THE MODEL OF RAMADAN DIURNAL INTERMITTENT FASTING: UNRAVELING THE HEALTH IMPLICATIONS - VOLUME I

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Editorial: The model of Ramadan diurnal intermittent fasting: Unraveling the health implications - volume I

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Editorial on the Research Topic
The Model of Ramadan Diurnal Intermittent Fasting: Unraveling the Health Implications - Volume I

Introduction

Intermittent fasting (IF) is rapidly gaining interest across various scientific disciplines and the general community. The practice of IF is a safe and costless intervention that offers health benefits and disease prevention, particularly related to chronic metabolic and aging diseases (1, 2). One of the most commonly practiced models of IF is the obligatory IF observed annually in the month of Ramadan by about 1.5 billion Muslim people. Much evidence gathered during the last two decades suggests that observing this 1-month religious fasting, lasting between 10 and 21 h a day depending on the location and season (3), provides metabolic and physiological benefits.

The many health benefits of Ramadan diurnal intermittent fasting (RDIF) include improvements in body weight and body composition (4–6), reducing complications of the metabolic syndrome (4), improving lipid profiles and other cardiometabolic risk factors (7–10). Further, in healthy people, RDIF helps improve glucose homeostasis (11), ameliorate inflammatory and oxidative stress markers (10), improve liver function tests (3), and modulate gene expression of various components of the anti-inflammatory and antioxidant defense and circadian rhythm systems (12, 13). In subjects with metabolic syndrome, RDIF was shown to improve several components of metabolic syndrome, upregulate tumor-suppressor proteins and downregulate tumor-promoter proteins (14). Despite being the most extensively studied form of IF, many gaps remain in our understanding of the many effects of RDIF in healthy people, including athletes. Further, it is unclear how the observance of RDIF affects patients with illnesses such as diabetes, cardiovascular disease, and cancer. More information is needed on the effects of RDIF on different body systems and the possible genetic expressions and epigenetic changes produced by this religious practice. A better understanding of RDIF will help optimize the practice of RDIF, maximize its health benefits, and guide healthcare providers to better advise their chronically ill patients on matters related to RDIF. Accordingly, variable aspects of RDIF are summarized in this Frontiers Research Topic "*The Model of Ramadan Diurnal Intermittent Fasting: Unraveling the Health Implications—Volume I*." This Research Topic aims to provide a comprehensive view of studies on RDIF, using observational and interventional studies in humans and animals.

Summary of selected articles from this Research Topic

About 530 proposed contributors were invited to participate in this Research Topic, from whom we received 14 abstracts and 26 manuscripts. After vigorous screening and a critical review, 15 articles were selected for this Research Topic. The contributing 80 authors were from 16 countries across five continents, including Lebanon, the United Arab Emirates (UAE), the United States of America, Germany, Tunisia, Italy, Canada, Jordan, United Kingdom, Libya, Australia, Singapore, Algeria, Netherlands, China, and Iran. This Research Topic received more than 33,400 views and downloads as of June 2022.

The study by Shatila et al. characterized food intake among Lebanese adults observant of RDIF and compared it to their intake for the remainder of the year. In a year-round observational study, the authors observed significant increases in dietary intakes for 12 out of 19 food groups such as intakes of cereals, cereal-based products, pasta, eggs, nuts and seeds, milk and dairy, and fats and oils were lower, while vegetables, dried fruit, Arabic sweets, cakes and pastries, and sugar-sweetened-beverages intakes during Ramadan. Such differences in the intakes of food groups were also reflected in nutrient intakes, where intakes of carbohydrates, cholesterol, calcium, beta-carotene, vitamin C, folate, and magnesium showed significant changes. This interesting study highlights important differences in dietary food groups and nutrient intakes during the fasting month compared to the rest of the year.

The authors of Riat et al. examined the association between mood-related symptoms and health-related quality of life and several biological parameters, including serum cortisol, brain-derived neurotrophic factor (BNDF), insulin growth factor-1 (IGF-1), interleukin (IL)-8, matrix metalloproteinase (MMP)-9 and myoglobin levels in 34 healthy adult subjects who practiced RDIF. They showed that the cortisol levels were significantly lower 1 week after RDIF compared with the levels measured 1 week before RDIF ($P < 0.001$), and BDNF levels were significantly lower during the last days of RDIF compared with the levels measured 1 week before RDIF ($P < 0.05$). The authors concluded that the effects of RDIF on mood-related symptoms were correlated with different biological parameters, specifically cortisol and BDNF levels.

The authors of Fekih et al. evaluated the effects of mental training through imagery on the competitive anxiety of adolescent tennis players fasting during Ramadan. They studied 38 male tennis players that were randomly allocated to the experimental group (EG) and control group (CG); the CG watched historical videos of the Olympics, while those in the EG performed mental training. There was a significant interaction for all competitive anxiety subscales, with higher intensity and direction scores for cognitive and somatic anxiety subscales during Ramadan for both groups. Higher intensity and direction scores for the cognitive and somatic anxiety subscales occurred during Ramadan for both groups; this increase in scores was greater for the control group than for the EG during the middle and at the end of Ramadan ($P < 0.001$). Intensity and direction scores were significantly lower during Ramadan for the two groups. Further, the score for the intensity of self-confidence was greater for the EG compared with the CG. The authors concluded that mental imagery training reduced cognitive and somatic anxiety and increased self-confidence in the intensity dimension of adolescent tennis players who fast during Ramadan.

The study by Al-Nawaiseh et al. investigated the impact of RDIF on runners' performances, using 15 trained male distance runners who observed Ramadan. Each participant reported to the human performance lab before and at the end of Ramadan. The participants performed graded exercise tests on a treadmill, and their VO_2, heart rate, time to exhaustion, and running speed were recorded. There were no significant effects of Ramadan fasting on body mass, body fat, lean body mass, VO_2max, energy availability, and protein intake. However, carbohydrate, lipid, water, and caloric intakes were significantly reduced during Ramadan. Daily training duration and exercise energy expenditure were also significantly reduced after Ramadan. Time to exhaustion and maximal running speed was improved, as were time to exhaustion and maximal running speed of the distance runners; these changes were independent of changes in nutrient intake observed during the study. The authors concluded that the performance of distance runners could be maintained or even slightly improved following the month of Ramadan fasting.

The genetic study by Madkour et al. examined RDIF-associated changes in the Fat mass and obesity-associated (*FTO*) relative gene expression in a group of 63 metabolically healthy subjects with overweight and obesity. The expressions of *FTO* were significantly decreased at the end of Ramadan by more than one-third of the pre-fasting gene expression levels (−32.30%, 95% CI−0.052 −0.981). Significant reductions occurred in body weight, BMI, fat mass, body fat percent, hip circumference, LDL, IL-6, TNF-α, and waist circumference, while there were increases in HDL and IL-10 at the end of Ramadan. Binary logistic regression analysis for genetic expressions indicated no significant association between high-energy intake, waist circumference, or obesity and *FTO* gene expression. The authors concluded that RDIF is associated with the downregulation of the *FTO* gene expression in subjects with obesity, which may explain, at least in part, the favorable metabolic effects of RDIF. Thus, RDIF presumably entails a protective effect on body weight gain and its adverse metabolic-related derangements in subjects with obesity.

In this narrative review, Elmajnoun et al. summarized the impact of the COVID-19 pandemic on children and young adults with type 2 diabetes (T2DM). The authors also explored the potential of intermittent fasting in reversing the pathogenesis of diabetes, highlighting how this could prevent these patients from developing chronic complications. The authors concluded that children and young adults with T2DM are not at risk of severe COVID-19 as is the case in adults with diabetes. However, more research is needed to identify the impact of COVID-19 in children and young adults with T2DM, particularly investigating the efficacy and safety of intermittent fasting, including Ramadan fasting. Moreover, the authors advised that implementing these cost-effective programs could greatly minimize diabetes in children and young adults. Furthermore, this could be particularly effective in patients with prediabetes.

Exercise and fasting confer health benefits independently, leading Zainudin et al. to propose that people who are fasting, especially those experiencing health and clinical challenges, continually engage in physical activity during the Ramadan fasting month. In this opinion piece, the authors recommended walking football (WF) as an exercise of choice among Muslims who are fasting. WF can be played by any individual regardless of their fitness level, skills, and age. WF elicits cardiovascular and metabolic stress responses, which can be beneficial in populations with low fitness levels. Most importantly, WF has the inherent characteristics of being a fun team activity requiring social interactions among participants and, hence, likely to encourage long-term consistent and sustainable participation.

The study by Muammar et al. investigated the outcomes of RDIF using multiple daily insulin injections and continuous subcutaneous insulin infusions to assess patterns of glycemic control and severity of complications during RDIF in older children and adolescents with type-1 diabetes mellitus (T1DM). The effects of dose adjustment, health professional teams, and parental support on safety and glycemic outcomes in children and adolescents with T1DM during Ramadan were also investigated. The results indicated no significant deterioration in indicators of overall glycemic control, which remained inadequate during RDIF. The authors concluded that RDIF should be discouraged in children with poorly controlled T1DM.

There are significant changes in sleep-wake patterns during Ramadan, which are largely caused by alterations in the timings of the two daily meals—one pre-dawn (*Sohor*) and the other at sunset (*Iftar*). The literature review by Bencharif et al. discusses the guidelines for drug treatment (Ramadan often requires altering treatment protocols for people with non-communicable diseases such as diabetes), physical exercise, body composition, and metabolic changes. The impact of the Covid-10 pandemic is also reviewed. The authors summarize international guidelines (from 1995 to 2021) that attempt to optimize the management of diabetes during Ramadan.

The authors previously reported that RDIF caused increases in the concentrations of short-chain fatty acids (SCFA) in the gut microbiome of healthy humans that were associated with improved metabolic parameters, an effect that could be due to a combination of psychological effects and microbiome remodeling. The current study by Su et al. examined changes in the gut microbiome in a mouse model of RDIF where the effects of these two variables (psychological effects and microbiome remodeling) could be isolated. The findings in the mouse model of RDIF confirmed the results of microbiome remodeling in humans of increases in bacteria that stimulate the production of SCFA (which are associated with reduced visceral fat mass in humans).

Using flash glucose monitoring (FGM) monitor in 24 patients with type 1 and types 2 diabetes on insulin therapy who were remotely connected to the diabetes clinics in the UAE; Helal et al. tried to examine the impact of COVID-19 lockdown on glycemic control pre- and post-lockdown and during RDIF. Analyses of data were performed on glucose management indicator (GMI), time in range (TIR), time in hyperglycemia, time in hypoglycemia, low blood glucose index (LBGI), and high blood glucose index (HBGI). Variables were calculated for each period: 30 days before lockdown, 30 days into lockdown and pre-Ramadan, and 30 days into lockdown and Ramadan, using the continuous glucose monitoring analysis package in R-studio software. Results revealed that the mean average glucose (MAG) remained steady before and during the lockdown. The significant difference in GMI and percentage of time in hyperglycemia were reported between Ramadan and pre-Ramadan during the lockdown period. The percentage of TIR was significantly lower in Ramadan as compared to pre-Ramadan. Mean absolute glucose (MAG) and HBGI were found to be significantly higher in Ramadan compared to the pre-Ramadan period. The authors concluded that the lockdown period did not significantly impact the markers of glycemic control in the population studied. However, the authors found that several changes were embedded during the Ramadan fasting period, including increased GMI, HBGI, and glycemic variability similar to what has been reported in other Ramadan studies.

In their study using liquid chromatography-mass spectrometry-based metabolomics technique, Chen et al. investigated the composition of fecal metabolites in Chinese and Pakistani individuals before and after RDIF. The distinct separation of metabolite profiles among ethnic groups as well as between pre-and post-fasting samples was performed. After RDIF, the whole population groups showed significant differences in their respective contents of various fecal metabolites, particularly L-histidine, lycofawcine, and cordycepin concentrations, which were higher after RDIF in the Chinese group. However, brucine was enriched in the Pakistani group. The Kyoto Encyclopedia of Genes and Genomes analysis suggested that metabolites related to purine metabolism, 2-oxocarboxylic acid metabolism, and lysine degradation were significantly enriched in the total subject population pre-RDIF vs. post-RDIF comparisons. Several bacterial taxa were found to be significantly correlated with specific metabolites unique to each ethnic group, suggesting that changes in fecal metabolite profiles related to RDIF may be influenced by associated shifts in gut microbiota. The authors concluded that RDIF-related differences in fecal metabolites, together with these group-specific correlations between metabolites and taxa, support their previous findings that ethnic differences in dietary composition drive the variation in gut microbial composition and diversity.

This systematic review analyzed insulin dosing recommendations that can reduce hypoglycemic events and improve glycemic control for patients with T2DM during the fasting month of Ramadan. Kieu et al. evaluated the findings of 14 eligible studies that included 2,969 study participants across four continents with an average age of 54.8 years. The studies consisted of five RCT studies, and nine observational cohort studies, of which six studies examined insulin dosage adjustment on glycemic control and hypoglycemia during Ramadan, three examined newer ultra-long-acting insulins, and another three compared insulin analogs. The systematic review indicates that insulin dose reduction could prevent hypoglycemia without causing subsequent hyperglycemia, and rapid-acting insulin analogs could improve post-iftar and overall blood glucose without incurring hypoglycemia. This systematic review exclusively analyzed insulin subtypes and dosing strategies for insulin-treated T2DM during Ramadan. The authors recommend more research to confirm the benefits of ultra-long-acting insulins as well as the use of flexible glycemic targets and recommend more randomized controlled trials out before more detailed conclusions could be made.

The authors Mousavi et al. used systematic reviews to summarize current findings on the impact of RDIF and non-RDIF on the gut microbiome. Several databases were used to identify 28 studies (of human and animal model) for this systematic review. The results indicate a significant shift in the gut microbiome, especially in increases of *Lactobacillus* and *Bifidobacteria* following fasting diets. Some studies reported increases in bacterial diversity and production of beneficial metabolites such as SCFA and decreases in inflammatory processes. However, other investigations reported adverse effects of fasting diets on the structure of the microbiome. The authors conclude that most animal and human investigations indicate positive effects of fasting on the composition and structure of the gut microbiome.

The impact of RDIF on the salivary flow rate (SFR) and metabolic parameters was addressed in this systematic review by Besbes et al.. After reviewing the PubMed database, six (06) original articles meeting the inclusion criteria were included in the systematic review. Several parameters were considered in this systematic review which displayed downward trends: SFR was decreased by 10%, the circadian pattern of melatonin remained constant, while melatonin levels, glucose, uric acid, and aspartate aminotransferase (AST) decreased. Levels of alkaline phosphatase (ALP) level increased significantly. The cortisol concentrations in saliva remain unchanged or increased during the fasting days of Ramadan. Salivary levels of immunoglobulin A (IgA) decreased during the last week of the Ramadan fasting month. The authors of this systematic review provided some recommendations for the design of future research studies based on the limitations of some studies e.g., different methodologies used to examine the impact of RDIF on SFR.

Conclusion

This Frontiers Research Topic contributes to our understanding of the impact of RDIF on various aspects of human nutrition, health, and disease. This Research Topic will hopefully open a venue for further studies and stimulate future discussions among researchers.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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ORIGINAL RESEARCH
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Impact of Ramadan Fasting on Dietary Intakes Among Healthy Adults: A Year-Round Comparative Study

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Religious rituals are considered among the principle factors that impact dietary behaviors and food selections. The main objective of this study is to characterize food intake among Lebanese adults observant of the fasting month of Ramadan and compare it to their intake of the rest of the year. During a year-round study, including the month of Ramadan, Lebanese adults ($n = 62$), completed multiple (9 to 13) 24-h dietary recalls. Information about sociodemographic and lifestyle characteristics was also obtained. Dietary intake was examined using food groups as well as energy, macro, and micronutrient consumption. Significant differences in dietary intakes were observed for 12 of the 19 food groups (expressed as a percent of total energy) during Ramadan as compared to the rest of the year. More specifically, the intakes of cereals, cereal-based products, pasta, eggs, nuts and seeds, milk and dairy, and fats and oils were lower, while vegetables, dried fruit, Arabic sweets, cakes and pastries, and sugar-sweetened-beverages intakes were higher during Ramadan as compared to the remainder of the year ($p < 0.05$). Such differences in food groups' intakes were reflected in nutrients intakes, including carbohydrates, cholesterol, calcium, beta-carotene, vitamin C, folate, and magnesium. The findings of this study highlighted major differences in dietary intakes between the fasting month as compared to the rest of the year. With the large number of adults who observe fasting during Ramadan, the particularities of dietary intake during Ramadan ought to be considered in the development of context and culture-specific dietary recommendations.

Keywords: fasting, dietary change, ethnic group, foodculture, intermittent fasting, Ramadan, religious affiliation

INTRODUCTION

Religious affiliation is one of the most distinguishing characteristics of the world population, with Islam as the second-largest affiliation after Christianity. Many of the major religions have their unique dietary rules, which may or may not be strictly adhered to by the followers (1). In essence, religion and religious rituals and feasts are considered among the principle factors that impact dietary behaviors and food selections (2). During Ramadan, food intake shifts drastically from diurnal to nocturnal eating time and practice, Ramadan fasting illustrates how religious beliefs affect human dietary behavior.

On average, one and a half billion Muslims around the world observe the fast during the holy month of Ramadan (3). Ramadan is the ninth month of the Islamic lunar calendar when Muslims fast from dawn to sunset for 29 or 30 consecutive days, abstaining from any food or water with a common practice of consuming one large meal after dusk and a lighter meal before dawn (4, 5). Between dusk and dawn, there are no dietary restrictions related to Ramadan as it is observed in other religions like Christianity fasting (e.g., avoidance of animal-based products, oil, or fish) (6). The duration of the daily fast during this month varies according to the geographical area and to its timing in the year, reaching in some countries and seasons to 19 h a day. This yearly fast, combined with many lifestyle modifications in physical activity (7, 8), sleep patterns and circadian rhythmic changes (9–11) have been shown to incur significant changes in dietary habits and food consumption patterns (12–14) leading ultimately to significant anthropometric, cardiometabolic, glucoregulatory, and inflammatory changes (15).

The nature of food intake during the month of Ramadan, with its particularities in terms of timing and frequency of meals, has been previously examined (16–18). More specifically, food intake during Ramadan has been associated with major changes in dietary patterns, food groups as well as energy, macro-, and micronutrient intakes. However, the available evidence concerning these changes is inconclusive (13, 14, 16, 19–26). While a few studies reported lower energy intakes (18, 19, 26), several studies comparing dietary intake during Ramadan to that during regular days reported higher energy consumption during Ramadan (16, 22, 27), mainly derived from a higher intake of carbohydrates (16, 23, 26, 27), particularly sweets (14), and fats (14, 21, 22). Such changes in dietary intake during this month are of significance, especially among individuals susceptible to metabolic diseases linked to these changes such as obesity, diabetes, and hypertension (14, 28–30). Thus these food practices are important to consider in countries with a high burden of non-communicable diseases (NCD) and where considerable proportions of the populations observe fasting during Ramadan.

Lebanon is a small country in the Middle East which, according to the World Health Organization, has surging rates of NCDs, estimated to account for 91% of all deaths in the country (31). Concomitant with the high prevalence of NCDs similar to its neighboring countries, Lebanon has been undergoing a nutrition transition characterized by dietary shifts toward a surplus of energy, fat, and sugars, resulting in increasing rates of weight gain and obesity (32). Studies examining dietary patterns in the country also reported a gradual erosion of the traditional Lebanese dietary pattern rich in fruits, vegetables, bulgur, legumes, olives, whole-fat dairy, and starchy vegetables (32–37). This latter pattern was identified as a variant of the Mediterranean diet, in light of both its composition and protective effects against metabolic diseases including obesity, hypertension, and type 2 diabetes (34, 35, 38–42).

With a large proportion of Muslims (over 61%) among the Lebanese (43), and the possibly high observance of fasting during the month of Ramadan, it is critical to understand the particularities of dietary intakes during this month to develop evidence-based recommendations for healthy adults and those living with the chronic disease during the holy month.

To our knowledge, there is a paucity of research that compares the dietary intake of adults who observe the month of Ramadan with their intake during the remainder of the year. Therefore, using multiple 24 h recalls over a year, this study aims to characterize food intake among Lebanese adults observant of the fasting month in terms of energy, macronutrients, micronutrients, and food groups during the month of Ramadan in comparison with their intake during the rest of the year. The results of this study will provide an evidence base for the development of culture-specific dietary guidelines during this month in Lebanon and its neighboring countries, which share with it many cultural, religious traditions, and food-related habits.

METHODS

Study Participants

Data for this study were derived from an earlier prospective investigation of dietary intake among Lebanese adults (18–65 years), the details of which are published elsewhere (44). In brief, subjects were recruited from the American University of Beirut (AUB), the largest employer in the Lebanese private sector (45, 46). The university is also a culturally diverse institution with employees from across the country and from various socioeconomic statuses. Flyers with invitations to participate in the study were posted on the advertisement boards across the AUB campus. The inclusion criteria were: (1) full or part-time employment at AUB, (2) Lebanese nationality, or residing in Lebanon for more than 10 years, (3) conversant in Arabic or English language, (4) not pregnant or breastfeeding, (5) not having any chronic health condition that requires dietary modifications and medication prescription. The Institutional Review Board of the Social and Behavioral Sciences at the AUB, Lebanon, approved the study protocol under the protocol number (NUT.FN.22). For this study, observant (fasting) participants of the month of Ramadan were included ($n = 62$ out of 107 who participated in the original study).

Data Collection

Face-to-face interviews were conducted in a private setting at the participant's office or, if not available, at the Nutrition and Food Sciences department at AUB. During the 1-year duration of the study, a total of 9–12 24-h dietary recalls (24HR) were collected, at least one of which was carried out during the month of Ramadan (26th of May until 24th of June). Given that the fast period during Ramadan is related to sunset and sunrise timings, it tends to be vary from day to day. Generally, during the year where data collection took place, the fast was between 3:30 a.m. and 8:00 p.m.

Every effort was exerted to secure 3 24 HR dietary recalls for each participants during each of the four seasons, totaling to

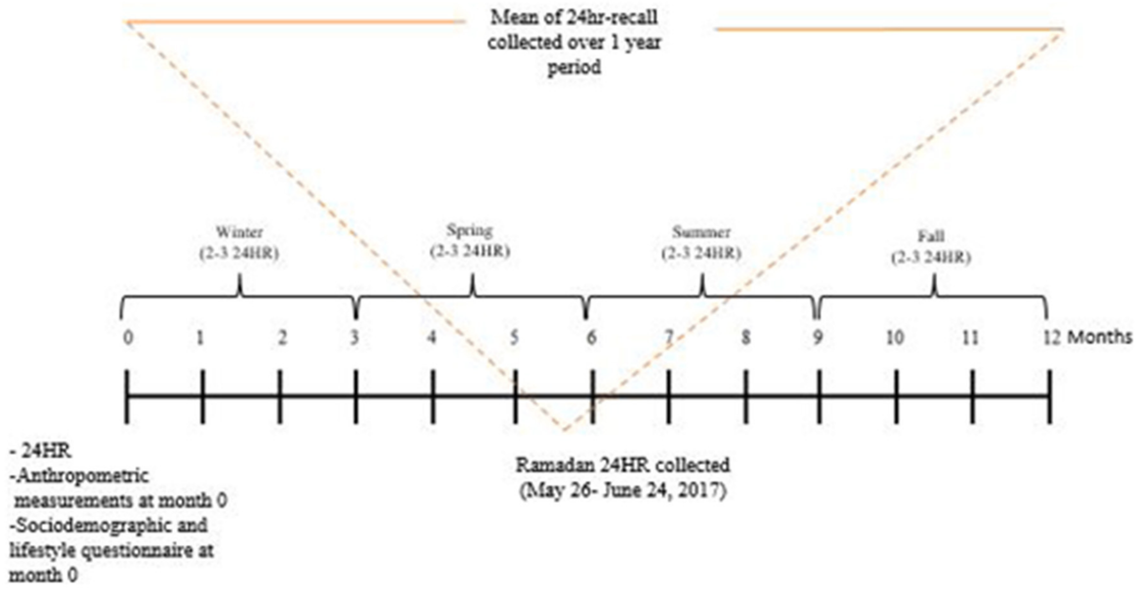

FIGURE 1 | Study protocol.

12 recalls. However, it was inevitable that access to participants be interrupted and only 2 per season were obtained in certain cases. Hence the range in the number of 24 HR dietary recalls. At baseline, a sociodemographic and lifestyle questionnaire was collected with information about the participant's age, sex, place of residence, marital status, smoking, physical activity (Arabic version of the International Physical Activity Questionnaire), education level, type of employment, monthly income, and crowding index (CI). The CI is calculated as the ratio of the number of household residents over the number of rooms in the house and used as a proxy indicator for socioeconomic status in Lebanon and neighboring countries (47–50). Further at the baseline visit, anthropometric measurements of participants were obtained using standardized techniques and calibrated equipment. Weight (kg) was measured to the nearest 0.1 kg using a beam scale. A stadiometer was used to measure the height (cm) to the nearest 0.1 cm. Study subjects were requested to remove their shoes and as much outerwear as possible before measuring the weight and height. The body mass index (BMI) was calculated as weight (kg)/height (m^2), and classified accordingly into normal (18.5–24.9), overweight (25.0–29.9), and obese (>30 kg/m^2) (51). All measurements were taken twice and the average of the two readings was used in the analysis. A representation of the study protocol is depicted in **Figure 1**.

The 24HR was collected using the Multiple Pass Food Recall (MPR) approach that involves a 5- step approach and was developed by the United States Department of Agriculture (USDA) (52, 53). The steps to collect the 24HR using the MPR approach include (1) quick food list recall, (2) forgotten food list probe (3) time and occasion at which foods are consumed, (4) detailed overall cycle, and (5) final probe review of the consumed foods. As a result, for each 24HR collected, the research dietitian obtained information about the time when the meal was consumed, the food consumed, beverages and/or supplement consumed, its portion size, and preparation method. Participants were not aware of the exact day on which the 24HR was collected to maintain the participants' regular dietary habits. The dietitian called the study participant on the same day to schedule the time for the recall. If the participant was not available on the same day (or unreachable), the dietitian would then try to contact the participant on another day. Attempts were repeated until a minimum of 2 24HR recalls were obtained for each season during the 1-year duration of the study. All interviews were administered to each participant by the same-trained research dietitian.

Food items collected from the 24HR were entered into the Nutritionist Pro™ (version 5.1.0, 2014, First Data Bank, Nutritionist Pro, Axxya Systems, San Bruno, CA) and were used to estimate dietary intake of food groups, energy, macro- and micronutrients. Lebanese composite dishes were formulated and added to the software database using standardized recipes based on single food items from the USDA database. All food items from Nutritionist Pro™ were extracted into an excel sheet. To combine food items into groups, a code was given to each food item consumed before entering the data into Statistical Package for Social Sciences (SPSS) (Appendix 1 in **Supplementary Material** describes the food groups included in the study and their corresponding food items). For each subject, the average calories from each food group consumed were estimated together with their micro-and macronutrients.

Statistical Analysis

Statistical analysis was computed using Statistical Package for Social Sciences (SPSS, version 25, 2017). The sociodemographic

TABLE 1 | Sociodemographic and lifestyle characteristics of the study population ($n = 62$).

Sociodemographic variable	*N* (%)
Age (year)	
23–34	25 (40.3)
35–44	17 (27.4)
45 and above	20 (32.3)
Sex	
Male	38 (61.3)
Female	24 (38.7)
Place of residence	
Beirut	35 (56.5)
Outside Beirut	27 (43.5)
Marital status[a]	
Single	25 (40.3)
Married	37 (59.7)
Education level	
Up to high school level	21 (33.9)
University/technical diploma	41 (66.1)
Type of employment	
Academic	9 (14.5)
Non-academic	53 (85.5)
Income per month (L.L.)[b]	
Below 3 million	30 (48.4)
3 million and above	32 (51.6)
Crowding index	
<1	31 (50.0)
≥1	31 (50.0)
Smoking[c]	
Non-smoker	30 (48.4)
Smoker	32 (51.6)
How long have you been a smoker (year) (mean ± SD)	9.54 ± 1.1
Physical activity level	
Low	14 (22.6)
Moderate	39 (62.9)
High	9 (14.5)
BMI (kg/m^2) (51)	
Normal (18.5–24.9)	17 (27.4)
Overweight (25.0–29.9)	25 (40.3)
Obese (≥30)	20 (32.3)

BMI, body mass index (kg/m^2).
[a]Single including divorced and widowed, [b]L.L. Lebanese Lira, at the time of data collection 1,500 L.L = 1USD.
[c]Non-smokers including never smoker and past smokers.

and lifestyle characteristics of the study population were expressed as frequencies and percentages, as well as means and standard deviations (SD) for categorical and continuous variables, respectively. The average intakes (mean ± SD) of the various food groups, energy, macro, and micronutrients were computed for Ramadan and intakes over the remainder of the year. The 24 HR dietary recall collected during Ramadan was excluded from the mean of the dietary intake in regular days. Paired *t*-tests were used to compare dietary intakes between the month of Ramadan and the rest of the year. Food group intakes were expressed as percent contribution to the total energy. To assess the adequacy of selected nutrients, their intake was categorized into those meeting and not meeting recommendations as set by the Institute of Medicine (IOM): the Acceptable Macronutrient Distribution Range (AMDR) for macronutrients and the Dietary Reference Intakes for micronutrients (54). Nutrient adequacy was compared between Ramadan and the rest of the year using the Chi-square test. A $p < 0.05$ was considered statistically significant.

RESULTS

Sociodemographic and Lifestyle Characteristics

Table 1 describes the sociodemographic and lifestyle characteristics of study participants ($n = 62$). More than two-thirds (about 67.7%) were <45 years old. Over half of the study participants (56.5%) were residing in Beirut, more than half (59.7%) were married and two-thirds (66.1%) had a university/technical diploma degree. The majority (85.5%) had non-academic employment and 51.6% of the study participants reported a monthly income of 3 million L.L and above. The participants were almost equally distributed between having a crowding index <1 (50%) vs. ≥1 (50%) and being smokers vs. non-smokers (48.4 and 51.6%, respectively). The proportion of normal weight, overweight, and obese participants was 27.4, 40.3, and 32.3%, respectively.

Comparison of Food Groups' Intakes Between Ramadan and the Rest of the Year

Mean intakes of the food groups, expressed as percent contribution to total energy, for Ramadan and regular days for the study participants are presented in **Table 2**. While the percent contribution of starchy vegetables and fries, and chips were similar between Ramadan and regular days (*p*-values: 0.410, and 0.311, respectively), that of cereals, cereal-based products, and pasta were significantly higher on regular days ($p < 0.001$). Percent contributions of vegetables and dried fruit intakes to total energy were significantly higher during Ramadan as compared to regular days (vegetables: 13.9 ± 11.3% vs. 8.2 ± 4.7%; $p < 0.001$; dried fruits: 4.8 ± 7.0 vs. 0.5 ± 1.1; $p < 0.001$) while those of fruits, and fruit juices intake were similar (p 0.218). Percent contribution of eggs, nuts and seeds, milk and dairy, fats and oils, and olive oil were all higher during regular days in comparison with Ramadan days, with corresponding *p*-values of <0.001, <0.001, 0.017, <0.001, 0.032, and <0.001, respectively. Additionally, chocolate, biscuits, candy, and sugar group and miscellaneous foods intakes (% contribution to total energy) were higher on regular days (*p*-values: 0.005 and 0.002, respectively), whereas intake of Arabic sweets, cakes and pastries, and sugar-sweetened-beverages (% contribution to total energy) were higher in Ramadan with corresponding *p*-values of 0.041 and 0.004, respectively).

TABLE 2 | Food groups (percent contribution to energy) during regular days vs. the month of Ramadan among study participants ($n = 62$).

Food group	Regular day intake Mean ± SD	Ramadan day intake Mean ± SD	Difference between Ramadan and regular days intakes Mean ± SD	*p*-value[a]
Cereals, cereal-based products, and pasta	30.5 ± 8.9	18.4 ± 15.2	−12.1 ± 15.5	**<0.001**
Starchy vegetables	0.8 ± 1.1	0.6 ± 1.9	−0.2 ± 2.1	0.410
Fries and chips	5.7 ± 4.6	7.5 ± 13.6	1.8 ± 14.0	0.311
Vegetables and vegetable-based dishes	8.2 ± 4.7	13.9 ± 11.3	5.7 ± 10.9	**<0.001**
Fruits and fresh fruit juice	4.9 ± 3.1	5.9 ± 7.3	0.95 ± 6.9	0.218
Dried fruit	0.5 ± 1.1	4.8 ± 7.0	4.3 ± 7.0	**<0.001**
Meats	7.3 ± 4.7	10.2 ± 13.7	3.0 ± 14.0	0.099
Poultry	5.2 ± 3.9	5.3 ± 8.7	0.1 ± 9.4	0.914
Eggs	1.5 ± 1.7	0.2 ± 0.9	−1.3 ± 2.0	**<0.001**
Fish and seafood	1.4 ± 1.9	1.1 ± 3.8	−0.2 ± 4.1	0.639
Pulses	4.0 ± 3.5	5.2 ± 8.7	1.3 ± 8.5	0.250
Nuts and seeds	2.5 ± 2.9	0.3 ± 1.5	−2.2 ± 3.2	**<0.001**
Milk and dairy products (with yogurt)	6.9 ± 3.8	4.5 ± 7.4	−2.4 ± 7.6	**0.017**
Fats and oils (without olive oil)	2.4 ± 2.2	1.4 ± 3.2	−1.0 ± 3.6	**0.032**
Olive oil	1.7 ± 1.9	0.6 ± 1.7	−1.1 ± 2.2	**<0.001**
Chocolate, biscuits, candies, and sugars (honey and sugar derivatives)	5.1 ± 3.4	2.6 ± 5.9	−2.5 ± 6.7	**0.005**
Arabic sweets, cakes, and pastries	5.9 ± 4.2	8.8 ± 11.7	2.9 ± 10.8	**0.041**
Sugar-sweetened beverages	4.7 ± 3.2	8.4 ± 10.1	3.7 ± 9.7	**0.004**
Miscellaneous	0.7 ± 1.4	0.2 ± 0.9	−0.5 ± 1.1	**0.002**

[a]p-value was derived from a paired sample t-test.
The numbers in bold are statistically significant ($p \leq 0.05$).

Comparison of Energy, Macro- and Micronutrients Intakes Between Ramadan and the Rest of the Year

The mean intakes of energy and nutrients on regular and Ramadan days for study participants are summarized in **Table 3**. The mean energy intake was slightly but not significantly higher during Ramadan. Similar percent macronutrient contributions to total daily calories were found during Ramadan and regular days and were within the acceptable macronutrient distribution ranges (AMDR) with values around 15% for protein (10–35%), 45% (45–65%) for carbohydrates, and 40% (20–35%) for fats. Cholesterol and saturated fats were significantly higher on regular than Ramadan days (*p*-values: 0.003 and 0.005, respectively). In contrast, sugar and calorie-adjusted dietary fiber intakes were significantly higher in Ramadan (*p*-values: <0.001 and 0.003, respectively). Vitamin A, β-carotene, vitamin C, folate and magnesium were also significantly higher in Ramadan (*p*-values: 0.022, 0.002, 0.014, 0.011 and 0.014, respectively) whereas calcium intake was significantly lower ($p < 0.001$). Further, intakes of sodium, α-carotene, iron, vitamin D, and α-tocopherol were similar between Ramadan and regular days (*p*-values: 0.762, 0.387, 0.180, 0.093, and 0.891, respectively).

With regards to nutrient adequacy, the proportions of study participants falling below or above the dietary reference intakes (DRIs) were calculated and compare between regular days and Ramadan (**Table 4**). Compared to regular days, the proportions of subjects meeting the recommendations for vitamin C, iron and magnesium were significantly higher during Ramadan (53 vs. 43%; 57 vs. 55%; 35 vs. 13%).

Figure 2 depicts the proportion of the study population meeting the AMDRs for daily macronutrients (regular days vs. Ramadan) for the fasting participants. All were within the AMDRs for protein (100.0%) during regular days, compared to 82.3% during Ramadan. Further, 53.2, 46.8, and 0% were below, within, or above AMDRs for carbohydrates during regular days compared to 45.2, 51.6, and 3.2% during Ramadan ($p < 0.05$). Fat intake was above the AMDRs for 88.7% during regular days and 66.1% in Ramadan.

TABLE 3 | Energy, macro, and micronutrient consumption during regular days vs. the month of Ramadan among study participants ($n = 62$).

Nutrient	Regular day intake Mean ± SD	Ramadan day intake Mean ± SD	Difference between Ramadan and regular days intakes Mean ± SD	*p*-value[a]
Energy (kilocalories)	2040.9 ± 611.2	2184.2 ± 2497.4	143.3 ± 2426.4	0.644
Protein (%)	15.0 ± 2.8	15.2 ± 5.4	0.2 ± 5.7	0.795
Carbohydrates (%)	44.1 ± 5.4	45.9 ± 9.0	1.9 ± 8.8	0.100
Fats, total (%)	40.7 ± 4.6	38.9 ± 7.5	−1.8 ± 8.1	0.078
Cholesterol (mg)	243.5 ± 138.1	175.5 ± 155.7	−68.0 ± 176.1	**0.003**
Saturated fats (%)	10.7 ± 1.9	9.5 ± 3.1	−1.2 ± 3.3	**0.005**
Monounsaturated fats (%)	15.2 ± 3.0	14.3 ± 3.8	−0.9 ± 4.7	0.126
Polyunsaturated fats (%)	9.2 ± 1.8	9.7 ± 3.8	0.5 ± 4.0	0.287
Dietary fibers, total (g)	18.7 ± 6.5	22.2 ± 29.8	3.5 ± 29.9	0.356
Dietary fibers (g/1,000 kcal)	9.3 ± 2.1	11.1 ± 5.2	1.8 ± 4.5	**0.003**
Sugars, total (%)	14.7 ± 4.7	25.5 ± 14.2	10.7 ± 13.7	**<0.001**
Sodium (mg)	2398.1 ± 737.9	2321.2 ± 2090.8	−76.9 ± 1988.8	0.762
Vitamin A (RE)	1041.4 ± 644.6	1697.1 ± 2144.7	655.7 ± 2189.9	**0.022**
Beta-carotene (μg)	3619.9 ± 2341.0	8087.6 ± 10406.8	4467.6 ± 10738.8	**0.002**
Alpha-carotene (μg)	420.7 ± 536.6	893.3 ± 4249.3	472.6 ± 4269.3	0.387
Vitamin C (mg)	84.0 ± 36.8	161.8 ± 237.9	77.7 ± 241.6	**0.014**
Calcium (mg)	806.5 ± 233.0	672.0 ± 264.9	−134.4 ± 255.5	**<0.001**
Iron (mg)	12.9 ± 5.1	14.5 ± 9.5	1.6 ± 9.0	0.180
Vitamin D (μg)	1.3 ± 1.1	0.9 ± 1.9	−0.4 ± 2.0	0.093
Alpha-tocopherol (mg)	10.7 ± 4.0	10.5 ± 13.5	−0.2 ± 14.2	0.891
Folate (total) (μg)	321.7 ± 115.6	413.4 ± 277.9	91.7 ± 275.2	**0.011**
Magnesium (mg)	283.2 ± 90.4	377.4 ± 304.0	94.2 ± 294.0	**0.014**

[a]*p-value was derived from a paired sample t-test.*
The numbers in bold are statistically significant ($p \leq 0.05$).

Gender Differences in the Impact of Ramadan on Dietary Intake

A comparison between dietary intakes of males and females showed that, while a few differences were observed during regular days, their intake was very similar during the month of Ramadan. More specifically, with regards to food groups, women tended to eat more vegetables and vegetable-based dishes and less meat as compared to males during regular days. These differences were not observed during the month of Ramadan (Appendix 2 in **Supplementary Material**).

The Impact of Ramadan on Dietary Intake Among Normal vs. Overweight and Obese Study Participants

The impact of Ramadan on dietary intake were analyzed separately or normal and overweight and obese study participants. Results are presented in Appendix 3 (**Supplementary Material**). Fewer differences in food groups intakes (as expressed in % contribution to total energy intake) were noted among normal as compared to overweight and obese subjects. Similarly, intakes of various nutrients tended to be less different between Ramadan and regular days among normal weight subjects as compared to overweight and obese subjects (Appendix 3 in **Supplementary Material**).

DISCUSSION

The current study is the first to examine the dietary intakes during Ramadan and to compare it to that of the rest of the year's months; with the majority of previous research examining dietary intakes before and after Ramadan for 1–2 months at the most (13, 14, 16, 19–26, 55). The year round approach used in this study provides a more precise estimate of the difference between the two time intervals to improve understanding of the impact of religious fasting on dietary behaviors and nutrient intakes. The study showed that Ramadan fasting was associated with significant changes in the intakes of 12 of the 19 food groups, implying that Ramadan has its unique food intake and dietary pattern as compared to the rest of year days. More specifically, percent contribution to energy intake of cereals, cereal-based products, and pasta were decreased, while vegetables and dried fruit intakes were increased during Ramadan. Concomitantly, percent contribution of eggs, nuts and seeds, milk and dairy, fats and oils, and olive oil to total energy were lower during Ramadan. Additionally, intakes of classical confectionaries and

TABLE 4 | Dietary adequacy during regular days vs. the month of Ramadan among study participants ($n = 62$).

Nutrient	DRI*	Regular day intake *N* (%)	Ramadan day intake *N* (%)	*p*-value[§]
Dietary fibers, total (g/day)				
Below recommendation	M:38	60 (96.8)	58 (93.5)	0.874
Above recommendation	F:25	2 (3.2)	4 (4.5)	
Sodium (mg/day)				
Below recommendation	1,500	7 (11.3)	23 (37.1)	0.244
Above recommendation		55 (88.7)	39 (62.9)	
Vitamin C (mg/day)				
Below recommendation	M: 90	35 (56.5)	29 (46.8)	**0.017**
Above recommendation	F:75	27 (43.5)	33 (53.2)	
Calcium (mg/day)				
Below recommendation	1,000	51 (82.3)	56 (90.3)	0.231
Above recommendation		11 (17.7)	6 (9.7)	
Iron (mg/day)				
Below recommendation	M:8	28 (45.2)	27 (43.5)	**<0.001**
Above recommendation	F:18	34 (54.8)	35 (56.5)	
Vitamin D (μg/day)				
Below recommendation	5	61 (98.4)	60 (96.8)	0.968
Above recommendation		1 (1.6)	2 (3.2)	
Folate (Total) (μg/day)				
Below recommendation	400	43 (69.4)	44 (71.0)	0.132
Above recommendation		19 (30.6)	18 (29.0)	
Magnesium (mg/day)				
Below recommendation	M:420	54 (87.1)	40 (64.5)	**<0.001**
Above recommendation	F:320	8 (12.9)	22 (35.5)	

[§]*p-value was derived from the chi-square test.*
The numbers in bold are statistically significant ($p \leq 0.05$).
[*]*DRI for age group 31–50y was used and calculated based on DRI (Institute of Medicine/Food and Nutrition Board. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids.2002/2005). https://www.nap.edu/catalog/10490/dietary-reference-intakes-for-energy-carbohydrate-fiber-fat-fatty-acids-cholesterol-protein-and-amino-acids-macronutrients (54).*
M, Males; F, Females.

sweets such as chocolate, biscuits, candies, and sugar groups and miscellaneous foods (% contribution to total energy) were lower during Ramadan and were replaced by traditional Arabic sweets, cakes, and pastries, and sugar-sweetened-beverages specific to this month of the year.

The observed marked changes in dietary intake in this study further highlight the previously reported profound impact of religious fasting on eating behavior. In fact, religion and religious rituals and fests have for long been considered among the fundamental factors that impact human dietary behaviors and food selections (2). Ramadan fasting represents one of the clearest examples of how religious beliefs impact human dietary behavior for three main reasons: first, the significant shift in food patterns from diurnal and nocturnal eating time to nocturnal eating for 29–30 consecutive days; second, dietary choices during this month are closely tied to traditions and customs with certain dishes consumed solely during Ramadan, and lastly particular eating habits during Ramadan whereby during month, meals are generally consumed together with family members (56, 57).

The results of this study revealed a clear tendency toward increasing intake of sugar-containing foods (dried fruit, Arabic sweets, and sugar sweetened-beverages) during night hours of Ramadan, which mirrors the recent reports from UAE (58, 59), and Ghana (17) indicating increased consumption of sugar-sweetened beverages after breaking the fast, and is in accord with other previous reports from different parts of the world (57, 60, 61). Such a higher consumption of these foods could be linked to common traditions and customs during this month, whereby certain sweets are produced, sold, and consumed solely during the month of Ramadan and not consumed during the rest of year. The marked increase in sugary food intake during this month could be explained by the reported feeling of dizziness and inactivity due to reduced blood sugar during the fasting hours prior to consumption, which drives the fasting individual to consume more sugars between dusk and dawn. This behavior is also closely linked to the traditions of consuming particular sweets that are specific to the month of Ramadan. In this context, it is important to highlight the philosophy of Ramadan fasting which stems from empowering self-control and contradicts with the prophetic guidance dictating avoidance of overeating; as noted in the following: "A human being fills no worse vessel than his stomach. It is sufficient for a human being to eat a few mouthfuls to keep his spine straight. But if he must (fill it), then one-third of food, one third for drink and one third for air"

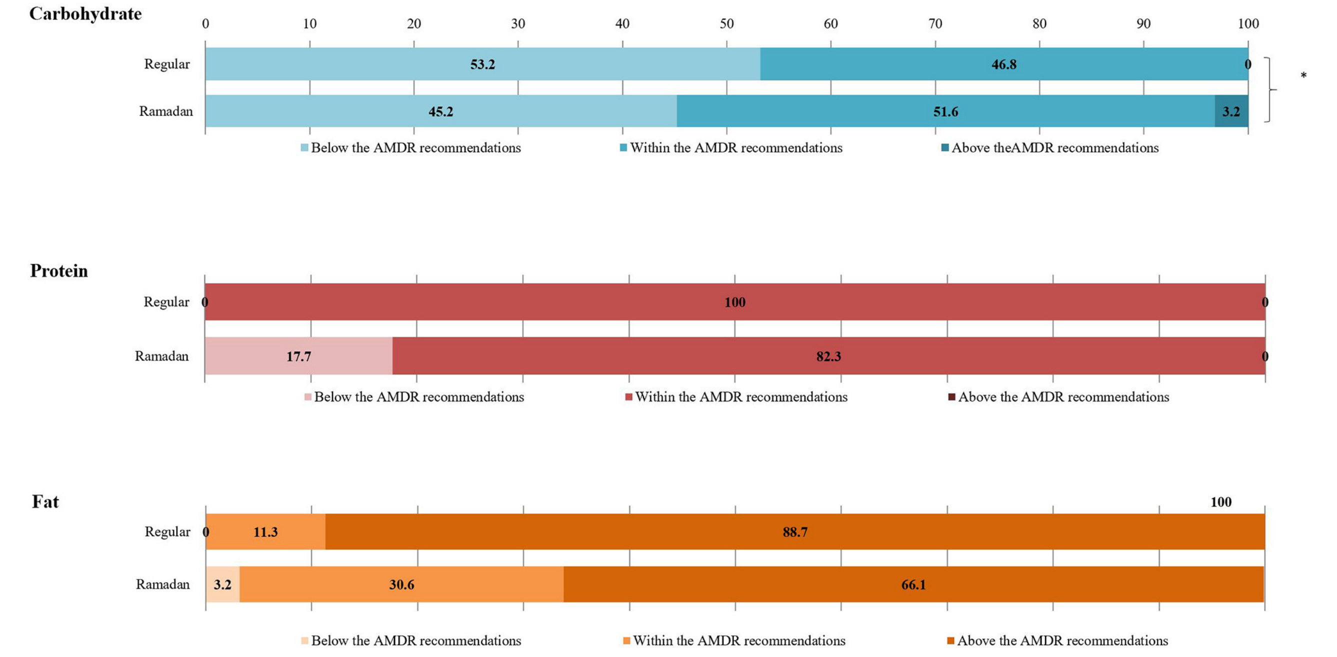

FIGURE 2 | Comparison of the proportions of participants meeting the AMDR recommendations for macronutrients between regular days and the month of Ramadan among study participants (*n* = 62). The '*' denoted a statistically significant difference at 0.05.

(62), and disagrees with the behaviors of the Messenger of Islam (Prophet Mohamad) who was reported to break his fast with water and few dates, and then taking small meal afterward (63).

Such an increased sugar intake during the month of Ramadan is important to be considered especially among patients with diabetes. While the effect of fasting (and its associated higher sugar intake) did not show any adverse glucometabolic impacts in healthy subjects (64), its effects on diabetic patients remains to be elucidated. Worldwide, it is estimated that 70 million people with diabetes observe fasting during Ramadan (65). Considering this large population of patients who mostly self-decide to fast during Ramadan, despite being exempted to do so as per the Islamic rules of Ramadan, the findings of this study (higher sugar consumption during the month of Ramadan) ought to be integrated into the formulation of culture-specific recommendations for dietary intake during the month of Ramadan for this group of patients. Furthermore, health care providers are encouraged to follow more closely the glycemic control of their diabetic patients during the month of Ramadan.

The current study showed a significant reduction in cereals and cereal-based starchy foods and pasta during the month of Ramadan compared to the rest of the year, which is consistent with the reported reduction in consuming breads and cereals in Iran by Nachvak et al. (26). While a slight, non-significant increase in protein sources of food (meats, poultry, and pulses) was reported in the current work, other reports indicated a significant increase in the consumption of protein foods during Ramadan (16, 23, 25, 27). It is arguable that the reported higher intakes of proteins during the month of Ramadan is related to its satiating effect. In fact, the results of a meta-analysis examining the effects of increased protein intake on fullness showed that higher protein meals increase fullness ratings more than lower protein meals (66). The increased intake of non-starchy vegetables and vegetable-based dishes reported in the current study in Lebanon echoes the reported increased consumption of vegetables in the United Arab of Emirates (UAE) during the holy month of Ramadan (14). In this study, the consumption of vegetables and dried fruit intakes was higher during Ramadan compared to the rest of the year. In fact, during this month, salads, dates, and dried apricots constitute an important cultural and traditional components of the meals (67) and hence their observed higher consumption in this study.

Studies examining the effect of Ramadan fasting on fat intake yielded inconsistent results. The findings of this study, together with another study from Spain (23), showed lower intake of fats and oils especially saturated fats. These findings are in line with the findings of Adlouni et al. who showed that fasting during Ramadan induces a marked increase in high-density lipoprotein cholesterol and decrease in low-density lipoprotein cholesterol (16). No such findings were reported in a study from the UAE which reported higher intakes of fats during Ramadan (14). Such inconsistency could be a reflection of the cultural context where the studies have been conducted.

In this study, the increased intake of dietary fibers, vitamin C, beta-carotene, and vitamin A (expressed as retinol equivalent) during Ramadan compared to the non-fasting remainder of the year could be explained by a higher intake of vegetables, and dried fruit during Ramadan night hours. This increased intake of fiber-rich foods could be looked at as a prophylactic measure for the anticipated and frequently experienced constipation during the fasting hours, which is exaggerated with the lack of water intake and reduced physical activity during Ramadan (68). Besides, fresh and dried fruits and vegetable salads are principal ingredients in many dishes during and out of Ramadan in the traditional Lebanese cuisine, which is part of the Mediterranean diet pattern (42).

The lack of significant change in total caloric intake reported in the current work is in line with the findings of many studies in different parts of the world (23, 58, 69–72). Accordingly, a few previous studies proposed Ramadan as a variant of intermittent fasting (73). In fact, the comparable total energy and macronutrient intakes during Ramadan and regular days in this study represents one of the distinguishing features of an intermittent fasting regimen (74). Having dietary intakes of macronutrients within the acceptable reference range for protein, carbohydrate, and fat, with higher intakes of many of the micronutrients (vitamin A, β-carotene, vitamin C, folate, and magnesium) and fiber, suggests that an intermittent fasting regimen, such as the one followed in Ramadan, may be a more practical dietary modification than caloric restriction (75).

Of note are the findings of this study showing that, while dietary intake of men and women tend to differ during the year, it is less different during the month of Ramadan. This finding could be due to the fact that during this month, families (including men and women) tend to eat together more frequently as compared to the rest of the year. The analysis by weight status in this study showed that more changes in dietary intake during Ramadan were observed among overweight and obese subjects as compared to those with normal weight. This finding is in line with the results reported by Fernando et al. and which showed a reduction of fat percentage in overweight and obese people during Ramadan fasting but not normal weight (76). Future larger studies are needed to further elucidated the differential effect of Ramadan on dietary intake among normal, overweight, and obese individuals.

The differences among various studies' findings regarding the effect of the month of Ramadan on dietary intake could relate to methodologic and cultural differences in study populations. One leading factor for discrepant results across studies examining changes in food and dietary intakes is the variation in dietary assessment tools. The latter being tightly related to the objective of the dietary assessment; whether assessing general patterns or explicit nutrient intakes for correlational purposes. More specifically, studies that aimed at providing a broader reflection of the dietary pattern generally tended to use food frequency questionnaires (FFQ); while those evaluating particular nutrients reported using repeated dietary recalls and records (58, 59, 77). In addition to the differences in their application, the use of various dietary assessment methods is bound to feasibility and budgetary issues. For instance, FFQ is a relatively inexpensive option and requires less commitment from the participants as compared to dietary recalls and records (78, 79). Therefore, caution ought to be exerted when comparing the results of various studies with different dietary assessment methods. Another major factor for the discrepancies among the studies addressing Ramadan eating is the unique cultural and traditional dietary behaviors adopted by different populations from various ethnic and cultural backgrounds, all within the regulations of the allowed *Halal* food system for Muslims.

To the best of our knowledge, this is the first study to examine the impact of Ramadan fasting on dietary intakes among healthy adults using a year round dietary assessment. As such, the results of this study were more reflective of the subjects' usual dietary habits around the year while those of other studies, which assessed the dietary intake 1 or 2 months before or after Ramadan, tend to be less representative to the overall dietary pattern of the individual. That said, the findings of this study ought to be interpreted in light of a few limitations. Dietary assessment was carried out using multiple 24 HR. Though this method is easy and simple, it relies on the memory of the participants, and hence results could be subject to recall error and bias. For that, project research assistants were trained to use the multiple pass technique to decrease such errors. Furthermore, the fact that only one of the 24 HR was carried out during the month of Ramadan may raise questions with regards to its representation of dietary intake during this month. That said, it is arguable that food intake during the fasting month tend to follow a consistent pattern especially with regards to the type and quantity of food intake. In addition, some of the changes observed in this study could be due to the time of the year Ramadan coincided with; perhaps future studies during years where Ramadan falls in different seasons will help decipher food habit changes due to Ramadan vs. those due to seasonal variations. Whether the observed impact of fasting on dietary intake is more pronounced during the months around Ramadan and fades through the year or such an impact is rather sustained for longer periods remains to be elucidated. Lastly, despite the advantages of an interview-based data collection, the possibility of an interviewer bias or a social desirable response could not be ruled out. Interviewers, however, were trained to maintain non-judgmental and neutral attitudes to avoid impacting the participants' responses.

CONCLUSIONS

In conclusion, the findings of this study suggested major changes in dietary intakes of Lebanese adults observant of the fast during the month of Ramadan as compared to the rest of the year. Such changes included a decrease in the usual intakes of cereals, cereal-based products, pasta, eggs, nuts and seeds, milk and dairy, fats and oils, and olive oil. On the other hand, fasting Lebanese adults were shown to consume more vegetables, dried fruit intakes, traditional Arabic sweets, cakes and pastries, and sugar-sweetened-beverages specific to the month of Ramadan. With the large number of adults who observe fasting during Ramadan, the particularities of dietary intake during Ramadan ought to be considered in the formulation of context and culture-specific dietary recommendations. To overcome the limitations of this study, future studies addressing the changes in dietary habits in Ramadan are encouraged to include more than one daily dietary intake collection and to further characterize dietary intake by eating occasions. Furthermore, given the subjectivity of most dietary assessment methods, if feasible, it will be optimal to collect biomarkers of various nutrients intake to compare between Ramadan and the rest of the year (80).

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 Institutional Review Board of the Social and Behavioral Sciences at the AUB. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

FN, MFo, and MFa contributed to the conception and design of the research. HS contributed to the study management. RE, MB, and HS contributed to data collection and preparation of the project data set. HS and NA undertook the analysis. FN, MFa, RC, and HS contributed to drafting the manuscript. All authors reviewed the manuscript and approved the final version intended for submission.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.689788/full#supplementary-material

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ORIGINAL RESEARCH
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Ramadan Fasting in Germany (17–18 h/Day): Effect on Cortisol and Brain-Derived Neurotrophic Factor in Association With Mood and Body Composition Parameters

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Ramadan fasting (RF) is a type of diurnal intermittent fasting. Previous studies reported the benefits of RF in healthy subjects on mood and health related to quality of life (QoL). Cortisol and brain-derived neurotrophic factor (BDNF) have been shown to play a role in mood, body composition parameters, and health-related QoL. This study aimed at elucidating the mechanism of the benefit of RF, particularly cortisol and BNDF and their association with mood and QoL. Insulin growth factor-1 (IGF-1), interleukin (IL)-8, matrix metalloproteinase (MMP)-9, and myoglobin were determined. Thirty-four healthy men and women were recruited. Serum from peripheral venous blood samples was collected at five time points: 1 week before RF (T1); mid of RF (T2), last days of RF (T3), 1 week after RF (T4), and 1 month after RF (T5). The amounts of biological mediators in the serum samples were determined by enzyme-linked immunosorbent assay (ELISA) and Luminex assays. BDNF and cortisol significantly decreased at T3 ($p < 0.05$) and T4 ($p < 0.001$) compared to T1, respectively. It seems the benefits of RF for mood-related symptoms are mediated by different biological mediators, particularly cortisol and BDNF.

Keywords: intermittent fasting, ramadan, cortisol, BDNF, mood, anthropometric, body composition

INTRODUCTION

Fasting has been practiced for centuries not only to fulfill religious obligations, but also as therapy and lifestyle (1). Ramadan fasting (RF) is one type of diurnal intermittent fasting that is performed by millions of Muslims worldwide. RF is performed from dawn to sunset for 29–30 days. Due to geographical differences, the time periods of RF vary from 9 to 22 h per day (2). In this study, RF was performed for 17–18 h/day. During RF, food, water, smoking, and sexual activities are forbidden, but not after breaking the fast (3). Travelers, the unwell, elderly, children, and pregnant and nursing people are exempt from RF (4).

Several studies have reported benefits of RF in healthy individuals (1, 5) and patients (6–9). Benefits of fasting were reported in different patient groups, including in diabetic (6, 7, 10), asthma (11), and allergic patients (11). In healthy men and women, the benefits of RF have been shown, such as improvement of body composition parameters, mood, fatigue, and health-related quality of life (1, 5).

Since the last decade, previous studies have reported the benefits of RF and aimed to show how such improvements are mediated by various biological factors. Fasting, in general, modifies immune systems and improves the symptoms of chronic inflammatory diseases (12). It can be mediated by monocyte metabolic activity and several cytokines (12, 13). However, the results concerning inflammatory markers are still inconclusive (13–15). A previous study elucidated the effects of RF on diabetic-related parameters, such as glucometabolic markers (16). Our previous study showed no differences with regard to the level of creatinine, as a biological marker for kidney, between fasting and non-fasting groups, although RF was conducted for about 19 h (17).

Our previous studies reported the benefit of RF in health-related quality of life and mood-related symptoms (1, 5). Furthermore, its benefit could still be observed up to 1 month after finishing RF (1). Therefore, it would be of interest to elucidate the biological mechanism related to mood-related symptoms.

Cortisol is a glucocorticoid class of hormone that is produced in the adrenal cortex (18, 19). Its release occurs in a circadian rhythm based on the regulation of the hypothalamic-pituitary-adrenal axis (HPA) as well as in stressful situations and during physical work (19). High cortisol levels are associated with stress-related disorders (18, 19). During fasting, the levels of cortisol alter. Alterations differ based on measurement time (20, 21). With regard to RF, there is a lack of information on how cortisol is altered during RF, particularly when fasting is performed in the summer time for about 17 to 18 h daily. Brain-derived neurotrophic factor (BDNF) is one of the neurotrophins that has been studied in mental-related disorders (22–25), cognition (26), physical activity, and nutrition (27, 28). Furthermore, a lower level of BDNF in the peripheral nervous system is associated with impairment of cognitive performance and higher body weight (26, 29). Therefore, this study aimed to determine cortisol and BDNF levels in serum samples during RF and their association with mood and health-related quality of life.

Additionally, other biological mediators associated with nutritional intake, body composition parameters, and mood such as insulin growth factor-1 (IGF-1), myoglobin, interleukin-8 (IL-8), and matrix metalloproteinase-9 (MMP-9) were also determined. IGF-1 also plays a role in mood and body composition (30, 31). IL-8, as one of the inflammatory markers, has been known to be associated with mood and nutritional intake (32, 33). The increase of IL-8 is correlated with positive mood (33). MMP-9 is correlated with obesity (34, 35) and muscle strength (36). Meanwhile, myoglobin has been known to be responsible for intramuscular oxygen transport (37) and can be used to measure the muscle breakdown in the body (38), as our previous studies showed a decrease in skeletal muscle mass during RF (1, 5).

MATERIALS AND METHODS

All procedures were authorized in accordance with the ethical standards and approval of Hannover Medical School (Ethics No. 7242; Registration code of the trial: DRKS00017640) and the Declaration of Helsinki 1964.

Participants

For this study, 34 healthy men and women were enrolled. Most of them were students and employees of Hannover Medical School. Inclusion criteria: healthy men and women, with no history of pain and mental health-related disorders; above the age of 18 years; intended to fast the whole month of Ramadan from sunrise to sunset. Exclusion criteria: participants who have interrupted their fasting for more than 7 days. Participation in this study was contingent on the signing of the informed consent in either English or German according to the preference of the participants.

Study Design

This was an explorative study to determine the mechanism of RF based on a previous study (1). This study consisted of five time points: 1 week before RF (T1), mid of RF (T2), last days of RF (T3), 1 week after RF (T4), and 1 month after RF (T5) (see **Figure 1**).

Biological Parameters

Blood Sampling

Peripheral venous blood samples were collected from all participants in serum tubes (Monovette®, Sarstedt, Germany) between 08:00 am to 10:00 am from T1 to T5. Serum samples were allowed to clot before centrifugation at 1,500 g (15 min) (Universal 320R, Hettich, Tuttlingen, Germany). They were stored at −80°C until analyses.

Detection of Biological Parameters

The blood serum concentrations of cortisol (Cortisol Pa-rameter Assay Kit, R&D Systems Inc.) was determined by employing a standard competitive enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. Myoglobin and human IGF-1 serum levels were detected by sandwich ELISA using a Human Myoglobin ELISA kit (Invitrogen) and Quantikine ELISA Human IGF-1 Immunoassay (R&D Systems Inc.) according to the manufacturer's instructions. BDNF, IL-8, and MMP-9 serum levels were detected using a Human Magnetic Luminex Assay (R&D Systems), following the protocol from the manufacturer.

Body Composition Parameters, Mood, and Health-Related Quality of Life

In this study, body composition parameters (body fat percentage (BFP), body weight (BW), body water mass (BWM), skeletal muscle mass (SMM), body fat mass (BFM) and fat-free mass

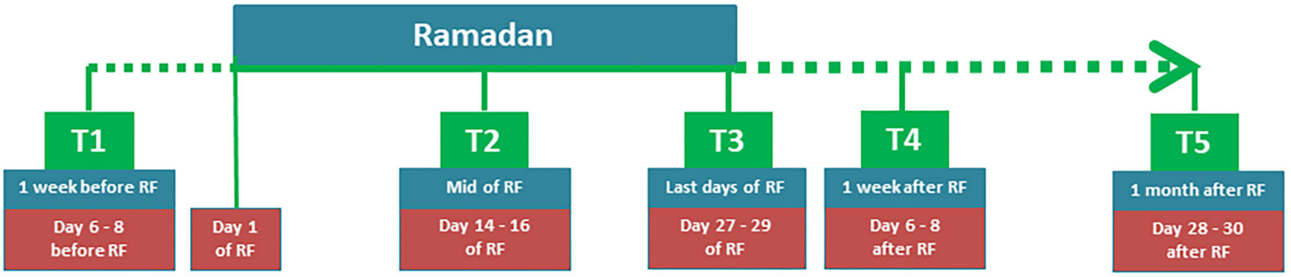

FIGURE 1 | Study design.

(FFM), and estimated basal metabolic rate (BMR)); mood (Beck's depression inventory (BDI)-II and hospital and anxiety depression scale-Anxiety/Depression (HADS-A/D), and health-related quality of life (QoL) [fatigue: fatigue severity scale (FSS); sleepiness (Epworth sleepiness score (ESS)] were determined. The methodology and results of these parameters were reported in a previous study (1). In this article, these parameters are only reported to support the correlation with the determined biological parameters.

Statistics Evaluation

The study objective aimed at assessing the level of cortisol, BDNF, and other biological parameters (IL-8, MMP-9, IGF-1, myoglobin) before (T1), during (T2 and T3) and after (T4 and T5) RF. The data were analyzed by using either Kruskal-Wallis or two-way Friedman ranked test. The *post hoc* tests were performed, and significances were adjusted by using Bonferroni correction. Spearman's correlation tests were performed to determine correlation between clinical parameters and biological mediators. Missing data were replaced by using a mean imputation method. The Kolmogorov–Smirnov test was used to determine the distribution of the data. Significance was set at $p < 0.05$. SPSS 26 (IBM, New York City, NY, USA) was used to analyze the data. Effect sizes were calculated by using following formula: $\eta^2 = Z^2/(N - 1)$.

RESULTS

Baseline Data

The baseline parameters of all, male, and female participants before RF (T1) are shown in **Table 1**. No significant differences could be seen between male and female participants in age, ethnicity, body mass index (BMI), and level of cortisol, IGF-1, MMP-9, and myoglobin. Whereas significant differences were identified in body weight (BW) and level of BDNF and IL-8.

Biological Parameters in All Participants

To study the effect of RF on biological parameters, different types of biological markers were analyzed at different time points (**Figure 2**). The cortisol level was significantly decreased at 1 week after RF (T4) in comparison to 1 week before RF (T1) and mid of RF (T2). However, it turned back to the baseline level 1 month after RF (T5) (**Figure 2A**). Next, a significantly lower level of BDNF was observed from the last days of RF (T3) to T1 (**Figure 2B**). Furthermore, chemokine IL-8 level was increased at T2 compared to T1, T3, T4, and T5 (**Figure 2C**).

IGF-1 level was also analyzed at different time points. Significantly increased level of IGF-1 at T2 compared to T1 was observed, although it was decreased at T3. Interestingly, 1 month after RF (T5), it was increased again compared to T1 and T3 (**Figure 2D**). Furthermore, the level of MMP9, an enzyme involved in the degradation of the extracellular matrix, was significantly decreased at T5 compared to T1 and T3 (**Figure 2E**). Lastly, the reduction of myoglobin level, an iron- and oxygen-binding protein in the skeletal muscle tissue, was observed at T3 compared to T1 (**Figure 2F**).

Biological Parameters in Male and Female Participants

Figure 3 demonstrates different types of gender based-biological markers (men and women) during RF at different time points. Interestingly, there was a significant reduction of cortisol level from T1 to T2 in comparison to T4 in male participants, but not in female participants (**Figure 3A**). Similar results were observed for BDNF level in that only men showed a reduction of BDNF level from T1 to T3, but not women. In addition, male participants showed a higher level of BDNF at T1, T2, and T4 compared to female participants (**Figure 3B**). For IL-8 level, it was shown that it significantly increased from T1 to T2 in women. Men showed a significant decreased IL-8 level from T2 to T5. The IL-8 level was significantly lower in women in comparison with men at T1, T2, T3, and T4 (**Figure 3C**).

