The effect of Ramadan fasting on body weight, fasting blood sugar and lipid profile of normal male healthy non obese male medical college students in Tikrit city.

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Abstract

Background: Fasting during the month of Ramadan is one of the five fundamental pillars of Islamic practices, mandatory for all healthy adult Muslims. In Ramadan, Muslim adults fast from sunrise to sunset and are required to refrain from oral intake of food, water, beverages, smoking and sexual intercourse. This type of fasting is defined as periodic food and water deprivation during daylight hours with free access during the night for the duration of one month. The aim of the study is to determine the effect of Ramadan on body weight, fasting blood sugar and lipid profile of normal healthy non-obese male medical college students in Tikrit city. Subjects and methods: A prospective study was carried out on thirty normal healthy male students aging between 20 to 22 years, in college of medicine-Tikrit university were participate in the present study. The volunteers were allowed to consume whatever they wanted except decrease fat intake in diet. The study was conducted in the month of Ramadan from beginning of May to 5th of June /2019. Average duration of the fast was about 13.5 hours & maximum temperature ranged from 25 to 35°C. Body weight was measured to the nearest 100gm. Fasting blood sugar and lipid profile were measured at beginning and the end of Ramadan and one hour before break fasting (one hour before sunset). Results: There were slight decrease in body weight and body mass index (BMI) at the end of Ramadan fasting as compare with beginning of Ramadan fasting. Also, in regard to blood parameters, there is no significant difference regarding blood glucose from at end of Ramadan as compare with the beginning of fasting. Also, there were significant reduction in serum TG, total cholesterol, LDL and VLDL at the end of Ramadan fasting as compare with beginning of Ramadan fasting. However, There was significant elevation in serum HDL at the end of Ramadan fasting as compare with beginning of Ramadan fasting.

Key words: Ramadan fasting, FBS, Body weight, lipid profile.


Introduction

Fasting during Ramadan is an Islamic rule and, therefore, Muslims fast a 29-30-day consecutive period per year. This Islamic rule is accepted for patients and whom fasting may be harmful to them, (1). Ramadan is a month of Islamic lunar calendar and, therefore, its duration varies in different seasons year to year. In fasting days, individuals do not eat anything from brightening to sunset. From sunset to brightening, Muslims can eat freely. Hence, time of sleeping and eating may be affected by Ramadan, (3).The experience of fasting teaches Muslims self-discipline and self-restraint, fasting is not obligatory for children, menstruating women, pregnant and lactating women are permitted to postpone the fasting during Ramadan (1,2).During Ramadan, the majority of Muslims have two good sized meals, one immediately after sunset and the other just before dawn, they are allowed to eat and drink between sunset and dawn but not after dawn, the month of Ramadan is either 29 or 30 days, (4). So, many studies have focused on the effect of Ramadan fasting on metabolic changes and health outcomes in different groups of Muslims population. Studies reported that total cholesterol (TC), low-density...
lipoprotein (LDL), high-density lipoprotein (HDL) and blood glucose have been improved after Ramadan compared to before Ramadan among athletes, (5). Although previous review studies have assessed the impact of Ramadan on cardiovascular risk factors (i.e., body mass index [BMI] and lipid profile), (6). Previous studies were done on patients with obesity, DM-type 2 and other cardiovascular diseases, (3, 4, 5).

The aim of the present study was to investigate the Effect of Ramadan fasting on lipid profile of normal healthy male medical college students in Tikrit city.

Subjects and methods

A prospective study was carried out on thirty normal healthy male students aging between 20 to 22 years, in college of medicine-Tikrit university were participate in the present study. The volunteers were allowed to consume whatever they wanted & decrease fat intake in diet. The study was conducted in the month of Ramadan from beginning of May to 5th of June /2019. Average duration of the fast was about 13.5 hours & maximum temperature ranged from 25 to 35 C.

Physical and metabolic data

Students completed the measurement of an anthropometric measurement (body weight, height and BMI) by trained staff in the laboratory of medical physiology, college of medicine. General physical examination and medical history were collected by medical Staff. Blood samples were taken after 14 hours of fasting before, during and 7 days after Ramadan fasting. The students were evaluated on their fasting blood sugar, total cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol, before and during and after Fasting of Ramadan. This study was approved by the research ethics committee of college of medicine, Tikrit university.

