

The Impact Of Ramadan Fasting On Adrenal Hormones And Cholesterol Among Healthy Iraqi Students

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ETHICAL DECLARATIONS

Ethics Approval and Consent to Participate

The students agreed to voluntarily participate in conducting this scientific research.

Consent for Publication

No specific personal information is included.

Availability of Data and Material

All data and materials have been published

Competing Interests

There are no disclosed conflicts of interest for the authors. The manuscript's contents have been reviewed and approved by each co-author.

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ABSTRACT

Background: The adrenal gland produces several hormones that help regulate the body's metabolism, blood pressure, and response to stress. It is expected that the shift to eating in the evening, the lack of sleep at night and lifestyle changes will have an effect on the circadian rhythm of hormones during Ramadan fasting. This

study's objective is to measure the levels of various adrenal hormones and cholesterol during and after Ramadan fasting in the blood of healthy Iraqi students. **Method:** This case-control study was conducted on forty healthy students from the Fallujah faculty of medicine. The blood was drawn on average at 1:00 p.m. Two times of blood samples were obtained; after the end of the third week of Ramadan fasting, and two weeks after the end of Ramadan for the year 1444 AH. Plasma epinephrine, aldosterone, and cortisol were measured immediately by ELISA, while cholesterol by spectrophotometer. **Results:** The blood levels of the adrenal hormones and cholesterol in both sexes, during and after Ramadan, were within the normal values. There are differences in the normal values of the studied compounds between males and females. In Ramadan, the results of adrenal hormones and cholesterol were statistically insignificant between males and females. And also after Ramadan, with the exception of epinephrine and aldosterone, which showed statistical significance between them. Cortisol results showed high statistical significance for both sexes, as well as epinephrine and aldosterone results for males between Ramadan and beyond. While the results of epinephrine, aldosterone, and cholesterol in females showed no statistical significance. **Conclusion:** Ramadan fasting does not appear to have a significant impact on level of adrenal hormones and cholesterol in the blood of healthy Iraqi students. Highly statistically significant ($P < 0.001$) results in the male adrenal hormones between Ramadan and after. Both genders display a notable degree of variability in normal values of these compounds.

Keywords: Adrenal; Cholesterol; Hormones; Ramadan; students

1. Introduction

Muslims around the world fast Ramadan, a type of intermittent fasting, during this holy month each year (the 9th months of the lunar calendar). According to the verse 183–187 of Sura Al-Baghara of the Holy Qur'an, fasting is the practice of not eating, drinking, sexual relations, or smoking between pre-sunrise and after sunset for 29 or 30 days. Depending on where in the world you live, the pre-sunrise to sunset fast lasts anywhere from 11 to 19 hours. In Iraq, approximately 15 hours were fasted during this year's Ramadan (March 23 – April 21, 2023, 1444 AH)^[1]. During this period, the body undergoes several changes, including adjustments in hormonal functioning.

Numerous prior studies on the impact of Ramadan fasting on diabetes and glucometabolic markers ^[2,3], cardiovascular disease ^[4], gastrointestinal disorders ^[5], Chronic Kidney Disease, Kidney Transplant and Dialysis Patients ^[6], and also on intakes of lipids, proteins, and sugars, body mass, percentage of body fat, visceral adiposity in people who are obese and overweight, healthy people's lipid profiles and fasting blood glucose ^[7,8]. In general, the effects of Ramadan fasting on diabetic patients, pregnant women, and body composition of diabetic patients were the frequently reported topics (between 2010 and 2021)^[9]. A review of the study on how Ramadan Fasting affects several aspects in 2022 was published by Hafidh et al. ^[10]. Few studies have been conducted on the impact of Ramadan fasting on patients with adrenal insufficiency and its management ^[11,12].

