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Insulin-Like Growth Factor-I Axis Changes in Fasting Among Healthy and Prediabetic Men: A Case-Control Study

ABSTRACT

Objective: Energy deprivation induces changes in various components of the "growth hormone"/"insulin-like growth factor-I" axis; however, it remains unclear whether Ramadan fasting could alter which ones in response to food deprivation. In this study, changes in the components of the mentioned axis during the fasting month of Ramadan are assessed.

Methods: Sixty-nine healthy men were studied while fasting during Ramadan. Participants are divided into 2 groups of control (without risk of diabetes mellitus [n = 30]) and cases (with risk factors of DM [n = 39]). Serum growth hormone, insulin-like growth factor-I, and "insulin-like growth factor-binding protein-3" were assessed using the enzyme-linked immunosorbent assay (ELISA) kit. The "homeostasis model assessment to quantify insulin resistance" was also calculated.

Results: Fasting increased circulating growth hormone, while decreased serum insulin and homeostasis model assessment to quantify insulin resistance index levels in both groups significantly. Plasma insulin-like growth factor-I and homeostasis model assessment to quantify insulin resistance index were found to be significantly higher in the cases group than in the controls at baseline. Fasting induced a more prominent reduction in serum insulin-like growth factor-I in the cases group than in the controls ($P = .014$ vs. $P = .257$). Although serum insulin-like growth factor-binding protein-3 level was not different between groups at baseline or 1 month after Ramadan fasting, the insulin-like growth factor-I:insulin-like growth factor-binding protein-3 ratio was decreased more in the cases group than the control group during fasting ($P < .001$ vs. $P = .070$).

Conclusion: Fasting plays an effective role in regulating the studied elements of the growth hormone/insulin-like growth factor-I axis which may help improve the metabolic status induced by insulin resistance in men at risk of diabetes mellitus.

Keywords: Fasting, growth hormone, insulin, insulin-like growth factor-I (IGF-I), insulin-like growth factor-binding protein-3 (IGFBP-3), Ramadan

Introduction

Many people practice fasting regardless of religion. Abstinence from eating and drinking starting at dawn and until sunset every day during the month of Ramadan which can occur in any season is the religious duty of all healthy adult Muslims. To tolerate the effects of dehydration and hunger, people intentionally have a break-of-fasting meal, consisting of fluids and significant amounts of food, just after the sun has set, at the end of the fasting period. Besides, another rich meal is consumed either at a late dinner before sleep or just before dawn, which marks the beginning of the new day of fasting. Pieces of evidence have shown determinant metabolic effects as a result of fasting on the physiological and pathological states of individuals, including the growth hormone (GH), serum insulin concentrations, and insulin-like growth factor-I (IGF-I) axis.[1] Moreover, IGF-I along with IGF-binding proteins, more specifically insulin-like growth factor-binding protein-3 (IGFBP-3), exerts a critical role in the regulation of human metabolism. The liver is where serum IGF-I is predominantly derived from. The IGF-I bound to the IGFBP-3 accounts for 90%-95% of the serum IGF-I.[2] Insulin-like growth factor-I production in the liver is under the influence of many factors, such as GH and portal insulin concentration.[3] Typically, a decrease in serum IGF-I levels is observed under conditions such as undernourishment, liver failure, hypothyroidism, or poorly controlled type 1 diabetes mellitus (DM), while in conditions such as acromegaly, or pregnancy, serum IGF-I levels are usually increased.[4] In the case of obesity, hyperinsulinemia leads to an increase in the number of GH receptors, and thus, hepatic tissue sensitivity toward GH is

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increased. This means smaller amounts of GH are needed to stimulate IGF-I release. Besides, elevated levels of serum insulin can also affect the levels of free IGF-I through inhibition of some of the IGF-I-binding proteins.[5] Fasting can cause GH resistance in the liver, contrary to elevated GH secretion, which leads to serum IGF-I reduction as an endocrine adaptation.[6] Diets with high glycemic loads also lead to chronic hyperinsulinemia which may contribute as a risk factor for type 2 diabetes.[7] Furthermore, mitogenic changes have been observed as a direct effect of IGF-I on endothelial cells' proliferation and angiogenesis associated with the cumulative incidence of severe retinopathy This may influence the progression to proliferative retinopathy, but non-proliferative retinopathy may not be attributed to IGF-I.[8] Alteration in daily eating patterns and meal frequency in Ramadan fasting may affect many metabolic and physiologic aspects of the human body, most importantly in GH/IGF-I axis.[9]

We considered the possibility of a regulatory interaction between fasting and circulating concentrations of serum IGF-I and IGFBP-3 in individuals with or without known risk factors of type 2 DM (T2DM). To explore this hypothesis, we evaluated the effects of a 30-day period of fasting on serum concentrations of fasting glucose, postprandial glucose, insulin, homeostasis model assessment insulin resistance (HOMA-IR), GH, IGF-I, and IGFBP-3.

