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Ramadan Fasting And Metabolic Changes Exploring The Impact On Insulin, Ghrelin, Leptin, Lipid Profile, Glucose Levels, SOD And LPO

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ABSTRACT

Background and Objectives: Ramadan fasting, a practice observed by millions worldwide, may induce metabolic changes. The current study aimed to investigate the effects of Ramadan fasting on insulin, ghrelin, leptin, lipid profile, glucose levels, superoxide dismutase (SOD) and lipid peroxidation (LPO) in Erbil, Iraq.

Methods: This observational survey study was conducted from March 12th to April 8th, 2024, involving 30 male lecturers aged 30-40 years. Blood samples were collected on the 2nd and 28th days of Ramadan. Serum lipid profiles, glucose, SOD, LPO, and hormonal levels (insulin, ghrelin, leptin) were measured using the COBAS INTEGRA 400 plus system and ELISA kits.

Results: The mean ± SD age of participants was 39.352 ± 1.347 years. There were no significant changes in weight and BMI during Ramadan. However, significant decreases were observed in insulin and leptin levels and ghrelin was significantly increased ($P \leq 0.05$). Fasting also resulted in significant changes in antioxidant enzyme activity and LPO ($P \leq 0.05$). All lipid parameters were affected by fasting. Triglycerides, cholesterol, LDL-C, VLDL-C, CH/HDL-C ratio, TG/HDL-C ratio, LDL-C/HDL-C ratio, TyG index, and HOMA-IR showed significant reductions, while HDL-C increased ($P \leq 0.05$).

Conclusion: Lifestyle changes, dietary habits, and physical activity during Ramadan had important and beneficial effects on fasting individuals. Fasting in Ramadan was associated with reductions in insulin and leptin levels and increasing ghrelin. It also led to changed antioxidant enzyme activity, LPO, and harmful lipids, alongside an increase in HDL-C.

Keywords: Glucose metabolism; Insulin resistance; Lipid profile; Ramadan fasting.

1. Introduction

Ramadan is the most sacred month in the Islamic calendar, characterized by a lunar duration of 29 or 30 days. Muslims observe a fasting period throughout this month by abstaining from consuming any food, water, drinks, oral medication, and smoking from dawn till dusk (1). The duration of fasting might diverge based on the season and geographic location of the nation. Hence, the duration of daytime fasting might range from about 11 to 18 hours, peaking during the summer season (2). Typically, food and liquids are taken in two meals during Ramadan: one in the morning, just before dawn, and one in the evening, just after sunset. This change in energy intake patterns places the meals within the hours of darkness (3). Modifications in the time of meal consumption and the nature of the diet may manipulate energy metabolism and potentially impact crucial enzymatic, hormonal, and metabolic reactions, as well as several

facets of human health (1).

During the fasting period, the body enters a state of ketosis, where it begins to utilize stored fat as a source of energy, leading to a reduction in blood glucose levels (4). This metabolic shift can result in weight loss, improved insulin sensitivity, and changes in lipid profiles (5). A study by Prasetya and Sapwarobol (2021), showed that fasting is associated with improved insulin sensitivity (6). In addition to insulin, other hormones such as ghrelin and leptin are also affected by fasting (7).

Ghrelin, often referred to as the "hunger hormone," stimulates appetite (8), while leptin is involved in regulating energy balance and inhibiting hunger (9). Research indicates that fasting during Ramadan can alter the levels of these hormones, potentially leading to changes in appetite and energy expenditure (7). Furthermore, Ramadan fasting has been associated with alterations in lipid profiles. Studies have reported variations in serum lipid levels, including triglycerides (TGs) and cholesterol, during Ramadan (10).

Oxidative stress markers like superoxide dismutase (SOD) and lipid peroxidation (LPO) are crucial indicators of cellular health. SOD is an antioxidant enzyme that protects cells from damage caused by free radicals (11), while LPO is a marker of oxidative stress (12). The balance between these two markers can provide insights into the oxidative state of the body during fasting. A study by Grundler et al. (2020), showed that fasting boosts antioxidant defenses, potentially leading to reduced oxidative stress and improved overall health (13).

Despite the growing body of literature on the metabolic impacts of Ramadan fasting, there remains a significant gap in understanding the comprehensive effects on various metabolic parameters, particularly in diverse populations. Most studies have focused on specific aspects of metabolism, often neglecting the interconnectedness of hormonal changes, lipid profiles, glucose levels, and oxidative stress markers. Therefore, the current study aimed to investigate the effect of Ramadan fasting on insulin, ghrelin, leptin, lipid profile, glucose, SOD, and LPO levels in Erbil, Iraq.

2. Methods and Materials

2.1 Study Design and Setting

This observational survey design study was conducted from March 12th to April 8th, 2024, the research took place at the College of Education, Salahaddin University-Erbil, located in Erbil, Iraq.

