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Effect of Ramadan Fasting on Intestinal Microbiota and Fatty Acid Binding Protein 4 in Overweight and Obese Individuals

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ABSTRACT

Background & Aims: Intermittent fasting is a nutritional strategy that focuses on when to eat, rather than what to eat. Although the effectiveness of intermittent fasting practices in many metabolic diseases is known, its effect on microbiota and its underlying mechanism has not yet been clarified. This study aimed to investigate the effect of Ramadan fasting, one of the intermittent fasting practices, on gut microbiota and fatty acid binding protein 4 (FABP4).

Methods: The study involved 10 male volunteers, 6 of whom were overweight and 4 were obese. They fasted for an average of 14-15 hours daily from dawn to sunset during the 29-day Ramadan month between 23 March - 20 April 2023 and met the inclusion criteria. The participants' nutritional and physical activity status before and during Ramadan, as well as their anthropometric measurements before and after Ramadan, intestinal microbiota, transaminases, gamma-glutamyl transferase, C reactive protein, total cholesterol (C), high-density lipoprotein (HDL) C, low-density lipoprotein C, triglycerides (TG), and FABP4 levels, were evaluated within the scope of the study.

Results: The study found a statistically significant increase in both alpha and beta diversity in the intestinal microbiota following Ramadan fasting ($p<0.05$). The F/B ratio, *Firmicutes* phylum, *Clostridia* class, *Clostridiales* order, and *Ruminococcaceae* family exhibited statistically significant decreases, while the *Bacteroidetes* and *Proteobacteria* phyla, *Bacteroidia*, *Alphaproteobacteria*, and *Erysipelotrichi* classes, *Bacteroidales, Erysipelotrichales*, and *Actinomycetales* orders, *Erysipelotrichaceae* family and *Prevotella* genus, demonstrated statistically significant increases ($p<0.05$). Participants who achieved an average weight loss of 2.3±0.99 kg at the end of Ramadan showed a significant increase in HDL-C and a significant decrease in TG levels ($p<0.05$). Although FABP4 levels decreased after fasting, this difference was not statistically significant ($p>0.05$).

Conclusion: Ramadan fasting induces weight loss, modifies gut microbiota, and improves blood lipid profile and FABP4 levels, suggesting the need for more extensive studies.

Key words: fatty acid binding protein 4 (FABP4) – human gut microbiota – intermittent fasting – obesity – overweight – ramadan fasting.

Abbreviations: BMI: body mass index; CRP: C-reactive protein; DM: diabetes mellitus; FABP4: fatty acid binding protein 4; F/B: Firmicutes/Bacteroidetes; GGT: gamma-glutamyl transferase; HDL-C: high-density lipoprotein cholesterol; LDL-colesterol: low density lipoprotein cholesterol; PAL: physical activity levels; TC: total cholesterol; TG: triglycerides.

INTRODUCTION

Obesity is defined by the World Health Organization as excessive fat accumulation in the body to the extent that it impairs health. According to the World Obesity Atlas 2023 data, 52% of the adult population in the world suffers from overweight (38%) or obesity (14%) as of 2020 [1]. It is known that obesity causes insulin resistance, type 2 diabetes mellitus (DM), hypertension, dyslipidemia, cardiovascular diseases and some types of cancer and increases the global disease burden [2]. Therefore, achieving and maintaining weight loss in obese individuals is the primary goal. It has been reported that even a weight loss of only 1 kg can reduce the risk of diabetes by up to 16% [3].

It has been reported that fatty acid binding protein 4 (FABP4), which is highly secreted from adipose tissue, is effective in the development of many chronic diseases [4]. Prentice et al. [5] suggested that FABP4, adenosine kinase (ADK) and nucleoside diphosphate kinase (NDPK) molecules may be the key regulator of pancreatic β-islet functions by forming a complex hormone called Fabkin, which FABP4 levels increased in the diabetic cohort group in sera obtained from patients with type 1 DM (<1 year) and that this hormone complex is a targetable molecule for metabolic diseases [5].

Obesity is also associated with gut microbiota composition. It is known that the gut microbiota of obese people differs in density and diversity compared to healthy controls [6]. Various strategies such as fecal transplantation, prebiotic and probiotic supplementation have been tried to obtain healthy microbiota in obese individuals. Although successful results have been obtained in the short term, it has been observed that the effects disappear or may cause different complications in the long term [7].

