	0.007	0.153	(0.092, 0.003)	0.774
Progesterone	−0.214*	(−0.045*, 0.0001)	0.033	−0.077	(−0.037, 0.006)	0.679	−0.127**	(−0.217**, 0.016)	0.004	−0.292	(−0.721, 0.085)	0.104
TAC	0.037	(0.010, 0.001)	0.842	0.084	(0.026, 0.007)	0.655	−0.099	(−0.045, 0.010)	0.591	−0.236	(−0.067, 0.056)	0.194
MDA	0.097	(0.014, 0.009)	0.603	0.151	(0.031, 0.023)	0.416	0.354*	(0.031*, 0.126)	0.047	0.289	(0.134, 0.084)	0.108
SOD	−0.025	(−0.001, 0.001)	0.894	−0.086	(−0.005, 0.007)	0.644	−0.125	(−0.007, 0.016)	0.496	−0.123	(−0.006, 0.015)	0.501
GPx	0.042	(0.007, 0.002)	0.824	−0.219	(−0.029, 0.048)	0.238	−0.228	(−0.030, 0.052)	0.210	−0.165	(−0.032, 0.027)	0.366
IL-10	0.058	(0.001, 0.003)	0.757	0.034	(0.034, 0.001)	0.855	−0.174	(−0.004, 0.030)	0.340	−0.236	(−0.004, 0.056)	0.194
TNF-α	0.039*	(0.021*,0.002)	0.036	0.037	(0.001, 0.001)	0.845	0.142**	(0.005, 0.020)	0.009	−0.179	(0.009, 0.032)	0.326
IGF-1	−0.068	(−0.042, 0.005)	0.716	−0.012*	(−0.007*, 0.002)	0.047	−0.122	(−0.075, 0.015)	0.505	−0.134*	(−0.089*, 0.018)	0.045

*$p \leq 0.05$; **$p \leq 0.01$; r, correlation coefficient; β, regression coefficient; partial r^2 for independent variables. TAC, Total antioxidant capacity; MDA, Lipid peroxidation as malondialdehyde; SOD, Super oxide dismutase; GPx, Glutathione peroxidase enzyme; IL-10, Interleukin-10; TNF-α, Tumor necrosis factor-alpha; IGF-1, Insulin-like growth factor-1.

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TABLE 5 Association between age and metabolic biomarkers before (R1) and at the end of Ramadan (R2) for PRE-M and POST-M healthy women (n = 62).

Independent variable/ dependent variable	PRE-M (n = 31)				POST-M (n = 31)			
	Age (years)							
	R1		R2		R1		R2	
	(β, SE)	p-value	(β, SE)	p-value	(β, SE)	p-value	(β, SE)	p-value
Estrogen	−0.001 ± 0.001	0.280	−0.050 ± 0.075	0.508	−0.019 ± 0.022	0.270	0.122 ± 0.110	0.772
Progesterone	−0.009 ± 0.004*	0.026	−0.071 ± 0.098	0.479	−0.017 ± 0.015	0.237	1.780 ± 1.040	0.564
TAC	−0.404 ± 0.555	0.474	−0.010 ± 0.053	0.859	−0.808 ± 0.446	0.959	0.110 ± 0.001	0.924
MDA	−0.135 ± 0.052*	0.017	−0.194 ± 0.110*	0.019	−0.089 ± 0.073	0.136	−0.444 ± 0.289	0.458
SOD	0.003 ± 0.018	0.875	0.013 ± 0.027	0.618	0.008 ± 0.034	0.849	−0.029 ± 0.011	0.487
GPx	−0.306 ± 0.218	0.174	−0.032 ± 0.030	0.312	−0.021 ± 0.011	0.950	−0.101 ± 0.001	0.989
IL-10	0.068 ± 0.025*	0.014	0.003 ± 0.013	0.828	0.054 ± 0.035*	0.048	0.014 ± 0.023	0.537
TNF-α	0.003 ± 0.028	0.909	0.014 ± 0.024	0.580	0.002 ± 0.043	0.826	0.043 ± 0.051	0.404
IGF-1	−0.221 ± 0.423	0.605	−0.166 ± 0.412	0.690	−0.100 ± 0.331*	0.046	−0.163 ± 0.351	0.047*

*$p \leq 0.05$; **$p \leq 0.01$; r, correlation coefficient; β, regression coefficient; partial SE = standard error for independent variables. TAC, Total antioxidant capacity; MDA, Lipid peroxidation as malondialdehyde; SOD, Super oxide dismutase; GPx, Glutathione peroxidase enzyme; IL-10, Interleukin-10; TNF-α, Tumor necrosis factor-alpha; IGF-1, Insulin-like growth factor-1.

diastolic blood pressure drastically changed. The reduction was greater in POST-M women than in PRE-M women. Lower blood pressure during RIF could be attributed to a variety of causes, including weight loss, age, and reduced catecholamine synthesis. These factors cause a decrease in sympathetic tone, which results in a reduction of cardiac output, blood pressure, and heart rate (21, 50). The present study showed that PRE-M women had significantly lower estrogen levels than POST-M women, although progesterone levels remained constant in both groups. Several investigations have produced contradictory findings about the effect of RF on reproductive hormones in healthy women (3, 4, 51). The current findings on lipid profile and cardiometabolic risk factors are also in line with the established findings reported in systematic reviews and meta-analyses on RIF in healthy people (21, 47).

Inflammatory and oxidative stress markers

Because of the differences in hormonal secretions between the two groups, RF has different health impacts on PRE-M and POST-M women. SOD is an antioxidant enzyme that catalyzes the dismutation of two superoxide anions ($^*O_2$) molecules to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) to help counteract the oxidative stress of superoxide anions (52). SOD levels were significantly greater within each group (PRE-M and POST-M); however, there was no significant difference between the two groups at the end of the RIF. However, GPx levels increased in both groups, with PRE-M women having greater levels of both GPx and IL-10. GPx is a cytosolic enzyme that catalyzes the conversion of hydrogen peroxide to water and then the conversion of peroxide radicals to oxygen and alcohol, which minimizes the risk of superoxide anion (53).

TAC and MDA biomarkers are used to assess oxidative stress, whereas IGF-1 serves as an aging biomarker. These biomarkers did not change in both groups (PRE-M and POST-M). MDA is an end product of lipid peroxidation that participates in lipid radical formation and oxygen uptake in animal tissues as an oxidative stress mechanism initiated by reactive oxygen species. MDA acts as an endogenous lipid peroxidation biomarker and is considered an important oxidative stress biomarker (54, 55). The results indicated reduced oxidative stress during RF in both groups that are facilitated by increased SOD levels and unchanged MDA levels. A significant increase in IL-10 and a decrease in TNF-α revealed an enhanced inflammatory response in PRE-M women as compared to POST-M women.