The endocrine glands known as adrenal glands are situated above the kidneys. The cortex and medulla make up the adrenal gland. The medulla generates amino acid-derived hormones such as catechol-amines (epinephrine and norepinephrine), whereas the cortex produces steroid hormones such as glucocorticoids (cortisol), mineralocorticoids (aldosterone), and androgens. These hormones are crucial for blood pressure control, salt and glucose homeostasis, metabolism and response to stress. It is fatal when these hormones are hypo- or hyper-secreted ^[13,14]. The secretion of hormones is not only related to metabolism, mood, stress, anxiety and depression, but other multiple biochemical compounds that have great importance in influencing these psychological and physiological conditions, including neurotransmitters ^[14]. Prior studies have demonstrated that Ramadan fasting has favorable effects on mood and health-related quality of life in healthy adults. In a meta-analysis of 11 research involving 1436 participants, Berthelot et al. found that post-Ramadan stress, anxiety, and depression levels were lower than pre-Ramadan scores ^[15].

The body's stress response heavily relies on the adrenal gland's capacity to adapt and regenerate in the face of harsh conditions ^[16]. Overall, fasting may activate particular stress-resistance and self-protective cellular systems to counteract any adverse consequences of elevated glucocorticoids and catechol-amines ^[14]. In healthy individuals, the studies show that intermittent fasting (one of three patterns of fasting regimens) may

improve systemic metabolic indices, the function and homeostasis of numerous organs and tissues, thereby creating a virtuous cycle and delaying aging^[17]. Our previous study showed no impact of Ramadan fasting on HOMA indexes, insulin, glucose and some minerals (Na, K, Ca, & PO₄) in the blood of healthy students^[18]. Cholesterol contributes to the maintenance of plasma membrane fluidity and serves as a precursor to all steroid compounds including steroid hormones (e.g. cortisol and aldosterone)^[19]. Aldosterone is crucial for maintaining the balance between liquid volume and electrolyte metabolism, and regulating blood pressure in the renin-angiotensin-aldosterone system^[20]. Cortisol (a crucial stress biomarker and sleep) has been shown to affect mood, body composition metrics, quality of life, and regulate blood sugar levels during fasting. The body receives immediate energy from cortisol's catabolic actions^[21,22]. It is critical to carry out research on how Ramadan fasting impacts the biochemical components of human blood. Our study's objective is to determine how Ramadan fasting affects adrenal hormones and cholesterol.

2. Methodology

This case-control study was conducted on medical students at the University of Fallujah (Fallujah City, Anbar Province-Iraq), in the period during and after the month of Ramadan, 1444 AH (March 23 – April 21, 2023). The period of fasting in Iraq is up to 15 hours every day (from pre-sunrise to sunset) during the aforementioned year.

The students agreed to voluntarily participate in conducting this scientific research, therefore the study was conducted on the ethical principles.

Blood samples were taken twice: the first time, blood samples were taken on the twenty-first day of Ramadan (after the end of the third week of Ramadan), and the second time, two weeks after the end of Ramadan. A 5 ml blood sample was taken from each student, each time at noon between the hours of 12:30 pm and 1:30 pm, approximately after nine to ten hours of fasting. Our study were conducted on 40 students (20 males and 20 females), apparently healthy, without female menstrual cycle, between the ages of 19 and 21 years. Each subject's age, gender, menstrual cycle for females, and sleeping pattern were all recorded.

Clinical analyzes were conducted in a private laboratory in Al Harthiya, Baghdad.

In this study, various tools, apparatuses and equipment were employed: A sandwich Enzyme-Linked ImmunoSorbent Assay (ELISA) for adrenal hormones (epinephrine, aldosterone, and cortisol), and a spectrophotometer for cholesterol.

All materials, tools, and devices used in the sandwich ELISA were from the German company -Human GmbH-Germany Products, while the spectrophotometer is from the American company Apple.

Normal value of: Epinephrine (0 to 140 pg/mL), Aldosterone (2-9 ng/dl), Cortisol (Varies according to different age, time, physical status...etc.: 0-690 nmol/l), and Cholesterol (0-200 mg/dl).

3. Statistical analysis:

The Statistical Analysis System - SAS (2018) program was used to analyze the results of the levels of adrenal hormones and cholesterol in the blood of healthy students, during and after Ramadan, and according to gender. T-test was used for significant comparison between means in this study^[23].

4. Results

Forty students (The number of females F equals the number of males M) from the Faculty of Medicine at the University of Fallujah-Iraq, participated in the study. Samples of blood were drawn on the twenty-first day of Ramadan, and the second time, two weeks after the end of Ramadan for the year 1444 AH (March-April, 2023 AD). The mean age of the participating students was 20 years, and the mean time of blood taking at 1:00 pm. Epinephrine, aldosterone, cortisol, and cholesterol were examined in the blood of students of both genders (F and M) during and after the Ramadan fasting period. The four tables shows the data of mentioned compounds as mean with the standard error (SE) and the T-test (P-value).