Next, we observed the increased level of IGF-1 from T1 to T2, but it came back to the baseline level at T3 in male participants. Meanwhile, no significant changes was observed in female participants (**Figure 3D**). Furthermore, there was no significant difference of MMP-9 levels observed at each time points in both men and women. However, the female participants showed a lower level of MMP-9 in comparison to male participants. Lastly, a significantly decreased level of myoglobin was observed from T1 to T3 in both female and male participants (**Figure 3F**).

Effect Size of Biological Parameters During RF

In order to determine the magnitude of effect of RF on biological parameters at different time points, effect sizes were computed

TABLE 1 | Baseline parameters (T1) in all, male, and female participants.

	All participants $N = 34$	Male $N = 19$	Female $N = 15$	P (male vs. female)
Age	25.1 ± 0.8	24.8 ± 1.0	25.5 ± 1.2	0.65
Caucasian/other	23/11	14/5	9/6	0.48§
BW (kg)	72.0 ± 2.3	77.31 ± 3.1	65.3 ± 2.3	<0.01
BMI (kg/m^2)	24.8 ± 0.6	25.10 ± 0.9	24.4 ± 0.8	0.56
Cortisol (Median(IQR))	225.5 (215.6–261.7)	229.3 (219.0–260.9)	218.7 (187.8–265.6)	0.228
BDNF (Median(IQR))	567.2 (345.7–970.9)	742.5 (516.8–1164.0)	440.4 (227.4–849.8)	0.021
IL-8 (Median(IQR))	2.5 (1.9–4.1)	3.7 (2.6–4.4)	1.9 (1.5–2.3)	0.002
IGF-1 (Median(IQR))	4579.5 (2058.5–6907.0)	4073.1 (1653.9–7514.6)	5474.0 (2999.7–6553.6)	0.784
MMP-9 (Median(IQR))	3783.3 (2463.5–7723.9)	4606.1 (3008.0–11028.2)	3177.0 (1882.0–4204.0)	0.056
Myoglobin (Median(IQR))	13.3 (8.0–21.1)	15.4 (11.2–25.5)	9.0 (6.9–15.8)	0.051

BW, body weight; BMI, body mass index; BDNF, brain-derived neurotrophic factor; IL-8, interleukin-8; IGF-1, insulin growth factor-1; MMP-9, matrix metalloproteinase-9. §Chi square test.

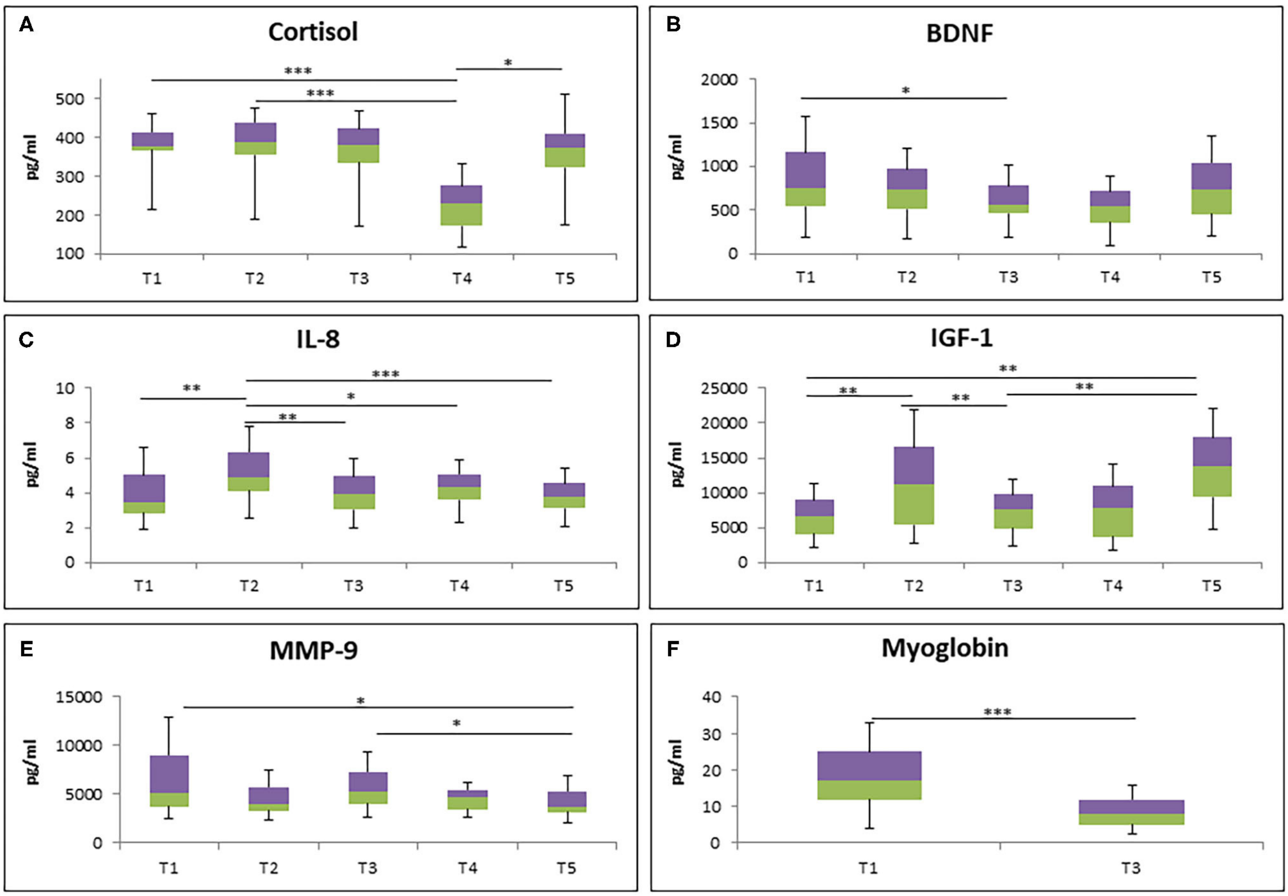

FIGURE 2 | The level of cortisol **(A)**, BDNF **(B)**, IL-8 **(C)**, IGF-1 **(D)**, MMP-9 **(E)**, and myoglobin **(F)** during RF in all participants ($N = 34$). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

(**Table 2**). The calculations were performed for T1–T3; T1–T4; and T1–T5. The large effect size of cortisol was observed particularly at T1-T4 of all participants and also in both men as well as women. In men, the large effect size of cortisol could also be observed between T1 and T3. The large effect size of BDNF in all, male, and female participants was only observed at T1-T3. Its effect sizes were medium at T1–T4. The large effect size of IGF-1 and MMP-9 could be observed at T1-T5 in all, male, and female

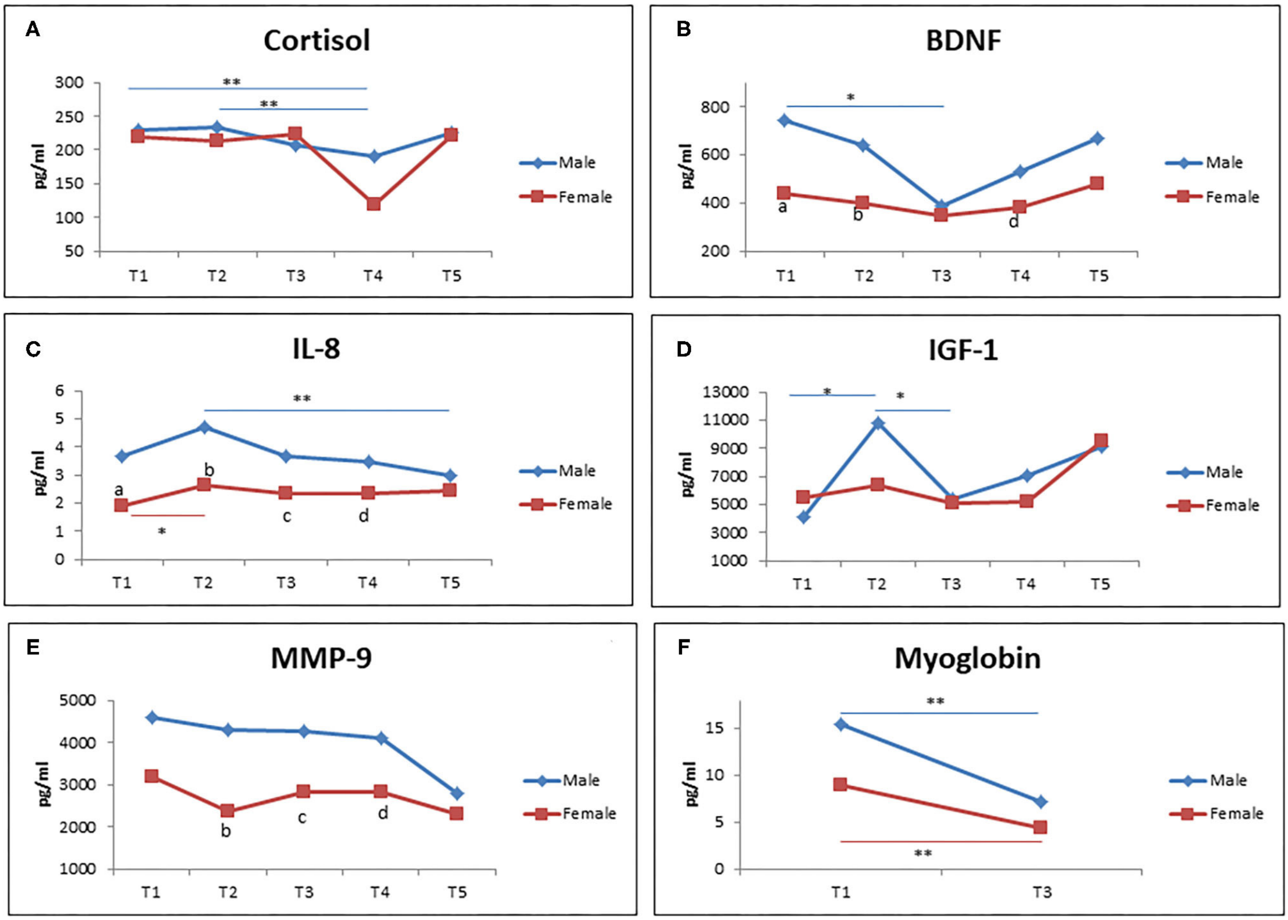

FIGURE 3 | The level of cortisol **(A)**, BDNF **(B)**, IL-8 **(C)**, IGF-1 **(D)**, MMP-9 **(E)**, and myoglobin **(F)** during RF (male = 19; female = 15). **(a–e)** significant difference between men and women at T1, T2, T3, T4, and T5. *$p < 0.05$, **$p < 0.01$.

participants. However in male participants, the large effect size of IGF-1 was also observed at T1–T4. Myoglobin showed a large effect size at T1–T3 in all, male, and female participants.

Correlations Between Biological Mediators and Clinical Parameters During RF

Table 3 shows the correlations between biological mediators and clinical parameters in all, male, and female participants. At T3, in all participants, IGF-1 and IL-8 levels showed positive correlations with body composition-related parameters. Cortisol showed a negative correlation with BW. Interestingly, in men, IGF-1 levels were only positively correlated with BW, meanwhile in women, a significant negative correlation occurred between cortisol and FSS; and cortisol positively correlated with BFP. At T4, in all participants, cortisol, IL-8, and MMP-9 levels were significantly correlated with body composition-related parameters. However, only cortisol levels showed a significant negative correlation with fatigue (FSS). In male participants, IGF-1 levels were significantly correlated with body composition-related parameters. Cortisol levels were correlated with mood (BDI score) and sleepiness (ESS score) in male and female subjects, respectively. In women, cortisol was also negatively correlated with BFM and positively correlated with FFM. At T5, in all participants, cortisol level was negatively correlated with BMI and BFP. Meanwhile, MMP-9 level was negatively correlated with BFP. BDNF levels were significantly correlated with mood (HADSD score). In men, BDNF was positively correlated with BFM, BFP, and WHR. In women, BDNF levels were negatively correlated with SMM, BW, FFM, and BMR. Additionally, in women only, BDNF levels were positively correlated with mood (HADSD score). Cortisol level was also negatively correlated with BFM and BFP in women only.

DISCUSSION

This study aimed to particularly determine cortisol and BDNF levels and their correlation with mood, health-related quality of life, and body composition parameters during RF. Additionally, other biological mediators including IGF-1, IL-8, MMP-9, and myoglobin were also studied. The RF was performed for 17–18 h/day for 1 month.

TABLE 2 | Effect size of biological parameters in all, male, and female participants between T1 and T3, T1 and T4, and T1 and T5.

	Effect size (η^2)								
	All			Male			Female		
	T1–T3	T1–T4	T1–T5	T1–T3	T1–T4	T1–T5	T1–T3	T1–T4	T1–T5
Cortisol	0.061	0.592	0.015	0.15	0.660	0.067	0.005	0.516	0.001
BDNF	0.296	0.098	0.027	0.307	0.077	0.111	0.327	0.114	0.008
IL-8	0.005	0.034	0.001	0.001	0.04	0.055	0.045	0.274	0.055
IGF-1	0.000	0.124	0.415	0.011	0.164	0.468	0.018	0.100	0.331
MMP-9	0.018	0.078	0.353	0.041	0.117	0.467	0.000	0.095	0.22
Myoglobin	0.634	–	–	0.573	–	–	0.774	–	–

Effect size (η^2: small: 0.01–0.05; medium: 0.06–0.12; large $\geq$ 0.13). BDNF, brain-derived neurotrophic factor; IL-8, interleukin-8; IGF-1, insulin growth factor-1; MMP-9, matrix metalloproteinase-9.

TABLE 3 | Correlation between biological mediators and clinical parameters in all, male, and females participants.

Time point	All participants			Gender	Parameters	R and *P*-value
		Parameters	R and *P*-value			
T3	IGF-1	BW BFM BMI	R:0.418;p:0.014 R:0.410; p:0.016 R:0.455; p:0.007	Male Female	IGF-1 vs. BW Cortisol vs. FSS Cortisol vs. BFP	R:0.480; p:0.038 R:−0.630;*p*: 0.012 R: −0.622; *p*:0.013
	Cortisol	BMI	R:−0.453; p:0.007			
	IL-8	SMM BWM FFM BMR	R:0.428; p:0.012 R:0.425;p:0.012 R:0.423;p:0.013 R:0.425;p:0.012			
T4	Cortisol	FSS SMM FFM	R:-0.358; p:0.038 R:0.345;p:0.046 0.355; p:0.040	Male	IGF-1 vs. BW IGF-1 vs. BFM IGF-1 vs. BMI IGF-1 vs. WHR Cortisol vs. BDI	R:0.545;p:0.016 R:0.566;p:0.011 R:0.463;p:0.046 R:0.531;p:0.019 R:-0.555; p:0.014
	IL-8	BFP BW SMM Bwater FFM BMR	R:-0.360;p:0.036 R:0.354;p:0.04 R:0.393;p:0.022 R:0.397;p:0.02 R:0.365;p:0.034 R:0.403;p:0018	Female	Cortisol vs. BFM Cortisol vs. ESS Cortisol vs. FFM	R:-0.534;p:0.04 R:-0.516; p:0.049 R:0.543; p:0.037
	MMP-9	SMM Bwater FFM BMR	R:0.446;p:0.008 R:0.454,p:0.007 R:0.458;p:0.006 R:0.455; p:0.007			
T5	Cortisol	BMI BFP	R:-0.471;p:0.005 R:-0.377;p:0.028	Male	BDNF vs. BFM BDNF vs. BFP BDNF vs. WHR	R:0.512,p:0.025 R:0.561; p: 0.012 R: 0.551; p:0.015
	BDNF	HADSD	R:0.438;p:0.010	Female	Cortisol vs. BFM Cortisol vs. BFP	R:-0.586; p:0.022 R:-0.650; p:0.009
	MMP-9	BFP	R:-0.342;p:0.048		BDNF vs. SMM BDNF vs. BW BDNF vs. FFM BDNF vs. BMR BDNF vs. HADSD	R:-0.597; p:0.019 R:-0.604; p:0.017 R:-0.599; p:0.018 R:-0.600; p:0.018 R:0.624; p:0.013

BDNF, brain-derived neurotrophic factor; IL-8, interleukin-8; IGF-1, insulin growth factor-1; MMP-9, matrix metalloproteinase-9; BW, body weight; BMI, body mass index; BFM, body fat mass; SMM, skeletal muscle mass; FFM, fat free mass; BMR, basal metabolic rate; BFP, body fat percentage; ESS, Epworth Sleepiness Scale; HADSD, Hospital Anxiety Depression Scale-Depression; BDI, Beck's Depression Scale-II; FSS, fatigue severity score.

Cortisol

Cortisol has been known to be associated with mental health-related problems (18, 30, 39, 40) and regulated by circadian rhythms (30). In healthy subjects, cortisol also plays a role in regard to physical activity, nutrition changes, and mood symptoms (24, 31). In this study, the level of cortisol decreased, particularly at T3 and T4 in all participants. However, the decrease at both time points could only be observed in male participants. It was confirmed by the correlation of cortisol with mood (BDI) at T4 in male participants. This study is in agreement with Bahijri et al. who showed the decrease of cortisol level after RF (32), but contradicts that of Al Rawi et al. (33). The difference could be explained by the fact that the latter study determined cortisol level in overweight and obese participants, whereas in this current study, the BMI of participants was in the normal range (1). In this study, the difference of cortisol level only occurred in male participants. This could be attributed to the fact that men and women manage stress differently (34). This result is in agreement with the mood level of men that has been reported in previous studies (1, 5). Interestingly, cortisol level is also significantly correlated with body composition parameters. These results are supported by several other studies that showed that cortisol played a role in body composition parameters (30, 39, 40).

BDNF

BDNF is the second neurotrophin that has been mostly determined in association with mood (17), stress-related disorders (35), nutrition (28), and chronic pain (22–24). RF has been reported to be associated with mood and alteration of nutritional intake. In this study, BDNF level of all participants tended to decrease until the last day of RF (T3) and it returned to baseline value (T1) at 1 month after RF (T5). The difference was particularly observed in male participants when compared at T1 and T3. This result cannot be directly compared with another study that did not show significant alterations, due to differing experimental time points (31); the latter study only observed BDNF level at day 3 of an intermittent fasting period. In another study, the result of the BDNF level was conflicting at T2 and T3 (17). It seems that gender could play a role in regard to this difference (36). Other reasons may include differences in body composition (1) and hormonal status (36). A significant correlation of BDNF with body composition parameters and mood supports the role of BDNF in mediating RF-derived health-related benefits and improvements (24, 27, 28).

IL-8

IL-8 is an inflammatory mediator, which is produced in different types of tissue (37). IL-8 has also been known to be associated with mood and nutritional intake (38, 41). In this study, the level of IL-8, particularly compared to T2, was gradually decreasing until T5. This result is different from other studies (14, 15). In one study, there was no significant alteration of IL-8 (14). Meanwhile, in another study, significant alterations were observed (15). However, in the latter study, if we check at a specific time point (morning time: 0600), there was no significant difference. The discrepancies of results with the latter study (15) could be that sleep/wake pattern, meal composition, and energy expenditure were controlled. In this study, we did not control such parameters, as we preferred to observe free-living participants. In this study, significant changes of IL-8 occurred only when comparing T2 and T5; and T1 and T2 in male and female participants, respectively. Interestingly, IL-8 was significantly correlated with body composition-related parameters. This study was in agreement with other study by Razmjou et al. (42), but contradicted another study by Ghashang et al. that did not show any correlation with body composition-related parameters (14). The reason could be that the latter study only included male participants.

IGF-1

IGF-1 is an important hormone, particularly in regulation of the pituitary growth hormone (43, 44). IGF-1 also plays a role in different clinical-related issues, such as mood and body composition (45, 46). In our study, the level of IGF-1 increased at T2 which then stabilized until 1 week after RF if we compare with before RF (T1). These data did not support other studies that showed a decrease of IGF-1 (47, 48). The differences could be the characteristics of the participants: one study observed the effect of RF on IGF-1 in overweight and obese participants, and another study only observed male participants. In our study, we had both male and female participants with normal BMI (1). These two studies only reported in two time points. Meanwhile, in our study, the measurements were performed at five different time points. In this study, the correlation of IGF-1 and body composition parameters were particularly observed at T3 (all participants and men) and T4 (in only men). This is in agreement with other studies that reported a correlation between IGF-1 and body composition parameters (46). These results support the role of IGF-1 in mediating RF-derived improvements in regard to body composition parameters (46).

MMP-9

MMP-9 is involved in different types of pathological remodeling including inflammatory mechanism, cardiovascular disease (49), and oncologic processes (50). It is known that fasting is recommended as a complementary therapy for cancer patients (51). In this study, the decrease of MMP-9 was observed until T5. Unfortunately, there was no comparison study available in regard to MMP-9, except one that measured MMP-9 from the ocular surface (52). Furthermore, it seems the benefit of fasting in different diseases could be mediated by the decrease of MMP-9. As in different type of diseases, MMP-9 was higher (e.g., in colorectal cancer, ulcerative colitis, and inflammatory bowel diseases) (50). In this study, correlation between MMP-9 and body composition parameters occurred, particularly with BFP and SMM. This interesting finding was supported by a previous study that reported a negative correlation between MMP-9 and obesity (53, 54) and muscle strength (55).

Myoglobin

Myoglobin is a muscle protein that is located in the cytoplasm of the heart and skeletal muscles and is responsible for intramuscular oxygen transport (56). Some studies showed that myoglobin can be used to measure the muscle breakdown in the body (57). Our previous studies showed a decrease of skeletal muscle mass (1, 5). Therefore, in this study, myoglobin was determined. Myoglobin was determined only at T1 (one week before RF) and T3 (last days of RF) as it was the most interesting time point that might lead to the highest effect during RF in regard to the decrease of SMM (1, 5). Interestingly, the level of myoglobin significantly decreased from T1 to T3. However, there was no correlation between myoglobin and SMM at all time points. It seems the decrease of myoglobin at T3 was due to the decreased level of SMM in the body. Therefore, the body would need less myoglobin. However, further studies are needed for this to be elucidated and other studies should also be performed in different time points.

Effect Size

The effect sizes of these biological parameters were determined to observe the magnitude alterations due to RF. Based on effect size calculations, it seems all determined biological parameters played a role in bringing benefits of RF on body composition parameters, mood, and QoL, as almost all parameters showed large effect sizes, either at T3, T4, and T5. These results were in line with the effect size of RF on mood and body composition parameters in our previous study (1).

LIMITATION

This study has some limitations. As mentioned above, all of these parameters were influenced not only by fasting, but also physical activities and nutrition intake. In this study, we also did not record the number of participants who skipped predawn breakfast and their sleeping pattern, which could also influence all the parameters. Therefore, further studies need to consider recording these parameters. This study was participated by young participants (mostly students in the Hannover area), which could influence the level of cortisol and other biological parameters. Future studies should be considered to include participants with a broader range of ages. This study was only performed in the fasting group. Future studies to compare fasting groups and non-fasting groups would also be of interest.

STRENGTHS

Some strong points of this study include the determination of RF in both male and female participants. Many RF studies only reported pre/during and post RF (9, 48, 58). However, this study determined the effect of RF in the mid of RF (T2); and extended the determination until 1 week (T4) and 1 month after RF (T5), in order to determine the longer effect of RF. Many RF studies have been conducted particularly at equatorial, subequatorial, and tropical zones which mostly perform RF between 13 to 16 h/day every year (9, 13, 33, 47, 58, 59). This study was performed in the northern part of the globe (Germany—temperate zones). In this zone, during summer, RF is performed longer than those aforementioned zones.

IMPLICATIONS OF THE FINDINGS AND FUTURE DIRECTIONS

The results of this study show that a prolonged duration of fasting (17 to 18 h/day) could still be tolerated and even show benefits for both physical and mental health in young and healthy participants. The benefits might be mediated by different biological mediators. Religious fasting, particularly RF, has been a concern of both patients and health professionals. Therefore, future studies with similar duration (17–18 h/day or even longer) of fasting should also be performed in different health conditions in order to give the best recommendations for patients who live in the temperate zone.

CONCLUSIONS

The benefits of RF include improvement of mood and body composition-related parameters. The benefit of RF in mood and body composition-related parameters might be mediated by different biological mediators, particularly cortisol and BDNF.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Hannover Medical School (Ethics No. 7242; Registration code of the trial: DRKS00017640). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

BN: conceptualization, formal analysis, visualization, and supervision. BN, AS, and MB: methodology and software. BN and AS: validation. Investigation: BN, AR, LE, SG, AS, and MB. Resources and funding acquisition: CG and GG. Data curation: BN, AR, LE, AS, and MB. Writing—original draft preparation: BN, AR, and SG. Writing—review and editing: BN, AS, SG, LE, MB, CG, and GG. Project administration: AR and LE. All authors have read and agreed to the published version of the manuscript.

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Time-Restricted Feeding and Aerobic Performance in Elite Runners: Ramadan Fasting as a Model

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A distance runner's performance is generally limited by energy availability when competing or training. Modifying meal frequency and timing by abstaining from eating or drinking, from dawn to dusk, during Ramadan fasting is hypothesized to induce hypohydration and reduced caloric and nutrient intake. The purpose of this study was to investigate the impact of Ramadan fasting on runners' performances. Fifteen trained male distance runners who observed Ramadan participated in this study (Age = 23.9 ± 3.1 years; Peak VO_2 = 71.1 ± 3.4 ml/kg/min). Each participant reported to the human performance lab on two testing occasions (pre-Ramadan and the last week of Ramadan). In each visit, participants performed a graded exercise test on the treadmill (Conconi protocol) and their VO_2, Heart Rate, time to exhaustion, RPE, and running speed were recorded. Detailed anthropometrics, food records, and exercise logs were kept for the entire period of the study. Repeated measure ANOVA, paired *t*-test, and Cohen's effect size analysis were carried out. Results indicated no significant influence for Ramadan fasting on body mass ($p = 0.201$), body fat ($p = 0.488$), lean body mass ($p = 0.525$), VO_2max ($p = 0.960$), energy availability ($p = 0.137$), and protein intake ($p = 0.124$). However, carbohydrate ($p = 0.026$), lipid ($p = 0.009$), water ($p < 0.001$), and caloric intakes ($p = 0.002$) were significantly reduced during Ramadan Fasting. Daily training duration ($p < 0.001$) and exercise energy expenditure ($p = 0.001$) were also reduced after Ramadan. Time to exhaustion ($p = 0.049$), and maximal running speed ($p = 0.048$) were improved. Overall, time to exhaustion and maximal running speed of the distance runners was improved during Ramadan fasting, independent of changes in nutrients intake observed during the current study. With proper modulation of training, distance runners performance can be maintained or even slightly improved following the month of Ramadan fasting.

Keywords: nutrition, running, intermittent fasting, time-restricted feeding, fasting (Ramadan)

INTRODUCTION

The popularity of endurance sports has increased globally in the last few decades, with the number of participants surpassing 5 million worldwide (1). A successful performance in such aerobic based events demands optimal nutritional regimens to allow for peak biochemical and physiological functions (2). Optimal physiological functions include energy production, transport of gases, removal of metabolic byproducts, and proper regulation of homeostasis. Competitive athletes under normal conditions strive to uphold proper nutrient intake, boost their energy storage, and maintain optimal hydration levels (3). Carbohydrate intake during exercise helps maintain high levels of carbohydrate oxidation, prevents hypoglycemia, and has a positive effect on the central nervous system.

Fasting for adults during the holy month of Ramadan is one of the mandatory practices in the Islamic religion; this annual event lasts for 30 consecutive days and mandates abstaining from eating or drinking from dawn to dusk. The number of daily meals is sometimes reduced, along with modified timing for such meals. Muslims taking part in Ramadan do not eat or drink anything during daylight hours, eating one meal (the "suhoor" or "sehri") just before dawn and another (the "iftar") after sunset. The modified meal timing is often referred to as intermittent fasting or time-restricted feeding (4–6). The restrictions on meal timing and frequency in conjunction with hypohydration during the daylight hours may impact Muslim athletes' ability to maintain proper nutrients and energy availability. Such conditions might be expected to add extra burden on athlete's ability to fulfill their nutritional requirements.

Meal consumption typically induces an increase in blood sugar level, which is regulated by insulin and glucagon. Recently, it has been reported that meal-consumption timing might influence energy balance (7, 8). Indeed, isocaloric meals with the same macronutrient content provided more calories when consumed at dinner when compared to breakfast. This might indicate that not only what we eat, but also when we eat, determines our physiological response to feeding and affects postprandial glucose levels.

Reduced energy and carbohydrate intake, impaired sleeping duration and timing, altered metabolic pattern, modified timing, and frequency of meal consumption, hypohydration, and oscillated circadian rhythm can all have unfavorable consequences on athletic performance (3). While some studies reported a detrimental effect of Ramadan fasting on athletes' performances, others showed minimal or no effects. However, in a recent study by Tinsley et al. (9) it is shown that, despite a reduction in caloric intake by 650 Kcal/day, the athletes revealed an increase in strength performance after 8 weeks of time restricted feeding (TRF). Furthermore, studies employing an intermittent fasting model for an extended period reported an enhanced neuromuscular performance in athletes (4, 9–11).

Studies that previously investigated the impact of Ramadan fasting on performance shared significant design and exercising protocol issues in common (12). Much of the available information on this topic has been collected from sedentary subjects or low-level competitors. Such design issues can be remedied by recruiting non-Muslim athletes and having them fast, or by recruiting Muslim athletes and having them only fast for a few days, which does not resemble fasting for the entire month of Ramadan. Additionally, to our knowledge, the impact of fasting on elite distance runners has not been investigated. The purpose of the current study is to investigate the effect of fasting during Ramadan (i.e., time-restricted feeding) on elite distance runners' performance. Moreover, the authors hypothesized that distance running performance will be positively impacted by Ramadan fasting in elite distance runners.

METHODS

Experimental Approach

Fifteen trained male distance runners who observed Ramadan participated in this study. Each participant reported to the human performance lab on two occasions (pre-Ramadan and the last week of Ramadan). In each visit, participants performed a graded exercise test on the treadmill (13) and their VO_2, Heart Rate, time to exhaustion, RPE, and running speed were recorded. Detailed anthropometrics, food records, sleep diary, and exercise logs were kept for the entire period of the study.

Subjects

Fifteen trained male distance runners were recruited by word of mouth to participate in the present study (age = 23.9 ± 3.1 years; height = 170.9 ± 7.2 cm; body mass = 60.8 ± 6.4 kg; body mass index = 20.8 ± 1.3; Rest HR = 48.8 ± 7.9 bpm; VO_{2Peak} = 71.1 ± 3.4 ml/kg/min). Review of the similar published studies (13, 14) suggested that a sample size ≥ 12 would be sufficient for the present investigation. All participants were active runners (i.e., engaged in running activity for a minimum of 150 min/day, at least 5 days a week); however, none of the participants regularly performed resistance training exercise. After being informed of the procedures and potential risks involved in the investigation, an IRB-approved informed consent document was signed by each participant before the commencement of the study. Participants were not instructed to alter their training duration, intensity, or frequency. The daily fasting period was equal to or exceeding 15 h. The study was carried out during summer with an average maximum temperature during daylight hours of 34 C° and a mean relative humidity of 20% for the entire period of the study. During the study, subjects consumed a meal before dawn at ∼0,500, with testing sessions beginning 4–4.5 h later.

Procedures

The study was an observational design. All procedures were approved by the Hashemite University Institutional Review Board (IRB number 1520162017). Before the study, participants were informed of the study's purpose, along with any associated risks and benefits. In accordance with the university institutional review board, and the Declaration of Helsinki, participants gave written informed consent and completed a health history questionnaire before the first test session. All data was collected in the Human Performance Laboratory at Hashemite University.

Participants completed three visits (a familiarization visit and two testing visits) on different days to undertake the study protocol as indicated below. On visit one (2 weeks before Ramadan), anthropometric characteristics, health status, blood pressure and heart rate were assessed. Briefly, participants were familiarized with the testing protocol and tools. Shortly after that, and following a 25–30 min resting period, the participants' blood pressure and resting heart rate (HR_{rest}) measurements were conducted while seated, three readings were recorded, and their average was used to determine blood pressure and HR_{rest}. On visit 2 (3 days before Ramadan: Pre-Fasting) and visit 33 (end of the fourth week of Ramadan: End-Fasting) preparations performed as described previously, and a Conconi (13) graded exercise test (GXT) to exhaustion began following a 15-min rest and a 5-to-7-min warm-up period. The initial speed was set at 8 km/h and increased by 1 km/h every 400 meters until exhaustion using a treadmill (Cosmos Saturn, Traunstein, Germany). All time to exhaustion running trials were performed at approximately the same time of day (between 10:00 am and 11:00 am), to avoid discrepancies in results caused by testing time. Oxygen consumption (VO_2), and carbon dioxide production (VCO_2) were calculated breath by breath using a respiratory metabolic cart (Quark PFT 2, COSMED. Rome, Italy). Heart rate and rate of perceived exertion (RPE) were obtained at the end of each stage (6–20, Borg scale chart). Participants' heart rate was monitored throughout the entire period of each trial using a chest strap heart rate monitor (Polar Electro, Oulu, Finland). Time to exhaustion was considered the point at which participants triggered the emergency stop, the termination signal, or failed to stay within safe range of the safety harness. Time to exhaustion was considered valid if participants achieved at least two of the following criteria at the point of exercise termination:

1. Plateau in VO_2 with increasing speed.
2. Heart rate within 10 beats of the age predicted maximal heart rate.
3. RPE value more than or equal to 17.

Body Composition

Body mass (BM) was measured with the use of a digital scale (SECA, Hamburg, Germany) to the nearest 0.1 kg wearing their running tops and shorts. Height was determined with the use of a stadiometer (SECA, Hamburg, Germany) to the nearest 0.1 cm. Measurements were compliant with the recommendations of the International Society for the Advancement of Kinanthropometry Guidelines. Body mass index (BMI) was calculated (kg/m^2). Body fat (BF), body fat percentage, fat free mass (FFM), and total body water (TBW) were assessed with the use of four electrode bioelectrical impedance system (InBody720, Inbody Seoul, Korea). Reliability and validity of the measurement system has been reported in the literature (6). Anthropometric measurements were obtained on each visit after subjects rested for a period of 30 min following their scheduled arrival time and before beginning the warmup.

Energy, Nutritional and Exercise Assessment

Participants were instructed to keep daily food records and exercise logs. The food records provided full details about ingested food and fluids along with time of consumption, preparation method, meal type, and perceived mood. Randomly selected complete food records were analyzed using NUTRITIONIST PRO (Axxya Systems; Woodinville, WA USA) and middle east food composition tables (http://www.fao.org/infoods/infoods/tables-and-databases/middle-east/en/). This software platform is routinely used in to validate other diet assessment tools (15).Exercise logs contained details about type of event, training duration, training intensity, and provided information about frequency of training. Exercise logs were analyzed using Sportlyzer (Sportlyzer LLC., Tartu, Estonia). Exercise energy expenditure (EEE) was estimated using the 2011 Compendium of Physical Activities (16). Energy availability (EA) was calculated as described by ACSM (17), and a good EA value was defined to be more than or equal to 45 kcal/kg FFM/day.

Sleep Assessment

The *Daily* Sleep Diary (Loughborough University, Loughborough, UK) was used to evaluate each participant's subjective daily sleep score. The sleep diary consisted of a brief eight item questionnaire, evaluating the sleep time and quality. This and similar instruments have been reported on in the literature (18). During this study, sleep diary records were collected at two different times, one before (at the start of the study visit) and one during (at the end of study visit). The questionnaire was completed by each participant for the entire period of the study. The two sets of records were treated separately (i.e., not averaged) in order to assess reliability and to analyze fasting effect on sleep characteristics. Sleep diaries that show bedtime and awake time and daytime napping during the weekdays and at weekends was obtained together with the total sleep duration in a 24-h period (i.e., one full day) for the interval of the study. Comparison was administered for the difference in sleep duration before and during Ramadan.

Statistical Analysis

All data is presented as means ± standard deviation (SD) and was analyzed using SPSS (version 25). Once the assumption of normality was confirmed using the Shapiro-Wilk test, parametric tests were performed. For anthropometric measures, sleep duration, and dietary variables, dependent sample *t*-test was used to detect significant differences between the two trials. For the RPE, VO2, and HR data, a two-way [Time (pre and end of Ramadan) x Speed] ANOVA with repeated measures was used. When appropriate, paired *t*-test was used for pair-wise comparisons. Cohen's *d* effect size for pairwise tests and partial eta squared (ηp^2) for repeated measures analyses were calculated to assess the magnitude of difference between the time points. Effect sizes were considered small at 0.2, medium 0.5 and large at >0.8 for Cohen's *d* and 0.01 for small, 0.06 for medium and 0.14 for large for partial eta squared tests. Coefficient of variation were calculated for all measures and were all below 10% for performance measures. Dietary variables had higher variability

TABLE 1 | Assessment of changes in body weight and body composition during Ramadan fasting.

Variable	Mean	SD	*t*	*P*-value	*d*
Body weight (kg)					
Pre-F	60.5	6.1	−1.347	0.201	0.36
End-F	60.8	6.4			
Body fat (kg)					
Pre-F	9.0	1.8	−0.714	0.488	0.19
End-F	9.1	1.9			
Lean body mass (kg)					
Pre-F	51.5	4.7	−0.653	0.525	0.17
End-F	51.7	5.1			

TABLE 2 | Assessment of changes in caloric, water, and macronutrients intake during Ramadan fasting.

Variable	Mean	SD	*t*	*P*-value	*d*
Carbohydrates intake gm/d					
Pre-F	446.0	74.3	2.511	0.026	0.67
End-F	379.3	77.9			
Proteins intake gm/d					
Pre-F	118.1	54.4	1.643	0.124	0.44
End-F	88.6	53.3			
Lipids intake gm/d					
Pre-F	81.5	15.6	3.063	0.009	0.82
End-F	72.2	16.7			
Energy intake Kcal/d					
Pre-F	2,989.7	359.9	3.869	0.002	1.03
End-F	2,523.2	454.4			
Daily water intake L/day					
Pre-F	4.2	1.1	5.472	<0.001	1.46
End-F	2.5	0.8			

(up to 53% for Protein intake), which was expected given the different size of the athletes and dietary preferences.

RESULTS

Body Weight and Composition

Assessment of changes in body mass and body composition are presented in **Table 1**. There were no significant changes in body mass ($p = 0.201$), body fat ($p = 0.488$), and/or lean body mass ($p = 0.525$) due to Ramadan fasting.

Dietary Intake

Table 2 shows changes in nutrients intake while fasting during Ramadan. As expected, a significant decrease in carbohydrate intake, lipid intake, water intake, and caloric intake was detected at the end of Ramadan when compared with pre-Ramadan values with effect size ranging from medium to large (*d*: 0.67–1.46). However, protein intake was not significantly affected by Ramadan fasting.

Physical and Training Performance

A significant decrease was detected in daily training duration ($p < 0.001$; *d:* =2.98, 95% CI −16.4 to −40.8 min), and exercise energy expenditure ($p = 0.001$; *d:* = 1.18, 95% CI −198.0 to −609.9 Kcal) at the end of Ramadan compared with pre-Ramadan. Furthermore, time to exhaustion (pre: 1,392.9 ± 126.8 s vs. post: 1,448.4 ± 108.8 s, 95% CI 1.0–110.0 s) along with maximum speed (pre: 18.6 ± 2.3 km/h vs. post: 19.7 ± 1.9 km/h, 95% CI 0.0–2.27 km/h) were significantly improved toward the end of Ramadan ($p = 0.04$ and $p = 0.048$, respectively), with a medium effect size (*d:* 0.58–0.59). However, maximum oxygen consumption, and energy availability were not significantly affected by Ramadan fasting ($p = 0.960$ and $p = 0.137$ respectively).

RPE, Oxygen Consumption, and Heart Rate Before and During Ramadan Fasting

Figures 1A–C shows the changes in RPE, Oxygen consumption, and heart rate, at different running speeds before and during Ramadan fasting. The repeated-measures analysis of variance indicated no significant main effects for time on VO2 values at all running speeds ($p > 0.05$). Additionally, no significant effect was observed for time on RPE ($p > 0.05$) at all running speeds. Repeated-measures ANOVA revealed a significant main effect for time [$F_{(1,4)} = 10.689$, $p = 0.031$, $\eta p^2 = 0.728$] on heart rate values. Heart rate values were significantly lower at low and medium-high speeds (8 km/h, $p = 0.001$ pre: 117.3 ± 12.0 vs. post: 107.9 ± 10.9; 11 km/h, $p = 0.016$ pre: 133.0 ± 10.8 vs. post: 127.9 ± 13.5; 14 km, $p = 0.030$ pre: 153.6 ± 10.4 vs. post: 148.5 ± 11.5) during Ramadan as compared to pre-Ramadan, however, the values were comparable at higher speeds.

Sleep

Figure 2 reports changes in sleep time because of Ramadan fasting. Sleep durations were comparable during Ramadan (437.6 ± 44.5 min/24 h) in comparison with before Ramadan (432.0 ± 59.0 min/24 h) ($t = -0.363$; $p = 0.722$; $d = 0.108$, 95% CI −27.5–38.7 min).

DISCUSSION

This study investigated the impact of Ramadan-Fasting on elite distance runners' performance. The results showed that time to exhaustion and running speed were improved under fasting conditions in comparison to non-fasting. However, the performance improvements observed among fasting athletes in the current study were independent of changes in Peak VO_2, oxygen consumption, or RPE. Furthermore, energy and water intake were significantly reduced during Ramadan. Macronutrients intake (except for protein) were also reduced during Ramadan.

In previous studies, Chtourou (14, 19) observed an attenuated physical performance in long-distance running during Ramadan fasting. A possible explanation for such a conflicting outcome with current results may be attributed to the employed testing protocol. Indeed, studies that employed a graded exercise test (20, 21), similar to current design, reported a better outcome in

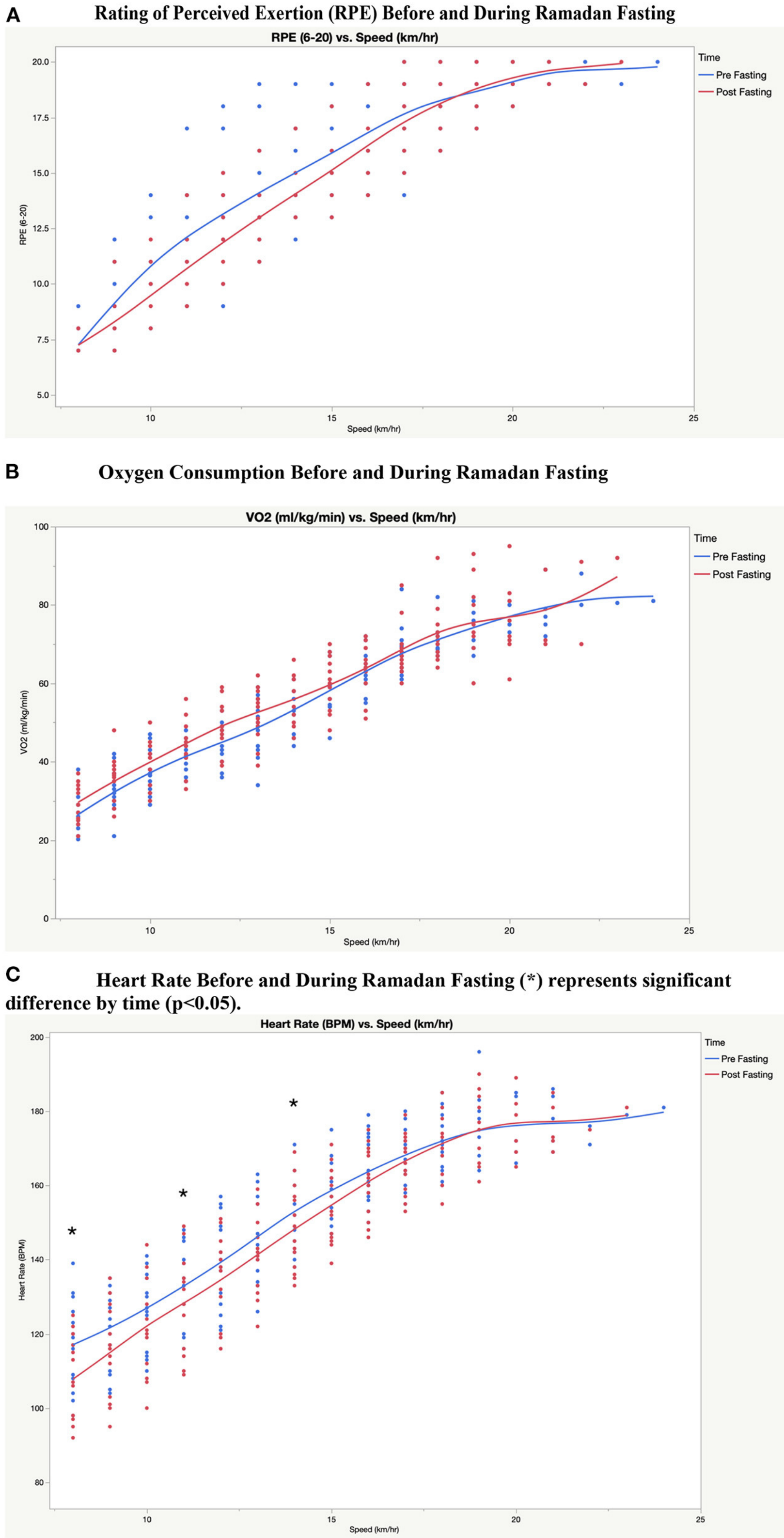

FIGURE 1 | (A) Rating of Perceived Exertion (RPE) before and during Ramadan fasting. **(B)** Oxygen Consumption Before and During Ramadan Fasting. **(C)** Heart Rate Before and During Ramadan Fasting (*) represents significant difference by time ($p < 0.05$).

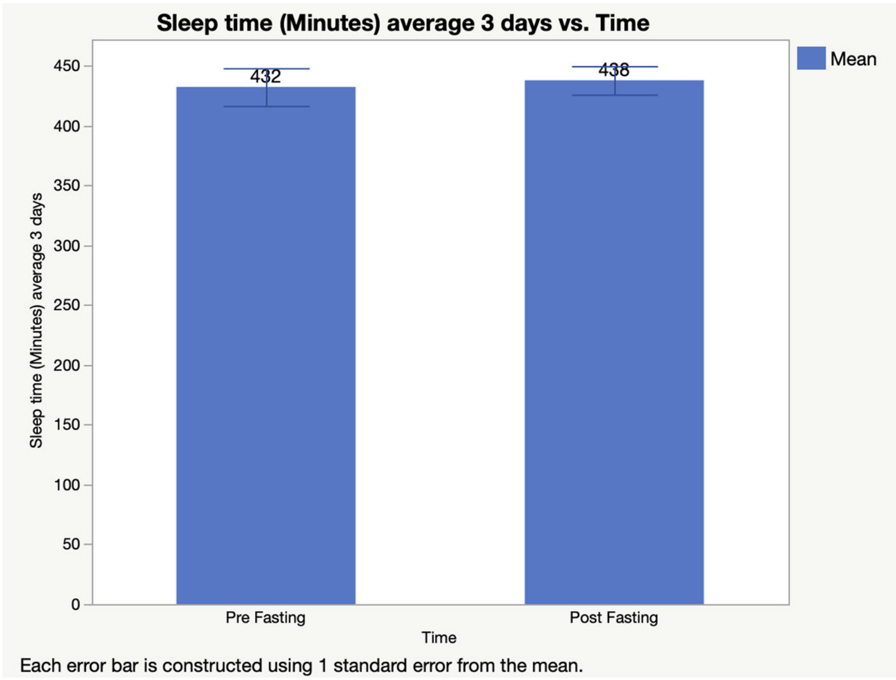

FIGURE 2 | Sleeping Duration Before and During Ramadan Fasting.

comparison to protocols comprised of mere constant intensity. Additional explanation for this disparity in results might also be attributed to athletic level and discipline (4, 21, 22). Elite athletes tend to react to the conditions of Ramadan fasting in a different manner compared with other categories of athletes, suggesting that a higher level of adaptation may protect against performance decay during extended periods of unfavorable conditions (23–27).

A significant improvement in time to exhaustion at the end of Ramadan fasting compared to pre-Ramadan was detected in this study. A similar result was observed in a study that was carried out during Ramadan fasting with Turkish soccer players (20), which showed an increase in total running time in the last week of Ramadan during the modified 20 m shuttle running test (MSRT) performed to volitional fatigue. Furthermore, the current study showed a pronounced reduction in daily training duration (∼48% compared with Pre-Ramadan) while maintaining the number of weekly training sessions. Thus, the improved time to exhaustion might be attributed to a form of maintenance-like training. In support, Garci (28) concluded that 2 weekly endurance training sessions would be enough to maintain aerobic power under conditions of reduced training.

The 6% improvement in maximum running speed observed in the current study was in agreement with previous findings reported by Güvenç (20). The researcher studied the effect of Ramadan fasting on aerobic performance in a sample of athletes comparable in age and fitness level to current study participants. The study reported an increase in peak running velocity toward the end of the fasting month compared with pre-Ramadan. The same author also reported a decrease in peak running velocity during the first week of Ramadan fasting compared with pre-Ramadan. These findings might suggest that athletes tend to positively adapt to Ramadan fasting toward the end of the month. Indeed, several published studies that investigated the effect of Ramadan fasting reported a negative influence on endurance performance when exercise testing was carried out within the first two weeks of Ramadan fasting (22, 29).

The current study showed a lack of a significant change in Peak VO_2 despite a significant reduction in training volume during the fasting period. The current results are consistent with the results of a previous study (28) that reported consistency in VO_2 max in world class non-fasting Kayakers following 5 weeks of reduced training, preceded by a period of tapering for 4 weeks. Such findings might suggest that a reduction in training volume for the entire period of the holy month of Ramadan should not alter maximal aerobic power from a scientific and training standpoint.

The current study results showed comparable values for oxygen consumption along with lower heart rate values when comparing pre-Ramadan with Ramadan fasting values while performing the graded exercise test. These findings agree with previously published results that also show no significant changes in oxygen consumption and lower heart rate because of Ramadan fasting (20, 30). In contrast, short-term fasting (i.e., overnight fasting) did not alter heart rate during the 6-min walk test in athletes in comparison with a non-fasting state (1, 31). The findings in Ramadan fasting athletes may suggest that prolonged fasting periods have different effects on aerobic performance indicators in comparison with either shorter fasting periods or non-fasting athletes. Similarly, RPE values in the current study were not altered across all running speeds due to Ramadan fasting, which was in contrast with previous studies. The disparity in RPE values between the current study and previous studies may be attributed to the fact that participants' fasting in the current study was voluntarily conducted, thus it may not have added an extra psychological burden. The lack of an increase in RPE during Ramadan fasting may also explain the improved time-to-exhaustion values achieved by fasting participants in the current study. This ability to outperform non-fasting values can be justified by psychological means such as Hormesis (32–34).

In the present study, body mass, body fat mass, and lean body mass, despite the long hours of Ramadan fasting, were not significantly changed (**Table 1**). Concerning dietary pattern and energy expenditure, the present study showed a significant reduction in carbohydrates, lipids, water, and caloric intake. The absence of changes in body mass and composition can be explained by our findings which identified a decline in daily training duration and intensity and the reduced exercise caloric expenditure which resulted in a comparable energy availability value. This result was in agreement with results from previous studies that reported no change in body mass during Ramadan fasting when comparable energy availability values were maintained (31, 35). Additionally, Mujika (36) suggested that athletes and trained subjects could maintain their lean body mass and muscular strength after reducing training loads. They also projected that the reduced training load could decrease the risk of dehydration (i.e., reduction in the sweat losses during training sessions). The reduced risk of dehydration may help athletes to preserve their energy levels for quality workouts and training (36).

As shown in **Figure 2**, sleeping duration was not changed between pre-Ramadan and during Ramadan for our group of athletes. This represents a difference between athletes and non-athletes who observed Ramadan from a similar age group that demonstrated reduced total sleeping hours during Ramadan (37). Muslim athletes managed to maintain their regular sleep duration, based on the results of the present study.

It is important to acknowledge the strengths and limitations of the current research. To date, the impact of fasting on elite distance runners has not been investigated. Limitations for the present work include sample size, lack of a control group, lack of data on training intensity and reliance on some self-reported data. A larger sample would have served to increase the statistical power for this research. A control group would have allowed for the effect of training to be determined on the study outcomes, however; given the mandatory nature of Ramadan fasting a control group of similar individuals was not feasible. The success of food records and exercise logs depends on the memory, cooperation, and the communication ability of the subject(s). Participants may have attempted to present themselves and their answers in a more positive light, and participants potentially may have worried about the perceived repercussions caused from their responses.

CONCLUSIONS

It is important that individual sport coaches are accommodating toward various cultural and/or religious values and are mindful of the potential impact certain dietary practices may have on an athlete. Fasting during the holy month of Ramadan is one of the mandatory practices in the Islamic religion. The restrictions on meal timing and frequency in conjunction with hypohydration during the daylight hours pose a challenge for the Muslim athlete to be able to maintain proper nutrients and energy availability for training. Given the results of the present study, a strategy of adjusting training intensity and duration may be considered to help maintain the quality of training during times of fasting. The present study also suggests that training might be optimized if it occurs during the morning hours approximately 4 h after the pre-dawn meal. The results of the present study showed a lack of a significant change in Peak VO_2, despite a significant reduction in training volume, and time-to-exhaustion and running speed were actually improved under fasting conditions. Despite the long hours of Ramadan fasting, body mass, body fat mass, and lean body mass, were not significantly changed. Therefore, a decline in daily training duration and intensity and the resultant reduced exercise caloric expenditure may explain the promising results in the present study. Coaches must closely monitor training results and make adjustments as needed to ensure their athletes are being properly prepared for training and competition in the most effective and safe manner.

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 HASHEMITE UNIVERSITY. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AA-N: conception and design of study and data acquisition. MB: data collection and design of study. HK: data interpretation and critical revisions. DB: statistical analysis and data interpretation. LJ: critical revisions and data interpretation. All authors contributed to the article and approved the submitted version.

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Impact of COVID-19 on Children and Young Adults With Type 2 Diabetes: A Narrative Review With Emphasis on the Potential of Intermittent Fasting as a Preventive Strategy

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Background: The world is still struggling to control the COVID-19 pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The level of uncertainty regarding the virus is still significantly high. The virus behaves differently in children and young adults. Most children and adolescents are either asymptomatic or have mild symptoms. They generally have a very good prognosis. However, it is not well-known whether children and young adults with type 2 diabetes are at risk of getting a severe infection of COVID-19. Many Muslim children with type 2 diabetes have been performing dawn to dusk fasting during the month of Ramadan, before and during the COVID-19 pandemic, and the impact of this on their health has not been well investigated. Previous studies in adults have suggested that intermittent fasting may be beneficial in different ways including reversal of type 2 diabetes and prevention of COVID-19 infection.

Objective: The primary aim of this narrative review is to summarise the impacts of the COVID-19 pandemic on children and young adults with type 2 diabetes, and to identify the knowledge gaps in the literature. It also explores the potential of intermittent fasting in reversing the pathogenesis of diabetes and highlighting how this approach could prevent these patients from developing chronic complications.

Methods: This narrative review has been produced by examining several databases, including Google Scholar, Research Gate, PubMed, Cochrane Library, MEDLINE (EBSCO), and Web of Science. The most common search terms used were "COVID-19 AND Children", "SARS-CoV-2 AND/OR Children", "COVID-19 AND Diabetes" "COVID-19 Epidemiology", "COVID-19 AND Ramadan fasting", "COVID-19 and Intermittent fasting." All the resources used are either peer-reviewed articles/reports and/or official websites of various media, governmental and educational organisations.

Results: Having reviewed the currently limited evidence, it has been found that the incidence of COVID-19 among children with type 2 diabetes seems to be not much different from children without diabetes. However, these patients are still vulnerable to any infection. Several studies have reported that prevention programmes such as intermittent fasting are effective to protect these groups of patients from developing any complications. Moreover, observing Ramadan fasting as a type of intermittent fasting could be beneficial for some children with established diabetes, prediabetes and people at risk.

Conclusion: Children and young adults with type 2 diabetes are not at risk of severe COVID-19 infection as the case in adults with diabetes. More research is needed to identify the impact of COVID-19 and to investigate the efficacy and safety of intermittent fasting, including Ramadan fasting, among these age groups. Implementing these cost-effective programmes may have a great impact in minimising the incidence of diabetes. Moreover, this could be effective particularly at prediabetes stage by preventing these people from going onto develop type 2 diabetes and taking medications for the rest of their life and protecting people from complications linked to disease and infection.

Keywords: type 2 diabetes, children, young adults, COVID-19, Ramadan fasting, intermittent fasting

INTRODUCTION

The global potential impacts of the coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 on children and young adults have been examined. It has been reported that the disease is less prevalent among these age groups, about 1–2% of the total cases (1, 2). They seem to have less risk of catching the infection and there is a very low mortality rate in comparison to adult people (3–5). In contrast, Dong et al. (4) have concluded that children and young adults are similar to adults in terms of their sensitivity or their risk to COVID-19 infection, however, the course of the disease is unusual. Typically, for children and young adults, the disease is mild and less severe compared to adults and infants (less than a year) and they often recover within 1–2 weeks (5). Moreover, it has been noticed that most children confirmed as having COVID-19 are asymptomatic (5). However, severe to moderate symptoms have been recorded among infants who are sensitive to the infection (4). Moreover, new onset of type 1 diabetes (T1D) related to COVID-19 among children have been reported in the UK and in the US (6, 7). However, this has not been noticed yet among children with type 2 diabetes (T2D).

Understanding the impacts of the COVID-19 infection on children and young adults with T2D is one of the aims of this narrative review article. It is widely assumed that these patients are at the same risk as their peers who do not have T2D (8). Even though pathological alterations develop in these patients, which suggest that they might be at risk of getting severe COVID-19, no evidence has been provided to support this theory. These particular patients have not been recognised as a high-risk group for developing severe COVID-19, which is opposite to the case among adults with T2D (8, 9). It is not well known yet why the disease is mild among children, however, there have been some theories to explain this (10). Moreover, there is a great deal of debate about whether asymptomatic children can transmit the infection to adults and other children (with or without health problems), and for how long the asymptomatic children could be considered as a source for transmission of the infection (11, 12). Statistically, T2D among children and young adults has significantly increased in recent years (13–15). The COVID-19 pandemic and its associated circumstances (recurrent lockdown measures and movement restrictions) could have a substantial impact on increasing the percentage of these patients in the world. Taken together, there remain some open questions about whether these patients are at risk or not and how these patients could be protected to prevent them from developing any complications due to the current COVID-19 pandemic.

The level of uncertainty regarding this pandemic is significantly high. Changing dietary and lifestyle behaviours, such as physical exercise, a healthy diet, and the practice of intermittent fasting (IF) may play a role in boosting immunity (16). Encouraging all the habits that help to boost immunity could improve the disease prognosis in general. One of these practices is Ramadan intermittent fasting (RIF) and other types of IF (17). This review will highlight the importance of implementing these strategies. The beneficial role of RIF and other types of IF in fighting infections and boosting immunity has been reported elsewhere (18–20). Moreover, Hannan et al. (21) have recently reviewed the importance of IF and how it could be used as a potentially protective approach to fight COVID-19. Furthermore, Faris et al. (22) indicated that RIF positively affects the body's immunity by changing different related elements, including oxidative stress and inflammation, metabolism, body weight, and body composition. Thus, this review will discuss and evaluate the current literature related to the effects of Ramadan fasting (RF) on human health and patients with T2D and how this could be applied during the current pandemic.

Several studies have shown that RF was associated with a positive impact in controlling blood glucose and weight loss among patients with T2D in adults (23–25). However, the findings of other studies suggested that RF could increase the risk of hypoglycaemia in some of these patients, while could not in others (26–28). This variation could be ascribed to many factors such as season of Ramadan month, fasting time duration, pre-fasting education, geographical location and the duration of time since diagnosis with the disease (29). Thus, this review has hypothesised that some children who are eligible to fast according to Islamic regulations on RF, which usually starts around 12 years old or reaching puberty, will benefit from RF and the effects could be the same as in adults. This is based on the fact that the pathogenesis of T2D in children is similar to that of adult patients (30). On the other hand, some may suffer due to the severity of their medical condition, poor diet, lack of activity, and anxiety. In addition, this narrative review has suggested several precautions could be taken before the month of Ramadan, such as intensive education programmes, adjusting medication, physical exercise, and avoiding missing follow-up appointments with the medical care professionals. This has to be implemented with clear communication from health care providers. To test this hypothesis, it is necessary to discuss the current scientific evidence on the risk of COVID-19 among children and young adults amongst patients with T2D compared to healthy people. Besides, the effects of RF and its long-term effects among these age groups of the population will be examined.

METHODS

This narrative review has been produced by examining several databases, including Google Scholar, Research Gate, PubMed, Cochrane Library, MEDLINE (EBSCO), and Web of Science. The most common search terms used were "COVID-19 AND Children", "SARS-CoV-2 AND/OR Children", "COVID-19 AND Diabetes" "COVID-19 Epidemiology", "COVID-19 AND Ramadan fasting", "COVID-19 and Intermittent fasting." All the resources used are either peer-reviewed articles/reports and/or official websites of various media, governmental and educational organisations.

EPIDEMIOLOGY OF COVID-19 AMONG CHILDREN AND YOUNG ADULTS

Based on the epidemiological summary, which has been published and is updated regularly by the Royal College of Paediatrics and Child Health, children and young adults can be affected by the COVID infection, however, the number is very small ($\leq$ 5%) in comparison to adults and adolescents who are more susceptible to the disease than younger children (31). Another UK study reported that children (less than 16 years old) testing positive for COVID-19, represented a very low percentage (1.1%) among over 35,000 children tested (32). This study was conducted between January and May 2020. Moreover, a retrospective study in Italy has reported that children who had COVID-19 were only 1% of the total cases at the beginning of the pandemic and that no deaths have been recorded among this age group (33). Similarly, a multicentre cohort study, involving 25 countries in Europe, has reported that the mortality rate was very small-−0.36% (4/582)—among children and teenagers with COVID-19 (34) including children with chronic medical problems. Generally, the course of the disease is mild, and very few numbers had moderate to severe symptoms. Moreover, the risk of mortality is extremely rare (0.01–0.1%), which is quite similar to the incidence of deaths due to seasonal flu per year (31). A systematic review was conducted worldwide during May 2020 and reported that children and young adults (up to 21 years old) with COVID-19 had a very good prognosis and most of the cases recovered completely, including people with pre-existing medical problems (35). They found that the mortality rate was just 0.09% among a total number of around 8,000 confirmed cases. This result was based on analysed data from healthy children and children with comorbidities (35). It seems that the younger the age, the better the outcome if someone has COVID-19.

According to Diabetes UK, children with diabetes can become infected with COVID-19 virus, however, the risk of developing severe illnesses is extremely rare (36). Nevertheless, these children and adolescents with diabetes are still vulnerable to the COVID-19 infection and careful precautions should be in place and close health care observations are highly recommended for these patients. This is particularly so in patients with uncontrolled blood glucose and who have a secondary complication of diabetes. Even though the COVID-19-related mortality rate has increased sharply among adults with diabetes, the risk of death in children with diabetes has not been recorded yet in the UK (36, 37). Furthermore, it has been reported that most of the hospital-admitted children (with comorbidities) who were confirmed to have COVID-19, were from ethnic minority groups, including Asian, and Black and other minor ethnicities (38), indicating that ethnicity could be considered as an independent risk factor for making the disease hard to control. Authors have suggested that this might be greatly influenced by the cultural and behavioural differences among these societies (38). Recently, it has been reported that many of the children and adolescents (less than 19 years old) who had developed paediatric multisystem inflammatory syndrome in children, were not of white ethnicity at 64% (39). This has also been proven by the multicentre prospective cohort study in the UK where around 651 patients with acute COVID-19 were admitted to the emergency departments. Only six died in the hospital, which is only 1 % of the total number and all of them had previous chronic illnesses (39).

It is known that children from different communities are not tested as frequently as adults. Therefore, it is expected that more children are affected by the SARS-CoV-2 virus in all societies. This has been clearly seen by the significant surge in the numbers of affected cases amongst pupils and staff members in the second week of returning to schools in the UK (40). Consequently, this had an impact on the sharp increase in the number of COVID-19 cases in the whole country (41). Thus, it has been noticed that the available data do not reflect the true picture of COVID-19 in children and young adults (42). Furthermore, at the early period of the outbreak, COVID-19 tests were restricted and were mainly for children with severe symptoms and who required hospital admission.

More recently, it has been observed that the incidence of COVID-19 has increased significantly and steadily among young adults (10–29 years old) in the UK (43). It has been suggested that this could be related to the fact that the young adults are not following COVID-19 protection rules in terms of wearing masks and maintaining the recommended social distance; there is no evidence to support this explanation though (43). In a month, the number of cases of those in their teens increased by four-fold and it has risen around three times among people in their twenties (43). However, it has not been established whether these identified cases are all healthy individuals or whether they have chronic diseases such as T2D. Therefore, epidemiologically, the accurate number of infected children either healthy or patients with T2D is not well known in most countries. For example, locally, how many children are affected at a school in the UK, how many teachers are affected by COVID-19 at a school in the UK, and how many children with diabetes had COVID-19 during the whole pandemic? Researchers and the general public have been struggling to find the answers to all of these questions. Apparently, governments around the world are experiencing great challenges in terms of collecting accurate data and classifying these data by age and sex. Moreover, there still remains a substantial deficit in capacity to test for COVID-19 and availability of the more accurate PCR testing. Identifying accurate statistics is essential to apply the right prevention, management, and control strategies to overcome this pandemic.

CHILDREN WHO HAVE BEEN CONFIRMED AS HAVING THE COVID-19 INFECTION EITHER MILD OR ASYMPTOMATIC - WHY?

There is great uncertainty regarding the effects of COVID-19 on children and young adults. The risk of the disease has not been recognised even in patients with chronic diseases such as diabetes. It could be argued that the biological, immunological, and physiological mechanisms in children could play a key role in how children's bodies are behaving with—and responding to—the virus as this might be determined and modulated by the developmental phases of the endocrine, muscle and nervous systems (44). Lingappan et al. (10) reviewed varied scientific pieces of evidence, which indicated that children have a significantly lower expression of the *Angiotensin-converting enzyme 2* (ACE2) receptors, which are required for SARS-CoV-2 binding to the cells. Besides, they found that the level of expression of these receptors is directly correlated with age. Moreover, it has been reported that the virus is competing with other viruses in children's airway mucosa, which is preventing the entry of the virus (45).

Another theory that has explained why children have mild COVID-19, is the maturity of the immune system in adults compared to children and adolescents (46). The innate immune system is weaker among children and this is further associated with the lower activity of the immune cells such as macrophages, dendritic cells, and neutrophils (10). These cells are involved in the proinflammatory state and trigger several cytokines among adults with COVID-19, which in turn indirectly damage the lung tissue (10). It has been suggested that this immune overreaction is subtle or does not develop in children and young adults. Supporting this hypothesis, a study investigated the pathogenesis of SARS-CoV-2 using a mouse model to explore the difference in the immune responses between adult and young mice (47). They noticed that the virus induced severe inflammatory reactions only in adult mice and this was associated with serious respiratory complications including alveolar damage and pulmonary oedema. This could be the same case in SARS-CoV-2, however more research-driven data are needed to confirm this.