Nutrition has been considered as a powerful strategy in the fight against obesity and several nutritional therapies have been applied to restrict the time of nutrient or energy intake [8]. Intermittent fasting diets, the effectiveness of which has been proven by many studies, are the most important of these treatments. Intermittent fasting is a broad term that encompasses a variety of programs in which eating time is manipulated by short-term fasting to improve body composition and overall health [9]. Various studies have reported that intermittent fasting protects against many diseases such as obesity, cancer, cardiovascular diseases, insulin resistance, type 2 DM, increases antioxidant function, protects against DNA damage and inflammation and even protects against brain damage by supporting new neuron formation [10-16]. In addition, the fact that intermittent fasting induces autophagy to maintain cellular homeostasis has increased intense interest in intermittent fasting practices [11]. Ramadan fasting, which is considered as a model of intermittent fasting, is the fasting period from the time of the beginning of the day until sunset in the month of Ramadan, which coincides with different periods of each year according to Muslim societies, without eating or drinking [17].

In this study, we evaluated the effect of 29-day Ramadan fasting on gut microbiota, anthropometric measurements, blood lipid profile, liver function tests, C-reactive protein (CRP) and FABP4. This study, which included overweight and obese individuals, shows that Ramadan fasting modifies gut microbiota, improves blood lipid profile, provides weight loss and may reduce FABP4 levels.

METHODS

This is a quasi-experimental study conducted in the Gastroenterology Department of Gazi University Faculty of Medicine Hospital in Ankara, Turkey.

Study Sample and Design

The study was conducted with 10 male volunteers who fasted for an average of 14-15 hours continuously from dawn to sunset during the 29-day Ramadan month between 23 March 2023 and 20 April 2023 and met the inclusion criteria.

Inclusion Criteria:

- Not having a chronic disease diagnosed by a doctor (DM, hypertension, dyslipidemia, cancer, chronic renal failure, gastrointestinal system problems etc.);
- Being a male between the ages of 20 and 50;
- Body mass index (BMI) ≥ 25 kg/m^2 and < 34.9 kg/m^2;
- Not having applied to any diet program in the last 6 months;
- Not taking prebiotic or probiotic supplements in the last 6 months;
- Not taking antibiotics, steroids or any medication that may affect gastrointestinal system functions in the last 6 months;
- Not using nutritional supplements;
- Not working in shifts.

Exclusion Criteria:

- According to Islamic belief, women do not fast during menstruation in Ramadan. Women were not included in the study because it was thought that they would not be able to keep the 29-day Ramadan fast completely due to the menstrual cycle.

Ethical Approval of the Study

Ethics committee permission dated 06.02.2023 and numbered 113 (Decision Amendment Date and Number: 03.04.2023 and numbered 295) was obtained from Gazi University Clinical Research Ethics Committee for the realisation of the study.

Collection of Study Data

All individuals who met the inclusion and exclusion criteria and agreed to participate in the study were read and signed the informed voluntary consent form and a copy was delivered to them.

Anthropometric Measurements

Body weight (kg) measurements of the individuals participating in the study were made with a weighing device sensitive to 0.1 kg and height (m) measurements were made with a digital display stadiometer device with a precision of 0.1 cm. Body mass index (kg/m^2) values [BMI = body weight (kg) / height^2 (m^2)] were calculated from body weight and height measurements and classified as (25.0-29.9 kg/m^2 "Overweight" and >30kg/m^2 "Obese") [18].

The waist circumference of the participants was measured at the midpoint between the lowest rib and the crista iliac, and hip circumference was measured with a non-flexible tape measure from the right side of the individuals, parallel to the ground at the highest point on the hip.

All anthropometric measurements were performed one day before the start of Ramadan and on the last day of Ramadan after at least 10 hours of fasting at full rest and without any metal jewellery, except for light clothing.

Food Consumption and Physical Activity

Participants were asked to record their food consumption for three consecutive days (2 days on weekdays + 1 day on

weekends) one week before the start of Ramadan fasting to assess their nutritional status before Ramadan fasting, and for three consecutive days (2 days on weekdays + 1 day on weekends) during the first, middle and last week of Ramadan to assess their nutritional status during Ramadan fasting. The data obtained from food consumption records were calculated with the Nutrition Information System (BeBiS) 9 program [19]. To evaluate the physical activity status of the participants, 24-hour physical activity records were also taken on the same days when food consumption records were taken.

Collection and Analysis of Blood Samples

One day before the start of Ramadan and on the last day of Ramadan, 8 ml blood samples were taken from the participants after 10 hours of fasting. Blood samples were analyzed for FABP4 (ng/L), CRP (mg/L), high-density lipoprotein cholesterol (HDL-C) (mg/dl), low-density lipoprotein cholesterol (LDL-C) (mg/dl), total cholesterol (TC) (mg/dl), triglycerides (TG) (mg/dl), alanin aminotransferase (ALT) (U/L), aspartate aminotransferase (AST) (U/L) and gamma-glutamyl transferase (GGT) (U/L) in a private medical laboratory and the samples were stored at -32°C until the analyses were performed. Serum FABP4 levels were measured using the Enzyme-Linked Immunosorbent Assay (ELISA) method (Bioassay Technology Laboratory, Birmingham, UK; Catalogue No: E7430Hu, Lot No: 202305012).