The decrease in cytokine levels during RF could also be attributed to reduced oxidative stress and reactive oxygen species, which participate in the activation of the transcriptional factor responsible for the expression of pro-inflammatory cytokines such as TNF-α (56). The reduction in C-reactive protein indicated significant suppression of inflammatory biomarkers in PRE-M women in comparison to POST-M women. This might be because of the substantial decrease in inflammatory biomarkers (C-reactive protein, IL-6) during IF (57). The reported results on the inflammatory and oxidative stress markers are consistent with the current literature supporting the beneficial effect of observing RIF (19, 24, 40).

Relationship between anthropometrics and age with cytokines and oxidative stress biomarkers

The relationship between BMI, percent body fat, and age as independent variables, and sex hormones and metabolic biomarkers as dependent variables revealed that as BMI increased, the estrogen levels also increased in POST-M women. In contrast, progesterone levels decreased significantly before and after Ramadan. However, an increase in the percentage of body fat boosted estrogen levels in POST-M women while lowering progesterone levels in both groups. MDA reduced as percent body fat dropped in POST-M women, indicating lower oxidative stress in these women. The BMI of POST-M women and the percent body fat of both groups increased TNF-α, which could be an indication of inflammation. During Ramadan, both BMI and percent body fat had a negative influence on IGF-1, resulting in reduced bone

density, less muscle mass, and changed lipid levels (48). Ramadan fasting did not affect women's sex hormones, regardless of age. However, before Ramadan, progesterone levels in PRE-M women decreased significantly with age. According to these findings, age had a negative impact on female sex hormones before Ramadan. This finding is consistent with those reported in other relevant studies (3, 4, 51). The strong negative connection between PRE-M women's age and lipid MDA before and after Ramadan could be because lipid MDA levels decrease with age, which may contribute to lower oxidative stress. The fundamental metric for evaluating the current potential of oxidative stress in aging and other age-related disorders is antioxidant capacity. The first step in anticipating oxidative stress in the aging process is to estimate the reducing power/antioxidant capacity since an imbalance between antioxidants and oxidants causes oxidative stress (58). Abbasi et al. reported that in subjects under a high-fat diet, both high-intensity interval training and IF may help to improve lipid profile (59). Still, their combination may have little synergistic effect. Before Ramadan, a positive association was found between the age of both groups and IL-10, indicating that the level of IL-10 increased with age, implying that the women will be protected against inflammation because IL-10 inhibits the activity of Th1 cells, natural killer cells, and macrophages during infection (60). The negative relationship between POST-M women's age and IGF-1 before and after Ramadan suggested that the hormone declined with age in POST-M women, resulting in low bone density, less muscular mass, and changed lipid levels (61).

The aforementioned findings of the current research are consistent with the broad literature supporting the health-improving effect of observing RIF by healthy people, particularly in terms of the improvements in cardiometabolic profile including anthropometric measures (BMI, body fatness, and waist circumference), lipid profile (increased HDL and reduced LDL, TG, and TC), and inflammatory and oxidative stress markers (increased anti-inflammatory IL-10, antioxidant SOD, GPx, decreased proinflammatory IL-6, TNF-α).

Study strengths and limitations

The strength of our study is that we examined both pre-menopausal and post-menopausal women from the same living community to ensure homogeneity and avoid inequalities. Extensive questionnaires, physical measurements, and laboratory tests were employed to quickly identify the variables impacting the patients' physiological and metabolic biomarkers before and after RIF. It is generally established that physiological and metabolic biomarkers differ significantly between the two groups; nevertheless, our findings show that the presence of RIF exacerbates this. Also, our findings suggest that RIF is associated with promoting positive metabolic alterations in both group states. Furthermore, variations in subjects' commitment to the diet or IF schedule, also referred to as compliance or adherence, are a problem for nutrition research. However, unlike studies done in non-Islamic nations, where fasting systems can be broken or violated in any way, our study was carried out in an Islamic country, where everyone follows the system strictly from 4 a.m. Fajr (dawn) until 6 p.m. Maghrib (sunset), on average, for a full month. This indicates that the study's strengths include its current research location and the caliber of its time-bound participants during the IF phase. However, the small sample size was the main limitation of this study, as it focused on a specific region in Saudi Arabia. In addition, all the participants were drawn from the same medium-income living community to ensure sample homogeneity and to avoid disparities and confounding socioeconomic and cultural factors. The non-fasting control group was excluded because it was difficult to find the same number of non-fasting Ramadan participants with similar criteria of fasting participants, and different nations varied in dietary and physiological habits as well. Measurements of women who are exempt from fasting during menstruation were taken on fasting days. Future research on the effects of RF on women with thyroid disease is required as this syndrome has become a public health concern: exploring fasting and peri-menopause will provide insight and valuable data; also, the effect of fasting on neurotransmitters are excellent topic to venture into; furthermore, the impact of fasting on autophagy, stem cell activation, and DNA telomere attrition delay are excellent topics to venture into; pursuing research in this field is cutting-edge, with a significant scientific contribution to postponing aging and increasing longevity.

Conclusion

It can be concluded that observing RIF is associated with reduced body weight, waist circumference, BMI, PBF, FM, FMI, TG, and DBP without affecting the other parameters. Furthermore, RIF might effectively help reduce inflammatory processes. This phenomenon was observed in PRE-M women with a significantly lower level of proinflammatory cytokine (TNF-α) and a substantially higher level of an anti-inflammatory biomarker (IL-10) as compared to POST-M women. The effect of RIF on cytokines provides biologically reasonable mechanisms regarding the beneficial aspects of fasting on lipid and carbohydrate balance, and autoimmune diseases. BMI and PBF changes were found to influence the cytokines and metabolic biomarkers, especially in POST-M women. Some metabolic biomarkers were observed with age changes in sex hormones.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

Ethics statement

The studies involving humans were approved by the regional research Ethics committee in Qassim and registered at the national committee of Bio & Med, Ethics (NCBE) with registration numbers H-04-Q-001 and No. (1109570-1443). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

NAZ: Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. AA-K: Conceptualization, Supervision, Investigation, Writing – review & editing. MAM: Formal analysis, Methodology, Supervision, Writing – review & editing.

SA: Resources, Supervision, Visualization, Writing – review & editing. MM: Conceptualization, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Writing – review & editing. MF: Investigation, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Validation of the IDF-DAR risk assessment tool for Ramadan fasting in patients with diabetes in primary care

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Introduction: In patients with diabetes intending to fast, Ramadan, risk assessment, and stratification are essential for an individualized treatment plan. It seems that the new IDF-DAR risk stratification tool (International Diabetes Federation - Diabetes and Ramadan Alliance) has become the primary tool in this setting. This study aims to validate this tool in the Abu Dhabi population.