Part2.2 icipants

Thirty male university lecturers aged 30-40 years participated in the study. Participants were selected based on specific inclusion criteria and were recruited using a convenience sampling method. Inclusion criteria required participants to be healthy males, with no chronic metabolic diseases and not taking any medications, and have written informed consent. Exclusion criteria included the presence of chronic illness, use of medication affecting metabolic parameters, or inability to comply with fasting requirements.

2.3 Data Collection

Blood samples were collected twice during the study: on the 2nd day and the 28th day of Ramadan. Participants were instructed to fast overnight, and blood collection was performed in the morning. Five milliliters of fasting blood was drawn from each subject using a sterile syringe and collected into a plain gel tube. The blood was allowed to coagulate at room temperature in order to guarantee the integrity of the samples. Within 30 minutes of sample collection, the samples were centrifuged for 15 minutes at 3,000 rpm. After centrifugation, the serum was carefully preserved at -20 °C awaiting further examination.

The serum lipid profile, including total cholesterol, TG, and high-density lipoprotein cholesterol (HDL-C), was measured using the COBAS INTEGRA 400 plus system analyzer, serviced by Roche Diagnostics (Deutschland). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation: LDL-C = TC − (HDL-C + total TG/5) mg/100 ml. Fasting blood sugar (FBS) levels were estimated using the glucose-oxidase colorimetric method with a kit supplied by Biolabo (France).

The triglyceride-glucose (TyG) index, a composite indicator of insulin resistance, was calculated for each participant. The index was determined using the formula: TyG index = Ln (Fasting TG (mg/dl) × FBS (mg/dl))/2 (14).

The levels of SOD activity and LPO were assessed using commercially available enzyme-linked immunosorbent assay (ELISA) kits from Sunlong Biotech CO., LTD, China. The assays were conducted following the manufacturer's protocols to ensure precision and reliability in measuring oxidative stress markers.

Hormonal levels, including serum fasting insulin (mU/L), total serum ghrelin (pg/mL), and serum leptin (pg/mL), were measured using ELISA kits from Sunlong Biotech CO., LTD, China. The tests were conducted following the manufacturer's guidelines to precisely assess the hormonal alterations linked to Ramadan fasting. Additionally, insulin resistance was assessed using the homeostasis model assessment-estimated IR (HOMA-IR). This was calculated using the formula: HOMA-IR = serum glucose (mg/dL) × plasma insulin (μU/mL)/405 (15).

2.4 Ethical Considerations

The research was performed in compliance with the Declaration of Helsinki and obtained ethical clearance from the Salahaddin University-Erbil Ethics Committee. Participants furnished signed informed permission after a briefing on the study's objectives, methodologies, and possible hazards. Confidentiality and anonymity were maintained throughout the study procedure.

2.5 Statistical Analysis

Data analysis was performed using SPSS software, version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were presented as mean ± standard error (SE). The Shapiro-Wilk test assessed data normality. A Student's t-test compared parameters between the two-time points (the 1st and 28th day of Ramadan). A p-value below 0.05 was deemed statistically significant.

3.Results

The present study examined 30 fasting men aged 35-45 years, with a mean ± SD age of 39.352 ± 1.347 years, to assess metabolic changes during Ramadan. The mean ± SE weight of these individuals was 81.891 ± 2.335 kg on the first day and 81.379 ± 2.482 kg on the 28th day, with no significant weight loss. The mean ± SE BMI was 27.635 ± 0.765 kg/m² on the first day and 27.441 ± 0.753 kg/m² on the 28th day, with no statistically significant change (Table 1).

Table 1: Demographic characteristics

Parameters	1st day	28th day	Mean Difference	P-value*
Body Weight (Kg)	81.891 ± 2.335	81.379 ± 2.482	-0.511	0.3395 ns
BMI	27.635 ± 0.765	27.441 ± 0.753	-0.1941	0.2492 ns

** P-value; t-test*

Table 2 illustrates the hormonal changes due to fasting. Significant decreases were observed in insulin and leptin levels and increases ghrelin level. The mean ± SE insulin level was 8.351 ± 0.457 mU/L on the first day and 6.711 ± 0.346 mU/L on the 28th day, with the reduction being statistically significant (P ≤ 0.0016). The mean ± SE leptin level was 1855.218 ± 294.799 pg/mL on the first day and 1245.692 ± 114.989 pg/mL on the 28th day, with a significant reduction (P ≤ 0.0096). The mean ± SE ghrelin level increased from 1471.269 ± 70.631 pg/mL on the first day to 1744.079 ± 74.891 pg/mL on the 28th day (P ≤ 0.0009).

