

## EFFECTS OF RAMADAN FASTING ON SOME BIOCHEMICAL PARAMETERS

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### Abstract

**Introduction:** Holy month Ramadan lasts for about 29-30 days. It is mandatory for all adult individuals of the Islamic faith to fast during the holy month of Ramadan. During this period, healthy Muslims do not eat or drink (i.e., total abstinence from food and fluids) and refrain from tobacco, daily from pre-dawn until dusk.

**Materials and Methods:** The present study included healthy adult male and female Muslim volunteers from different towns in the Republic of North Macedonia. The number of subjects in the study was 195. Blood samples from all subjects were collected twice during the study - once 2-3 days prior to the beginning of Ramadan and then again, the last day of Ramadan fasting. The following biochemical parameters were taken into consideration: total cholesterol, triglycerides, HDL cholesterol, glucose, insulin, total proteins, albumin, total and direct bilirubin, urea, creatinine and uric acid.

**Results:** It was observed that compared to pre-fasting levels, total cholesterol (TC) and triglycerides (TG) were significantly decreased ( $P=0.000$ ) and high-density lipoprotein cholesterol (HDL-C) level had significantly increased ( $P=0.000$ ); total proteins were significantly increased ( $P=0.000$ ); albumins were significantly increased ( $P=0.004$ ); urea was significantly increased ( $P=0.004$ ); total and direct bilirubin were significantly increased ( $P=0.000$ ), glucose was significantly increased ( $P=0.000$ ); there was no change in insulin levels ( $P<0.1$ )

**Conclusion:** This study showed that Ramadan fasting has an effect on biochemical parameters. We can conclude that reduction in total cholesterol and triglycerides along with a rise in levels of HDL-C have beneficial effects on lipid profile post-Ramadan fasting period.

**Keywords:** biochemical parameters, Ramadan, fasting

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\* contributed equally

### **Introduction**

Fasting is defined as voluntary total or partial abstinence from all foods or a selected abstinence from forbidden foods and may be practiced for religious reasons, for health reasons, or for the primary purpose of exercising spiritual strength.

There are three forms of abstinence from food:

1. caloric restriction,
2. alternative fasting,
3. dietary restriction.

In caloric restriction (CR), the total amount of kilocalories (kcal) (on average 20-40%) of food consumed *ad libitum* is reduced.

Alternative day fasting (ADF) consists of alternating 24-hour periods: the so-called feast period "feasting period", during which food can be consumed *ad libitum*, and the fast period "fasting period", during which no food is consumed at all. Ingestion of water is allowed during this period.

Dietary restriction (DR) is a form of fasting in which the intake of one or more nutrient components (usually macronutrients) is reduced (or eliminated altogether) with minimal (or no) reduction in total kcal intake.

### **Characteristics of the Ramadan fast**

According to the Islamic calendar, fasting lasts during the holy month of Ramadan. Since the Islamic calendar (Hijra) is a lunar calendar, the holy month of Ramadan "falls" in a 33-year cycle at different times of the year. In a period of 28-30 days, from sunrise (Sahur) to sunset (Iftar) lasts the period of abstinence from food, water, cigarettes and physical pleasures - a period of "fasting". The period of "feast" begins at sunset.

Depending on the month in which the fast begins and the latitudinal distance at a given location relative to the equator, the fasting period can last from 12 to 22 hours [1, 2].

Ramadan fasting is similar to ADF in that it alternates between "fasting" and "feasting" periods, with two major differences:

The first difference is the duration of the fasting and feasting periods. In Ramadan, they do not last 24 hours but alternate in a day [1-4].

The second major difference is in water intake. In ADF, intake of water is allowed, while during Ramadan, in the "fasting" period, intake of water and other liquids is not allowed.

Abstaining from food and water during the day results in less frequent meals and less food consumed during the day, less physical activity during the day, and less sleep during the night.

### **Effects of Ramadan fasting on health**

Fasting has been the subject of scientific research since it has been demonstrated that fasting can improve health.