Lipid Profile Analysis

Lipid profiles of blood were analyzed using CHOD-PAP method for Total Cholesterol (TC) and HDL-Cholesterol; and GPO-PAP method for triglycerides (TG).

Serum lipid profile:

Lipid profile was carried out for each patient who had received simvastatin treatment before onset of treatment and at the end of the treatment period which extended for four months. Thirty healthy fertile subjects served as a control group had also been investigated for their lipid profile for the purpose of comparison.

a) Total Cholesterol

For the quantitative in vitro determination of cholesterol in serum. Randox kit (Cat. No.NH 1530). Randox laboratories Ltd. Ardmore. Diamond Road. Crumin. Co. Antrim. United Kingdom BT 29 4QY was used.

1) Assay Principle: Enzymatic end point method is used to determine the cholesterol after enzymatic hydrolysis and oxidation. The indicator quinonimine is formed from hydrogen peroxide and 4aminoantipyrine*+in the presence of phenol and peroxidase+

\[
\text{Cholesterol ester} + \text{H}_2\text{O}_2 \xrightarrow{\text{cholesterol esterase}} \text{cholesterol} + \text{fatty acid}
\]

\[
\text{Cholesterol} + \text{O}_2 \xrightarrow{\text{cholesterol oxidase}} \text{cholestene 3-0ne} + \text{H}_2\text{O}_2
\]

Frozen serum of each patient was taken for assay.

2) Procedure (Appendix 1)

3) Calculation
Conc. of cholesterol in sample = \( \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times \text{conc. standard} \)

### a) Triglycerides

For the quantitative in vitro determination of triglycerides in serum, Randox kit, Randox laboratories Ltd. CO. Antrim, United Kingdom, BT 29 4 QY were utilized.

1) **Assay Principle:**

A colorimetric method (GPO- PAP) was used to determine the triglycerides after enzymatic hydrolysis with lipases. The indicator is a Quinoneimine formed from hydrogen peroxide, 4-amino phenzone and 4-chlorophenol under the catalytic influence of peroxidase.

\[
\text{Triglycerides} + H_2O \rightarrow \text{glycerol} + \text{fatty acide}
\]

\[
\text{Glycerol} + \text{ATP} \rightarrow \text{glycerol -3- phosphate} + \text{ADP}
\]

\[
\text{Glycerol 3- phosphate} + O_2 \rightarrow \text{dihydroxyacetox phosphate} + H_2O
\]

\[
2H_2O_2 + 4 \text{aminophenzone} + 4 \text{cholesterol} \rightarrow \text{quinoneimine} + HCl + 4H_2O
\]

2) **Procedure (Appendix 2).**

3) **Calculation**

\[
\text{Triglycerid concentration} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times 2.29 \text{ (standard conc.)} = \text{mmol/L}.
\]

### b) HDL- cholesterol

Randox kit was used, Randox laboratories Ltd. CO. Antrim United Kingdom BT 29 4 QY for in vitro quantitation of HDL- cholesterol.

1) **Principle:**

Low density lipoproteins (LDL and VLDL) and chylomicron fraction are precipitated quantitatively by addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL (high density lipoprotein) fraction, which remains in the supernatant, is determined.

2) **Procedure (Appendix 3)**

3) **Calculations**

Concentration of HDL cholesterol in supernatant = \( \frac{A_{\text{sample}}}{A_{\text{standard}}} \times \text{conc. of standard} \).

### c) LDL cholesterol

In mmol/ l

\[
\text{LDL} = \text{Total} - c - \frac{\text{triglycerides}}{2.2} - \text{HDL} - c
\]

CECIL- 1011 – England spectrophotometer was used in all measurements of the lipid profile.

### D) Calculation of Serum Very Low Density Lipoprotein-cholesterol (VLDL-c):

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Serum VLDL-C was calculated using the equation (S. S. Martin et al., 2013).

\[ \text{VLDL-c (mmol/l)} = \frac{\text{Triglycerides}}{2.2} \]

### Appendix (1)

- **Wavelength**: 500 nm
- **Cuvette**: 1 cm light path
- **Temperature**: 37°C
- **Measurement**: against reagent blank

<table>
<thead>
<tr>
<th>Pipette into corvette:</th>
<th>Reagent blank (μ L)</th>
<th>Standard (μ L)</th>
<th>Sample (μ L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled H_2O</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>Reagent</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
</tbody>
</table>

Mix incubate for 5 min at 37°C. Measure the absorbance of the sample (A sample) against the reagent within 60 minutes.