Method: The assessment was performed before Ramadan, followed by an evaluation of any significant outcome after Ramadan through tele-interview and an electronic medical records review. Patients were included if the attending physicians used the tool in the risk assessment of the patients within 6 weeks before Ramadan 1,444 (CE 2022) in the AHS healthcare center.

Results: The study included 435 patients. Half (51.7%) were in the low-risk category of the IDF-DAR risk stratification tool, 28.5% were in the moderate-risk category, and 19.8% were in the higher-risk category. Of the total patients, 81.3% fasted during the entire Ramadan period and 18.7% attempted to fast. A total of 14 (3.8%) patients were admitted at least once, and 56 (12.9%) had at least one significant event, including admission to the hospital. Using univariable logistic regression, the occurrence of adverse events was significantly associated with more days not fasted, $B = -0.126$, $p < 0.001$, OR = 0.88 (0.839–0.927). Using multivariable logistic regression, and after controlling for all variables studied, other risk factors identified with the occurrence of adverse events in this study were as follows: being in the low-risk category of the DAR risk assessment tool, $B = -1.1$, OR = 0.34 (0.157–0.744), $p = 0.0072$; being in the frail category compared to the reference category, the robust category, $B = 1.54$, OR = 4.6 (1.3–16.6), $p = 0.018$; and older age $B = -0.034$, OR = 0.966 (0.938–0.995). There was no significant difference between moderate- and high-risk categories in the occurrence of significant adverse events (SAEs). Similar determinants of fasting were identified during the entire Ramadan period using multivariable logistic regression.

Conclusion: According to the IDF-DAR risk assessment, patients with diabetes in the low-risk category had a better outcome than those in the moderate- or high-risk categories regarding SAEs. Another independent risk factor is if the patient is frail, according to the FRAIL scoring.

KEYWORDS

diabetes mellitus, Ramadan, fasting, adverse events, risk assessment

Background

The health effects of changes in the diet and lifestyle of patients with diabetes during Ramadan are of increasing interest in terms of estimating the possible risks and benefits to better inform management guidelines, including recommending exemptions from fasting and optimal medication adjustments. For the whole month of Ramadan, Muslims must abstain from eating and drinking from dawn to sunset, and these fasting hours are accompanied by a change in sleep and primary meal times. Accumulating evidence, including a recent systematic review, confirms that the incidences of complications during Ramadan are minimally higher than at other times of the year in high-risk patients with diabetes (1). However, the ability of patients' bodies to adjust to these changes is variable, and many factors, such as comorbidities and diabetic complications, may influence it. Therefore, many international societies have issued guidelines to guide physicians and patients to fast Ramadan safely (2).

In patients with diabetes, risk assessment and stratification are essential for an individualized treatment plan. It seems that the new IDF-DAR risk stratification tool (International Diabetes Federation - Diabetes and Ramadan Alliance) has become the primary tool in this setting. It was developed based on the available evidence and the consensus of experts who included factors thought or found to increase the risk of adverse events in patients with diabetes. A score is generated as collective points given to the different factors if these risk factors were present (2). It was found to be valid in predicting both the ability to fast during Ramadan and the likelihood of getting hypoglycemia, hyperglycemia, or significant adverse events (SAEs) (3–9). Validating this calculator in different settings will facilitate more perception and the applicability of the tool.

The Abu Dhabi Emirates context represents a high-resource Muslim country with a high prevalence of diabetes (2). Providing appropriate, effective, quality care for this large population in preparation for Ramadan fasting is a priority for better quality of life and less risk. Globally, fasting was found to be efficient for the management of diabetes (10). Assessment tools that can assist in stratifying patients with diabetes are critical in avoiding stressing high-risk patients.

For a few years, Ambulatory Healthcare Services had an initiative to counsel and adjust the care plan for patients with chronic diseases who intend to fast during Ramadan. The IDF-DAR practical guidelines, including the IDF-DAR risk stratification tool published in 2021, were used to assess risk and assist in decision-making for people with diabetes. The tool was built into the electronic medical records (EMR) system. Physicians were encouraged to use it to help stratify patients with diabetes and guide their counseling on decisions regarding fasting. This study aimed to validate the IDF-DAR risk stratification tool by comparing the patients' outcomes after Ramadan to the assessed baseline, the IDF-DAR risk stratification score before Ramadan. This outcome-based risk stratification is important for guiding clinical patient care decisions.

Method

Ambulatory Healthcare Services has structured chronic disease clinics. Within these clinics, an annual initiative targeted patients with chronic disease to counsel them and adjust their care plan in preparation for fasting during Ramadan if medically appropriate. Integrating Ramadan fasting-related counseling in chronic disease patient visits is preceded by an educational event targeting physicians on new updates in this area. The IDF-DAR practical guidelines were included in the educational event, including the IDF-DAR risk stratification tool published in 2021. The AHS team requested to use the tool within the EMR system, which was built internally within CERNER EMR. The training was conducted, and physicians were encouraged to use it to help stratify patients with diabetes and guide their counseling on decisions regarding fasting.

Data collection

This is a prospective observational study. Assessments were performed for all participants at two time points: 6 weeks before Ramadan 1,444 (CE 2022) in the AHS healthcare center and again after Ramadan. Patients were included if the attending physicians used the IDF-DAR risk stratification tool as a pre-Ramadan assessment. As per the IDF-DAR practical guidelines, all patients with diabetes who intend to fast during Ramadan should be screened using this tool. An EMR report was extracted before

Ramadan. All patients in the EMR report were contacted and asked for consent, and an assessment of frailty using the FRAIL scale was carried out. Besides the IDF-DAR risk assessment and FRAIL score, no other clinical data were collected except those routinely ordered before Ramadan. Therefore, patients from the AHS centers assessed as part of their routine visits were included.

After Ramadan, family medicine residents collected data through tele-interview. They called the patients, and if they consented to participate, data were collected regarding fasting and significant history during Ramadan. As Abu Dhabi has integrated electronic health records, data collected through patient tele-interviews could be validated through the EMR chart review. Patient privacy was maintained during tele-interviews as it was done from the AHS centers, and consent for the interview and EMR review was taken.

Review of EMR was done after Ramadan. Important demographics and medical history not included in the IDF-DAR risk assessment were collected in addition to clinical data such as laboratory results within 3 months before Ramadan and 3 months after Ramadan that were determined as part of the patient's routine care. These laboratory results included HBA1C, renal function, systolic blood pressure (SBP), diastolic blood pressure (DBP), and body mass index (BMI). Frailty assessment was performed for patients 60 years or older, and the FRAIL tool was used. It is a validated tool with five questions demonstrating strong evidence for predicting clinical outcomes (11–14). The five questions were related to fatigue; resistance or climbing stairs; ambulation, i.e., walking a couple of blocks; number of chronic illnesses; and losing weight by more than 5%.