Collection of Fecal Samples and Gut Microbiota Analysis

Fecal samples of the participants were collected for gut microbiota analysis by giving sterile disposable spoon stool collection tubes to the participants one day before the start of Ramadan and on the last day of Ramadan. Samples for gut microbiota analysis were stored at -80°C within 30 minutes after collection. Stool samples were analyzed using DiaRex® Stool Genomic DNA Extraction Kit (Cat No: SD-0323, Ankara).

The stool samples were frozen at -20°C the day before analysis and then thawed at room temperature. Once fully thawed, the samples achieved a homogeneous structure. For the analysis, 25 mg stool samples were taken and weighed on an Isolab 602.31.001 precision balance with a sensitivity of 0.001 g. After adding 250 µl of Lysis Solution (LBD) to the samples, 15 mg of glass and 10 zircon beads were added. The mixture was homogenized using a Thermo Fisher Fastprep Fp120 homogenizer device at 4000 rpm for 2 x 20 seconds. Following homogenization, 25 µl of Proteinase K (PKD) solution was added and incubated at 56°C for 60 minutes in a Hotplate Benchmark Scientific BSH1002 device. After the incubation period, the entire mixture was centrifuged at 5000 g for 5 minutes using a Hettich Mikro 220 R centrifuge. The resulting supernatant was then transferred to a new tube. Following this, 200 µl of Stool Lysis Solution (SLD) was added to the supernatant and incubated at 70 °C for 10 minutes. Once the incubation was complete, 250 µl of absolute (96%) ethanol was added to the lysate, pipetted, and the entire contents were transferred to the column. The column was then centrifuged at 8000 g x 1 min and the resulting mixture was transferred to a new tube. 500 µl of washing solution-2 (WBD-2) was added and centrifuged at 8000 g for 1 minute. The waste tube was then discarded and the contents were transferred to a new column. The mixture was then centrifuged at 8000 g for 1 minute. Next, 500 µl of wash solution-2 (WBD-2) was added and centrifuged at 8000 g for 2 minutes. The waste tube was discarded and the filter was placed in a new tube. Genomic DNA was obtained by adding 100 µl of elution solution (EBD) to the filter and incubating at room temperature for 1-3 minutes. The DNA was amplified using 16S V3-V4 341F-806R primer sets. Library preparation was performed with the Nextera XT DNA library preparation kit and indexes from Illumina. Cleaned pooled libraries with specific size selections were applied following the manufacturer's protocol (AMPure XP, Beckman Coulter). After library preparation, Illumina MiSeq was used for sequencing.

Bidirectional (2x250) Illumina reads were loaded into the QIIME2 system. All samples were found to occur at a depth of over 100X in close proximity to each other, and none were excluded at this stage. Quality clean-up and chimera detection were performed using the DADA2 algorithm in qiime2. Amplicon Sequence Variants (ASVs) were generated by excluding parts with a quality score mostly below 30. The resulting ASVs were mapped to the Silva 138 database, and taxonomic tables were created.

Statistical Analysis Methods

The alpha diversity assessment used to evaluate the diversity of the relevant taxonomic units in the sample was interpreted using three different indices, namely Chao1, Simpson and Shannon. The p values between groups were calculated by the Kruskal-Wallis test. Beta diversity analysis used to assess taxonomic differences between individuals was calculated based on Jaccard, Bray-Curtis and Unweighted Unifrac. Specific differences between groups were determined by differential abundance analysis, Deseq2 R package. Linear discriminant analysis effect size (LEfSe) analysis was performed between groups to show statistically significant taxonomies.

IBM Statistical Package for the Social Sciences (SPSS) 25® package program was used for statistical analysis of data other than gut microbiota. The assumption of normal distribution of the data was checked by the Kolmogorov-Smirnov test. Descriptive statistics were given as mean ± standard deviation for normally distributed variables and median (25th-75th percentile) for non-normally distributed variables. In the comparisons of dependent measurements, a paired t-test was used for normally distributed variables and the Wilcoxon T-test was used for non-normally distributed variables. Since the parametric test assumptions were not met, the relationships between variables were analyzed by Spearman Correlation Analysis and a value of $p<0.05$ was considered statistically significant.