Moreover, children could be protected by the trained immunity that had developed due to some vaccines such as the bacillus Calmette-Guérin (BCG) vaccine (48, 49). Several previous researchers have reported that the BCG vaccination was associated with a significant decline in the incidence of respiratory tract infections and decreased the infant mortality rate [reviewed by O'Neill and Netea (49) and Pandit et al. (50)]. They showed that children could possibly have a powerful innate immune system as they are used to having recurrent viral infections. Consequently, the level of immunoglobulins is expected to be high and it is protecting them from getting the infection and developing severe illnesses (51). Also, it has been reported that the severity of pneumonia in children was significantly connected to the immune response (47). Cases of mild pneumonia in children were associated with the activation of CD8+ T cells and the adaptive immune response of the IL-10 (52, 53). Thus, understanding the mechanisms/reasons behind the mildness of the disease among children will pave the way for developing the means of tackling the disease and in creating preventive approaches against COVID-19, which could be applied among children, adults and people with chronic disorders (54).

All the above hypotheses could be applied to children with diabetes as well. However, these patients are still at risk of developing severe proinflammatory complications due to COVID-19 and on top of this most children with T2D are associated with obesity (55). Furthermore, high levels of proinflammatory cytokines in obese children have been reported such as IL-6 and IL-15 (56). This in fact could worsen the disease prognosis among these patients by increasing the risk of cytokines damaging surge. Therefore, theoretically, there is still a concern regarding children with obesity who have T2D diabetes, even though, currently this has not been recognised as is the case in adult patients. Furthermore, at the early stages of the pandemic, cytokine storm has been reported in eight critically ill children (ranged from 5 months to 15 years old) with no previous chronic diseases (57). Most of these children had direct contact with COVID-19-infected cases. Furthermore, Cho et al. (58) have shown that the dysregulation of some cytokines [resistin and plasminogen activator inhibitor 1 (PAI-1)] was associated with developing a new-onset of T2D among adults with prediabetes. However, this has not been identified in children and young adults yet. Therefore, precaution and well-controlled diabetes are inevitable among this group of population. In addition, several protective and preventive strategies to reverse T2D could be applied, such as introducing healthy diet programmes, practising IF, and encouraging physical activities. These will be discussed below in more detail.

THE RISK OF COVID-19 TRANSMISSION FROM AND ON CHILDREN

The risk of COVID-19 infection transmission from children to adults has been a significant concern for many people and researchers. Moreover, much of the research up to now has been descriptive in nature. Wongsawat et al. (59) have investigated the risk of spreading the infection from children with COVID-19 to their household/carers. They concluded that there was no risk of the transmission of the COVID-19 from children (4 and 8 years old) to adult carers. However, this study was designed as a case series in which the number of cases was very limited, and the cases had mild symptoms (mild cold and with no fever) (59). On the other hand, another study in China has shown that children (mean age was 6 years) with non-severe symptoms of COVID-19 were associated with a risk of transmission to their parents, even though the risk was only 1% of the total studied cases (60). This was defined as 'intrafamily transmission' (60). Besides, they noticed that about 50% of patients had SARS-CoV-2 RNA identified in their stool samples within 1 month of the start of the illness (60). Therefore, the authors have raised the warning that children could be a source of infection to others, adults and children, even after the symptoms have completely resolved. This could be related to the fact that the incubation period of COVID-19 infection among children is slightly longer than in adults (60, 61). Recently, evidence has reported that children are infectious to others even if they are asymptomatic or having mild symptoms (62, 63).

Thus, in terms of preventing the public transmission of this current pandemic, more investigations are vital. Furthermore, most of the infected children were secondary cases as a result of being exposed to adult cases (households) or travel-associated (60, 64). Therefore, it seems that children could be involved either way in human-to-human transmission and this will have an important role in Infection-Prevention-Control strategies for this pandemic. In a retrospective study using data from three hospitals in China, Qiu et al. (65) reported that 36 patients, under 16 years old, were confirmed to have COVID-19 within 2 months. The sources of infection for most of these cases (approximately 90 %) were from household contacts (65). Also, most of the patients in this study were admitted with moderate to mild symptoms and around 30 % were asymptomatic (65). Importantly, this highlights the point that a substantial number of asymptomatic children are hard to identify among communities as they lack the typical clinical and epidemiological features to tackle the disease transmission. Consequently, this feature could seriously increase the risk of making COVID-19 one of the community-acquired infections (57, 65). However, the ability of asymptomatic cases to transmit the infection to others remains unclear and further investigation is needed.

It has been reported that a considerable number of children with confirmed COVID-19 had typical radiographic features during the first few days of the infection or since they had been in contact with an infected person or a household (60). For this reason, all children who are asymptomatic and/or have mild symptoms and have a history of contact with infected people should be followed closely by their carers (parents and health care providers). However, such an approach might be hard to apply in some countries. Therefore, all these findings could have a negative impact on patients with chronic illnesses such as children and adolescents with T2D.

DIABETES EPIDEMIOLOGY

All over the world, the incidence of diabetes has increased tremendously throughout the last decade. According to the International Diabetes Federation (IDF), it has been estimated that the number of patients with several types of diabetes, aged between 18 and 99 years, reached 451 million in 2017, and in 2045 this figure is projected to expand to 693 million worldwide (66). Furthermore, they estimated that there are around 352 million people worldwide who are pre-diabetic (who have impaired glucose tolerance) and this number is predicted to grow up to 531.6 million by 2045. These figures give an estimate that nearly half of all populations are either at prediabetes stage or undiagnosed cases and about 5 million deaths among the same age groups were due to diabetes during 2017 (66, 67). Globally, it has been predicted that 90% of patients who are diagnosed with diabetes have type 2 diabetes (68–70). Moreover, based on the last report that was published by the World Health Organization (WHO), the global number of diabetes (T1D and T2D) among young adults and adults, $\geq$ 18 years old, in 1980 stood at 4.7% and had remarkably grown to 8.5% by 2014 (71). This rise was associated with the increased incidence of numerous risk factors such as obesity and a sedentary lifestyle. Additionally, it was reported that in 2016, diabetes was the seventh cause of death in the world (71). Therefore, these warning statistics are expected to get worse during the current COVID-19 pandemic with the consequences of the recurrent lockdown measures.

According to the National Paediatric Diabetes Audit (2018–2019), it has been reported that the recent update for the prevalence of patients with T2D among children and young adults (< 25 years old) in the UK was 790 (72). They indicated that this number was based only on the patients who were under the Paediatric Diabetes Units (PDUs) and did not include the patients who had been followed by primary care and private clinics. Besides, it was most predominant among girls whose ethnicities are non-white (72). Moreover, according to Diabetes UK, it has been reported that 'there are more than 7,000 children and young adults under 25 with T2D in England and Wales' (73). Therefore, all these statistical findings confirm the issue that the number of children with T2D has substantially increased in comparison to other types of diabetes during recent years. It could be argued that compared to the total population in the UK, which is around 66 million, the incidence of T2D would be expected to be much higher than this figure (74). In addition to the current COVID-19 pandemic, the number of cases with diabetes and prediabetes among this age group is anticipated to be doubled by the end of the year. However, no recent statistics have been announced yet. Another important point to mention is that T2D at a younger age is associated with significant risks of vascular morbidity, recurrent fracture, and high mortality rate (75, 76). Therefore, highlighting these statistics is extremely important to provide valuable evidence to create new government policies/guides in agreement with the health care professionals. For instance, IF could be recommended for children who are at prediabetes stage as it has been recommended for adults (77). However, more research is needed in order to apply this to the medical practice. In addition, providing the optimal health care to this group of the population (during the current pandemic) should be seen as an urgent matter. For example, providing/sponsoring free virtual education events for parents and children in different societies would be beneficial. This could importantly prevent or minimise the epidemic rise of T2D.

EFFECTS OF THE COVID-19 PANDEMIC ON PATIENTS WITH TYPE 2 DIABETES AMONG CHILDREN AND YOUNG ADULTS

It has been reported that the risk of death and comorbidity progression is at the same rate as the population without diabetes (78). Moreover, according to the Juvenile Diabetes Research Foundation (JDFR), there were no COVID-19 deaths recorded among children with diabetes and the incidence of hospitalisation has been very low during the pandemic period (79). However, there are no available data regarding the incidence of cases with COVID-19 among patients with T2D. Curiously, this was completely the opposite of the situation among adults with diabetes, either T2D or T1D, who have been identified as one of the highest risk groups with an increased rate of hospitalisation (80). The risk of death due to COVID-19 in adults was about three times higher than the rest of the population as a whole (81). This could be related to the fact that children are less prone to serious COVID-19 infection as has been discussed earlier in this review.

Furthermore, it is well-known that diabetic ketoacidosis (DKA) rarely presents in new-onset cases with T2D, however, the COVID-19 pandemic has had a significant impact on increasing the risk of DKA among new-onset cases of T2D in adults (82, 83). The reason behind this might be that people are avoiding visiting medical centres and seeking medical advice (84). It is not clear whether COVID-19 has impacted the incidence of DKA among children with T2D and more scientific evidence is needed. DKA is an inflammatory condition associated with increased levels of several inflammatory factors including interleukin 6 (IL-6), interleukin-1β (IL-1β), and tumour necrosis factor (83). Therefore, this could have a worse impact by increasing the incidence of severe COVID-19 in patients with high risks, such as those who are obese and have a family history of T2D.

Even though the pathophysiological changes in diabetes patients with COVID-19 are not clear yet, this infection could lead to severe inflammatory cascade culminating in serious comorbidities (85). Moreover, it may trigger diabetes in many prediabetes cases or those at risk of developing diabetes, due to an increase in the levels of cytokines (86). This will be based on the fact that several viral infections increase insulin resistance, and as a result, the risk of developing diabetes (T1D and T2D) is very high (87). A good example of this is the hepatitis C virus, which has been found to be associated with a disturbance in β-cell function and inhibits the mechanism of glucose-stimulated insulin, *in vitro* (88). Furthermore, Yang et al. (89) have shown that the other coronaviruses, such as SARS-CoV, caused significant damage to different organs, including the lungs, kidneys, and the endocrine organs. This was directly related to a significant increase in the ACE2 expressions (the SARS coronavirus receptors) which explains the reason behind the development of acute diabetes in patients with SARS-CoV-2 who were previously healthy individuals (89). It has also been noticed that most of the cases recovered completely and that their diabetes reversed and only a few cases continued with chronic diabetes. Similarly, this was reported in some patients who had been affected by COVID-19 (85). It has been suggested that COVID-19 could trigger diabetes and thus indicates that there are significant complicated pathophysiological changes caused by COVID-19, concerning diabetes (85). There are reports that these cases were associated with poorer outcomes in comparison to patients with established T2D (9). For this purpose, there is currently a large international project known as CoviDIAB, organised by diabetes researchers worldwide (90). This could answer the most asked questions related to the risk of COVID-19 among children with diabetes, where most of the cases are mild.

It is not clear yet whether these risks could occur among children and adolescents with T2D or not. For this reason, vaccination against flu infections is recommended for people at risk such as people with obesity or with a strong family history of T2D and patients with diabetes during the current COVID-19 pandemic (91, 92). Although no scientific evidence has been provided yet, these groups of patients who are asymptomatic and have uncontrolled diabetes could be at risk of developing the symptoms of COVID-19. This could be triggered by increasing stress hormones and blood pressure, which could be developed due to the pandemic circumstances (93). Thus, psychological support for these patients could play a key role in protecting them. Patients with diabetes need to be reassured that their medical providers are accessible and available at any time either by phone or by email (94). Garge and his group (95) have found that during the COVID-19 pandemic, using telemedicine technologies to manage diabetes in new-onset T1D in children and adults is effective and feasible. Patients can share their data remotely with their physicians who can advise them and adjust insulin doses, accordingly, using emails, phones, and via video calls. Thus, identifying the feasibility of the virtual tools could be considered as one of the beneficial impacts of the pandemic as it will allow patients to seek medical advice at their convenience and is less stressful in terms of social distancing, travel, and missing school for some children (95, 96).

However, these facilities may not be available in some areas where the internet is not available. Therefore, other prevention approaches such as exercise and fasting for some patients could play a key role in reducing or eliminating hospitalisation and comorbidities. Advising patients to go outside for walks and practising light to moderate exercise would have a great impact (97). In addition, IF has been studied for years (98, 99). It has been indicated that the implementation of several fasting programmes into practice has the potential to improve the disease prognosis and can reverse the disease condition, particularly in patients with T2D and prediabetes (20, 100, 101). While this has been reported among adults with T2D (102), this approach has not been investigated widely among children and young adults. This article will discuss several types of fasting and it will introduce the importance of Ramadan fasting in more depth. Fasting in general is a cost-effective measure to treat and prevent several chronic illnesses such as diabetes. Authors of this article propose that applying this approach among children and young adults with T2D or at prediabetes stage, could be beneficial and a preventive and protective approach in terms of minimising the integrated risks of the two epidemics: diabetes and COVID-19. It is like any other approach that might work more for some people than others, but could save lives until accurate evidence/data regarding the effects of COVID-19 infections in these focused groups are identified and published. Furthermore, IF and changing life-style may prevent these young people from taking medications for the rest of their life.

INTERMITTENT FASTING

Intermittent fasting (IF) has been defined as periodic fasting where people are fasting and eating for certain hours during the day (103). Extensive research showed that IF is associated with numerous health benefits including extending life span, cognitive function, intellectual performance, and metabolic regulation among healthy adults and patients with different disorders (100, 104). Several studies suggested that IF could have the profound potential to be used as a preventive/therapeutic tool for chronic illnesses (100, 104). This is based on the fact that naturally and genetically, the human body system is programmed on periods of intermixture cycles: active and rest cycle, feast and famine cycle, where these intermittent periods are critical for the human physiology to be able to modulate all the metabolic and biological processes required (105). In addition, it has been proven that the other metabolic processes including the shift in energy sources during the fasting period are essential in providing the optimal energy for cellular functions and regeneration (106). The abolishing of these cycles, caused by eating frequently without proper physical activities as in a sedentary lifestyle, results in metabolic and biological deregulations and the development of different metabolic disorders, such as diabetes and obesity (100, 106). Various approaches of IF have been widely studied including alternate day fasting (ADF) and time-restricted feeding (TRF). Moreover, Ramadan fasting is also a kind of IF and it is often referred to as Ramadan intermittent fasting or Ramadan diurnal IF in the scientific literature (107, 108).

Alternate day fasting has been identified by fasting every other day and during the fasting day, the followed protocol is either to limit the food intake to only 25% of the daily food intake (500 calories/day) or to consume zero calories, while returning to the normal healthy diet during the eating day (101). On the other hand, TRF is characterised by the limitation of the daily consumed food over a specific period during the day with no calorie restriction and this time limit varies from 4 to 12 h (109). Considerable research attention has been paid to these kinds of fasting in humans and animals (98, 99, 110). It has been reported to be associated with a significant improvement in glucose homeostasis, blood pressure, decreased lipid biomarkers, lowering of inflammation, body weight reduction, insulin level, fasting blood glucose (FBG), and insulin sensitivities (20, 109, 111, 112). However, some scholars reported that ADF was associated with a remarkable rise in hunger during the fasting day making this approach unpleasant or inconvenient for a longer period (113). Another negative consequence of ADF is that people who are food lovers or heavy eaters did not lose much weight on this regime as they might be eating a large amount of food during the feasting day leading to hyperphagia (114). To prevent these drawbacks, this approach was replaced with TRF for some people.

Gow et al. (115) suggested that an intensive low-calorie diet could be used as a therapeutic tool for T2D among children and adolescents and it might be more efficient and able to cure the disease than standard medications. In their study, eight patients with T2D had a very low calorie/energy diet (VLED) at less than 3,360 kJ/day for 8 weeks followed by a hypo-caloric diet at about 6,300 kJ/day for 34 weeks. They reported that there were significant reductions in insulin level, weight, cholesterol level, HbA1c with a noticeable improvement in insulin sensitivity in all participants (115). Furthermore, three participants on insulin were able to stop their medication by week 8 and the other participants who were on metformin achieved T2D reversal by week 34 (115). However, in the opinion of this author, this extremely low-calorie diet pattern (including 3 to 4 meals of a low carb diet for 8 weeks, which is gradually restricted to one meal per day) might be considered as a tough lifestyle regime and it would probably not be followed by most of the patients of a younger age. This regime has also been evaluated among adults and up to now many studies have suggested that the main pathophysiological changes in diabetes; beta-cell failure and insulin sensitivity could be reversed by just following the VLED, consequently, disease remission was achieved in approximately half of the patients who adhered to this protocol (116–118).

Furthermore, an important study conducted in the UK by Lean et al. (119) reported that complete remission of T2D among young adults and adults was successfully achieved by following diet replacement over 12 months. This study conducted over 4 years was known as DiRECT (119). Thus, even though research among children and young adults with T2D is limited, specific diet regime such as VLED still has the potential to be used as a therapeutic approach for these patients who would like to avoid the use of medications and their adverse effects such as insulin. From this point of view, the diet pattern during RF could have the same potential positive impact, and research studies related to this are necessary as the diet approach could prevent disease complications, decrease health care costs, and positively influence the quality of patients' lives in the long term.

IMPORTANCE OF FASTING IN REVERSING THE PATHOGENESIS OF TYPE 2 DIABETES AND THE NEED FOR STUDIES IN CHILDREN

Various theories have been reported to identify the reasons behind the disturbance in glucose homeostasis resulting in increased blood glucose, insulin level, and HbA1c, and consequently the development of diabetes (120). This includes environmental factors, a stressful life, sleep deprivation, and genetic factors (121, 122). However, it has been shown that this epidemic rise is strongly related to a substantial alteration in diet or lifestyle in general, where people tend to consume a great amount of processed foods, fast foods, and refined sugars (120). Dalgaard (120) has proposed that cells are protecting themselves from the high level of glucose by shutting off the glucose uptake to prevent any cellular damage that could take place due to auto-catalytic glycation. This was based on the theory of epigenetics by which the cells can regulate the expression and suppression of different genes and modify them according to the intracellular biological function, for instance when the cells are exposed to increased amounts of glucose (123). These genetic modifications are preventing the cells from taking more glucose from the blood, and this might be mediated by decreasing the expression of glucose transporter type 4 (GLUT4) and/or impairing the insulin receptors/insulin signalling pathway (124). Furthermore, several studies have shown that people with diabetes have certain epigenetic variations in comparison to healthy individuals (124, 125). This explains the improvements in insulin sensitivity that have been observed in some studies that are based on IF and calorie restriction approaches (115). Thus, changing diet by consuming low to no carbohydrates could reverse the condition and reactivate the genes and transcription factors that are necessary for glucose uptake. Therefore, in the case of insulin resistance and based on the above theory, T2D could be cured/reversed by just modulating diet such as by consuming fewer carbohydrates, and this has been already proved in some studies (115, 116, 126).

In recent decades, it has been shown that the incidence of insulin resistance has substantially increased among children (specifically at around 12 years old), adolescents, and young adults. This substantial rise was strongly associated with obesity and overweight epidemics among these age groups (127). Further, the negative effect of puberty on insulin sensitivity plays a role in the rapid progression of this disorder (128). This could be pertinent to hormonal and metabolic alterations among adolescents, where insulin sensitivity is significantly declined, and this alteration is automatically reversed later by the end of puberty (129). However, in children/adolescents who experienced obesity during their growing periods, this condition might remain and cause diabetes (129). Once β-cells fail to compensate for the insulin resistance, high-risk individuals progress gradually to pre-diabetes and eventually go on to develop diabetes (130). Moreover, it has been observed that the pathogenesis of T2D among adolescents and/or young adults (< 20 years old) who are obese is somewhat similar to the pathological changes in adults, in terms of the reduction of β-cell function about a significant decline in insulin sensitivity (131). In addition, a failure in insulin secretion was observed even within overweight youth with a normal FBG and oral glucose tolerance test (127). Furthermore, Sjaarda et al. (132) found that in adolescents who had prediabetes, HbA1c between 5.7 and 6.5% had significant impairment in β-cell function.

Therefore, all these observations indicate that the administration of new dietary modification approaches such as IF among younger age groups could have a profound potential as a therapeutic and preventive regime. This could be an effective strategy for people who are at risk such as obese children/adolescents, in combination with physical activities and dietary interventions. Soliman et al. (133) have recently suggested the effects of IF in switching host metabolism. However, more scientific research is required in the near future in order to apply this in clinical practice. The standard treatment of these groups of the population starts with lifestyle alterations including nutritional advice and the encouragement of physical activities as it has been reported that loss of body weight by around 6 % has a significant impact on blood glucose control (134). A randomised controlled trial study conducted for around a year among obese 8–16 years old children found that an intensive family-based programme (nutrition, exercise, and changing behaviour) had a positive impact on insulin sensitivity and body composition indices such as weight, BMI, and body fat (134). Furthermore, Marcus et al. (135) have conducted the most popular study known as Treatment Options for T2D in Adolescents and Youth (TODAY) investigating the best therapeutic approach for those with T2D who are obese. They have noticed that apart from the medical treatment that has been prescribed, reduction in body weight is critical and associated with substantial effects on C-peptides, HbA1c, and lipid parameters (135). However, another study reported that dietary intervention by introducing low-calorie food was not effective among adolescents (136). It could be argued that this perhaps relates to the physiological and the biological variations among humans. Similarly, fasting programmes in general could be more beneficial for some people than others.

The therapeutic approach in early-onset T2D is based mainly on the hyperglycaemic state and the metabolic parameters, where patients are advised to start with metformin tablets either alone or in combination with insulin (127). Furthermore, the evidence displayed that different kinds of bariatric surgery such as laparoscopic adjustable gastric banding, Roux-en-Y gastric bypass, could be effective as a preventive and therapeutic approach for both early and late-onset T2D associated with severe obesity (137, 138). Bariatric surgery has profound useful impacts on regulating glucose homeostasis biomarkers in obese youth with and without diabetes, reducing coronary heart disease risk, and also giving complete remission to patients with T2D among adolescents compared to other medical treatments (139). The remission rate reached up to 90% in some surgery types, for instance, biliopancreatic-diversion (140). However, like any other surgery, it has some drawbacks or complications including hypoglycaemia, hernia, anastomotic leaks, ischemia, and pulmonary embolism (141). Thus, it will be more sensible to introduce safer programmes/approaches such as fasting to avoid all these risks and achieve the same results.

Another point to mention is that compared to T2D in adults, early-onset T2D has an aggressive nature and is associated with serious complications leading to an increase in rates of mortality and morbidity (142, 143). These include macrovascular complications, cardiovascular risk, and renal function disturbance; most of these complications are age-related meaning they tend to develop at an early age (143). This might be due to many factors such as psychological/social factors and the rate of response to the medications. In addition, it has been anticipated that this will get worse during the current pandemic circumstances due to the effects on the mental health of children and young adults (144). Therefore, new approaches including preventives and therapeutics are essential in order to reduce this epidemic and to provide a healthier life for this group of the population. RF is one of the most common types of IF that has been investigated among adults and mainly within Muslim communities, constituting around 1.9 billion worldwide (145). Early intervention in children and young people, through a combination of intermittent fasting, dietary guidance and physical activity may prevent or reverse diabetes and ensure that poor health does not persist into adulthood. RF where children fast for a month is a good opportunity that should not be missed. A "prevention is better than cure" approach is particularly important with childhood obesity reaching epidemic levels (146).

EFFECTS OF RAMADAN FASTING ON PATIENTS WITH TYPE 2 DIABETES AMONG CHILDREN AND YOUNG ADULTS

Most of the research that has been conducted pertaining to the effects of RF on glucose biomarkers in T2D patients was among adults and young adults (23, 147). The findings were controversial with wide variations in the study design and methods that were used to measure and assess the metabolic parameters (148). It has been reported that RF is safe and has a significant impact on weight reduction among adult patients with T2D, without a significant increase in the frequency of hypoglycaemia/hyperglycaemia when compared to controls (149, 150). Furthermore, RF is associated with a remarkable improvement in glucose lipid biomarkers including HbA1c, FBG, fractosamine, TG, and LDL (23, 151). All these findings indicate that RF could prevent/decrease cardiovascular disease risk in T2D patients. In addition, several studies reported that intensive education programmes before and during Ramadan have had a significant impact on improving and preventing the complications of diabetes such as hypoglycaemia, and this was in comparison to standard health care (152–154). Interestingly, it has been reported that the high similarity between RF and TRF makes it reasonable to translate the effect of TRF to RF (155).

To date, no attention has been paid to the effects of RF among children with T2D even though it is well known that children participate in RF. The impact of RF in glucose biomarkers among children and adolescents with T2D has not been examined yet. However, it has been reported that the effects of RF on children and adolescents have been examined mainly for T1D (156, 157). Evidence supported the fact that a majority, around 60 per cent of children and teenagers with T1D, can fast for more than half of the month of Ramadan and that they can fast safely in association with proper and focused education before Ramadan and close medical care during Ramadan, where patients are advised to break their fasting during hypo/hyperglycaemia (154, 158, 159). Similarly, and more recently Zabeen et al. (160) have concluded that children and adolescents with T1D and have uncontrolled blood glucose can observe Ramadan safely if they have been provided with close medical care. However, this kind of support may not be provided for Muslim children and adolescents who are interested to fast during Ramadan in Western countries. Misconceptions between paediatrics medical professionals and parents of fasted children in Michigan, US, has been reported (161). It has been found that no medical advice being provided for fasted children (161). In addition, differences in complication frequencies in people with T1D on an insulin pump compared to those on multiple-dose injections (MDI) were not be identified (159). Supporting this, Eid et al. (154) found that intensive/focused education programmes before/during Ramadan, are associated with a significant improvement in glucose homeostasis biomarkers (FBG and HbA1C).

On the other hand, other studies considered that children and young adults with diabetes as a high-risk group that should not fast during the month of Ramadan as it may increase the incidence of Diabetic ketoacidosis (DKA), dehydration, and hypoglycaemia among T1D in these age groups [reviewed by Beshyah et al. (157)]. Furthermore, most of the recent studies reported that RF was not associated with an increased risk of DKA (157). Therefore, even though the pathogenesis of T1D varies from T2D, the above evidence strongly support that RF could be very effective for some patients with T2D in children and adolescents. Examining how fasting could affect children's health is vital and more research is needed. However, based on the currently available literature among adults, it might be safer to implement fasting programmes among healthy young adults and patients with controlled diabetes under close observation either to support them to practice RF or apply some previously studied programmes of IF.

RAMADAN FASTING DURING COVID-19 AND ITS IMPACT ON CHILDREN AND YOUNG ADULTS WITH DIABETES

Recently, several reviews have examined the impact of fasting (IF and RF) on healthy people and patients with chronic problems; considering the influence of the COVID-19 pandemic, they reported that RF is safe among healthy people and some people with controlled diabetes among adults (162, 163). In addition, several beneficial effects have been reported among healthy adolescents, for instance decreasing the incidence of obesity, preventing infections, and mental disorders (164). Furthermore, the importance of combination of RF, exercise and good nutrition was recommended to boost the immune system among Muslims societies during COVID-19 pandemic before Ramadan 2020 (165). No study so far has identified the impact of COVID-19 pandemic during RF on children and young adults with T2D. Regardless, it can be argued that due to the presence of lockdown measures such as school closures during the previous Ramadan, these children may have fasted more safely where they had more rest and increase in sleeping hours in the morning. This could outweigh the health outcomes of RF and improve the blood glucose parameters and the disease prognosis in general. However, they might struggle to obtain the appropriate medical support needed. Therefore, more scientific studies are required to identify how these patients manage their fasting during Ramadan and whether they have been provided with medical advice or not.

Despite the fact that children with T2D have not been identified as a high-risk group for COVID-19, precaution is essential among these patients, and focus group education would be beneficial before the month of Ramadan. For example, advice for patients on how to keep hydrated, consuming healthy food, adjusting medications and physical activities during Ramadan. This will avoid any risk of hypoglycaemia that may occur, which has been reported to be related to the age group among children (166, 167). Moreover, the physiological mechanisms of maintaining the normal blood glucose level during the fasting period between children and adults are slightly different (166, 168). However, this case could be only among younger children, less than 10 years old who have a rapid reduction in blood glucose level and increase in the ketones levels compared to older children during fasting (168). Children who are expected to fast during Ramadan are usually at the age of puberty, between 12 and 15 years old. Recently Diabetes and Ramadan (DAR) International Alliance (https://daralliance.me/) community has published an updated practical guideline to help the medical professionals to support patients with diabetes who are interested to fast during Ramadan (169). Unfortunately, this does not cover guidelines for children and there is, as yet, no guideline that proposes IF as a preventive and protective strategy against diabetes. Furthermore, the guideline is not implemented in all medical practices in western countries, and the most common practice is to discourage these people from fasting, but not all patients are following this advice. Thus, there is an urgent need for supporting these patients medically as this will help to understand and classify who could have benefits or drawbacks of fasting, particularly in children and young adults with T2D at their early stage of the disease.

CONCLUSION

In general, it is evident that there is great reassurance about the impact of COVID-19 infections on children and young adults. Most of the cases have milder symptoms and have an excellent prognosis. Even patients with diabetes, have the same risk of infection as those without diabetes. However, according to the pathophysiological changes from diabetes, some patients with T2D could be at risk of comorbidity in the case of any infection including SARS-CoV-2. Moreover, the data available are very limited in terms of new-onset diabetes in relation to the COVID-19 infection and the risks of DKA among children with T2D. The impact of the pandemic circumstances on the rates of identifying new cases among people at risk, such as those who have prediabetes, has not been well investigated. The current data highlight the importance of introducing and implementing preventive and protective tools during the current period of uncertainty. This could include encouraging physical exercise, healthy diet, and practising IF and RIF by some patients who have well-controlled diabetes or at prediabetes stage. These kinds of preventive and protective approaches will be paramount in improving public health, and significantly decrease the burden on health care providers. Unfortunately, there is a lack of studies on IF in children with T2D even though it is known that many of these patients fast during the month of Ramadan. There is, therefore, a definite need for patients who are willing to observe the month of Ramadan so they can achieve the benefit of fasting safely under medical supervision and potentially reverse their diabetes. In addition, more studies are required in order to obtain a clearer understanding of the biological effects of COVID-19 among children and young adults with T2D. Greater efforts are needed to ensure the effectiveness of fasting in patients with T2D among children and young adults and how this may help people who are at risk of developing diabetes during stressful situations such as pandemics. Another important practical implication is that even though conducting experimental studies is a great research challenge during the pandemic restrictions, using virtual tools such as survey-based studies or video interview-based studies, may have a great influence on clinical care, patient support, and in developing novel and effective guidelines. These kinds of studies could be conducted in all paediatric centres among T2D cases in children and young adults. Furthermore, they could also explore the percentage of patients who developed diabetes due to COVID-19, the risk of DKA and comorbidity in patients with established diabetes and confirmed COVID-19.

AUTHOR CONTRIBUTIONS

HE suggested the idea and the importance of the whole review and carried out most of the work, including searching, structuring, writing up and editing the review article. She contacted all the authors and discussed the importance of all the suggested points with them. MF provided detailed review of the article and helping in the editing process of the final drafts. He has also provided several recent studies/information to support some insights in the review. SU provided detailed review to this piece of work and has suggested several ideas in reorganising the article. SG provided a comprehensive overview of this work and suggested some ideas to make it more sensible and easier to read by many people from different fields. JG provided a deep review of the article and updated information to support some points. PH is a Ph.D. supervisor of HE and provided many updated resources to enhance the quality of this work. In addition, he has helped in reviewing and editing of the final drafts. A-BA-M is a Ph.D. supervisor of HE and is the corresponding author responsible of the publication process. He contributed in the restructuring, editing process, and reviewing of the article. All authors contributed to the article and approved the submitted version.

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Effects of Mental Training Through Imagery on the Competitive Anxiety of Adolescent Tennis Players Fasting During Ramadan: A Randomized, Controlled Experimental Study

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This study aimed to analyze the effects of mental training through imagery on the competitive anxiety of adolescent tennis players fasting during Ramadan. This is an experimental study conducted with 38 male tennis players, randomly allocated to two groups: an experimental group (EG, $n = 18$), aged 16.9 ± 0.6 years, and a control group (CG, $n = 20$), aged 16.7 ± 0.8 years. The study was designed as a randomized, controlled experimental trial (registration code PACTR 202006847771700). CG watched historical videos of the Olympics, while EG performed mental training. The competitive anxiety state assessment was recorded four times. The first measurement was carried out 1 week before Ramadan, the second measurement during the first week of the month, the third measurement at the end of the second week, and, finally, the fourth measurement during the fourth week of Ramadan. Our results revealed a significant interaction (time × groups) for all competitive anxiety subscales. Higher intensity and direction scores for the cognitive and somatic anxiety subscales during Ramadan compared with before Ramadan for both groups could be reported at $P < 0.001$. Higher intensity and direction scores for the cognitive and somatic anxiety subscales during Ramadan compared with pre-Ramadan for both groups could be found at $P < 0.01$. This increase in scores was greater for the CG than for the EG in the middle and at the end of Ramadan at $P < 0.001$. Finally, for the self-confidence subscale score, results revealed that intensity and direction scores were lower during Ramadan compared with pre-Ramadan for the two groups at $P < 0.01$. The score for the intensity of self-confidence was higher for the EG compared with the CG at the end of Ramadan at $P < 0.001$. It was concluded that mental imagery training was effective in reducing anxiety (cognitive and somatic) and increasing self-confidence in the intensity dimension of adolescent tennis players who fast during Ramadan.

Keywords: Ramadan fasting, tennis, mental imagery training, cognitive and somatic anxiety, self-confidence, intensity, direction, sport psychology

INTRODUCTION

Anxiety is a complex, multidimensional construct that deals with the individual's disposition and response to stressors and also with the tendency to perceive and cope with stressful situations. One of the main research topics in the field of sports psychology is competition and precompetition anxiety, in terms of the key factors and underlying psychological mechanisms that influence it in sports environments (1). Despite a growing body of studies and empirical observations, athletes' state of anxiety and the way they face such a psychological state remain not fully elucidated and understood with regard to some particularities. These include, for example, the dimensions and the determinants of competition and precompetition anxiety as a psychological state in general or in specific situations, such as those of athletes who fast during the month of Ramadan. Indeed, this month is a period which appears to be difficult for all Muslims, and, in particular, for the majority of Muslim sportsmen who are willing to fast. This difficulty is reflected in the physical, mental, and psychological state of athletes (2–4), and, of course, this may have a profound impact on sports performance and related results/outcomes.

Some studies have tried to subjectively assess the general psychological state during Ramadan. The most commonly used tools were self-designed questionnaires. Most studies have shown that the scores obtained were higher during Ramadan compared with the control period. Indeed, Chtourou et al. (5) found that fatigue assessed by means of the "Profile of Mood States" (POMS) questionnaire was highest in the afternoon in 17-year-old footballers who were fasting during Ramadan. Leiper et al. (6) demonstrated that the ability to concentrate decreased in footballers who are fasting. Zerguini et al. (7) found an increase in the incidence of headaches and a decrease in mood and motivation during Ramadan among footballers. On the one hand, according to the literature, no study has been done on the state of anxiety of athletes during Ramadan. Regarding the relationship between sports discipline and anxiety levels, athletes competing in individual sports appeared to show a higher level of anxiety compared with athletes in team sports (1).

Tennis is considered as an individual sport which has certain peculiarities, which can result in competitive anxiety in athletes. For example, during a tennis match, the player carries out an intermittent activity, composed of high intensity interspersed with periods of active or passive recovery, on the physical level, that can be very hard on the psychological and emotional levels (8, 9). During a match, the player may experience a wide variety of emotions such as anger, fear, or negative thoughts. A number of mental qualities and skills are essential for a tennis player, such as emotional control, confidence, perseverance, and focus, among others. To the best of our knowledge, no study has been done on the state of anxiety for tennis players facing the consequences of fasting in the month of Ramadan. Nevertheless, stress, anxiety, and self-confidence were the elements most investigated under usual conditions. The most widely adopted theoretical model is the multidimensional theory of competitive anxiety (10), which subdivides competitive anxiety into two dimensions: cognitive and somatic. Some models also consider a third dimension called self-confidence. Studies have found a negative relationship between cognitive anxiety and athletic performance (11, 12). Likewise, the results of previous research have shown that somatic anxiety negatively affects the performance of competitive athletes (13, 14). In contrast, according to Millet et al. (12), increased self-confidence can enhance and maximize athlete performance outcomes. In this sense, it is important to identify conditioning/training strategies that can reduce anxiety (somatic and cognitive) and increase the self-confidence of athletes. According to Wang et al. (15), mental training can be an effective strategy to improve cognitive variables of athletes. Mental training refers to the creation of mental images from sensory processes stored in the memory and accessed without external stimuli (16). According to symbolic learning theory, a person is able to create a "mental sketch" that helps deal with a particular task (17). Brick et al. (18) stated that imagination can elicit the motor cortex and generate neuromuscular activation like performing a mental task. The same authors demonstrated that there are four major mental training techniques: motivation-specific, motivation-general, cognition-specific, and cognitive-general (18). The first two are used to improve motivation and emotional control ability, respectively. Specific- and general-cognitive mental training techniques are adopted by athletes to maximize the performance outcomes of a motor task or to resolve a situation that arises in competition, respectively. Regardless of the mental training technique adopted, studies have shown that mental training can be a good strategy for maximizing the performance of an athlete (19).

Even though some scientific findings have revealed that mental training improves the cognitive and/or physical performance of athletes (18, 19), it should be noted that none of these studies was specifically devised to examine the effect of mental training on competitive anxiety among tennis players during Ramadan. From a practical standpoint, this type of research may identify the effects of mental training on competitive anxiety in tennis players who fast during this month. In this sense, the results can be extremely important for the athletes and for the managers and coaches of this sport. In this context, the aim of the study was to analyze the impacts of mental training through imagery on the competitive anxiety of adolescent tennis players fasting during Ramadan. Even though mental training appears to be able to improve the control of an athlete, two hypotheses have been formulated: (a) Fasting in the month of Ramadan is expected to increase the scores for the intensity and direction of cognitive and somatic anxiety and decrease the scores of intensity and direction of self-confidence; (b) Training with mental imagery reduces the cognitive and somatic anxiety scores and increases the intensity and the self-confidence direction scores.

MATERIALS AND METHODS

The study protocol was reviewed in depth and fully approved by the "Ethical Committee for the Protection of Southern People" (C.P.P.SUD), Sfax, Tunisia (protocol reference C.P.P.SUD No. 0032/2017). The present study, a 4-week randomized controlled experimental study conducted during the month of Ramadan in 2017, was carried out based on the latest version of the Helsinki Declaration and its subsequent amendments.

The registration code for the trial is PACTR 202006847771700.

Sample Size

H_0 is the null hypothesis, which was formulated as H_0: $m_1 = m_2$, while the alternative hypothesis is H_a: $m_1 = m_2 + d$, where d is the difference between the two means. $N = n_1 + n_2$ is the total sample size, where n_1 and n_2 are the sample sizes for the experimental and control groups, respectively.

The total sample size was estimated using the following formula (1) (20):

$$N = \frac{(r+1)\cdot(Z_{\frac{\alpha}{2}} + Z_{1-\beta})^2 \cdot \sigma^2}{r \cdot d^2}$$

where Z_α is the normal deviate achieving statistical significance = 1.64 (5% level of significance), $Z_{1-\beta}$ is the normal deviate at 1–β% power with β the % of type II error (0.84 at 80% statistical power), and r is calculated as the n_1/n_2 ratio ($r = 0.67$ gives the sample size distribution as 1:1.5 for two groups). Here σ and d are the pooled standard deviation (SD). These values were computed based on a similar hypothesis formulated in studies carried out in similar settings (21).

Participants

The non-probabilistic sample is made up of 38 tennis players competing at the national level and participating in the Tunisian national tennis championship. To be included in the research, given the inclusion criteria utilized for other surveys with racquet sports athletes (15, 22), participants must: (a) have been tennis athletes for at least 2 years; (b) systematically be training for at least 6 h per week; and (c) register for the National Tennis Championship. They were recruited because they said they would be willing to fast for the entire month of Ramadan. These participants were randomly allocated to two groups: an experimental group (EG, $n = 18$), aged 16.9 ± 0.6 years old and a control group (CG, $n = 20$) aged 16.7 ± 0.8 years old. There were no significant differences for age ($P = 0.34$) and experience in the discipline ($P = 0.28$) between GE and GC before the survey (i.e., at the baseline). The anthropometric data for both groups (EG and CG) are presented in **Table 1**.

TABLE 1 | Mean and standard deviation of descriptive research variables.

Variables	Control group	Experimental group	*P*
	Mean (SD)	Mean (SD)	
Height (m)	1.76 ± 0.1	1.77 ± 0.1	0.36
Body mass (kg)	66.2 ± 9.3	67.4 ± 5.9	0.32
BMI (kg/m^2)	21.73 ± 0.87	22.02 ± 0.58	0.31

SD, standard deviation; BMI, body mass index.

Procedure

This is a 4-week, randomized, controlled, experimental survey carried out during the month of Ramadan in 2017, recruiting adolescent male tennis athletes. Both groups (experimental and control) attended the same physical/technical training plan during Ramadan for 2 h per training session with a frequency of three times a week in the afternoon from 5 p.m. to 7 p.m.

The CG watched advertisement videos, whereas the EG underwent mental imagery training. Three mental imagery training sessions were performed per week, for a total of twelve sessions over a 4-week period during Ramadan. The sessions were held at 30-min intervals between the end of the mental imagery training session and the start of the technical/physical training session. No mental training session has been conducted without having completed physical/technical training. All mental imagery training sessions lasted approximately for 10 min in a quiet environment (near the tennis court), where the athletes wore the outfits they used to wear when competing (23). Videos of tennis players who were successful in competitions were used before each mental training session to aid the imagination of the athletes in the experimental group.

The recommendations of Brick (18) were used for the development and implementation of the mental training protocol. Therefore, the general type of cognitive imagination was adopted, asking athletes to imagine themselves being engaged in a competition. The following instructions were given to the athletes: (1) to create a mental situation using the first person; (2) to imagine the task at a speed close to reality; (3) to imagine positive situations during a competition; (4) to generate emotions (anxiety and mood) similar to those experienced during the competition. Two trainers expert in training in motor imagery were responsible for leading the interventions for the experimental and the control groups, with the principle of avoiding any bias between the groups. During this study, the assessment of dimensions of competitive anxiety was collected four times. The first measurement was carried out 1 week before Ramadan, the second measurement during the first week of the month, the third measurement at the end of the second week and, finally, the fourth measurement during the fourth week of Ramadan. To avoid any disturbance, the measurement points

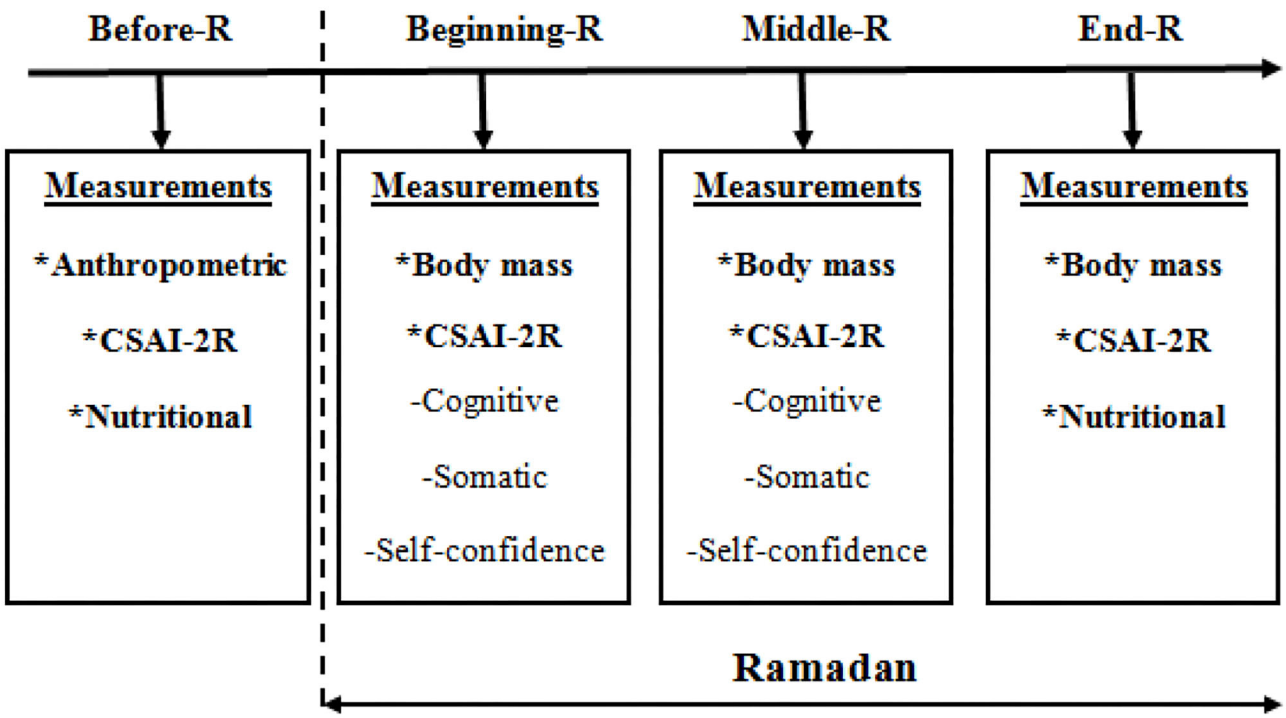

FIGURE 1 | Experimental protocol adopted in the present study. CSAI-2R, The Tunisian version of the Competitive State Anxiety Inventory (24).

TABLE 2 | Daily nutritional intake before and at the end of the fourth week of Ramadan.

Variables	Before Ramadan	End Ramadan
	Mean (SD)	Mean (SD)
Protein (%)	19.9 ± 2.6	20.2 ± 3.8
Lipids (%)	32.1 ± 5.81	30.6 ± 4.3
Carbohydrate (%)	50.4 ± 5.6	50.1 ± 3.6
Calorie intake (kcal/day)	3,147 ± 141.3	3,133 ± 182.2

SD, standard deviation.

were recorded within 30 min before the commencement of the standard training. The evaluation of the anthropometric data was carried out a week before Ramadan, as pictorially represented in **Figure 1**.

Measures

The food consumption record was taken by the participants over a period of three consecutive days using a food consumption log. Each participant was asked to note all the types of food ingested during three consecutive days. The average daily calorie intakes as well as the percentages of carbohydrates, fats, and proteins in the food were calculated (**Table 2**).

To study the symptoms of competitive anxiety a few minutes before athletic competition, we used the Tunisian version of the revised competition anxiety state inventory (24). The CSAI-2R has been validated for Tunisian athletes and has demonstrated excellent psychometric properties (24). This tool allows the measurement of three subscales of competitive anxiety (cognitive, somatic, and self-confidence) which comprises of 16 items on a Likert scale. Each subscale includes three dimensions measuring intensity [from 1 (not at all) to 4 (a lot)], direction [from −3 (very unfavorable) to +3, (very favorable)] and frequency [from 1 (not at all) to 7 (always)]. After obtaining the consent of the coaches and parents/legal guardians, the athletes were asked to answer the items of the Tunisian version of CSAI-2R, 1 h before the competition (25). This psychometric tool can be used multiple times during a competitive season because its reproducibility is well established (10). We did not opt to use the frequency dimension of the CSAI-2R because this dimension analyzes the extent of competitive anxiety at the time of its filling. In addition, also other investigations have not adopted the frequency dimension of CSAI-2R (1, 26). For this sample, the internal consistency in the CSAI-2R ranged from 0.73 to 0.91 on the intensity scale and from 0.84 to 0.89 on the direction scale as identified (assessed by Cronbach's alpha) for the cognitive anxiety, somatic anxiety, and self-confidence subscales.

Measurements of height, body mass, and body mass index (BMI) were made using a board and electronic scale (Tanita, Tokyo, Japan).

Statistical Analyses

Before proceeding to statistical analyzes, normality (Shapiro-Wilk test) and homogeneity of variance (Mauchly's sphericity test) were checked. For this sample, the internal consistency of intensity and direction dimensions was verified (assessed by

TABLE 3 | Averages (± SD) of body mass and BMI recorded before and during Ramadan.

Parameters	Groups	Before Ramadan	Beginning of Ramadan	Middle of Ramadan	End of Ramadan	Ramadan × Group interaction		
		Mean (±SD)				$F_{(3.108)}$	P	η_p^2
Body mass (kg)	CG	66.2 ± 9.3	65.9 ± 8.7	64.2 ± 7.9*	63.8 ± 9.1*	5.99	0.017	0.08
	EG	67.4 ± 5.9	67.2 ± 5.2	65.3 ± 4.9*	65.1 ± 5.3*			
BMI (kg/m^2)	CG	21.73 ± 0.87	21.58 ± 0.94	21.47 ± 0.93*	21.33 ± 0.94*	5.44	0.023	0.08
	EG	22.02 ± 0.58	21.91 ± 0.59	21.80 ± 0.57*	21.68 ± 0.57*			

SD, standard deviation; EG, experimental group; CG, control group; BMI, body mass index.
**Significantly different from Bef-R at P < 0.05.*

TABLE 4 | Variation in the dimensions of cognitive anxiety recorded before and during Ramadan.

Dimension	Groups	Before Ramadan	Beginning of Ramadan	Middle of Ramadan	End of Ramadan
				Mean (±SD)	
Intensity	CG	2.0 ± 0.2	2.7 ± 0.3**	3.4 ± 0.2**	3.5 ± 0.1**
	EG	2.2 ± 0.2	2.6 ± 0.2**	2.9 ± 0.2**##	2.8 ± 0.2**##
Direction	CG	−1.8 ± 0.5	−2 ± 0.5**	−2.3 ± 0.4**	−2.5 ± 0.3**
	EG	−1.6 ± 0.4	−1.8 ± 0.4*	−2.1 ± 0.3**	−2.4 ± 0.2**

SD, standard deviation; EG, experimental group; CG, control group.
**, **Significant difference from before Ramadan respectively at P < 0.05 and at P < 0.001.*
##Significant difference from GC, at P < 0.001.

Cronbach's alpha) for the cognitive anxiety, somatic anxiety, and self-confidence subscales. Then, the scores obtained from the responses to the different items of the competitive anxiety questionnaire were analyzed by means of the analysis of variance (ANOVA) on repeated measures [period (before Ramadan/start, middle, and end of Ramadan) × imagery (without/with)]. When the latter test showed a significant effect, the Wilcoxon test was utilized. If there was a significant effect, the Fisher's least significant difference (LSD) *post-hoc* test was used to compare the pairwise means. All observed differences were considered statistically significant for a probability threshold <0.05 ($P < 0.05$).

RESULTS

The descriptive data are reported in **Table 1**. No significant differences between the experimental and control groups before starting the intervention could be found, indicating the homogeneity of the two groups. **Table 2** shows the means (±SD) of the daily calorie intake and the percentages of carbohydrates, fats, and proteins contained in consumed foods, recorded before, during, and the end of Ramadan. No significant differences could be identified for daily nutritional intake during the two periods.

Table 3 presents the means (±SD) of body masses and BMI of the two groups (experimental and control) at different times of the experiment (before, at the beginning, at the middle and at the end of Ramadan). Significant differences were observed for body mass and BMI, in the middle and at the end of Ramadan compared with before Ramadan for both the groups.

Statistical analysis showed a significant interaction between Ramadan period (before/at the beginning/in the middle/at the end) × body mass [$F_{(3.108)} = 5.99$; $P = 0.017$]. A similar trend could be observed for BMI [$F_{(3.108)} = 5.44$; $P = 0.023$].

Regarding cognitive anxiety, the ANOVA results showed a significant interaction of the Ramadan period (before/at the beginning/in the middle/at the end) × groups for the dimensions of cognitive anxiety [intensity: $F_{(3.108)} = 25.23$, $P = 0.001$; direction: $F_{(3.108)} = 2.73$, $P = 0.048$] (**Table 4**). Comparison analyzes for the periods of Ramadan vs. before Ramadan revealed that the intensity and direction of cognitive anxiety during Ramadan were higher compared with before Ramadan for both the groups (all, $P < 0.001$). Regarding the differences between groups in the same time periods, the means of intensity dimension scores were higher for CG compared with EG in the middle and the end of Ramadan ($P < 0.001$).

Regarding somatic anxiety, the ANOVA results showed a significant interaction of the Ramadan period (before/at the beginning/in the middle/at the end) × groups for the dimensions of somatic anxiety (intensity: $F_{(3.108)} = 23.9$, $P = 0.001$; direction: $F_{(3.108)} = 5.01$, $P = 0.003$) (**Table 5**). Comparison analyzes for the periods of Ramadan vs. before Ramadan revealed that the intensity and direction of somatic anxiety during Ramadan were higher compared with before Ramadan for both groups respectively at $P < 0.001$ and $P < 0.003$. Regarding the differences between the groups in the same time periods, the means of intensity dimension scores were higher for CG compared with EG in the middle and the end of Ramadan ($P < 0.001$).

Regarding self-confidence, the ANOVA results showed a significant interaction of the Ramadan period (before/at the

TABLE 5 | Variation in the dimensions of somatic anxiety recorded before and during Ramadan.

Dimension	Groups	Bef-R	Beg-R	Mid-R	End-R
				Mean (±SD)	
Intensity	CG	1.6 ± 0.2	2.3 ± 0.2**	3.1 ± 0.3**	3.3 ± 0.3**
	EG	1.6 ± 0.3	2.2 ± 0.2**	2.6 ± 0.3**##	2.7 ± 0.3**##
Direction	CG	−0.9 ± 0.4	−1.3 ± 0.3**	−1.8 ± 0.3**	−2.3 ± 0.3**
	EG	−0.7 ± 0.3	−1.3 ± 0.3**	−1.9 ± 0.3**	−2.2 ± 0.3**

SD, standard deviation; EG, experimental group; CG, control group.
***Significant difference from before Ramadan at P < 0.001.*
##Significant difference from GC, at P < 0.001.

beginning/in the middle/at the end) × groups for the dimensions of self-confidence [Intensity: $F_{(3.108)} = 8.75$, $P = 0.001$; direction: $F_{(3.108)} = 2.69$, $P = 0.05$] (**Table 6**). Comparison analyzes for the periods of Ramadan vs. before Ramadan revealed that the intensity and direction of self-confidence during Ramadan were lower compared with before Ramadan for both groups respectively at ($P < 0.001$ and $P < 0.05$). Regarding the differences between groups in the same time periods, the means of intensity dimension scores were higher for EG compared with CG at the end Ramadan ($P < 0.001$).

TABLE 6 | Variation in the dimensions of self-confidence recorded before and during Ramadan.

Dimension	Groups	Bef-R	Beg-R	Mid-R	End-R
				Mean (±SD)	
Intensity	CG	2.6 ± 0.4	2.2 ± 0.3**	1.9 ± 0.3**	1.6 ± 0.2**
	EG	2.3 ± 0.2	2 ± 0.2**	2 ± 0.1**	1.9 ± 0.2**##
Direction	CG	1.9 ± 0.3	1.4 ± 0.2**	1.3 ± 0.2**	1.1 ± 0.2**
	EG	2.2 ± 0.3	1.6 ± 0.4**	1.4 ± 0.4**	1.1 ± 0.4**

SD, standard deviation; EG, experimental group; CG, control group.
***Significant difference from before Ramadan at P < 0.001.*
##Significant difference from GC, at P < 0.001.

DISCUSSION

The aim of this study was to analyze the effects of mental imagery training on the competitive anxiety of adolescent tennis players who fast during Ramadan. Two hypotheses have been formulated. First, fasting in the month of Ramadan increases the scores for the intensity and direction of cognitive and somatic anxiety and decreases the scores of intensity and direction of self-confidence. Second, training with mental imagery reduces the cognitive and somatic anxiety scores and increases the intensity score and the self-confidence direction.

Effects of Fasting During Ramadan on Variation in Competitive Anxiety State

The results of this research indicated an observable increase in the dimensions of intensity and direction of cognitive and somatic anxiety for the CG during the fasting of Ramadan. Moreover, a decrease in the dimensions of intensity and direction of self-confidence indicates a reasonable probability that this conclusion may be true for tennis players who fast during Ramadan and who have similar characteristics reported in the present study. The state of anxiety of athletes and the way they deal with this psychological state warrant research with regard to a few particularities, for example, the state of competitive anxiety for athletes who fast during the month of Ramadan, which is relatively overlooked in the currently available scholarly literature. Indeed, a few studies have tried to subjectively assess the general psychological state during Ramadan. Most of these studies have shown that the scores obtained are higher during Ramadan compared with the control period. Moreover, Chtourou (5) found that fatigue, assessed by the POMS, questionnaire was highest in the afternoon in 17-year-old footballers fasting during Ramadan. A study by Leiper (6) found that the ability to concentrate decreased in fasting footballers. Zerguini (7) also reported an increase in the incidence of headaches and a decrease in the mood and motivation during Ramadan among footballers. On the other hand, according to the literature, no study has been done on competitive anxiety state for athletes who fast during Ramadan. Regarding the state of competitive anxiety measured outside the period of Ramadan, we can see that a tennis player is involved in an intermittent activity, consisting of great intensity efforts interspersed with active or passive recovery periods, which can be very hard on the physical, psychological, and emotional level (8, 9). For this, a certain number of mental qualities are essential for the tennis player to be successful, such as emotional control, confidence, perseverance, and concentration, among others. According to Di Rienzo and Fernandes (26, 27), high cognitive anxiety on the eve of a competition leads to increased muscle tension which, in turn, can lead to increased muscle stress, resulting in decreased performance and anaerobic resistance. Moreover, recently Fekih et al. (28) have shown a decrease in tennis service performance outcomes in terms of accuracy and stroke speed for tennis players fasting during Ramadan.

An increase in cognitive and somatic anxiety scores (in terms of intensity and direction), and also a decrease in self-confidence due to competitive anxiety during Ramadan fasting may be associated with the change in lifestyles and daily habits during this particular period. More specifically, one of these effects may be associated with the change in the sleep–wake cycle. In this context, the necessary duration of sleep of our players has been reduced compared with the shift in the times of food intake during Ramadan. Waterhouse (29) demonstrated that Muslims during Ramadan continue to eat and drink until late at night, which is likely to prevent them falling asleep. Then, they wake up for the last meal before dawn. These disturbances could reduce the duration of nighttime sleep. The fatigue, induced by this partial sleep deprivation, could explain the increase in cognitive and somatic anxiety scores in the dimensions of intensity and direction during Ramadan. On the other hand, dehydration may also be one of the factors responsible for the increase in cognitive and somatic anxiety scores during Ramadan. Indeed, the fasting of Ramadan is associated with a reduction of fluid, especially if carried out in a hot environment, which can strengthen the onset of dehydration. Based on our results, we found a decrease in body mass at the end of Ramadan compared with before Ramadan. This reduction could be related to a loss of body water, which is in line with the study by Sweileh (30). The negative effects of hypo-hydration on athletic performance outcomes are well documented in the literature (31). The increase seen in intensity and direction for cognitive and somatic anxiety during Ramadan is probably not related to changes in calorie intake, as we have not observed any difference in calorie intake and the percentages of lipids, carbohydrates, and proteins contained in food consumed during Ramadan compared with before Ramadan. This is in agreement with previously published studies (28, 32), which failed to observe any difference in the level of nutritional intakes during Ramadan compared with before Ramadan.

Finally, the increase in competitive anxiety during Ramadan can be attributed to a decrease in the arousal and motivation of participants (29, 33). Additionally, lower mood has been suggested as a factor responsible for lower performance outcomes during Ramadan, as a result of lowered self-confidence (34, 35).

Effects of Mental Imagery Training on Changes in Competitive Anxiety State During Ramadan Fasting

The results of this research indicated a slight stabilization in intensity for the three subscales of competitive anxiety for the EG in the middle and at the end of Ramadan, which was not observed in the CG. In this sense, it seems that 30 min of training with the technique of mental imagery per week (three sessions of 10 min separated by 48 h during the month of Ramadan) can modify the dimension of intensity for the three cognitive anxiety subscales. According to Fernandes (1), cognitive anxiety is responsible for an impairment in decision-making related processes and concentration. Thus, given that tennis matches require a good stimulation strategy (8), which in turn requires maximum concentration, the high magnitude of cognitive anxiety could, maybe, generate early fatigue in tennis players due to poor preparation of the stimulation. Training with mental imagery, may, in a way, be effective in reducing the effect of fasting and stabilizing the intensity dimension for the three subscales of cognitive anxiety in fasting tennis players during Ramadan. This finding is in agreement with the results found by another work (36).

Regarding the somatic anxiety dimension, the results of this study showed a slight stabilization in the intensity dimension in the middle and at the end of Ramadan for the EG. This stabilization was not observed in the CG. In addition, a significant difference was observed between the two groups in favor of the EG in the middle and at the end of Ramadan. Based on a literature review, somatic anxiety can increase cardiovascular and neuromuscular stress (12). Thus, the increase in organic stress just before competition is linked to a decrease in cognitive and muscular performance outcomes (14). As such, somatic anxiety can lead to a decrease in the performance of tennis players during Ramadan. Thus, indirectly, training with mental imagery during the Ramadan fast can be adopted as a strategy of stabilizing the intensity dimension for somatic anxiety, thereby stabilizing the performance outcomes of adolescent tennis players also. According to Patel (14), the increase in somatic anxiety is linked to increased psychophysiological fatigue. For this, stabilization of or reduction in somatic anxiety resulting from mental imagery training appears to be essential to inhibit/counteract the early fatigue of tennis players, which can result in improved performance during competitions. Regarding self-confidence, results also showed stabilization in intensity for the EG in the middle and the end of Ramadan. This stabilization was not observed for the CG. The comparison between CG and EG by means of *post-hoc* tests suggests that the training with mental imagery may delimit the effects of the fasting by stabilizing the intensity dimension of the subscale of self-confidence in our tennis players. From this perspective, studies have shown that increased self-confidence can have a positive effect on athlete performance outcomes (26). Therefore, considering our results, training with mental imagery combined with physical and technical training during Ramadan may be an effective strategy to stabilize the dimension of intensity for the subscale of the self-confidence and, therefore, stabilize tennis performance.

Thus, coaches need to know their athletes to identify the magnitude required to increase self-confidence to maximize performance during training or competition in the month of Ramadan. It should be noted that confident tennis athletes often performed well in sports compared with athletes with low levels of self-confidence (1). In addition, the results of some studies have shown a positive relationship between self-confidence and athletic performance (12, 14). Thus, constructing a mental situation in the first person, imagining the task at a speed close to reality and positive situations during a competition and generating emotions (anxiety and mood) similar to the competition seem to be an essential cognitive strategy for mitigating the effects of fasting during Ramadan, and therefore stabilizing the intensity of competitive self-confidence in tennis players. This can lead to stability or increased performance during tennis training or in competitions (28, 36).

Strengths of the Study

The present study has some strengths, including its novelty, its methodological rigor (in terms of study design and implementation, and statistical analyses conducted), and the wealth of indicators and data collected at various time-points.

Limitations of the Study

Although revealing interesting results, this study has some limitations that should be mentioned. Brain and electromyographic signals were not assessed during mental training sessions for both groups (EG and CG). The use of the questionnaire is also a limitation. According to Fortes (37), the use of Likert scale-based questionnaires in surveys with repeated measures can generate a learning effect. Also, the Hawthorne effect, which is a type of effect linked with behavioral reactivity when individuals are aware of being observed, cannot be ruled out. Further, biopsychological indicators such as cortisol and testosterone were not evaluated during the tests. Finally, the results of this study must be interpreted with caution.

CONCLUSIONS

This study represents a first attempt to examine the effects of mental imagery training on changes in the dimensions of competitive anxiety during fasting during Ramadan. Results showed that fasting during Ramadan increases both dimensions (intensity and direction) of cognitive and somatic anxiety and decreases both dimensions (intensity and direction) of self-confidence. A training program with mental imagery after regular training sessions can only stabilize the intensity dimension for the three subscales of competitive anxiety (cognitive, somatic, and self-confidence).

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 author.

ETHICS STATEMENT

The study protocol was reviewed in depth and fully approved by the Ethical Committee for the Protection of Southern People (C.P.P.SUD), Sfax, Tunisia: protocol reference C.P.P.SUD No. 0032/2017. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SF, MZ, NB, and MJ conceived the study and wrote the manuscript. AK, AB, and JH critically revised the manuscript. All authors contributed to the article and approved the submitted version.

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Walking Football During Ramadan Fasting for Cardiometabolic and Psychological Health Benefits to the Physically Challenged and Aged Populations

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Concurrent exercise and intermittent fasting regimens for long periods have been shown to enhance cardiometabolic health in healthy individuals. As exercise and fasting confer health benefits independently, we propose that Muslims who are fasting, especially those experiencing health and clinical challenges, continually engage in physical activity during the Ramadan month. In this opinion piece, we recommend walking football (WF) as the exercise of choice among Muslims who are fasting. WF can be played by any individual regardless of the level of fitness, skills, and age. WF has been shown to elicit cardiovascular and metabolic stress responses, which are suitable for populations with low fitness levels. Most importantly, WF has the inherent characteristics of being a fun team activity requiring social interactions among participants and, hence, likely to encourage long-term consistent and sustainable participation.

Keywords: intermittent fasting, walking football, well-being, social activity, health benefits

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INTRODUCTION

The search for a dietary intervention with the most optimal health benefits is ongoing. One such intervention that is gaining popularity is intermittent fasting. Intermittent fasting is when energy consumption in the form of food or drinks is interrupted and markedly reduced for a period of time (1). This is commonly practiced in various regimens such as alternate day fasting, 5:2 diet, and time-restricted feeding. Alternate day fasting involves a fasting day alternating with ad libitum "feast day." On the other hand, the 5:2 diet includes 5 ad libitum feast days followed by 2 days of fasting. Meanwhile, time-restricted feeding uses the concept of limiting the number of hours for eating each day to between 4 and 8 h usually in the daytime while fasting for the remaining hours.

Fasting and Health

Intermittent fasting alone may lead to cardiovascular and metabolic benefits (2). A meta-analysis found that intermittent fasting reduces body weight by 1.1 to 6.5 kg and improves lipid profile (3). Four- and 6-h time-restricted feedings can result in similar weight loss by 3.2% and reduce insulin resistance by 12–29% in obese individuals (4). Moreover, time-restricted feeding for 5 weeks has been found to reduce systolic and diastolic blood pressure by 11 ± 4 mmHg and 10 ± 4 mmHg, respectively, in men with impaired glucose tolerance (5).

A recent systemic review and meta-analysis also found that Ramadan fasting (RF) improved cardiometabolic risk factors that may confer short-term protection from cardiovascular disease (6). In another systemic review, all fasting regimens revealed strong evidence to support intermittent fasting as a feasible diet to improve glycemia and body composition measures in obese people with type 2 diabetes mellitus (T2DM) within 12–24 weeks (6) and redistribution of abdominal fat, although a follow-up of 12–18 months after intermittent fasting did not show promising results for continued weight loss and improved glycemic control (6). Intermittent fasting has beneficial effects on lipid profile, and it is associated with weight loss and modification of the distribution of abdominal fat in people with obesity and T2DM as well as improvement in the control of glycemic levels (7).

In a systemic review by Horne et al., there were 3 randomized controlled clinical trials of fasting in humans with results published in 5 articles; all of which evaluated the effects of fasting on surrogate outcomes with improvements in weight and other risk-related outcomes. Improvements in weight and other risk-related outcomes were found in the 3 trials. Two observational clinical outcomes studies on humans were found in which fasting was associated with a lower prevalence of CAD or diabetes diagnosis. No randomized controlled trials of fasting for clinical outcomes were identified (8).

In short, chronic intermittent fasting alone can accrue health and metabolic benefits.