### Appendix (2)

- **Wavelength**: 500 nm
- **Cuvette**: 1 cm light path
- **Temperature**: 37°C
- **Measurement**: against reagent blank

<table>
<thead>
<tr>
<th>Pipette into test tubes</th>
<th>Reagent blank (UL)</th>
<th>Standard (UL)</th>
<th>Sample (UL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Reagent</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
</tbody>
</table>

Mix, incubate for 10 minutes at 37°C for 5 minutes, measure the absorbance of the sample (A) and standard (A standard) against the reagent blank within 60 minutes.

### Appendix (3)

1. **Precipitation**

<table>
<thead>
<tr>
<th>Pipette into centrifuge tubes:</th>
<th>Macro</th>
<th>Semi Micro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>500 μl</td>
<td>200 μl</td>
</tr>
<tr>
<td>Precipitant</td>
<td>1000 μl</td>
<td></td>
</tr>
<tr>
<td>Diluted precipitant</td>
<td></td>
<td>300 μl</td>
</tr>
</tbody>
</table>
Mix and allow to sit for 10 minutes at room temperature. Then centrifuge for 10 minutes at 4000 rpm, or 2 minutes at 12000 rpm.
Separate off the clear supernatant within two hours and determine the cholesterol content by CHOD-PAP method. The supernatant may be stored up to five days at + 2°C.

2. Cholesterol CHO-PAP

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>500 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cuvette</td>
<td>1 cm light path</td>
</tr>
<tr>
<td>Temperature</td>
<td>37°C</td>
</tr>
<tr>
<td>Measurement</td>
<td>against reagent blank</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Reagent blank (μ L)</th>
<th>Standard (μ L)</th>
<th>Sample (μ L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>100 μ L</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Supernatant</td>
<td>-</td>
<td>100 μ L</td>
<td>-</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>100 μ L</td>
<td>-</td>
</tr>
<tr>
<td>Reagent</td>
<td>1000 μ L</td>
<td>100 μ L</td>
<td>1000 μ L</td>
</tr>
</tbody>
</table>

Mix incubate for 10 minutes at 37°C for 5 minutes.
Measure the absorbance of the sample (A sample) and standard (A standard) against the reagent blank with 60 minutes.

**Statistical Analysis**
The data of body weight, body mass index, and plasma lipids (TC, TG, HDL and LDL) was analyzed using repeated ANOVA followed by LSD test. The data of pro-inflammatory cytokines (IL-6 and TNF-α) was analyzed using paired t-test. The correlation between pro-inflammatory cytokines and other parameters observed was analyzed using Rank Spearman Correlation. Probability of 0.05 was used as significant value.

**Results**

**Table 1** Body weight, and body mass index and fasting blood sugar (FBS) before at end of Ramadan fasting in male students.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before</th>
<th>End</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>86.3 ± 8.7</td>
<td>64.0 ± 4.7</td>
<td>0.05</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.1 ± 3.2</td>
<td>21.4 ± 2.1</td>
<td>0.05</td>
</tr>
<tr>
<td>FBS (mmol/l)</td>
<td>3.78 ± 0.51</td>
<td>3.45 ± 0.31</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Table 1** summarized the effect of Ramadan fasting on blood parameters & body weight. There was slight decrease in body weight and body mass index (BMI) at the end of Ramadan fasting as compare with beginning of Ramadan fasting. In regard to blood parameters, there is no significant difference regarding blood glucose from at end of Ramadan as compare with the beginning of fasting. Moreover, table 2 show the results of lipid profile of fasting students at the beginning and at the end of Ramadan fasting.