RESULTS

The comparison of energy and nutrient intakes and physical activity values of the participants before and during the fasting period is given in Table I. In general, the energy and nutrient intakes of the participants were similar before and during the fasting period. Although protein, vitamin A, thiamine, riboflavin, niacin, vitamin B6, folate, vitamin B12, phosphorus, magnesium, iron, zinc, copper, and potassium intake decreased during the fasting period ($p<0.05$), this may be due to a few specific foods (liver, roasting, milk etc.) eaten at the time when

Table I. Comparison results of energy and nutrient intakes and physical activity values of individuals before and during the fasting period

Energy and Nutrients	Before Fasting	Fasting Period	p
Energy (kcal)	1936±461	1783±506	0.245[t]
Carbohydrate (g)	220.3±64.1	204.1±59.9	0.367[t]
Carbohydrate (%)	46.5±7.6	46.9±6.1	0.886[t]
Protein (g)	83.9±19.0	69.8±17.5	**0.008[t]**
Protein (%)	18.0±2.9	16.0±2.2	0.058[t]
Oil (g)	78.0±25.3	74.3±26.4	0.681[t]
Oil (%)	35.6±7.6	36.6±6.4	0.747[t]
Saturated fatty acids (g)	29.1±8.8	27.9±10.6	0.755[t]
Monounsaturated fatty acids (g)	26.6±9.8	25.5±9.8	0.728[t]
Polyunsaturated fatty acids (g)	15.4±7.1	14.2±4.5	0.502[t]
Cholesterol (mg)	441.8±148.8	418.9±207.8	0.814[t]
Soluble fibre (g)	6.18±2.19	5.61±2.06	0.395[t]
Insoluble fibre (g)	12.11±3.75	11.08±4.01	0.197[t]
Vitamin A (µg)	7397 (801-13325)	785 (577-1330)	**0.013[w]**
Vitamin E (mg)	15.6±7.7	14.3±4.6	0.573[t]
Vitamin K (µg)	141.4±60.9	109.5±40.5	0.130[t]
Vitamin C (mg)	75.1±42.7	81.0±45.6	0.560[t]
Thiamine (mg)	0.91±0.30	0.74±0.23	**0.044[t]**
Riboflavin (mg)	2.38±1.14	1.25±0.47	**0.018[t]**
Niacin (mg)	21.1±6.9	13.2±4.1	**0.001[t]**
Vitamin B6 (mg)	1.59±0.57	1.05±0.33	**0.002[t]**
Total folate (µg)	490 (222-632)	214 (187-345)	**0.022[w]**
Vitamin B12 (µg)	25.5 (4.8-41.2)	4.0 (3.0-6.1)	**0.009[w]**
Calcium (mg)	480 (460-700)	456 (379-617)	0.508[w]
Phosphorus (mg)	1196±301	967±282	**0.010[t]**
Magnesium (mg)	274.7±83.7	235.9±74.1	**0.018[t]**
Iron (mg)	12.57±3.74	9.44±3.11	**<0.001[t]**
Zinc (mg)	12.21±1.93	10.29±3.07	**0.021[t]**
Copper (mg)	2.66±1.16	1.49±0.54	**0.001[t]**
Potassium (mg)	2383±794	2104±686	**0.036[t]**
Sodium (mg)	3313±757	3506±2308	0.824[t]
PAL	1.63±0.18	1.62±0.18	0.835[t]

PAL: physical activity level.Descriptive statistics are given as mean±standard deviation or median (25^{th}-75^{th} percentile). t: Paired t-test, w: Wilcoxon T-test.

food consumption records were taken. At the same time, no statistically significant difference was observed between the physical activity levels (PALs) measured before and during the fasting period (p>0.05).

The anthropometric measurement results of the participants before and after fasting are given in Table II. Body weight, BMI, waist circumference and hip circumference values measured after fasting were statistically significantly lower than the

Table II. Comparison results of anthropometric measurements of individuals before and after fasting

Anthropometric Measurements	Before Fasting	After Fasting	p
Body Weight (kg)	90.2±7.2	87.9±6.9	**<0.001[t]**
BMI (kg/m^2)	28.9±2.1	28.2±2.2	**<0.001[t]**
Waist Circumference (cm)	102.9±7.7	100.6±7.6	**0.001[t]**
Hip Circumference (cm)	110.8±4.0	109.5±4.4	**0.006[t]**
Waist/Hip Ratio	0.9 (0.9-1.0)	0.9 (0.9-1.0)	0.317[w]

BMI: body mass index. Descriptive statistics are given as mean±standard deviation or median (25^{th}-75^{th} percentile). t: Paired t-test, w: Wilcoxon T-test.

pre-fasting values (p<0.05). The comparison results of the biochemical and FABP4 findings of the participants before and after fasting are given in Table III. A significant increase in HDL-C and a significant decrease in TG were observed after fasting (p<0.05). Although FABP4 levels decreased after fasting, this difference was not statistically significant (p=0.169). Similarly, the changes in ALT, AST, GGT, CRP, LDL-C and TC after fasting were not statistically significant (p>0.05).