Materials and Methods

Study Design

This investigation was designed as a case-control study and conducted based on the Declaration of Helsinki. The ethical committee at the Mazandaran University of Medical Sciences approved this study. Written informed consent was also obtained from all participants.

Participants

Healthy men practicing complete fasting during the entire month of Ramadan without any dietary restrictions were included in the study. Females were not enrolled because their requirements during the menstrual cycle would prohibit them from fasting for some days during the month of Ramadan.

MAIN POINTS

- This study was conducted to assess the changes in serum insulin, homeostasis model assessment insulin resistance (HOMA-IR) index, growth hormone (GH), insulin-like growth factor-I (IGF-I), and insulin-like growth factor-binding protein-3 (IGFBP-3) levels during the common practice of fasting in the month of Ramadan in healthy men and men at risk of diabetes.
- The results determined a decrease in body mass index scores in both groups along with changes in serum cholesterol, high-density lipoprotein-C, and low-density lipoprotein-C levels.
- Furthermore, the results showed an increase in GH levels along with a decrease in serum insulin levels, HOMA-IR index, and serum IGF-I levels, over the period and until the end of the fasting month, Ramadan.
- This study demonstrates the potential of fasting in regulation of serum hormones, insulin availability, and IR index as a means of better prevention against type 2 diabetes and the promotion of healthcare in society.

Individuals with underlying medical conditions that might influence metabolic response to fasting (e.g., diabetes, kidney diseases, thyroid diseases, or cardiovascular diseases) were excluded along with the individuals using hormones, medications, or tobacco. Participants' unwillingness to continue with the study was also considered an exclusion criterion.

The enrollment process started at the beginning of the month of Ramadan in May 2019 and ended after a month of fasting in June 2019. Participants from the general population referring to the general clinic at Bu-Ali Sina hospital, Sari, Iran were enrolled in the study.

Study Groups

Participants were allocated into 2 groups based on the presence or absence of risk factors of T2DM. Participants were all Muslim adults of northern Iranian origin. The length of the fasting was 15-16 hours per day and the temperature averaged between 18°C and 30°C. Participants in the case group consisted of healthy men who have had all 3 risk factors of T2DM including being overweight (body mass index [BMI]>25), age ≥30 years, and a sedentary lifestyle.

Any waking behavior with an energy uptake of less than 1.5 metabolic equivalents (METs) while in a sitting, reclining, or lying posture is considered sedentary behavior. Thus, physical activity of shorter than 20 minutes per day (with an equivalent of less than 150 minutes per week) with frequencies of less than 3 times a week was considered as a sedentary lifestyle in this study.[10] A calibrated electronic scale and a stadiometer (manufactured by Seca Germany) were used to measure the heights and weights of participants and data were used to calculate the BMI, the ratio of weight (in kilograms) to height (in square meters) [weight (kg)]/[height (m^2)] to the nearest decimal place.

Data Collection

A self-designed questionnaire was used to collect the baseline demographic data of the participants (i.e., age, previous medical history, habitual, diet, and physical activity). Serum biochemical and endocrine parameters were assayed in the biochemistry laboratory at the Bu-Ali Sina University Hospital, Sari, Iran.