Table 2: Hormonal changes during Ramadan fasting (Mean ± SE)

Parameters	1st day	28th day	Mean Difference	P-value*
Insulin (mU/L)	8.351 ± 0.457	6.711 ± 0.346	-1.640	0.0016 **
Ghrelin (pg/mL)	1471.269 ± 70.631	1744.079 ± 74.891	272.8	0.0009 ***
Leptin (pg/mL)	1855.218 ± 294.799	1245.692 ± 114.989	-609.5	0.0096 **

** P-value; t-test*

Significant changes were also observed in antioxidant enzyme activity and LPO due to fasting. The mean ± SE T-SOD (total superoxide dismutase) level was 25.822 ± 0.902 U/mL on the first day and 28.721 ± 0.664 U/mL on the 28th day, with a statistically significant increase ($P \leq 0.0026$). The mean ± SE LPO level was 39.879 ± 1.547 pg/mL on the first day and 44.927 ± 1.537 pg/mL on the 28th day, showing a significant increase ($P \leq 0.0026$) (Table 3).

Table 3: Changes in antioxidant enzyme activity and LPO during Ramadan fasting (Mean ±SE)

Parameters	1st day	28th day	Mean Difference	P-value *
T-SOD (U/mL)	25.822 ± 0.902	28.721 ± 0.664	2.900	0.0026 **
LPO (pg/ml)	39.879 ± 1.547	44.927 ± 1.537	5.047	0.0017 **

** P-value; t-test*

Table 4 presents the changes in glucose and lipid profiles. There were no significant changes in mean ± SE glucose levels; however, significant changes in the lipid profile were observed from the first to the 28th day of fasting. The mean ± SE TG level decreased from 164.706 ± 8.998 mg/dL on the first day to 131.753 ± 6.767 mg/dL on the 28th day ($P \leq 0.0001$). The mean ± SE cholesterol level decreased from 196.506 ± 4.733 mg/dL on the first day to 182.666 ± 4.962 mg/dL on the 28th day ($P \leq 0.0001$). The mean ± SE LDL-C level decreased from 155.532 ± 5.712 to 145.192 ± 6.260 mg/dL ($P \leq 0.0157$). The mean ± SE VLDL-C level decreased from 32.941 ± 1.800 to 26.350 ± 1.353 ($P \leq 0.0001$).

The mean ± SE CH/HDL-C ratio decreased from 15.110 ± 1.668 on the first day to 11.435 ± 1.112 on the 28th day ($P \leq 0.0001$). The mean ± SE TG/HDL-C ratio decreased from 13.471 ± 1.721 to 7.357 ± 0.525 ($P \leq 0.0001$). The mean ± SE LDL-C/HDL-C ratio decreased from 11.901 ± 1.378 to 9.186 ± 1.120 ($P \leq 0.0023$). The mean ± SE TyG index decreased from 4.758 ± 0.042 to 4.605 ± 0.040 ($P \leq 0.0001$). The mean ± SE HOMA-IR decreased from 1.746 ± 0.115 to 1.326 ± 0.072 ($P \leq 0.0001$). The mean ± SE HDL-C increased from 15.729 ± 1.098 mg/dL on the first day to 182.666 ± 4.962 mg/dL on the 28th day, with a statistically significant increase ($P \leq 0.0001$) (Table 4).

Table 4: Changes in serum glucose and lipid profile during Ramadan fasting (Mean ±SE)

Parameters	1st day	28th day	Mean Difference	P-value*
Glucose (mg/dL)	84.066 ± 1.511	80.064 ± 2.398	-4.002	0.0877 ns
TG (mg/dL)	164.706 ± 8.998	131.753 ± 6.767	-32.95	<0.0001****
Cholesterol (mg/dL)	196.506 ± 4.733	182.666 ± 4.962	-13.84	<0.0001****
HDL-C (mg/dL)	15.729 ± 1.098	18.555 ± 0.878	2.826	0.0007 ***
LDL-C	155.532 ± 5.712	145.192 ± 6.260	-10.34	0.0157 *
VLDL-C	32.941 ± 1.800	26.350 ± 1.353	-6.591	<0.0001****
CH/HDL-C Ratio	15.110 ± 1.668	11.435 ± 1.112	-3.674	<0.0001****
TG/HDL-C Ratio	13.471 ± 1.721	7.357 ± 0.525	-6.114	<0.0001****
LDL-C/HDL-C Ratio	11.901 ± 1.378	9.186 ± 1.120	-2.715	0.0023 **
TyG index	4.758 ± 0.042	4.605 ± 0.040	-0.1525	<0.0001****
HOMA-IR	1.746 ± 0.115	1.326 ± 0.072	-0.4205	0.0013 **

** P-value; t-test*

4. Discussion

In the present study, the metabolic changes resulting from fasting during Ramadan were examined. The hormonal changes, antioxidant enzyme activity, LPO, and lipid profile showed significant and beneficial alterations. Hormonal variations indicated that the mean levels of insulin and leptin decreased and mean level of ghrelin increased due to fasting during Ramadan. Additionally, the activity of the antioxidant enzyme T-SOD and LPO also increased during this month, showing an increase in their mean levels. The lipid profile changes were noteworthy as well, with a decrease in all parameters except HDL-C, which increased.