Table III. Comparison results of biochemical findings of individuals before and after fasting

Biochemical Findings	Before Fasting	After Fasting	p
ALT (U/L)	25.1±11.0	23.8±10.8	0.613[t]
AST (U/L)	19.6±5.9	18.2±5.7	0.274[t]
GGT (U/L)	30.0±12.3	24.4±8.5	0.096[t]
CRP (mg/L)	0.35±0.18	0.42±0.37	0.429[t]
HDL-C (mg/dl)	53.9±9.5	56.4±9.2	**0.027**[t]
LDL-C (mg/dl)	90.5±22.5	89.7±20.9	0.719[t]
TG (mg/dl)	81.2±59.2	50.8±28.3	**0.038**[t]
TC (mg/dl)	170.8±29.4	167.5±27.9	0.215[t]
FABP4 (ng/L)	1637.5 (788.9-3068.0)	1532.0 (836.2-2678.0)	0.169[w]

CRP: C-reactive protein; FABP4: fatty acid binding protein 4; GGT: gamma-glutamyl transferase; HDL-C: high-density lipoprotein cholesterol; LDL-colesterol: low density lipoprotein cholesterol; TC: total cholesterol; TG: triglycerides. Descriptive statistics are given as mean±standard deviation or median (25th-75th percentile). t: Paired t-test, w: Wilcoxon T-test.

Evaluation of Intestinal Microbiota Analyses of Individuals

Fig. 1 shows the phylum and genus level changes in the relative abundances of the gut microbiota of the participants before and after fasting. Ramadan fasting caused a significant difference in gut microbiota.

Fig. 2 shows the alpha (Chao1, Simpson and Shannon) and beta (Jaccard, Bray-Curtis, Unweighted Unifrac) diversity graphs of intestinal microbiota samples of individuals before and after fasting. A statistically significant increase in alpha and beta diversity was observed after fasting (Chao1 p<0.001; Simpson p=0.028; Shannon p=0.01; Jaccard p=0.049; Bray-Curtis p=0.032; Unweighted Unifrac p=0.001).

As a result of the evaluation of these data by LEfSe analysis, the units showing a statistically significant increase or decrease after fasting are given in Fig. 3. Accordingly, there was a statistically significant decrease in *Firmicutes* phylum (p=0.002), *Clostridia* class (p=0.001), *Clostridia*les order (p=0.001), and *Ruminococcaceae* family (p=0.041). Additionally, there was a statistically significant increase in the *Bacteroidetes* (p=0.012) and *Proteobacteria* (p=0.001) phyla, *Bacteroidia* (p=0.028), *Alphaproteobacteria* (p=0.001), and *Erysipelotrichi* (p=0.019) classes, *Bacteroidales* (p=0.028), *Erysipelotrichales* (p=0.019), and *Actinomycetales* (p=0.005) orders, *Erysipelotrichaceae* (p=0.019) family and *Prevotella* (p=0.049) genus.

In addition, the *Firmicutes*/*Bacteroidetes* (F/B) ratio decreased from 12.01 before fasting to 3.93 after fasting and this decrease was statistically significant (p=0.004).