Trained staff conducted interviews and measured the subjects' data on 4 separate occasions to obtain venous blood samples from the participants exactly on the 12th hour of fasting in the following days: 3 days before Ramadan (Bef-R), the 15th day of Ramadan (Mid-R), the 29th day of Ramadan (End-R), and 21 days after Ramadan (Post-R). On each occasion, subjects provided fasting blood samples and underwent anthropometric measurements. Venous blood samples were collected into 10 mL ethylene diamine tetra-acetic acid (EDTA) K2-Gel tubes, which were immediately centrifuged at 20°C. The obtained plasma was stored at −80°C for further analysis. In each session, approximately 7 mL of the subjects' venous blood samples were collected from the antecubital vein and stored in a plain vacutainer tube. An aliquot of subjects' blood was used in a mixture with the anticoagulant EDTA to evaluate serum hemoglobin, hematocrit, and platelets count using an automated analyzer (Beckman Coulter, UK). The remainder of the blood was allowed to clot and then was centrifuged at 1500 g for 10 min at 4°C. A sample of the serum after the centrifugation was used to measure the blood glucose level, while the remainder was stored at −20°C until subsequent analysis. The concentrations of biochemical parameters were measured by an automated analyzer (Beckman Coulter Cx9, UK). An enzymatic colorimetric method (Biomérieux, France) was used to assess blood

glucose, triglycerides, and total cholesterol. Glycerine phosphate oxidase peroxidase (GPO-PAP) and cholesterol oxidase-peroxidase aminophenazone (CHOD-PAP) methods were used to measure blood triglyceride (TG) and cholesterol levels, respectively (Par-sazmun kit, Karaj, Iran). Human kits (Human Gesellschaft fur Biochemica und Diagnostica GmbH, Max-Planck-Ring 21—D-65205 Wiesbaden, Germany) using the direct method were used to evaluate the high-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels. GOD-PAP method (Parsazmun kit, Karaj, Iran) was used to determine fasting glucose level by glucose oxidase. Serum total GH values were determined using the ELISA technique (Kavoshyar™, Iran). The inter-assay coefficient of variation (CV) for GH was determined at 4%, with an intra-assay CV of 6.1%. Furthermore, the sensitivity of the report was 0.2 ng/mL, with a detection limit of 1.25 ng/mL.

Insulin-like growth factor-I, IGFBP-3, and insulin levels were assessed using the ELISA technique. All laboratory studies including GH, insulin, IGF-I, and IGFBP-3 were performed at the Medical School Research Center's immunoassay Toba laboratory and were quantified by a 2-site assay technique using a Tosoh 2000 auto-analyzer. The intra-assay CV was 4.4%. The midpoint between 0 and 2.0 μU/mL (1.0 μU/mL) was reported for insulin concentration levels below the detectable range of 2.0 μU/mL. The homeostasis model assessment of insulin resistance (HOMA-IR), as originally described by Matthews et al[11] was used to estimate the insulin resistance and was calculated using the following equation: [fasting insulin concentration (μU/mL) × fasting glucose concentration (mmol/L)]/22.5.[12]

Data Analysis

Statistical Package for Social Sciences Program version 18.0 (SPSS Inc.; Chicago, IL, USA) was used in data analysis. A general linear mixed model following the Bonferroni comparisons post hoc analysis of variance (ANOVA) test was used to compare the variables between groups collected at the 4 studied checkpoints, as well as the within-group differences at the end of the fasting period. Quantitative data are expressed as mean ± standard deviation for all parameters. Statistical significance was assumed when the *P*-value was calculated to be smaller than.05.

Results

Eighty participants took part in this study, from which 11 participants were lost due to inconsistency with the follow-up protocol, resulting in 69 males who voluntarily and entirely completed the follow-up procedure. The mean age of the participants was 42.94 ± 8.93 years ranging from 20 to 57 years. The fasting group with predisposing factors of T2DM (n = 39) and the control fasting group (n = 30) were well-matched. The clinical and biochemical characteristics of the 2 studied groups are demonstrated in Table 1. As reported, no significant differences regarding BMI, total cholesterol, triglyceride, and lipoproteins were observed in either of the studied groups, between men with or without risk factors of T2DM.

Table 1. Baseline Characteristics of 69 Study Participants

Parameter	Cases (n = 39)	Controls (n = 30)	*P*
Age (years)	43.3 ± 9.23	42.53 ± 8.66	.741
BMI (kg/m^2)	28.61 ± 3.67	27.57 ± 2.96	.212
Sedentary lifestyle	39 (100)	9 (30)	**<.001**
Glucose (mg/dL)	97.10 ± 6.93	83.27 ± 6.51	**<.001**
TC (mg/dL)	189.46 ± 21.62	191.80 ± 19.97	.647
TG (mg/dL)	142.05 ± 73.52	150.50 ± 76.93	.644
HDL-C (mg/dL)	53.69 ± 7.19	53.40 ± 6.19	.860
LDL-C (mg/dL)	108.28 ± 21.52	109.97 ± 20.75	.744

BMI, body mass index; HDL-C, high-density lipoprotein-C; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglyceride.
P-values are for comparison between 2 groups. Data are demonstrated in mean ± SD or *N* (percentage).