LIFESTYLE CHANGES IN RAMADAN FASTING

During the annual month of Ramadan, Muslims practice consecutive days of intermittent fasting. This religious practice is frequently accompanied by lifestyle changes and includes altered dietary habits and meal timings and differing sleep and rest patterns, with greater emphasis on religious routines such as increased night time prayers (9). In addition, there are regional differences in RF duration due to differences in daylight and night time hours. Given these drastic changes in circadian rhythm, exercising during Ramadan may be even more challenging for Muslims while fasting, especially for those with prior poor physical fitness and pre-existing health issues, and the frail elderly. It is, thus, not surprising that the evidence points toward greatly reduced physical activity and poorer physical fitness level (10) during the RF month.

Exercise During Fasting

Exercise and diet, either low energy intake or fasting, have positive effects on health through different pathways and mechanisms (11). There is evidence of increased fat metabolism when exercise is performed in the fasting state rather than post feeding, hence supporting the health benefits for exercise performed when fasting (12). To promote and encourage physical activeness during the RF month, we thus propose in this narrative review that low- to moderate-intensity exercise be performed in the form of WF during the daytime near breaking of fast hours in regions with long daytime fasting such as the equatorial region and summer periods. We reasoned that the combination of RF and WF will potentially augment the health benefits of fasting individuals.

Why specifically WF and not some other types of exercise?

WALKING FOOTBALL (WF)

Walking football is a variant of football first introduced in England in 1932 for players 65 years and older but recently re-emerged in England aimed at providing more exercise and social networking opportunities for older adults, with rapid recognition globally, especially in Europe, Australia, North America, and Asia (13). In WF, players cannot run and are only allowed to walk throughout the match, defined as one foot in contact with the ground at all times (14). There should not be any physical contact between players and tackling is not allowed during WF to avoid injuries; the ball cannot be above head height. It is a skilled activity that could be performed by more physically challenged individuals either due to older age or restricted mobility, or those with medical conditions restricting physical activities, irrespective of gender. WF is usually played on an indoor court or outdoor field space which have dimensions equivalent to a basketball court but smaller than the soccer pitch. The intensity of WF tends to vary widely from low to high, depending on the duration of each match session and age of the participants, with increased intensity for longer matches and older age groups.

Effects of WF

To our knowledge, there have been only five studies published on the physiological and psychological outcomes measured from WF sessions, as shown in **Table 1** (15–19).

In **Table 1**, WF matches were mostly played 5-a-side, suggesting the potential association of the number of players with exercise intensity. In this aspect, a smaller number of players could imply greater distance covered and, consequently, a higher level of muscle activation, since individuals are expected to cover a wider ground and be engaged with the play to a greater extent. In addition, a WF match also involves a high volume of turning and twisting and many spurts of immediate change of direction actions (i.e., very short accelerations and decelerations). Indeed, these movements could have positive effects on bone health, which is clearly beneficial to older aged participants (15).

Cardiovascular Health Benefits of WF

Harper et al. found that participants developed a mean percentage of maximum heart rate of 76 $\pm$ 6% during sessions, with a rating of perceived exertion across all sessions at 13 $\pm$ 2. Blood lactate significantly increased by $\sim$157% from pre-session to post-session. There were $\sim$100 changes of direction per session. Hence, WF is a moderate- to vigorous-intensity activity (15).

Ayabe et al. found that the average heart rate was 127 $\pm$ 20 beats per min (82 $\pm$ 14% of the age-predicted maximum HR) in a 10-min game of WF, with a significant association to the number of plays after adjusting for age. The estimated metabolic cost was 8 $\pm$ 1.6 metabolic equivalents with a significant correlation to the maximal oxygen uptake, number of plays with a ball, and

TABLE 1 | Acute responses and chronic adaptations of studies on walking football.

Study	Subjects' characteristics	Duration of session/ match	Key variables measured	Results of acute responses	Results of chronic adaptations	Remarks
Harper et al. (15)	*N* = 17; F 66 ± 6 y	60 min per session of 5- or 7-a-side matches	Mean match peak HR = 95 ± 8% HR_{max} Mean match HR = 76 ± 6% HR_{max} Mean post-match blood lactate = 3.2 ± 1.7 mmol Mean Players' Load = 353 ± 67 au Mean nos. of Change of direction = 95 ± 11 Mean nos. of Accelerations = 13 ± 3 Mean nos. of Decelerations = 30 ± 4 Mean RPE = 13 ± 2 (somewhat hard)	WF elicit a moderate-to-high intensity stimulus; with significant involvement of anaerobic glycolytic contribution (based on blood lactate). Biomechanically, WF exercise session is equivalent to 25 min of normal running football.	Not applicable	Data was collected over 25 sessions. Participants were experienced in playing WF. HR_{max} was estimated from formula (208–0.7 × age).
Ayabe et al. (16)	*N* = 20; M+F 65 ± 5 y 56 ± 9 kg Some with mild metabolic disorders	2 × 5 min of 5-a-side matches	Mean match peak HR = 92 ± 13% HR_{max} Mean match HR = 82 ± 15% HR_{max} Estimated METs = 8.0 ± 1.6 Step rate per min = 85 ± 18 Nos. of plays with ball = 12 ± 4	WF was deemed of vigorous intensity.	Not applicable	HR_{max} was estimated from formula 220—age. METs was estimated from exercise HR rather than measured directly.
Heil et al. (17)	*N* = 22; F 40 ± 10 y 75 ± 17 kg	2 × 15 min per match with 5–10 min break between half	Mean match HR = 77–80% HR_{max} Mean distance covered during match = 1,650–3,500 m Duration where exercise was > 3 METs = 10–20 min	Exercising HR during WF exceed the physical activity intensity threshold for minimizing non-communicable diseases risks.	Not applicable	Environmental conditions: 26–30°C & 85–90% RH. HR_{max} was estimated from formula 220—age.
Reddy et al. (18)	*N* = 11 in WF group and *N* = 9 in CON group; M+F of betw 50–60 y old	1 × 45–60 min session per wk for 12 wk Each session 5-a-side game	Mean match HR = 76 ± 7% HR_{max} Mean distance covered per match = 2,386 ± 309 m Mean RPE = 13 (somewhat hard)	WF was deemed of moderate intensity.	High levels of enjoyment & individuals were keen to participate in WF regularly. Blood pressure showed enhanced improvement in WF vs control group. No differences in cognitive executive functions between groups.	Control group maintained their normal routine.
Arnold et al. (19)	*N* = 10 M 66 ± 7 y 89 ± 9 kg Possessed some comorbidities	1 × 2 h session per wk for 12 wk Each session consisted of several 15–20 min of 5-a-side game	No physiological data was reported	Not applicable	Body fat ↓ 9%. Body fat mass ↓11%. Systolic blood pressure ↑ 4%. Exercise to exhaustion time ↑ 11 % (but with no change in VO_{2max}).	No control group was included.

M, males; F, females; wk, week; METs, metabolic equivalent; WF, walking football; RPE, ratings of perceived exertion; HR, heart rate; HR_{max}, maximum heart rate; CON, control; au, arbitrary unit; VO_{2max}, maximal oxygen uptake.

stepping rate. Hence, cardiorespiratory responses could be above the desirable levels of exercise prescribed in middle to old-aged adults with mild metabolic disorders (16). However, the authors found that their study was limited by the lack of familiarity of participants to WF, which could account for physiological stress and change with experience.

Heil et al. found that mean relative HRs exceeded the 65% threshold for improving cardiovascular fitness for both teams competing in a match. Both teams also maintained an average metabolic intensity that was statistically similar to the 3.0 MET threshold that decreases one risk for non-communicable diseases and walked an average of 2.2–2.4 km/match. Hence, this is supportive evidence for competitive WF being of sufficient intensity to promote positive changes in both cardiovascular and metabolic fitness in Southeast Asian women (17).

Arnold et al. studied a population with medical conditions such as hypertension, T2DM, knee osteoarthritis, spinal stenosis, atrial fibrillation, bronchitis, and other medical comorbidities with results that prove to be beneficial, including a significant reduction of 11% in body fat mass and in percentage body fat of 9%, and improvement in other anthropometric measures such as 2% reduction in whole body mass, 4% increase in lean body mass, and 3% reduction in body mass index (BMI). However, there was a significant increase in systolic blood pressure by 4% after the 12-week WF program, attributed to lack of medication adherence (19).

In contrast, Reddy et al. found significant greater improvement in blood pressure for players when compared to the control group (18).

In summary, playing WF has been shown to elicit sufficient exercise intensity and duration that would promote cardiovascular fitness, muscular health, and possibly bone health among regular participants of the game.

There are only two studies examining the training-induced adaptations as a result of chronic WF exercise (**Table 1**). The study of Arnold et al. showed that 12 weeks of WF (2 h per session once a week) among the elderly has had a positive impact on lowering body fat percentage and increasing exercise tolerance (19). However, the study was limited because no physiological data during the WF sessions were reported, and no control group was included in the study (18). The study of Reddy et al. on playing WF once a week between 45 and 60 min, on the other hand, did not show any clinical or health improvements in WF participants relative to controls, although a small positive impact on blood pressure was noted in the WF group (16). Nonetheless, the limited prevailing data on acute responses and chronic training-induced adaptations to WF activity seem to support the potential cardiovascular and clinical health benefits of WF when performed consistently or regularly across a prolonged period of time.

Psychological Benefits of WF

It should be noted that many older adults had reported a dislike for structured exercise programs (20). During WF, the movements are unstructured, unplanned, and varied. Thus, an interesting and important finding in all the five reviewed studies was that all the participants of WF have reported elevated levels of enjoyment and keen participation in this exercise form (20). Indeed, in a recent review on WF, the authors found that almost all participants in this activity believed that WF provides beneficial mental and physical effects (13). The review also reported that the factors valued the most by the participants were the collaborations among team-mates and team identity during game. The participants of the review also found that compared to visiting the gym or engaging in other regimental physical exercise programs, WF is the preferred form of physical activity. It was concluded that WF has a major positive impact on the overall sense of well-being and social connections of participants (15).

Reddy et al. surveyed a group of elderly who played WF for 12 weeks and found a very positive impact from participation with individuals experiencing high levels of excitement and enjoyment when playing (17). They also highlighted that the ability to meet and make new friends contributed to the self-reported overall improvement in their physical health and well-being (17).

In another study, McEwan et al. similarly showed high adherence to participation in a 12-month WF exercise program among middle-aged obese men (21). When evaluated for a personal perspective of the program, social interaction, group interaction to improve health, and new lease of life were the 3 main themes for continued involvement (21). The opportunity to engage in football and the link to a professional football club 21 were the top 2 factors to continued involvement. Those who participated were overweight, sedentary, exhibited blood pressures outside normal ranges, and all but two were hypertensive. Adherence to the program was 90% over 8 weeks, and of those contacted after a year, all had maintained engagement in WF. Hence, WF is a feasible and cost-effective method of recruiting and retaining men aged at least 50 years old to a physical activity program, although attrition is to be expected.

DISCUSSION

In view of the commonly reported reduced physical activity during Ramadan, we strongly believe that WF would be the ideal choice of physical activity for populations with varying levels of physical fitness, hence, encouraging fasting Muslims to be active and remain physically active thereafter.

No studies have yet been conducted that have specifically examined the chronic effects of concurrent training on WF and RF. However, studies that have examined the impact of chronic exercise training in the fasted state (but non-Ramadan specific fasting) have shown positive outcomes.

Continuous endurance exercise training (3 days per week, progressively from 25 to 40 min at 60–75% HR_{max}) for 12 weeks showed a significantly greater reduction in body weight and favorable lipid profile when the endurance training was performed in conjunction with an alternate day fasting regimen relative to performing either alternate day fasting or exercise on their own among obese subjects (22). This study by Bhutani et al. (22) was among the first to show experimental evidence that the combination of alternate day fasting and exercise produces superior changes in clinical and health markers when compared to either modality alone.

Likewise, Edinburgh et al. (23) showed that in obese participants, 6 weeks of chronic exercise (3 sessions per week cycling at 50–55% peak power output) improved insulin sensitivity and increased skeletal muscle glucose transporter type 4 levels by two times more when performed in the fasted state (overnight fasting) relative to being performed in the fed state.

With regard to cardiovascular exercise performance, Stannard and colleagues showed a significantly greater magnitude of improvement of 9.7% in VO_{2max} among healthy participants who underwent 4 weeks of endurance exercise (5 $d \cdot wk^{-1}$, 25–100 min incrementally at 65% VO_{2max}) in the overnight fasted state group relative to the 2.5% improvement in VO_{2max} in the fed group (24). Indeed, in their review, Knuiman and colleagues suggested that exercise training in the fasted state can lead to a much greater metabolic stimulus to the working muscles which could amplify training-induced adaptations relative to the same exercise training performed in a well-fed state (25).

While WF does elicit many cardiovascular and metabolic health benefits to participants, we reiterate that the main advantage of WF relative to other forms of exercise and physical activity programs is its inherent social attractiveness. WF is played in small groups of individuals requiring close teamwork to be successful. The integral socially friendly format of this exercise mode fits nicely and naturally appeals to the physically and metabolically challenged older age group. Indeed, it has been argued critically that WF is likely to be a sustainable form of exercise for older adults (18).

Ramadan Fasting and WF

To our knowledge, there have not been studies on WF during RF. Future studies looking into health outcomes of WF in RF and the non-fasted state as well as studies comparing WF with other low-intensity exercises during RF in an age-adjusted and gender-adjusted manner would be useful to promote exercise in a more physically active Ramadan to reap the maximal health benefits from RF.

CONCLUSION

Fasting and exercise can independently provide health benefits. Hence, we hypothesize that the combined effects of RF and WF training will likely show relatively greater cardiovascular and metabolic health benefits than either RF or WF alone. While more studies directly examining the effects of WF on health in the Ramadan fasted state is needed to provide evidence to support our proposed hypothesis, we propose to encourage fasting Muslims to engage in physical activity, specifically in WF, for cardiometabolic and psychological benefits, in keeping with the spirit of discipline and social interactions encouraged in the Ramadan month.

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 author/s.

AUTHOR CONTRIBUTIONS

SZ, DS, and AA: contributed to conception and design. SZ: wrote the first draft of the manuscript. SZ and AA: wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Ramadan Fasting and NCDs-Example of the Diabetes

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Although Ramadan lasts only for 1 month each year, it can be accompanied by significant changes in: both energy and nutritional intake; in the diet composition; in the working hours; and the usual way of life. The majority of practitioners consume two meals, one after sunset (*Iftar*) and one before dawn (*Sohor*). During this month, it is also an opportunity to share a meal with family and friends, a period of highly intensified socialization. In parallel with the nutritional changes brought about by this unique pattern of fasting in Ramadan, other metabolic and physiological changes may occur, such as fluctuations in body weight and/or disturbance in the quantity and quality of the sleep-wake circadian rhythm. In the verses of the Qur'an, the exemption from fasting in certain situations such as illness is clearly stated. Despite this religious tolerance, many faithful who are eligible for the exemption observe the fast of Ramadan either for the spiritual aspect it provides by performing it, by religious guilt or to mark a normalization in the Muslim community for fear of the gaze of others. The world is experiencing an increase in the emergence of non-communicable diseases (NCDs); leading cause of the global mortality. Environmental and behavioral risk factors related to lifestyle, such as smoking, excessive alcohol consumption, unhealthy diet, and sedentarity have a causal association with NCDs. Other factors, such as genetic and physiological factors may also be associated (overweight, high blood pressure, dyslipidemia). Diabetes is one of the highest prevalent NCDs in the world and it continues increasing year by year. This chronic disease can lead to significant potential complications (degenerative, dermatological, and acute) to the patient's health. This requires an individual and appropriate care, both dietetic and therapeutic and over the long term will at best make it possible to sensitize the diabetic patient to the adverse effects related to his disease and thus improve its quality of life. Performing the Fast of Ramadan for a diabetic is a common situation. Diabetes is the only chronic disease widely studied in relation to Ramadan fasting. In the literature, many studies have investigated the effects of Ramadan intermittent fasting on diabetic patients. This article aims to provide a general overview and highlight if there are many effect of Ramadan fasting on diabetes, as an example of a NCDs.

Keywords: Ramadan fasting, NCDs, diabetes, lifestyle habits, nutritional education, Muslim patient

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INTRODUCTION

Ramadan is one of the five pillars of Islam. It constitutes the month of fasting, a holy month for Muslims who have the duty to fast from sunrise to sunset. Ramadan lasts between 29 and 30 days depending on the lunar cycle, shifts from year to year and gradually changes from one season to another. This religious rite concerns all healthy Muslims in good health, and only those who are at risk of harm from fasting are exempted from this obligation such as people with non-communicable diseases (NCDs) or other disabling diseases. In the Muslim community, there is an intense desire to participate in fasting, even among those who are eligible for the religious exemption (1). Despite this, many diabetics fast despite the risk of complications and decompensation (2). Indeed, in the literature, several studies have been interested in fasting Ramadan related to diabetes as NCDs, and various works have been published on this subject around the world: in Lebanon (3), in Morocco (4), in France (5), in Tunisia (6), in Algeria (7, 8). The interest of this theme also lies in the fact that there are many millions of Muslims in the world and the proportion of people with diabetes who are diagnosed or not. Therefore, we have chosen in this literature review to provide a thematic overview of the problem ≪ Ramadan and diabetes ≫ by exposing research and recent advances in relation to behavioral and lifestyle changes (diet, sleep, physical activity), body composition (anthropometry), metabolic changes, the link with Covid-19 pandemic and the role of nutritional education, as well as the recommendations issued on this subject.

RESEARCH METHODS

The PubMed and Google Scholar databases were searched. We also used a national online documentation system of the algerian university (SNDL.cerist). The key words used were "Ramadan fasting," "fasting" and we have associated with each of these word "diabetes," "NCDs," "circadian rhythm," "complications," "dietary habits," "physical activity," "body composition," "nutritionnal education." We mainly took the recent and most relevant studies focused on human studies. The **Figure 1** summarized the studies and works included and rejected according to the literature search carried out from these three databases.

EATING HABITS DURING RAMADAN

Ramadan comes without transition, and practitioners move from one way of life to another overnight. The whole rhythm of everyday life is upset, thus reflecting a reversal of activities characterizing day and night. Normally, people eat during the day, and most socializing activities take place during the day. During Ramadan, the schedule of meals and activities is changed. These changes vary according to the seasons, geographical and socio-economic situations as well specific traditions of each country. In these conditions, the fasting body tries to adapt twice in the space of a month: at the beginning and end of Ramadan (9). The management of the diabetic patient during this month is of particular importance because of the crucial changes in lifestyle and diet both qualitatively and quantitatively. The change in eating habits has important implications for the physiological process, such as the impact on blood glucose control. People with diabetes must maintain a healthy and balanced diet during Ramadan. However, dietary intakes are exclusively nocturnal and are most often characterized by an overconsumption of carbohydrate products and fat preparations. These dietary changes have a direct impact on the health of both fasting (DMF) and non-fasting diabetics (DMNF) (10–12). A multicenter study was carried out in 13 cities in Algeria with 2,819 type 1 (T1DM) and type 2 (T2DM) diabetics showed that DMNF approached the topic of diet with their physician more than DMF (50.6 vs. 25.9%; $P = 0.0000$). Probably due to their diet during this month because, in fact, 94.6% of DMNF declared an increased consumption of carbohydrates foods, fat meals (91.6%), higher rations (90.5%) in the evening and heavy meals than usual (81.1%) (13). Throughout the month of Ramadan, there is an abundance and a variety of preparations on tables and can be sold in the market level, all rich in saturated fatty acids and carbohydrates (honey cakes, pastries, traditional dishes, etc.) that are difficult to resist; a self-control for both DMF and DMNF. Moreover, in another Algerian study carried out in 276 obese women with T2DM, showed that the fast-breaking meal (Iftar) alone provided 74% of the energy intake during the month of Ramadan, which is probably not without disadvantages (glycemic variations) in diabetic patients (7). A priori in the literature research results have shown either an increase in total daily energy intake associated with an increase in the intake of carbohydrates [Tunisian prospective study, (14)], or a decrease in these intakes with an increase in the intake of carbohydrates and saturated fatty acids [Moroccan study, (15)], or no significant change in energy intake [Ajman-UEA, (16)]. The heterogeneity of the results of the various studies can be attributed to the methods of estimating dietary intake, the survey season, socio-economic status, and other lifestyle variations of the different populations studied.

CHANGE IN THE NYCTHEMERAL CYCLE

Food deprivation, in general, is deemed to have an influence on a number of physiological functions (17). According to Khalfallah et al. (18), chronobiological inversion of nutritional habit is responsible of several functional disorders. Ramadan is a chronobiological desynchronization model which is characterized by a reversal of the foodboorne and waterboorne rhythm. The fasting of Ramadan has the particularity of constantly changing the synchronizers of the central clocks for a month. On healthy volunteers, a comparative study was carried out under identical observation conditions (Winter Ramadan and summer Ramadan). Researchers found that sleep debt could accumulate without compensation through daytime naps and that Ramadan alters the circadian organization of the sleep-wake rhythm, and this, most importantly, in summer when the photoperiod is the longest (19). Sleep can appear simply as a state of rest. It, nevertheless, constitutes a complex physiological state, necessary for the survival of the organism, characterized by different phases of deep sleep (20). The rhythm of life imposed by

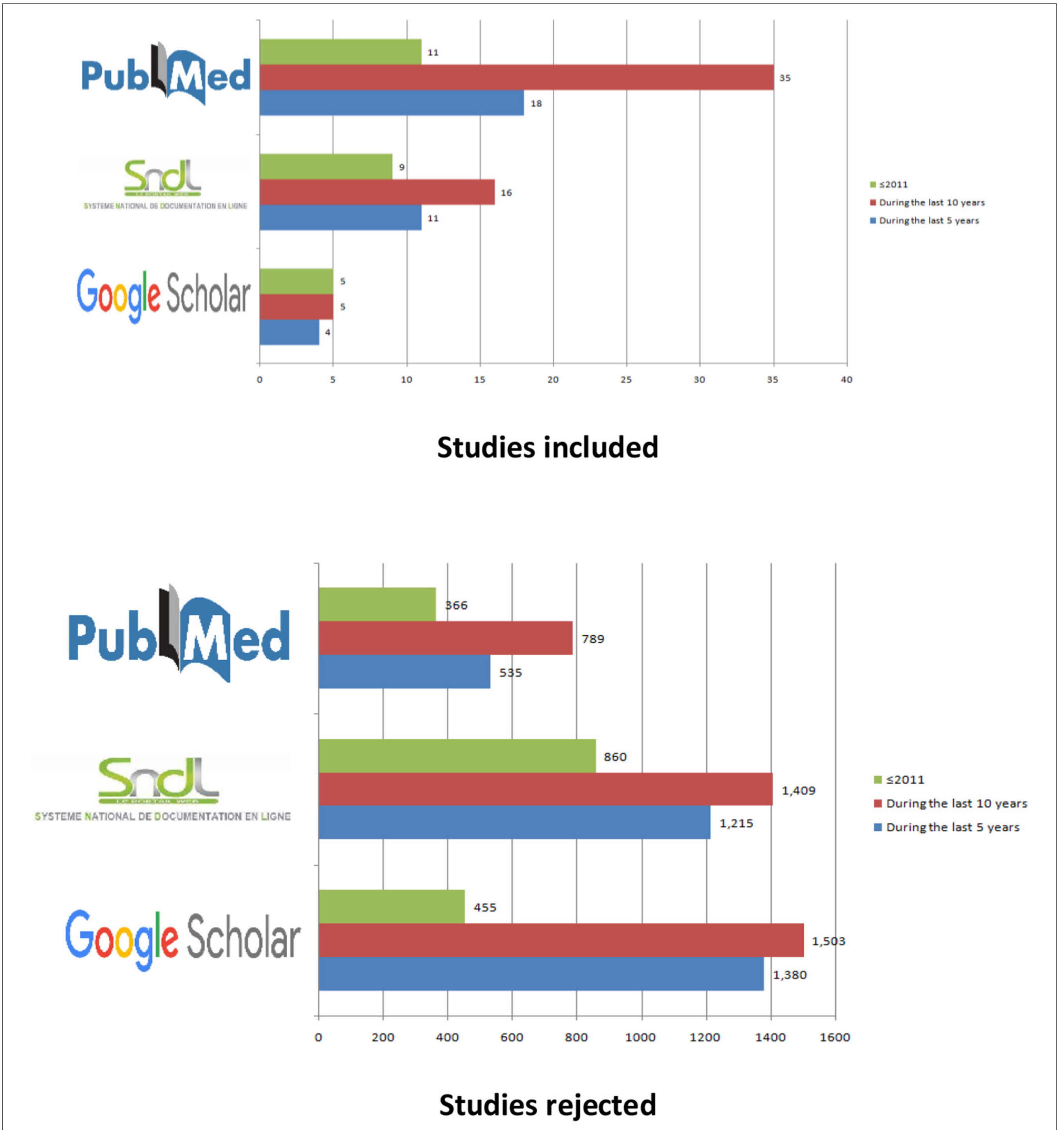

FIGURE 1 | Studies included and rejected according to the literature search.

the month of Ramadan systematically leads to a decrease in the duration in sleep time. In a study of 2,708 diabetics (21), a sleep-wake cycle disturbance was observed, where sleep decreased during the month of Ramadan, while napping increased ($p < 0.05$). The results of another study carried out on 1,266 diabetic patients showed an average sleep duration between 5 to 8 h in 70.3% of T2DM and 65.5% of T1DM. The same results both for the DMF and DMNF (22). In an epidemiological study related to the sleep-wake rhythm during Ramadan, researchers found a significant increase in sleep latency, intra sleep warkining and daytime naps. These disturbances appeared from the first week of fasting, persisted throughout the month, and disappeared after the post-Ramadan period. This sleep-wake rhythm disturbances also persisted on weekends (23).

PHYSICAL ACTIVITY PRACTICE

Physical activity can help people with diabetes achieve a variety of goals, like improving their cardiorespiratory health; increasing their physical endurance; better controlling their blood glucose levels; reducing their insulin resistance; improving their lipid profile; lowering their blood pressure; and maintaining weight loss (24). Sedentary lifestyle in an individual can increase the risk of early mortality (25). In patients with T2DM, regular physical activity reduces the risk of all-cause mortality (between −30 and −40%), as well as cardiovascular mortality (−25 to −40%) (26). Indeed, the study of Kooiman et al. (27) in people with T2DM, showed an increase in the physical activity that was correlated with decreased levels of glycated haemoglobin (HbA1c) and blood glucose and decreased cardiovascular complications. The continuation of religious practices, as well as the disturbances of the circadian rhythm that are associated with the fasting of Ramadan, such as the perceived feeling of subjective fatigue, drowsiness, thirst and/or even mood swings, can lead to a significant decrease in the level of physical activity of Muslims (28). Results from a Moroccan study on healthy athletic adults showed a significant reduction in physical performance and a significant effect of Ramadan fasting on blood glucose and memory (29). Paradoxically, the results of a study on Algerian basketball players from a sports club showed no variations in training during the month of Ramadan and no significant effect on sports activity (30). According to Ghrici (31), the practice of moderate sport promotes physiological adaptation to fasting, since some changes during Ramadan are observed in sedentary people and not in athletes. This is the case with variations in blood glucose and triglycerides. Studies of substitution of sedentary time by physical activity (iso-temporal substitution) confirm the importance of replacing this sedentary time with physical activity, regardless of its intensity (25). During the month of Ramadan, physical activity levels must be maintained, whether for the general population or the sick population such as diabetics, but this is not always the case. In a study of Saudi diabetics, a high prevalence of physical inactivity and an increase in sedentary time were shown during Ramadan (32). While the work of Sfar et al. (33) showed a non-significant increase in physical activity in Tunisians with T2DM during Ramadan compared to the pre-Ramadan period. With longer intervals between the two main meals of the day, there can be a reduction in physical activity and exercise during the day (34). During a summer Ramadan, as in recent years, it is difficult to get around in the heat wave all day except to go to work and shopping in the market. Only the movement to the mosque to fulfill the voluntary evening prayers made in groups during the holy month of Ramadan ≪ *Tarawih* ≫ and/or social gatherings between family and friends allow to maintain a minimum level of daily physical activity throughout the month of Ramadan. Being physically active during Ramadan can help the individual to maintain his/her level of conditioning, as well as better deal with intermittent fast (28). However, excessive physical activity can increase the risk of hypoglycemia and hence should be avoided (10).

BODY COMPOSITION

The diet during the holy month of Ramadan should be the same as throughout the year, that is, a healthy and balanced diet from qualitative and quantitative perspectives. It should aim to maintain a constant body mass. In the meta-analysis of Tahapary et al. (35), on the impact of Ramadan fasting on the metabolic profile in the 2TDM patients, a change was found in body weight in 15 studies with 2,511 patients. Overall, there was a slight overall reduction in body weight of 0.71 kg (CI at 95%—.45–0.003 kg). Similarly, six studies involving 2,289 T2DM subjects reported a decrease in waist circumference of 0.62 cm (CI at 95%–1.31–0.08 cm) before and after the fast of Ramadan. As for the effect of Ramadan fasting on the body composition of the diabetic patient, the results were discordant. The researchers Aloulou et al. (36), Khaled and Belbraouet (7), and McEwen et al. (37) reported changes, in other studies some changes were found (16, 38, 39) and for others no change was found (6, 21, 40–43).

FASTING, METABOLIC CHANGES, COMPLICATIONS, AND CLINICAL ASPECTS

Fasting induces a secondary adaptive metabolic cascade to hormonal changes. The challenge is to make the best use of stored energy substrates during feeding periods, by keeping blood glucose levels as low as possible in the normal range, while preserving the protein capital (44). Metabolic adaptation to fast is designed to spare fat free mass (mainly muscle mass) or active cell mass. At the initial phase of fasting and in normal conditions, the body mobilizes fat reserves which are oxidized into ketone bodies then used by the brain; at this point, neo-glucogenesis of protein origin is reduced, allowing some saving of the free-fat mass (45). In the case of diabetes, lack of insulin promotes metabolism to ketone body production, which can cause diabetic ketoacidosis. This situation is compounded by difficulties in adjusting therapeutic doses during this month of fasting, which is not without consequence. Indeed, in T1DM there is no endogenous production of insulin by the pancreas. Therefore, insulin intake is entirely dependent on injections. While in the T2DM, endogenous insulin is still secreted, but inadequately or inappropriately, and glucagon is preponderante (46). During Ramadan, fasting-related complications for diabetics can include hyperglycemia, hypoglycemia, acidoketosis, dehydration, or thrombosis (2, 21, 47–51). These consequences are subject in most cases to hospitalization and complete interruption of fasting until the end of the holy month of Ramadan. In the literature, the variation of certain biochemical and clinical parameters, significant or not, contradicted, has been reported in relation to the effect of fasting in diabetics. The study results of Ouhdouch et al. (52) and of Abdessadek et al. (53) reported a significant decrease in HbA1c, while the opposite was noted in the study of Halimi et al. (54) and of Mbainguinam et al. (55) explaining this inequality of HbA1c would be the cause of hypertriglyceridemia. Also, an improvement in the lipid profile (53, 56, 57) was found, a significant decrease in fasting blood glucose during Ramadan compared to outside (53, 57, 58), hyperglycaemic episodes with or without ketoacidosis and severe hypoglycaemia (59–61), the presence of higher hypoglycemia in patients treated with insulin ($p = 0.002$), followed by those treated with oral agents including sulfonylureas as compared to oral agents excluding sulfonylureas (57). Also saved, a non-significant reduction in plasma creatinine, uric acid (53), an improvement in blood pressure figures (57), while in another study no change was found (6, 56).

COVID-19 PANDEMIC, DIABETES, AND RAMADAN FASTING

As for last year, Ramadan fasting took place in a particular context; in the midst of the COVID-19 pandemic. According to international iterim guidelines (62), healthy people should be able to fast during Ramadan, as in previous years, While COVID-19 patients should consider not doing so, following religious exemptions, in consultation with their physician, as with any other illness. According to Khan et al. (63), fasting during the COVID-19 crisis was a challenge for diabetic Muslims during Ramadan. A study of 829 diabetics, 34 fasting patients developed symptoms of COVID-19 before or during Ramadan. Ten patients (four fasters and six non-fasters) were admitted with symptoms related to COVID-19 (nine of whom were confirmed COVID-19 positive), however, none required intensive care. In the study of the DAR 2020 global survey on Ramadan fasting during the COVID-19 pandemic aiming to describe the characteristics and care of participants with T2DM showed that in 5,865 participants (recruited from 20 predominantly Muslim cities) concern over the COVID-19 pandemic affected the decision to fast by 7.6% from ≥65 years old vs. 5.4% from <65 years old, while 94.8% fasted ≥15 days and 12.6% had to break the fast due to diabetes-related illness (64). As reported by Chowdhury et al. (65), if a diabetic develops symptoms of COVID-19 during fasting, he/she must be told to break the fast immediately, to hydrate, to regularly monitor capillary blood glucose, because according to the study of Li et al. (66), there is evidence that ketosis and ketoacidosis were more common in people with diabetes and COVID-19. In their article Chowdhury et al. (65) continued that this applies particularly to patients on sodium-glucose 2 transporter inhibitors (SGLT2i) (67). If a patient is not feeling well with symptoms of COVID-19, it may require hospitalization as it is at high risk of deterioration. There are guidelines suggesting that metformin and SGLT2i should be discontinued in all diabetic and suspected COVID-19 patients requiring hospitalization (65, 68).

NUTRITIONAL EDUCATION

Ramadan is a special time in the life of Muslim diabetic patients leading to a major and sudden change in their rhythm of life having an impact on the management of their diabetes (69, 70). In a study highlighting the point of view of health professionals on the difficulties encountered during medical consultations during Ramadan, it was noted: treatment management (69.1%), glycaemic variations (52.1%), venous thrombosis (8.8%), weight gain (2.8%), changes in eating habits (42.9%) and food intake schedules (50.7%). The results of this study also showed that out of 1,975 diabetic patients, 924 of them did not know about the conditions for interruption of fasting whether T1DM or T2DM (53.0 vs. 46.4%; $p = 0.2984$) (13). In parallel, in another study highlighting the role of nutritional education in the management of T2DM during Ramadan, the researchers showed that 96% of the patients who received educational sessions were able to fast more than 21 days with a frequency of hypoglycemia 9 times lower compared to the control group (71). The patient wishing to fast must also be followed nutritionally; pair a balanced and varied diet (coverage of recommended nutritional intakes and respect for food preferences), appropriate treatment (in molecule, dose and frequency of intake), added to glycaemic control, will allow the diabetic patient to better manage his/her disease (13, 69, 70). Pre-Ramadan education is crucial to fast safely during the holy month. Education on fasting and diabetes management is also useful beyond Ramadan (72) having regard to the number of fasters after Ramadan [Example: CREED study (73) between 8 and 46%] and its important place in Muslim society. Admittedly, it is not an obligation, but an accomplishment of a pious act and an inner comfort that can be achieved throughout the year, but also the possibility and not the obligation (especially for the sick patients) to catch up for the unfasted days of the month of Ramadan (13).

ESSENTIALS POINTS IN TERM FOR RECOMMENDATIONS

Several international recommendations and consensus of experts have resulted in proposals to optimize diabetes management during the month of Ramadan:

- The first international recommendations for a good practice of fasting were those of the Hassan II Foundation in Morocco in 1995. This consensus proposed criteria authorizing and prohibiting fasting and reinforced clinical-biological surveillance before, during and after Ramadan, a monitoring of glycemic control, adaptation of treatment, education of diabetics, and their families on the contraindications of fasting, the risk of acute complications and the means of prevention and treatment (74).
- Other recommendations follow those of Morocco, including those of the American Diabetes Association (ADA) published in 2005 (75). In these recommendations, the term “indications” or “counter-indications” of fasting was avoided because the authors considered Ramadan fasting to be a spiritual religious issue for which patients make their own decisions after receiving appropriate advice from religious teachings and their own health professionals. In response to numerous requests and comments on important issues that were not discussed in the previous 2005 document, these recommendations were subsequently revised by regular updates every 5 years and were also the subject of other publications.
- In 2010 (76), research highlighted once again that fast in T1DM patients with poor glycemic control is associated with multiple risks. In the revised document, some issues were

discussed, concerning voluntary fasting outside Ramadan, discussion of the effect of prolonged fasting (more than 18 h per day) in remote areas of the equator during Ramadan when it occurs in summer, on the lower risk of hypoglycaemia, such as incretin based therapies and, on the limits of drugs and their use as thiazolidinediones.

- The third update to the recommendations of the ADA Working Group (77) was also published following numerous requests for information on education, dietary habits and new oral and injectable agents that may be useful for the management of diabetic patients during Ramadan.
- In the 2020 update (78), the research group aimed to apply the principles of ADA/European Association for the Study of Diabetes (EASD) type 2 diabetes management guidelines to Ramadan. New sections on the management of T1DM children and gestational diabetes were included. They carefully reviewed the literature and published data on medical nutrition therapy and the use of oral diabetes medication during Ramadan.
- The International Diabetes Federation (IDF) and the Diabetes and Ramadan (DAR) International Alliance met to provide comprehensive guidance on this topic. This guide (79) highlights relevant contextual information and practical recommendations to help diabetic patients to fast during Ramadan while minimizing the risk of complications. The guidelines cover several key topics, including epidemiology, physiology of fasting, risk stratification, nutritional advice, medication adjustment, and implementation of recommendations. One of the recurring themes in the guidelines is the importance of individualization and education as part of a diabetes management plan.
- The update of the IDF-DAR International Alliance Practical Guidelines features new guidance based on a greater and more recent body of evidence (80). This includes an updated set of criteria for risk stratification; information on the impact of fasting on physical and mental wellbeing; specific guidance on the management of T1DM and T2DM in special populations such as pregnant women and the elderly; and information on changes to the risk of comorbidities such as cardiovascular disease, stroke, and renal impairment.
- In addition, the Asian recommendations of the South Asian Consensus Guideline, where researchers (81) indicate that it is possible for people with diabetes to fast safely during Ramadan, but requires careful planning in order to avoid problems that could be serious and have long-term effects. The choice of insulin therapy is decided by the previous therapy that the patient is taking and also the blood glucose profiles. The major objective of insulin therapy during Ramadan is to provide adequate insulin to prevent the post meal (After *Iftar*) hyperglycemia and also prevent hypoglycaemia during the period of fast. With the use of analoges, these objectives may be met more easily.

The importance of the socio-cultural context of the holy month of Ramadan associated with the culturally-religious identity to which the diabetic patient is or wants to adhere are parameters to be included in the decision to fast. The advice of health professionals and members of religion will only reassure the diabetic in the accuracy of their decision. Each patient must be monitored individually, taking into account in particular the evolution of his disease, his therapeutic treatment, his socio-economic level, his level of education, his age, and his family status.

CONCLUSION

Fasting is a very sensitive period for the glycemic control of DMF and DMNF patients, requiring multidisciplinary preparation and expert advice. This work gave a brief visualization of the effect of Ramadan fasting on a NCDs which is diabetes. The few studies cited reflecting the various points raised (diet, sleep, physical activity, body composition, metabolic changes, nutrition education) showed heterogeneity of the results found probably due to the number of days of fasting, climatic conditions, cultural variations in eating habits, etc. Some slight advantages were noted. These particularities must be taken into account in the development of any study project on this topic. Effective management of diabetes with regular glycemic control will allow the patient to maintain an appropriate metabolic profile. Education on fasting and diabetes management is useful beyond Ramadan. It is important that recommendations have to be based on the gaps in existing data.

AUTHOR CONTRIBUTIONS

MBenc: study design, literature searching, and article draft writing. IS, MBent, and FB: draft revision and literature searching. YB: draft revision. All authors agree to be accountable for the content of the work.

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Ramadan Diurnal Intermittent Fasting Is Associated With Attenuated FTO Gene Expression in Subjects With Overweight and Obesity: A Prospective Cohort Study

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Aim and Background: A growing body of evidence supports the impact of intermittent fasting (IF) on normalizing body weight and that the interaction between body genes and environmental factors shapes human susceptibility to developing obesity. *FTO* gene is one of these genes with metabolic effects related to energy metabolism and body fat deposition. This research examined the changes in *FTO* gene expression upon Ramadan intermittent fasting (RIF) in a group of metabolically healthy subjects with overweight and obesity.

Methods: Sixty-three (63) subjects were recruited, of which 57 (17 males and 40 females, mean age 38.4 ± 11.2 years) subjects with overweight and obesity (BMI = 29.89 ± 5.02 kg/m^{2}were recruited and monitored before and at the end of Ramadan month), and 6 healthy subjects with normal BMI (21.4 ± 2.20 kg/m^2) recruited only to standardize the reference for normal levels of *FTO* gene expression. In the two-time points, anthropometric, biochemical, and dietary assessments were undertaken, and *FTO* gene expression tests were performed using RNA extracted from the whole blood sample.

Results: In contrast to normal BMI subjects, the relative gene expressions in overweight/obese were significantly decreased at the end of Ramadan (−32.30%, 95% CI–0.052 −0.981) in comparison with the pre-fasting state. Significant reductions were found in body weight, BMI, fat mass, body fat percent, hip circumference, LDL, IL-6, TNF-α ($P < 0.001$), and in waist circumference ($P < 0.05$), whilst HDL and IL-10 significantly increased ($P < 0.001$) at the end of Ramadan in comparison with the pre-fasting levels. Binary logistic regression analysis for genetic expressions showed no significant association between high-energy intake, waist circumference, or obesity and *FTO* gene expression.

Conclusions: RIF is associated with the downregulation of the *FTO* gene expression in subjects with obesity, and this may explain, at least in part, its favorable metabolic effects. Hence, RIF presumably may entail a protective impact against body weight gain and its adverse metabolic-related derangements in subjects with obesity.

Keywords: caloric restriction, intermittent fasting, obesity, nutrigenomics, Ramadan, time-restricted eating, gene expression, Middle East

INTRODUCTION

Obesity is one of the most common prevalent chronic diseases, with its comorbidities and long-term consequent mortality has become a major challenge to global health (1). Obesity is a complicated multifaceted disease that develops from the interaction of cellular, molecular, genetic, metabolic, physiologic, behavioral, cultural, and socioeconomic, influences (2, 3). Notably, cardiovascular disease, diabetes, renal disorders, and neoplasms were the major causes of high body mass index (BMI)-related disability-adjusted life years (DALYs), accounting for 89.3 percent of all high-BMI-related DALYs, with the BMI-related disease burden varying significantly, depending on the Socio-Demographic Index (SDI) (4). Despite the tremendous efforts in the MENA region to combat the problem, yet obesity is still a major health problem due to several factors including the adopted dietary patterns and physical inactivity (5, 6). Genetic predisposing factors represent one of the major contributing factors in the etiopathogenesis of obesity and its consequent complications, with some studies unraveling that high BMI is 25–40% heritable (7). However, to affect body weight, genetic predisposing factors often need to be coupled with environmental and behavioral triggering factors (8, 9).

With the progressive advancement of genome-wide association studies, more than 100 loci have been identified to be associated with obesity and its related traits (10). One of these genetic loci with a strong effect on obesity and related biological functions such as adipogenesis and energy balance regulation, fat mass, and obesity-associated (*FTO*) gene has emerged as one of the influential genes with remarkable impact (11). Recent large-scale analyses found that the obesity-risk allele (rs9939609 A allele) of the *FTO* is associated with increased food intake (12, 13), and previous studies also reported that the *FTO* obesity-risk allele was associated with a reduced response to hunger and satiety after meals in adults and children (14–16).

Nowadays, intermittent fasting (IF) has been looked at as an emerging effective, and costless dietary intervention that helps to promote health and prevent disease and aging (17). Several reports showed the benefits of different styles of IF, including time-restricted eating (TRE, a form of IF that involves confining the eating window to 4–10 h and fasting for the remaining hours of the day) (18), modified fasting regimens allowing 20–25% of energy needs to be consumed on scheduled fasting days, alternate-day fasting. The benefits are well evident for metabolic disorders, as well as cancer, obesity, diabetes, and neurological disorders (17, 19, 20).

Among the widely observed and extensively examined types of IF is the religious form observed during the month of Ramadan (RIF) (21). Ramadan is the ninth month of the lunar calendar, during which healthy adults are mandated to abstain from dawn to sunset, and to refrain from eating and drinking (including water) for a period that extends from 12 to 17 h, depending on the solar season and geographical location (22). This pattern of fasting is associated with dietary (including both food quality and quantity) (23), lifestyles (including sleep quality and quantity) (24) as well as circadian rhythm hormonal changes (25) that may harbor changes in gene expression.

Original research, systematic reviews, and meta-analyses unraveled that observing RIF entails a plethora of beneficial improvements in variable health and metabolic aspects, including reducing the cardiometabolic risk factors such as waist circumference, very low-density-lipoprotein cholesterol, low-density lipoprotein cholesterol (LDL), high-density-lipoprotein cholesterol HDL, total cholesterol (TC), triglycerides (TG), heart rate (HR), systolic blood pressure (SBP), and diastolic blood pressure (DBP) (26, 27). Moreover, RIF is associated reduce body weight (28), body fat content (29) with emphasis on visceral adiposity (30), and ameliorating inflammatory and oxidative stress (31–33), and non-alcoholic fatty liver disease markers in healthy people (34).

With the expansion of the nutrigenomic studies, growing attention has been directed toward examining the effect of different regimens of IF on gene expression of variable genes related to human health and diseases (16, 35). However, there is a paucity of studies tackling the effect of RIF and the associated dietary and lifestyle changes on the expression of specific genes related to human health and disease. Among these, only two studies examined the impact of RIF on the anti-oxidative stress genes (*TFAM, Nrf2, SOD2*) and metabolism-controlling genes (*SIRT1, SIRT3*) (36), the Circadian Locomotor Output Cycles Kaput (*CLOCK)* gene, and other genes related to circadian rhythmicity (37). However, the relationship between RIF and obesity- and body fat-controlling gene expressions is still to be investigated. In the former study, RIF was associated with significant increases in the relative expressions of the antioxidant genes (TFAM, SOD2, and Nrf2) in obese subjects in comparison to counterpart expressions of healthy weight subjects, with percent increments of 90.5, 54.1, and 411.5% for the three genes, respectively. However, the metabolism-controlling gene (SIRT3) showed a highly significant downregulation accompanied by a clear trend for reduction in the SIRT1 gene at the end of Ramadan month, with percent decrements of 61.8 and 10.4%, respectively (36). For the latter, profound changes were reported in the diurnal expression of *CLOCK*, a central component of the circadian molecular clock, during Ramadan compared to the non-fasting month of *Sha'aban* (the month before Ramadan) (37). One study assessed the association of common polymorphisms in the CLOCK and FTO gene polymorphisms (SNPs) (rs1801260 and rs9939609, respectively) with standardized BMI scores, and the impact of dietary and lifestyle modification in school-age children (38). It was found that sex is a potential modifier for the association between the CLOCK polymorphism and BMI z-scores in school-age children (38) and that the FTO SNP, rs9939609, did not significantly modify the effect of the intervention on BMI z-scores at the follow-up or changes of BMI z-scores (38).

Given the proven impact of RIF in lowering body weight (28), body fatness (29), visceral fat content (30), and satiety and eating-controlling hormones (leptin, adiponectin, ghrelin) (25); it becomes rational to examine the relationship between the observance of RIF and the expression of *FTO* gene. Considering the preventive effect of IF, and RIF in particular as a unique diurnal TRE model (39), on the above-mentioned obesity-related indicators, and the principal role of *FTO* in controlling satiety, food intake, body fatness, and obesity risk (11, 13–16); the current work stemmed from the hypothesis that observing RIF will be associated with reduced expression of the *FTO* gene in fasting people with obesity. Therefore, the current work was designed to find out how the observance of RIF by fasting people with obesity will be associated with changes in the genetic expression of *FTO*.

METHODS

Participant Selection

In total, 63 subjects were recruited, of which 57 (17 males and 40 females, mean age 38.4 ± 11.2 years) subjects with overweight and obesity (BMI = 29.89 ± 5.02 kg/m^2 (were recruited and monitored before and at the end of Ramadan month, and 6 healthy subjects with normal BMI (21.4 ± 2.20 kg/m^2) recruited only to standardize the reference for normal levels of *FTO* gene expression. All subjects who visited the University Hospital Sharjah (UHS), UAE, for screening were recruited for this study. All subjects were Arabs from the Arabian Gulf, Iraq, Egypt, Sudan, Tunisia, and the Levant countries (Syria, Jordan, Lebanon, and Palestine). The study protocol was designed and conducted following the Declaration of Helsinki and approved by the UHS Research Ethics Committee (Reference no. REC/16/12/16/002). All enrolled subjects (n = 63) were provided with an information sheet describing the research plan, objectives, and requirements of participation. Subjects were recruited using personal communication, social media, and institutional emails. All subjects attended the UHS for screening and investigations and provided signed informed consent to participate in this study. Subjects were men and women who were of either normal weight or overweight/obesity (BMI >25 kg/m^2) and decided to fast Ramadan and were willing to participate in this study. We have collected basic and sociodemographic data using a self-report questionnaire that covered the medical history and demographic information. The questionnaire was administered in individual face-to-face interviews. A trained research assistant conducted all interviews. The exclusion criteria were a history of metabolic syndrome, diabetes, or cardiovascular disease, taking regular medications for any chronic disease, following a weight-reducing diet, a history of bariatric surgery within the last 6–9 months before commencing Ramadan fasting, and being a pregnant or peri-menopausal woman.

Study Design

A prospective observational study design was used to investigate the effect of RIF on *FTO* gene expressions along with variable anthropometric, metabolic, and inflammatory markers in subjects with overweight and obesity. Data were collected at baseline (2–7 days before RIF) and after completing 28–30 consecutive days of diurnal RIF. During the fasting month of Ramadan, individuals abstain from all foods and drinks (including water) from dawn to sunset, with the average fasting duration being 15 h per day. Subjects were not requested to follow any dietary or physical activity regimens or recommendations during any stage of this study. All subjects were asked to pursue habitual lifestyle patterns during both fasting and non-fasting hours. According to Islamic laws of fasting, menstruating women are exempted from observing Ramadan fasting during their period; hence, the fasting period for participating women was less than that for men (23–25 vs. 28–30 days).

Anthropometric Assessment

Anthropometric measurements were taken at two time points (before and at the end of commencement of 28–30 fasting days). Anthropometric measures of body weight, fat mass, and body fat percentage, and fat-free mass were measured using segmental multi-frequency bioelectrical impedance analysis (DSM-BIA; TANITA, MC-980, Tokyo/Japan) before and at the end of the fasting month. The DSM-BIA machine measured the visceral fat rating (from 0 to 100), and this value was converted into a visceral fat surface area by multiplying the obtained value by 10, consistent with the manufacturer's instructions. Height was measured using a fixed stadiometer to the nearest 0.1 cm. BMI was calculated as weight (kg) divided by height in m^2. Waist and hip circumference were measured to the nearest 0.01 m using a non-stretchable measuring tape (Seca, Hamburg/Germany), and their ratio was calculated accordingly.

Dietary Intake Assessment

No special dietary recommendations or food regimens were given to the study subjects during any stage of the study, and all the subjects were asked to pursue their habitual dietary patterns during the eating period before and during Ramadan. Dietary intakes were assessed by trained nutritionists using the 24-h recall technique on 3 days (one weekend day and two weekdays) at the two-time points (before and at the end of Ramadan fasting). Printed two-dimensional food models were used to help study subjects approximate the eaten portion sizes. Dietary intakes of energy (calories), macronutrients (carbohydrates, protein, fats, and water), and micronutrients (vitamins and minerals) were estimated using the Food Processor software (version 10.6 ESHA Research, Salem, OR/USA).

Physical Activity Level

The Dietary Reference Intakes classification for general physical activity level was used to assess subjects' level of physical activity

(40). This classification depends on the general physical exercise pattern. Subjects were considered highly active if they performed at least 2 h per day of moderate-intensity physical exercise or 1 h of vigorous exercise in addition to daily living activities. Subjects were considered moderately active if they performed more than 1 h per day of moderate-intensity exercise in addition to daily living activities. Subjects that performed 30 mins to 1 h per day of moderate-intensity physical exercise in addition to daily living activities were considered to have low activity. Finally, subjects who performed daily living activities without other physical exercise were considered sedentary (40).

Blood Sampling

A sample of 10 ml of blood was collected from subjects at baseline in 3 different tubes including red top (plain) for serum, purple top (EDTA) for plasma and RNA extraction, and gray top (sodium fluoride) for glucose level (before commencing fasting) and at the end of the fasting month. At both time points, blood samples were collected after at least 8 h of fasting. The samples were collected between 11 a.m. and 1 p.m. to eliminate the effect of timing and dietary intake on the measured biochemical parameters and ensure consistency in the duration of fasting for the two-time points. Collected blood samples were divided into two aliquots. One aliquot was centrifuged at 2,500 rpm for 15 min within 1 h of the collection; the serum was aliquoted, coded, and stored at −80°C until it was used for biochemical analysis. The second aliquot was used for RNA extraction, as explained below.

Biochemical Assay

In this study, we used chemiluminescent immunoassay (CLIA) based on a fully automated clinical chemistry analyzer (Adaltis, Pchem1, Italy) to quantify fasting glucose, total cholesterol (TC), LDL-cholesterol, HDL-cholesterol, and triglycerides (TG) at the two-time points. The pro-and anti-inflammatory cytokines (IL-6 and TNF-α; and IL-10, respectively) were quantified using a multiplex assay (Luminex, Bio-Plex Pro™ Human Cytokine plex Assay).

Blood Pressure Measurement

Blood pressure was measured before blood sampling using a digital blood pressure monitor (GE, USA), with subjects in an erect, seated position after a 5-min resting period.

FTO Gene Expression, RNA Extraction, Reverse Transcription, qPCR

RNA was extracted using the column-based, Total RNA Purification kit from Norgen, (Thorold, Canada) and reverse transcribed to cDNA by the QuantiTect Reverse Transcription kit from Qiagen (Hilden, Germany), according to the manufacturer's instructions. cDNA and primer concentrations were optimized to obtain a single amplification peak. qPCR reaction was performed at a volume of 20 μl, including 10 ng of cDNA with 5x HOT FIREPol EvaGreen qPCR Mix Plus (ROX) (Solis Biodyne). 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, annealing at 55°C for 30 s followed by extension at 72°C for 30 s. The forward and reverse primers used in the study are presented in **Table 1**. For each sample, the expression of each gene was normalized to the housekeeping gene ribosomal protein L18 subunit (*RPL*18s); The *RPL*18s was chosen as it showed less variation among *GAPDH* and actin housekeeping genes during the initial optimization; at the same time point. (10ng of cDNA and 0.1 uM of each primer per reaction). Three different negative controls were used at this analysis; control (1) no enzyme was added, control (2) no mRNA was added, and control (3) water was added instead of cDNA (NTC control). Therefore, the minimum amount of cDNA, primers and SYBR green have been used per reaction to obtain the specific signal avoiding false amplification. The relative expression was shown as fold change according to Livak and Schmittgen (41) and was presented as mean and standard deviation as described elsewhere (42). Considering the lack of reference range for *FTO* gene expression, six subjects with normal BMI (21.4 ± 2.20 kg/m^2) were recruited only to get the normal *FTO* expression levels at the two points (before and after Ramadan fasting). For overweight/obese subjects, at each time point, the *FTO* gene expression was first calculated relative to the housekeeping (*RPL18*) gene, then as a fold-change compared to the gene expression for the normal reference levels obtained from subjects with normal BMI.

TABLE 1 | Forward and reverse primers were used in genetic analysis for the *FTO* and housekeeping genes tested.

Gene	Forward primer	Reverse primer
FTO	CCAGAACCTGAGGAGAGAATGG	CGATGTCTGTGAGGTCAAACGG
RPL18	GATGTCGGATTCTGGAAGTTCC	GGTCAAAGGTGAGGATCTTACCC

Statistical Analyses

The statistical analyses were done using Statistical analyses were performed using SPSS 24 (IBM, Armonk, NY, USA). and reported based on the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (43). The primary outcome measure was the change in the genetic expression of the *FTO* gene between the two-time points. We estimated that 51 subjects would provide 80% power to detect a significant difference of 5% in genetic expression between baseline (pre-fasting) and post-fasting using a two-tailed paired-samples *t*-test with $\alpha = 0.05$. With an expected dropout rate of 10%, 56 subjects were planned for enrollment. Tests for normality were included in the model. The variables were expressed as the mean ± standard deviation (SD). Independent sample *t*-test comparing baseline characteristics between males and females. Two-tailed Paired sample *t*-tests were used to compare within-subject changes from baseline (pre-fasting) to post-fasting time points. Binary logistic regression [the odds ratio (OR), 95% confidence interval (CI)] was calculated considering genetic expression as dependent variables, and sex (male vs. female), caloric intake (high, >2,000 Kcal vs. low, <2,000 Kcal), waist circumference as independent variables. We recoded the waist circumference variable as high waist circumference or low waist circumference as per the corresponding sex of the participant.

TABLE 2 | Subjects' sociodemographic characteristics of the overweight/obese subjects (n = 57).

Characteristic	*n* (%)
Age, years (Mean ± SD)	38.42 ± 11.18
Sex	
Male	40 (70.2)
Female	17 (29.8)
Nationality	
UAE and other GCC countries	5 (8.8)
Non-GCC (Palestine, Jordan, Syria, Egypt, Iraq, Tunisia,Sudan)	52 (91.2)
Marital status	
Married	47 (82.5)
Single	10 (17.5)
Educational level	
Basic education	5 (8.8)
Undergraduate studies	44 (77.2)
Postgraduate studies	8 (14)
Physical activity	
Sedentary	52 (91.2)
Low activity	2 (3.5)
Moderately active	3 (5.3)
Highly active	-

GCC, Gulf Corporation Council; UAE, the United Arab Emirates.

TABLE 3 | Basic anthropometric and cardiac function characteristics of the overweight/obese subjects according to sex variable (n = 57).

Parameter	Males (n = 17)		Females (n = 40)		Significance between males and females
	Mean	SD	Mean	SD	
Weight (kg)	92.52	12.78	78.46	19.44	NS
BMI (kg/m^2)	29.99	4.20	29.66	6.71	NS
FM (kg)	25.39	8.01	29.15	12.19	NS
BFP (%)	26.86	5.19	35.73	7.17	NS
FFM (kg)	67.13	5.81	49.31	7.56	NS
MM (kg)	63.80	5.54	46.81	7.19	NS
TBW (kg)	47.70	4.30	35.38	5.35	NS
VFA (cm^2)	114.25	45.79	66.47	38.07	NS
WC (cm)	101.67	11.04	91.53	16.79	NS
HC (cm)	109.64	7.39	111.12	13.34	**0.007***
WHR	0.93	0.06	0.82	0.08	NS
SBP (mmHg)	123.47	10.35	124.12	16.13	**0.016***
DBP (mmHg)	72.19	8.22	73.00	10.83	NS
Pulse rate (pulse/min)	72.47	8.99	70.00	10.11	NS

P-value: Independent samples t-test comparing baseline variables between men and women.
BMI, Body mass index; BFP, Body fat percent; DBP, Diastolic blood pressure; FM, Fat mass; FFM, Fat-free mass; HC, Hip circumference; MM, Muscle mass; SBP, Systolic blood pressure; TBW, Total body water; VFA, Visceral fat area measured by DSM-BIA; WC, Waist circumference; WHR, Waist: hip ratio.
**P < 0.05, significant difference.*

The following criteria were used: High, ≥ 102 cm vs. Normal <102 cm for men and High, ≥ 88 cm vs. Normal <88 cm for women. Linear regression was used to determine the relationship between the change in *FTO* gene expression (dependent variable) before and at the end of Ramadan and biochemical and anthropometric variables (as independent variables). All data were tested at a 5% level of significance ($P < 0.05$).

The intra- and inter-assay coefficient variation (CVs) for the tested biomarkers are found through the repository link: https://www.dropbox.com/scl/fi/pghy4x01f6crvy7boihby/Inter-intra-assays-CVs-DNA-15.9.2021.xlsx?dl=0&rlkey=s5j7v8jj5gstdl878fd2k7rgc.

RESULTS

Fifty-seven (17 males and 40 females, mean age of 38.42 years ± 11.18) overweight/obese subjects (BMI = 29.89 ± 5.02 kg/m^2) were recruited and monitored before and at the end of fasting the whole month of Ramadan. The majority of subjects were females (about 70%), and most subjects were married (about 83%), university graduates (around 77%), and sedentary (about 91%). About 91% of the study population were from non-Gulf Cooperation Council (GCC) countries (**Table 2**).

The basic and anthropometric characteristics of the subjects are shown in **Table 3**. Bodyweight and composition, glucose homeostasis, blood pressure, and inflammatory markers significantly varied between pre-and post-Ramadan fasting, as shown in **Tables 4–6**. By the end of the Ramadan fasting month, body weight, BMI, fat mass, body fat percent, waist circumference, and hip circumference significantly ($P < 0.05$) reduced when compared to pre-fasting levels. LDL-C, IL-6, and TNF-α were significantly reduced as well ($P < 0.05$) at the end of the fasting month, while HDL-C and interleukin 10 were significantly increased ($P < 0.05$) (**Table 5**). Changes in dietary intake are shown in **Table 4**. Significant increases were reported in the dietary intake of total sugars, PUFA, vitamin C, omega-3 fatty acids, lycopene, and vitamin E in comparison with the pre-fasting intakes, while the intake of protein and cholesterol decreases in comparison with the pre-fasting intakes (**Table 6**). Results of relative genetic expressions in subjects with overweight/obesity showed significant downregulation in the *FTO* gene expression at the end of Ramadan in comparison with the pre-fasting level, with a percent reduction of about−32% (95% CI–0.052 −0.981) (**Figure 1**).

Binary logistic regression analysis for genetic expressions showed no significant ($P > 0.05$) association between high-energy intake (≥2,000 kcal vs. <2,000 kcal), waist circumference (High, ≥ 102 cm vs. Normal <102 cm for men and High, ≥ 88 cm vs. Normal <88 cm for women), obesity (BMI ≥ 30 vs. BMI < 30) and gene expressions of *FTO* gene (**Supplementary Table 1**). Linear regression analysis showed a significant, but weak, positive association between the hip circumference and the *FTO* gene expression at the end of Ramadan fasting days (**Supplementary Table 2**).

TABLE 4 | Changes in anthropometric and cardiac functions measured before and at the end of Ramadan for the overweight/obese subjects (*n* = 57).

Parameter	Before Ramadan (T1)		At the end of Ramadan (T2)		Significance as compared to baseline
	Mean	SD	Mean	SD	
Weight (kg)	88.32	16.24	86.73	15.74	**0.001****
BMI (kg/m^2)	29.89	5.02	29.40	4.94	**0.001****
FM (kg)	26.51	9.49	25.25	9.40	**0.001****
BFP (%)	29.51	7.09	28.58	7.34	**0.001****
FFM (kg)	61.81	10.37	60.94	10.99	NS
MM (kg)	58.73	9.88	58.41	9.57	NS
TBW (kg)	44.02	7.31	43.84	7.01	NS
VFA (cm^2)	100.00	48.59	95.72	45.11	NS
WC (cm)	98.64	13.69	97.23	13.03	**0.030***
HC (cm)	110.08	9.46	108.55	8.87	**0.001****
WHR	0.89	0.08	0.89	0.08	NS
SBP (mmHg)	123.66	12.21	125.64	12.84	NS
DBP (mmHg)	72.43	8.99	73.53	10.26	NS
Pulse Rate (pulse/min)	71.73	9.32	72.13	9.25	NS

Paired t-test comparing the end of RIF (T2) with pre-fasting baseline (T1).
BMI, Body mass index; BFP, Body fat percent; FM, Fat mass; BFP, Body fat percent; FFM, Fat free mass; MM, Muscle mass; TBW, Total body water; VFA, Visceral fat area measured by DSM BIA; WC, Waist circumference; HC, Hip circumference; WHR, Waist: hip ratio; SBP, Systolic blood pressure; DBP, Diastolic blood pressure.
**$P < 0.05$, significant difference.*
***$P < 0.001$, highly significant difference.*

TABLE 5 | Changes in glucose homeostasis and inflammatory markers before and at the end of Ramadan for the overweight/obese subjects (*n* = 57).

Parameter	Before Ramadan (T1)		At the end of Ramadan (T2)		Significance as compared to baseline
	Mean	SD	Mean	SD	
FBG (mg/dl)	99.91	20.54	105.70	24.28	NS
TC (mg/dl)	173.95	39.07	175.88	35.81	NS
HDL-C (mg/dl)	45.65	6.77	58.35	11.70	**0.001****
TG (mg/dl)	93.63	53.83	97.42	44.10	NS
LDL-C (mg/dl)	109.58	32.55	98.02	33.91	**0.001****
Interleukin-6 (pg/dl)	29.86	16.75	18.21	1.29	**0.001****
TNF-α (pg/dl)	28.17	4.40	21.24	1.49	**0.001****
Interleukin-10 (pg/dl)	18.25	0.71	19.44	1.21	**0.001****

Paired t-test comparing the end of RIF (T2) with pre-fasting baseline (T1).
***$P < 0.001$, highly significant difference.*

TABLE 6 | Changes in the dietary intake before and at the end of Ramadan for the overweight/obese subjects (*n* = 57).

Nutrient	Before Ramadan (T1)		At the end of Ramadan (T2)		Significance as compared to baseline
	Mean	SD	Mean	SD	
Energy (kcal/d)	2,123.08	754.20	2,150.43	847.11	NS
Fat calories (kcal/day)	694.50	396.38	687.07	366.23	NS
Protein (g/d)	108.35	36.61	89.93	39.25	**0.002***
Total carbohydrates (g/d)	253.66	94.84	282.74	122.88	NS
Total sugars (g/d)	65.90	31.33	107.73	53.71	**0.001****
Total fats (g/d)	77.32	44.08	76.76	41.10	NS
Saturated Fat	23.49	12.55	22.66	12.20	NS
Total water (ml/day)	1,397.02	690.34	1,518.62	808.16	NS
MUFA (g/d)	20.23	11.24	23.05	13.78	NS
PUFA (g/d)	10.47	8.09	16.32	16.81	**0.016***
Trans fat (mg/d)	0.50	0.81	0.47	1.21	NS
Cholesterol (mg/d)	394.85	176.89	272.56	181.90	**0.001****
Vitamin C (mg/d)	73.50	50.63	97.43	66.71	**0.006***
α-Carotene (μg/d)	12.61	22.82	16.24	33.30	NS
β-Carotene (μg/d)	393.88	657.97	576.24	938.61	NS
Omega 3 (mg/d)	0.66	0.56	1.82	2.29	**0.001****
Omega 6 (mg/d)	7.84	7.08	10.06	10.70	NS
Lycopene (μg/d)	1,484.37	3,493.41	4,234.36	9,700.64	**0.048***
Vitamin E (mg/d)	5.75	3.57	8.49	9.44	**0.042***
Selenium (μg/d)	85.84	48.50	70.98	47.22	NS

Paired t-test comparing the variables at the end of RIF (T2) with pre-fasting baseline (T1).
MUFA, Monounsaturated fat; PUFA, Polyunsaturated fat.
**$P < 0.05$, significant difference.*
***$P < 0.001$, highly significant difference.*

DISCUSSION

The current study provides the first evidence of a link between RIF and the *FTO* gene expression in a cohort of overweight/obese subjects who observed Ramadan fasting (28 days for an average of 15 h/day). There was an association between reduced *FTO* expression and favorable effects, as demonstrated by suppression of pro-inflammatory markers and improvement of the lipid profile. Above all, such an association was also accompanied by a reduction of BMI and waist/hip ratio denoting that the effect of RIF may be explained, at least in part, by its link to the *FTO* expression. Several human studies showed beneficial effects of IF (17, 19, 21, 44, 45). Recently, a growing body of evidence suggests substantial health implications for the religious form of IF, with the Ramadan model as one of the most extensively studied forms with variable anthropometric, metabolic, and inflammatory impacts (25–29, 31, 32). The main distinctive features of RIF in comparison to other patterns of IF models are presented in the fact that RIF involves diurnal, dawn to sunset, IF for 29–30 consecutive days with complete abstinence from food and drink, including water. Other models of IF include modified fasting regimens (involves consumption of 20–25% of energy needs on scheduled fasting days such as 5:2 diet), TRE (allows *ad libitum* nutrient and energy intake within specific time frames, and inducing regular, extended fasting intervals, mostly nocturnal fasting), and alternate-day fasting (allows alternating fasting days with eating days) (45).