Outcome assessment after Ramadan included fasting status, significant health events, and time of admission into care collected after Ramadan. Only events occurring during Ramadan were considered in this study and for those who did fast after their fast. Surveillance started on the first day of Ramadan and continued until the end of the holy month. Events included unplanned admissions, a history of hypoglycemia, and significant symptoms that required breaking fasting, such as dizziness, fainting, and fever.

Data analysis

Out of the 610 patients with diabetes who had the IDF-DAR risk assessment score calculated, 435 were included in the analysis (Figure 1). Twenty patients who did not attempt to fast for a single day were excluded. Patients with type 1 diabetes were also excluded due to their small number in primary care (21 patients in total), which can result in a heterogeneous sample. There were 134 patients with no response or who refused to participate.

Data analysis was performed using SPSS v27. Frequencies, cross-tabulation, and regression analysis were used. Logistic regression was used to test the dependent variable outcomes studied, significant event occurrence, and hypoglycemia, with all variables collected and included to test possible significant associations. Hosmer and Lemeshow chi-square was used to test for goodness of fit and calibration of logistic regression models.

Results

According to the IDF-DAR Assessment tool, half of the study participants (51.7%) were in the low-risk category, of which 89.8% fasted during Ramadan. Among the study participants, 28.5% were categorized as moderate risk and 84.7% fasted during Ramadan; 19.8% were in the higher-risk category, and those who fasted during Ramadan were 80.2%.

Table 1 shows the patients' demographics, with almost two-thirds of the patients belonging to the 50 years or above age group and 16.1% belonging to the above 70 age group. Those between 40 and 50 comprised 18.4%. Older patients were in the higher-risk category per the DAR stratification. For example, nearly one-quarter of them were in the higher-risk group in the age groups above 50 years compared to 2.3% and 11.6% in the age groups 31 to 40 years and 41 to 50 years, respectively. Male patients comprised 43.4% of the sample, and UAE nationals comprised the majority (74.5%). Of the included patients, 57% had diabetes for more than 10 years. Diabetes control became progressively worse as the DAR risk category increased, with an average HbA1C of 7.18, 8.2, and 9 for the low-, moderate-, and high-risk categories, respectively. Table 1 shows the distribution of comorbidities and the medications used by patients with diabetes.

Regarding outcome, from the entire sample, 14 (3.8%) were admitted at least once and 56 (12.9%) had at least one significant event, including hospital admission. This was progressive among the three risk groups as per the DAR tool with 2 (1.1%), 3 (2.7%), and 9 (12%) admissions in the low-, moderate-, and high-risk groups, respectively, and 18 (8%), 21 (16.9%), and 17 (19.8%) among low-, moderate-, and high-risk groups, respectively. Those who did not fast had an event rate of 8 (42.1%) compared to 56 (12.9%) among those who fasted or attempted to fast.

Using univariable logistic regression, the occurrence of adverse events was significantly associated with more days not fasted, $B = -0.126$, $p < 0.001$, OR = 0.88 (0.839–0.927). Using

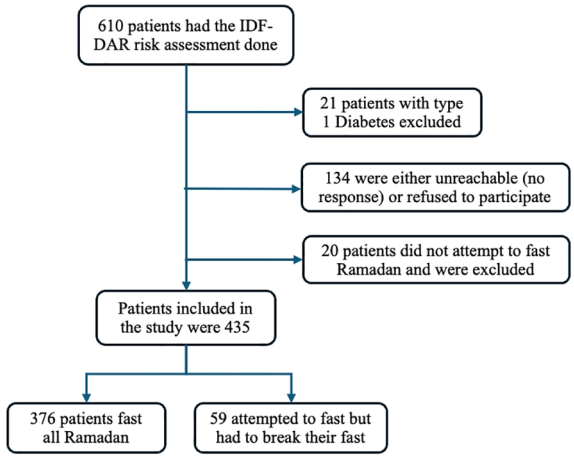

FIGURE 1
Flow diagram for the study sample with excluded and included subjects.

TABLE 1 Study subjects' characteristics.

		Low Risk	Moderate Risk	High Risk	All
Age groups	≤30	2	1	0	3
		0.90%	0.80%	0.00%	0.70%
	31–40	16	8	2	26
		7.10%	6.50%	2.30%	6.00%
	41–50	49	21	10	80
		21.80%	16.90%	11.60%	18.40%
	51–60	74	30	22	126
		32.90%	24.20%	25.60%	29.00%
	61–70	60	47	23	130
		26.70%	37.90%	26.70%	29.90%
	>70	24	17	29	70
		10.70%	13.70%	33.70%	16.10%
Gender	Female	130	64	52	246
		57.80%	51.60%	60.50%	56.60%
	Male	95	60	34	189
		42.20%	48.40%	39.50%	43.40%
Nationality	Non-UAE	53	29	29	111
		23.60%	23.40%	33.70%	25.50%
	UAE	172	95	57	324
		76.40%	76.60%	66.30%	74.50%
Duration of diabetes (years)	A duration of <10	140	35	12	187
		62.20%	28.20%	14.00%	43.00%
	A duration of ≥10	85	89	74	248
		37.80%	71.80%	86.00%	57.00%
Renal complications/ comorbidities	eGFR < 30 mL/min	0	0	4	4
		0.00%	0.00%	4.70%	0.90%
	eGFR >60 mL/min	222	110	56	388
		98.70%	88.70%	65.10%	89.20%
	eGFR 30–45 mL/min	0	3	15	18
		0.00%	2.40%	17.40%	4.10%
	eGFR 45–60 mL/min	3	11	11	25
		1.30%	8.90%	12.80%	5.70%
MVD complications/ comorbidities		1	1	0	2
		0.40%	0.80%	0.00%	0.50%
	No MVD	213	92	38	343
		94.70%	74.20%	44.20%	78.90%
	Stable MVD	11	31	40	82
		4.90%	25.00%	46.50%	18.90%

(Continued)