**Table 2** shows the result of lipid profile before and at the end of Ramadan fasting in male non obese healthy student.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before</th>
<th>End of fasting</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG</td>
<td>205 ± 9</td>
<td>121.8 ± 18.9</td>
<td>0.01</td>
</tr>
</tbody>
</table>
As table 2 show, there is a significant decrease in serum triglycerides from the beginning of Ramadan (205 ± 9 mg/dl) as compare to the end of Ramadan, (121.8 ± 18.9 mg/dl). Moreover, a significant decrease in serum LDL-cholesterol at the end of fasting from (153.7 ± 20 mg/dl) as compare to the beginning of Ramadan fasting (111.9 ± 3.9 mg/dl). Moreover, it was found a significant reduction in serum cholesterol at the end of fasting from (169.4 ± 17.5), as compare with that at the beginning of Ramadan fasting, (219 ± 8.2 mg/dl). Also, there is a significant reduction in serum VLDL-c at the end of fasting from (15.1± 3.1), as compare with that at the beginning of Ramadan fasting, (49.1 ± 2.3 mg/dl). However, there is a significant elevation in serum HDL- at the end of Ramadan fasting (46.3 ± 3.1) as compare with that at the beginning of Ramadan to 33.4 mg/dl at the end of Ramadan, (28.2 ± 4.2 mg/dl).

**Discussion**

In the present study, there is non significant reduction in body weight and body mass index. So, there is clear effect of Ramadan fasting on body weight and body fat. The present study agrees with previous finding, (8). However, The present study result does not agree with previous works done on normal healthy subjects (9,10). The first study was conducted on older normal male healthy doctors of Samara hospital, (9). While the second study was done on young women, (10). In the present study, fasting blood glucose level showed a non significant change during Ramadan fasting, (RF). This result agrees with previous studies (9). In the present study, decreasing food intake could be attributed to the changing of eating patterns from three times to two times a day. An overweight subject who has fasting eat fewer calories after 14 days and 21 days of Ramadan fasting, (3). However, the present result does not agree with those studies who reported a significant decrease in bloodsugar toward the end of Ramadan. These differences may attribute to the fact that they gave a hypocaloric diet to the Volunteers (11). Whereas in our study, the volunteers were free to consume anything they wanted. Other possible explanations may be the gender differences of volunteers& environmental / climatic factors (10).

In the present study, there were a significant reduction in serum triglycerides, total cholesterol& LDL-c levels at the end of Ramadan. While, there was a significant elevation in HDL-c concentration at the end of Ramadan fasting as compare with before Ramadan. It appears that as if the quality & quantity of fat intake in Ramadan govern blood cholesterol level (6, 7, 11). In the present study, all volunteers were instructed to consume whatever they wanted & decreasefat intake in diet. The increased in HDL-cholesterol at the end of Ramadan in the present study can be explained by decreased saturated fatty acid intake & decrease in circulating insulin& arise in catecholamine concentration from lipolysis in adipose tissue in response tohypoglycemia of Ramadan fasting (12). Previous study found that TC and TG were decreased in men and HDL was increased among women. In both genders, a significant reduction in LDL was observed, (13). There is a controversy in existing literature about the lipid profile during Ramadan fasting. A previous result agree with the present finding which found that fasting during Ramadan led to a significant decrease in serum total cholesterol, triglyceride and LDL-C, (14). However, a significant increase in the serum HDL-cholesterol during the fasting month. On the other hand, noted that LDL-C, very-low-density lipoprotein (VLDL) and total-cholesterol have not changed, while the authors found a significant increase in HDL-cholesterol levels and a reduction in the LDL/HDL and TC/LDL ratio at the end of Ramadan, (15). This result not agree with the present result which done on normal healthy male students. HDL- cholesterol removes excess cholesterol from body cell & transport to liver by preventing accumulation of cholesterol in blood, so HDL is associated with a decreased risk of coronary heart disease. For this reason, Ramadan fasting is a good protection of cardiovascular system, (1, 2).

**References**

<table>
<thead>
<tr>
<th>Cholesterol</th>
<th>219 ± 8.2</th>
<th>169.4 ± 17.5</th>
<th>0.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-c</td>
<td>153.7 ± 20</td>
<td>111.9 ± 3.9</td>
<td>0.01</td>
</tr>
<tr>
<td>VLDL-c</td>
<td>49.1 ± 2.3</td>
<td>15.1± 3.1</td>
<td>0.01</td>
</tr>
<tr>
<td>HDL-c</td>
<td>28.2 ± 4.2</td>
<td>46.3 ± 3.1</td>
<td>0.01</td>
</tr>
</tbody>
</table>