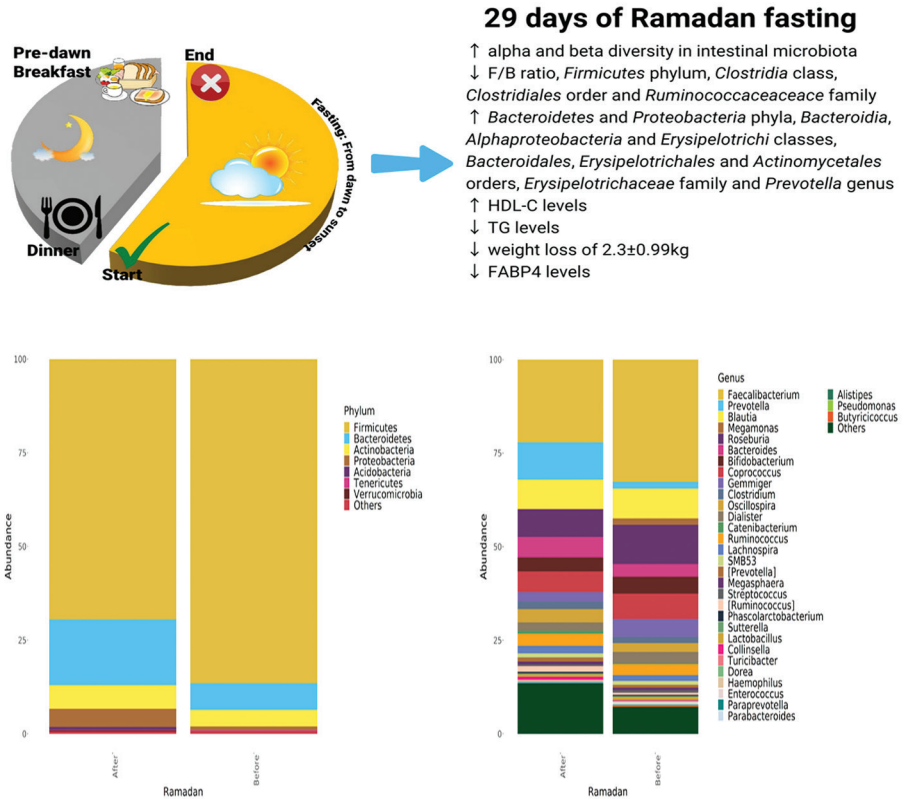

Fig. 1. Phylum and genus level changes in relative abundances of gut microbiota of individuals before and after fasting.

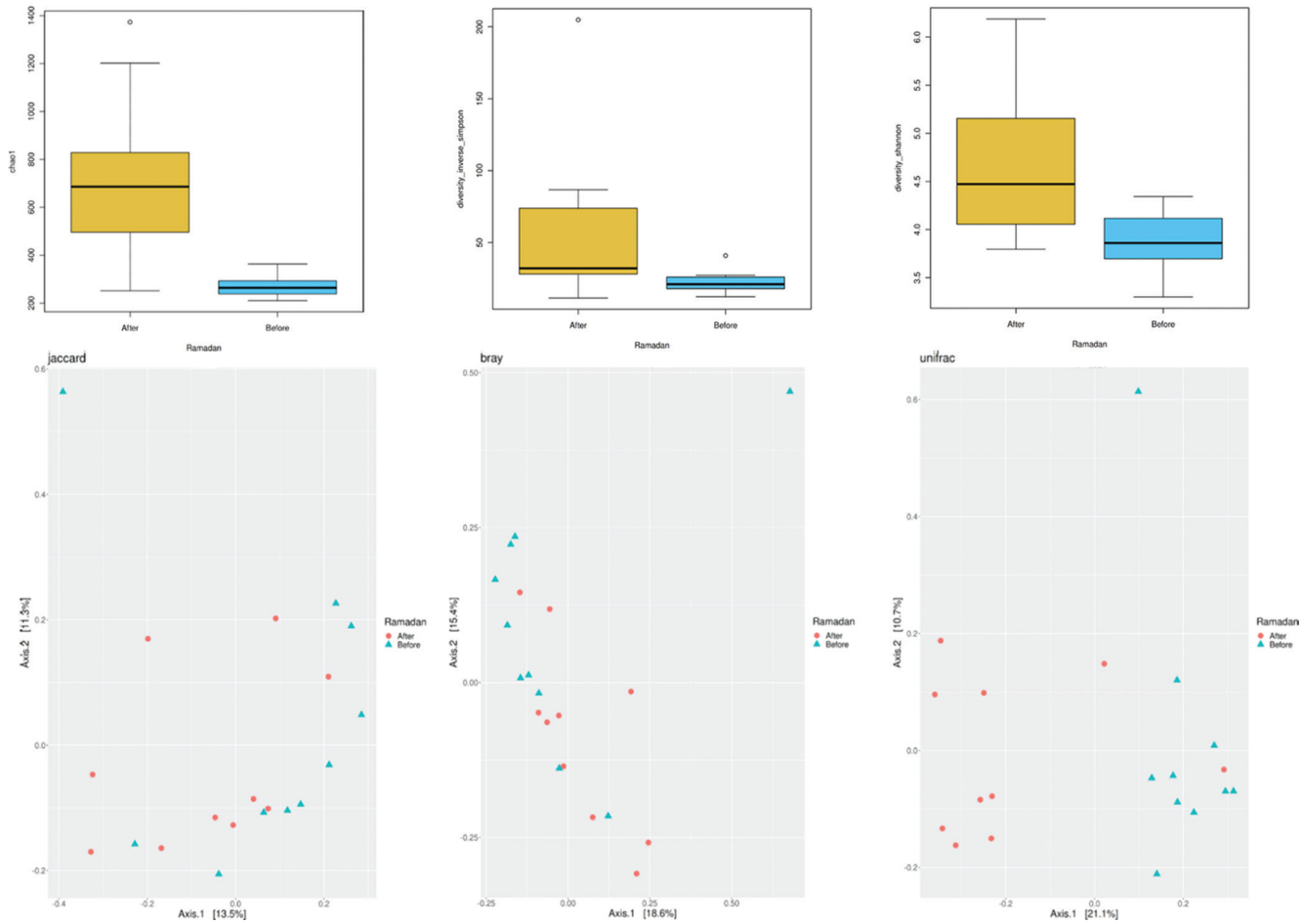

Fig. 2. Box plots of alpha and beta diversity of intestinal microbiota samples of individuals before and after fasting.