TABLE 1 Continued

		Low Risk	Moderate Risk	High Risk	All
	Unstable MVD	0	0	8	8
		0.00%	0.00%	9.30%	1.80%
Presence of hypoglycemia	Hypoglycemia less than 1	13	8	19	40
		5.80%	6.50%	22.10%	9.20%
	Hypoglycemia unawareness	0	1	3	4
		0.00%	0.80%	3.50%	0.90%
	Multiple weekly hypoglycemia	0	1	6	7
		0.00%	0.80%	7.00%	1.60%
	No hypoglycemia	212	114	57	383
		94.20%	91.90%	66.30%	88.00%
	Recent severe hypoglycemia	0	0	1	1
		0.00%	0.00%	1.20%	0.20%
Pregnancy	No	142	72	56	270
		100.00%	97.30%	98.20%	98.90%
	Yes	0	2	1	3
		0.00%	2.70%	1.80%	1.10%
Frailty and cognitive function as assessed by physicians	>70 years old with no home support	0	5	10	15
		0.00%	4.00%	11.60%	3.40%
	Impaired cognitive function or frail	0	0	10	10
		0.00%	0.00%	11.60%	2.30%
	No frailty or loss in cognitive function	224	118	66	408
		99.60%	95.20%	76.70%	93.80%
HbA1C	<7.5	144.00	38.00	20.00	202.00
		72.00	34.00	26.00	57.00
	7.5–9	41.00	44.00	25.00	110.00
		20.00	0.39	0.33	28.00
	>9	16.00	31.00	32.00	79.00
		8.00	27.00	42.00	20.00
Physical labor		0	1	0	1
	0.00%	0.80%	0.00%	0.20%	
	Moderate to intense pain	14	24	12	50
		6.20%	19.40%	14.00%	11.50%
	No physical labor	211	99	74	384
		93.80%	79.80%	86.00%	88.30%

(Continued)

TABLE 1 Continued

		Low Risk	Moderate Risk	High Risk	All
Self-monitoring of blood glucose	Conducted as indicated	162	62	32	256
		72.00%	50.00%	37.20%	58.90%
	Indicated but conducted sub-opt	53	46	32	131
		23.60%	37.10%	37.20%	30.10%
	Indicated but not conducted	9	16	22	47
		4.00%	12.90%	25.60%	10.80%
Fasting hours	<16 h	206	108	74	388
		91.60%	87.10%	86.00%	89.20%
	≥16 h	19	16	12	47
		8.40%	12.90%	14.00%	10.80%
Ischemic heart diseases	No IHD	218	110	67	395
		97.80%	90.20%	78.80%	91.90%
	IHD	5	12	18	35
		2.20%	9.80%	21.20%	8.10%
Stroke	No stroke	222	121	82	425
		99.60%	98.40%	96.50%	98.60%
	Stroke	1	2	3	6
		0.40%	1.60%	3.50%	1.40%
Hypertension	No hypertension	96	45	20	161
		43.60%	36.60%	23.80%	37.70%
	Hypertension	124	78	64	266
		56.40%	63.40%	76.20%	62.30%
GLP-1 receptor agonist	Not on GLP-1 receptor agonist	117	50	26	193
		52.00%	40.30%	30.20%	44.40%
	On GLP-1 receptor agonist	108	74	60	242
		48.00%	59.70%	69.80%	55.60%
DPP-4 inhibitor	Not on DPP-4 inhibitor	74	26	17	117
		32.90%	21.00%	19.80%	26.90%
	On DPP-4 inhibitor	151	98	69	318
		67.10%	79.00%	80.20%	73.10%
SGLT2 inhibitor	Not on SGLT2 inhibitor	96	38	17	151
		42.70%	30.60%	19.80%	34.70%
	On SGLT2 inhibitor	129	86	69	284
		57.30%	69.40%	80.20%	65.30%

(Continued)

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TABLE 1 Continued

		Low Risk	Moderate Risk	High Risk	All
Sulfonylurea	Not on sulfonylurea	95	33	18	146
		42.20%	26.60%	20.90%	33.60%
	On sulfonylurea	130	91	68	289
		57.80%	73.40%	79.10%	66.40%
Insulin	Not on insulin	125	48	11	184
		55.60%	38.70%	12.80%	42.30%
	On insulin	100	76	75	251
		44.40%	61.30%	87.20%	57.70%
TZDs	Not on TZDs	122	54	29	205
		54.20%	43.50%	33.70%	47.10%
	On TZDs	103	70	57	230
		45.80%	56.50%	66.30%	52.90%
CKD	No CKD	221	109	62	392
		98.20%	87.90%	72.10%	90.10%
	CKD	4	15	24	43
		1.80%	12.10%	27.90%	9.90%
Days not fasted coded	Did fast all days	202	105	69	376
		89.80%	84.70%	80.20%	86.40%
	−1 to −4	10	10	7	27
		4.40%	8.10%	8.10%	6.20%
	−5 to −9	5	3	3	11
		2.20%	2.40%	3.50%	2.50%
	−10 to −14	2	1	1	4
		0.90%	0.80%	1.20%	0.90%
	−15 to −19	2	3	3	8
		0.90%	2.40%	3.50%	1.80%
	−20 to −24	1	0	1	2
		0.40%	0.00%	1.20%	0.50%
	−25 to −30	3	2	2	7
		1.30%	1.60%	2.30%	1.60%
Admitted to hospital	No	184	107	66	357
		98.90%	97.30%	88.00%	96.20%
	Yes	2	3	9	14
		1.10%	2.70%	12.00%	3.80%
Significant event or admitted to hospital	No	207	103	69	379
		92.00%	83.10%	80.20%	87.10%
	Yes	18	21	17	56
		8.00%	16.90%	19.80%	12.90%

(Continued)

TABLE 1 Continued

		Low Risk	Moderate Risk	High Risk	All
Frail cat	Robust	47	29	19	95
		52.80%	45.30%	36.50%	46.30%
	Prefrail	38	33	19	90
		42.70%	51.60%	36.50%	43.90%
	Frail	4	2	14	20
		4.50%	3.10%	26.90%	9.80%
Total		225	124	86	435
		51.7%	28.5%	19.8%	100.00%

multivariable logistic regression, and after controlling for all variables studied, other risk factors identified with the occurrence of adverse events in this study were as follows: being in the low-risk category of the DAR risk assessment tool, $B = -1.1$, OR = 0.34 (0.157–0.744), $p = 0.0072$; being in the frail category compared to the reference category, the robust category, $B = 1.54$, OR = 4.6 (1.3–16.6), $p = 0.018$; and older age, $B = -0.034$, OR = 0.966 (0.938–0.995). Interestingly, there was no significant difference between moderate- and high-risk categories in the occurrence of SAE in Table 2A and Figure 2. Hosmer and Lemeshow chi-square = 3.36, $p = 0.91$.

Similar determinants of fasting during Ramadan were identified using multivariable logistic regression (Table 2B). Being in the lower-risk category of IDF-DAR doubles the possibility of fasting during Ramadan, $B = 0.76$, OR = 2.1 (1.01–4.5), compared to the high-risk group, and there is no difference between the moderate- and high-risk groups in completing fasting during Ramadan. Similar to the occurrence of ASE, being frail was a significant risk factor for not fasting during the entire Ramadan period, while older age was marginally significant, $p = 0.076$. Hosmer and Lemeshow chi-square = 8.597, $p = 0.377$.

The performance of the developed model from the logistic regression analysis is described in Table 3.