In the current study, RIF reduced body weight, BMI, body fat percent, waist circumference; RIF also resulted in a reduction of LDL, with increased HDL. This is in support of the previous reports on the favorable effect of RIF on the cardiometabolic risk factor profile. In our recent meta-analysis, we demonstrated the favorable effect of IF on reducing total cholesterol, LDL, triglyceride levels as well as diastolic blood pressure and heart rate (26). The study included subgroup analysis of age, sex, and duration of fasting (as confounding factors) and the significant favorable effect of RIF was constantly evident in all subgroups. Concordant with our findings in the current and previous studies, Mindikoglu et al. showed that RIF resulted in a significant reduction of BMI, waist circumference, and improvement in blood pressure, with an anti-cancer, anti-diabetes, and anti-aging serum proteome response, providing another dimension for the benefits of IF that is likely to be promoted by cytokine modulation (46).

The observed changes in the total energy and dietary intakes from different macro and micronutrients are repeatedly shown in several studies (30, 32), and consistent with the recent work that compared dietary intakes from different food groups and macronutrients in a comparative study using the year-round dietary intakes (23).

FTO expression is different in underfeeding and fasting conditions and displays tissue-specific differences in mouse models of obesity, but it is not known whether these differences are the cause or the consequence of obesity (47). *FTO* mRNA expression in mice and humans is broadly distributed in many organs, with notably high levels of expression in the brain and hypothalamus, which regulate energy balance and hunger (48, 49). Previous studies showed that tissue-specific genes may be expressed in a wider variety of tissues. When transcriptomics analysis of peripheral blood mononuclear cells (PBMCs) was compared to that of liver, kidney, stomach, spleen, prostate, lung, heart, colon, and brain, more than 80% of shared differentially expressed genes (50). Also, other reports suggested that PBMCs can represent a surrogate indicator in dietary investigations to identify differentially expressed genes in population studies (51, 52). In mice, the expression of FTO may be influenced by their dietary condition (53). When mice are fasting, there is a strong stimulus to eat, and their hypothalamus *FTO* mRNA expression is significantly reduced compared to their fed counterparts. Supplementation with the anti-hunger hormone leptin does not reverse this effect, which suggests that the reduced hypothalamic FTO expression observed during fasting is independent of leptin levels (54). These findings show that FTO is downregulated during fasting and increased during feeding and that a decrease in FTO expression or activity might be a signal that encourages overeating and obesity. Noteworthy, the results in rats are different; possibly because of inconsistency in the conditions of different studies and the different sensitivity to starvation among different species. Murine FTO gene expression was shown to be downregulated under fasting conditions, suggesting that obese mouse models mimic the fasted state, possibly contributing to their over-eating (47).

Interestingly, *FTO* was highly expressed in the cerebellum, salivary gland, and kidney of adult pigs, whereas it was not detected in blood (55). The latter study showed *FTO* was positively associated with energy intake in the pancreas, and with age in the muscle, adding to the multiple factors that affect FTO expression. Such variation can be explained by different metabolic and secretion activities of different tissues at different age groups. Moreover, as previously described with leptin, diurnal variation may also affect the level of expression of *FTO* (56). The link of *FTO* expression to fasting and obesity is not yet fully elucidated in humans, where more factors may interplay to determine this effect. Such factors include the effect of food predilection, dietary patterns, and complexity of gut-brain networking including leptin, ghrelin, among other key players (57). Our current study highlights the reduction of *FTO* in overweight/obese subjects as a consequence of observing diurnal IF for four consecutive weeks.

Furthermore, the current study showed a reduction in both pro-inflammatory cytokines IL6, TNF-α. Concordantly, in a study by Faris et al. significant reductions in IL-6, IL-1β, and TNF-α were reported in fasting subjects during Ramadan of both sexes, when compared to basal pre-fasting values obtained 1 week before Ramadan (32). Furthermore, this finding is consistent with the results of a meta-analysis and original research showing that RIF is associated with significant reductions in serum proinflammatory cytokines (IL-6, IL-1β, and TNF-α) and *hs*-CRP, and the oxidative stress marker malondialdehyde and urinary 15-f(2t)-isoprostane (32, 33). The current findings on the significant reductions in lipid profile components (TC, LDL, and TG) and increased HDL are consistent with the systematic reviews and meta-analyses showing that RIF is associated with such improvements in the cardiometabolic risk factors (26, 27). As shown by Faris and colleagues (30), these reductions in the proinflammatory cytokines and other inflammatory adipokines were reported to be associated with significant reductions in visceral adiposity in obese subjects who observed the 4-week dawn to sunset IF of Ramadan. Experimentally, fasting reduced TNF-α in visceral white adipose tissue, IL-1β in subcutaneous tissue, as well as insulin and leptin in the plasma in stressed rats (58).

The current study showed that RIF increased IL-10, which is consistent with a previous study by Faris et al. among obese subjects observing RIF when compared with the pre-fasting levels (30). IL-10 has a strong immune-modulation activity (59). It is thought of as an anti-inflammatory cytokine that can suppress cytokine production from macrophages and the function of neutrophils (60, 61) but can activate CD8+ T cells and natural killer (NK) cells for anti-viral immunity, denoting its dual role in immunity (62, 63). Intriguingly, the IL-10 signaling pathway was one of the top Differentially Expressed Genes (DEGs) in COVID-19 infected normal epithelium vs. mock-infected cells (64) and could be, along with the reduction in other metabolic and inflammatory risk factors, involved in the plausible protective effect of Ramadan fasting against the COVID-19 infection (65).

FTO expression did not correlate with high-energy intake, waist circumference, or obesity as shown by the binary logistic regression analysis performed. These findings denote that RIF exerts its beneficial effects independently from the dietary and anthropometric factors, through different pathways that may or may not involve weight reduction and lower energy intake. Such dissociation between the beneficial effect of IF and caloric

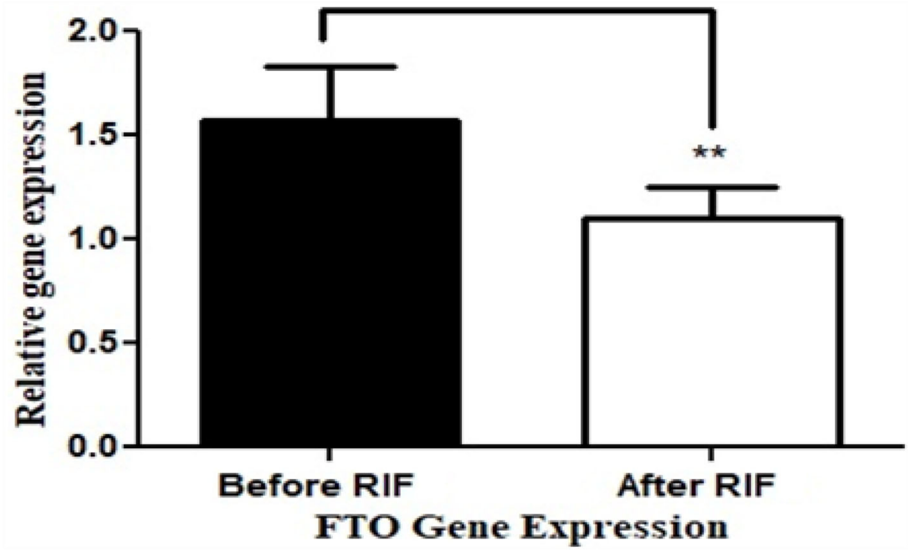

FIGURE 1 | Relative (in comparison with healthy subjects with normal BMI) *FTO* gene expression before and at the end of Ramadan intermittent fasting (RIF) month for the overweight/obese subjects. $^{**}P < 0.001$.

restriction is supported by previous work on rodents (66) who found that IF has beneficial effects in experimental mice reported on glucose regulation and neuronal resistance to injury that are independent of caloric intake. Several proteins interact with the *FTO*; the most significant of which is Melanocortin receptor 4 (MCR4) that is co-expressed with the *FTO* in some species. The MCR4 is involved in energy balance as well as somatic growth (67).

Pharmacologic treatments cannot reset the circadian clock rhythm; thus, there is an urgent need for an effective intervention to reset the circadian clock and prevent metabolic syndrome and metabolic syndrome-induced cancers (68, 69). However, IF practiced exclusively during human activity hours can reset the circadian rhythm. Therefore, resetting the disrupted circadian clock in humans by consecutive daily IF could provide a primary strategy to improve metabolic syndrome and reduce the incidence of metabolic syndrome-induced cancer (68, 69). RIF upregulated several key regulatory proteins that play a key role in tumor suppression, DNA repair, insulin signaling, glucose, and lipid metabolism, circadian clock, cytoskeletal remodeling, immune system, and cognitive function (70).

Nonetheless, our results indicate a lack of association between *FTO* gene expression and caloric intake by the fasting people. This notion may appear inconsistent with the evident association between intakes of calories, carbohydrates, and fats with the *FTO* genotype (67), given some studies that showed a correlation of the *FTO* risk allele and high *FTO* gene expression (68, 69). Moreover, it has been reported that the *FTO* genotype may influence dietary macronutrient intakes, body weight, energy balance, appetite, and hormone secretion (70, 71). The SNPs of the *FTO* gene are likely associated with food intake and obesity through modifying the expression of other genes (72). Until now, there is no strong evidence for the association of *FTO* A risk allele and level of gene expression. There is a recognized association of the A risk allele of *FTO rs9939609* and overweight worldwide (48, 71) in several Arab populations (72–75). In this study, we did not investigate the subjects' genotypes, as the study group is not from the same ethnicity. In our previous study on the Emirati population, the rs9939609 AA genotype was significantly associated with higher BMI; in females, but not in males (76). In another study by our group, subjects with *rs9939609* AA genotype showed significantly higher fasting glucose compared to other genotypes, with a trend of higher insulin levels and HOMA2-IR (77). We recently correlated the *FTO* genotypes, as well as FGF21 genotypes to dietary patterns in the Emirati population (78). Whether the outcome of a caloric intervention is affected by *FTO* rs 9939609 A risk allele is weakly evident (79).

A few limitations should be considered when interpreting the findings of the current work. First, causality cannot be inferred, as the design is observational prospective in nature. Hence, undetected confounding factors could be involved in the downregulation or upregulation of the tested *FTO* gene upon RIF. Changes in circadian rhythm and sleep patterns that have been reported to affect the expression of some genes (37) are among the factors that may be implicated in changing *FTO* gene expression. Tissue- and age-specific variations of *FTO* expression also add to the complexity of the interpretation of its expression in the blood. Although the practice of physical exercise did not change during Ramadan month in comparison with the pre-fasting stage, still this factor may be of paramount effect, and objective measurements have to be applied in measuring physical exercise levels in the forthcoming studies.

CONCLUSIONS

RIF is linked to the downregulation of *FTO* gene expression in subjects with obesity, which might explain, at least in part, its beneficial metabolic benefits. Consequently, RIF may have a preventive effect against body weight increase and associated negative metabolic-related derangements in overweight/obese people, possibly through modulation of *FTO* gene expression.

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 author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Research Ethics Committee, University of Sharjah. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MF, MS-A, and MM: conceptualization and validation. MF, MM, and LM: data curation and methodology. DA: formal analysis. MF, MS-A, and WA-R: funding acquisition, supervision, and project administration. MF, MS-A, MM, and LM: investigation. MF and WA-R: resources. MF and WA-R: software. LM, MM, and DA: visualization. MF and MS-A: writing—original draft. MF, MS-A, and WA-R: writing—review and editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.741811/full#supplementary-material

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Effects of Ramadan and Non-ramadan Intermittent Fasting on Gut Microbiome

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Background: In recent years, intermittent fasting (IF) has gained popularity in the health and wellness in the world. There are numerous types of IF, all of which involve fasting periods that last longer than an overnight fast and involve limited meal time-windows, with or without calorie restriction. The objective of this review is to summarize the current evidence for the effects of Ramadan and non-Ramadan IF on gut microbiome.

Methods: We explored PubMed, Scopus, Web of Science, and Google Scholar according to the PRISMA criteria (Preferred Reporting Items for Systematic Reviews and Meta-Analysis). Animal and human studies were screened and reviewed separately by two researchers.

Results: Twenty-eight studies were selected after screening. Some of the studies were performed on animal models and some on humans. The results of these studies indicate a significant shift in the gut microbiota, especially an increase in the abundance of *Lactobacillus* and *Bifidobacteria* following fasting diets. The results of some studies also showed an increase in the bacterial diversity, decrease inflammation and increased production of some metabolites such as short-chain fatty acids (SCFAs) in individuals or samples under fasting diets. Moreover, Ramadan fasting, as a kind of IF, improves health parameters through positive effects on some bacterial strains such as *Akkermansia muciniphila* and *Bacteroide*. However, some studies have reported adverse effects of fasting diets on the structure of the microbiome.

Conclusion: In general, most studies have seen favorable results following adherence from the fasting diets on the intestinal microbiome. However, because more studies have been done on animal models, more human studies are needed to prove the results.

Keywords: fasting, intermediate fasting, Ramadan, gut microbiome, review

INTRODUCTION

People can calorically restrict while feeling hungry, and this approach has already been demonstrated in various mammalian species to enhance life span, increase numerous physiological indicators, and lower metabolic parameters for chronic illness (1, 2). There are numerous types of intermittent fasting (IF), all of which involve fasting periods that last longer than an overnight fast and involve limited meal time-windows, with or without calorie restriction (3, 4).

The Islamic lunar calendar's ninth month, Ramadan, is 11–12 days shorter than the Gregorian solar calendar. This indicates that the month of Ramadan revolves around the four seasons and the 12 months of the year. Fasting during Ramadan is an obligatory duty for all healthy adult Muslims, as stated in the Holy Quran where ALLAH says, "O you who believe, fasting is prescribed for you as it was prescribed for those before you, that you may develop God-consciousness" (Surat Al-Baqarah 2:183). Ramadan fasting is one of the most common types of fasting diets in which millions of Muslims around the world do not receive any food or drink for a daily time varies between 12 and 22 h (mean 12–14 h), depending on the geographical location and season during a special month for a month. Ramadan also spelled Ramazan, Ramzan, Ramadhan, or Ramathan, is the ninth month of the Islamic calendar, observed by Muslims worldwide as a month of fasting (sawm), prayer, reflection and community (5). According to Islamic law, during the days of Ramadan, healthy adults must fast at certain times of the day, while fasting is not required for premature children, the elderly, the sick, and pregnant and lactating women (6) #42; (7) #32.

In addition to Ramadan fasting diets, in recent years, there has been an increased interest in following modified fasting diets aimed at weight loss or the management of some chronic diseases among people in different countries (8). IF have greatly increased in recent decades as weight loss and some other metabolic benefits (9). The effectiveness of these diets in weight loss or management of metabolic parameters has varied depending on the type and duration of fasting diets (10).

The human gastrointestinal microbiome, which contains millions of organisms can be affected by various environmental factors such as diet. On the other hand, various studies have shown that adverse changes in the intestinal microbiome can be associated with the development of various chronic diseases (11). Some findings have revealed that fasting diets can also cause changes in the microbiome (12, 13).

The objective of this review is to summarize the current evidence for the effects of Ramadan and non-Ramadan IF on gut microbiome. We first review the evidence from pre-clinical studies to provide a background on the purported mechanisms by which fasting diets induces changes in gut microbiome and then focused on human studies.

METHODS

The PubMed, Web of Science, Scopus, and Google Scholar databases were searched from their inception until December 2021 according to the PRISMA criteria (Preferred Reporting Items for Systematic Reviews and Meta-Analysis). We used from the keywords included "gut microbiome" OR "Fecal microbiota" OR "Gut microbial profile" OR "Gut microbiota" OR "gut flora" OR "intestinal flora" OR "intestinal microbiota" in combination with Fasting OR "Intermittent fasting" OR "Ramadan Fasting" OR "Islamic fasting." Additional items were added after examining the referenced articles (**Figure 1**). Two authors (MR and SM) independently assessed the abstract and full text of the articles, and animal and human studies, which evaluated the effect of different types of fasting on the microbiome, were screened (**Table 1**). Disagreements were resolved by consensus. Studies were included in this review article if the following conditions were met: (1) animal and human studies investigating the effect of fasting diets on the gut microbiota, (2) in order to evaluating the gut microbiota alterations in various fasting conditions and probable mechanisms in improving overall metabolic health, types of fasting regimens were classified into two main subgroups (time restricted fasting including Ramadan fasting and 8/16 h fasting program and calorie restricted fasting including alternate day fasting, water only fasting and the Buchinger program). Studies were excluded if the main text was not available or was not in the English language. Reviews, protocols, conference papers and case reports were also excluded. Therefore, only original researches with original data on animal models or human patients exploring any kind of fasting regimes on gut microbiota were included in the present study.

RESULTS

Twenty-eight articles were included in the qualitative synthesis. The characteristics of the evaluated studies and their results, including the results of animal studies and human studies, are listed in **Tables 2**, **3**. The following are the results of animal studies and then the results of human studies.

EXPERIMENTAL STUDIES

Various animal studies have evaluated changes in the gut microbiome following a variety of fasting diets. Most animal studies on this interaction have been conducted in the past 5 years. Liu et al. in an experimental study compared the effect of intermediate fasting (IF) with melatonin administration on clinical variables and changes in the intestinal microbiome. They found that IF compared to the control group led to a significant increase in the abundance of *Lactobacillus*, *Ruminococcus*, and *Akkermansia* strains. Also, they found a significant reduction in the abundance of *Helicobacter*, *Prevotella*, and *Parasutterella* in the IF group (14). In another study on farmed mink (Neovision vision), the gut microbiota load and diversity showed no change after 3 days of fasting. Firmicutes were as the major phylum in the gut of these animals, however the Proteobacteria and Fusobacteria also were seen in another study (15). The rapid movement of food through the gastrointestinal tract may not allow enough time for bacterial metabolism to provide an environment that is suitable for growth of anaerobes (16).

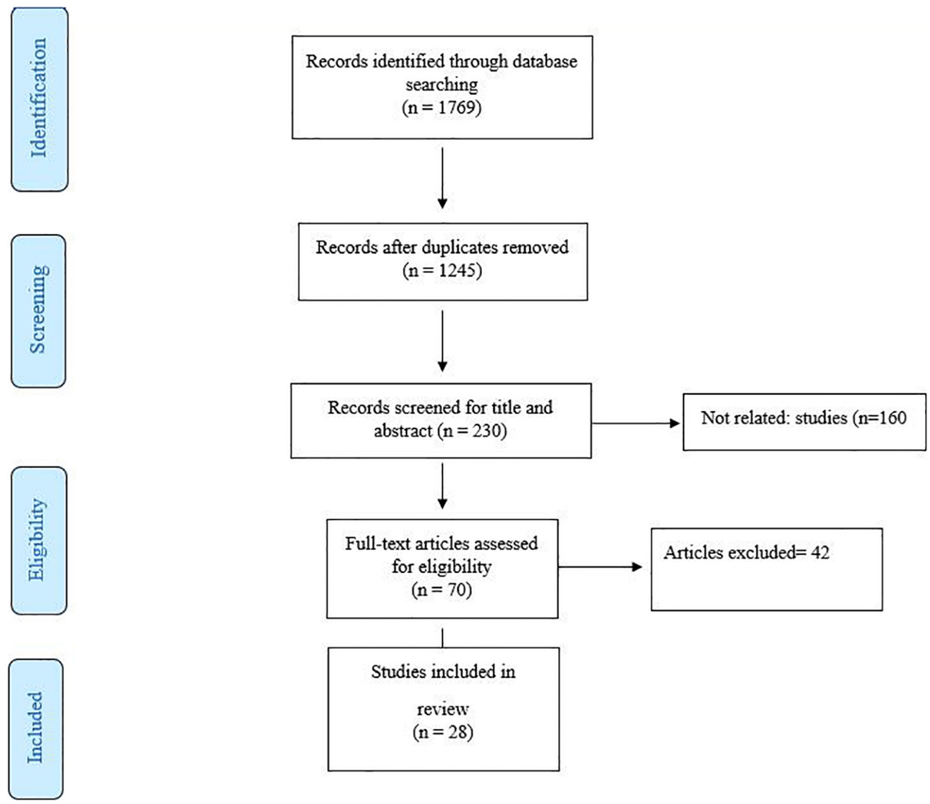

FIGURE 1 | Flow diagram of study.

Beli et al., evaluated the effects of long-term IF on gut microbiome, retinopathy and prolongs survival in db/db mice. The animals were fed *ad libitum* (AL) before the IF was initiated at 4 months of age. The db/db mice in the intervention group were then exposed to IF daily for up to 7 months. Microbiome analysis revealed increased levels of Firmicutes and decreased levels of Bacteroidetes and Verrucomicrobia in the IF group than control. Compared to the db/db mice on AL feeding, microbiome changes in the fasting group were associated with an increase in the gut mucin, goblet cell number, villi length, and reductions in plasma peptidoglycan (12). It has been reported in the previous studies that higher Firmicutes to Bacteroidetes ratio is associated with obesity (16, 17), as well as improve energy harvesting capacity (18). In this study, researchers used measurement of plasma peptidoglycan levels as an indicator of damage to the blood-brain barrier, and the results showed that IF regimen reduced plasma peptidoglycan levels and improved blood-brain barrier integrity. It has also been shown that a decrease in peptidoglycan concentration following IF is consistent with a reduction in endotoxemia (12). Therefore, fasting diets effect on weight loss through changes in the gut microbiota diversity and number, as well as peptidoglycan production. Gut microbiota involves major energy metabolic processes (19). Some studies have found a significant association between intestinal dysbiosis and energy dysmetabolism-induced chronic diseases such as diabetes, metabolic syndrome, and obesity (20). The positive results of the IF regimen on animal models with hypertension have also been shown in some studies (21).

Another part of animal studies has evaluated the effect of fasting on intestinal microbiome in animal models of neurodegenerative diseases. Cignarella et al. evaluated the effects of IF on gut microbiome and clinical symptoms of animal models of multiple sclerosis (MS), which named experimental autoimmune encephalomyelitis (EAE). They found that IF led to a significant improvement in the gut bacteria richness, enrichment of the Lactobacillaceae, Bacteroidaceae, and Prevotellaceae families and enhanced antioxidative microbial metabolic pathways. The results of this study also showed that the IF reduced the differentiation of native T cells into T17 cells, which secrete proinflammatory cytokines, and, conversely, increased the differentiation into regulatory T cells. Interestingly, the results of this study showed that fecal microbiome transplantation from mice under the fasting diet to mice with EAE ameliorated the symptoms, which could indicate the positive effect of the fasting diet (22).

On the other hand, some studies have shown that IF cause weight loss, reduce lipid peroxidation, and hepatic steatosis on obese mice through changes in microbial profile. Also, it has been reported in this study that IF led to a significant increase in the intestinal flora community diversity [Firmicutes to Bacteroidetes (F/B ratio) and relative increase in the *Allobaculum* abundance] (23). Increasing the abundance of Firmicutes following fasting diets can increase the production of short-chain fatty acids (SCFAs). SCFAs have the ability to increase the integrity of gut barrier, strengthen the immune system, reduce weight and insulin resistance (24). Moreover, fasting diets effect on the α-diversity (richness) and β-diversity (variety) of gut microbiota (12). Some pre-clinical studies have shown that IF could increase β-diversity, but the results on the effect of fasting diets on α-diversity are contradictory (12, 22, 25). Seven months IF on mice gut microbiota increased β-diversity compared in animals (12). Furthermore, weight loss introduced as the important and effective factor on α-diversity of gut microbiota (22), however it varies greatly during the day and dependents to dietary content (26, 27).

On the other hand, some studies have evaluated the effect of fasting diets on gut microbiota changes. Li et al. evaluated the effect of 12, 16, or 20-h fasting diets on the gut microbiome for 1 month. The results of this study showed that the composition of the gut microbiome changed in all types of fasting diets. At genus level, 16 h fasting led to increased level of *Akkermansia* and decreased level of *Alistipes*, but these effects disappeared after the cessation of fasting. No taxonomic differences were identified in the other two groups (28). In some previous findings, an increase in *Akkermansia* strains has been associated with metabolic benefits such as a reduction in the severity of fatty liver and intestinal inflammation (29). Increased levels of *Alistipes* can also exacerbate gut inflammation (30, 31).

Given that different metabolites are produced by the gastrointestinal microbiome, some other studies have evaluated these metabolites produced by microbiota following fasting diets. It has been reported an increased plasma levels of some metabolites such as tryptophan, serotonin, tryptophan, various bile acids, propionate, and acetate following the administration of fasting diets in animal samples (25, 32, 33). These results have also been confirmed in some human studies (34). Changes in the production of some metabolites can affect processes such as inflammation in the body. For example, some preclinical studies have shown that fasting diets exert inhibitory effects on the biosynthesis pathways of lipopolysaccharides by altering the intestinal microbiome. Lipopolysaccharides are among the major constituents of the outer membrane of Gram-negative bacteria, and studies have shown that increased production of lipopolysaccharides can induce toll like receptor-4 (TLR-4). TLR4 represents a key receptor on which both infectious and non-infectious stimuli converge to induce a proinflammatory response (35).

TABLE 1 | Table of inclusion and exclusion criteria following the PICOS approach[1].

PICOS	Inclusion and exclusion criteria	Data extraction
Participants	Adult population's ≥18 and ≤65 years or animal samples were included.	Age, sex, gender, sample size, location, inclusion and exclusion criteria
Intervention	Types of fasting regimens were classified into two main subgroups (time restricted fasting including Ramadan fasting and 8/16 h fasting program and calorie restricted fasting including alternate day fasting, water only fasting and the Buchinger program).	Types of fasting regimens, fasting duration
Comparators	Only studies with control group were included, participants or animal models with normal diet.	Type of comparator, compliance
Outcomes	Changes in the gut microbiota.	Outcomes measured, evaluation methods and side effects.
Study design	Studies were excluded if the main text was not available or was not in the English language. Reviews, protocols, conference papers, and case reports were also excluded. Therefore, only original researches with original data on animal models or human patients exploring any kind of fasting regimes on gut microbiota were included in the present study.	Design of the study, loss of the study

[1] *PICOS, participants, intervention, comparator, outcome, study type.*

HUMAN STUDIES

According to the positive results of pre-clinical studies, in recent years, various human studies have evaluated the association between intestinal microbiome and fasting. In some human studies, fasting diet of Ramadan type on intestinal microbiome has been evaluated (13, 36–39). The duration of fasting time was 12–18 h per day in these studies. The results of these studies mainly showed changes in the intestinal microbiome following adherence to Ramadan fasting, some of which are mentioned below. In a clinical study in 2021, Mohammadzadeh et al. evaluated the effect of Ramadan fasting on serum levels of butyrate, intestinal microbiome and lipid profile. The results of this study showed that the serum level of butyrate in the fasting group increased significantly after 1 month. There was also a significant increase in the bacteroides and filminus strains in the intervention group (39). In another study, which conducted on Pakistani and Chinese participants, researchers evaluated the effect of a 29-day Ramadan fasting on alpha and beta diversity. The results of this study showed that the population of some bacterial strains such as Bacteroidetes and Firmicutes increased in the Pakistani population following fasting, however no noticeable changes were observed in the Chinese population. In addition, it has been reported that fasting in both populations affects beta diversity. Moreover, lower levels of genus *Coprococcus* observed after Ramadan fasting suggesting that fasting could have implications on health. On the other hand, fasting could also have harmful effects on health (38). A study of two cohort data showed that following a Ramadan-associated IF increased microbiome diversity and was specifically associated with upregulation of the Clostridiales order–derived Lachnospiraceae. In fact, the fasting diet in this study increased the expression of butyric acid-producing Lachnospiraceae. These alternations were independent of living area, body weight and diet composition and disappeared again when fasting was stopped (13). Various studies have shown that changes in the intestinal microbiome cause changes in physiological functions and reduce energy intake.

TABLE 2 | Summary of the animal studies investigating the effects of fasting on gut microbiota.

Study	Dietary restriction regimen	Study model	Gut microbiota variations induced by dietary restrictions	Potential health benefits	Microbiota analyzing methods
Shi et al. (21)	IF for 4 days in two cycles	Hypertensive rat	*Lactobacillus* and *Bifidobacterium* abundance increased in the IF group than control.	Rats in the IF group had significantly lower blood pressure than control group.	Shotgun sequence analysis of the microbiota and untargeted metabolomics
Zhang et al. (42)	(1) Fed *ad libitum*, (2) 30% CR, (3) 5:2 IF regimen	7-week-old C57BL/6 male mice	30% CR led to a significant increase in the *Lactobacillus*, and significant reduction in the Bacteroidetes. 5:2 IF regimen led to increase in the *Bacteroides*, *Alloprevotella* and significant reduction in the *Lactobacillus*.	IF group consume more energy than *ad libitum* and CR groups in the first 4 days after refeeding. Both of the CR and IF group had lower body weights, white adipose tissue and serum cholesterol than *ad libitum* group.	16S rRNA gene sequencing
Liu et al. (14)	Four groups: control (C), intermittent fasting (F), melatonin (M), and intermittent fasting plus melatonin (MF)	Male C57BL/6J mice	The F and M groups had significantly lower alpha diversity than the MF group. Increase in the abundance of *Lactobacillus*, *Ruminococcus*, and *Akkermansia* in the F group than control group. Reduction in the abundance of *Helicobacter*, *Prevotella*, and *Parasutterella* in the F group than C group.	There was no difference between the groups in the cumulative food intake. IF group had lower body weight, serum glucose and TG than control or melatonin groups.	16S rRNA gene v3–v4 amplicon
Deng et al. (23)	*Ad libitum* (AL) group or an IF group for 30 days	Male C57BL/6J mice	IF did not change the bacterial community richness Reduction in the Firmicutes to Bacteroidetes (F/B ratio) and relative increase in the *Allobaculum* abundance.	Weight was significantly reduced in the fasting group, but the cumulative energy intake was not different. IF reduced liver steatosis and lipid metabolisme.	16S rDNA gene amplicon sequencing
Li et al. (28)	*Ad libitum* control group or intermittent fasting groups.	C57BL/6JLvri mice	There were not significant differences between two groups in alpha diversity Mice in the 16 h fasting had increased level of *Akkermansia* and decreased level of *Alistipes*.	Cumulative food intake was not changed in the 12 h fasting but changed in the 16 and 20 h fasting.	16S rRNA gene amplicon sequencing
Park et al. (43)	IF vs. ketogenic diet	Male Sprague Dawley rats: Alzheimer's disease (AD) model	In the IF group than keto group: Clostridiales abundance decrease and Lactobacillales increase.	IF than keto improved memory function.	16S rRNA amplicon sequencing
Kim et al., (44)	Fasting: the ruminal fluids feeding and 24 h after fasting	Three ruminally cannulated Holstein steers	Reduced abundance of *Anaerovibrio lipolytica*, *Eubacterium ruminantium*, *Prevotella albensis*, *Prevotella ruminicola*, and *Ruminobacter amylophilus*.	Increase in the gas, ammonia, and microbial protein production.	Denaturing gradient gel electrophoresis and quantitative polymerase chain react
Cignarella et al. (22)	In the IF mice, food pellets were provided or removed at 9 a.m. each day. Control group had unrestricted access to food	Mice	Lactobacillaceae, Bacteroidaceae, and Prevotellaceae families increased in the IF group. Fecal transplantation from mice in IF group to control, reduced the severity of EAE in this group.	IF reduced the differentiation of native T cells into T17 cells.	16S rRNA gene sequencing
Catterson et al. (45)	A 40-day course includes 2-day fed and 5 fasting days	Fruit flies (*Drosophila melanogaster*)	Reduced bacterial abundance in IF group than control Reduction in age-related pathologies and improved gut barrier function in the IF group.	Increases Stress Resistance, not changed cumulative food intake.	qPCR quantification of bacterial load
Beli et al. (12)	*Ad libitum* diet vs. intermittent fasting *ad libitum* diet as 24 h feeding-24 h fasting	db/db mice	Increased levels of Firmicutes and decreased Bacteroidetes and Verrucomicrobia in intermittent fasting group.	Glycated hemoglobin levels were not affected by the IF regimen, survival rate was significantly improved in the IF group.	16S rRNA sequencing with the MiSeq platform
Wei et al., (46)	Fasting diet with 30% restriction of calorie for 1 week	6-week-old male C57BL/ksJ-db	Increase in the Lactobacillaceae, Bacterioidaceae, and Prevotellaceae abundance.	Increase in the ketone production Decrease in the proinflammatory cytokines.	16 s rRNA sequencing
Bahl et al. (15)	3 days of food deprivation (fasting)	Farmed mink (Neovision vision)	The bacterial load and community structure within the mucus was not severely impacted by 3 days of fasting.	–	16S rRNA gene sequencing

(Continued)

TABLE 2 | (Continued)

Study	Dietary restriction regimen	Study model	Gut microbiota variations induced by dietary restrictions	Potential health benefits	Microbiota analyzing methods
McCue et al. (47)	21 days of fasting	Mice, quail, tilapia, toad, geckos	Alteration in Bacteriodetes, Firmicutes, Proteobacteria, Fusobacteria, and Verrucomicrobia.	Changes in distal intestine morphology.	16S rRNA sequencing
Sonoyama et al. (48)	96 h fasting compared to the control group	Male Syrian hamsters	Increase in the proportions of injured bacterial Cells Increase *Akkermansia muciniphila*, a mucin degrader, in fasting group Clostridia increased in the fed group	Reduction of total SCFA concentration in the fasted group than fed group.	16S rRNA clone library and species specific real-time quantitative PCR

AD, Alzheimer's disease; CR, calorie restriction; IF, Intermediate fasting; SCFA: short chain fatty acid.

TABLE 3 | Summary of the human studies investigating the effects of fasting on gut microbiota alterations.

References	Fasting model	Study type/duration	Study population	Results
Su et al. (49)	1 month of intermittent fasting	Longitudinal physiologic data in 2 cohorts, sampled in 2 different years	Healthy non-obese young and middle-aged men	Ramadan-associated intermittent fasting increased microbiome diversity and was specifically associated with upregulation of the Clostridiales order–derived Lachnospiraceae
Mohammadzadeh et al. (39)	Hour time restricted feeding intervention (8-h feeding window/16-h fasting window)	Before/after the cross-sectional study	Healthy adult volunteers (*n* = 30)	Butyrate significantly increases, the gut Bacteroides and Firmicutes increased by 21 and 13% after Ramadan.
Gabel et al. (40)	A daily 8-h time restricted feeding (8-h feeding window/16-h fasting window) for 12 weeks	Pilot study/12 weeks	Adults with obesity (*n* = 14)	Gut microbiota phylogenetic diversity remained unchanged.
Maifeld et al. (50)	Ramadan fasting	Clinical trial	Healthy subjects (*n* = 30)	Fasting alters the gut microbiome, impacting bacterial taxa and gene modules associated with short-chain fatty acid production.
Maifeld et al. (50)	5-days with a daily nutritional energy intake of 300–350 kcal/day, derived from vegetable juices and vegetable broth, followed by a modified Dietary Approach to Stop Hypertension diet	Randomized-controlled bi-centric/12 weeks	Patients with Metabolic Syndrome (*n* = 32–31)	Fasting alters the gut microbiome, impacting bacterial taxa and gene modules associated with short-chain fatty acid production.
Lilja et al. (51)	Buchinger fasting: 250 kcal/day for 5 days	RCT	154 healthy adults	↑ Distribution of Proteobacteria, ↓ Firmicutes/Bacteroidetes ratio fasting mimetic
Guo et al. (41)	"Two-day" modified IF	Clinical trial, 8 weeks	Adults with Metabolic Syndrome (*n* = 39)	Changes in gut microbiota communities, increase the production of short-chain fatty acids, and decrease the circulating levels of lipopolysaccharides.
He et al. (52)	Water-only fast or juice fast for 7 days	Intervention pre-post design	16 healthy individuals, age: 18–40 years	Water-only fasting changed the bacterial community, ↑ more homogenous gut microbiomes, ↓ *Fusobacterium*. ↓ Colorectal cancer
Ali et al. (38)	Ramadan fasting	Cohort	Healthy adult participants (*n* = 34)	↑ *Klebsiella*, *Faecalibacterium*, *Sutterella*, *Parabacteroides*, and *Alistipes* ↓ *Coprococcus*, *Clostridium_XlV*, and *Lachnospiracea*
Balogh et al. (53)	Buchinger fasting protocol followed by DASH diet	RCT/5 days	Control (*n* = 36), fasting (*n* = 35)	↑ *Clostridial Firmicutes* ↓ *Butyrate producers*
Ozkul et al. (37)	Ramadan fasting	Pilot study/29 days	Healthy adult participants (*n* = 9)	*Butyricicoccus, Bacteroides, Faecalibacterium, Roseburia, Allobaculum, Eubacterium, Dialister*, and *Erysipelotrichi* were significantly enriched genera after the end of Ramadan fasting.
Mesnage et al. (54)	Buchinger fasting (daily energy intake of about 250 kcal and an enema every 2 days	Clinical study/10-day	Healthy men (*n* = 15)	Decrease in the abundance of Lachnospiraceae and Ruminococcaceae increase in Bacteroidetes and Proteobacteria (*Escherichia coli* and *Bilophila wadsworthia*).
Remely et al. (55)	A fasting program with laxative treatment for 1 week followed by a 6-week intervention with a probiotic formula	One week	Overweight people (*n* = 13)	Fasting group had higher abundance of *Faecalibacterium prausnitzii*, *Akkermansia*, and *Bifidobacteri*

Thus, human microbiome can be an effector for physiologic effects of IF (13). In another preliminary study, it was found that following the Islamic fasting diet caused significant changes in the intestinal microbiome, so that the number of *A. muciniphila* and *B*acteroides *fragilis* group members increased, however, *Lactobacillus* spp. and *Bifidobacterium* spp. remained relatively unchanged perhaps due to low fiber intake (36).

In addition to Ramadan fasting, some studies have examined the effect of restricted feeding in a form of IF on the intestinal microbiome. One of the major problems seen in these studies is the low sample size. Therefore, it is difficult to generalize the results of these studies to large populations. Gabei et al. in a pilot study evaluated the effect of fasting in a form of IF on the intestinal microbiome in adults with obesity. They found that IF led to a significant weight loss. Baseline evaluation of fecal microbiome by 16 S rRNA (ribosomal ribonucleic acid) gene sequencing showed that the predominant strains included Firmicutes and Bacteroidetes. However, at the end of 12 weeks of fasting diet, no significant change was observed in the abundance and distribution of dominant bacterial strains (40). However, the results of some other studies were inconsistent with this study. Guo et al. in a RCT study were evaluated the effects of 8 weeks of "2-day" modified IF in patients with metabolic syndrome. The results of this study revealed that 8 weeks of "2-day" modified IF led to a significant reduction in fat mass, oxidative stress, inflammatory cytokines, and improved vasodilatory parameters. On the other hand, the results of this study showed that following the 8 weeks of "2-day" modified IF caused a significant change in the composition of the intestinal microbiome, increased the production of SCFA and decreased lipopolysaccharide levels (41).

CONCLUSION

In this review study, we evaluated the effects of Ramadan and non-Ramadan IF on gut microbiome. The results of most animal and human studies indicate the positive effects of fasting on the composition and structure of the gut microbiome. In addition to the positive role of fasting on the composition and abundance of intestinal microbiome, in some studies, other positive results have been observed following the observance of fasting regimes. Positive alterations in gut microbiota, such as overexpression of *A. muciniphila*, *B. fragilis*, *Bacteroides*, and butyric acid-producing Lachnospiraceae, were found to be associated with improved health indicators and decreasing disease development during Ramadan fasting. However, factors such as the duration of fasting diets, the presence of chronic diseases and obesity can affect the results. Considering the role of intestinal microbiome changes in the management of various diseases, future studies, especially clinical studies, should evaluate the impact of fasting regimes, especially Ramadan, on the management of various diseases through changes in the intestinal microbiome.

AUTHOR CONTRIBUTIONS

MR and ER: conception and design, and systematic search. SM and JH: screening and data extraction. MR and RT: manuscript writing. All authors contributed to the article and approved the submitted version.

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Ramadan Fasting Among Older Children and Adolescents With Type 1 Diabetes Mellitus: A Real-World Study From the UAE

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Background: Ramadan fasting (RF) is a religious obligation for all healthy adult Muslims. The sick and pre-pubertal children are exempt, but many choose to fast for various reasons. In this "real world" study, glycaemic control has been investigated in the context of RF in children and adolescents with type 1 diabetes mellitus (T1DM) and compared multiple daily injections (MDI) and continuous subcutaneous insulin infusion (CSII) outcomes.

Methods: Children and adolescents with T1DM seen at Imperial College London Diabetes Centre who decided to fast in the ensuing Ramadan were educated with their families about diabetes mellitus management during RF using an adapted CHOICE (Carbohydrate, Insulin, and Collaborative Education) educational programme. Pertinent data including hypoglycaemia episodes and diabetic ketoacidosis (DKA) were obtained through patient/family interviews. Information on weight, glycated hemoglobin (HbA1c), and blood glucose levels from continuous glucose monitoring (CGM)/flash glucose monitoring (FGM) before (1 month prior), during, and after (1 month afterwards) Ramadan were retrieved retrospectively from the electronic database. Data are presented as mean ± SD.

Results: Forty-two patients [age 13.5 ± 2.4 years; 27 (64.3%) males; T1DM duration 4.9 ± 3.1 years] were included in the study and were able to fast for 22 ± 9 days during Ramadan. Twenty-three (54.8%) of the patients were on MDI and 19 (45.2%) were on CSII. No statistically significant differences were seen in CGM/FGM generated mean blood glucose level before, during, and after Ramadan [one-way ANOVA ($F_{(2, 80)}$ =1.600, $p = 0.21$)]. HbA1c and weight after Ramadan did not change significantly compared to baseline (paired *t*-test; $p = 0.02$ and $p = 0.08$, respectively). Between MDI and CSII groups, there was no significant difference in fasting days ($p = 0.49$), frequency of hypoglycaemia episodes ($p = 0.98$), DKA frequency ($p = 0.37$), HbA1c level ($p = 0.24$), and weight ($p = 0.11$) after Ramadan.

Conclusion: Data show no significant deterioration in indicators of overall glycaemic control which remained inadequate. RF should be discouraged in children with poorly controlled T1DM.

Keywords: Ramadan fasting, type 1 diabetes mellitus, glycaemic control, flash glucose monitoring, continuous glucose monitoring

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INTRODUCTION

Fasting during the Muslim holy month of Ramadan is one of the five pillars of Islam for all healthy adult Muslims. The fast entails abstinence from eating and drinking from dawn to sunset for a whole lunar month (29 or 30 days). There are no restrictions on food or fluid intake between sunset *(iftar*) and early dawn (*sohour*) (1). The main meal, *iftar*, is taken at sunset and is usually a heavy meal served with deeply fried food and dessert. The other meal, *sohour*, which is taken before sunrise is normally lighter and is primarily composed of complex carbohydrate (2).

Children, the elderly, travelers, pregnant or nursing women, and sick individuals including those with diabetes mellitus, are exempted from fasting. However, many Muslims, both adults and children, including those with diabetes mellitus opt to undertake Ramadan fasting (RF) even when religiously not required, for cultural, social and personal reasons (3). Fasting in people with diabetes mellitus has certain risks including susceptibility to hyperglycaemia after excessive consumption of sweet and fried foods during and after *iftar*, risk of diabetic ketoacidosis (DKA), and hypoglycaemia (4, 5).

Management of type 1 diabetes mellitus (T1DM) among children and adolescents is a challenge, even outside RF periods (6). However, children and adolescents with T1DM are often keen to fast during Ramadan and are able to do so for a significant number of days (6). Amongst users of continuous subcutaneous insulin infusion (CSII) or multiple daily insulin injections (MDI), the main treatment regimens used for older children and adolescents with T1DM, reports indicate no significant difference in glycaemic control with "reasonable risk" of hypoglycaemia and hyperglycaemia (7, 8).

The United Arab Emirates (UAE) has one of the world's highest prevalence rates of diabetes mellitus at 16.3% according to the report of International Diabetes Federation (IDF) in 2019 (7). There are over 1 million people living with diabetes mellitus in the UAE, placing the country in the 15th place worldwide for age-adjusted comparative diabetes mellitus prevalence (9). Studies further point out that the incidence of diabetes mellitus in the UAE is rising at a faster rate than both the MENA (Middle East and Northern Africa) region and the rest of the world (10). Risk factors including rapid economic growth, sedentary lifestyles and unhealthy diets are characteristic to the UAE, and it is expected that there will be 2.2 million individuals with diabetes mellitus in the country by 2040 (7). The incidence of T1DM in the UAE has also risen, making it one of the countries with higher prevalence worldwide (11). In 2019, the new cases were estimated at 20.8 per 1,000 in children and adolescents aged 10–19 years old (12).

Over the last few years, several studies have investigated diabetes mellitus outcomes with RF in adults; studies in the pediatric population in this context have been more limited. This often poses challenges to health care providers in providing medical advice to patients of this age group on the feasibility of RF partly because little is known about the safety or metabolic effects of fasting in this group of patients (13).

The current cohort study investigates the outcomes of RF using MDI and CSII to assess patterns of glycaemic control and severity of complications during RF amongst older children and adolescents with T1DM seen at the Imperial College London Diabetes mellitus Center (ICLDC), UAE. The effects of dose adjustment, health professional team and parental support on safety and glycaemic outcomes in children and adolescents with T1DM during Ramadan were also investigated.

PATIENTS AND METHODS

Study Design and Participants

This was a retrospective study on children and adolescents with T1DM and their families seen at ICLDC who were routinely evaluated in the year 2019. Ramadan began on May 6th, 2019 and ended on June 4th, 2019; RF lasted for 30 days in the UAE with around 14 hours of fasting per day. Study population age range was between 9–18 (13.5 ± 2.4) years and as such included older children and adolescents as defined by the World Health Organization (14).

Approximately one month before Ramadan, participants who resolutely intended to fast during Ramadan were educated about diabetes mellitus management during fasting. Medical team included physicians, nurses, diabetes mellitus educators, dieticians, and telemedicine staff. The CHOICE (Carbohydrate, Insulin, and Collaborative Education) educational programme has been adapted by the pediatric diabetes mellitus and endocrinology clinic in ICLDC and was used to guide before Ramadan education during this study. The programme recommends insulin dose adjustments according to diet and lifestyle changes in Ramadan (15, 16).

The programme focuses firstly on insulin dose adjustments. For insulin pump users who normally have a follow up visit and before Ramadan preparation, patients have their pumps adjusted to a new setting in which insulin dose is decreased during daytime, increased after *iftar*, and decreased before *sohour* to avoid hypoglycaemia. Patients on MDI are instructed to decrease their long-acting insulin by 20%, decrease *sohour* meal short acting insulin by 10–20%, and increase short acting insulin for *iftar* meal by 0–10% according to their needs.

The second arm of the educational programme before Ramadan is dietetic advice. Diet plans are individually designed by dietitians to target consumption of complex carbohydrate rich food in *iftar* meal, with strict recommendations to avoid high sugar containing food and snacks. Patients are further instructed to start their plan of decreasing the long-acting insulin 1 day before the start of Ramadan. Parents' proactive role is essential in closely tracking the blood glucose level of their children in the first couple of days of RF especially after 4 pm (around 2 h before *iftar* meal).

Patients and their parents are strictly advised to break the fast if hypoglycaemia occurs, even if it is only a few minutes before *iftar*, to avoid complications. If the first day passed safely and easily, patients are allowed to continue fasting provided they will follow the recommendations. If the first day had episodes of hypoglycaemia, further reduction of long-acting insulin is recommended with close monitoring, and the physician and/or diabetes mellitus educator can be contacted if needed. The flow chart of the methods is outlined in **Figure 1**.

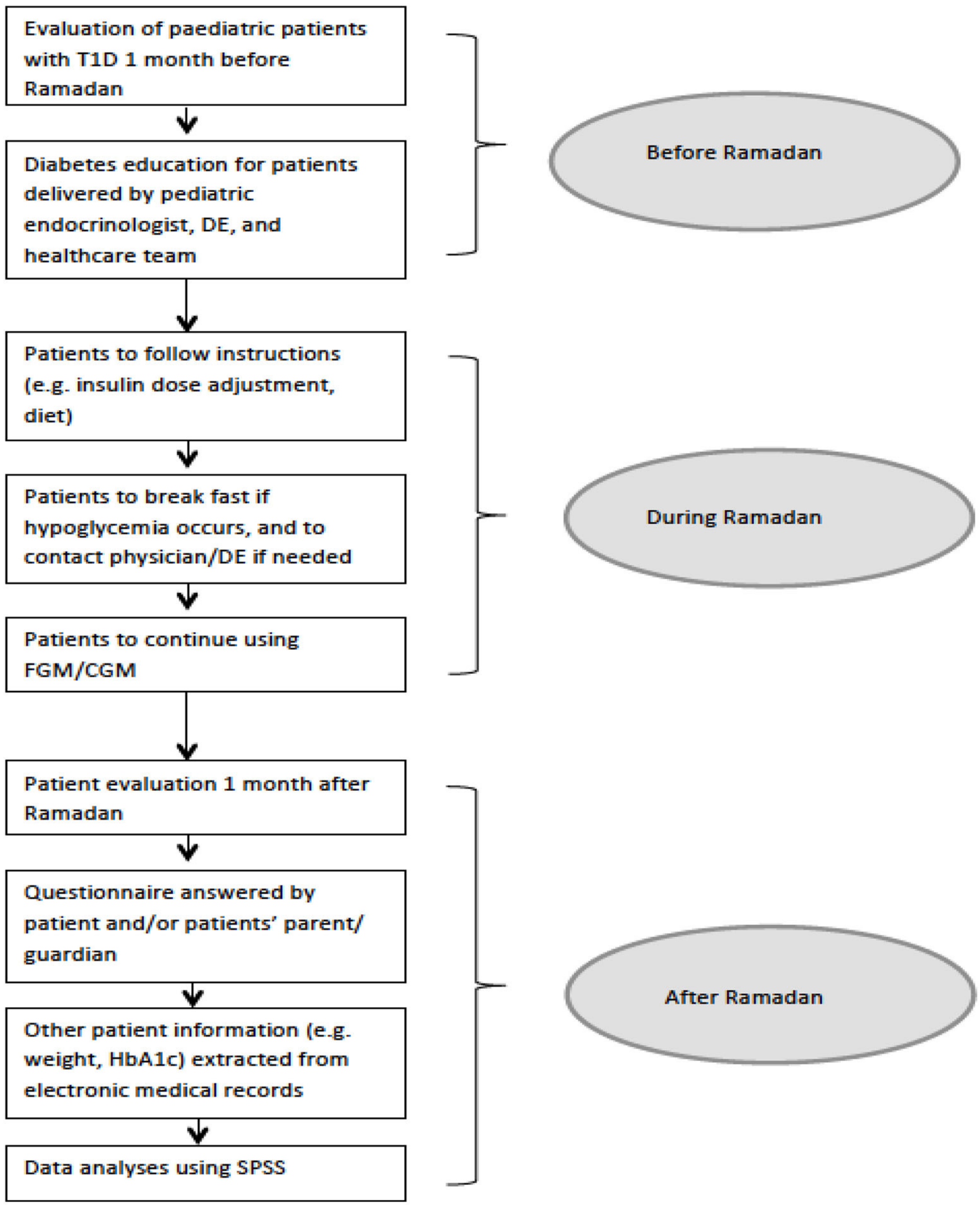

FIGURE 1 | Study flowchart: an overview of study design. T1DM, type 1 diabetes mellitus; DE, diabetes mellitus educator; CGM, continuous glucose monitoring; FGM, flash glucose monitoring; SPSS, Statistical Package for the Social Sciences.

Outcome Measures

The mean fasting hours was 14 h and 40 min as it was at the summer season in UAE. Average temperature was 31°C, while humidity ranged between 45 and 55%.

Approximately one month after RF, a questionnaire was given to the participants (and their families) on their clinic visit after Ramadan. The questionnaire was in English (Arabic translation included) with eight items; questions included age, type of treatment, continuous glucose monitoring/flash glucose monitoring (CGM/FGM) sensors used, days fasted, hypoglycaemia episodes, frequency of DKA, best support noted during the fast, and willingness to fast in the next Ramadan 2020. Information from the centre's database was retrieved for weight, insulin dose, glycated hemoglobin (HbA1c), and blood glucose levels based on uploaded sensor readings. Blood samples collection and weight assessment were usually done during clinic attendance between 7 am-2 pm after 6–8 hours fasting. Patients were grouped and compared according to insulin regimen (MDI vs. CSII) and outcome variables (fasting days, HbA1c and blood glucose levels, weight, hypoglycaemia episodes, insulin dose, and DKA) were analyzed. Written general consent including use of data for research use was provided by the participants and/or

TABLE 1 | Baseline characteristics of the study cohort.

Parameters	Total (n = 42)
MDI	23 (54.8%)
CSII	19 (45.2%)
Sex (male)	27 (64.3%)
Age (years)	13.5 ± 2.4
Duration of T1DM (years)	4.9 ± 3.1
Insulin, basal (units/day; n = 14)	23.9 ± 10.4
Insulin, bolus (units/meal; n = 20)	13.3 ± 7.8

MDI, multiple daily injections; CSII, continuous subcutaneous insulin infusion; T1DM, type 1 diabetes mellitus.

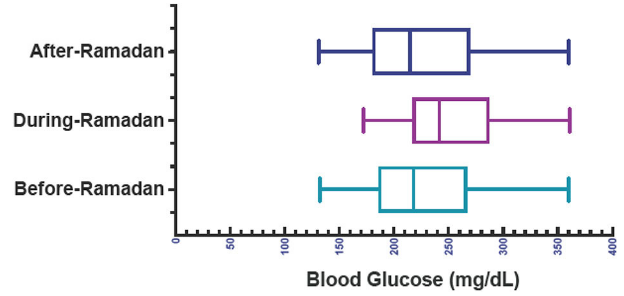

FIGURE 2 | Blood glucose levels before-, during-, and after-Ramadan.

participants' parents/legal guardian upon patient registration in the centre.

Statistical Analysis

Frequency (percent) and mean ± standard deviation (SD) was computed for the numeric and categorical variables. Student's t-test and one-way analysis of variance (ANOVA) were used to compare the outcome variables as appropriate. For evaluation of "reasonable" RF risk on glycaemic control, statistical significance was reported for a p-value < 0.01. Data analyses were performed using Statistical Package for Social Sciences (SPSS) version 24.

RESULTS

Forty-seven patients signified intention to fast during Ramadan; data of five (11%) patients were excluded in the analyses because of fasting plans withdrawal. Forty-two (89%) patients were included in the study; fasting days of 22 ± 9 days during Ramadan. All patients' pubertal stage was within normal range for age based on routine clinical assessment. Baseline characteristics of the study cohort are summarized in **Table 1**. Majority (46.8%) of the participants used flash glucose monitoring FGM flash glucose monitoring (Freestyle Libre) as their glucose monitoring system (36.2% Enlite, 14.9% Accu-chek).

For all participants (n = 42), there was no statistically significant difference in mean blood glucose levels before- (226.5 ± 54.6 mg/dL), during- (226.5 ± 54.6 mg/dL), and after- (225.7 ± 58.8 mg/dL) Ramadan [one-way ANOVA; $F_{(2, 80)} = 1.600$, $p = 0.208$; **Figure 2**]. HbA1c levels (9.0 ± 1.7 vs. 8.7 ± 1.3) and weight (53.3 ± 18.0 vs. 54.9 ± 19.3) before and after Ramadan did not change significantly compared to baseline (paired t-test; $p = 0.02$ and $p = 0.08$, respectively).

In the MDI group, there was no significant difference in paired p-values for before- and during-Ramadan (p = 0.132), before- and after-Ramadan (p = 0.865), and during- and after-Ramadan blood glucose levels; similarly, no statistically significant difference was found for the CSII group (p = 0.957, p = 0.964, p = 0.225, respectively). Between the MDI and CSII groups, there was no significant difference in unpaired p-values for before- and during-Ramadan (p = 0.368), before- and after-Ramadan (p = 0.872), and during- and after-Ramadan (p = 0.434) (**Table 2**).

In addition, between the two groups, there was no significant difference in fasting days (p = 0.49), HbA1c levels (p = 0.24), and weight (p = 0.11) after Ramadan; as well as reported frequencies of hypoglycaemia episodes (p = 0.98) and DKA (p = 0.37) during Ramadan (**Table 3**). More than half (57%) of the patients and their families noted dose adjustment as the main support during the fast, followed by parents' support (45%). All (100%) of the patients who completed the fast were willing to fast in the next Ramadan.

DISCUSSION

Previous studies have shown that effective counseling before RF delivered by health care providers reduces the incidence of hypoglycaemia (17). Patient education before Ramadan provides a platform to remind patients about the importance of diet, exercise, dose adjustment and that regular glucose monitoring is vital to avoid complications while reassuring them that this does not invalidate the fast (9). Patient education is certainly a cornerstone of Ramadan diabetes mellitus management (18), and is stringently applied in the ICLDC pediatric clinic. Patients are instructed that the first day of Ramadan is the most important day to carefully assess glycaemic control as it gives a provisional prediction of glucose excursions in the upcoming month of fasting. Other topics discussed during before Ramadan education sessions include prior fasting experiences, ways to reduce blood glucose levels fluctuations and complications, how to improve self-reliance and confidence, tailored change plan for dose and pump programming adjustments, and more importantly, informed decision-making about fasting. This education plan offers an imperative opportunity to allow those who wish to fast to do so more safely (9).

The recent 2021 recommendations of the (IDF)/Diabetes and Ramadan (DAR) Alliance (19) discourage children and adolescents with T1DM to practice RF. However, many children and adolescents choose to fast (20), presenting a challenge to health care providers. Children and adolescents can be even more eager to fast compared to their adult family members, a phenomenon related to self-esteem and happiness (21, 22). The Islamic norm on RF in this age group is for Muslim children to fast when specific features of puberty are attained (21, 23). The challenge to physicians is primarily in providing intensive

TABLE 2 | Changes and comparisons in blood glucose levels before-, during-, and after-Ramadan.

Cohort	Average blood glucose level (mg/dL) before 4-week Ramadan fasting		Average blood glucose level (mg/dL) during Ramadan compared with level before 4-week Ramadan fasting				Average blood glucose level (mg/dL) after Ramadan compared with level before 4-week Ramadan fasting				Average blood glucose level (mg/dL) during Ramadan compared with level after 4-week Ramadan fasting			
	Mean	SD	Mean paired difference[a]	SD	Paired p-value[b]	Unpaired p-value[c]	Mean paired difference[a]	SD	Paired p-value[b]	Unpaired p-value[c]	Mean paired difference[a]	SD	Paired p-value[b]	Unpaired p-value[c]
MDI $n = 23$, girls/boys = 8/15, mean (SD) age = 13.1 years (2.6)	222.5	57.9	24.4	40.4	0.132 ($n = 8$)	0.368	−2.3	50.1	0.865 ($n = 14$)	0.872	−9.9	52.9	0.64 ($n = 7$)	0.434
CSII $n = 19$, girls/boys = 7/12, mean (SD) age = 14.1 years (2.1)	230.6	52.7	−1.3	66.1	0.957 ($n = 8$)		0.5	40	0.964 ($n = 13$)		−36.5	56.9	0.225 ($n = 5$)	

[a]Paired difference indicates change in blood glucose level for each subject (i.e., positive and negative values indicate increase and decrease in blood glucose level, respectively); [b]Paired p-value compares the mean paired differences within cohorts; [c]Unpaired p-value compares the mean paired differences between the two cohorts. CSII, continuous subcutaneous insulin infusion; MDI, multiple daily injections.

TABLE 3 | After Ramadan values on fasting days, hypoglycaemia and DKA frequency, HbA1c, and weight: MDI and CSII compared.

After Ramadan	Total ($n = 42$)	MDI ($n = 23$)	CSII ($n = 19$)	*P*-value
Fasting days	22.3 ± 8.7	23.2 ± 8.1	21.3 ± 9.5	0.49
Hypoglycaemia frequency*	1.1 ± 2.3	1.1 ± 2.2	1.1 ± 2.5	0.98
DKA frequency*	0.02 ± 0.15	0.04 ± 0.21	0 ± 0.00	0.37
HbA1c (%)	8.7 ± 1.3	8.9 ± 1.4	8.4 ± 1.1	0.24
Weight (kg)	54.9 ± 19.3	50.5 ± 20.4	60.1 ± 16.9	0.11

*DKA, diabetic ketoacidosis; HbA1c, glycated hemoglobin; MDI, multiple daily injections; CSII, continuous subcutaneous insulin infusion; *reported for during Ramadan. Data is presented as mean ± SD.*

pre-fasting education and emphasizing the need for closer blood glucose monitoring during the fast (2).

Although several studies have investigated different aspects of RF in individuals with diabetes mellitus, most exclude children and adolescents. The issues and challenges in this age group are, in fact, often more pronounced and, in some ways, different. During childhood, the responsibility on managing diabetes mellitus falls on the parents who usually decide on whether to proceed with the full RF for their children. The parents try to monitor blood glucose fluctuations and make adjustments of insulin doses with primary aim of avoiding hypoglycaemia. As a result, exaggerated hyperglycaemic response may happen and is more pronounced at the time when fasting is broken (*iftar*). In adolescents, the problems can be even more challenging because at this age, they prefer to take charge of their disease management and are often less predictable with their dietary and medication compliance (20, 24).

International guidelines on diabetes mellitus management for adults during RF confirm that poor control of diabetes mellitus is considered as one of the main contraindications for fasting (17, 25–27). Well-controlled children and adolescents with T1DM are expected to have less complications making RF feasible for them (28). Studies on the effects of RF in children with diabetes mellitus have been few and results have been inconsistent. While some studies showed stable HbA1c before and after Ramadan (22, 29), some other studies showed deterioration of glycaemic control in the form of elevated HbA1c after Ramadan (24, 30). Bin-Abbas et al., suggested that fasting can be safe for well-controlled adolescents with T1DM who are using CGM devices (29), whereas other investigators have asserted that RF might predispose to acute complication in patients of this age group (30, 31). Further diabetes mellitus management challenges exist for poorly controlled children and adolescents with T1DM who fast against medical advice, as in some patients in the cohort. Study showed no significant differences were observed on mean blood glucose levels, HbA1c, and weight before- and after-Ramadan. These results need to be interpreted with caution. Our previous studies in adults indicate that HbA1c is a poor indicator of changes in glycaemic control when comparing a one-month time window such as Ramadan with another. A similar principle may be applicable in the pediatric population and that a CGM/FGM-derived glucose management indicator (GMI, or estimated HbA1c) may be better suited for looking at any such potential change more closely. Furthermore, adult studies show definite differences in glucose profiles of individuals with diabetes mellitus during Ramadan when compared with non-Ramadan periods, with a significant glucose excursion at *iftar* time which is more pronounced in people with poorer glycaemic control (32).

The results of this study also support previous studies which show that outcomes of CGM/FGM use with both MDI and CSII during RF are not significantly different. A recent similar study in Kuwait (33) is also in agreement with the present study that intensive monitoring along with patients' education before Ramadan are crucial for safe RF in the cohort. Another study conducted in Saudi Arabia reported that children and adolescents with T1DM using FGM could fast Ramadan without severe hypoglycaemia or DKA risks (34).

Limitations

This was a single centre study conducted in a specialized diabetes mellitus management centre, and therefore some selection bias may have influenced the glycaemic profile excursion. Moreover, the small sample size of the study might skew the data, as it was done on older children and adolescents with T1DM who decided to fast Ramadan. Importantly, studies established with more CGM data had shed more light on RF-induced effect on glycaemic profiles in adults; such research may also give more depth and breadth in studies on children and adolescents.

CONCLUSION

This study sheds light on the practice of RF in children and adolescents which is often a personal decision supported by family, social and cultural values which may not necessarily be supported by religious recommendations. The challenge to healthcare professionals is significant; and this study shows the impact of a modified CHOICE before Ramadan education programme (16) guided by the IDF-DAR recommendations (19) in addition to the expertise of a diverse health care team. More evidence from future studies is needed to improve medical guidelines and help health care providers take proper clinical decisions.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written general consent including use of data for research use was provided by the participants and/or participants' parents/legal guardian upon patient registration in the centre.

AUTHOR CONTRIBUTIONS

TM: study concept, data acquisition, and manuscript editing. EF: statistical analyses and manuscript writing. RH: data interpretation and manuscript writing. NL: study design, data interpretation, and manuscript editing. All authors contributed to the article and approved the submitted version.

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The Effect of Coronavirus Disease-19 Pandemic Lockdown and the Overlapping Ramadan Fasting Period on Glucose Control in Insulin-Treated Patients With Diabetes: A Flash Glucose Monitoring Study

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Background: A strict lockdown was enforced during coronavirus disease (COVID-19) pandemic in many countries including the UAE. Lockdown period overlapped with Ramadan which is accompanied by its own drastic changes in lifestyle that include meal timings.

Aims: We report the impact of COVID-19 lockdown (between 22/3/2020 and 24/6/2020) on glucose control pre- and postlockdown and during Ramadan, in patients with type 1 diabetes (T1D) and type 2 diabetes (T2D) on insulin therapy.

Methods: A number of twenty-four patients (19 men, 6 women) who were monitoring their glucose levels using flash glucose monitoring (FGM) and remotely connected to the diabetes clinic in Imperial College London Diabetes Centre (ICLDC), Abu Dhabi, UAE were included. Using the international consensus on the use of continuous glucose monitoring guidelines, analyses of data were performed on glucose management indicator (GMI), time in range (TIR), time in hyperglycemia, time in hypoglycemia, low blood glucose index (LBGI) and high blood glucose index (HBGI). Variables were calculated for each period: 30 days before lockdown 14/2/2020–14/3/2020, 30 days into lockdown and pre-Ramadan 20/3/2020–18/4/2020, and 30 days into lockdown and Ramadan 24/4/2020–23/5/2020, using cgmanalysis package in R-studio software.

Results: Mean average glucose (MAG) remained steady before and during lockdown, and no significant differences were observed in TIR, time in hypoglycemia, and LBGI between prelockdown and lockdown periods. However, there was a statistically significant difference in GMI and percentage of time in hyperglycemia (>10.0 mmol/L) between Ramadan and pre-Ramadan during the lockdown period in $p = 0.007$, 0.006, and 0.004, respectively. Percentage of TIR (3.9–10.0 mmol/L) was significantly lower in Ramadan as compared to pre-Ramadan (50.3% vs. 56.1%; $p = 0.026$). Mean absolute glucose (MAG) (182.0 mmol/L vs. 166.6 mmol/L, $p = 0.007$) and HBGI (10.2 (6.8, 14.8) vs. 11.9 (7.9, 17.8), $p = 0.037$) were significantly higher in Ramadan

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compared to pre-Ramadan period. There was no statistically significant difference in percentage of time in hypoglycemia (<3.9 mmol/L) and LBGI between Ramadan and pre-Ramadan periods.