Analysis showed a significant decrease in the subjects' BMIs ($P < .001$) at the end of Ramadan in both groups. Total cholesterol (TC), HDL-C, and LDL-C concentrations underwent significant changes over the month of Ramadan; however, serum TG levels did not change significantly, as demonstrated in Table 2.

As shown in Table 3, serum GH levels increased after the initiation of fasting and peaked at significantly different levels compared to their baseline values at the end of Ramadan. Afterward, GH levels decreased to lower values, despite remaining significantly more than the baseline levels at the last checkpoint of measurement ($P < .001$ and $P = .014$, respectively) in both groups. Between-group analysis showed the difference in GH levels was more prominent at the middle and end of Ramadan between cases and control ($P = .002$ for both).

Analysis using ANOVA showed that the insulin level declined with a moderate slope in both groups after fasting and stayed at slightly lower levels than the baseline during all time points. Between-group analysis of insulin concentrations showed a statistically significant difference at the end and after Ramadan in subjects with risk factors of T2DM compared to the control group ($P<.001$).

The HOMA-IR index had considerably reduced in both groups over the period of the study ($P < .001$ and $P = .048$, respectively). The differences between the 2 subject groups were significant at all

Table 2. Comparison of Clinical and Biochemical Parameters Before and After Ramadan Between 2 Groups

	Cases (n = 39)			Controls (n = 30)		
Parameter	Pre-Ramadan	Post-Ramadan	*P*	Pre-Ramadan	Post-Ramadan	*P*
BMI (kg/m^2)	28.61 ± 3.67	27.82 ± 3.69	**<.001**	27.57 ± 2.96	26.80 ± 3.05	**<.001**
Glucose (mg/dL)	97.10 ± 6.93	90.79 ± 8.63	**.020**	83.27 ± 6.51	88.57 ± 8.60	**<.001**
TC (mg/dL)	189.46 ± 21.62	185.59 ± 19.73	**.038**	191.80 ± 19.97	185.47 ± 18.13	**.049**
TG (mg/dL)	142.05 ± 73.52	138.51 ± 40.52	.733	150.50 ± 76.93	149.10 ± 45.85	.914
HDL-C (mg/dL)	53.69 ± 7.19	56.23 ± 6.86	**.026**	53.40 ± 6.19	56.53 ± 5.38	**.033**
LDL-C (mg/dL)	108.28 ± 21.52	102.13 ± 19.6	**.014**	109.97 ± 20.75	102.83 ± 21.82	**.029**

BMI, body mass index; HDL-C, high-density lipoprotein-C; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglyceride.
P-values are for comparison among time points before and after Ramadan between 2 groups. Data are demonstrated in mean ± SD.

Table 3. Effect of Ramadan Fasting on GH, IGF-I, and IGF-Binding Protein Level

Parameter	Cases (n = 39)	Controls (n = 30)	*P*
GH (ng/mL)			
Before Ramadan	0.41 ± 0.75	0.32 ± 0.43	.341
Middle of Ramadan	0.95 ± 0.96	0.39 ± 0.52	**.002**
End of Ramadan	1.46 ± 1.16	0.75 ± 0.62	**.002**
After Ramadan	0.92 ± 0.85	0.68 ± 0.58	.111
P	**<.001**	**.014**	
Insulin (μU/mL)			
Before Ramadan	11.52 ± 2.74	11.17 ± 2.06	.639
Middle of Ramadan	10.28 ± 2.66	10.44 ± 3.07	.732
End of Ramadan	6.55 ± 1.96	8.74 ± 3.02	**<.001**
After Ramadan	7.19 ± 2.30	9.80 ± 2.92	**<.001**
P	**<.001**	**<.001**	
HOMA-IR (units)			
Before Ramadan	2.73 ± 0.56	2.28 ± 0.35	**<.001**
Middle of Ramadan	2.19 ± 0.58	2.16 ± 0.59	.940
End of Ramadan	1.40 ± 0.39	1.86 ± 0.64	**<.001**
After Ramadan	1.60 ± 0.48	2.12 ± 0.56	**<.001**
P	**<.001**	**.048**	
IGF-I (ng/mL)			
Before Ramadan	128.89 ± 32.47	105.57 ± 35.86	**.006**
Middle of Ramadan	109.90 ± 25.20	108.60 ± 58.19	.793
End of Ramadan	76.64 ± 20.57	84.14 ± 34.44	.313
After Ramadan	97.86 ± 31.13	97.79 ± 36.64	.923
P	**<.001**	.051	
IGFBP-3 (ng/mL)			
Before Ramadan	3342.28 ± 414.24	3293.04 ± 399.79	.539
Middle of Ramadan	3333.73 ± 418.11	3282.58 ± 407.20	.508
End of Ramadan	3193.51 ± 628.58	3231.59 ± 421.63	.838
After Ramadan	3354.03 ± 425.14	3289.91 ± 402.54	.434
P	.657	.371	
IGF-I/IGFBP-3			
Before Ramadan	0.039 ± 0.011	0.032 ± 0.012	**.016**
Middle of Ramadan	0.034 ± 0.009	0.033 ± 0.018	.865
End of Ramadan	0.028 ± 0.029	0.026 ± 0.011	.633
After Ramadan	0.029 ± 0.010	0.029 ± 0.011	.996
P	**<.001**	.070	