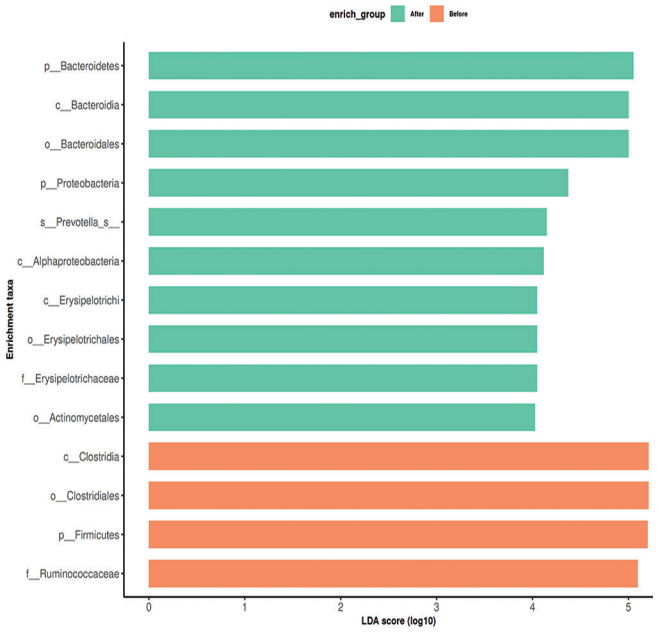

Fig. 3. Taxonomic units showing a statistically significant increase or decrease after fasting according to LEFse analysis.

However, no significant relationship was found between changes in microbiota and anthropometric measurements, nutrient intake and blood parameters (p>0.05).

DISCUSSION

In our study, since the effect of fasting on the variables was aimed to be evaluated, it was desired to maintain the existing energy and nutrient intake and physical activity of the participants, and in this context, the general nutritional status of the participants before and during Ramadan was similar. In a study in which healthy individuals were evaluated, it was reported that the food intake of the participants decreased during Ramadan, but information on physical activity was not analyzed [20]. In a study conducted on 33 healthy men in our country, the nutrition and physical activity values of the participants were recorded before, during the first and last week of Ramadan fasting. In the last week of Ramadan fasting, total energy, carbohydrate and protein intake decreased significantly compared to the pre-Ramadan period, whereas there was no change in fluid intake and fat intake of the participants. Physical activity levels remained consistent before and during the final week of fasting, but decreased during the first week [21].

We observed that the participants lost a mean weight of 2.3±0.99 kg despite similar nutrition and PALs with the pre-fasting period. Yeoh et al. [22] observed that participants lost weight and improved their lipid profile with intermittent fasting despite their routine diet and physical activity habits [22]. In a study involving 115 adults, it was observed that weight loss occurred despite the participants taking more energy and

carbohydrates during Ramadan than before Ramadan [23]. Khan et al. [24] reported that intermittent fasting reduced BMI in overweight and obese individuals and provided weight gain in thin women without any change in normal individuals. It has been shown in previous studies that intermittent fasting practices reduce body weight, but it was reported for the first time in this study that it normalized body weight [24].

In this study, which is important as it is the first human study in the literature in which intermittent fasting and FABP4 were associated, it was observed that the participants lost an average of 2.3±0.99 kg weight during the month of Ramadan compared to the pre-Ramadan period without any change in energy and nutrient intake and physical activity status. Considering that FABP4 is correlated with body fat, the decrease in serum FABP4 levels after fasting, although not statistically significant, is consistent with the literature.

We noticed a significant increase in HDL-C and a significant decrease in TG, but no significant change was observed in ALT, AST, GGT, CRP, LDL-C and TC. Ramadan fasting was observed to produce a statistically significant decrease in BMI, LDL-C, HDL-C, LDL/HDL ratio and TC, and a non-statistically significant decrease in fasting blood glucose and TG levels [20]. In another study, it was reported that intermittent fasting increased HDL-C and decreased TC, TG, LDL and VLDL levels [24].

We depicted a statistically significant increase in both alpha and beta diversity in the intestinal microbiota following Ramadan fasting. There was a statistically significant decrease in the F/B ratio, *Firmicutes* phylum, *Clostridia* class, *Clostridia*les order, and *Ruminococcaceae* family. Additionally, there was a increase in the *Bacteroidetes* and *Proteobacteria* phyla, *Bacteroidia*, *Alphaproteobacteria*, and *Erysipelotrichi* classes, Bacteroidales, *Erysipelotrichales*, and *Actinomycetales* orders, *Erysipelotrichaceae* family and *Prevotella* genus.