During Ramadan, the type and amount of energy intake for most fasting individuals can change. Furthermore, sleep

habits and daily physical activities also undergo modifications. These changes can influence biochemical and hormonal factors in individuals (16). Changes in body weight during Ramadan have been reported differently in various studies. In the study by Khan et al. (2024), it was shown that nearly half of the participants experienced no change in weight, while 30% reported weight loss and 13% reported weight gain (17). Additionally, Fernando et al. (2019) indicated that fasting was associated with weight loss and a reduction in BMI. However, the examination of the mean weight and BMI of fasting individuals showed that fasting had little effect on reducing weight and BMI (18). In a study conducted in Iran, it was shown that fasting led to weight loss but did not significantly affect BMI (19). Another study conducted by Hasan et al. (2022) demonstrated that fasting resulted in weight and BMI reduction among fasting individuals (1). The differences in outcomes appear to be due to variations in diet, physical activity levels, and calorie intake.

Among fasting individuals, it was shown that fasting led to hormonal changes and an increase in ghrelin level and a decrease in insulin and leptin levels from the first to the 28th day of Ramadan, which provided substantial health benefits. Fasting improves insulin sensitivity, allowing body cells to respond better to insulin and utilize glucose more effectively, leading to a reduction in insulin levels (20). Furthermore, fasting reduces ghrelin production, diminishing hunger and appetite throughout the day, resulting in lower calorie consumption (21). Fasting also enhances leptin sensitivity, helping to regulate appetite and increase satiety (22), thereby improving metabolic health through hormonal optimization. Other studies have reported varying effects of fasting on insulin, ghrelin, and leptin levels. Studies by Prasetya et al. (2021) (6), and Almadhidi et al. (2021) (23), showed that fasting improved insulin sensitivity, resulting in decreased insulin levels during Ramadan, whereas a study in the United Arab Emirates reported an increase in insulin levels during Ramadan fasting (1). In studies by Harder et al. (2017), (24), and Al-Rawi et al. (2020) (7), intermittent fasting was shown to decrease ghrelin and leptin levels, while studies by Akan et al. (2023) (25), and Zouhal et al. (2020) (26), found that fasting was associated with an increase in these hormone levels.

It has been demonstrated that the activities of T-SOD and LPO enzymes, by causing DNA damage and cell death, create a predisposition for various diseases (27). According to the results, fasting during Ramadan was associated with an increase in the mean levels of T-SOD and LPO, thereby decreasing the activity of these enzymes. In a review by Adawi et al. (2017) (28), it was shown that fasting during Ramadan did not affect antioxidant enzyme activity and LPO. However, research conducted in Turkey revealed that fasting correlates with reduced antioxidant enzyme activity and LPO (29).

Given the link between elevated lipid levels and various diseases (30), the current study results indicated that all lipid parameters were affected by fasting. TGs, cholesterol, LDL-C, VLDL-C, CH/HDL-C ratio, TG/HDL-C ratio, LDL-C/HDL-C ratio, TyG index, and HOMA-IR showed significant reductions. In a study conducted by Ahmed et al. (2022), it was also shown that fasting during Ramadan was associated with a reduction in blood lipids (31). Another study in India confirmed that fasting improves the lipid profile and reduces lipid levels (10). The decrease in total caloric intake and saturated fatty acids during Ramadan, along with reduced energy intake, contributed to the decrease in blood lipids (32). Moreover, fasting was found to increase HDL-C levels among participants. Correspondingly, a study by Madkour et al. (2023) examining lipid profile changes during Ramadan found that HDL-C levels increased after the month of fasting among participants (33).

The results of this study indicated that fasting had no significant impact on mean glucose levels, although there was a reduction from the first to the 28th day of fasting, which was not statistically significant. In a study by Faris et al. (2020), fasting was shown to reduce glucose levels among fasting individuals (34), while in a study by Al-Rawie et al. (2020), glucose levels increased after Ramadan (7).

5. Conclusions

Changes in lifestyle, diet, and physical activity during Ramadan have important and beneficial effects on fasting individuals. Fasting during Ramadan increased sensitivity to insulin and leptin and increased ghrelin production.

Fasting was demonstrated to decrease antioxidant enzyme activity and LPO, contributing to the maintenance and improvement of individual health. Additionally, fasting was associated with a reduction in harmful lipids and an increase in HDL-C. Further research with adequate sample sizes and in different regions are essential to explore other health effects of fasting.

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Data availability: The corresponding author is prepared to provide the study's data upon a fair request.

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