The *c* statistics of the performance of the developed logistic regression model was better, 0.676, than the IDF-DAR alone, with 0.61 to predict outcomes studied. In addition, the sensitivity of the IDF-DAR alone was better in predicting SAE than a prediction of not fasting during the entire Ramadan period, 67.3% compared to

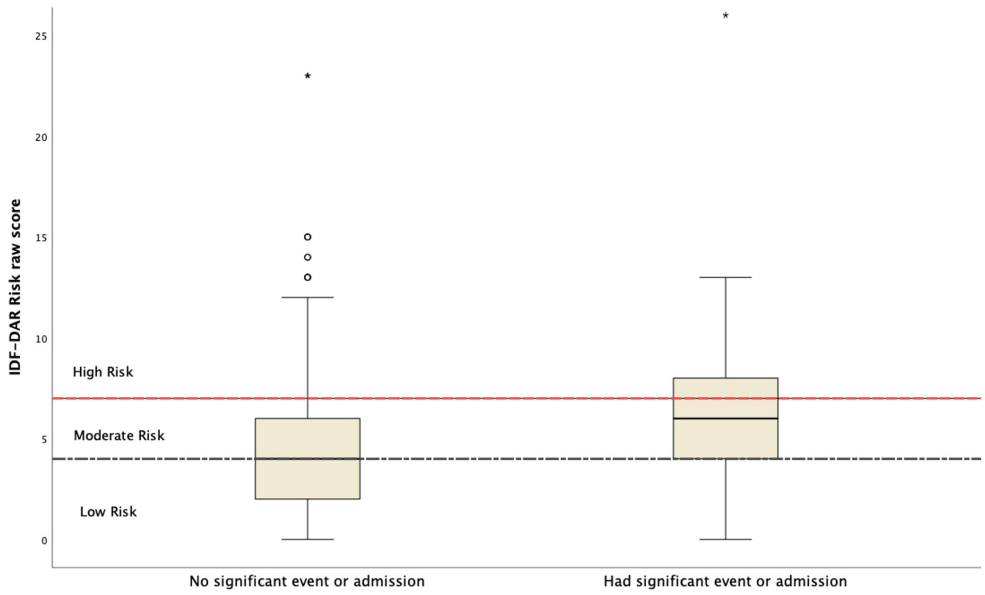

FIGURE 2
The IDF-DAR raw risk score in relation to the occurrence of significant adverse events (reported significant symptom or hospital admission).

TABLE 2 Predictors of (A) significant adverse events occurring during Ramadan and (B) fasting during Ramadan among the whole cohort of 435 participants.

Predictors of significant adverse events (SAE) during Ramadan.					
	B	*p*-value	OR	95% CI for OR (*B*)	
Age	−0.034	0.023	0.966	0.938	0.995
Robust frailty category (Reference)					
Prefrail	0.170	0.692	1.185	0.511	2.747
Frail	1.537	0.018	4.649	1.305	16.558
High-risk IDF-DAR risk category (Reference)					
Low-risk IDF-DAR risk category	−1.074	0.007	0.342	0.157	0.744
Moderate-risk IDF-DAR risk category	−0.124	0.749	0.883	0.412	1.893

Logistic regression

Predictors of fasting during Ramadan					
	B	*p*-value	OR	95% CI for OR (*B*)	
AGE	0.026	0.076	1.026	0.997	1.056
Robust frailty category (Reference)					
Prefrail frailty category	−0.166	0.686	0.847	0.380	1.889
Frail frailty category	−1.340	0.034	0.262	0.076	0.904
High-risk IDF-DAR risk category (Reference)					
Low-risk IDF-DAR risk category	0.758	0.045	2.134	1.017	4.478
Moderate-risk IDF-DAR risk category	0.230	0.558	1.259	0.583	2.715

Logistic regression.

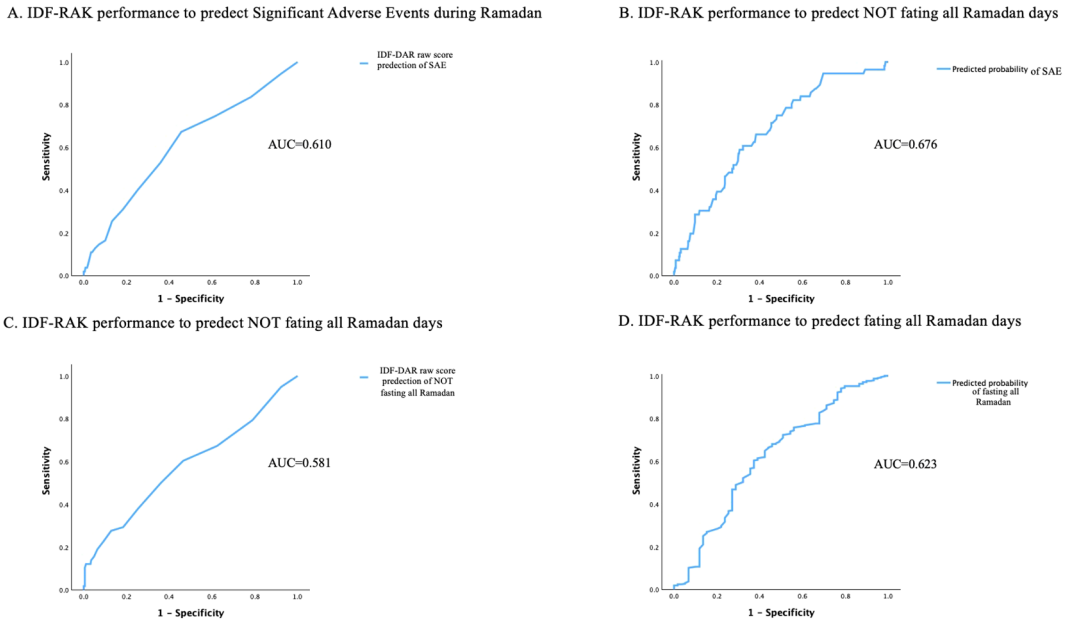

FIGURE 3
The IDF DAR raw risk score in relation to the occurrence of significant adverse events. (reported significant symptom or hospital admission). **(A)** IDF-RAK performance to predict Significant Adverse Events during Ramadan. **(B)** IDF-RAK performance to predict NOT fasting all Ramadan days. **(C)** IDF-RAK performance to predict NOT fasting all Ramadan days. **(D)** IDF-RAK performance to predict fasting all Ramadan days. AUC, Area Under the Curve; SAE, Significant Adverse Events.

TABLE 3 Performance of the IDF-DAR tool and the model developed and from the logistic regression in predicting (A) SAE and (B) fasting during Ramadan.