GH, growth hormone; HOMA-IR, homeostasis model of assessment of insulin resistance; IGF-I, insulin-like growth factor-I; IGFBP-3, IGF-binding protein-3.
Data are demonstrated in mean ± SD. $P < .05$ is significant.

time points except in the middle of Ramadan ($P < .001$ vs. $P = .940$, respectively).

In the cases group, the serum concentration of IGF-I started to decrease until the end of the month of Ramadan, and then increased to a higher level, but did not return to its primary measures over the last studied time point. In the control group, the IGF-I level raised initially in a non-significant manner after fasting in the middle of Ramadan and then decreased to smaller values than the basic values and remained at its lower levels over the time. Between-group analysis of IGF-I concentrations revealed a statistically significant difference at basal levels ($P = .006$). The final measurements of IGF-I levels were lower than the baseline measures in both groups.

Participants in the cases group had higher levels of IGFBP-3 than the control group in a non-significant manner. After the start of fasting, IGFBP-3 levels decreased in both studied groups, which continued to decrease until the end of the fasting month of Ramadan. Subsequently, a sudden rise was observed in IGFBP-3 levels until the last measurement. None of these changes were statistically significant. No statistically significant differences were observed between studied groups in IGFBP-3 levels at any recorded checkpoint.

One-month fasting resulted in decreased IGF-I ($P < .001$ and $P = .051$, respectively, Table 3) without significant change in IGFBP-3 levels in both groups. Because of these changes, the ratio of IGF-I:IGFBP-3 also decreased over time in these groups until the last measurement. However, the between-groups comparison of the mentioned ratio was not concluded as statistically significant.

Discussion

It was revealed in the current study that 1-month fasting during Ramadan is associated with a significant increase in serum GH level among men particularly among those with risk factors of T2DM. It has been reported that an increase in serum GH adapts the GH/IGF-I axis with the new endocrine balance.[13] Various studies have reported similar results to those of this study. It has been suggested that exercise induced a more predominant GH response in individuals with predisposing factors of DM than in healthy subjects.[14] The significant difference in GH levels compared to baseline can be explained by the greater GH responses in these individuals compared to the control group.

Another noteworthy finding reported in this study was a decrease in serum insulin levels following fasting, which has also been observed in other reports.[15-17] Furthermore, decreased serum insulin and IGF-I levels during and after fasting promote an increase in glucose transport sensitivity.[18] The mentioned elevation of sensitivity toward insulin along with the decrease in insulin levels keeps at a balance during the period of fasting. This balance will perhaps prevent the fasting-induced hypoglycemia. As a consequence, a reduction in insulin levels is needed to be able to properly keep the glucose homeostasis.[6]