Conclusion: The lockdown period had no significant effects in the markers of glycemic control in the population studied. However, Ramadan fasting period embedded within this time was associated with several changes that include increase in GMI, HBGI, and glycemic variability similar to what has been reported in other Ramadan studies.

Keywords: Ramadan fasting, type 1 diabetes, type 2 diabetes, flash glucose monitoring, COVID-19, lockdown

INTRODUCTION

Fasting during the lunar month of Ramadan is a religious responsibility for adult Muslims which entails daytime fasting for 29–30 days. Ramadan fasting is one of the five pillars of Islam. Several groups are exempted from this obligation (including acute and/or chronic illnesses). However, people included in these exemptions, which include some patients with diabetes often choose to proceed with Ramadan fasting for personal, social and cultural reasons, and their individual perceptions of religious law (1). People with diabetes are generally confronted with serious risks such as hypoglycemia and hyperglycemia. The Epidemiology of Diabetes and Ramadan (EPIDIAR) survey of people with diabetes in 13 Islamic countries revealed that around 43% of people with type 1 diabetes mellitus (T1D) and 79% of people with type 2 diabetes mellitus (T2D) fast during Ramadan despite the presence of exemptions (2).

Ramadan in the year 2020 was very different from previous years due to the outbreak of coronavirus disease (COVID-19). The COVID-19 pandemic response in many countries included several restrictions. In the United Arab Emirates (UAE), a strict lockdown was enforced between 22 March 2020 and 24 June 2020. The lockdown period overlapped with Ramadan fasting which lasted for 30 days in UAE and with around 14 h of fasting per day. Ramadan began on 23 April 2020 and ended on the 23 May 2020.

Ramadan is inevitably associated with its own drastic changes in lifestyle that include sudden change in mealtimes, sleep, and routine daily activity. The first meal in Ramadan day (*Iftar*) consumed after a period of fasting is usually of high-calorie diet which might contribute to hyperglycemia (3). In normal individuals, there is a slight drop in average blood glucose levels in the beginning of Ramadan followed by stabilization through the second half of the month. Yet, all variations are within physiological range (4).

Patients with diabetes who tend to continue their routine medications usually build up episodes of hypoglycemia depending upon medication compliance, type of consumed food, modifications in physical activities, or binge eating after the *Iftar* meal (5, 6). Some previous studies showed conflicting changes in the overall glycemic control during Ramadan as compared to pre-Ramadan period (3, 7–10).

Our previous study (11) reported no significant differences in markers of overall glycemic control and in number of high or low glucose excursions between pre-Ramadan and Ramadan periods. Moreover, the absolute differences in CGM parameters during the pre-Ramadan and Ramadan periods were very small. Cultural, personal, social, nutritional, and medical factors may contribute to this variation. This emphasizes the implication of tailored individualized plan for patients which focuses on meal types and timing, and also making appropriate medication and dose changes during Ramadan fasting period.

Although some studies noted a decrease in food consumption and healthier diet practices during the lockdown period (12–15), many studies found either an increase in snacking and meal numbers or an increase in unfavorable food choices and dietary habits (16–19). Therefore, COVID-19 lockdown resulted in both favorable and unfavorable changes in eating practices, and this may have both short- and long-term consequences on health. The positive diet practices included an increase in the consumption of fresh produce, mostly fruits and vegetables, and an increase in home cooking during lockdown. However, poor food habits were seen in most studies, which include increased snacking and meal frequency, reduced fresh production, and increased comfort foods. Reasons for changes in behavior predominately included limited availability and increased price. This was associated with mental health conditions that include depression and anxiety, sedentary time, and weight gain (20). For patients with diabetes, stable rhythm of lifestyle proposes improvement in glucose control with less need to dosing changes in antidiabetic treatment (21).

Recently, some studies presented the effect of COVID-19 lockdown in glycemic control in patients with diabetes using CGM; nevertheless, majority of these studies done in countries do not practice Ramadan fasting, which make that their results on lockdown impact are independent from Ramadan fasting influence on glycemic control (22, 23). The fact that diabetes has been reported in the media as a risk factor for COVID-19 prognosis may have contributed to the improvement in glycemic management into lockdown.

The purpose of this study is to assess to what extent the lockdown overlapped with Ramadan affected ambulatory glucose metrics measured by FGM devices, as defined by the international consensus recommendation guidelines on clinical targets for continuous glucose monitoring data interpretation (24). To assess this, we compared the glycemic profile of patients with type 1 and insulin-treated type 2 diabetes using flash glucose monitoring (FGM), before and during lockdown including the holy month of Ramadan. In addition, we explored the clinical and demographic factors associated with a decline in glycemic control across this period through retrospective access of electronic medical records (EMRs) on Imperial College London Diabetes Centre's database (ICLDC). Results and conclusion from this study could be used in future for better diabetes management in Ramadan during the COVID-19 restrictions, which considers the sociocultural issues relevant to eventual circumstances.

PARTICIPANTS AND METHODS

Study Design and Participants

This is a retrospective observational single-centre study based on data retrieved from EMRs for patients using FGM (FreeStyle Libre, Abbott, Witney, United Kingdom) who have linked their glucose data to ICLDC using the LibreView online platform.[1] Of the patients with diabetes who are LibreView users, we identified a cohort of 50 patients with glucose profile data uploaded within the period of 12/2/2020–23/5/2020 to include at least 30 days before COVID-19 lockdown, 30 days into lockdown and pre-Ramadan, and 30 days into lockdown and Ramadan. Of note, during the period of lockdown, regular lifestyle was maintained since COVID-19 infection spread was very limited in the UAE.

Outcome Measures

The main study outcomes were the assessment of FGM glycemic variables and to compare them in between the 3 time periods (before lockdown, into lockdown and pre-Ramadan, and into lockdown and Ramadan). These variables include mean average glucose (MAG), glucose management indicator (GMI), estimated A1c, interquartile ranges of glucose, coefficient of variation (CV), time spent in range (TIR) (70-180 mg/dL, 3.9-10.0 mmol/L), time spent in hypoglycemia level 1 (54–70 mg/dL, 3.9–3.0 mmol/L), time spent in hypoglycemia level 2 (<54 mg/dL, <3.0 mmol/L), time spent in hyperglycemia level 1 (180–250 mg/dL, 10.0–13.9 mmol/L), time spent in hyperglycemia level 2 (>250 mg/dL, >13.9 mmol/L), mean amplitude of glycemic excursions (MAGE), mean of daily differences (MODD), low blood glucose index (LBGI), high blood glucose index (HBGI), and area under the curve (AUC). These variables were calculated using cgmanalysis package in R-studio software (25). In addition to this, we compared change across a range of glycemic variables between February and May 2020 for each of the following periods: 30 days before COVID-19 lockdown 4/2/2020–14/3/2020 (period 1), 30 days into lockdown and pre-Ramadan 20/3/2020–18/4/2020 (period 2), and 30 days into lockdown and Ramadan 24/4/2020–23/5/2020 (period 3).

Statistical Analysis

A number of three time periods were compared using repeated measures of ANOVA, with Greenhouse-Geisser correction for significance level (p = 0.05) using STATA version 15.0. All pairwise comparisons were adjusted with Bonferroni method in *post hoc* analyses. *Post hoc* multiple comparisons were reported for the significant outcomes only, whereas no pairwise comparisons were assessed for measures, where null hypothesis could not be rejected.

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RESULTS

A total of 24 patients with diabetes (19 men and 6 women) had complete data [≥70% FGM sensor data captured (24, 26)] and were included in the study. A total of eighteen had T1D, 5 had insulin-treated T2D, and one patient had maturity onset diabetes of the young (MODY). All patients with T1D were on multiple daily insulin (MDI) regimen, with mean total daily insulin dose of 90 ± 26 units. Patients with T2D were on insulin together with other agents as follows: metformin (n = 1), gliptin with metformin (n = 3), or dapagliflozin (n = 1). The mean total daily insulin dose in this group was 56 ± 24 units. The patient with MODY was on MDI regimen with a daily insulin dose of 46 units.

Data were categorized and analyzed primarily to compare FGM metrics before COVID-19 lockdown 1/1/2020–11/3/2020 and during COVID-19 lockdown 12/3/2020–15/5/2020 (which included pre-Ramadan and Ramadan month) within 60 days for each period (**Table 1**). MAG remained steady before and during lockdown, with no significant differences observed in TIR, time in hypoglycemia, and LBGI between prelockdown and lockdown periods.

Further analysis of the data was performed based on the comparison of 3 time periods: pre-COVID-19 lockdown, pre-Ramadan (into lockdown), and Ramadan (into lockdown) with 30 days for each period **Tables 2**, **3**.

TABLE 1 | Comparison of FGM metrics between *prelockdown and lockdown period.

FGM metrics	Prelockdown	Lockdown	*p*-value
**MAG (mg/dL)	169.4 (45.7)	173.6 (42.9)	0.334
GMI (mmol/L)	7.4 (1.1)	7.5 (1.0)	0.319
Estimated A1c	7.5 (1.6)	7.7 (1.5)	0.358
CV	0.38 (0.1)	0.39 (0.1)	0.184
Percentage of TIR	54.3 (20.9)	53.0 (18.6)	0.508
Percentage of TBR level1, median (IQR)	4.9 (1.8, 10.2)	4.0 (2.5, 8.3)	0.903
Percentage of TBR Level 2	14.5 (6.0, 45.5)	16.0 (9.0, 47.8)	0.269
Percentage of TAR level 1, median (IQR)	39.1 (24.4)	40.7 (22.0)	0.48
MAGE	113.6 (31.5)	118.6 (38.4)	0.291
MODD	18.1 (4.7)	18.1 (4.6)	0.885
LBGI	5.4 (2.8)	5.7 (2.8)	0.473
HBGI, median (IQR)	9.8 (6.5, 15.7)	13.1 (7.2, 16.4)	0.326

**COVID-19 prelockdown period: 1/1/2020–11/3/2020 and lockdown 12/3/2020–15/5/2020 (60 days in each period). Data presented as mean (SD), or median (interquartile range – IQR) as stated.*
***MAG, mean average glucose; GMI, glucose management indicator; CV, coefficient of variation; TIR, time in range (defined as 70–180 mg/dL); TBR, time below range (level 1: 54–70 mg/dL; level 2: <54 mg/dL); TAR, time above range (level 1: 180–250 mg/dL; level 2: >250 mg/dL); MAGE, mean amplitude of glycemic excursions; MODD, mean of daily differences; LBGI, low blood glucose index; HBGI, high blood glucose index.*

TABLE 2 | Comparison of FGM metrics between *pre-Ramadan and Ramadan during lockdown.

FGM Metrics	pre-Ramadan	Ramadan	*p*-value
Estimated A1c	7.4 (1.7)	8.0 (1.5)	0.007
**MAG (mg/dL)	166.6 (47.4)	182.0 (41.9)	0.007
GMI (mmol/L)	7.3 (1.1)	7.7 (1.0)	0.006
CV	0.4 (0.1)	0.4 (0.1)	0.155
TIR	390.8 (136.3)	352.4 (153.1)	0.19
Percentage of TIR	56.1 (19.0)	50.3 (20.4)	0.027
TBR level 1	33.2 (31.8)	22.9 (23.3)	0.07
Percentage of TBR level 1	4.6 (4.5)	3.2 (3.2)	0.05
TBR level 2	25.1 (32.1)	15.7 (22.0)	0.21
Percentage of TBR level 2	3.5 (4.4)	2.2 (3.0)	0.193
TAR level 1	149.6 (76.9)	177.5 (65.1)	0.022
Percentage of TAR level 1	21.3 (10.2)	25.5 (8.4)	0.01
TAR level 2	103.5 (118.3)	132.1 (112.1)	0.097
Percentage of TAR level 2	15.2 (17.5)	19.5 (17.3)	0.045
AUC	115476.0 (33427.3)	125698.4 (30057.3)	0.09
MAGE	115.6 (39.9)	122.8 (36.0)	0.42
MODD	17.8 (5.1)	18.0 (4.2)	1
J_index	57.1 (33.2)	64.8 (29.7)	0.047
LBGI	5.8 (2.8)	5.5 (3.5)	1
HBGI	11.8 (7.8)	13.6 (7.4)	0.037

**During lockdown: pre-Ramadan 20/3/2020–18/4/2020, Ramadan 24/4/2020–23/5/2020 (30 days in each period). Data are presented as mean (SD).*
***MAG, mean average glucose; GMI, glucose management indicator; CV, coefficient of variation; TIR, time in range (defined as 70–180 mg/dL); TBR, time below range (level 1: 54–70 mg/dL; level 2: <54 mg/dL); TAR, time above range (level 1: 180–250 mg/dL; level 2: >250 mg/dL); AUC, area under the curve; MAGE, mean amplitude of glycemic excursions; MODD, mean of daily differences; LBGI, low blood glucose index; HBGI, high blood glucose index.*

Table 2 shows pairwise comparisons during lockdown in pre-Ramadan and Ramadan periods, where estimated a1c was higher in Ramadan as compared to pre-Ramadan, with Bonferroni adjustment (8.0 (1.5) vs. 7.4 (1.7), $p = 0.007$). Moreover, statistically significant difference was observed in MAG, GMI, and time in hypoglycemia level 1, time in hyperglycemia level 1, and j-index.

Percentage of time in range (TIR) was significantly lower as 50.3% in Ramadan as compared to 56.1% pre-Ramadan. Higher MAG was reported in Ramadan (182.0 mmol/L) with significant statistical difference than its level in pre-Ramadan period (166.6 mmol/L). HBGI was also higher during Ramadan whereas no statistically significant differences were observed in percentage of time in hypoglycemia level 2 and LBGI.

In **Table 3**, which displays pairwise comparisons between pre-COVID-19 lockdown and Ramadan (into lockdown), a similar trend is observed. There is no significant difference intime in range, time in both hypoglycemia and hyperglycemia (levels 1 and 2), AUC, MODD, and LBGI, whereas statistically significant difference was reported for estimated a1c, MAG, GMI, j-index, and HBGI.

Overall comparisons of the three time period studies show that average glucose measure was statistically higher in Ramadan vs. pre-Ramadan and pre-COVID period. Similar trend was reported for GMI, median, 25 and 75th percentiles. Parameters for glucose variability did not show a statistically significant increase in Ramadan as compared to pre-Ramadan or pre-COVID, which include standard deviation ($p = 0.178$) and CV ($p = 0.145$).

There was a significant decline in percentage of TIR in Ramadan as compared to pre-Ramadan only ($p = 0.027$); however, statistical difference was not noted for absolute number of minutes in TIR in both groups ($p = 0.101$). Risk of hyperglycemia using HBGI was significantly higher in Ramadan period ($p = 0.047$) using Friedman's test for comparison. LBGI, an indicator of risk of hypoglycemia, did not show statistical association in three time periods.

Time below range (TBR) (hypoglycemia levels 1 and 2) was not associated with the changes in three time periods. There is a slight significance in percentage of time above range (TAR) (hypoglycemia level 1). TAR (hyperglycemia level 1) was statistically higher in Ramadan, and the significance was maintained in pairwise comparisons between Ramadan and pre-Ramadan only.

For further exploratory analysis, we evaluated the glycemic effect of the lockdown by displaying the overall 24-h glucose profile for patients ($n = 24$) in the 3 periods. Similar trend is observed in **Figure 1**, which shows median glucose level,

TABLE 3 | Comparison of FGM metrics during *prelockdown and Ramadan during lockdown.

FGM metrics	Prelockdown	Ramadan (during lockdown)	*p*-value
Estimated A1c	7.5 (1.5)	8.0 (1.5)	0.014
**MAG (mg/dL)	167.8 (43.2)	182.0 (41.9)	0.013
GMI (mmol/L)	7.3 (1.0)	7.7 (1.0)	0.013
CV	0.4 (0.1)	0.4 (0.1)	0.729
TIR	392.7 (153.9)	352.4 (153.1)	0.156
Percentage of TIR	55.1 (20.1)	50.3 (20.4)	0.092
TBR level 1	30.0 (27.1)	22.9 (23.3)	0.225
Percentage of TBR level 1	4.4 (4.0)	3.2 (3.2)	0.133
TBR level 2	20.5 (28.2)	15.7 (22.0)	1
Percentage of TBR level 2	2.9 (3.8)	2.2 (3.0)	0.918
TAR level 1	165.5 (75.9)	177.5 (65.1)	0.701
Percentage of TAR level 1	23.1 (10.1)	25.5 (8.4)	0.281
TAR level 2	107.1 (113.4)	132.1 (112.1)	0.179
Percentage of TAR level 2	15.1 (16.1)	19.5 (17.3)	0.045
AUC	119404.8 (33181.5)	125698.4 (30057.3)	0.525
MAGE	113.8 (34.4)	122.8 (36.0)	0.2
MODD	18.1 (4.7)	18.0 (4.2)	1
J_index	56.4 (28.4)	64.8 (29.7)	0.027
LBGI	5.3 (2.9)	5.5 (3.5)	1
HBGI	11.8 (6.9)	13.6 (7.4)	0.038

**Prelockdown: 14/2/2020–14/3/2020 and Ramadan (during lockdown): 24/4/2020–23/5/2020, 30 days in each period. Data are presented as mean (SD).*
***MAG, mean average glucose; GMI, glucose management indicator; CV, coefficient of variation; TIR, time in range (defined as 70–180 mg/dL); TBR, time below range (level 1: 54–70 mg/dL; level 2: <54 mg/dL); TAR, time above range (level 1: 180–250 mg/dL; level 2: >250 mg/dL); MAGE, mean amplitude of glycemic excursions; MODD, mean of daily differences; LBGI, low blood glucose index; HBGI, high blood glucose index; AUC, area under the curve.*

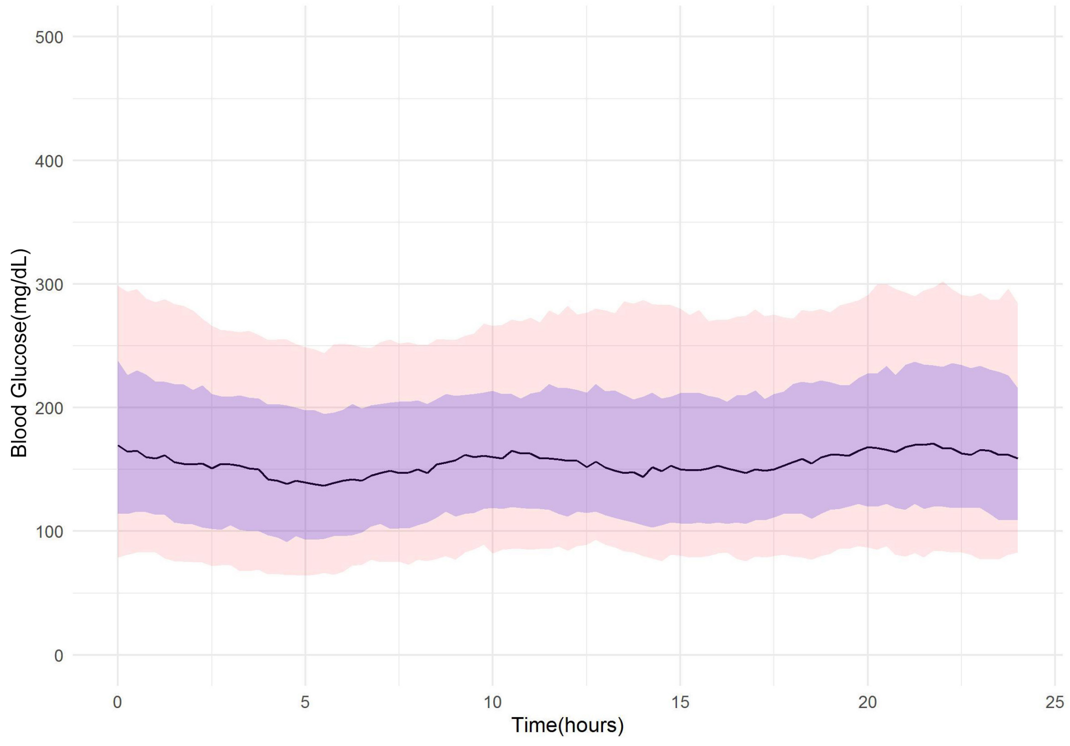

FIGURE 1 | FGM glucose profile (*n* = 24) in 30 days pre-COVID-19 lockdown and pre-Ramadan (14 February 2020–14 March 2020). Line indicates median glucose and purple shaded area shows 25 and 75th percentiles. Pink area denotes 10 and 90th percentiles.

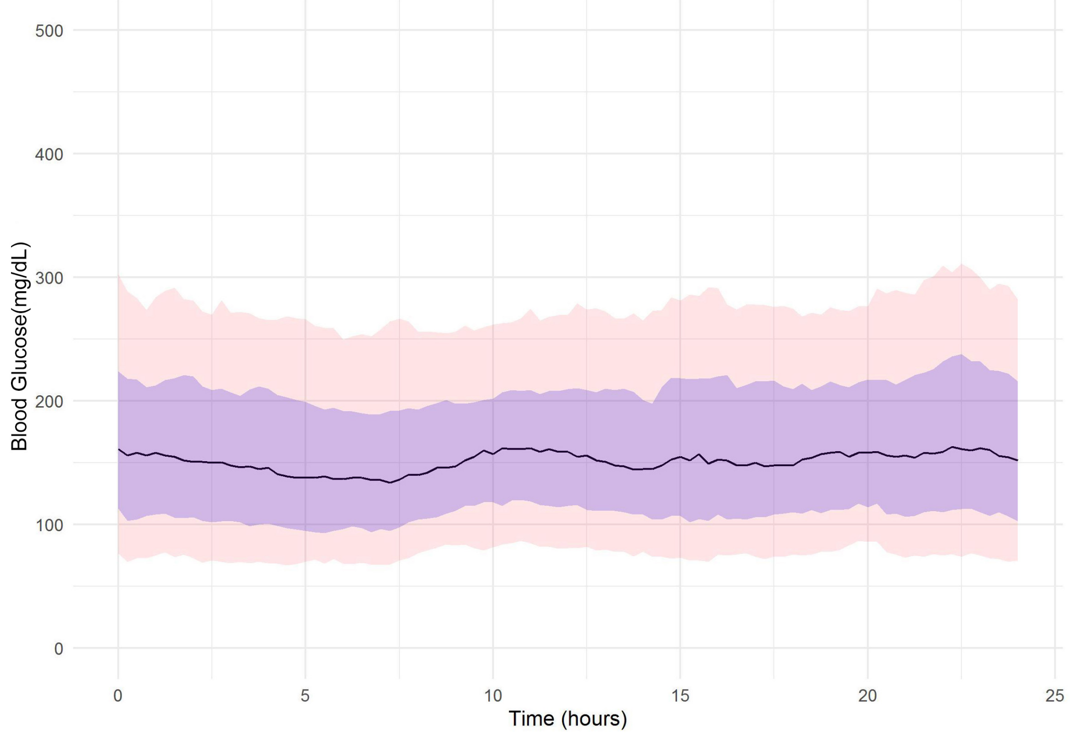

FIGURE 2 | FGM glucose profile (*n* = 24) in 30 days during COVID-19 lockdown and pre-Ramadan (20 March 2020– 18 April 2020). Line indicates median glucose and purple shaded area shows 25 and 75th percentiles. Pink area denotes 10 and 90th percentiles.

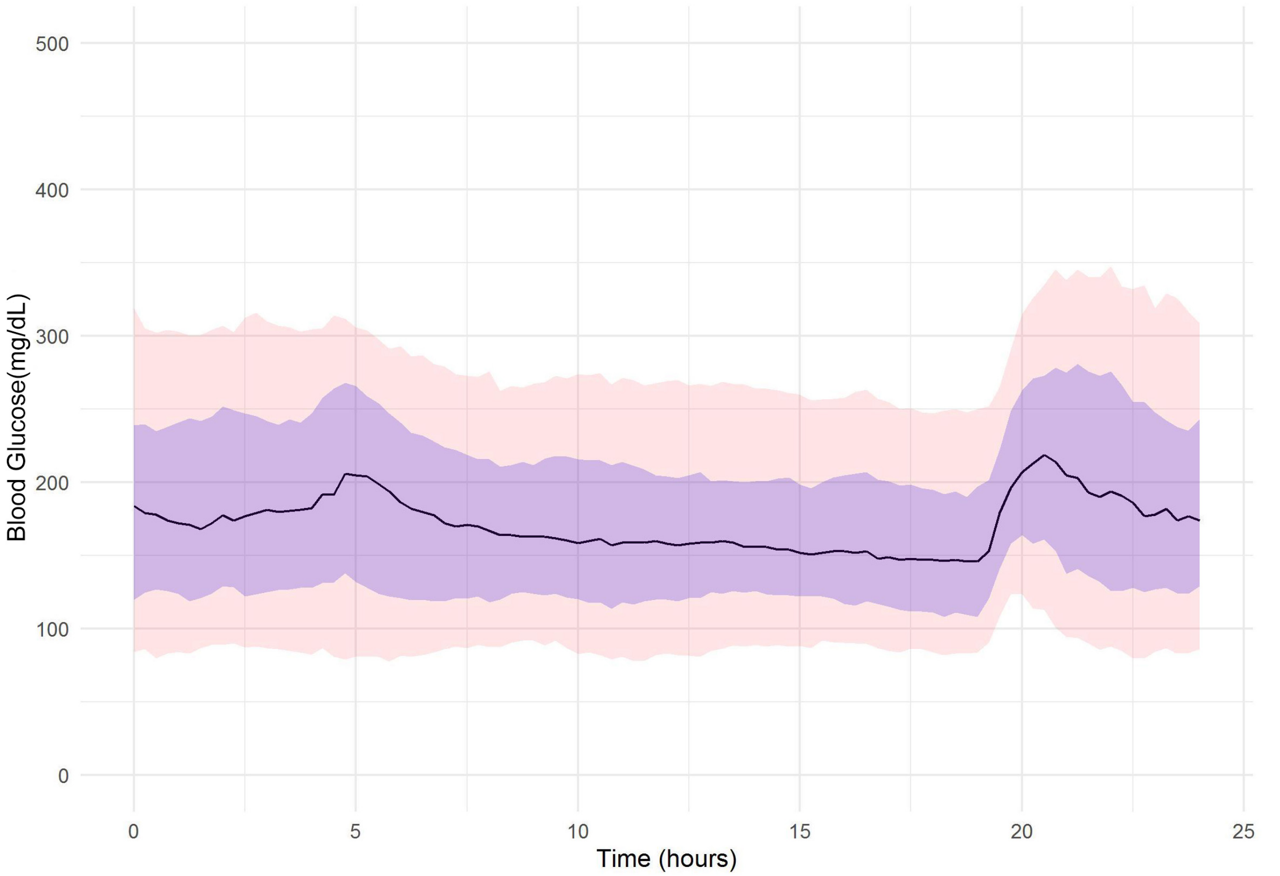

FIGURE 3 | FGM glucose profile (*n* = 24) in 30 days during Ramadan and during COVID-19 lockdown (24 April 2020 – 23 May 2020). Line indicates median glucose and purple shaded area shows 25 and 75th percentiles. Pink area denotes 10 and 90th percentiles.

10, 25, 75, and 90th percentiles in pre-COVID lockdown, and **Figure 2** shows glucose level in pre-Ramadan and lockdown. **Figure 3** shows consistent late-night elevation of median glucose level, 10, 25, 75, and 90th percentiles in Ramadan and COVID lockdown period. This indicates the occurrence of hyperglycemia after iftar meal.

Figure 4 shows the comparison of percentage of TIR, TAR (hyperglycemia levels 1 and 2), and TBR (hypoglycemia levels 1 and 2) between before lockdown, pre-Ramadan, and Ramadan after lockdown. Slight decrease occurs in TIR in Ramadan (50.3%) as compared to pre-COVID (55%) and pre-Ramadan (56.1%) ($p = 0.026$). Whereas TAR showed greater increase 45% for hyperglycemia level 1 and 19.5% for hyperglycemia level 2 in Ramadan, with almost the same percentages in pre-COVID and pre-Ramadan, ANOVA test showed statistically significant difference in both levels; hyperglycemia level 1 ($p = 0.015$) 1 and hyperglycemia level 2 ($p = 0.027$).

DISCUSSION

Coronavirus disease-19 pandemic is an unprecedented healthcare crisis. For the first time in recorded human history, total lockdown and stay-at-home restrictions were imposed and resulted in extreme disruption to lives of people worldwide. The accompanying anxiety was much exaggerated in patients with chronic diseases. This anxiety was further exacerbated in people with diabetes who were shown to be a high-risk group. As a direct unfavorable effect on glycemic control, the COVID-19 lockdown could lead to inactivity and indirect negative effects on glucose control. On the other hand, this lack of activity may be accompanied by changes in food intake and meal patterns with its own effects on glycemia, which may in fact go in either direction.

In this study, we have compared FGM-derived indicators of glycemic control and variability before and during the COVID-19 lockdown period.

The overlap of the Ramadan fasting with the lockdown period was an important consideration in analyzing our data. Therefore, further data analysis has been performed to compare FGM metrics pre-Ramadan and Ramadan, during the lockdown period. Furthermore, a separate analysis was performed to see any differences between prelockdown and Ramadan (into lockdown).

Contrary to our expectation, we found no significant difference in FGM-derived glucose metrics between these two periods. There was no statistical difference in MAGE (mean

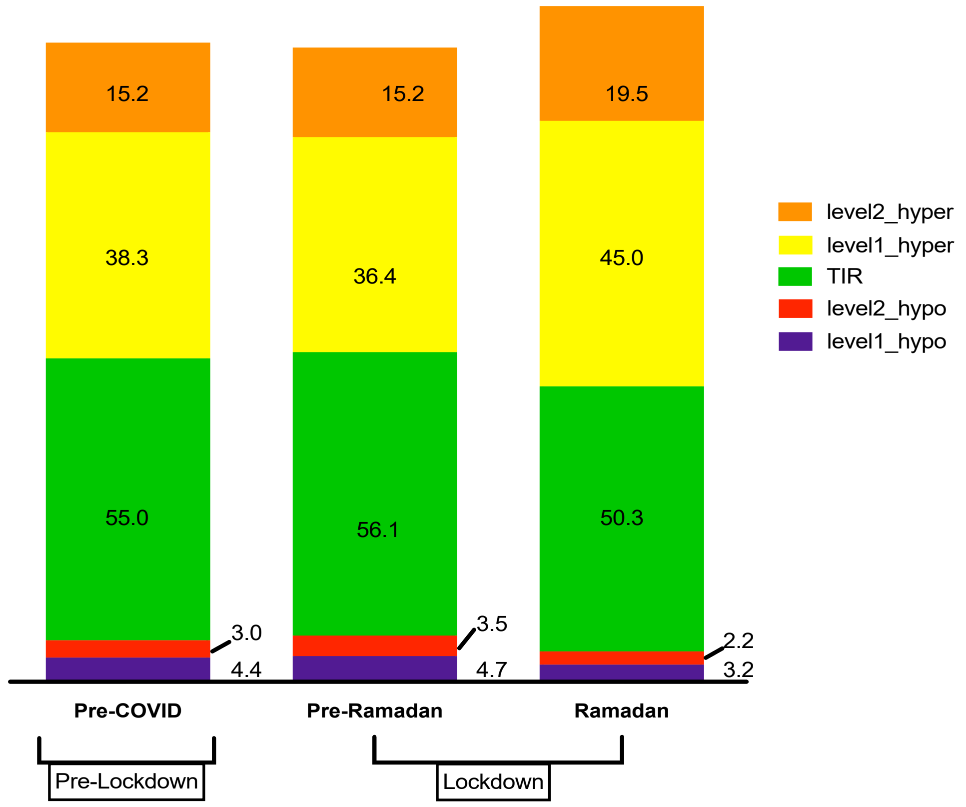

FIGURE 4 | Comparison of percentage of TIRs according to COVID-19 lockdown time points: before lockdown 4/2/2020–14/3/2020 (pre-COVID), during lockdown and pre-Ramadan 20/3/2020–18/4/2020 (pre-Ramadan), and during lockdown and Ramadan 24/4/2020–23/5/2020 (Ramadan). TIR = percentage of TIR (70–180 mg/dL, 3.9–10.0 mmol/L), Level1_hypo = percentage of TBR level 1 (<70–54 mg/dL, <3.9–3.0 mmol/L), Level2_hypo = percentage of TBR level 2 (<54 mg/dL, <3.0 mmol/L), Level1_hyper = percentage of TAR level 1 (>180–250 mg/dL, >10.0 mmol/L), Level2_hyper = percentage of TAR level 2 (>250 mg/dL, >13.9 mmol/L).

amplitude of glycemic excursions) between prelockdown, pre-Ramadan, or Ramadan periods in our studies sample. MAGE considers glycemic peaks and nadirs occurring daily without counting the total number of fluctuations (27). Likewise, LBGI did not show statistically significant differences between these three time period studies whereas HBGI was significantly higher in Ramadan than prelockdown and pre-Ramadan periods. Additionally, when we compared Ramadan 2020 and Ramadan 2019, there was no statistically significant differences in FGM-derived indicators of glycemic control and variability.

Ramadan is accompanied by its own sudden and drastic changes in lifestyle that include meal timings. These are accompanied by alterations in sleeping schedules and circadian rhythm of various hormones. For patients with diabetes, there are changes in glycemic variability patterns which are more pronounced in patients on insulin secretagogues and insulin therapy, which includes multiple daily injections (MDIs) of insulin or continuous subcutaneous insulin infusion (CSII). These have been well-described in work by several groups (28), including our own (29). The paradoxical risk of excessive eating after hours of fasting and also reduction in total sleep duration may lead to the increase in glycemic variability (5).

In keeping with the previous work, in this study, we have shown a significant reduction in time spent in range (3.9–10.0 mmol/L), time spent in hypoglycemia and hyperglycemia during Ramadan as compared to pre-Ramadan period, with late-night-time surge of mean blood glucose after *Iftar* time (**Figure 3**). Moreover, j-index (which represents a measure of the quality of glycemic control based on the combination of information calculated from the mean and SD) was significantly higher in Ramadan than pre-COVID and pre-Ramadan periods. As such, even during the COVID lockdown period, the effects of Ramadan fasting were apparent.

Our results are in concurrence with the study performed in Spain by Beato–Víbora (22), patients with type 1 diabetes using CGM and FGM reported no deterioration in glycemic control related to the prolonged COVID-19 lockdown, and TBR remained unchanged, whereas TIR and estimated HbA1c improved. On the other hand, Bonora et al. (23) and Verma et al. (30) suggested that changes in routine daily activities and having more time for self-management had beneficial effects on glycemic control and consequently diabetes management during lockdown in patients with diabetes, at least in the short term.

Another study reported an improvement in glycemic control after 8 weeks of lockdown, especially in patients with reduced baseline control (31).

What about the lockdown effect with Ramadan period dissected out? Our data showed no significant differences in multiple glucose metrics between pre-COVID-19 lockdown and pre-Ramadan periods. This underlines the drastic effect of Ramadan on glucose control and glycemic variability in Muslim patients with diabetes who strictly practice Ramadan fasting. Moreover, in year 2020, time synchronization of COVID-19 lockdown and Ramadan accentuated this influence.

CONCLUSION

Our study did not find any relevant significant effect of the lockdown itself on glycemic control in this group of patients. This study also highlights the effects of Ramadan fasting in insulin-treated patients with diabetes, which results in major changes in glycemic profiles, particularly pronounced in the evening hours after the fast is broken. These changes were apparent even within the COVID-19 lockdown period which had its own dramatic lifestyle changes. These findings may have important lessons in designing appropriate lifestyle strategies for management of insulin-treated patients.

LIMITATIONS

This was a single-centre study conducted in specialized diabetes management centre. Moreover, the selection bias may have influenced the glucose excursions, as FGM users in our center tend to have better glycemic control. Therefore, it remains unclear whether these results can be generalized to patients with diabetes with poorer control or not. In addition, only a small sample size of patients was studied, and this might skew the data.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The study protocol was approved by Institutional Research Ethical committee in ICLDC (IREC055) and Department of Health (DOH), Abu Dhabi, United Arab Emirates. The study was conducted according to the ethical principles of the Declaration of Helsinki (32).

AUTHOR CONTRIBUTIONS

RH: data acquisition, data interpretation, and manuscript writing. TA: data interpretation and manuscript writing. MM: statistical analyses. NL: study design, data interpretation, and manuscript editing. All authors contributed to the article and approved the submitted version.

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SYSTEMATIC REVIEW
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The Impacts of Ramadan Intermittent Fasting on Saliva Flow-Rate and Metabolic Data: A Systematic Review

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The aim of this systematic review was to report the impacts of Ramadan intermittent fasting (RIF) on salivary flow-rate (SFR) and metabolic parameters. A thorough literature search was carried out using the databases *PubMed* and *Scopus* from their inception up to 15 July 2021. The Boolean connectors used in *PubMed* were (Saliva [Mesh] AND Fasting [Mesh]). The same keywords were used in *Scopus*. Inclusion criteria were defined using PICOS. The research included all original studies involving "healthy" adults and published in English. Methodological quality assessment was performed utilizing the Joanna Briggs Institute Critical Appraisal Tool, which allows attributing scores from 1 to 11 to the selected studies. Two authors carried out the literature search, study selection, and data extraction. Differences on issues were resolved by a third author if necessary. The systematic review protocol was registered within the "Open Science Framework" (Doi: 10.17605/OSF.IO/DE7BH). Six articles met the inclusion criteria. All studies were heterogeneous and had a high score of bias and several methodological differences. The following parameters were collected: SFR, melatonin, cortisol, glucose, immunoglobulin A (IgA), uric-acid, alkaline phosphatase (ALP), and aspartate aminotransferase (AST). The SFR decreased by 10% during Ramadan in fasting subjects. The circadian pattern of melatonin remained unchanged during Ramadan, but melatonin levels dropped significantly from baseline. The salivary cortisol levels were unchanged or increased during Ramadan. The salivary glucose levels were decreased. ALP increased significantly, whilst uric-acid and AST decreased significantly. Salivary IgA decreased during the last week of Ramadan. To conclude, there is a trend toward a decrease in SFR and the content of the majority of the biomarkers investigated, with the exception of ALP and uric-acid. These changes cannot be easily attributed to any single factor (hydration status, dietary habits, physical activity, or hygiene habits).

Systematic Review Registration: [https://osf.io/de7bh/], identifier [Doi: 10.17605/OSF.IO/DE7B].

Keywords: cortisol, oral health, melatonin, Ramadan fasting, salivary biomarkers, salivary flow rate

INTRODUCTION

Human saliva is a biofluid produced and secreted by the major and minor salivary glands (1). The major salivary glands are the parotid, submandibular, and sublingual glands, responsible for more than 90% of salivary secretions, and the minor glands are distributed throughout the oral mucosa surfaces (1). Saliva plays an essential role in oral cavity maintenance and functionality (1), and it represents a mirror reflecting both oral and systemic health (2). Salivary secretions are composed of water, electrolytes, and several biomolecules, including proteins, enzymes, exosomes, nuclear acids, hormones, and cellular components (2). Many studies have demonstrated that the composition of saliva varies depending on the type of stimulation (2), the short-term acute mental stress (3), the taste and smell (4), and the daily and seasonal circadian rhythms (5). Hence, recurrent circadian fasting during Ramadan [i.e., Ramadan intermittent fasting (RIF)] may modify the salivary parameters.

Ramadan is the ninth month of the Muslim lunar calendar and it lasts 29 or 30 days depending on the actual observation of the moon's crescent (6). The synodic nature of the Muslim calendar means that Ramadan occurs 10–11 days earlier each Gregorian year, migrating across all four seasons over approximately a 33-year cycle (6). Therefore, the fasting daytime duration can vary accordingly with longer fasting durations during summer. At any time point, the geographical situation will have an impact on the daylight. The higher the latitude is, the longer the fasting duration will be (7). Recurrent circadian fasting during Ramadan is practiced by around two billion Muslims every year (8), and healthy adult Muslims are asked to refrain from eating and drinking during this month between *Sahur* (dawn meal just before the start of fast) and *Iftar* (sunset meal marking the end of the fast) as a religious duty (6). Since food and water intake takes place from sunset to dawn, this modification in Muslims' lifestyle for 1 lunar month may have an impact on oral health. A Muslim may be exempt from fasting during Ramadan (DR) for several reasons, including pregnancy, breastfeeding, diabetes mellitus, and mental disability, however; despite these exemptions, many Muslim patients with chronic medical conditions still choose to fast (9).

Several systematic reviews have studied the effects of RIF on general health (10–12), notably on the immune system (13), cardiovascular function (14), dietary intake and body composition or weight (15, 16), glycemic control (17), kidney function (18), and sleep (19). However, to the best of the authors' knowledge, no previous systematic review has investigated the impacts of RIF on salivary secretion [e.g., salivary flow-rate (SFR)] and metabolic parameters such as cortisol, glucose, melatonin, and uric-acid. The aim of this paper was therefore to systematically review the impacts of RIF on SFR and saliva metabolic parameters.

Abbreviations: ALP, alkaline phosphatase; AR, after-Ramadan; AST, aspartate amino transferase; BR, before-Ramadan; DR, during-Ramadan; IgA, immunoglobulin A; JBI, Joanna Briggs Institute; RIF, Ramadan intermittent fasting; SFR, salivary flow-rate.

METHODS

Protocol and Eligibility Criteria

The systematic review protocol was registered within the "Open Science Framework" (OSF, DOI 10.17605/OSF.IO/DE7BH). This systematic review followed the "Preferred Reporting Items for Systematic Reviews and Meta-Analyses" (PRISMA) guidelines (20). The inclusion criteria were formulated based on the following PICOS tool questions (21): ***P*** (population) = healthy Muslim adults willing to fast DR; ***I*** (intervention/exposure) = exposure to RIF; ***C*** (Comparison): DR and outside Ramadan [i.e., before-Ramadan (BR) and after-Ramadan (AR)]; ***O*** (Outcome): SFR and saliva metabolic parameters; and ***S*** (Study design): all original articles written in English. No restrictions were applied in terms of study design, setting, country, or period. Publications not in compliance with the purpose of this systematic review as well as those not representing original research (i.e., reviews, editorials, qualitative papers, case reports, case series, and letters to editors) were not included.

Literature Search

An online literature search was performed using two databases: *PubMed* and *Scopus* from their inception up to 15 July 2021. For *PubMed*, the search was carried out using a strategy employing the combination of the following two "Medical Subject Headings" (MeSH) terms: *Saliva* AND *Fasting*. As for *Scopus*, the previous two terms were searched for in the article titles, abstracts, and/or keywords. In addition, the reference lists of the included articles were checked. All the authors involved in this review agreed on the articles to be included in this systematic review.

Study Selection

The process of articles selection is outlined in **Figure 1**. Duplicate articles were eliminated using End-Note X9 library. Titles of the remaining articles were independently appraised during the initial online literature search for studies by two of the authors (*AB* and *MK* in the authors' list) to check for their relevance to the searched topics. Abstracts of these titles were then read to determine if the studies met the inclusion criteria. The studies whose abstracts met the inclusion criteria were then read in full-text format to determine their eligibility and therefore retention. Two authors (*AB* and *MK* in the authors' list) conducted the study selection process for this review, with discrepancies being checked by a third author (*HBS* in the authors' list), if necessary.

Data Extraction

Data from the retained studies were extracted using a format including the population, the parameters being investigated, the periods during which the parameters were collected, and the significant findings. Data were extracted, reviewed, and analyzed by two authors (*AB* and *MK* in the authors' list). Extracted data were then verified by a third author (*HBS* in the authors' list). Discrepancies in data collection were resolved through discussion.

Methodological Quality Assessment

Methodological quality assessment was performed using the Joanna Briggs Institute (JBI) critical appraisal tool, precisely the checklist for cohort studies (https://joannabriggs.org/last visit: 4 March 2022). The checklist appraises the following areas: recruitment, exposure measurement, reliability of exposure measurement, confounding factors identified, strategies to deal with confounding factors, participants free of outcome at the onset of the study, validity and reliability of outcome measurement, follow-up timeframe reported, follow-up completion, strategies utilized to deal with incomplete follow-up, and appropriate statistical analysis. The checklist included the following 11 items: ***1.*** Were the two groups similar and recruited from the same population? ***2.*** Were the exposures measured similarly to assign people to both exposed and unexposed groups? ***3.*** Was the exposure measured in a valid and reliable way? ***4.*** Were the confounding factors identified? ***5.*** Were the strategies to deal with confounding factors stated? ***6.*** Were the groups/participants free of the outcome at the start of the study (or at the moment of exposure)? ***7.*** Were the outcomes measured in a valid and reliable way? ***8.*** Was the follow-up time reported and sufficient to be long enough for outcomes to occur? ***9.*** Was follow-up complete, and if not, were the reasons for loss to follow-up described and explored? ***10.*** Were the strategies to address incomplete follow-up utilized? and ***11.*** Was the appropriate statistical analysis used? These items are scored as either yes, no, unclear, or not applicable. Two reviewers (*AB* and *MK* in the authors' list) independently scored the retained studies, with discrepancies being resolved through discussion. If discrepancies could not be resolved through discussion, a third author (*HBS* in the authors' list) intervened to reach consensus. The risk of bias in the studies was judged to be low ("yes" scores $>$ 70%), moderate (50 $\leq$ "yes" scores between $\leq$ 69%), and high ("yes" scores $<$ 49%) (22).

RESULTS

Search Results

The search process yielded 675 articles, of which 202 were duplicated. Among the 473 remaining papers, 465 were excluded based on the title and the abstract. When screening the references lists of the remaining eight articles (23–30), one additional paper was added (31). After assessing full-text articles for eligibility, three articles were excluded (23–25). Consequently, six articles were retained (26–31). The search results are presented in **Figure 1**.

Methodological Quality Assessment Results

The retained six studies were assessed for methodological quality (**Table 1**). All the studies have a high score of bias (i.e., final score ranging from 9.1 to 36.4%). *Items 2, 5,* and *10* were rated as not applicable for all the studies. No study reported data regarding *items 4, 7,* and *11*. Six (26–31), five (26–29, 31), four (27–29, 31), one (29), and one (31) studies included information regarding *items* 6, *8*, *3, 9,* and *1,* respectively.

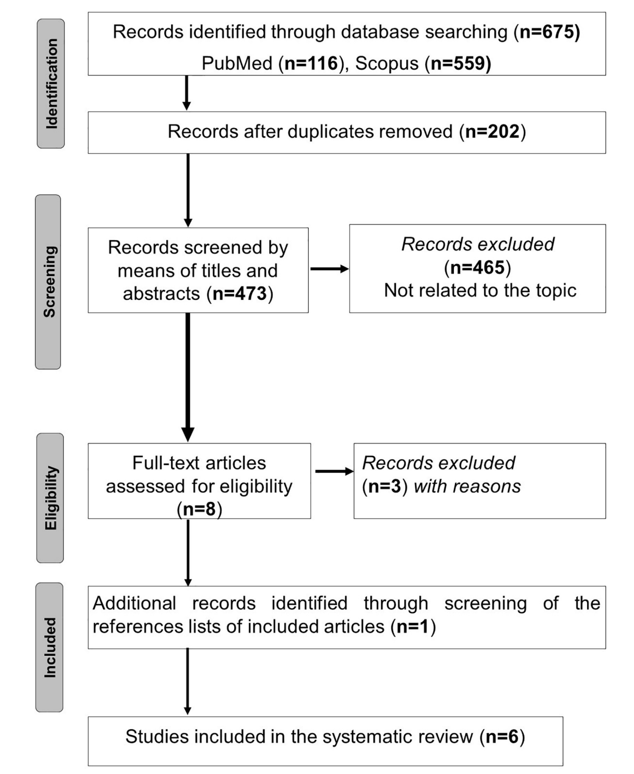

FIGURE 1 | Study flowchart.

Study Selection and Characteristics

Table 2 exposes the main characteristics and methodological points of the retained studies. The latter were published between 2004 (26) and 2020 (29, 30). The studies were conducted in Saudi Arabia (26), Turkey (27), United Arab Emirates (29), and Iran (28, 30, 31). The study design was not reported in three studies (27–29). In the remaining studies, three designs were applied: observational design with repeated measures (26), case-control (31), and descriptive analytical research design (30). All the studies (26–31) opted for convenience samples. The Ramadan year was omitted in three studies (28, 30, 31). Only one study (29) mentioned the number of fasting days DR. Only one study (28) reported the average ambient temperature, which was around 15°C. Only three studies reported the mean fasting duration [i.e., 12 h (26), 15 h (29), 17 h (27)]. No study reported data with regard to the Ramadan season, the average ambient pressure, or the average ambient humidity.

The number of evaluation sessions was two (27, 29, 30), three (26, 28), and four (31). Five studies (26–30) opted for a session BR with different periods applied [i.e., 1 day BR (28), 1 week BR (27, 29, 30), 2 weeks BR (26)]. Only one study opted for a session AR (i.e., 7 days AR) (31). The number of sessions DR was one (27, 29, 30), two (26, 28), and four (31), and different periods were retained [i.e., 1 week (26), first 10 days (31), 10–20 second

TABLE 1 | Quality scoring of the retained articles according to Joanna Briggs Institute critical appraisal checklist.

First author	Reference	1	2	3	4	5	6	7	8	9	10	11	Score (%)	Study risk of bias
Bahammam	(26)	N	N/A	N	N	N/A	Y	N	Y	N/A	N/A	N	18.2	High
Sariri	(31)	N	N/A	Y	N	N/A	Y	N	Y	N/A	N/A	N	27.3	High
Develioglu	(27)	N	N/A	Y	N	N/A	Y	N	Y	N/A	N/A	N	27.3	High
Khaleghifar	(28)	N	N/A	Y	N	N/A	Y	N	Y	N/A	N/A	N	27.3	High
Dehaghi	(30)	N	N/A	N	N	N/A	Y	N	N	N/A	N/A	N	9.1	High
Al-Rawi	(29)	N	N/A	Y	N	N/A	Y	N	Y	Y	N/A	N	36.4	High

N, no; N/A, not applied; U, unclear; Y: yes.
Item 1. Were the 2 groups similar and recruited from the same population?
Item 2. Were the exposures measured similarly to assign people to both exposed and unexposed groups?
Item 3. Was the exposure measured in a valid and reliable way?
Item 4. Were the confounding factors identified?
Item 5. Were the strategies to deal with confounding factors stated?
Item 6. Were the groups/participants free of the outcome at the start of the study (or at the moment of exposure)?
Item 7. Were the outcomes measured in a valid and reliable way?
Item 8. Was the follow-up time reported and sufficient to be long enough for outcomes to occur?
Item 9. Was follow-up complete, and if not, were the reasons for loss to follow-up described and explored?
Item 10. Were the strategies to address incomplete follow-up utilized?
Item 11. Was the appropriate statistical analysis used?

days (31), third week (26, 30), 21–29 last days (31), 25th day of Ramadan (27, 28), last day of Ramadan (28, 29)].

Two-hundred twenty-nine participants fasting DR were included. The sample sizes varied from 8 (26) to 75 (30). Three studies included mixed population of males and females (27, 28, 30), two studies included only males (28, 31), and the participants' sex was not reported in one study (26). Four studies included healthy participants (26–28, 31), one study involved both overweight and obese participants (29), and one study omitted to report the health status of the included participants (30). The included participants were: students (31), employees in a factory (28), staff of a training and research hospital (27), and nurses (30). Several non-inclusion/exclusion criteria were applied. They were related to habits [e.g., smoking (26, 28), alcohol-use (26), addiction to caffeinated beverages (26)], medication-use (26, 27, 29), some health complaints [e.g., sleep complaints (26)], acute diseases [e.g., upper respiratory tract infections (27), severe infections (31)], chronic conditions [e.g., unhealthy teeth or mouth (28), oral diseases (28, 31), internal diseases (28), endocrine diseases (29), diabetes mellitus (29, 30), metabolic disorders (30), cardiovascular diseases (29, 30), hearing impairment (30), headache (30), psychiatric shock (30), unspecified (27)], previous surgeries [e.g., bariatric surgery (29), head surgery (30)], pregnancy (29), weight management program (29), and job experience <1 year (30). Only one study highlighted that no participant practiced fasting as routine and voluntary rituals before the month of Ramadan (29). In one study (29), participants were asked to continue their regular diet during non-fasting hours, and not to alter their habitual physical exercise levels BR or DR. Participants' ages varied from 24.2 ± 2.3 (31) to 59 (27) years. Participants' weight and body mass index were reported in two (27, 29) and three (26, 27, 29) studies, respectively.

Eight different saliva parameters were evaluated: SFR (31), glucose (31), melatonin (26), cortisol (29, 30), immunoglobulin A (IgA) (27), uric-acid (28), alkaline phosphatase (ALP) (28), and aspartate amino-transferase (AST) (28). The numbers of saliva sampling were one (27, 28), two (29, 30), and three (26, 31). Different times of saliva sampling were chosen. In some studies, fixed times were applied [e.g., mid-night (26), 6h00 (30), 8h00 (26), between 11h00 and 13h00 (29), 16h00 (26)]. In some other studies, a minimum of hours of fasting was needed [e.g., 6 (31), 8 (28), 12 (27)]. Four studies reported that they opted for unstimulated saliva (27–29, 31), and only two studies reported the duration of saliva collection [e.g., 2–5 (31) and 5 (27) min]. The volume of the collected saliva (in mL) was highlighted in four studies [e.g., 2 (30), 3 (28, 31), and 5 (26)]. One study omitted to report the equipment used to analyze the saliva outcomes (29). Before saliva collection, participants were asked to rinse their mouths with water (26–31) and to avoid: ***(i)*** coughing or throat clearing into the collection tube (26), ***(ii)*** consuming caffeine and substances containing melatonin or melatonin precursors (26), ***(iii)*** eating, drinking, and smoking (29, 30), ***(iv)*** brushing (30), and ***(v)*** using oral hygiene products (29).

Impact of Ramadan Intermittent Fasting on the Salivary Flow-Rate and Saliva Metabolites

Table 3 presents the main results of the six retained studies.

Salivary Flow-Rate

The only study evaluating the SFR reported its decrease by 10% DR compared to controls (31).

Salivary Hormones: Melatonin and Cortisol

Khaleghifar et al. (28) reported that melatonin keeps the same circadian pattern DR, but its level drops significantly from baseline. At midnight, melatonin level has a flatter slope and a significantly lower peak in the first and the third weeks of Ramadan compared to BR. At 8 a.m., there is no significant difference between BR and the first or third weeks of Ramadan. At 16 a.m., there is a significant decrease of melatonin from baseline for BR vs. the first or third weeks of Ramadan.

Regarding salivary cortisol levels, studies reported different results (29, 30). One study reported no change in salivary cortisol levels DR compared to BR (29). Another study reported that RIF has a significant effect on salivary cortisol secretory levels (30). The latter increases during fasting when it is combined with noise as another stress factor (30).

Salivary Metabolic and Immunologic Data

Sariri et al. (31) reported a significant decrease in salivary glucose during the first 10 days of Ramadan (by 25% compared to controls), the 10–20 days of Ramadan, and 21–29 days of Ramadan (by 17% compared to controls). Khaleghifar et al. (28) reported that compared to BR, on the 15th day of Ramadan, ALP significantly increases, and uric-acid and AST significantly

TABLE 2 | Main characteristics and methodology points of the published studies aiming to evaluate the impacts of Ramadan intermittent fasting (RIF) on saliva parameters.

First author (ref)	**Bahammam (26)**	**Sariri (31)**	**Develioglu (27)**	**Khaleghifar (28)**	**Al-Rawi (29)**	**Dehaghi (30)**
Town (country)	Riyadh (Saudi Arabia)	Tehran (Iran)	Istanbul (Turkey)	Rasht (Iran)	Sharjah (United Arab Emirates)	Ahvaz (Iran)
Yr of publication	2004	2010	2012	2017	2020	2020
Ramadan Yr	2002	2007	2012	NR	2017	2018
Study design	Observational study with repeated measures	Case-control study	NR	NR	NR	Descriptive and analytical study
Evaluation sessions' number	2 weeks BR 1st week of R 3rd week of R	1–9 first days of R 10–20 second days of R 21–29 last days of R 7th day AR	BR (1 week BR) 25th day of R (last week of R)	1 day BR (used as control) 25 day of R Last day of R	1 week BR 28 day of R	1 week BR 3rd week of R
Inclusion criteria	No regular medications No alcohol	Healthy Males Students	Healthy Males	Healthy non-smokers Male Healthy teeth Healthy mouth No oral disease No internal disease	Overweight/obese Muslims	Nurses Fasting during the study period
Non-inclusion and exclusion criteria	Sleep complaints Smoking Addiction to caffeinated beverages	Severe infection Oral and/or dental diseases	Acute diseases Chronic disease Medication-use	NR	Diabetes-mellitus Endocrine disease Cardiovascular diseases Medication-use Pregnancy Bariatric surgery Weight management program	Hearing impairment Headache Head surgery Psychiatric shock- last 6 months Cardiovascular disease Metabolic disorders Diabetes-mellitus Job experience <1 year
Participants' number (M/F)	8 (NR/NR)	Fasting group: 30 (30/0) Control group: 30 (30/0)	24 (19/5)	35 (35/0)	57 (40/17)	75 (39/36)
Age (years)	31.8 ± 2.0[a]	24.2 ± 2.3[a]	35.9 ± 11.1[a] 20–59[b]	30–50[b]	38 ± 11[a]	Hospital 1: 36.3 ± 8.8[a] (M), 35.5 ± 7.6[a] (F) Hospital 2: 37.2 ± 9.1[a] (M), 37.7 ± 6.2[a] (F)
Weight (kg)	NR	NR	77.2 ± 1.4[a] (BR) 76.0 ± 11.6[a] (DR)	NR	88.3 ± 16.2[a] (BR) 86.7 ± 15.7[a] (DR)	NR
BMI (kg/m^2)	25 ± 2.2[a]	NR	25.5 ± 3.5[a] (BR) 25.1 ± 3.5[a] (DR)	NR	29.9 ± 5.02[a] (BR) 29.4 ± 4.9[a] (DR)	NR
Collected saliva parameters and time	Melatonin 3 times (08:00; 16:00; 00:00)	SFR (= time required to collect 1 ml of saliva in 1 minute) Glucose 3 samples at mid-day (after 6 h of fasting)	Immunoglobulin A Saliva samples were taken after a 12-h overnight fast BR and 12-h after the last meal during-R	Uric-acid ALP AST Saliva sample at noon (after 8 h of fasting)	Cortisol Fixed times of the day (11:00–13:00).	Cortisol 2 times (06:00, 16:00)
Type of saliva (volume)	NR (5 mL)	Timed (2–5 min) unstimulated saliva (3 mL)	Timed (5-min) unstimulated saliva (NR)	Unstimulated saliva (3 mL)	Unstimulated saliva (NR)	NR (2 mL)
Used equipment	Highly sensitive radioimmunoassay kit	Enzymatic assay glucose kit	Behring Nephelometer	Enzymatic uric-acid assay kit Kits for assay of AST and ALT	NR	Cobase radioimmunoassay kit with electro-chemiluminescence
Participant instructions	Rinse the mouths with water before collection Avoid coughing or throat clearing into the collection tube Avoid consuming caffeine and substances containing melatonin or melatonin precursors	Gargling the mouth with about 5 ml of distilled water for 2 min	Mouth rinsed by distilled water	Gaggling the mouth with about 5.0 ml of distilled water for about 1 minute.	Avoid eating, drinking, and smoking Not to practice oral hygiene at least 1 h before No special dietary recommendations Continue a regular diet during non-fasting hours. Do not alter the habitual physical exercise levels BR or DR	In the morning brushing, eating, drinking and/or smoking was forbidden before taking the saliva sample.

(Continued)

TABLE 2 | (Continued)

First author (ref)	Bahammam (26)	Sariri (31)	Develioglu (27)	Khaleghifar (28)	Al-Rawi (29)	Dehaghi (30)
Other details	Fixed daytime working hours Regular sleep-wake schedule during week-ends Same type of work, tasks, and working hours during the study period	Mouth and teeth were examined before saliva collection	The content of the participants' diets was similar BR and DR No URTIs during the study period	NR	No sleep problems Regular sleep/wake schedule No participant practiced fasting as routine, and voluntary rituals BR	NR

ALP, alkaline phosphatase; AR, after-Ramadan; AST, aspartate amino-transferase; BMI, body-mass-index; BR, before-Ramadan; DR, during-Ramadan; F, females; h, hour; Ig, immunoglobulin; M, males; NR, not-reported; R, Ramadan; SFR, salivary flow-rate; URTIs, upper respiratory tract infections; Yr, year.
Data were: [a]Mean ± SD; [b]Minimum-maximum.

decrease. Develioglu et al. (27) noted that salivary IgA decreases significantly during the last week of Ramadan compared to BR.

DISCUSSION

The present systematic review included six studies, all having a high score of bias (26–31). In these studies, eight saliva parameters were evaluated (SFR, melatonin, cortisol, glucose, IgA, uric-acid, ALP, and AST). The main results were: ***(i)*** the SFR decreased by 10% DR in fasting participants compared to controls (31), ***(ii)*** the circadian pattern of melatonin was unchanged DR, but melatonin level dropped significantly from baseline (28), ***(iii)*** the salivary cortisol levels were unchanged DR compared to BR (29), or increased DR (30), ***(iv)*** the salivary glucose levels were decreased DR (31), ***(v)*** compared to BR, on the 15th day of Ramadan, ALP significantly increased, and uric-acid and AST significantly decreased (28); ***(vi)*** the salivary IgA decreased during the last week of Ramadan compared to BR (27). All the retained studies were heterogeneous and had several methodological differences. This heterogeneity limited the ability of the present review to perform any data synthesis *via* meta-analysis. It also challenged the researchers' ability to identify trends in the data. Research reports in this area are few and they were almost limited to the changes of glucose concentrations in plasma (31). To the best of the authors' knowledge, this is the first systematic review investigating the effects of RIF on SFR and saliva parameters.

Impacts of Ramadan Intermittent Fasting on Salivary Flow-Rate

SFR decreased by 10% DR (31). DR, the lack of gustatory stimulation decreases the stimulation of salivary glands, therefore, SFR may decline. The autonomic nervous system controls SFR and the secretion of various salivary compounds (32). Stimulation of this system induces modifications in salivary secretions and SFR (33). In Ramadan, sedentary activity with minimal orofacial movement and metabolism slowing down in body tissues cells, including oral cavity cells, may explain the low stimulation of the autonomic nervous system (28). This hyposalivation can cause malodor, especially DR (34). Since saliva works to moisten the mouth, to neutralize acids produced by plaque, and to clean bacteria and food particles from the mouth, any salivary modifications create a suitable environment for aerobic and anaerobic bacteria that coat several sites in the oral cavity, notably the dorsum of the tongue (35). Overall, it has been shown that oral microflora modifications taking place DR may lead to malodor, even if other factors are involved (36).

Impact of Ramadan Intermittent Fasting on Salivary Hormones: Melatonin and Cortisol

Melatonin in saliva or plasma is an indicator of the timing of the circadian clock (37). According to Bahammam (26), the sleep hormone follows the same circadian rhythm both BR and DR. This means that melatonin secretion is low during the daytime, while the highest levels are released at night, but its level drops significantly from baseline (26). This variation may be due to the sleep habits modification DR (36). Nevertheless, this outcome should be considered with caution because of the small sample size in the study ($n = 8$) (26).

Cortisol is a hormone produced by the adrenal glands (38). Cortisol plays an essential role in balancing blood glucose and releasing sugar from the body's stores in response to increased energy demands (39). Cortisol has an important role in the metabolism of fats and proteins as well as in the circadian rhythm regulation (38). This hormone is usually measured in the morning (7–9 a.m.) because it reaches a peak at this time (40). DR, external sources of glucose are reduced due to fasting. Consequently, salivary glucose concentration drops significantly (31). Thus, we can "speculate" that cortisol levels in saliva may rise to regulate glucose levels, however; the latter mechanism is not that straightforward and has to be elucidated by further research.

Dehagi et al. (30) reported that when participants are exposed to RIF and noise, which is another stress source, salivary cortisol levels increase. In addition to its glycemic effects, cortisol is also liberated during the stress periods in order to allow the body to adapt to an emotional or physical shock by mobilizing additional energy sources. The contradictory results of the studies of Al-Rawi et al. (29) and Dehagi et al. (30) may be due to methodological reasons, notably the study design and population,

TABLE 3 | Main results of the published studies aiming to evaluate the impacts of Ramadan intermittent fasting (RIF) on saliva parameters.

First author (ref)	Data	BR			During Ramadan								AR
					Period 1			Period 2	Period 3			Period 4	
Bahammam (26)					1st week of R				3rd week of R				–
	Timing	Midnight	8 a.m.	16 a.m.	Midnight	8 a.m.	16 a.m.	–	Midnight	8 a.m.	16 a.m.	–	–
	Mel[a]	18.1 ± 5.5	2.01 ± 1	0.62 ± 0.37	5.9 ± 8.0*	1.2 ± 1.1	0.14 ± 0.1*	–	4.1 ± 7.0†	3.9 ± 2.7	0.21 ± 0.1†	–	–
	Main aim	To assess the effect of RIF on sleep architecture, daytime sleepiness and the circadian cycle of Mel level											
	Conclusion	Midnight: Mel level has a flatter slope and a significantly lower peak for periods 1 and 3 compared to BR (BR > period 1 and BR > period 3). 16 a.m.: significant decrease of Mel from baseline for BR vs. period 3 and BR vs. period 1. 8 a.m.: no significant difference between BR vs. period 1 and BR vs. period 3. Although Mel keeps the same circadian pattern during Ramadan, its level drops significantly from baseline.											
Sariri (31)			–			R: 1–9 days		R: 10–20 days		R: 21–29 days		–	7th day after-R
	Glu[a]		–			54.5 ± 0.74[α]		58.8 ± 1.25[β]		63.6 ± 9.43[W]		–	68.5 ± 1.22
						(decrease by 25 ± 2% compared to controls)				(increase by 17 ± 2% compared to controls)		–	
	SFR		0.08–1.4					NR (10% decrease in Ramadan)				–	
	Main aim	To evaluate the influence of RIF on the level of Glu in the saliva of healthy individuals											
	Conclusion	An important decrease in salivary Glu occurred during period 1 followed by rises in periods 2 and 3. Salivary Glu decreased/decreases during fasting, mainly at the beginning of the month compared with non-fasting period.											
Develioglu (27)			1 week before			–		–		–		25th day of R (last week of R)	–
	IgA[a]		11.15 ± 6.82			–		–		–		8.98 ± 6.85[$]	–
	Main aim	To investigate the effects of RIF on serum concentrations of IgG and IgM, and salivary IgA concentrations											
	Conclusion	Salivary IgA decreased/decreases significantly during Ramadan compared to BR.											
Khaleghifar (28)			1 day BR			–		–		15th day of R		–	–
	UA[c]		4.86			–		–		3.18†		–	–
	ALP[c]		14.51			–		–		17.47†		–	–
	AST[c]		26.33			–		–		19.66†		–	–
	Main aim	To identify the influence of RIF on saliva of healthy individuals											
	Conclusion	ALP significantly increased/increases in period 3. UA and AST significantly decreased/decreases in period 3 compared with BR.											
Al-Rawi (29)			1 week BR			–		–		–		28 day of R	–

(Continued)

TABLE 3 | (Continued)

First author (ref)	Data	BR		During Ramadan					AR
				Period 1	Period 2	Period 3		Period 4	
	Cor[a]	2.2 ± 0.40		–	–	–		2.1 ± 0.40$	–
	Main aim	To examine the effect of RIF on daytime levels of ghrelin, leptin, Mel, and Cor hormones in a group of overweight and obese participants							
	Conclusion	No salivary Cor levels changes during fasting compared to BR.							
Dehagi (30)		1 week BR				3rd week of R			
	Timing	Morning	Evening			Morning	Evening		
	Cor M[b] H_1	1.41 (0.12–2.02)	0.86 (0.11–1.00)	–	–	1.61 (0.52–2.62)	1.28 (0.43–1.09)	–	–
	Cor M[b] H_2	1.16 (0.81–2.43)	0.75 (0.11–0.91)			1.55 (0.83–2.46)	1.11 (0.71–1.77)		
	Cor F[b] H_1	1.54 (0.32–2.31)	0.94 (0.10–1.02)	–	–	1.78 (0.62–2.91)	1.04 (0.35–1.42)	–	–
	Cor F[b] H_2	1.54 (0.32–2.31)	0.83 (0.10–0.98)			1.64 (0.44–2.53)	1.53 (0.88–2.18)		
	Cor[b]	–	0.81 (–0.1 to 1.13)			–	1.32 (0.29–2.32)†		
	Main aim	To investigate the combined effects of noise exposure and RIF on salivary Cor levels in nurses							
	Conclusion	Salivary Cor increased/increases during fasting when it was/is combined with noise as another stress factor. Contradictory results: BR vs. period 3							

ALP, alkaline phosphatase (U/L); AR, after-Ramadan; AST, aminotransferase (U/L); BR, before-Ramadan; Cor, cortisol (pg/mL); F, female; Glu, glucose (mg/100 ml); H, hospital; Ig A, immunoglobulin A (mg/dl); M, male; Mel, melatonin (pg/ml); R, Ramadan; SFR, salivary flow-rate (ml/min); UA, uric-acid (mg/100 ml).
Data were: [a]Mean ± SD; [b]Mean (95% confidence interval); [c]Mean.
P-value < 0.05.
**BR vs. period 1 (Bahammam).*
†BR vs. period 3 (Bahammam, Khaleghifar, Dehagi).
$BR vs. period 4 (Develioglu,Al-Rawi).
αAR vs. period 1 (Sariri).
βAR vs. period 2 (Sariri).
WAR vs. period 3 (Sariri).

and the lack of information about the timing and duration of sleep in one study (30). It should be highlighted that many people in various Islamic countries may change their sleep rhythm during the Holy month. Indeed, their nighttime sleep duration is reduced compared to non-fasting days (41), in addition to the dietary patterns' changes (42).