A. Predicting SAE						
	Cut-off	Sensitivity	Specificity	*c* statistics	CI	
IDF-DAR score	3.5	67.3	54.4	0.610	0.527	0.692
Predicted probability	13.8	60.7	67.8	0.676	0.603	0.748
B. Prediction of fasting during Ramadan						
	Cutoff	Sensitivity	Specificity	*c* statistics	CI	
IDF-DAR score	7.5	27.6	87.2	0.581	0.495	0.667
Predicted Probability	86.7	60.4	67.7	0.623	0.541	0.705

27.6%. The developed model, which included the FRAIL score, age, and the IDF-DAR score, performed better in detecting both studied outcomes. Sensitivity and specificity were similar, approximately 60% and 67%, respectively (Figure 3).

Another important outcome is the incidence of hypoglycemia, with 46 patients reporting hypoglycemic episodes during the month of Ramadan (11.7%). The high-risk group, as per the DAR stratification tool, had 10 patients experiencing these episodes during Ramadan (12.7% of the high-risk patients); the moderate-risk group had 18 episodes (15.8%), and the low-risk group had 18 episodes (9%) (Table 4). These hypoglycemia episodes were more likely among those who needed to break their fast, which was the only significant determinant of hypoglycemia as per logistic regression, $B = -0.11$, CI (0.85–0.94), SE = 0.027, $p < 0.001$ (Figure 4).

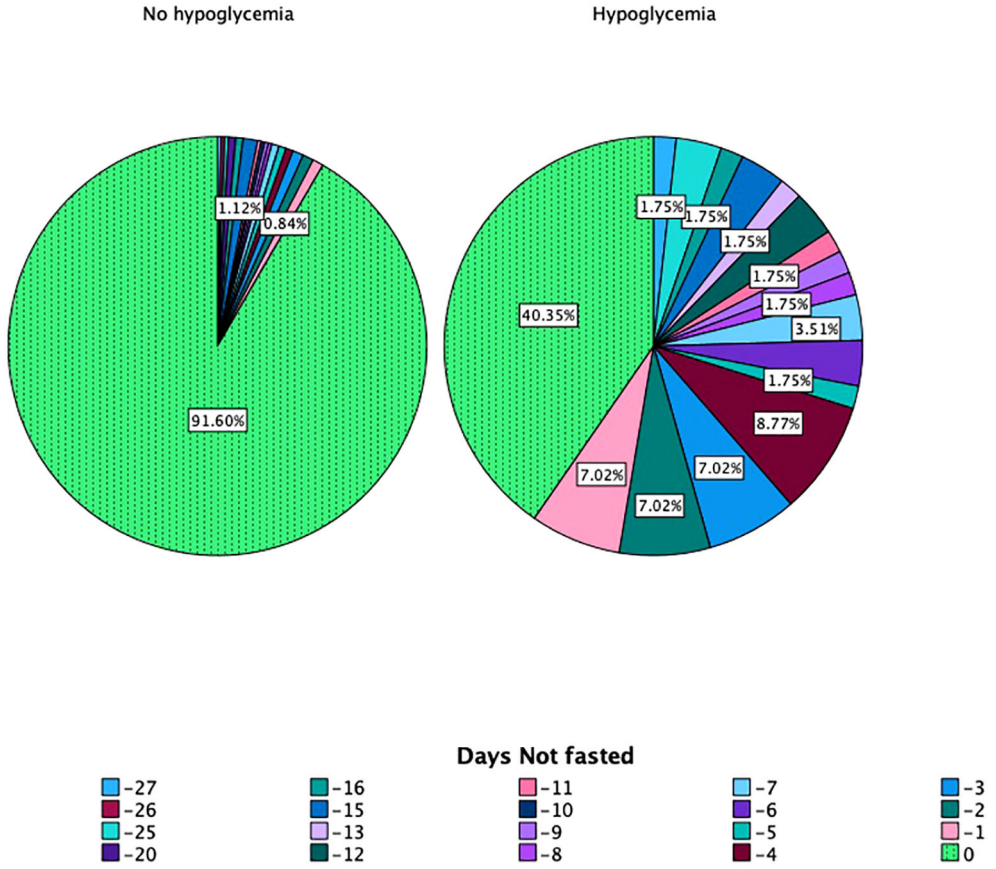

FIGURE 4
The incidence of hypoglycemia in relation to days fasted during Ramadan.

Discussion

Patients in the low-risk category, as per the DAR risk assessment tool, who fasted during Ramadan or attempted to fast had a significantly better outcome than those in the moderate- or high-risk categories during Ramadan regarding the occurrence of SAEs. They were at 70% less risk of developing an adverse event than those from the moderate- or high-risk categories. Those in the moderate- and high-risk categories were nearly close to each other in predicting patient outcomes during fasting. Although there was a noticeable increase in the risk of adverse events with increasing IDF-DAR risk category scores from low to moderate to high, as seen from the logistic regression controlling for other factors, it is suggested that counseling be provided for patients in the moderate- and high-risk groups equally.

This study reported more adverse events than a similar study in the same period in a diabetes center in Abu Dhabi-AlAin (3). Mohammad et al.'s study reported outcomes related to mainly hypoglycemic and hyperglycemic episodes. Only one patient in their cohort had been admitted. Therefore, this study adds an outcome that matters: fewer hospitalizations and incidence of illness in the lower-risk group. This observation was higher in this study than in Mohammad et al.'s. Surprisingly, their study had more hypoglycemic episodes than this study, 15% compared to 9.6%, respectively. However, other variables, such as diabetes control, insulin use, or diabetes duration, did not differ much between the two cohorts in explaining this difference. Such differences between cohorts warrant an investigation of the possible factors influencing outcomes. For example, this study setting is a primary care center, while the Mohammad et al. study is based in a diabetes-specialized center. There are potential cultural and healthcare system differences between the two settings that could explain such differences. Patients choosing to be followed in a specialized center could represent a different socioeconomic status or disease risk category. Moreover, differences in practice resources and medication use can influence such outcomes.

The risk of hypoglycemia may be related to other factors, such as the healthcare setting, health literacy, and self-management, but it is not associated with the patient's risk profile. In another similar study to validate the DAR risk assessment tool in Bangladesh, Kamrul-Hasan et al. found that hypoglycemia and hyperglycemia risks were 3.74-fold and 3.86-fold higher in the high-risk group than in the low-risk group (5). Similar to this study, the episodes of hypoglycemia were lower than those of Mohammed et al.

TABLE 4 Hypoglycemia incidence distributed by the IDF risk assessment score.