According to the findings of the tables, the blood levels of epinephrine, aldosterone, cortisol, and cholesterol, during and after Ramadan, within the normal values in the both sexes.

The results of epinephrine were presented in the table 1. They were during Ramadan showed that the mean \pm SE (F:71.26 \pm 4.08 and M: 73.61 \pm 3.70 pg/mL) and after Ramadan (F:70.60 \pm 3.47 and M: 55.78 \pm 4.25 pg/mL). The P value is higher than 0.05 between F and M during Ramadan (0.602), which indicates that it is not statistically significant. While the statistic was highly significant ($P < 0.01$) after the month of Ramadan (0.0076). And also highly statistically significant in the males between Ramadan and after (0.0034). Non-significant result in the female between Ramadan and after (0.902).

Table 2 show the results of aldosterone during Ramadan (F: 2.95 \pm 0.23 and M: 3.06 \pm 0.34 ng/dl) and after Ramadan (F: 3.49 \pm 0.38 and M: 4.76 \pm 0.52 ng/dl). The P value between F and M during Ramadan (0.517), which indicates that it is non-statistically significant. While there is statistical significance at the level ($P < 0.05$) after the month of Ramadan (0.0227). It is highly statistically significant for males between Ramadan and beyond (0.0094). Non-significant result also in the female between Ramadan and after (0.233).

The results of cortisol (Among students aged 19-21 at noon between 12:30 p.m. and 1:30 p.m.) during Ramadan (F: 114.23 \pm 10.35 and M: 104.70 \pm 9.08 nmol/l) and after Ramadan (F: 58.17 \pm 1.98 and M: 57.56 \pm 3.17 nmol/l). The P value between F and M during and after Ramadan is not statistically significant (in Ramadan: 0.415 and after Ramadan: 0.761). It is highly statistically significant for females (0.0001) and males (0.0001) between Ramadan and after, as shown in table 3.

Analysis of total cholesterol during Ramadan fasting (F: 166.35 \pm 9.05 and M: 154.55 \pm 5.72 mg/dl), while the results after Ramadan (F: 174.25 \pm 6.09 and M: 170.00 \pm 10.34 mg/dl) as shown in table 4. Non-statistically significant results for cholesterol levels during Ramadan and after Ramadan fasting for both genders (in Ramadan: 0.371 and after Ramadan: 0.816). Non-significant result also in the female and male, between Ramadan and after (F: 0.463 and M: 0.199).

5. Discussion

The impact of Ramadan fasting on adrenal hormones was examined in this study, which is the first to examine, according to the previous studies^[8,9,10], how Ramadan fasting affects adrenal hormones in healthy subjects. Ramadan fasting causes changes to the frequency, timing, and makeup of meals during the night, as well as sleep patterns. Lack of physical activity and sleep at night is the general phenomenon among the students under study.

Our results on the effect of fasting on total cholesterol level were consistent with international research, as it was found at its normal value in the blood. Many studies have been carried out on the impact of Ramadan fasting on lipid profile (including cholesterol), showing their moderate improvement during and after fasting^[24,25].

In our study, ELISA was used to analyze adrenal hormones. When the samples were subjected to ELISA and numerous other techniques, there were noticeable variances in the results of cortisol^[22]. There is no international study with the same conditions as our current study.

Those who fast typically get less sleep throughout Ramadan. Previous study has highlighted the need for additional investigations on the connection between cortisol and male sleep outcomes, indicating a lucrative area of investigation for future research^[21].

Cortisol levels and circadian rhythmicity are highly correlated. The cortisol levels fluctuate according to a circadian cycle, rising in the early morning, falling throughout the day before dropping to their lowest point at night. The findings suggest that intermittent fasting elevates the quantity and regularity of cortisol secretion^[26]. In the study by Roelfsema et al., serum cortisol levels were above physiological values (about 800 nmol/L), and sex, age, or BMI had no impact on the mean 24-hour cortisol concentration^[27]. However, the relationship of fasting with circadian rhythms is currently unknown^[16]. Cortisol results showed high statistical significance for females and males between Ramadan and after. A decrease in blood cortisol levels was observed after Ramadan in both genders due to the removal of the stress occurring during the month of Ramadan, such as changing lifestyle, lack of sleep, etc.. While salivary cortisol measurements taken before fasting and at the end of Ramadan were identical^[28].