Fasting is of great importance to the individual from a spiritual point of view, but there is evidence that during fasting certain organs can regenerate, giving fasting a medical dimension as well as a spiritual one.

It is known to detoxify the body and restore functions that are impaired due to overeating, malnutrition or inadequate nutrient intake.

Studies show that calorie restriction has many benefits, such as a lower risk of cancer, cardiovascular disease, diabetes, insulin resistance, immune disorders, and a reduction in the aging process with the potential to prolong life.

According to the American Academy of Sciences, other benefits of fasting include increased stress resistance, increased insulin sensitivity, decreased morbidity, and increased life expectancy.

Physiological effects of fasting include lowering blood sugar levels, lowering cholesterol levels, and lowering systolic blood pressure.

People with impaired glucose regulation but without insulin dependence, obesity and essential hypertension can fast.

However, there are also many conflicting results regarding the effects of Ramadan fasting on biochemical parameters.

Variability in the fasting period is one of the few confounding variables affecting research findings on the effects of Ramadan fasting on biochemical parameters.

Other "confounding" variables include: cigarette smoking, use of *per os* medications, or use of IV infusions, which are prohibited during the "fasting" period of Ramadan [5-7]. Many studies have not analyzed these "confounding" variables, although the number of respondents who smoke, take medications, and/or receive intravenous infusions can be a large contributor to study results.

For this reason, the results of the effects of Ramadan fasting on metabolic processes are highly inconsistent [8-12].

The largest differences in literary data are in terms of: group size, age, gender, ethnicity, climatic conditions, cultural influence, physical activity, genetic predisposition, and dietary habits of the groups studied [13-15].

The results of studies on healthy subjects [16-25], overweight subjects [26], metabolic syndrome [27-29], hypertension [30], hypercholesterolemia [31], and patients with cardiovascular disease [32,33], type 2 diabetes mellitus [34-39], and chronic kidney disease [40] have varied widely.

The motive and objectives of the study emerged from the conflicting literature data.

### **Objective of the study**

To assess the impact of Ramadan fasting on the health of the respondents by analyzing the biochemical parameters:

- lipid status: total cholesterol, triacylglycerols, HDL cholesterol, LDL cholesterol;
- protein status: albumin, total proteins;
- serum glucose;
- insulin;
- urea, creatinine, uric acid, direct and total bilirubin.

### **Material and methods**

The study included 195 respondents of Muslim faith who practiced Ramadan fasting. The respondents were aged between 20 and 40 years with equal representation of both genders. Only healthy individuals were included in the study. Individuals with diabetes mellitus, cardiovascular, renal, liver, thyroid, or other chronic diseases, or individuals receiving certain therapies were not included in the study. Pregnant women, lactating women, and women taking birth control pills were not included in the study.

Hematological and biochemical parameters, insulin and electrolytes were determined in each subject according to the following protocol: 2-3 days before the beginning of the fast and the day before the end of the fast, 12 hours after the last morning meal.

All subjects completed a questionnaire with information on dietary habits, physical activity, smoking, and other topics of interest. Each respondent signed an informed consent form to participate in the study.

The study was approved by the Ethics Committee of the Faculty of Medicine in Skopje.

A venous blood sample was collected from all subjects (vacuum tubes without anti-coagulant for serum separation).

Lipid status, protein status, direct and total bilirubin, glycemia, degradation products were determined by photometric methods; insulin by the electrochemiluminescence immune assay (ECLIA).

Biochemical parameters were determined at the Institute of Medical and Experimental Biochemistry - Faculty of Medicine, Skopje.

### **Statistical analysis**

For description of patient's data, we provide statistical summaries of their demographics and baseline measurements. To assess differences between post- and pre-fast measurements, we calculated the differences between the second and first measurement, with graphic and numeric check for the assumption for normality of the distribution with the Shapiro-Wilk test. Continuous variables that violated the distribution assumption were tested for differences with the Kruskal Wallis test, while the rest were tested with the T test for paired sample. The provided p-values were adjusted for multiple comparisons with the Benjamini-Hochberg procedure in R. As significant, we accepted results with associated p-value below 0.05 after adjustment. All other analyses were conducted using IBM SPSS, v.24.