Our results revealed an elevated HOMA-IR index in the basal level in cases with predisposing factors to T2DM group as compared to the control group. Fasting caused the HOMA-IR index to considerably decline and return to the optimal level in both groups. Consistent with the results of our study, some recent studies have suggested that the caloric restriction on Taiwanese obese subjects has led to significant attenuation of insulin level and HOMA-IR index.[19] Mitra et al showed that a regimen with 6 months of energy-restricted diet in Malaysian adults had a bigger reduction in IR indexes and fasting insulin as compared with the control group.[20] Hołowko et al[21] found a significant decrease in homeostasis model assessment of IR in subjects with 6 weeks of calorie restriction in both obese and overweight athletes. Unalacak et al[22] also indicated a significant decline in the HOMA-IR index in subjects with Ramadan fasting in both healthy and obese individuals, whereas other investigators reported the HOMA-IR index to remain unchanged before and after Ramadan.[23] Nachvak

et al[24] showed that HOMA-IR elevated significantly at the end of the month of Ramadan in healthy men. The possible explanations for the discrepant results may relate to the non-comparative design of studies, case selection, and time of tests after the period of fasting. In the current study, HOMA-IR was used as a marker of IR,[25] besides, our results are also supported by most of the studies assessing the changes in the metabolic substrate due to energy deprivation.[26]

With regard to IGF-1 levels, significantly different levels were observed following the food deprivation caused by fasting in both cases and control groups compared to their baseline levels. However, results from previous studies are controversial. Some investigators reported that the IGF-I level in fasting was not changed.[6] Aksungar et al[27] reported a gradual increase in IGF-I levels during the calorie restriction period. However, on the seventh day of the intermittent fasting period among obese subjects, a constant decrease was observed until the end of the 12 months of follow-up. In this study, however, the possibility of a decrease in IGF-I levels after Ramadan fasting can be assessed due to the continued 1-month duration of fasting in the study protocol. Furthermore, it has been explained in other studies that fasting affects the circulating IGF-I levels by diminution of the free IGF-I levels.[23] This could explain the significant decrease in serum concentrations of IGF-I after Ramadan fasting in the current study.

The levels of IGFBP-3 demonstrated a decrease over time in this study. However, by the end of the study, statistically insignificant different levels were reported compared to their baseline values. The rather long half-life of this carrier protein, the most crucial regulator for the availability of IGF-I,[28] may account for the rather delayed detection of significant changes in IGFBP-3 levels, more toward the final studied time points in this study. It has also been theorized that changes in the levels of IGFBPs in energy deprivation occur mostly due to the decrease in total IGFBP-3; however, inconclusive reports have been observed so far.[29]

Serum IGF-I and IGFBP-3 levels reflect the mean daily levels of endogenous serum GH. Besides, their ability to be reproduced on regular tests creates an ideal opportunity to assess the GH/IGF-I axis by examining their changes.[30]

With regard to the molar ratio of IGF-I and IGF-binding protein 3, a decrease was observed in fasting in cases with predisposing factors toward T2DM, but not significantly different during fasting in the control group. The complex relationship between fasting and IGF-I concentrations may affect this significant decrease.[28]

High IGFBP-3 coupled with elevated IGF-I may act as a risk factor for IR in the development of T2DM.[31] Findings in this study provide evidence to support the change/adaptation in the studied components of the GH/IGF-I axis with the goal of offering partial prevention against the course T2DM through fasting.

Some limitations of our study can be acknowledged. The most important limitation of this study was our inability to assess the impact of dietary patterns and the sleep patterns of fasted individuals on the components of the GH/IGF-I axis. Another limitation of our study was the small population of male healthy participants which may diminish the generalizability of the results on the whole population. Nevertheless, one significant strength of our study was the measurement of the variables like BMI and physical activity, which contribute to changes in some of the GH/IGF-I axis components, as well.

Conclusion

In conclusion, the calorie restriction resulting in fasting leads to regulatory responses in components of the IGF-I system, which can be studied as changes in serum concentrations. However, this response can vary, depending on the metabolic status among different individuals. In addition, the changes in the HOMA-IR index and IGF-I level after fasting were significantly different between the healthy group and the cases, as a more significant decrease was observed among the prediabetic participants. It can be suggested according to these findings that Ramadan fasting may influence the GH/IGF-I axis, leading to a reduction of the burden of disease in the predisposed individuals toward DM in comparison with healthy peers.

Ethics Committee Approval: The study has been approved by the ethics committee of the Mazandaran University of Medical Sciences.

Informed Consent: A written informed consent was signed by all participants and was obtained before the start of the enrollment process.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - A.A.A., S.A., M.R.S.R., P.I.P.; Design - A.A.A., S.A., M.R.S.R., P.I.P.; Acquisition of data - A.A.A., S.A., F.D.; Analysis and interpretation of data - A.A.A., P.I.P.; Drafting - A.A.A., M.R.S.R., S.A.; Revising - A.A.A., F.D.

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