In studies conducted in our country to investigate the effects of 29-day Ramadan fasting on intestinal microbiota composition, it was observed that *Butyricicoccus, Bacteroides, Faecalibacterium, Roseburia, Allobaculum, Eubacterium, Dialister* and *Erysipelotrichi* genus bacteria significantly increased after Ramadan fasting, and in another study, a significant increase was observed in *Akkermansia muciniphila* and *Bacteroides fragilis* species [25, 26]. In a study conducted on healthy volunteers outside our country, an increase in the butyric acid-producing *Lachnospiraceae* family was detected after Ramadan fasting, but the intestinal microbiota composition returned to its previous state after the cessation of fasting [27]. A 30-day time-restricted diet increased alpha diversity in intestinal microbiota, but no statistically significant difference was observed in alpha and beta diversity [28]. Ferrocino et al. [29] pointed out that time-restricted feeding increased short-chain fatty acid (SCFA) producing bacteria such as *Lachnospiraceae, Parasutterella* and *Romboutsia*, and bacteria such as *Christensenellaceae* and *Escherichia*. In a study in which the effect of Ramadan fasting on two different ethnic groups, Chinese and Pakistani individuals, *Firmicutes* and *Actinobacteria* were predominant in the Pakistani group before fasting, while a decrease in *Firmicutes* and an increase in *Bacteroidetes* and *Proteobacteria* were observed after fasting. In the Chinese group, while *Bacteroidetes* was dominant before fasting, *Bacteroidetes* and *Proteobacteria* increased after fasting [30]. In a 12-week randomised controlled study in which intermittent fasting or energy restricted diet intervention was applied to 55 participants with BMI between 27-45 kg/m^2, *Subdoligranulum, Collinsella* decreased in relative abundance while *Parabacteroides, Alistipes* and *Bacteroides* increased in both groups. While no significant change was observed in alpha and beta diversity in both groups in general, *Akkermansia* increased in the intermittent fasting group and did not change in the energy restriction group [31].

High *Firmicutes* and F/B ratios and low *Bacteroidetes* ratios have been associated with obesity [32]. Bacteria belonging to the phylum *Firmicutes* are effective in the fermentation of indigestible polysaccharides ingested with food. It has been reported that a 20% increase in *Firmicutes* and a decrease in *Bacteroidetes* at the same rate provides 150 kcal/day more energy [33]. Although it is not fully understood whether the changes in microbiota composition are a consequence or cause of obesity, the increase in *Firmicutes* and *Bacteroidetes* with a decrease in F/B ratio with body weight loss in our study is consistent with the literature.

In our study, the *Clostridia* class belonging to the phylum *Firmicutes* and *Clostridia*les, a clade of this class, decreased after fasting. While some bacterial species belonging to the *Clostridia* class produce SCFAs from the fermentation of indigestible carbohydrates, some species may be pathogenic [34]. However, in our study, no significant change was observed in bacteria belonging to this class at the genus or species level. In contrast to the study of Mohr et al. [35], a decrease in the *Ruminococcaceae* family was observed in our study after Ramadan fasting. The *Ruminococcaceae* family generally promotes intestinal health by producing butyrate and SCFAs [35]. The class *Bacteroidia* belonging to the phylum *Bacteroidetes*, the order *Bacteroidales* belonging to this class and the genus *Prevotella*, a sub-member of this order, have increased. Bacteria belonging to the phylum *Bacteroidetes* are bacteria responsible for the digestion of carbohydrates and some subspecies are known to be pathogenic [36]. However, our findings did not show a significant change in the genera belonging to the phylum *Bacteroidetes* except *Prevotella*. An increase was observed in *Erysipelotrichaceae* family belonging to *Firmicutes* phylum, *Alphaproteobacteria* class belonging to *Proteobacteria* phylum and *Actinomycetales* order belonging to *Actinobacteria* phylum after fasting. Although there are not enough human studies on the clinical importance of these bacteria, we have shown that there is an increase in starvation such as fasting.

Similar to the study by Ferrocino et al. [29], the fact that there was no significant relationship between changes in microbiota and anthropometric measurements, nutrient intake and blood parameters in our study suggesting that changes in microbiota are related to meal timing.

CONCLUSIONS

This study is significant as the first human study investigating the effect of intermittent fasting on FABP4, highlighting the need for further research in this area. However, the limited number of studies investigating the

effects of intermittent fasting on the microbiota have produced variable results due to the limited number of samples, the lifestyle and dietary habits of participants, and methodological differences between studies. There is a need for large-scale randomized controlled studies that investigate the benefit/loss effects of intermittent fasting practices on intestinal microbiota composition according to health and disease conditions and evaluate the long-term efficacy of these practices.

Conflicts of interest: None to declare.

Authors' contribution: H.S., A.S.K., T.K. and K.M. designed the study. H.S. collected the data. H.S., A.S.K., T.K and K.M. provided the statistical analysis, drafted the manuscript and revised it. T.K. revised it critically for the scientific content. All the authors read and proved the final version.

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