Most of the recent studies on the aldosterone and epinephrine are on diseases and their uses as a treatment. Our research showed that Ramadan and after Ramadan had no effect on female blood aldosterone levels. For male, however, it has a high statistical significance both during and after Ramadan due to the difference in sex hormones^[29]. Aldosterone regulates the blood's sodium and potassium levels to help keep blood

pressure under control. Previous studies confirm that Ramadan fasting has a positive effect on blood pressure and minerals in the blood of those fasting [18,30]. The normal range for aldosterone levels varies depending on age [13].

The epinephrine result in males was similar to the cortisol result. A decrease in blood epinephrine levels was observed after Ramadan in male only. The two hormones (cortisol and epinephrine) are stress hormones. Epinephrine levels in male, like cortisol, fluctuate according to the circadian cycle. However, the pattern of epinephrine excretion varied across the sexes: although both stressors significantly increased epinephrine excretion in males, it stayed at the same level in females throughout both stress conditions and relaxation [31].

Consequently, the effects of Ramadan fasting as recorded in the literature were inconsistent and often conflicting. The number of days of fasting, the environment, hereditary variables, and the kind of food consumed are probably all important. However, further research is required to better understand how Ramadan fasting impacts adrenal hormones in order to clarify these issues. It is necessary to conduct extensive studies using large samples of females and males to determine the normal levels of cholesterol and adrenal hormones in the blood of healthy Iraqi.

Conclusion

In healthy Iraqi students, Ramadan fasting does not appear to have a significant impact on adrenal hormones and cholesterol levels in the blood. Significant high statistical results (P < 0.001) were observed among male adrenal hormone levels between Ramadan and beyond. Both sexes show a marked degree of variation in normal values of adrenal hormones and cholesterol.

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Table 1: Comparison of epinephrine levels during and after Ramadan according to gender.

Ramadan	Mean ± SE of Epinephrine (pg/ml)		T-test (P-value)
	Female	Male	
In Ramadan	71.26 ±4.08	73.61 ±3.70	8.45 NS (0.602)
After Ramadan	70.60 ±3.47	55.78 ±4.25	10.51 ** (0.0076)
T-test (P-value)	10.83 NS (0.902)	11.507 ** (0.0034)	---

** (P≤0.01), NS: Non-Significant.

Table 2: Comparison of aldosterone levels during and after Ramadan according to gender.

Ramadan	Mean ± SE of Aldosterone (ng/ dl)		T-test (P-value)
	Female	Male	
In Ramadan	2.95 ±0.23	3.06 ±0.34	0.611 NS (0.517)
After Ramadan	3.49 ±0.38	4.76 ±0.52	1.194 * (0.0227)
T-test (P-value)	0.907 NS (0.233)	1.253 ** (0.0094)	---

* (P≤0.05), ** (P≤0.01).

Table 3: Comparison of cortisol levels during and after Ramadan according to gender.

Ramadan	Mean ± SE of Cortisol (nmol/L)		T-test (P-value)
	Female	Male	
In Ramadan	114.23 ±10.35	104.70 ±9.08	16.03 NS (0.415)
After Ramadan	58.17 ±1.98	57.56 ±3.17	9.54 NS (0.761)
T-test (P-value)	20.87 ** (0.0001)	21.47 ** (0.0001)	---
** (P≤0.01), NS: Non-Significant.			

Table 4: Comparison of cholesterol levels during and after Ramadan according to gender.

Ramadan	Mean ± SE of Cholesterol (mg/dl)		T-test (P-value)
	Female	Male	
In Ramadan	166.35 ±9.05	154.55 ±5.72	19.38 NS (0.371)
After Ramadan	174.25 ±6.09	170.00 ±10.34	15.02 NS (0.816)
T-test (P-value)	21.60 NS (0.463)	23.94 NS (0.199)	---
NS: Non-Significant.			

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