	Low Risk	Moderate Risk	High Risk	Total
No hypoglycemia	183	96	69	348
	91.00%	84.20%	87.30%	88.30%
Hypoglycemia	18	18	10	46
	9.00%	15.80%	12.70%	11.70%
Total	201	114	79	394
	100.00%	100.00%	100.00%	100.00%

This study supports Mohammad et al.'s and Kamrul-Hasan et al.'s study in that the DAR risk assessment tool helps stratify the risk of patients with diabetes intending to fast during Ramadan. Two additional independent risk factors were identified: previous fasting experience and patients' frailty. The more days the patients did not fast, the more adverse events there were, which may indicate that they could not complete fasting due to poor health, and those who completed fasting are in a better health status. This highlights the importance of patients' previous fasting experience, validating the DAR risk assessment tool question.

Frailty was found to be another independent risk factor in this study. No study used the FRAIL score to assess the outcome of fasting during Ramadan; therefore, this is an important addition discussed in another part of this study (15). This highlights the importance of studying new risk factors for adverse events, such as social factors, healthcare literacy, income level, and access to medical care. In the United States, for example, integrating social context into healthcare delivery has become a priority strategy due to accumulating evidence of it being determinantal in the outcome of patients with diabetes (16–18). The main limitation of this study is the possible variation among physicians who performed the clinical judgment and utilized the risk estimation calculator. This was found to be true in a study by Afandi et al., where classifying the risk of patients with diabetes during Ramadan fasting was found to be widely varied (19). They used case scenarios presented in a survey distributed to physicians and asked to evaluate patients' risk utilizing the same DAR calculator. The variation was particularly higher in moderate-risk cases. Investigators attributed this to factors other than clinical judgment, such as personal, spiritual, and social factors. They recommended that risk categorization be customized to empower physicians to stratify each patient. Nevertheless, these factors, although important, in support of this risk assessment tool is the significant prediction of key outcomes that support the physicians' judgment.

It is empirically recommended that validating this tool in different cultures and geographical regions will strengthen the prediction of outcomes. Important factors, such as healthcare literacy, self-management, and socioeconomic status, could significantly influence a patient's ability to fast and manage diabetes. Future studies need to control for these social determinants of health.

Variations in individual physicians' practice may influence the outcome, and it is also a limitation and possibility for future research. The medication change and review were not addressed during data collection. However, it was one of the aims of the counseling visits before Ramadan as part of routine care in preparation for Ramadan.

Another limitation could be the lack of blinding of patients and healthcare providers of the assessment result and subsequent counseling and change in management effect on outcome. Unfortunately, this limitation in assessing the risk of Ramadan fasting may not be possible to overcome; since the IDF-DAR tool was introduced a year before the study, it became a required assessment during the routine care of patients with diabetes before Ramadan.

Depriving patients from knowing the risk and having physicians target it with appropriate interventions is unethical. Nevertheless, in support of the fact that the lack of blinding may not have significantly affected the study, the aim is that the influence of counseling targeted all participants and not selective to a group. In addition, a higher risk could have more intense counseling and possibly reduced the number of SAE. Fasting decisions were not much affected as only 7 out of the 93 high-risk patients did not fast compared to 5 out of 128 in the moderate-risk group. This is explained by the cohort's firm intention to fast as Muslims and the shame and disappointment they feel by not fulfilling this major pillar of Islam. Therefore, they are counseled, but most of them fast as per this cohort; 96.4% did fast the whole month of Ramadan or attempted to do so. Patients intend to observe fasting during all Ramadan days and will only refrain from doing so in case of an illness to prevent a serious adverse effect.

Another area for improvement is the potential biases introduced through tele-interviews, with the risk of data collection being compromised by less rapport, probing, and interpretation of responses. Yet, tele-interviews contribute to better accessibility to participants, more effortless follow-up, and lower costs. In support of its use, evidence is lacking that they produce lower-quality data (20).

Finally, this study's practical recommendation for clinicians is to incorporate evidence-based tools in risk assessment, such as the IDF-DAR risk assessment tool and FRAIL, into practice to aid in decision-making when assessing recommendations for added potential stress on patients, such as fasting.

Conclusion

Fasting is not associated with a higher risk of adverse events, but the patient's higher-risk category is. According to the IDF-DAR risk assessment, patients with diabetes in the low-risk category had a better outcome than those in the moderate- or high-risk categories regarding SAEs during Ramadan. Another independent risk factor is if the patient is frail, according to the FRAIL scoring. Future IDF-DAR risk assessment tool validation studies related to fasting need to control for social determinants of health and medication adjustment during fasting. Additionally, future research should aim at prospective and multi-regional validation to enhance the generalizability of the findings.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The study was approved by the Ambulatory Healthcare Services- Abu Dhabi IRB. All methods were carried out under relevant guidelines and regulations. Informed consent was obtained from all subjects, and data was anonymized at analysis. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

LB: Conceptualization, Formal analysis, Methodology, Project administration, Supervision, Validation, Writing – original draft. BA: Conceptualization, Methodology, Project administration, Writing – review & editing. NN: Formal analysis, Writing – review & editing. HA: Data curation, Writing – review & editing. RA: Data curation, Writing – review & editing. MA: Data curation, Writing – review & editing. FA: Data curation, Writing – review & editing. NSA: Data curation, Writing – review & editing. AMA: Data curation, Writing – review & editing. NMA: Data curation, Writing – review & editing. AAA: Data curation, Writing – review & editing. SA: Data curation, Writing – review & editing. MH: Conceptualization, Methodology, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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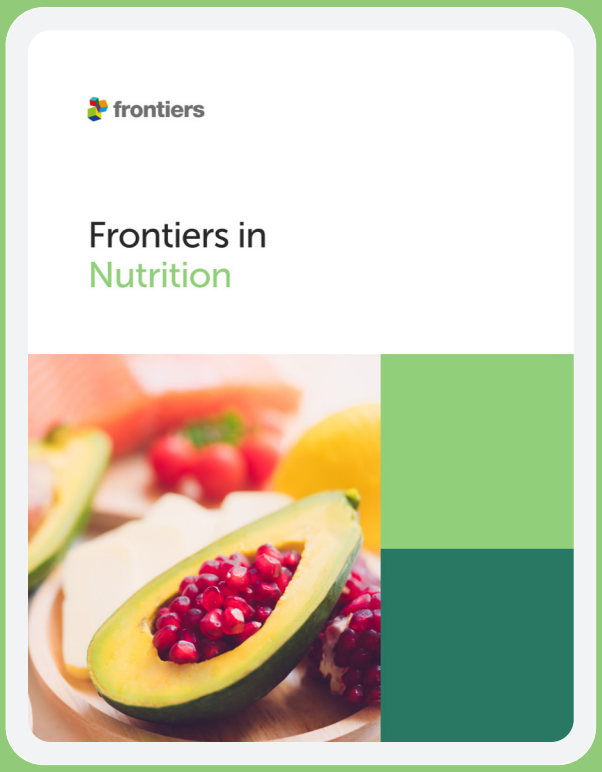

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