### **Results**

Baseline biochemical parameters of the study group are presented in Table 1.

**Table 1.** Baseline biochemical parameters

Variable	Unit of measurement	Measurements	Mean	SD
Glucose	mmol/L	195	4.65	0.60
Urea	mmol/L	195	4.24	1.12
Creatinine	μmol/L	195	83.91	13.03
Uric acid	μmol/L	195	248.71	59.68
Insulin	μIU/mL	195	11.34	6.41
Total bilirubin	μmol/L	195	13.68	5.07
Direct bilirubin	μmol/L	195	4.80	1.68
Total cholesterol	mmol/L	195	5.77	1.04
HDL	mmol/L	195	1.13	0.30
LDL	mmol/L	195	3.62	1.01
Triglycerides	mmol/L	195	1.27	0.63
Total proteins	g/L	195	72.56	7.10
Albumin	g/L	195	43.16	3.81

SD Standard Deviation

Results from the repeated measurements and calculated differences (M2-M1) for the sample are presented in Table 2.

### **Lipid Status**

Our results showed that the value of total cholesterol decreased statistically significantly after the end of fasting ( $5.34 \pm 1.11$  mmol/L) compared to the period before the start of fasting ( $5.77 \pm 1.04$  mmol/L),  $p=0.000$ .

Fasting subjects had an increase in HDL cholesterol ( $1.24 \pm 0.34$  mmol/L) one month after the start of fasting compared to baseline values before fasting ( $1.13 \pm 0.30$  mmol/L),  $p=0.004$ .

Serum levels of triacylglycerols in the group of subjects before the start of fasting were  $1.27 \pm 0.63$  mmol/L, while after the end of fasting we found a significant decrease in levels ( $1.09 \pm 0.70$  mmol/L),  $p=0.000$ .

**Table 2.** Results from the repeated measurements and calculated differences (M2-M1) for the sample

Variable name	Unit of measurement	Measurement 2, M2		M2 - M1		Test for differences Adjusted p-value *
		Mean	SD	Mean	SD	
Glucose	mmol/L	4.91	0.69	0.26	0.59	0.000*
Urea	mmol/L	4.63	1.15	0.38	1.18	0.000*
Creatinine	$\mu\text{mol/L}$	81.86	14.33	-2.06	13.66	0.197
Uric acid	$\mu\text{mol/L}$	244.05	67.23	-5.25	55.47	0.218
Insulin	$\mu\text{IU/mL}$	10.37	5.12	-0.18	4.33	0.574
Total bilirubin	$\mu\text{mol/L}$	14.00	4.31	-4.33	5.81	0.000*
Direct bilirubin	$\mu\text{mol/L}$	3.98	1.48	-0.78	1.78	0.000*
Total cholesterol	mmol/L	5.34	1.11	0.51	1.03	0.000*
HDL	mmol/L	1.24	0.34	0.10	0.38	0.004*
LDL	mmol/L	3.84	1.27	0.22	1.53	0.058
Triglycerides	mmol/L	1.09	0.70	-0.15	0.61	0.000*
Total proteins	g/L	76.72	4.01	4.26	7.66	0.000*
Albumin	g/L	44.05	2.43	0.87	3.79	0.004*

\*Adjusted for multiple testing with Benjamini Hochberg procedure; SD Standard Deviation

### Protein Status

The values of total serum proteins before the beginning of the fast were  $72.56 \pm 7.10$  g/L, while after the end of the fast we found a statistically significant increase in the values ( $76.72 \pm 4.01$  g/L),  $p=0.000$ .

Serum albumin levels before fasting were  $43.16 \pm 3.81$  g/L, while after fasting we observed a statistically significant increase in levels ( $44.05 \pm 2.43$  g/L),  $p=0.004$ .