Impact of Ramadan Intermittent Fasting on Salivary Metabolic and Immunologic Data

Alkaline phosphatase and aminotransferase are usually measured together to investigate the hepatic, cardiovascular, and renal functions (43). ALP is a protein produced by various cell types (e.g., polymorphonuclear leukocytes, osteoblasts, macrophages, and fibroblasts) within the alveolar bone and/or the salivary glands (44, 45). ALP can be a salivary biomarker of periodontal diseases and caries (46), as it interferes in the balance of the remineralization-demineralization cycle since it is primarily involved in calcium and phosphate binding (47). It seems that the function of ALP relatively depends on the salivary pH and buffering capacity (48). Khalighefar et al. (28) reported that ALP rebounds during the middle of Ramadan compared to BR. Although ALP increase may suggest much more susceptibility to dental caries and/or oral diseases, it is believed that this fluctuation is not so critical to lead to an illness. AST is an enzyme involved in the metabolism of several tissues and organs (49). Khaleghifar et al. (28) indicated that AST activity in fasting volunteers decreases significantly DR. This decrease can be related to the fact that fasting reduces the metabolism of body tissues cells, including oral cavity cells, thus leading to reduced SFR during fasting (28). Uric-acid is the ultimate product of the metabolic breakdown of purines, which are the nitrogenous bases in DNA and RNA (50). It is involved in healing and defense (50). Khaleghifar et al. (28) reported that uric-acid decreases DR since the metabolism is reduced (28). In contrast, several studies have shown that blood uric-acid increases during RIF (51–54). According to studies reported in the literature, despite the shifts in metabolic interactions among the organs producing uric-acid, AST or ALP, we cannot conclude on the effects of RIF on these enzymes because of the scarcity of these studies in addition to the limitations of the unique retained study investigating those parameters (28).

Salivary glucose DR plunges from baseline, especially in the first 10 days (31). First, this is expected because of food restriction for 4 weeks. Secondly, this fact is interesting and beneficial for oral health. Actually, both cariogenic bacteria and *Candida* use glucose for their development and survival (55, 56). This dysbiosis enhances the proliferation of these bacteria and dental biofilm development (46, 57). A recent study investigated the effect of different salivary glucose concentrations on dual-species biofilms of *Candida albicans* and *Streptococcus mutans* (58). The authors reported that higher salivary glucose increases counts of *Candida albicans* (58). It is possible that the higher levels of IgA detected in saliva BR can be attributed to the greater colonization of the oral cavity by *Candida albicans* due to the higher salivary glucose levels during that period compared to DR.

Salivary IgA has an important role in mucosal immunity. Its levels increase in case of oral mucosa infection, such as candidiasis. It allows inhibiting the adherence of *candida* to epithelial cells (59, 60). In contrast, the decrease in those salivary IgA levels does not necessarily suggest that the participant is more susceptible to oral infection onset, since a salivary IgA concentration threshold is absent (27). Subsequently, authors suggested that RIF results in neither severe immunological disturbances nor adverse impact on health (27). Some remarks related to the usefulness of salivary IgA in real practice should be highlighted. First, there are some concerns regarding the usefulness of salivary IgA as a biomarker in the detection of respiratory tract infection due to lack of reproducibility, low specificity, and sensitivity (61). Secondly, there are conflicting data in the literature regarding salivary IgA levels induced by exercise, with some studies reporting a decrease whilst others have reported an increase or no change (62). Thirdly, previous studies have reported a decrease in systemic IgA levels without leading to an increase in infection (63). Fourthly, exposure to pathogenic microbes may be reduced DR, possibly due to consumption of more fresh foods DR compared to other months (64). It is possible that oral health and microbial exposure from foods are poorer BR, which may explain the higher IgA levels detected in the saliva DR (65). In this context, a recent study involving mice reported that oral colonization by *Candida albicans* increases IgA production (65). Another study suggested that an increase in salivary IgA is an attempt by the immune system to counter the accumulation of microorganisms (64). Considering the aforementioned studies (63–65), the decrease in IgA levels DR may reflect a lower microbial colonization of the oral cavity DR. This is plausible since the number of hours when the mouth is exposed to foods and beverages is reduced DR compared to other periods when one considers the number of hours spent fasting and sleeping.

Overall, it seems that fluctuations in salivary parameters in Ramadan are not as significant as blood changes. These alterations are not enough to cause diseases in healthy participants. Nevertheless, we believe that further studies using other salivary biomarkers are needed in order to investigate correlations with the risk of oral disturbances or infections, such as caries, malodor, periodontal disease, or candidiasis in Ramadan.

In view of the absence of evidence about the impacts of RIF on oral health, we recommend the following four advices for people observing Ramadan: ***(i)*** adopt a well-balanced diet with sufficient hydration before *Sahur* and after *Iftar*; ***(ii)*** brush teeth, at least after *Iftar* and just after *Sahur*, before the dawn; ***(iii)*** rinse mouth without swallowing water for a better biofilm control and reduction of halitosis; and ***(iv)*** take care of the oral cavity, particularly for patients with chronic systemic diseases, especially with metabolic disorders (e.g., diabetes mellitus) in order to avoid the progression of a preexistent pathology (e.g., periodontal disease, dental caries). Finally, it is recommended that dentists carry out "dental procedures" with special precautions [e.g., administer intramuscular or trans-dermal treatment instead of oral agents] (36).

TABLE 4 | Some recommendations for designing future studies related to the impact of Ramadan intermittent fasting on salivary parameters.

Issue	Authors are encouraged to:
General remarks	·Report information about the following points: season of Ramadan, ambient temperature and humidity during the study period, elapsed fasting time, and number of fasting days during the Ramadan month. ·Report the exact timing of the saliva samples.
Study protocol/design	·Opt for a cohort design. ·Include a non-fasting control group, if possible. ·Select participants using a probability sampling method. ·Perform at least three evaluation sessions: before-Ramadan (e.g., 1 week), during-Ramadan (e.g., during the last 7–10 days of Ramadan) and after-Ramadan (e.g., 7–10 days AR).
Population characteristics	·Avoid the combination of males and females in one sample. ·Systematically report the following confounding factors which interact with saliva parameters: age, smoking status, alcohol drinking, hydration status, total fluid intake (coffee, tea, juice, etc.), dietary habits, sleeping habits, teeth brushing, physical activity, obesity, *Miswak* use, fasting ritual. ·Spitting out or not (some people do not want to swallow their saliva, mistakenly thinking that it will break their fast). ·Determine how often the mouth is rinsed with water (some people avoid rinsing their mouth with water thinking this will break their fast).
Saliva collection and analysis	·Use standardized and reliable methods of saliva sampling. ·Use standardized methods of biological analysis (e.g., concentration of biomarkers should be adequately adjusted by factors, such as osmolality, total protein concentration, saliva flow-rate, and saliva secretion rate). ·Opt for unstimulated saliva rather than stimulated saliva. ·Report the normal range of saliva parameters. ·Adjust the metabolites by factors, such as total protein content, saliva osmolality, saliva flow-rate, and saliva secretion rate.
Sample size and statistical analysis/methods	·Calculate the sample size. ·Report and interpret the effect size measurement (if needed). Clearly distinguish the "clinical" significance approach from the "statistical" significance approach.

Discussion of Methodology

According to the JBI critical appraisal tool, precisely the checklist for cohort studies, the methodological quality is considered as "low." In fact, no study succeeded to get the average score and items related to "confounding factors" and "sample size calculation." Moreover, "salivary collection methods" were not reported in any of the six retained studies (**Table 1**). First, non-inclusion of a non-fasting control group can be considered as a "bias" since the variations in the assessed parameters cannot be exclusively attributed to RIF. However, it is important to note that including non-fasting participants is still problematic, due to religious considerations in Muslim countries. For that reason, the non-fasting control groups could be the participants themselves outside the Ramadan period (e.g., BR and/or AR). Given the circumstances of the Ramadan observance, and for practical reasons, the authors think that is more feasible and easier to control the parameters than to arrange a separate group of participants who do not observe Ramadan. Secondly, selecting participants by a convenience sample may be considered as a major confounding factor (66). Convenience sampling is a type of non-probability sampling methods based on the judgment of the investigator (66). Its low cost and comfort of use make it an easy choice for investigators. Nevertheless, it can lead to under/over representation of specific groups inside the sample (66). Thus, it may be impossible to make generalizations in the whole population. For these reasons, convenience sampling should be treated with caution. Thirdly, calculation of an optimal size is a crucial point since it helps avoid an inadequate power to detect statistical effects (67). Using few participants in a study may lead to lower "precision" in findings. A large sample size is, however, expensive and exposes more participants to procedures (67). Fourthly, the procedure of saliva collection was not well-described (**Table 1**). In fact, it is very important to standardize saliva sampling in order to make comparison between studies possible. Since saliva collection should be made at least one time DR, unstimulated saliva might be preferred. In fact, stimulated saliva must be collected by chewing sterile paraffin (68). Then, a minimum duration for sufficient saliva collection may be defined to ensure efficient analysis.

Additional limitations should be highlighted. For example, information about the season, the average ambient pressure, and/or the average ambient humidity was lacking in the included studies (**Table 1**). The average ambient temperature as well as the fasting duration were mentioned in some studies (26–29) (**Table 1**). Consequently, both climatic conditions and geographical locations strongly influence RIF (69). Also, the inclusion of patients with obesity (i.e., body mass index $\geq$ 30 kg/m^2) may be considered as a limitation. In fact, a lower SFR was observed among obese compared to non-obese participants (70, 71). In addition, the inclusion of females and old participants could complicate the interpretation of saliva parameters (72, 73). Indeed, Mahesh et al. (72) reported significant changes in the pH and the buffer-capacity in post-menopausal females' saliva compared to regularly menstruating ones. Besides, it is known that females do not fast all the month of Ramadan. Subsequently, the comparison with males may not be valid because they are not exposed to the same fasting period. With regard to age, changes in salivary pH, buffering-capacity, calcium, and proteins concentrations were reported (73). Finally, the number of evaluation sessions was heterogeneous. Therefore, saliva collection should be performed at least three times as follows: BR (e.g., 1 week BR), DR (e.g., during the last 7–10 days of Ramadan), and AR (e.g., 7–10 days AR). In future studies aiming to evaluate the effects of RIF on oral health, three important points should be reported. The first is related to the practice of fasting as a routine (e.g., some Muslims fast on

Mondays and Thursdays during all the year). The inclusion of some participants who practice this ritual may influence some saliva parameters. The second point concerns the chewing stick, called "*Miswak*," which is widely used in some Arab states of the Persian Gulf (74, 75). In fact, it seems that "*Miswak*" use increases SFR (76). The third point concerns the hydration status. The role of the hydration status BR and DR were not considered in the six retained studies and the differences observed in the concentrations of the different metabolites may be partly due to the hydration status, which can alter the salivary composition and SFR (77). The six studies involved in this review did not adjust the concentrations of the different salivary biomarkers before comparing the data obtained BR and DR. Therefore, in the future, it would be interesting to see if the differences observed are still present after adjusting the metabolites by factors, such as total protein content, saliva osmolality, SFR, and saliva secretion rate (78).

The critical limitation of this Systematic Review is our inability to make a strong clinical case for the impacts of RIF on saliva parameters. **Table 4** summarizes some recommendations for designing future studies aiming to investigate the impacts of RIF on saliva parameters. It is recommended that researchers assess the antimicrobial, anticancer, and wound healing properties of fasting saliva (collected just before *iftar*) and compare it with non-fasting saliva. Moreover, it would be great to compare the fasting saliva proteome with the non-fasting saliva, and to see if the fasting saliva can be a source of novel peptides that display health benefits (79). This will address the "myth/superstition" in medieval Europe where fasting saliva was used as a medicine (80, 81).

CONCLUSION

There is a general trend toward a decrease in SFR and a decrease in the content of the majority of the biomarkers investigated, with the exception of ALP and uric-acid. These changes cannot be easily attributed to any single factor, especially because of the lack of information on the hydration status, dietary habits, physical activity, and hygiene habits. Although the findings of this systematic review are interesting, scientific evidence should be interpreted carefully because studies of the impact of RIF on saliva parameters are scarce. This is mostly due to the lack of accurate methodological details or variations in the investigated saliva parameters and the employed methodologies. Furthermore, the authors have provided some recommendations for designing future studies related to the impact of RIF on salivary parameters.

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 author.

AUTHOR CONTRIBUTIONS

AB, MK, and HB performed bibliographic research, collected published manuscripts, and helped to draft the manuscript. NB helped draft the manuscript. All authors read and approved the final manuscript.

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Shifts in Fecal Metabolite Profiles Associated With Ramadan Fasting Among Chinese and Pakistani Individuals

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The human gut microbiota has been proposed to serve as a multifunctional organ in host metabolism, contributing effects to nutrient acquisition, immune response, and digestive health. Fasting during Ramadan may alter the composition of gut microbiota through changes in dietary behavior, which ultimately affects the contents of various metabolites in the gut. Here, we used liquid chromatography–mass spectrometry-based metabolomics to investigate the composition of fecal metabolites in Chinese and Pakistani individuals before and after Ramadan fasting. Principal component analysis showed distinct separation of metabolite profiles among ethnic groups as well as between pre- and post-fasting samples. After Ramadan fasting, the Chinese and Pakistani groups showed significant differences in their respective contents of various fecal metabolites. In particular, L-histidine, lycofawcine, and cordycepin concentrations were higher after Ramadan fasting in the Chinese group, while brucine was enriched in the Pakistani group. The KEGG analysis suggested that metabolites related to purine metabolism, 2-oxocarboxylic acid metabolism, and lysine degradation were significantly enriched in the total subject population pre-fasting vs. post-fasting comparisons. Several bacterial taxa were significantly correlated with specific metabolites unique to each ethnic group, suggesting that changes in fecal metabolite profiles related to Ramadan fasting may be influenced by associated shifts in gut microbiota. The fasting-related differences in fecal metabolite profile, together with these group-specific correlations between taxa and metabolites, support our previous findings that ethnic differences in dietary composition also drive variation in gut microbial composition and diversity. This landscape view of interconnected dietary behaviors, microbiota, and metabolites contributes to the future development of personalized, diet-based therapeutic strategies for gut-related disorders.

Keywords: fasting, metabolite, gut microbiota, diet, ethnic groups

INTRODUCTION

Ramadan fasting is a religious rite of Muslims which is also recognized as a form of intermittent fasting. More than 1.5 billion Muslims reportedly refrain from eating or drinking from sunrise (Sahur) to sunset (Iftar) during the holy month of Ramadan each year, which lasts between 28 and 30 days (1, 2), and thus practice intermittent fasting and caloric restriction. Intermittent fasting has been shown to have various beneficial health effects, including improved immune system function (3, 4), enhanced cognitive function (5), improved body composition and reduced obesity (6, 7), and even reduced episodes of seizures in some patients with epilepsy (8). However, the mechanisms mediating the effects of intermittent fasting remain largely obscure, which hamper the adoption of intermittent fasting as a strategy for improving health or as a disease intervention. Experiments in mice and clinical observations suggest that the benefits of intermittent fasting, such as positive effects on body weight and metabolism, can be explained by reduced energy intake (9). In addition, intermittent fasting regimens can influence metabolic regulation through changes in modifiable lifestyle behaviors, circadian rhythms, and gut microbiota (10). Our previous study has revealed that Ramadan fasting can significantly influence gut microbiota through dietary changes and has highlighted the enrichment of specific bacterial taxa, such as *Sutterella* and *Parabacteroides*, with implied health benefits in some individuals (11).

Gut microbiota have been described as a new metabolic organ that participates in regulating host metabolism (12, 13). Accumulating evidence suggests that changes in metabolic levels can affect health status and are correlated with some diseases, such as Type 2 diabetes (14), pulmonary tuberculosis (15), obesity (16), epilepsy (17), and others. Recent studies have reported that both environmental and host factors, such as environmental contaminants (18), lifestyle (19), disease conditions (20), and drug use (21), could all influence gut metabolism. To explore the role of caloric restriction-induced microbiome changes in ischemic stroke rehabilitation, Huang et al. (22) applied metabolite profiling in a mouse model. Their results showed that caloric restriction increased the levels of several metabolites, such as prostatin B1 and 3β-hydroxy-5-cholic acid, and that the upregulation of these metabolites was correlated with the abundance of bifidobacteria, suggesting that caloric restriction leads to enrichment for both specific gut microbiota and their associated metabolites. Previous studies (23, 24) have also reported that intermittent fasting can confer protective effects against diabetes by impacting gut microbiota, which consequently modulates the levels of circulating microbial metabolites. Intermittent fasting can also regulate the composition of intestinal microbial communities in diabetic patients, ultimately affecting the levels of circulating microbial metabolites. Wang et al. (25) also proposed that the beneficial effects of caloric restriction on health and metabolism might be attributable to shifts in the composition and diversity of gut microbiota. However, changes in gut metabolism driven by Ramadan fasting, with consideration for host- or ethnicity-related factors, remain poorly characterized, indicating that a detailed analysis of correlations among fasting, ethnicity, dietary composition, fecal metabolites, and gut microbiota is warranted. In light of our previous data that showed diet-related differences in gut microbiota are affected by Ramadan fasting in Pakistani and Chinese groups living in close proximity, we proposed that the month-long Ramadan fasting could affect gut metabolite profiles through the response by gut microbiota to intermittent fasting.

In this study, we investigated the influence of fasting during Ramadan on the gut metabolic profiles in fecal samples obtained from healthy Chinese and Pakistani subjects living in Lanzhou, China, using liquid chromatography–mass spectrometry (LC-MS)-based metabolomics analysis. This study was conducted with three main objectives. First, we sought to better understand whether caloric restriction during Ramadan fasting is a determining factor for metabolite profiles. Second, this study aimed to identify differences in metabolite profiles between the Pakistani and Chinese Hui ethnic groups, and to investigate whether these differences were diet-related. The third objective of this work was to test whether the diversity of intestinal flora is correlated with metabolite profile.

MATERIALS AND METHODS

Ethical Approval, Consent to Participate, and Recruitment

All procedures performed were approved by the Medical Ethics Committee of the School of Public Health at Lanzhou University (GW-20171013). Participants received the detailed information about the study and informed consent before sampling.

Participants' mental and physical health were included as criteria. We excluded volunteers in case of (1) gastrointestinal diseases, chronic diseases, anorexia nervosa, and cachexia, (2) insufficiency of liver and kidney, (3) smoking or drinking alcohol, (4) Bristol Stool Form Scale (<3/>4), and (5) antibiotics usage within last 3 months (26). According to the inclusion and exclusion criteria, 34 healthy adults (aged 18–40 years) living in a proximity area in Lanzhou city (16 Chinese adults and 18 Pakistani adults) were recruited. All participants signed the consent letter and attended Ramadan fasting from 15 May 2018 to 15 June 2018; both dietary survey (3 days 24-h food dietary recall) and biosamples (fecal) were collected before and after Ramadan fasting (27). To figure out the gut metabolic profile driven by fasting, all the participants were subject to three groups, namely, (1) total fasting group: total before fasting (TBF) vs. total after fasting (TAF); (2) Pakistani fasting group: Pakistani before fasting (PBF) vs. Pakistani after fasting (PAF); and (3) Chinese fasting group: Chinese before fasting (CBF) vs. Chinese after fasting (CAF). In contrast, group comparisons between ethnic groups were carried out by CBF vs. PBF and CAF vs. PAF.

Sample Collection and 16S rRNA Gene Sequencing

Fecal samples were collected for all participants on the morning of 15 May 2018 (before fasting) and 15 June 2018 (after fasting). Samples were frozen in liquid nitrogen immediately upon receipt

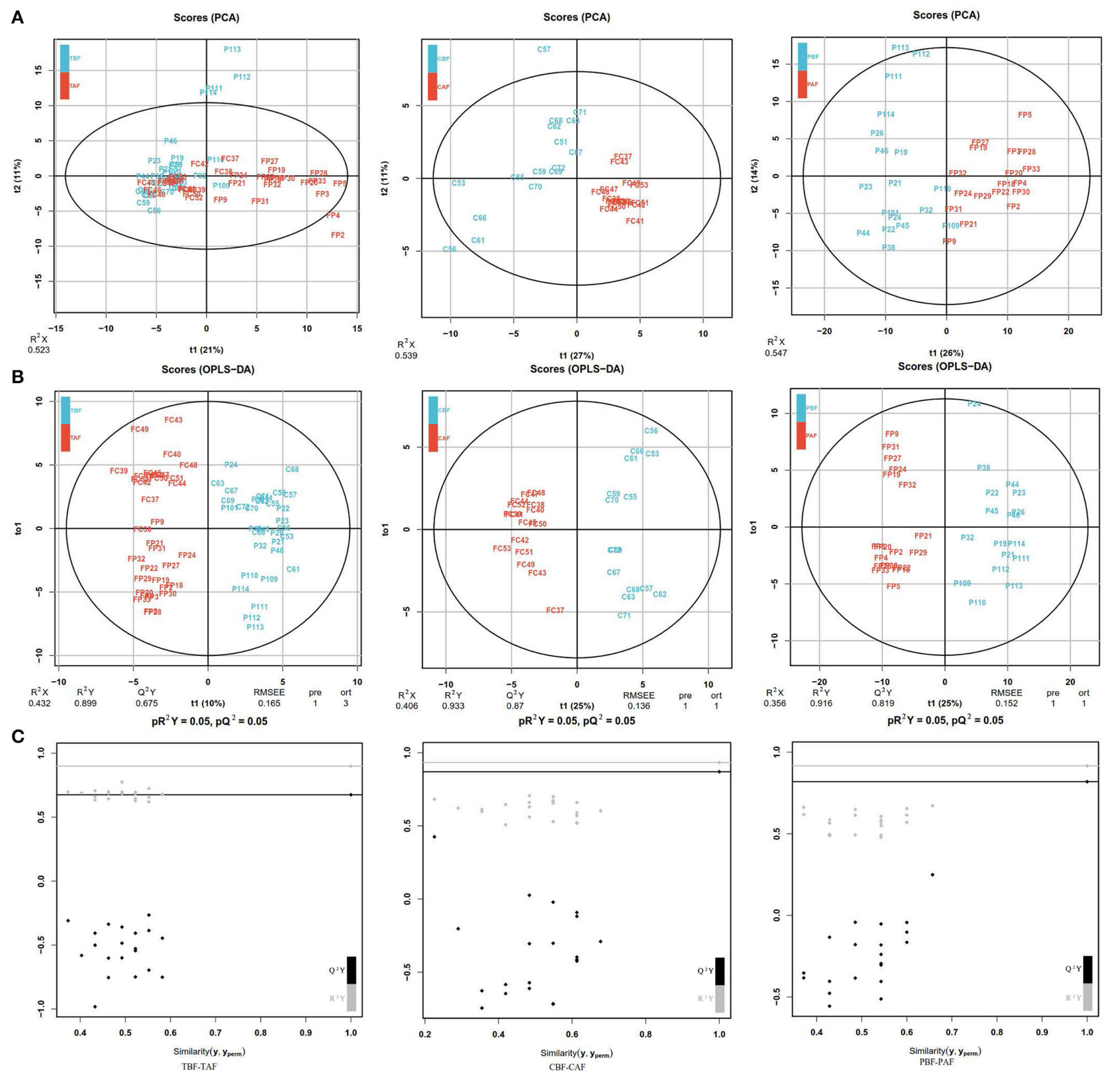

FIGURE 1 | (A) Principal component analysis (PCA) analyses comparing metabolic profiles of subjects within each ethnicity group before and after fasting (CBF vs. CAF, PBF vs. PAF) and among the total study subjects (TBF vs. TAF). Each numbered datapoint represents an individual subject. Blue, before fasting; Red, after fasting; CBF/CAF, Chinese before or after fasting; PBF/PAF, Pakistani before or after fasting; TBF/TAF, Total subjects before or after fasting. **(B)** Orthogonal partial least squares discrimination analysis (OPLS-DA) scores of fasting groups showed significant differences in clustering between samples collected before and after fasting (TBF vs. TAF; CBF vs. CAF; PBF vs. PAF). The abscissa represents the predicted principal component score of the first principal component and the ordinate represents the variability within the grouping. R^2 represents the explanatory power of the model to variables, and Q^2 represents the predictability of the model. **(C)** Model validation of OPLS-DA (TBF vs. TAF; CBF vs. CAF; PBF vs. PAF) ($p < 0.05$). The abscissa indicates similarity with the original model, and the ordinates represent R^2Y and Q values. Gray and black points on the respective lines in the upper right corner indicate the actual values, while points on the left represent simulated values. When all simulated Q^2 (black) and R^2 values (gray) are lower than the respective original points, the model is robust, without over-fitting.

to maintain sample stability, then transferred to the laboratory, and further stored at −70°C for future experiments.

As described by Ali et al. (11), QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany) was used for DNA extraction and subjected to DNA sequencing using MiSeq Reagent Kit version 3 (Illumina, San Diego, CA, USA). Raw reads were loaded into the European Nucleotide Archive under the succession number PRJEB38231 (http://www.ebi.ac.uk/ena/

data/view/PRJEB38231), and the results have been presented in previous studies (11).

LC-MS Experiments

The samples were slowly thawed on the ice. A 50-mg sample was added into a 1.5-ml centrifuge tube, followed by adding 800 μl of 80% methanol, grinding for 90 s at 65 Hz, thorough mixing using eddy oscillation, and treating using ultrasound at 4°C for 30 min. The mixture was kept at −40°C for 1 h, followed by mixing using eddy oscillation for 30 s at 4°C and allowing to stand for 0.5 h. All supernatants were placed into a centrifuge tube, allowed to stand at −40°C for 1 h, and centrifuged at 4°C and 12,000 rpm for 15 min. A 200 μl of supernatant was removed and 5 μl of internal standard (0.14 mg/ml dichlorophenylalanine) was added prior to transfer into injection vial. The treated samples were used for untargeted metabolomics analysis of metabolic characteristics, assisted by Shanghai Tianhao Biotechnology Co., Ltd.

The ACQUITY UPLC system (Thermo, Q Exactive) was used to analyze metabolic profiles in Electrospray ionization (ESI)-positive and ESI-negative modes. ACQUITY UPLC HSS T3 (2.1 × 100 mm, 1.8 μm) column was used for positive and negative modes. The binary gradient elution system consists of (A) water (containing 0.05% formic acid, V/V) and (B) acetonitrile, separated using the following gradient: 0 min, 5%B; 1 min, 5%B; 12 min, 95%B; 13.5 min, 95%B; 13.6 min, 5%B; and 16 min, 5%B. The flow rate was 0.3 ml/min, and the column temperature was 40°C.

Quality control (QC) samples that were included in batches of analytical samples during the course of the study were considered to monitor the overall quality of the sample pretreatment and mass spectrometry analyses. The data were collected using feature extraction and preprocessed using the Compound Discoverer software (Thermo), and then normalized and edited into two-dimensional data matrix using the Excel 2010 software, including retention time (RT), compound molecular weight (compMW), observations (samples), and peak intensity.

Data Analysis

Unsupervised principal component analysis (PCA) was used to observe the overall distribution among samples and the degree of dispersion between groups. Then, supervised orthogonal partial least squares discrimination analysis (OPLS-DA) was used to distinguish the overall differences of metabolic profiles among groups and to search for the metabolites that differed between groups. In the OPLS-DA analysis, the Variable Importance for the Projection (VIP) > 1 was set as the difference variable. *T*-test combined with multivariate analysis OPLS-DA was used to screen out the metabolites that differed between groups (VIP > 1 and $p < 0.05$). Differential metabolites obtained from the comparison between groups were input into MetaboAnalyst 3.0. Cluster analysis was performed on different substances in different groups to check the relative changes in the contents of these different substances in different groups, and pathway attribution was carried out to locate the differential metabolites into metabolic pathways. Metabolite data were entered into the KEGG database using comprehensive analysis (e.g., enrichment analysis and topological analysis) of the pathways where the differential metabolites were located, and we could further screen the pathways and find the key pathways with the highest correlation based on the difference in metabolites.

The Wilcoxon signed-rank tests were used to compare the "before vs. after Ramadan fasting" groups (CBF vs. CAF, PBF vs. PAF, TBF vs. TAF), and the Mann–Whitney *U* test was used for comparisons between ethnic groups (CBF vs. PBF, CAF vs. PAF). In addition, Pearson's correlation coefficient was used to analyze the correlation between fecal metabolite and the relative abundance of intestinal microorganisms.

The SPSS version 23.0 was used for all statistical analyses. R (phearmap) was used to analyze the correlation between fecal metabolites and intestinal flora, and Pearson's correlation coefficient (the Spearman statistical analysis) was used to indicate the degree of correlation (28). The correlation coefficient is presented in the form of heat map, and the correlation is reflected by a color gradient.

RESULTS

Demographic and Dietary Intake Features of Study Participants

The dietary intake and demographic characteristics of all participants in this study were previously described by Ali et al. (11). A total of 34 volunteers (16 Chinese and 18 Pakistanis) were included in this study, aged between 20 and 33 years, with a male-to-female ratio of approximately 8:2. Body mass index (BMI) of subjects before and after fasting was calculated based on the height and weight, with significantly greater BMI in the Chinese group before fasting ($p = 0.01$) but no significant difference between groups after fasting (**Supplementary Table 1**). Before fasting, the Chinese group had higher daily intake of grains (CBF range = 537.35–921.35 g vs. PBF range = 487.33–666.68 g) and soybeans (CBF range = 27.91–226.67 g vs. PBF = 0.00 g), but a lower daily intake of livestock meat (CBF range = 10.11–87.83 g vs. PBF range = 41.67–127.09 g), fruits (CBF range = 0.00–82.92 g vs. PBF range = 0.00–251.10 g), and poultry (CBF = 0.00 g vs. PBF range = 79.16–244.99 g), compared to that consumed by the Pakistani group. The proportions of energy sources consumed by each group showed that carbohydrate intake was significantly greater among Chinese participants (CBF range = 61.50–70.18% vs. PBF range = 45.24–54.73%), while the Pakistani group consumed a significantly greater proportion of fats (PBF range = 31.86–38.23% vs. CBF range = 16.71–25.61%) and proteins (PBF = 13.15–17.81% vs. CBF = 11.41–14.49%). No significant differences were identified between groups in beverage consumption, including caffeinated and non-caffeinated beverages (11).

Shifts in Fecal Metabolite Profiles Associated With Fasting or Ethnicity

To identify the differences in fecal metabolite profiles attributable to either Ramadan fasting or ethnicity, we used untargeted LC-MS for metabolomics analysis of fecal supernatants from before and after Ramadan fasting. Metabolite profiles were then subjected to PCA modeling to identify differences between ethnic

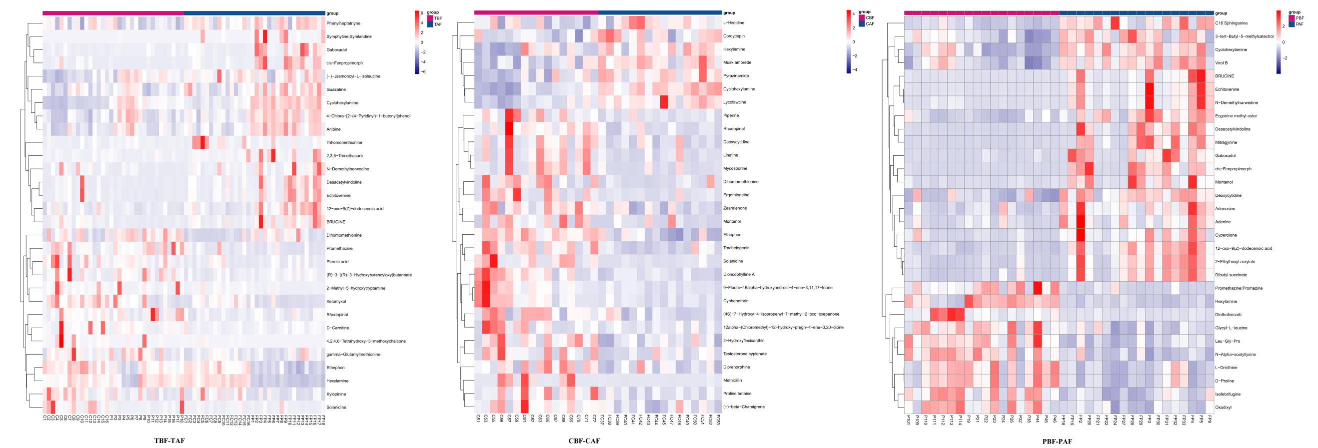

FIGURE 2 | Heat maps of metabolites of fasting groups (TBF vs. TAF; CBF vs. CAF; PBF vs. PAF) show the first 30 metabolite that was significantly different before and after fasting ($p < 0.05$).

groups (CBF vs. PBF and CAF vs. PAF) or within ethnic groups before and after Ramadan fasting (CBF vs. CAF and PBF vs. PAF), and among total subjects before and after fasting (TBF vs. TAF). A total of 257 different metabolites were detected between before and after samples from total subjects (VIP > 1, $p < 0.05$). In contrast, 118 metabolites were different after fasting in the Chinese groups (CBF vs. CAF) and 500 metabolites were unique to pre- or post-fasting Pakistani groups (PBF vs. PAF) (VIP > 1, $p < 0.05$, **Supplementary Figure 2**). Furthermore, 547 metabolites differed between Chinese and Pakistani ethnic groups before fasting (CBF vs. PBF), while 796 metabolites differed between ethnic groups after fasting (CAF vs. PAF) (VIP > 1, $p < 0.05$, **Supplementary Figure 2**).

Fasting Groups

The PCA analysis showed significant differences between pre- and post-fasting metabolite profiles for both ethnic groups (CBF vs. CAF and PBF vs. PAF) and in the total subject population (TBF vs. TAF) (**Figure 1A**). Total subjects clustered into distinct before and after fasting groups along PC1 (21%)/PC2 (11%) axes, with some overlap in the groups. In comparison with each ethnic group, the pre- and post-fasting samples also formed distinct clusters that were largely explained by the PC1(27%)/PC2(11%) in CBF vs. CAF comparisons and PC1(26%)/PC2(14%) in PBF vs. PAF comparisons (**Figure 1A**). Modeling by OPLS-DA (**Figure 1B**) showed more obvious separation in metabolite profiles before and after Ramadan fasting for both ethnic groups, which was supported by model validation (**Figure 1C**) for each of these OPLS-DA plots. These results showed that, despite ethnic differences, fecal metabolite contents and composition were changed after fasting.

Ethnic Groups

We next identified the differences in fecal metabolite profiles before and after fasting that were attributable to ethnicity. PCA showed that the significant separation of metabolite profiles between Chinese and Pakistani groups before fasting (CBF vs. PBF) could be largely explained by PC1 (31%)/PC2 (8%), as were differences in metabolite profiles between ethnic groups after fasting PC1 (32%)/PC2 (9%) (CAF vs. PAF). Moreover, the separation was more obvious after fasting (**Supplementary Figure 1A**). OPLS-DA modeling (**Supplementary Figure 1B**) further revealed significant differences in the clustering of samples, indicating that fecal metabolite profiles were distinct to each ethnic group, both before (CBF vs. PBF) and after (CAF vs. PAF) fasting, which was supported by model validation (**Supplementary Figure 1C**). Taken together, these results showed that fecal metabolites were distinct to each ethnic group, and that these differences persisted after fasting.

Differential Enrichment for Metabolites and KEGG Pathways After Fasting

To explore the potential mechanisms responsible for changes in fecal metabolites before and after fasting, we analyzed the significantly different metabolites and their respective metabolic pathways. Heat map visualization of differentially abundant metabolites between pre- and post-fasting groups showed that the levels of ethephon, zearalenone, trachelogenin, deoxycytidine, and rhodopinal were higher before fasting than after among Chinese participants, while L-histidine, cordycepin, hexylamine, pyrazinamide, and cyclohexylamine were increased after fasting. Among Pakistani subjects, metabolites, such as promethazine, diethofencarb, hexylamine, D-proline, and *N*-α-acetyl-lysine, were all higher before fasting, whereas cyclohexylamine, virol B, ecgonine methyl ester, deoxycytidine, and adenine levels were all significantly higher after fasting compared to their pre-fasting levels (**Figure 2**). The KEGG analysis suggested that purine metabolism, 2-oxocarboxylic acid metabolism, and lysine degradation were all significantly enriched among the total subject population in pre- and post-fasting comparisons (TBF vs. TAF). In contrast, 2-oxocarboxylic acid metabolism, biosynthesis of amino acids, and arginine biosynthesis pathways were significantly enriched in comparisons of metabolites before and after fasting specifically among Chinese subjects (CBF vs. CAF). Among Pakistani participants, pathways related to biosynthesis of amino acids, purine metabolism, and 2-oxocarboxylic acid metabolism were significantly enriched in before and after fasting comparisons (PBF vs. PAF) (**Figure 3**).

Comparisons of significantly different metabolites between ethnic groups before fasting (CBF vs. PBF) indicated that ethephon, diethofencarb, sodium oleate, montanol, and nafcillin were enriched in samples from Chinese subjects, while picolinic acid, rehmaionoside C, homobaldrinal, cyclohexylamine, and N6-trimethyl-L-lysine were all higher in Pakistani subjects. After fasting (CAF vs. PAF), diethofencarb, soyasapogenol E, norcodeine, ethephon, and hexylamine were higher in the Chinese group, while ergothioneine, dicrotophos, nabilone, bufadienolide, and luffariellolide were enriched in the Pakistani group (**Supplementary Figure 3**). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis showed that biosynthesis of amino acids, caffeine metabolism, arginine, and proline metabolism were significantly enriched in comparison with pre-fasting metabolites between ethnic groups (CBF vs. PBF), while pathways related to biosynthesis of amino acids, arginine and proline metabolism, and ABC transporters were significantly enriched after fasting (CAF vs. PAF) (**Supplementary Figure 4**).

Fecal Metabolites Show an Obvious Correlation With Gut Microbiota Before and After Fasting

We then evaluated possible correlations between fecal metabolites and intestinal microbiota between ethnic groups before and after fasting using Pearson's correlation analysis. Correlations between the top 30 most abundant metabolites and gut microbiota (genus level) in different groups (1. TBF vs. TAF; 2. CBF vs. CAF; 3. PBF vs. PAF; 4. CBF vs. PBF; 5. CAF vs. PAF) are shown in **Figure 4** and **Supplementary Figure 5**.

Among the total subjects (TBF vs. TAF), *Prevotella* was positively correlated with cyclohexylamine (cor $\geq$ 0.5, $p < 0.01$) but negatively correlated with ethephon (cor ≤ -0.5, $p < 0.01$), whereas *Bacteroides* was positively correlated with ethephon (cor $\geq$ 0.5, $p < 0.01$) and negatively correlated with cyclohexylamine (cor ≤ -0.5, $p < 0.01$). *Catenibacterium* was positively correlated with cyclohexylamine (cor $\geq$ 0.5, $p < 0.01$). In the pre- and post-fasting Chinese groups (CBF and CAF), *Escherichia/Shigella* was positively correlated with musk ambrette and cordycepin (cor $\geq$ 0.5, $p < 0.01$), while *Faecalibacterium* was positively correlated with cordycepin (cor $\geq$ 0.5, $p < 0.01$) and negatively correlated with zearalenone (cor ≤ -0.5, $p < 0.01$). *Gemmiger* showed a positive correlation with lycofawcine (cor $\geq$ 0.5, $p < 0.01$), and *Oscillibacter* was positively correlated with 12α-chloromethyl-12-hydroxy-pregn-4-ene-3, 20-dione (cor $\geq$ 0.5, $p < 0.01$).

Comparisons of Pakistan groups before and after fasting (PBF and PAF) revealed a positive correlation between *Prevotella* and 12-oxo-9Z-dodecenoic acid, 2-ethylhexyl acrylate, montanol, and cyperolone (cor $\geq$ 0.5, $p < 0.01$). *Coprococcus* was positively correlated with L-ornithine, D-proline, and Isofebrifugine (cor $\geq$ 0.5, $p < 0.01$), but was negatively correlated with 12-oxo-9Z-dodecenoic acid, 2-ethylhexyl acrylate, dibutyl succinate, and cyperolone (cor ≤ -0.5, $p < 0.01$). *Blautia* abundance was positively correlated with hexylamine (cor $\geq$ 0.5, $p < 0.01$) and negatively correlated with brucine (cor ≤ -0.5, $p < 0.01$), while *Dialister* was positively correlated with oxadixyl (cor $\geq$ 0.5, $p < 0.01$), and *Bacteroides* was negatively correlated with montanol (cor ≤ -0.5, $p < 0.01$) (**Figure 4**).

Comparisons of pre-fasting ethnic groups (CBF vs. PBF) showed a negative correlation between *Prevotella* and nafcillin, 9-fluoro-16α-hydroxyandrost-4-ene-3,11,17-trione, and cyphenothrin (cor ≤ -0.7, $p < 0.01$). *Oscillibacter* was negatively correlated with trans-trans-farnesyl phosphate and sodium oleate (cor ≤ -0.7, $p < 0.01$), and *Faecalibacterium* was negatively correlated with ethephon (cor ≤ -0.7, $p < 0.01$). *Catenibacterium* shared a positive relationship with azacitidine (cor $\geq$ 0.7, $p < 0.01$), but was negatively correlated with 17α-methyl-5α-androstane-3β-11β-17β-triol and trans-trans-farnesyl phosphate (cor ≤ -0.7, $p < 0.01$). *Bacteroides* was positively correlated with 9-fluoro-16α-hydroxyandrost-4-ene-3-11-17-trione (cor $\geq$ 0.7, $p < 0.01$) and negatively correlated with leonuridine, cyclohexylamine, theobromine, N6-trimethyl-L-lysine, cis-acetylacrylate, picolinic acid, gaboxadol, calystegin A3, gabaculine, methionine sulfoxide, and carteolol (cor ≤ -0.7, $p < 0.01$).

Correlation analysis after fasting (CAF vs. PAF) showed that romboutsia was negatively correlated with N6-trimethyl-L-lysine and calystegin A3 (cor ≤ -0.7, $p < 0.01$). *Prevotella* was positively correlated with N6-trimethyl-L-lysine, methylone, calystegin A3, dicyclomine, kikkanol B, and deoxycytidine (cor $\geq$ 0.7, $p < 0.01$). *Oscillibacter* had positive correlations with homobaldrinal, cyclohexylamine, nabilone, methylone, cis-acetylacrylate-2-hydroxy-2,4-pentadienoate, dicrotophos, dioctyl phthalate, cassaidine, bufadienolide, and kikkanol B (cor $\geq$ 0.7, $p < 0.01$), but was negatively correlated with norcodeine (cor ≤ -0.7, $p < 0.01$). *Escherichia/Shigella* was negatively correlated with N6-trimethyl-L-lysine, rehmaionoside C, and testosterone enanthate (cor ≤ -0.7, $p < 0.01$), while *Bacteroides* was negatively correlated with 6-eenzylaminopurine (cor ≤ -0.7, $p < 0.01$) (**Supplementary Figure 5**).

DISCUSSION

To date, several studies have examined the effects of Ramadan fasting on gut microbiota. However, the results have been inconsistent, largely due to heterogeneity in age, sex, ethnicity, and/or health status, as well as in methodology. In addition, the effects of dietary behavior (e.g., Ramadan fasting) on the relationship between gut microbiota and fecal metabolites have not yet been investigated. In this study, we applied untargeted metabolomics to detect differential metabolites between Chinese and Pakistani ethnic groups before and after fasting for Ramadan to identify characteristic fecal metabolites and enriched KEGG pathways that are potentially influenced by either dietary behavior (fasting) or host factors (ethnicity), or both.

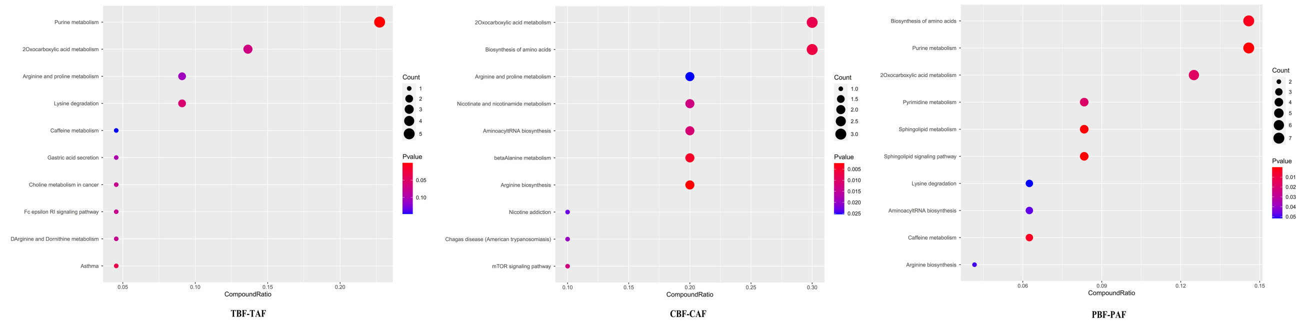

FIGURE 3 | Dot plot of top 10 metabolite pathway of fasting groups (TBF vs. TAF; CBF vs. CAF; PBF vs. PAF. TBF/TAF, Total subjects before or after fasting; CBF/CAF, Chinese before or after fasting; PBF/PAF, Pakistani before or after fasting). The abscissa is the proportion of metabolites, and the ordinate is the path. The redder the color of the point, the smaller the *p*-value, and the size of the point represents the quantity of metabolites.

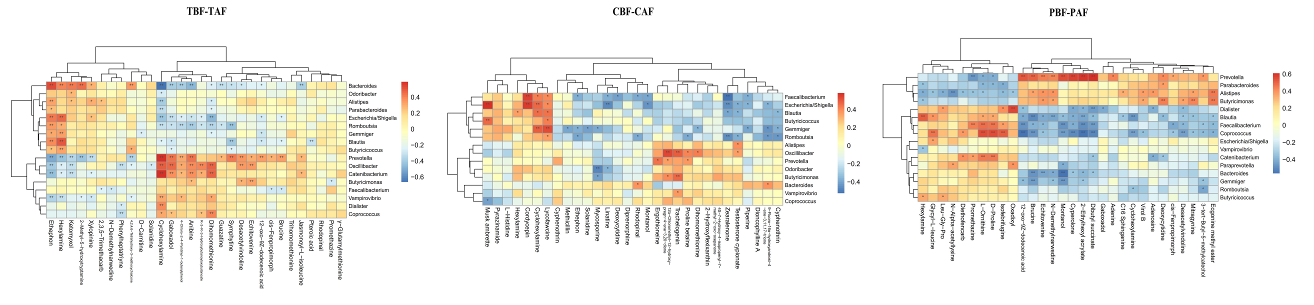

FIGURE 4 | Correlation between fecal microbiota members and metabolites between fasting groups (TBF vs. TAF; CBF vs. CAF; PBF vs. PAF. TBF/TAF, Total subjects before or after fasting; CBF/CAF, Chinese before or after fasting; PBF/PAF, Pakistani before or after fasting). The abscissa represents the top 30 differential metabolites, and the ordinate represents the highest abundance species of intestinal microflora (genus level). Black stars in the box indicate significant results (*$p < 0.05$; **$p < 0.01$).

In previous study, we investigated whether Ramadan fasting leads to changes in gut microbiota composition in 34 healthy participants from China or Pakistan by conducting high-throughput 16S rRNA gene sequencing of fecal samples before and after fasting. We detected a total of 1,074 operational taxonomic unit (OTUs) in the full study population and identified several taxa as indicators of host ethnicity and/or changes in dietary behavior. Notably, *Dorea*, *Klebsiella*, and *Faecalibacterium* were more abundant after fasting in the Chinese group, while *Sutterella*, *Parabacteroides*, and *Alistipes* were significantly increased after fasting in the Pakistani group. Further analysis of gut microbiota and dietary composition by principal co-ordinates analysis (PCoA) showed similar patterns of slight separation of samples across fasting groups, but dramatic distinctions between ethnic groups. These findings, together with obvious correlations between dietary intake and microbial composition, strongly suggested that differences in gut microbiota among the two ethnic groups were likely driven by differences in dietary intake (11).

Intermittent fasting has been shown to trigger substantial remodeling of the gut microbiota (29). For example, Ozkul et al. reported significant enrichment of *Lachnospiraceae* and *Erysipelotrichaceae* after fasting, which was associated with the accumulation of short-chain fatty acids (30). However, our previous study has showed enrichment for butyric acid-producing bacteria, such as *Clostridium_XlVa* and *Lachnospiraceae incertae sedis*, before Ramadan fasting rather than after. We are inclined to speculate that regional differences in the sources of food consumed during and after Ramadan fasting (i.e., different food sources than those in other published studies) led to a decline in this population, rather than enrichment, but further evidence examining microbiota in food sources from geographically isolated regions is necessary to test this possibility.

In this work, fecal metabolomics profiles from before and after fasting in Chinese and Pakistani groups revealed that L-histidine, lycofawcine, and cordycepin, among others, were significantly higher after fasting in Chinese participants. L-histidine is reported to provide potentially ameliorative effects on atopic dermatitis and was shown to exhibit some neuroprotective activity, notably in the treatment of vascular dementia (31, 32). Intestinal dysfunction is commonly reported in the studies on calorie restriction, especially intermittent fasting (33). However, the accumulation of functional metabolites produced by gut bacteria with anti-inflammatory effects, such as propionic acid, has been proposed to possibly mitigate intestinal dysfunction during intermittent fasting (34). Lycofawcine is a bioactive alkaloid obtained from *Lycopodium* that reportedly exerts anti-inflammatory and anti-cytotoxic biological activities (35), while cordycepin exerts significant protective effects against hepatic steatosis, inflammation, liver injury, and fibrosis in mice under metabolic stress through activation of the Adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) signaling pathway (36).

Among the Pakistani participants, brucine content was significantly higher after fasting than before. Brucine is an anti-inflammatory and analgesic drug used to relieve arthritis and traumatic pain (37). It was also shown to inhibit tumor angiogenesis, growth, and bone metastasis by downregulating the expression of vascular endothelial growth factor (VEGF), and it can inhibit the growth and migration of Human colon cancer cell (LoVo) colorectal cancer cells by regulating the Wnt/β-catenin signaling pathway (38, 39). While Brucine increased, the alkaloid isofebrifugine decreased after fasting, which can significantly inhibit the proliferation, migration, and invasion of SGC7901 gastric cancer cells (40). It is unlikely that the observed enrichment for this compound was due to medical treatment and exclusion criteria for this study.

Metabolomics analysis in this study showed that diethofencarb, rehmaionoside C, soyasapogenol E, and bufadienolide levels, among other metabolites, differed between Chinese and Pakistani individuals. Moreover, the contents of caffeine and caffeine-specific metabolites, such as theobromine, paraxanthine, and 1,7-dimethyluric acid, were significantly higher in the fecal metabolites of Pakistani samples before fasting than in Chinese samples, which could reflect differences in dietary intake between the two groups (41), potentially reflecting differences in the consumption of chocolate, tea, soda, or coffee, the main dietary sources of caffeine (42).

Metabolites produced by a healthy gut microbiota, such as short-chain fatty acids (e.g., acetic, propionic, and butyric acids), play important roles in maintaining the intestinal barrier, (43) providing energy (44) and immune homeostasis (45). It is well-recognized that gut microbiota also participate in modulating the intestinal microenvironment and host metabolism, while the gut microecology is vulnerable to the effects of unhealthy diet (46) and antibiotic treatments (47). Furthermore, the disruption of gut microbial homeostasis may result in metabolic shifts, ultimately leading to the pathological development of metabolic diseases. Thus, identifying correlations between intestinal microbiota and fecal metabolites can enhance our understanding of intestinal microbiota function and regulatory effects on human health and disease. For example, a lifestyle intervention study and screen of gut microbiota with metabolite profiling by Jang and colleagues (48) showed that choline and betaine, which contribute to the risk of obesity, could serve as reliable biomarkers of metabolic changes driven by lifestyle interventions, and that their levels were affected by diet and gut microbiota. In our study, we observed a wide range of betaine levels after fasting, particularly in the Chinese group, but found no correlation between betaine and specific taxa, although other studies reported significant correlations between betaine levels and certain potentially beneficial bacteria. We thus speculated that this discrepancy may be related to the increased consumption of some betaine-rich foods during Ramadan, although further study is required to determine the dietary source of betaine in this group.

In summary, our findings suggest that intermittent fasting can affect the composition and diversity of intestinal microbiota, and hence microbial metabolites, possibly resulting in different effects on human health. Other studies have also shown that intermittent fasting can have profound beneficial effects on animal and human health (49–53), although fasting-based interventions most commonly focus on young, healthy participants and do not consider age- or disease-related differences in metabolism and other factors. For example, severe protein restriction results in weight loss in older, but not younger, mice; conversely, low protein intake is associated with reduced mortality in people aged 65 years and younger, but not in individuals aged 66 years and older (54). Currently, the majority of fasting-related studies suggest that this dietary behavior confers potentially beneficial effects on the human health. For example, in animal models, intermittent fasting can improve metabolic function and help control hormonal changes, inflammatory responses, lipid metabolism, and insulin sensitivity (55, 56). Although some studies show promising effects of intermittent fasting, the long-term effects of caloric restriction through this dietary method remain unclear (57). Some studies examining the effects of Ramadan fasting have shown that alertness is decreased in individuals during fasting (58, 59), while sleepiness and irritability are increased (60), or that fasting for Ramadan is associated with impairment of cognitive function (61). Thus, while most studies have shown that fasting is beneficial to human health, specific fasting strategies should be personalized based on an individual's current health status, dietary preferences, social environment, and other relevant factors.

CONCLUSION

Our results suggest that fasting leads to changes in metabolite profiles specific to each ethnic group in a manner dependent on dietary components. These changes are correlated with dynamic shifts in microbiota composition and diversity which, in conjunction with dietary changes during Ramadan fasting, lead to enrichment or depletion of various functional metabolites. Future work should examine whether these changes in metabolite profiles are also correlated with the expression of biomarkers to determine if there are ethnicity-specific differences in adaptive response to Ramadan fasting. In addition, future work with more stringent controls will examine whether and to what extent environmental factors also contribute to shifts in metabolite profiles during Ramadan fasting.

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 human participants were reviewed and approved by Medical Ethics Committee of School of Public Health in Lanzhou University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XH and RL: study design. XH: funding acquisition. SC and IA: investigation. SC, IA, XL, and DL: data analysis. SC: writing. XH, RL, and YZ: manuscript review and editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.845086/full#supplementary-material

Supplementary Figure 1 | (A) PCA analyses comparing metabolic profiles of subjects within Ethnic groups (CBF vs. PBF; CAF vs. PAF. CBF/PBF, Chinese or Pakistani before fasting; CAF/PAF, Chinese or Pakistani after fasting). Each numbered datapoint represents an individual subject. Blue, Pakistani; Red, Chinese. **(B)** OPLS-DA scores of fasting groups showed significant differences in clustering between ethnic groups (CBF vs. PBF; CAF vs. PAF). The abscissa represents the predicted principal component score of the first principal component and the ordinate represents the variability within the grouping. R^2 represents the explanatory power of the model to variables, and Q^2 represents the predictability of the model. **(C)** Model validation of OPLS-DA (CBF vs. PBF; CAF vs. PAF) ($p < 0.05$). The abscissa indicates similarity with the original model, and the ordinates represent R^2Y and Q values. Gray and black points on the respective lines in the upper right corner indicate the actual values, while points on the left represent simulated values. When all simulated Q^2 (black) and R^2 values (gray) are lower than the respective original points, the model is robust, without over-fitting.

Supplementary Figure 2 | (A) The volcano figure of fasting groups (TBF vs. TAF; CBF vs. CAF; PBF vs. PAF. CBF/CAF, Chinese before or after fasting; PBF/PAF, Pakistani before or after fasting; TBF/TAF, Total subjects before or after fasting) shows the number of different metabolites detected in different fasting groups (VIP > 1, $p < 0.05$), with red representing up-regulated metabolites and blue representing down-regulated metabolites. **(B)** The volcano figure of ethnic groups (CBF vs. PBF; CAF vs. PAF. CBF/PBF, Chinese or Pakistani before fasting; CAF/PAF, Chinese or Pakistani after fasting) shows the number of different metabolites detected in different ethnic groups (VIP > 1, $p < 0.05$), with red representing up-regulated metabolites and blue representing down-regulated metabolites.

Supplementary Figure 3 | Heat maps of metabolites of ethnic groups (CBF vs. PBF; CAF vs. PAF. CBF/PBF, Chinese or Pakistani before fasting; CAF/PAF, Chinese or Pakistani after fasting) show the first 30 metabolite that was significantly different between Chinese and the Pakistanis ($p < 0.05$).

Supplementary Figure 4 | Dot plot of top 10 metabolite pathway 619 of ethnic groups (CBF vs. PBF; CAF vs. PAF. CBF/PBF, Chinese or Pakistani before fasting; CAF/PAF, Chinese or Pakistani after fasting). The abscissa is the proportion of metabolites, and the ordinate is the path. The redder the color of the point, the smaller the *p* value, and the size of the point represents the quantity of metabolites.

Supplementary Figure 5 | Correlation between fecal microbiota members and metabolites between of ethnic groups (CBF vs. PBF; CAF vs. PAF. CBF/PBF, Chinese or Pakistani before fasting; CAF/PAF, Chinese or Pakistani after fasting). The abscissa represents the top 30 differential metabolites, and the ordinate represents the highest abundance species of intestinal microflora (genus level). Black stars in the box indicate significant results (*$p < 0.05$; **$p < 0.01$).

Supplementary Figure 6 | Graphical Abstract: Bridging human health, gut microbiota & metabolites by fasting.

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Investigating Ramadan Like Fasting Effects on the Gut Microbiome in BALB/c Mice

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Recently we reported that in healthy volunteer Ramadan-associated intermittent fasting (RAIF) remodels the gut microbiome and resulted in an increase in small chain fatty acid producing bacteria concomitant with improved metabolic parameters. As interpretation of these results is hampered by the possible psychological effects associated with the study, we now aim to investigate RAIF in experimental animals. To this end, 6-week male BALB/c mice were subjected to RAIF (30 days of a 16-h daily fasting; $n = 8$) or provided with feed *ad libitum* ($n = 6$). Fecal samples were collected before and the end of fasting and bacterial 16S rRNA sequencing was performed. We found that RAIF remodeled the composition of gut microbiota in BALB/c mice ($p < 0.01$) and especially provoked upregulation of butyrate acid-producing Lachnospireceae and Ruminococcaceae ($p < 0.01$), resembling the effects seen in human volunteers. Hence we conclude that the effects of RAIF on gut microbiome relate to the timing of food intake and are not likely related to psychological factors possibly at play during Ramadan.

Keywords: Ramadan, intermittent fasting, BALB/c mice, gut microbiome, human

INTRODUCTION

Intermittent fasting is voluntary temporal partial abstinence or reduction from food consumption, usually showing a daily pattern. As a popular form of temporal dieting, intermittent fasting is widely practiced for a variety of medical, societal, or religious reasons. A growing body of research has shown that intermittent fasting exerts a wide spectrum of impacts on improving indicators of health (including weight management, insulin sensitivity, and inflammation), protecting normal cells or stem cells, delaying aging, and reducing common side effects associated with cancer treatment (1–4).

The most widely practiced form of intermittent fasting is that relating to the Ramadan, in which adherents of the Islamic faith refrain from food and fluid consumption between dawn and sunset for approximately 30 days (5). Recently, we showed that RAIF remodels the gut microbiome in healthy humans, with emphasis on the increase in short-chain fatty acids (SCFAs) producing bacteria. We interpreted these findings as a possible explanation for health effects associated with intermittent fasting (6). As Ramadan is likely to have a plethora of other consequences (psychological and physical) it is possible that the effects observed related to other parameters apart from the timing of food consumption (7). Experimental animals are an obvious way of addressing these concerns and might also be useful for performing mechanistic studies on the effects seen with RAIF.

Prompted by the considerations mentioned above we aimed to establish whether RAIF effects exist in experimental animals. The results show that the effects of RAIF in BALB/c mice resemble those seen in humans, providing support for the notion that spatial timing of food intake *per se* drives the alteration in microbiome composition associated with RAIF in humans.

MATERIALS AND METHODS

Mice and Husbandry Conditions

Male BALB/c Mice (6 weeks old) were randomly grouped into a control group (n = 6) and Ramadan model of fasting group (n = 8). All mice were individually housed and provided with feed *ad libitum* for 2 weeks prior to study initiation, allowing the animals acclimation to the animal facility. Mice in fasting group were fed every day with 8-h period of free access to food and water followed by 16-h of fasting, and the control group were fed *ad libitum*. The temperature in the animal facility was maintained at approximately 24°C degrees. The water and the diet were sterilized by elevated temperature and irradiation, respectively. Unique fecal samples for each mouse were collected on day 0 and 30. Briefly, mice were individually and temporally placed in a sterilized cage without bedding. Four to five freshly evacuated fecal pellets per mouse were collected in a 1.5 ml sterile tube. All samples were stored at −80°C until further analysis. Animal care was performed according to the Animal Ethics Procedures and Guidelines of the People's Republic of China, and the experimental protocol was approved by the local committee on animal use and protection, Kunming University of Science and Technology.

Metagenome Sequencing and Data Processing

Fecal DNA was extracted using the QIAamp DNA Stool Mini Kit (Qiagen, Germantown, MD, United States), according to the manufacturer's instructions. The DNA quality was monitored on 1% agarose gels. Reagent microbiome and bacterial environmental contamination during DNA library preparation were managed as described elsewhere (6). The quality of the library was assessed using the Qubit@ 2.0 Fluorometer (Thermo Scientific, Waltham, MA, United States). The metagenome sequencing of V3–4 variable regions of the bacterial 16S ribosomal RNA was carried out by the NovoGene Company (Beijing, China). Data processing and analysis were performed according to routine procedures, which have been described elsewhere in detail (8–11).

Statistical Analysis

Gut microbiome diversity was assessed by the Shannon and Simpson indices, which calculated using alpha-diversity.py in QIIME, and the Mann–Whitney test was used to determine statistical significance between groups. Principal coordinates analysis (PCoA) of Bray–Curtis distance was performed for establishing the shift of the gut microbiota composition after intermittent fasting according to a previously described method (6). Changes in gut microbial shifts were determined by using multivariate data analysis according to analysis of similarities (ANOSIM). A P-value of less than 0.05 was considered statistically significant. To identify bacterial taxa whose sequences were differentially abundant between groups, linear discriminant analysis (LDA) coupled with effect size measurements (LEfSe) analysis was applied with the significance level of 0.05 and the logarithmic LDA score threshold equal to 4.[1]

RESULTS

Study Execution and Next-Generation Sequencing

We previously showed in healthy humans that RAIF remodels the gut microbiome and enriches SCFAs-producing microflora, potentially providing a mechanistic explanation for health-promoting effects of intermittent fasting in general and those associated with Ramadan in particular. Further mechanistic studies, however, would require an model in experimental animals, also to avoid the psychological component associated with human studies. In addition such a model would open experimental possibilities not available in human studies, like gnotobiotic experimentation, studies in genetically modified animals and studies independent of the Islamic calendar. Thus prompted, here we aimed to prospectively investigate RAIF effects in BALB/c mice (**Figure 1A**), generally considered to be a versatile animal model. After fecal DNA isolation, the bacterial V3-V4 region of 16S rRNA gene was sequenced to map the gut microbiota. As a result, a total of 2,287,043 valid sequences were generated and the average for each sample was 68740 ± 4769 (mean ± SD) sequences. Rarefaction curves were plotted to determine the sequence number per group at the same depth. The number of OTUs reached a saturation plateau at a level of 55,000 sequences for each group (**Figure 1B**), indicating that the sequencing depth was sufficient to represent the majority of bacterial species for each group, although the mice in fasting group show evidence for increased presence of low-abundant bacterial species. Rank-abundance curves were generated to visualize both species richness and evenness of the microbiome in each group at different time points (**Figure 1C**). There was no clear difference between two groups either before or after IF with respect to species richness and evenness, suggesting that these parameters are not markedly influenced by RAIF in BALB/c mice.