### Degradation Products

Serum urea levels before fasting were  $4.24 \pm 1.12$  mmol/L, while after fasting we observed a statistically significant increase ( $4.63 \pm 1.15$  mmol/L),  $p=0.004$ .

There was no difference in uric acid and creatinine levels between the two measurements. The values of total serum bilirubin were  $13.68 \pm 5.07$   $\mu\text{mol/L}$  before the beginning of the fast, while after the end of the fast we found a statistically significant increase in the values ( $14.00 \pm 4.31$   $\mu\text{mol/L}$ ),  $p=0.000$ .

Direct serum bilirubin levels before fasting were  $4.80 \pm 1.68$   $\mu\text{mol/L}$ , while after fasting we observed a statistically significant decrease in levels ( $3.98 \pm 1.48$   $\mu\text{mol/L}$ ),  $p=0.000$ .

### Glycemia and insulin

Serum glucose levels before fasting were  $4.65 \pm 0.60$  mmol/L, while after fasting we observed a statistically significant increase in levels ( $4.91 \pm 0.69$  mmol/L),  $p=0.000$ .

The results from the sample revealed 5 patients with extreme measurements for insulin (above 60); further analysis was done after removing these outlier measurements.

Due to unexpected shortages, insulin measurements were done only in 46% of the sample. Taking this into account, the groups of patients with repeated insulin measurements were compared to patients without repeated insulin measurements in terms of age (Mann Whitney U test) and gender (Chi-square test), with noted statistically insignificant difference ( $r < 0.1$ ).

## Discussion

During the conduct of our study, the average duration of fasting was 15-16 hours (on the first day from 4:14-19:25, on the last day from 3:19-19:52). During Ramadan, there is no restriction on calorie intake. The difference between total abstinence from food and Ramadan fasting is the timing of food intake. Those who practice the Ramadan fast do not eat at noon, eat breakfast early in the morning, before sunrise, and do not take food and water until sunset. Fasting in Ramadan has spiritual, social, physiological and psychological significance for those who fast.

But it can have a negative impact on human health if not practiced properly. It is not necessary to eat too much food during Iftar (the meal to break the fast immediately after sunset) or Sahur (a light meal in the early morning, half an hour to an hour before sunrise).

The diet should be balanced to maintain a good psychophysical condition during the holy month of Ramadan.

So far, there are conflicting findings in the literature about the effects of Ramadan fasting on biochemical parameters [23, 24, 41, 42]. This is the first study to examine the effects of Ramadan fasting in our setting, taking into account the cultural, social and dietary habits of Muslims living in the Republic of North Macedonia.

Our study showed a significant decrease in total cholesterol after fasting ( $5.34 \pm 1.11$  mmol/L) compared to the period before fasting ( $5.77 \pm 1.04$  mmol/L). Our results are in agreement with the results of Akhtar *et al.* [43], who showed a statistically significant decrease in total cholesterol concentration after fasting. But the results of Ziaee V *et al.* [44] and Nomani MZA [45] showed no change in cholesterol concentration.

There are conflicting results in the literature on whether fasting during Ramadan decreases [46,47] or increases [48] the LDL-C/HDL-C ratio.

It has been found that the TC / HDL-C ratio decreases during fasting [47,49].

It is believed that the body develops adaptive mechanisms during Ramadan fasting and that oxidation of fats is increased and oxidation of carbohydrates is decreased.

Plasma HDL-C concentration is a protective factor against the development of atherosclerosis and cardiovascular disease. In our study, we observed an increase in the value of HDL cholesterol ( $1.24 \pm 0.34$  mmol/L) one month after the start of fasting compared to the baseline values before fasting ( $1.13 \pm 0.30$  mmol/L),  $p = 0.004$ . Since there was no change in physical activity, smoking habits or consumption of alcohol (which is prohibited for religious reasons), the increase in HDL-C concentration cannot be due to any of these factors.

The finding of an increase in HDL-C at the end of the Ramadan fast in this study can be explained by a decrease in saturated fat intake and a decrease in insulin levels, as well as an increase in catecholamine concentration, increased lipolysis in adipose tissue due to hypoglycemia during fasting [50, 51]. Our results are consistent with the findings of other authors who also found an increase in HDL-C during Ramadan fasting [52]. Although the mechanisms by which fasting affects HDL-C concentration are unclear, weight loss may increase HDL-cholesterol levels. Changes in lipid status are thought to be the result of altered diet or a biochemical response to starvation. It is known that there is an inverse correlation between HDL concentration and cardiovascular disease. Increased HDL-C levels should have a beneficial effect because HDL particles have the ability to take up esterified cholesterol

from peripheral tissues and transport it to the liver and intestines, thereby lowering serum cholesterol levels. In our study, the serum levels of triacylglycerols in the group of subjects before the beginning of fasting were  $1.27 \pm 0.63$  mmol/L, while after the end of fasting we found a significant decrease in the levels ( $1.09 \pm 0.70$  mmol/L). Statistical analysis revealed  $p = 0.000$ , which was statistically significant. Our results are in agreement with those of other studies [53, 54]. However, there are opposite results in which an increase in serum concentrations of triacylglycerols has been observed after Ramadan fasting when the consumption of carbohydrates is increased during fasting. During fasting, the biosynthesis of triacylglycerols is reduced due to the reduced availability of the precursor molecules acetyl-CoA and glycerol during starvation due to reduced glucose oxidation [55]. Pentose phosphate dehydrogenase activity has also been shown to be reduced during fasting, and reducing equivalents are necessary for the synthesis of fatty acids and cholesterol [56]. This results in a decrease in the concentration of cholesterol, triacylglycerols, and LDL during fasting.

However, with the end of the fast, most fasters return to their old eating habits. Therefore, it is difficult to assess how long the effects of Ramadan fasting last and what are the chronic effects of fasting each year. In our study, serum glucose levels before fasting were  $4.65 \pm 0.60$  mmol/L, while after fasting we observed a statistically significant increase in levels ( $4.91 \pm 0.69$  mmol/L),  $p = 0.000$ . We found no difference in insulin levels before and after fasting.

During Ramadan fasting, glucose homeostasis is mainly maintained by the pre-sunrise meal and glycogen stores in the liver, but in this regard, the results of the studies are not consistent [3, 10, 25, 35, 36].

### Conclusions

It is very difficult to compare the results of different studies on the effects of Ramadan fasting on human health because there are several "confounding" variables:

- The duration of abstinence from food and water (in hours) depends on the period/season in which the holy month of Ramadan falls, as well as the geographical distance from the equator.
- The proportion of subjects who are smoking, taking medications, and/or intravenous fluids may greatly affect the results of the study, as these activities are prohibited during the fasting period.
- Dietary habits vary greatly from culture to culture.

All these variables taken together may explain why there is no consensus on the effects of Ramadan fasting on biochemical parameters.

Although there are many conflicting results in studies that have examined the effects of Ramadan fasting on human health, from a metabolic perspective, fasting can be divided into three main phases:

**Phase 1:** Post-absorption phase, ~6-24 hours after fasting begins, when the central nervous system (CNS) and many other tissues use glucose derived primarily from glycogenolysis. Lipolysis, ketogenesis, and gluconeogenesis increase, albeit gluconeogenesis to a lesser extent. Glycogenolysis decreases.

**Phase 2:** The process of gluconeogenesis occurs ~1-10 days after fasting begins. Protein breakdown is used to provide energy sources for the CNS (glucose), while other tissues use ketones and fats for energy. Lipolysis and ketogenesis increase until a plateau is reached, while gluconeogenesis begins to decrease and glycogenolysis does not occur.

**Phase 3:** In this stage, protein preservation occurs if the fast lasts longer than 10 days. Protein catabolism is reduced to a minimum, and fatty acids and ketones are used extensively as

energy sources for the CNS. Lipolysis and ketogenesis reach a plateau, while gluconeogenesis plateaus, but to a lesser extent than ketogenesis [57, 58].

**Conflict of interest statement.** None declared.

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