Intermittent Fasting Alters the Composition of the Gut Microbiome

For further analysis of the diversity in the gut microbiome in animals subjected to RAIF and the appropriate controls,

[1] http://huttenhower.sph.harvard.edu/galaxy

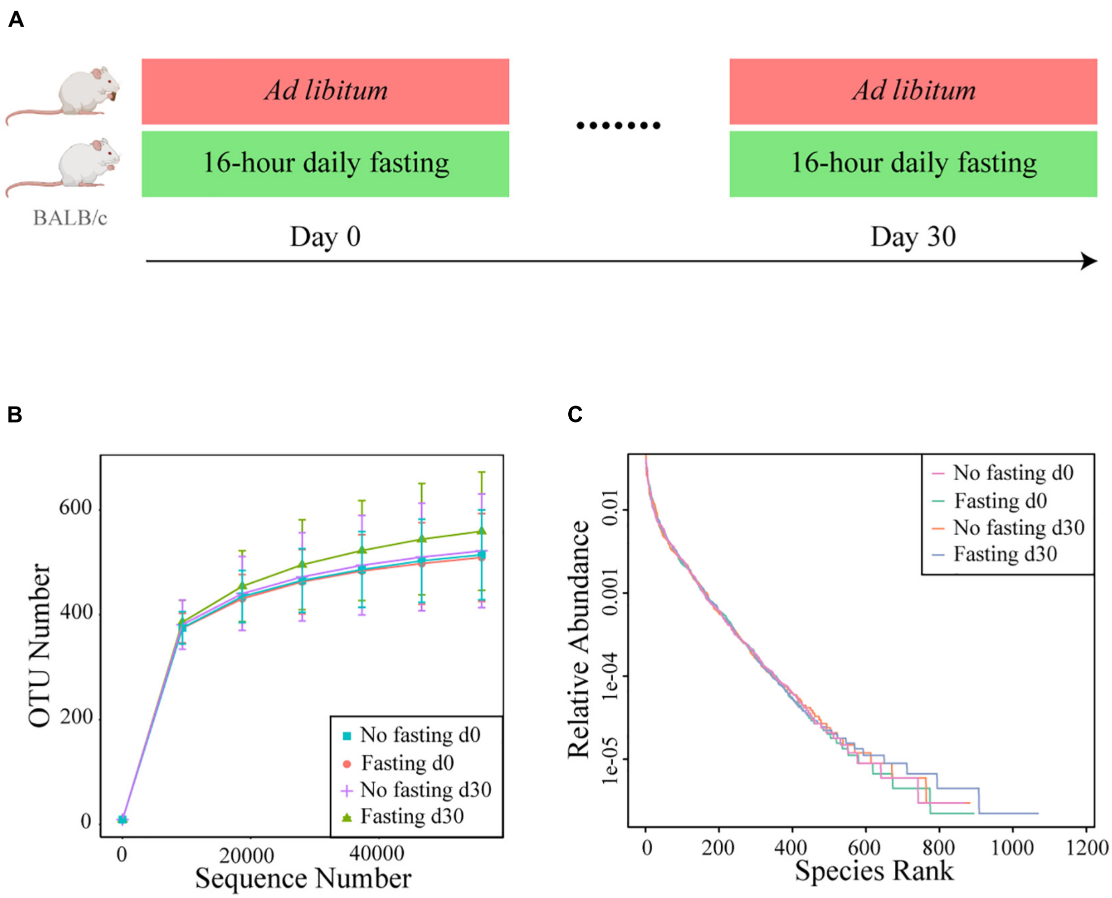

FIGURE 1 | Study design and bacterial 16S rRNA sequencing. **(A)** Experimental design for the 16-h daily fasting group (n = 8) and the no fasting group fed *Ad libitum* (n = 6). Food and water are only freely available during the other 8 h. The fecal samples were collected at the beginning (Day 0) and end (Day 30) the fasting. After fecal DNA isolation, the bacterial V3-V4 region of 16S rRNA gene was sequenced to map the gut microbiota. The images of mouse model were generated with assistance of Biorender.com. **(B)** Rarefaction curves of detected bacterial OTUs of the gut microbiome from no fasting and fasting groups each reach saturation stage with increasing sequencing depth. Each vertical bar represents standard error. **(C)** The rank abundance curve depicting the bacterial species richness and evenness in the gut microbiome of fasting vs. no fasting mice before or after fasting.

the Shannon and Simpson indices, which are important and commonly used diversity indicators of an ecosystem, were calculated. As shown in **Figure 2A**, gut microbiome diversity was not significantly changed after the fasting, showing that fasting of these animals has no major effect on mean species diversity. Next, changes in microbiome composition were visualized using Bray–Curtis distance-based principal co-ordinates analysis (PCoA). Before the onset of fasting begun (Day 0), microbiome composition was not different between the fasting and non-fasting groups of mice according to Analysis of Similarities (ANOSIM) (R = 0, p = 0.46) (**Figure 2B**). After 30 days of IF, however, a remarkable difference was found between the two groups (R = 0.74, p = 0.002 by ANOSIM) (**Figure 2C**), indicating that the relative abundance of certain bacterial taxa had changed during the fasting. To further identify the bacterial taxa influenced by fasting, LEfSe analysis was performed. Multiple taxonomic differences were found when comparing the results of fasting mice to the no fasting controls (**Figure 2D**). At phylum level, an increase of the phylum Firmicutes and depletion of the phylum Bacteroidetes was associated with fasting.

Intermittent Fasting Elevates the Lachnospiroceae and Ruminococcaceae Families

For further confirmation of the fasting-provoked alterations in the microbiome identified, differences in the bacterial taxa identified by the LEfSe were reanalyzed using unpaired t-tests. In agreement with the findings of the LEfSe, we again observed that the phylum Firmicutes was indeed increased as a result of RAIF, while the Bacteroidetes was decreased (**Table 1**). No significance was found between two groups before fasting. At the family level, it was evident that the abundance of both Lachnospiroceae and Ruminococcaceae was significantly increased by RAIF (**Table 1**). This may relate to the beneficial effects of IF has on gastrointestinal tract physiology, because these two families are known as important contributors to

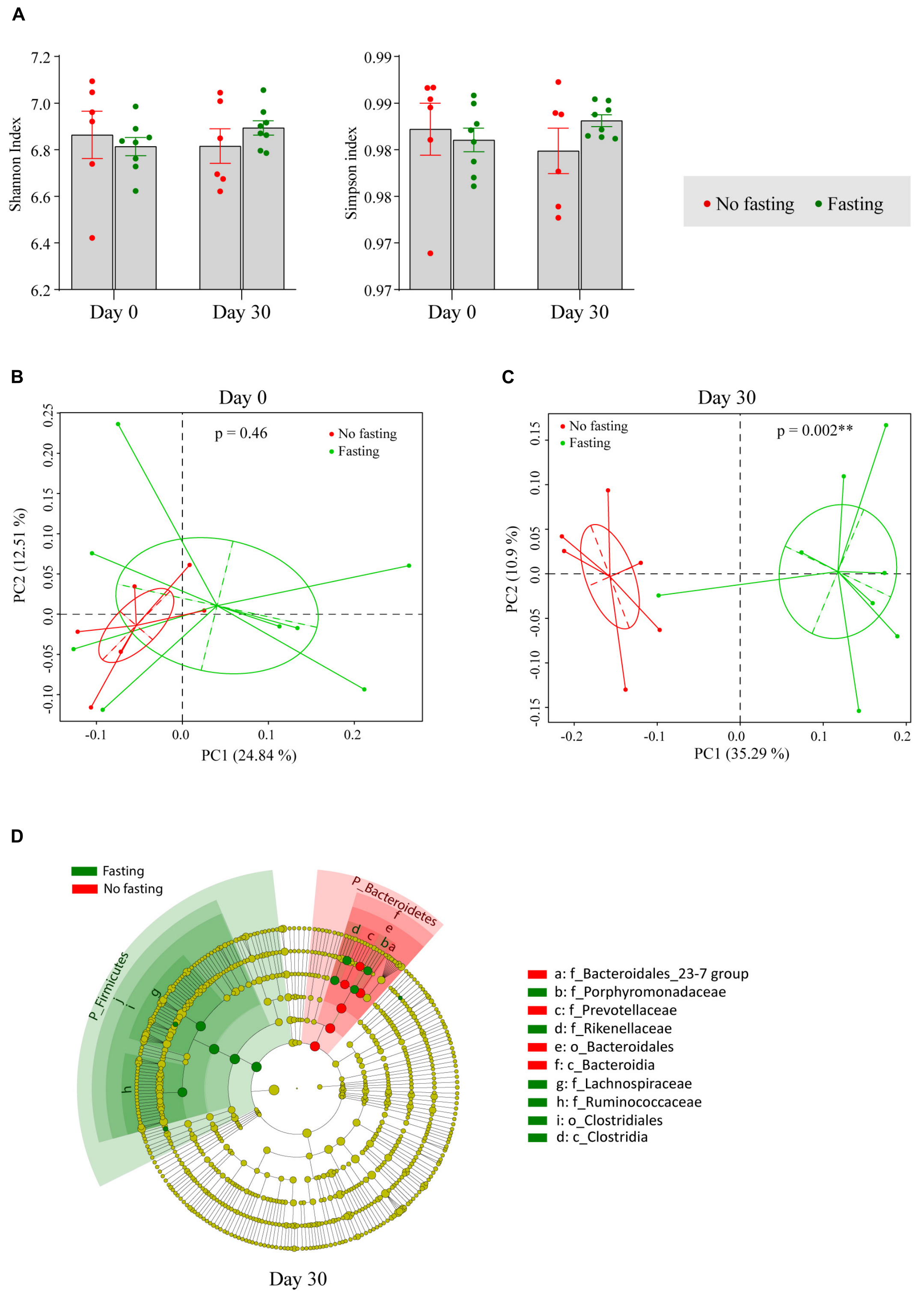

FIGURE 2 | IF shapes the composition of the gut microbiome in BALB/c mice. **(A)** Alpha diversity indexes were shown as Shannon and Simpson. The significance was calculated by unpaired student's *t*-test. Bray–Curtis distance based Principal Co-ordinates Analysis (PCoA) of the gut microbiome in fasting and no fasting mice at day 0 **(B)** and day 30 **(C)**. Different color represents different groups or time points of sample collection. Each point corresponds to a community from a single individual. Colors indicate community identity. Ellipses show the 95% confidence intervals. ** if *p*-value is less than 0.01 by ANOSIM test. **(D)** Taxa that show alternative abundance before and after fasting are depicted. Taxa with a log linear discriminant analysis (LDA) score above 4.00 as determined by using linear discriminant analysis coupled with effect size measurements (LEfSe). The hierarchy of the discriminating taxonomic levels was visualized as cladograms allowing taxonomic comparisons before and after fasting.

TABLE 1 | Changes in relative abundance of LefSe-identified taxa during fasting.

	Day 0	Day 30		*P-value*		
	Baseline (A) (*n* = 14) Mean ± SD	Non-fasting (B) (*n* = 6) Mean ± SD	Fasting (C) (*n* = 8) Mean ± SD	A vs. B	A vs. C	B vs. C
Phylum						
Firmicutes	56.68 ± 9.77	52.79 ± 7.48	67.53 ± 4.84	0.40	0.008	<0.001
Bacteroidetes	33.32 ± 10.96	38.79 ± 4.93	24.39 ± 6.12	0.26	0.048	<0.001
Family						
Lachnospiraceae	37.46 ± 7.36	34.40 ± 7.36	43.99 ± 3.90	0.41	0.031	0.008
Ruminococcaceae	15.07 ± 2.90	13.98 ± 2.52	19.45 ± 1.99	0.43	0.001	<0.001
Rikenellaceae	6.64 ± 1.58	5.46 ± 1.19	7.92 ± 1.85	0.12	0.10	0.015
Porphyromonadaceae	2.02 ± 0.48	1.51 ± 0.60	3.88 ± 1.57	0.06	<0.001	0.005
Bacteroidetes_S24-7_group	20.26 ± 9.64	22.14 ± 3.33	9.77 ± 6.21	0.65	0.012	<0.001
Prevotellaceae	2.90 ± 2.20	8.63 ± 4.13	2.12 ± 1.44	<0.001	0.38	0.001

SD, standard deviation. The significance was calculated by two-sided unpaired student's t-test.

short-chain fatty acid production in the intestine. Interestingly, the Rikenellaceae and Porphyromonadaceae families were also enriched after the fasting (**Table 1**). Since upregulation of these two families have been associated with reduced visceral adipose tissue compartment size and improved metabolic profiles in humans (12) and thus the effects seen in mice may reflect a beneficial microbiological shift provoked by IF in general. In contrast, the levels of the Bacteroidetes S24-7 and Prevotellaceae families decreased following fasting as compared to controls, whereas no difference between the groups was found in any of these families before fasting.

DISCUSSION

Ramadan-associated intermittent fasting is a form of periodic fasting adhered to by millions of adherents of the Islamic faith. A growing number of studies have been conducted in humans to investigate the health effects of RAIF, and we recently characterized the chances in gut microbiome in this respect (6). Volunteer studies in humans suffer, however, from specific cofounders (psychology, altered physical activity during Ramadan, altered circadian rhythms *etcetera*) and studies in humans are less suited for mechanistic investigation (e.g., it is not possible to conduct studies involving genetic modification of the study subjects). A study in experimental animals would show that the effects of RAIF observed indeed relate to the timing of food intake and would allow future mechanistic studies. Prompted by these consideration we characterized the effect of RAIF in BALB/c mice. We observe that changes in microbiome resemble those seen in RAIF in our earlier study (6). The most straightforward interpretation of these observations is that indeed timing of food intake drives the effects seen of RAIF in humans and thus the present study provides further support for the notion that IF can provoke beneficial changes in gut microbiome. In addition, the alterations observed within the bacterial communities might also be provoked by metabolic or immune modifications within host cells such as the production of hormones or ketone bodies, as these factors are known to shape the gut microbiota composition and its function (13, 14). Among the effects, the upregulation of Lachnospiraceae and Ruminococcaceae during the fasting was the most notable and this aligns well with the studies of others associating these bacteria as important drivers for health effects of IF (8, 9).

Over last decade, gut microbiota has attracted great scientific and public attention, and is widely recognized as the nexus of the interaction between life style (e.g., dietary habits) and health status (10). The Lachnospiraceae and Ruminococcaceae families are two most abundant and are seen as the major contributor to luminal biochemistry, while these families are also described as the health-associated core microbiome (11). Both are major SCFAs-producers in our gut and by providing nutritional energy and positional signals to the epithelial compartment play a crucial roles in maintaining the physiological homeostasis of the host. Dysbiosis of these two families is associated with various diseases, especially obesity (15), type 2 diabetes (16), liver cirrhosis (17) and aging (11). Lachnospiraceae are significantly reduced in obesity and levels do not return to those seen in healthy controls even after gastric bypass (15), while Ruminococcaceae were seen to increased following a 2-day modified IF intervention and appear to be negatively associated with the severity of cardiometabolic disease in metabolic syndrome (18). In the present study, we showed that both Lachnospiraceae and Ruminococcaceae families were upregulated in their abundance in the RAIF group, while the abundance of Prevotellaceae was decreased. These changes all echo our previous findings on the alterations of the gut microbiome in healthy subjects who underwent RAIF (6). These data are also in line with previous studies with 7 month of every other day fasting (8, 19). Collectively, this study indicates that experimental rodents can be successfully employed to model RAIF.

Preclinical studies in experimental animals are often essential for understanding disease and therapy and direct design of subsequent clinical study (20). As an extremely important and useful experimental animal, BALB/c mice have been shown to be exceedingly useful for studying both immunology and cancer and many tools tailored for the mouse strain are available (21–23). A great number of studies have been performed to identify the role of the gut microbiome and their implications in health and disease using BALB/C mouse models (22, 23). Successful mimicking the effects of RAIF in this mouse model provides an novel avenue for preclinical studies on how RAIF influences immunology and perhaps cancer progress, especially in gut microbiome dysbiosis-associated conditions.

In summary, we have demonstrated that RAIF shapes the gut microbiota in BALB/c mice, characterized with an increase in SCFAs-producing bacteria. These results support the notion that RAIF in humans works through the temporal spacing of nutrient consumption and provide an experimental model that will allow dissection of the underlying mechanisms.

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: https://www.ncbi.nlm.nih.gov/bioproject/, accession ID: PRJNA789722.

ETHICS STATEMENT

The animal study was reviewed and approved by the Local Committee on Animal Use and Protection, Kunming University of Science and Technology.

AUTHOR CONTRIBUTIONS

JS collected and processed the fecal sample, involved in the DNA extraction, performed the PCR reaction, and data analysis. YW, FL, YS, and AV involved in the data interpretation. JS, ZM, and MP involved in the study design and wrote the manuscript. All authors have revised and approved the manuscript.

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A Systematic Review of Insulin Management Recommendations to Improve Glycemic Control and Reduce Hypoglycemic Events During Ramadan Fasting in Patients With Insulin-Requiring Type 2 Diabetes

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Background: Muslims with insulin-requiring type 2 diabetes are at high risk of hypo- and hyperglycemia while fasting during the month of Ramadan. Although a few reviews on diabetic management during Ramadan have been published, surveys reveal knowledge gaps remain among physicians.

Aim: This systematic review qualitatively analyzes what insulin dosing recommendations are likely to reduce hypoglycemic events and improve glycemic control during the Ramadan fasting for this high-risk group.

Methods: A comprehensive search in six databases and gray sources was performed from August 10, 2001, to August 10, 2021, for studies assessing which types of insulin and/or what dosing recommendations reduce hypoglycemic events and improve glycemic control during Ramadan. We excluded studies focusing mainly on oral antihyperglycemic medications, type 1 diabetes, persons with insulin pumps, and studies older than 20 years. Hypoglycemic event rates, pre-, and post-iftar blood glucose levels, overall average blood glucose, and hemoglobin A1c were analyzed, and a narrative synthesis was performed.

Results: Out of 1,101 collected articles, 14 eligible studies including 2,969 participants with an average age of 54.8 years, we found that insulin dose reduction may prevent hypoglycemia without causing subsequent hyperglycemia, and rapid-acting insulin analogs may improve post-iftar and overall blood glucose without incurring hypoglycemia.

Conclusions: Though initial findings are promising, more research is needed to confirm the benefits of insulin dose reduction, rapid-acting insulin analogs, and ultra-long-acting insulins.

Systematic Review Registration: https://www.crd.york.ac.uk/prospero/, identifier: CRD42021268943.

Keywords: type 2 diabetes, Islam, insulin, hypoglycemia, hyperglycemia

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INTRODUCTION

The burden of diabetes mellitus continues to rise globally across all regions of the world (1). Approximately 463 million adults are living with diabetes worldwide, and this figure is projected to increase by 51% in the next 25 years (2). Of all the people with diabetes globally, 150 million are estimated to be Muslim (3). One of the five pillars of Islam, central to the Muslim faith, is annual fasting during the holy month of Ramadan. During this month, all healthy Muslims who have reached puberty are required to fast from dawn to sunset, which includes refraining from eating, drinking, use of oral medications, and smoking (4). Exemptions are available for certain populations, such as Muslims who are elderly, traveling, expecting, or nursing mothers and Muslims with serious medical conditions including diabetes. However, many Muslims with these conditions still voluntarily choose to observe the practice of fasting during Ramadan (5). Epidemiologic studies have shown that Muslims with diabetes fast for an average of 27–28 days in the month of Ramadan (4, 6–8). Even 43.9% of Muslims with a "high" or "very high" risk classification of diabetes (according to the American Diabetes Association, ADA) fasted for an average of 28 days during Ramadan despite medical advice (9).

Hypoglycemia during Ramadan is a major concern, particularly for those who fast for up to 20 h consecutively (4). Recurrent hypoglycemia may increase the risk of cognitive impairment and mortality (10). This concern is heightened for patients with insulin-requiring diabetes, many of whom are categorized as very high risk by the ADA risk index (9). One study showed this very high-risk cohort has a 13.8% incidence of hypoglycemia compared to 4.2% for low-risk individuals (9). The same study showed that persons with type 2 diabetes taking only insulin during Ramadan had a greater incidence (16.8%) of hypoglycemia than those treated with only oral hypoglycemic agents (5.3%) (9).

In addition to hypoglycemia, hyperglycemia during Ramadan is also a major concern. One large epidemiologic study revealed a significantly increased risk of severe hyperglycemia or ketoacidosis during Ramadan (0.05 ± 0.35) compared to before Ramadan (0.01 ± 0.05) (4). A possible explanation for this may be because the meal that breaks the fast after sunset (iftar) is typically larger than average, and it has been shown that the risk of hyperglycemia is consequently higher (6). A more recent study that used flash-glucose monitoring on insulin-treated patients during Ramadan showed an increase in time in hyperglycemia and a reduced time in the target range (11). The long-term effects of elevated uncontrolled blood glucose are well-known including increased risk of cardiovascular disease, nephropathy, neuropathy, and ophthalmopathy.

Although the Diabetes and Ramadan International Alliance (DaR) and the International Diabetes Federation (IDF) collaboratively published practice guidelines (5, 12), healthcare providers continue to have knowledge gaps pertaining to diabetes management during Ramadan. Beshyah et al. conducted a survey on 260 physicians from 27 countries—almost all Muslim majority countries. Many physicians surveyed (54.1%) admitted to having a knowledge gap in the practical management of high-risk diabetic groups, and 49.2% believed there is limited data on high-risk patients. Additionally, respondents most desired knowledge on how to best organize healthcare before, during, and after Ramadan, and if newer pharmacological agents are better than older ones if used correctly. Most physicians agreed that the two most appropriate types of articles to disseminate knowledge about Ramadan fasting are original research (73.3% of respondents) and systematic reviews (64.3%) (13).

A few reviews have analyzed both glycemic control and adverse events during Ramadan for insulin-requiring diabetes, specifically. However, to our knowledge, the original research from which these reviews and guidelines derived their recommendations have not been critically appraised. Additionally, there is no systematic review dedicated exclusively to insulin management in type 2 diabetes during Ramadan. Given the growing body of literature on Ramadan and diabetes, this systematic review seeks to answer the question: what insulin dosing recommendations are likely to reduce hypoglycemia and improve glycemic control for persons with insulin-requiring type 2 diabetes who participate in the Ramadan fast?

MATERIALS AND METHODS

The review is reported in accordance with the 2020 Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement and informed by the Cochrane Handbook for Systematic Reviews of Interventions (14, 15). A review protocol was prospectively registered online in PROSPERO (Prospective, International Register of Systematic Reviews) under the registration number: CRD42021268943.

Literature Search

A medical librarian specialized in systematic reviews (LÖ) conducted a comprehensive search for literature in six electronic databases: PubMed (NML), EMBASE (Elsevier), CINAHL (Ebscohost), Scopus (Elsevier), Web of Science (Clarivate), and Cochrane Library (Cochrane Collaboration). Gray literature sources were located via OAlster Gray Repository, World Health Organization Institutional Repository (WHO IRIS), ClinicalTrials.gov, BASE, and Open Gray. The search was performed in August 2021. Pre-searches in PubMed and PubMed's MeSH to identify relevant search term variations and to develop the search string was conducted by LÖ in May-July 2021 in close collaboration with subject specialists (AK and MK). The search strategy developed in PubMed was later systematically repeated in all selected information sources. A combination of the search fields title, abstract, keywords (Text Word, TOPIC, or similar), and "MeSH"/"thesaurus" (when available) was used to ensure that the best possible evidence was located. The search was conducted without any language or geographical restrictions. Because of the recent advances in diabetic management, studies older than 20 years were excluded. Search details, dates, keywords, results, and notes for all databases and gray sources are reported in **Appendix A**.

TABLE 1 | PICOS criteria applied for the systematic review.

Parameter	Inclusion criteria	Exclusion criteria
Population	Persons with insulin-requiring type 2 diabetes	Type 1 diabetes, Persons with an insulin pump
Intervention	All insulin types and insulin dosing strategies during Ramadan	Studies focusing mainly on oral antihyperglycemic medications
Comparison	Studies comparing insulin subtypes or insulin dosing strategies	
Outcomes	The difference in hypoglycemic event rate, pre-and post-iftar blood glucose, overall blood glucose, and HbA1c between the insulin types and/or insulin dosing strategies	Studies that do not quantify glycemic control or adverse events or report any outcomes.
Study type	Studies focusing on insulin type and/or dosing strategies in insulin-requiring type 2 diabetes during Ramadan	Reviews, epidemiological studies, editorials, case reports, conference abstracts, comments, and letters to the editors; physiologic focused articles
Time	The cut-off date limit of 2001–2021 was applied	Studies published before 2001
Language	All countries and all languages	
Setting	All settings	

Study Selection

All records identified in the database search were uploaded to the systematic review software Covidence (Veritas Health Innovation, 2021) for automatic de-duplication and prepared for blinded screening and data extraction (LÖ) (16). The results from the gray sources were de-duplicated by hand. Cabell's Predatory Report (Cabell's Scholarly Analytics, 2021) was consulted to verify the scientific status of included studies published in open access journals (17). Two independent reviewers (AK and MK) screened the titles and abstracts of unique records against the pre-set inclusion and exclusion criteria, which is summarized in **Table 1**.

Because this review aims to guide insulin management for type 2 diabetes during Ramadan, a concerted effort was made to include studies focusing on insulin and exclude studies with an emphasis on oral hypoglycemic agents. If studies were able to control for oral hypoglycemic agents and isolate the effects of insulin on hypoglycemia and glycemic control during Ramadan fasting, they were included for review. Additionally, if studies evaluated a specific insulin dosing strategy and measured its effects on blood glucose levels, they were included for review. If a study included persons with insulin-requiring type 2 diabetes during Ramadan, but the primary objective of the study was to evaluate the effects of an oral hypoglycemic agent, this study was excluded. If it was difficult to ascertain the effects of insulin specifically on diabetic management during Ramadan because an oral hypoglycemic agent was an uncontrolled variable in the study, this study was excluded.

A third independent reviewer (AI) resolved the conflicts identified by the software. Full-text papers were sought and uploaded to Covidence for blinded screening (AK and AI) and conflict resolution (MK). A PRISMA flow diagram with details of the screening and selection process is illustrated in **Figure 1**.

Data Extraction

Two independent reviewers (AK, AI) used the Covidence software to extract study characteristics and outcomes. The primary outcome assessed was the difference in hypoglycemic incidence or event rate between the insulin types and/or insulin dosing recommendations. Hypoglycemia was defined in most studies as a serum blood glucose level < 70 mg/dL (3.9 mmol/L) except in two studies it was <63 mg/dL (3.5 mmol/L), and one study defined hypoglycemia as <60 mg/dL (3.3 mmol/L). The secondary outcomes obtained reflected glycemic control which was measured by the changes in pre-and post-iftar blood glucose (mg/dL, mmol/L), overall blood glucose (mg/dL, mmol/L), and HbA1c (mmol/mol) between the insulin subtypes and/or dosing recommendations. Additionally, insulin dosing strategies were examined and compared between studies. Type 2 diabetes-associated adverse events, such as DKA or HHS was also extracted when available in addition to each study's funding sources. A third reviewer (MK) was available to resolve any conflicts if necessary.

Quality Assessment

Two independent reviewers (AK, MK) used the Newcastle-Ottawa Scale (NOS) and the National Heart, Lung, and Blood Institute's (NHLBI) quality assessment tools to assess the quality and risk of bias of each observational cohort study and controlled intervention study, respectively (18, 19). A third reviewer (DB) resolved conflicts. The Covidence software enabled a systematic and blinded approach.

Data Synthesis

The characteristics and outcomes of each study were summarized in a comprehensive table, allowing us to group and compare similar findings relevant to our primary and secondary outcomes. Our primary outcome, the hypoglycemic incidence or event rate, was expressed as a difference in the percentage between different insulin types and/or dosing recommendations, and the *p*-values from each study were noted. The CREED epidemiologic study reported a hypoglycemia incidence rate of 13.8% for insulin-requiring type 2 diabetes during Ramadan, so this was the benchmark value that we used in our analysis. Our secondary outcomes concerning glycemic control were measured as the mean difference in blood glucose levels between different insulin types and/or dosing recommendations while considering the *p*-value of each respective measurement. If a study found that a specific insulin subtype or dosing recommendation decreased the hypoglycemia incidence rate to <13.8% without

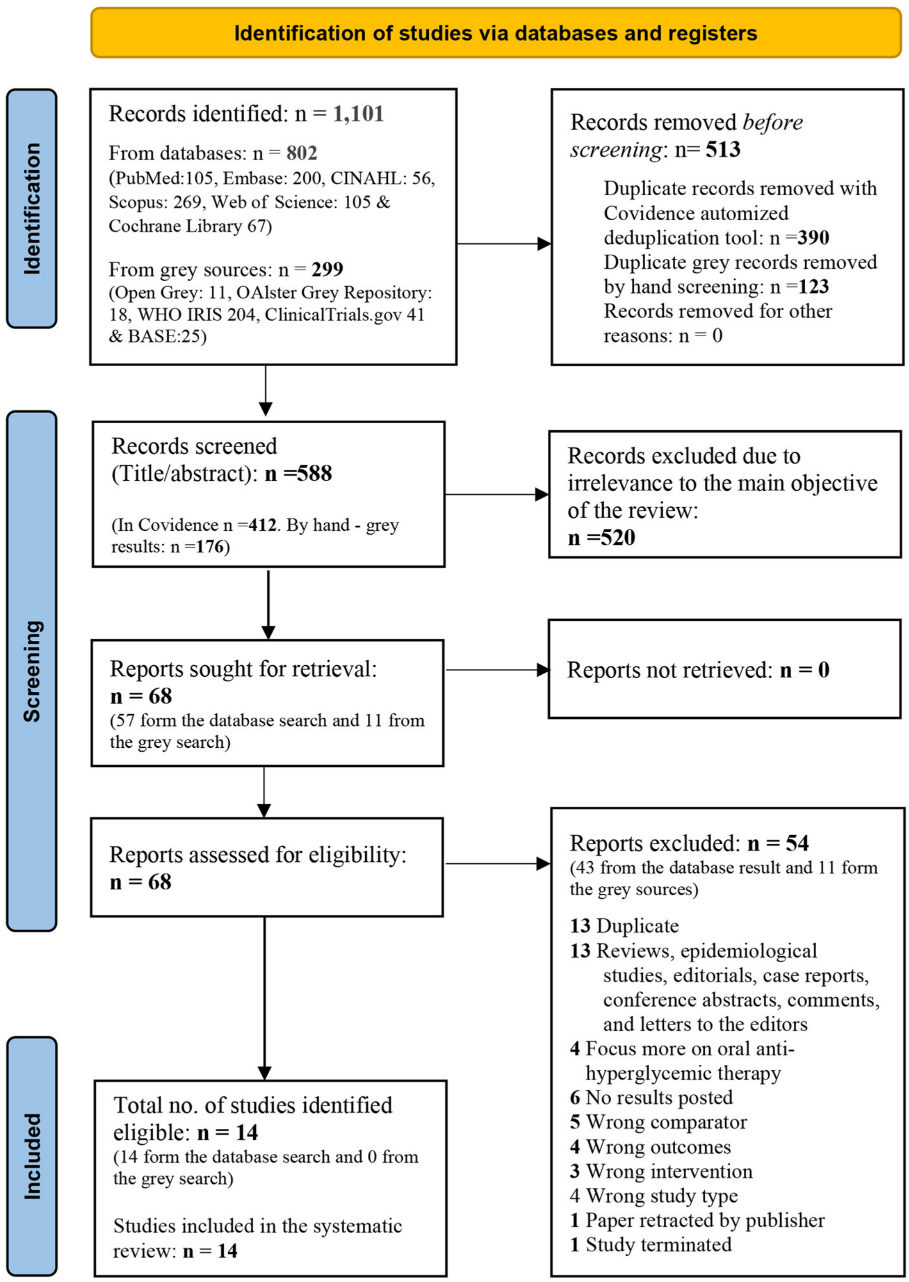

FIGURE 1 | PRISMA 2020 flow diagram for new systematic reviews which included searches of databases and registers only. From: Page et al. (14). For more information, visit: http://www.prisma-statement.org/.

significantly increasing post-iftar blood glucose, overall blood glucose, or severe hyperglycemia (blood glucose > 300 mg/dL, 16.7 mmol/L), this would be an acceptable finding. A narrative synthesis was done to analyze which insulin types and dosing recommendations are superior for improving glycemic control and reducing hypoglycemic events during Ramadan. Due to the high heterogeneity of study designs, interventions, and outcomes, we did not perform a meta-analysis.

RESULTS

Of the 1,101 records located in the literature search, 588 unique studies remained for the title and abstract screening after de-duplication. All studies were found to be in the English language. Sixty-eight papers were selected for full-text screening, of which fourteen were identified as eligible to be included in the systematic review. A PRISMA 2020 flow diagram detailing the search, de-duplication, screening, and selection process is summarized in **Figure 1**.

The total number of study participants was 2,969 between all fourteen studies reviewed. These studies were conducted across four continents in 25 different countries; there were six in Africa: Algeria ($n = 1$), Egypt ($n = 3$), Libya ($n = 1$), Morocco ($n = 2$), South Africa ($n = 2$), and Tunisia ($n = 1$), seventeen in Asia: Bangladesh ($n = 1$), China ($n = 1$), India ($n = 5$), Indonesia ($n = 1$), Iraq ($n = 1$), Israel ($n = 1$), Jordan ($n = 4$), Kuwait ($n = 1$), Lebanon ($n = 3$), Malaysia ($n = 3$), Oman ($n = 1$), Pakistan ($n = 3$), Qatar ($n = 2$), Saudi Arabia ($n = 3$), Singapore ($n = 1$), Turkey ($n = 1$), the United Arab Emirates ($n = 1$), one in Europe: the United Kingdom ($n = 1$), and one in North America: Canada ($n = 1$). Among all fourteen studies, the average age is 54.8 years, the average duration of diabetes is 11.1 years, 52.3% were female, and 47.7% male.

Five studies were RCTs, and the remaining nine studies were observational cohort studies. A comprehensive summary of all included studies is organized in **Table 2** with RCTs in the top five rows. Six studies analyzed how insulin dosage adjustment affects glycemic control and hypoglycemia during Ramadan, three examined newer ultra-long-acting insulins, three compared insulin analogs (synthetic insulin designed to mimic the body's natural insulin release pattern) to regular human insulin, and two studied specific medical regimens for the Ramadan fast. We have grouped these studies according to the previously mentioned categories concerning hypoglycemia and glycemic control. These findings are summarized in **Tables 3**, **4**.

Hypoglycemia

Insulin Dosage Adjustment

Three studies (two RCTs) out of four demonstrated that insulin dosage reduction of between 25 and 40% TDD decreases rates of hypoglycemia for some participants (23, 24, 28). The average hypoglycemic incidence rate from these two RCTs was 22.5% [21.4% in Shehadeh et al. (23), 23% in Zaghlol et al. (24)] in the control group and 4.5% [4.8% from Shehadeh et al. (23), 4.2% in Zaghlol et al. (24)] in the intervention group. Another study compared premixed twice-daily dosing to short-acting insulin at iftar with intermediate at suhur (the meal consumed early in the morning before dawn and before fasting commences) and found no difference in hypoglycemia (26). Ahmedani et al.'s strategy of flexible glycemic targets (100–200 mg/dl during fasting hours and 100–180 mg/dl during non-fasting hours) reported only a 0.6% rate of hypoglycemia (25).

Long and Ultra-Long-Acting Insulins

Ultra-long-acting insulins (IDegAsp twice daily) demonstrated a 62% reduction in overall hypoglycemia compared to BIAsp 30 twice daily ($p = 0.007$) in one RCT (21) and reported no severe hypoglycemic episodes in another cohort study (32). Utilization of Gla-300 resulted in no severe hypoglycemic episodes during or post-Ramadan (30). Participants taking basal + Non-SU-OHA reported zero episodes of hypoglycemia (27). The use of nighttime Levemir with reduced total daily insulin dosing resulted in a lower adverse event rate (0.04 vs. 0.07, $p = 0.010$) and a lower hypoglycemic event rate (4.8 vs. 21.4%, $p < 0.001$) compared to usual care (23). Salti et al. found that using insulin glargine with glimepiride resulted in minimal severe hypoglycemic episodes and that keeping fasting blood glucose > 120 mg/dL (6.7 mmol/L) had a protective effect on hypoglycemia (33).

Insulin Analogs vs. Human Insulin

Although three studies (2 RCTs) found that rapid-acting insulin analogs can improve glycemic control, each study comparing insulin lispro/protamine to human mixed insulin found no statistically significant difference in hypoglycemic event rate between the two arms (20, 22, 31). Hajjaji et al. reported three minor hypoglycemic episodes in both the intervention (rapid-acting insulin analog) and control (human insulin) groups (20), Mattoo et al. recorded a similar number of hypoglycemic episodes (0.4 episodes per participant) for both groups (22), and Hui et al. did not record a statistically significant difference in hypoglycemic events between the group using a rapid-acting insulin analog (0.04 events per participant reduction) vs. the group using human insulin (0.15 events per participant increase) (31).

Glycemic Control

Insulin Dosage Adjustment

Two RCTs and four observational cohort studies analyzed how insulin dosage adjustment affects glycemic control. Three of the studies reduced the total insulin daily dose by 25% in the experimental groups, and the result was no difference in hyperglycemia compared to regular dosing (24), no episodes of DKA or NKHS (no comparator) (28), and no difference in A1c or average blood glucose compared to regular dosing (29). Shehadeh et al. reduced TDD by 40% in their intervention group, giving 60% of the reduced dose as biphasic insulin 70 for iftar and 40% as Levemir at suhur, and their result showed no difference in A1c compared to usual care. In fact, although the insulin TDD was reduced, there was a decreased event rate of >300 mg/dL (16.7 mmol/L) blood glucose in the intervention group ($p = 0.026$) (23). Altemimi compared human premixed (NPH/regular) insulin dosed as 2/3 TDD pre-iftar and 1/3 TDD pre-suhur vs. human regular insulin and NPH dosed as ½ TDD pre-iftar and ½ TDD pre-suhur, respectively, and the result was no statistical difference in average A1c or hyperglycemic events (26). Ahmedani et al. allowed for flexible targets: 100–200 mg/dl during fasting hours and 100–180 mg/dl during non-fasting hours. This resulted in an A1c reduction of 0.88 ($p < 0.0001$) without any DKA or HHS (25).

Long and Ultra-Long-Acting Insulins

Five studies examined long and ultra-long-acting insulins and their effects on glycemic control. IDegAsp was found to significantly lower pre-iftar glucose by −8 mg/dL (−0.54 mmol/L, $p = 0.0247$) compared to BIAsp 30 (21). Similarly, Gla-300 decreased A1c (−0.4%) and fasting plasma glucose (−13.5 mg/dl) during Ramadan (30). Another small study found that 5 out of 6 participants who switched from premixed or NPH to IDeg or IDegAsp resulted in 12–25% insulin dose reduction (32). For long-acting insulin, one study used Levemir and a reduced

TABLE 2 | Comprehensive summary of included studies.

References	Journal name	Countries	Study design, #participants, study duration	Average participant age, % female, duration of diabetes	Aim of study	Funding sources
Hajjaji et al. (20)	Int'l Jour of Clinical Practice	Libya	RCT $n = 40$ 5 weeks	53.5 years 52.5% 12.3 years	To test if changing the Iftar insulin to a 50:50 mixed analog insulin from a 30:70 human insulin improves postprandial glucose	Eli Lilly for insulin mix
Hassanein et al. (21)	Diabetes Research and Clinical Practice	Algeria, India, Lebanon, Malaysia, and South Africa	RCT $n = 248$ 34 weeks	55.1 years 55.9% 12.2 years	Compare the efficacy and safety of insulin degludec/insulin aspart (IDegAsp) and biphasic insulin aspart 30 (BIAsp 30) before, during, and after Ramadan	Novo Nordisk A/S
Mattoo et al. (22)	Diabetes Research and Clinical Practice	India, Pakistan, Malaysia, Singapore, Egypt, South Africa, Morocco	RCT $n = 151$ 10 weeks	53 years 54.3% 12.5 years	To compare the effects of insulin lispro Mix25 and human insulin 30/70 on the daily BG profiles, specifically the postprandial BG control	Eli Lilly
Shehadeh et al. (23)	Int'l Jour of Clinical Practice	Israel	RCT $n = 238$ 2 months	59.8 years 61.4% 12.7 years	Comparing insulin detemir (Levemir) and biphasic insulin (NovoMix70) to standard care	Grant from Novo Nordisk
Zaghlol et al. (24)	Frontiers in Endocrinology	Jordan	RCT $n = 365$ on insulin 5 months	58.0 years 50.4% 8.2 years	Investigate the effect of dosage reduction of four hypoglycemic multidrug regimens on the incidences of acute glycemic complications	Not stated
Ahmedani et al. (25)	Diabetes Research and Clinical Practice	Pakistan	Cohort $n = 54$ 2 months	54.7 years 51.9% 13.8 years	To observe the effect of keeping flexible glycemic targets during fasting and tighter targets during non-fasting hours in insulin-treated people with type 2 diabetes during Ramadan	Unspecified
Altemimi et al. (26)	Cureus	Iraq	Cohort $n = 30$ 10 weeks	53 years 54.0% 9.3 years	To compare the degree of glycemic control, tolerability, and the existence of dysglycemic events from the use of either human premixed insulin or basal plus short-acting insulin regimens	Unspecified
Ba-Essa et al. (27)	Diabetes Research and Clinical Practice	Saudi Arabia	Cohort $n = 360$ 3 months in 2015 and 2016	53.8 years 54.7% 12.5 years	To determine the safety and effect of diabetes medication on glycemic control and the risk for hypoglycemia and to find some predictors associated with increased risk for hypoglycemia during fasting.	None reported
Beano et al. (28)	Endocrinology and Metabolism	Jordan	Cohort $n = 301$ 2 months	58.7 years 52.6% 7.0 years	To assess the safety of a protocol involving dose adjustments to four different anti-diabetic drug regimens in T2DM patients who chose to fast during Ramadan.	Unspecified
Elhadd et al. (29)	Diabetes Research and Clinical Practice	Qatar	Cohort $n = 12$ (on basal insulin) 20 weeks	50.8 years 15.2% 13.1 years	To assess the effect of structured education and medication dose adjustment, according to the PROFAST Protocol on the risk of hypoglycemia captured using FGM in patients on sulfonylurea or basal insulin and at least 2 other diabetes medications, before and during Ramadan.	Medical Research Council, Ministry of PH, Qatar
Hassanein et al. (30)	Diabetes Research and Clinical Practice	Kuwait, Qatar, Saudi Arabia, UAE, Jordan, Lebanon, Turkey, Egypt, India, Pakistan, Canada	Cohort $n = 466$ 5 months	54.4 years 48.3% 9.1 years	To prospectively evaluate the safety and effectiveness of Gla-300 in participants with T2DM prior to, during, and after Ramadan.	Sponsored by Sanofi (Gla-300 manufacturer)
Hui et al. (31)	Int'l Jour of Clinical Practice	United Kingdom	Cohort $n = 6$ 12–14 weeks	62.1 years 65.4% 9.7 years	Compare hypoglycemic events, HbA1c, and changes in body weight between Humalog Mix 50 and human Mixtard 30 twice daily	None reported

(Continued)

TABLE 2 | Continued

References	Journal name	Countries	Study design, #participants, study duration	Average participant age, % female, duration of diabetes	Aim of study	Funding sources
Kalra et al. (32)	Indian Jour of Endocrinology and Metabolism	India	Cohort $n = 349$ 6 months	46.3 years 66.7% N/A	Document the utility and safety of insulin degludec (IDeg) and insulin degludec aspart (IDegAsp)	None
Salti et al. (33)	Diabetic Medicine	Bangladesh, China, Egypt, Kuwait, Oman, UAE, Indonesia, Lebanon, India, Jordan, Malaysia, Morocco, Saudi Arabia, Tunisia	Cohort $n = 349$ 6 months	54.5 years 49.0% 11.3 years	To determine the safety and efficacy of the combination of insulin glargine and glimepiride in patients with T2DM before, during, and after Ramadan.	Sanofi-Aventis

RR, relative risk; R, Ramadan; TDD, total daily dose (of insulin); BG, blood glucose; ERR, Estimated Relative Risk; AE, adverse events; FBG, Fasting Blood Glucose; PP, post-prandial; Exp, experimental; DKA, diabetic ketoacidosis; NKHS, non-ketotic hyperosmolar syndrome.

total daily dosing strategy, which resulted in a significantly reduced > 300 mg/dL (16.7 mmol/L) event rate (23). Salti et al. found that insulin glargine with glimepiride helped improve FBG (176–124 mg/dL, 9.8–6.9 mmol/L, $p < 0.0001$) and A1c (8.6–7.7%, $p < 0.0001$) in non-insulin naïve participants (33).

Insulin Analogs vs. Human Insulin

Rapid-acting insulin analog mixes, such as lispro/protamine, improved glycemic control in two RCTs and one cohort study when compared to human mixed insulin. During Ramadan, lispro/protamine improved pre-iftar blood glucose 6 mg/dL (0.4 mmol/L, $p = 0.034$) (22), 2-h post iftar blood glucose 20 mg/dL (1.1 mmol/L, $p = 0.0001$) (20), mean postprandial blood glucose 21.1 mg/dL (1.2 mmol/L, $p < 0.001$) (20), mean A1c 0.4% ($p = 0.01$) (31), and overall blood glucose 9 mg/dL (0.6 mmol/L, $p = 0.004$) compared to human insulin 30/70 (22). Another cohort study demonstrated improved A1c by 0.48% ($p = 0.0001$) when using lispro/protamine mix 50 at iftar as opposed to an increase in A1c by 0.28% ($p = 0.007$) in the comparator group using human insulin mix 30 at iftar (31).

Risk of Bias and Quality Assessment

The NOS was used for the risk of bias and quality assessment for the nine included observational cohort studies. Five out of nine cohort studies were rated as *Good* according to the NOS scale, and the remaining four cohort studies were rated as *Poor*. Most cohort studies rated as *Poor* were rated as such because the analytic design of the study did not control for confounders. The NHLBI quality assessment was used for the five RCTs with two studies rated as *Fair,* and three studies rated as *Good.* **Tables 5**, **6** summarize our ratings for the nine observational cohort studies and the five RCTs, respectively.

DISCUSSION

Despite the risks involved, up to 43.9% of Muslims with high-risk diabetes choose to fast during Ramadan (9, 10). Although large epidemiologic studies have demonstrated increased hypo- and hyperglycemia during the fasting month (6–9), most physicians acknowledge inexperience with managing diabetes during Ramadan (13). It is incumbent upon all physicians to identify safe and effective insulin dosing recommendations during Ramadan for Muslims, a cohort claiming almost one-third of all persons with diabetes worldwide (3). In this review, we found that insulin dosing adjustment and long and ultra-long acting insulins can reduce hypoglycemic events, and rapid-acting insulin analogs can improve post-iftar and overall blood glucose during Ramadan.

Insulin Dosage Adjustment

Reducing the pre-Ramadan TDD of insulin by 25–40% during the fasting month appears to effectively decrease the rate of hypoglycemia (23, 24, 28) from a combined average incidence rate of 22.5–4.5% (23, 24, 28). By comparison, the CREED epidemiologic study recorded a hypoglycemia incidence rate of 13.8% in a similar demographic during Ramadan (7). Theoretically, lowering the insulin dose may consequently increase rates of hyperglycemia, however, studies have shown that decreasing the pre-Ramadan TDD of insulin does not subsequently increase the rate of hyperglycemia (23, 24), DKA (28), or A1c (23, 29). In fact, the large epidemiologic EPIDIAR study reported a severe hyperglycemic event rate of 4% during Ramadan compared to a 3% event rate in the intervention group in Shehadeh et al.'s study (23). This finding can be explained by the strategy of administering the long-acting insulin at suhur prior to the daytime fast, thus preventing hypoglycemia, and giving a higher insulin dose with the larger meal (iftar), which helps mitigate a large glucose load.

Previous reviews have similar recommendations: the IDF/DAR guidelines (5), the South Asian Health Foundation (UK) guidelines (34), Ibrahim et al. applied principles of the ADA/EASD consensus guidelines in 2020 (35), and Sadikot et al. (36) all recommend reducing the basal dose by 15–30% and the suhur dose by 25–50%. After a comprehensive systematic review and critical appraisal of the original research, we have also found that reducing the TDD of insulin is a dosing recommendation that may help reduce hypoglycemic events during Ramadan.

TABLE 3 | The effects of different insulin subtypes and dosing strategies on hypoglycemia.

References study design	Intervention	Comparator	Effect on hypoglycemia	Dosing recommendations
Hajjaji et al. (20) RCT	Humalog Mix 50/50 at iftar (lispro/protamine) Humalog Mix 75/25 at suhur	Human mixed insulin 30:70 (Human Mixtard 30)	3 hypoglycemic episodes in each group, considered "minor" (BG of ≤70 mg/dL)	Decrease short-acting dose at Suhur
Hassanein et al. (21) RCT	IDegAsp BID	BIAsp 30 BID	During R: 62% reduction in overall hypoglycemia in the IDegAsp arm (ERR 0.38, $p = 0.007$); During treatment period: IDegAsp rate of overall (ERR 0.26, $p < 0.0001$, 74% RR) and nocturnal (ERR 0.17, $p < 0.0001$, 83% RR) hypoglycemia lower. Severe hypoglycemia lower (44%, $p = 0.5801$)	Use dose adjustment and titration algorithm for dosing insulin for both efficacy and safety. IDegAsp may be safer than BIAsp 30 in Ramadan fasting (lower risk of hypoglycemia)
Mattoo et al. (22) RCT	Lispro Mix 25 × 2 weeks then human insulin 30/70 × 2 weeks	Human insulin 30/70 × 2 weeks then Lispro Mix 25 × 2 weeks	Similar rate between the two groups (0.49 ± 0.9 for lisproMix25 and 0.49 ± 0.8 for insulin 30/70; $P = 0.725$)	Insulin Lispro Mix25 had better glycemic control (overall, pre-iftar and 2 h post iftar) without increasing hypoglycemic risk compared to human insulin 30/70
Shehadeh et al. (23) RCT	60% TDD pre-R split: 40% Levemir at suhur, 60% biphasic 70 at iftar	Standard care per ADA recommendations	AE rate significantly lower in intervention group (0.04 vs. 0.07, $p = 0.010$). Hypoglycemia more common in control [6 (4.8%) vs. 24 (21.4%), p,â§0.001]	Insulin dose (intervention) was 60% of the usual, of this 40% was dosed as Levemir at sunrise and 60% as biphasic 70 before dinner
Zaghlol et al. (24) RCT	75% insulin dosage reduction	Regular dosing	Low dosage vs. regular: M+IG 3.9 vs. 20.6% [odds ratio 0.16 (0.05–0.46), $p < 0.001$], M + IG + HRI 5.2 vs. 27.6% [odds ratio 0.14 (0.05–0.39), $p < 0.001$]. No incidence of DKA or HHS	Dose decreases (75% tested in this study) did decrease hypoglycemia without increasing hyperglycemia or its adverse sequela.
Ahmedani et al. (25) Cohort	Insulin dose adjustments (100-200 mg/dl fasting, 100–180 mg/dl non-fasting	None	6 (0.6%) episodes of hypoglycemia reported; no hospitalizations for hypoglycemia.	1) switch insulin usual morning and evening doses 2) use flexible targets (100–200 mg/dl fasting, 100–180 mg/dl non-fasting hrs
Altemimi et al. (26) Cohort	Human premixed (NPH/regular) 2/3 before iftar; 1/3 before suhur	1/2 TDD of human regular short-acting before iftar, and 1/2 basal NPH	Hypoglycemic events were reported with both groups (35.7 and 43.8% of participants from premixed and basal+ short-acting, respectively), with no statistical difference.	Both regimens are effective for glycemic control and can be used safely for fasting "if the treatment is personalized on a case-by-case basis."
Ba-Essa et al. (27) Cohort	Ramadan focused diabetes education, diet counseling	None	Insulin only (13.6%) = 46.9% hypo; Insulin + OHA (30%) = 35.2% hypo; Basal + SU (13.1%) = 29.8% hypo; Basal + Non-SU-OHA (3.3%) = 0 hypo; MDDI ± Non-SU-OHA (13.6%) = 49% hypo	Insulin increased the risk of hypoglycemia during Ramadan except when using basal + non-SU-OHA
Beano et al. (28) Cohort	Reduction by 75% in all doses (if bid, 45% iftar, 30% suhur)	None	Reduced # hypoglycemic episodes in all groups vs. preceding month, Group C (metformin+insulin): $p = 0.008$, Group D (insulin alone): $p = 0.02$	75% TDD (if bid, 45% Iftar, 30% suhur)
Elhadd et al. (29) Cohort	Reduction of basal insulin 25% and SU by 50%	Compared to sulfonylureas and itself (full dose)	No difference before or during Ramadan	Reduce insulin dose by 25% per PROFAST Ramadan protocol
Hassanein et al. (30) Cohort	Gla−300, patient education and dosing adjustment	None	No significant difference in pre, post, during R in # of episodes. No severe hypoglycemia episodes during and post-Ramadan. Daytime hypoglycemic events are more common than nocturnal.	People with T2DM using Gla-300 during R had a low risk of severe/symptomatic hypoglycemia and improved glycemic control
Hui et al. (31) Cohort	Humalog Mix 50 at iftar, Mixtard 30 suhur	Mixtard 30 bid	No statistically significant difference between rates of hypoglycemia in Mix 50 vs. Mix 30 groups.	Changing to Humalog Mix 50 for Iftar improved glycemic control without increasing hypoglycemia (maybe)
Kalra et al. (32) Cohort	IDeg or IDegAsp	None	No severe hypoglycemic episodes were reported in patients. 3 total episodes of hypoglycemia were reported in the non-fasting period and were self-treated successfully.	IDeg dose may need reduction by 25% for Ramadan. Dose reduction of 25-30% at Suhur with IDegAsp "May" switch morning dose of IDegAsp to evening meal with changing dose amt.

(Continued)

TABLE 3 | Continued

References study design	Intervention	Comparator	Effect on hypoglycemia	Dosing recommendations
Salti et al. (33) Cohort	Insulin glargine and glimepiride	None	Minimal severe hypoglycemic episodes, mild hypoglycemic episodes increased from 156 pre-R and 153 post-R vs. 346 during R ($p <$ 0.001, $p = 0.0002$). FBG > 6.7 mmol/L had a protective effect on hypoglycemia	This combination may be useful in some patients, provided glimepiride is given at the time of breaking the fast and insulin glargine titrated to provide FBG > 6.7 mmol, ÂÑl.

RR, relative risk; R, Ramadan; TDD, total daily dose (of insulin); BG, blood glucose; ERR, Estimated Relative Risk; AE, adverse events; FBG, Fasting Blood Glucose; PP, post-prandial; Exp, experimental; DKA, diabetic ketoacidosis; NKHS, non-ketotic hyperosmolar syndrome.

However, it must be acknowledged that we found and reviewed only four studies relating to insulin dose adjustment, limiting our confidence in this conclusion.

Novel insulin dosing strategies may lead to improved outcomes for persons with diabetes during Ramadan. The IDF/DAR guidelines in 2021 relied on standard glycemic targets through the suhur, pre-iftar, and post-iftar periods (90–130 mg/dL) (5). However, the study done by Ahmedani and colleagues opens the door for more research regarding the possible superiority of flexible glycemic targets up to 200 mg/dL during fasting hours and tightened glycemic targets to <180 mg/dL during non-fasting hours (25). Their result of only a 0.6% hypoglycemia incidence rate appears very promising compared to the 9.2% incidence rate in a similar demographic in the CREED study (7). Future studies will need to examine the long-term impact of annually recurring permissive hyperglycemia as the study by Ahmedani et al. was only 2 months in duration.

Long and Ultra-Long-Acting Insulins

Long and ultra-long-acting insulins, when given at sunrise, may reduce the risk of hypoglycemia during Ramadan (27, 30, 33), particularly when compared to intermediate-acting insulins (21, 23, 30). Long-acting insulins have a more attenuated peak and can accommodate fasting hours lasting as long as 20 h (37), thus resulting in fewer episodes of hypoglycemia. However, regarding hyperglycemia, each study recorded a beneficial, but different, outcome [lower pre-iftar glucose (21), lower A1c (30), insulin dose reduction (32), and reduced hyperglycemia > 300 mg/dL, 16.7 mmol/L (23)]. Because we cannot compare these outcomes to each other, we are unable to conclude long and ultra-long-acting insulins and their effects on hyperglycemia. Additionally, three of the six studies examining long and ultra-long-acting insulins had insufficient quality per the NOS, further limiting our confidence (27, 30, 32). More high-quality RCTs need to confirm whether long and ultra-long-acting insulins reduce hypoglycemic events and improve glycemic control during Ramadan.

Rapid-Acting Insulin Analogs

Between two RCTs and one cohort study, rapid-acting insulin analogs significantly improved post-iftar blood glucose (20, 31) and overall blood glucose compared to regular human insulin without the risk of increased hypoglycemia (20, 22, 31). In 2015, Lessan et al. demonstrated that persons with insulin-treated diabetes (with/without oral antidiabetic drugs) recorded a significant difference in the mean amplitude of glycemic excursion during Ramadan (176 mg/dL, 9.8 mmol/L) compared to persons without diabetes (44 mg/dL, 2.4 mmol/L) (38). Other groups on only oral antidiabetic drugs did not show a significant difference (38). Physiologically, rapid-acting insulin analogs can better counteract these post-iftar excursions than regular-acting ones. Our findings are consistent with the previous reviews (3, 34–36), but because our review included only three relevant studies, two of which funded by industry, further research is necessary to confirm that rapid-acting insulin analogs improve glycemic control without increasing hypoglycemic events during Ramadan.

The strength of this review is that it is, to our knowledge, the only systematic review exclusively analyzing insulin subtypes and dosing strategies for insulin-treated type 2 diabetes during Ramadan. A comprehensive search of relevant literature was conducted, including gray literature, and a critical appraisal of the original research was performed. We reviewed recent literature which includes an investigation of second-generation basal insulin and the use of flexible glycemic targets. Acknowledged limitations include heterogeneity of study designs and study outcomes which precluded rigorous meta-analysis. We were able to group similar interventions and comparators between studies but given the variety of insulin types and dosing recommendations, our conclusions are limited. Additionally, six of the fourteen included studies were funded by industry which may introduce bias.

This review did not examine how combining insulin with other non-insulin antidiabetic medications affects hypoglycemic events and glycemic control. Abdelrahim et al. performed a large comprehensive review in 2021 and found that certain oral hypoglycemic agents combined with insulin are preferable in preventing hypoglycemic events and improving glycemic control during Ramadan, namely non-sulfonylureas such as incretin mimetics (39). In this review, we have shed light on which types of insulin and dosing strategies are beneficial during Ramadan. However, practically, many regimens include both insulin and oral hypoglycemic agents, so further research on the superiority of insulin subtypes and dosing strategies in combination with oral hypoglycemic agents during Ramadan would help close the knowledge gap which physicians have expressed (14).

CONCLUSIONS

The research and body of literature pertaining to insulin-requiring diabetes and Ramadan remain sparse. Many reviews

TABLE 4 | The effects of different insulin subtypes and dosing strategies on hyperglycemia.

References study design	Intervention	Comparator	Effect on hyperglycemia	Dosing recommendations
Hajjaji et al. (20) RCT	Humalog Mix 50/50 at iftar (lispro/protamine) Humalog Mix 75/25 at suhur	Human mixed insulin 30:70 (Human Mixtard 30) ($n = 20$)	During R, mean pp BG in Exp group lower by 21.1 mg% ($p < 0.001$) compared to control, using ANCOVA to adjust for pre-R values of age, gender, duration of diabetes. After R, mean A1c in Exp group lower 0.4% ($p = 0.01$) vs. control. Mean fasting BG no sig difference	Insulin analog mix 50:50 is preferred for glycemic control post iftar compared to intermediate insulin (human insulin mix 30:70).
Hassanein et al. (21) RCT	IDegAsp BID ($n = 121$)	BIAsp 30 BID ($n = 127$)	Significantly lower pre-iftar (−0.54 mmol/L, $p = 0.0247$). Similar A1c reduction between 2 arms	Use dose adjustment and titration algorithm for dosing insulin for both efficacy and safety.
Mattoo et al. (22) RCT	Lispro Mix 25 x 2 weeks then human insulin 30/70 x 2 weeks	Human insulin 30/70 × 2 weeks then Lispro Mix 25 × 2 weeks	For LisproMix25 vs. Human insulin 30/70: pre iftar BG 7.19 ± 2.2 vs. 7.59 ± 2.6 mmol/l (adjusted $P = 0.034$); 2 h post dinner 10.59 ± 3.2 mmol/l vs. 11.69 ± 3.4 mmol/l ($p = 0.0001$) Evening 2 h pp excursion 3.49 ± 2.9 vs. 4.09 ± 3.2 mmol/l (adjusted $p = 0.007$)	Insulin Lispro Mix25 had better glycemic control (overall, pre-iftar and 2 h post iftar) without increasing hypoglycemic risk compared to human insulin 30/70
Shehadeh et al. (23) RCT	60% TDD pre-R: 40% Levemir at suhur, 60% biphasic 70 iftar	Standard care per ADA recommendations	No significant difference in A1c. Intervention arm non-inferior to the control. Blood-glucose > 300 mg/dL event rate mean 0.01 in intervention vs. 0.02 in control ($p = 0.026$)	Insulin dose (intervention) was 60% of the usual, of this 40% was dosed as Levemir at sunrise and 60% as biphasic 70 before dinner
Zaghlol et al. (24) RCT	75% insulin dosage reduction	Regular dosing	No statistically different difference in hyperglycemia incidence in low vs. regular dose groups	Dose decreases (75% tested in this study) did decrease hypoglycemia without increasing hyperglycemia or its adverse sequela.
Ahmedani et al. (25) Cohort	Insulin dose adjustments (100–200 mg/dl fasting, 100–180 mg/dl non-fasting	None	A1c reduction 9.21 +/- 2.05 to 8.33 +/- 1.45 (p< 0.0001); 352 (30%) hyperglycemic episodes reported, no DKA or HHS	1) switch insulin usual morning and evening doses 2) use flexible targets 100–200 mg/dl fasting, 100–180 mg/dl non-fasting hrs
Altemimi et al. (26) Cohort	Human premixed (NPH/regular) 2/3 s before iftar; 1/3 before suhur	1/2 TDD of human regular short-acting before iftar, and 1/2 basal NPH	Hyperglycemic events were reported with both groups, with no statistical difference. Both groups decreased average A1c (more in premixed), but no significant difference	Both regimens are effective for glycemic control and can be used safely for fasting "if the treatment is personalized on a case-by-case basis."
Ba-Essa et al. (27) Cohort	Ramadan focused diabetes education, diet counseling	None	A1c reduced from 8.79 before R to 8.59 post R, ($p = 0.022$) overall (not insulin specific)	NA
Beano et al. (28) Cohort	Reduction by 75% in all doses (if bid, 45% iftar, 30% suhur)	None	No episodes of DKA nor NKHS	75% TDD (if bid, 45% Iftar, 30% suhur)
Elhadd et al. (29) Cohort	Reduction of basal insulin 25% and SU by 50%	Compared to sulphonylureas and itself	No difference in A1c or average BG before or during Ramadan	Reduce insulin dose by 25% per PROFAST Ramadan protocol
Hassanein et al. (30) Cohort	Gla−300, patient education and dosing adjustment	None	A1c fell 0.4% (±1.0%) pre to post R, Gla-300 daily dose reduced 25.6 to 24.4. Fasting plasma glucose decreased (mean change −13.5 ± 44.1)	People with T2DM using Gla-300 during R had a low risk of severe/symptomatic hypoglycemia and improved glycemic control
Hui et al. (31) Cohort	Humalog Mix 50 at iftar, Mixtard 30 suhur	Mixtard 30 bid	Mix50 mean A1c reduction 0.48% ($p = 0.0001$) Mix30 A1c increase 0.28% ($p = 0.007$). Significant after adjusting for baseline factors ($p = 0.0004$, 95% CI (0.19%, 0.62%)	Changing to Humalog Mix 50 for Iftar improved glycemic control without increasing hypoglycemia (maybe)
Kalra et al. (32) Cohort	IDeg or IDegAsp	None	5 persons who switched from either premixed or NPH resulted in a 12–25% dose reduction after 14–20 days.	IDeg dose may need reduction by 25% for R; a dose reduction of 25-30% at Suhur with IDegAsp. "May" switch morning dose of IDegAsp to evening meal
Salti et al. (33) Cohort	Insulin glargine and glimepiride	None	FBG and A1c improved for insulin naive (10.9–7.0, 8.7–7.7, $p = 0.0002$) and non-insulin naive (9.8–6.9, 8.6–7.7, $p < 0.0001$)	The combination may be useful, provided glimepiride was given at iftar; insulin glargine titrated to provide FBG > 6.7 mmol, ÅÑl.

RR, relative risk; R, Ramadan; TDD, total daily dose (of insulin); BG, blood glucose; ERR, Estimated Relative Risk; AE, adverse events; FBG, Fasting Blood Glucose; PP, post-prandial; Exp, experimental; DKA, diabetic ketoacidosis; NKHS, non-ketotic hyperosmolar syndrome.

TABLE 5 | Newcastle-Ottawa Scale for the risk of bias and quality assessment of cohort studies.

No.	References	Selection	Comparability	Outcome/exposure	Total (out of 9)	Quality
1	Ahmedani et al. (25)	4	0	2	6	Poor
2	Altemimi et al. (26)	4	1	3	8	Good
3	Ba-Essa et al. (27)	4	0	3	7	Poor
4	Beano et al. (28)	3	1	2	6	Good
5	Elhadd et al. (29)	3	2	2	7	Good
6	Hassanein et al. (30)	4	0	2	6	Poor
7	Hui et al. (31)	3	2	3	8	Good
8	Kalra et al. (32)	3	0	1	4	Poor

TABLE 6 | NHLBI risk of bias and quality assessment for controlled intervention studies.

Question	Hajjaji et al.	Hassanein et al.	Mattoo et al.	Shehadeh et al.	Zaghlol et al.
1	Yes	Yes	Yes	Yes	Yes
2	No	Yes	Yes	No	Yes
3	No	Yes	NR	No	Yes
4	No	No	No	No	No
5	No	No	No	No	Yes
6	Yes	Yes	Yes	No	Yes
7	Yes	Yes	Yes	Yes	Yes
8	Yes	Yes	Yes	Yes	Yes
9	Yes	Yes	Yes	Yes	Yes
10	NR	Yes	Yes	Yes	NR
11	Yes	Yes	Yes	Yes	Yes
12	No	NR	NR	Yes	Yes
13	Yes	Yes	Yes	Yes	Yes
14	Yes	Yes	Yes	No	NR
Total	8	11	10	8	11
Quality Rating	**Fair**	**Good**	**Good**	**Fair**	**Good**

NR, Not reported.
Questions for the NHLBI risk of bias and quality assessment:
1. Was the study described as randomized, a randomized trial, a randomized clinical trial, or an RCT?
2. Was the method of randomization adequate (i.e., use of randomly generated assignment)?
3. Was the treatment allocation concealed (so that assignments could not be predicted)?
4. Were study participants and providers blinded to treatment group assignment?
5. Were the people assessing the outcomes blinded to the participants' group assignments?
6. Were the groups similar at baseline on important characteristics that could affect outcomes (e.g., demographics, risk factors, co-morbid conditions)?
7. Was the overall drop-out rate from the study at the endpoint 20% or lower than the number allocated to treatment?
8. Was the differential drop-out rate (between treatment groups) at the endpoint 15 percentage points or lower?
9. Was there high adherence to the intervention protocols for each treatment group?
10. Were other interventions avoided or similar in the groups (e.g., similar background treatments)?
11. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?
12. Did the authors report that the sample size was sufficiently large to be able to detect a difference in the main?
the outcome between groups with at least 80% power?
13. Were outcomes reported or subgroups analyzed prespecified (i.e., identified before analyses were conducted)?
14. Were all randomized participants analyzed in the group to which they were originally assigned, i.e., did they use an intention-to-treat analysis?

and guidelines have been published, but the original research had not been critically appraised, which we have done here. Insulin dose reduction may prevent hypoglycemic events, and rapid-acting insulin analogs may improve glycemic control without incurring subsequent hypoglycemia during Ramadan. However, more randomized controlled trials need to be performed before conclusions can be made. Though initial findings are promising, more research is needed to confirm the benefits of ultra-long-acting insulins as well as the use of flexible glycemic targets. While certain types of insulin and particular dosing strategies demonstrate some advantages, these recommendations should be tailored to the context of each person with diabetes to make the appropriate regimen adjustments in preparation for intensive fasting practices during the month of Ramadan.

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 author/s.

AUTHOR CONTRIBUTIONS

AK conceived of the presented idea. AK, AI, MK, and LÖ designed the review. LÖ conducted the literature search and prepared the articles for screening. AK and MK conducted the title/abstract screening while AI resolved conflicts. AK and AI did the full-text screen with MK resolving conflicts. AK and AI extracted data. AK and MK performed the quality assessment with DB resolving conflicts. AK, AI, and LÖ wrote the manuscript. DB and MF designed tables. All authors edited the manuscript, discussed the results, analyzed the data, and contributed to the final manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.846600/full#supplementary-material

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