

## Impact of Ramadan Fasting on Healthy Adult Males: Immuno-Bio-Chemical Study

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### Abstract

**Background:** The mandatory month is Ramadan on Muslim's fasting. Muslims cease from ingestion of food and water starting onset to grass widower to who wants to be fasting in this month that's according to the lunar calendar; so many immunological, physiological and biochemical changes may happen.

**Objective:** To evaluate some vitamins, hormones and immunological markers in the first and the 28th day of fasting.

**Patients and Methods:** Twenty five healthy adult male who were subjected to this study. The age range was 24-49 years with mean  $\pm$  SD (31.6 $\pm$ 7.07). Body weight, BMI, serum glucose, total cholesterol, high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C), triglyceride (TG), aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total serum bilirubin (TSB), serum creatinine, blood urea, serum electrolytes (Na, K, Cl,) haemoglobin concentration (Hb), haematocrit, vitamin-D, testosterone and C-reactive protein (CRP) were estimated in the 1st day and the 28th day of Ramadan month. The while-interval of study was 27 days. Human privacy, statistical analyses and P value were used.

**Results:** Serum glucose, LDL, ALT, TSB, serum creatinine, blood urea, vitamin D and CRP were significantly decreased in 28th day of fasting (P=0.0002, P=0.0005, P=0.0009, P=0.00004, P=0.013, P=0.0074, P=0.0109, respectively). There were no significance change in total cholesterol, VLDL-C, TG, AST, ALP, K, Cl, Hb, haematocrit, testosterone (P>0.05). While HDL-C and serum Na were increased significantly in this study (P= 0=0.004, P=0.0214, respectively).

**Conclusion:** Consuming two meals per a day during month of Ramadan has a balancing effect on biochemical, enzymes and CRP in fasted men.

**Key words:** Ramadan Fasting, Lipid Profile, Vitamin and Hormone Profile, CRP.

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### Introduction

Since more than a billion people worldwide follow Islam, it is assumed that every year a hundreds of millions of people

keep on fasting during the Ramadan [1][2]. Ramadan is muslim month of fasting, during which a whole month is devoted to

fasting is a particular to islam, which is carefully can saw by a great numbers of Muslims around the world [3].

The month is a good way to develop body health and personal life, fasting has its own spiritual, physical, physiological and social benefits but the problem may occur if fasting is not properly practiced [4]. As a rule fasting individuals take two meals, one before sunrise and other is after grass widower [5]. Over and above individuals take lots of carbohydrate, protein and lipids attribution. As a result Ramadan fasting awards best results by changing the lipid profile and blood contents [6]. In Fasted individuals ever after the concentration of blood is low, fat oxidation increases more significantly compared to carbohydrates [7]. As it is in prospect there are decreasing in body fat percentage and body weight [8][9].

According to Kamal (2012) there was significant decrease in body weight, triglyceride, LDL, and systolic blood pressure but there was no significant change in diastolic blood pressure [10]. Then Adlouni et al., (1997) with 32 healthy men, there are different before and during Ramadan [11]. According to Ismail and Haron (2014), there was a significant decrease in serum cholesterol and triglycerides in fasting Ramadan [12]. Likewise there are significant increase in HDL-cholesterol and decrease in LDL-cholesterol too. This dietary restriction conducts to metabolic changes and liver is the central organ for all major metabolic pathways [13].

Ebrahimi (2016) reported that preponderance of the researches in this field have distilled on the impact of fasting on inflammation through investigating the serum levels of inflammatory markers, such as C-reactive protein, leukocyte count, cytokines, and chemokines, as a marker to differentiate between healthy and inflammatory diseased individuals; as many

studies reported that Ramadan fasting has a beneficial effect on inflammatory diseased patient by down regulating pro-inflammatory molecules [7].

Various studies applied to investigate Ramadan fasting on healthy adults as well as patients, liver function and blood parameters but in addition to these several study tested some immunological, hormonal changes and serum vitamin-D level [14][15] [16].

The purpose of our study is to rectify the effect of muslim fasting and determine the association serum glucose, lipid profile, liver function, renal function, some haematological parameters, hormones, vitamins and C-reactive protein between first day and 28th day of Ramadan on fasting adults.

## Patients and Methods

Twenty-five adult, apparently healthy male lecturers and teaching assistants, aging 24-49 years old (mean  $31.6 \pm 7.07$ ) was randomly invited from the department of biology, college of education, Salahaddin University-Erbil.

The study was conducted in the month of Ramadan from 6th of June to 3rd of July 2016. Average duration of the fast was 16 hours. Anthropometric assessment (body weight, body mass index-BMI) was measured throughout the two periods (1st and 28th day of Ramadan). The body weight (kg) was measured with an electronic balance (Beurer GS 27 black flower Design-Glaswaage). The height was measured with a body height scale (measuring range: 0-220cm, Graduation Length: 1mm). The BMI was calculated as:  $BMI (kg/m^2) = \text{weight (kg)}/\text{height (m)}^2$ .

Following an anthropometric assessment provided a blood sample for the measurement of haematocrit, haemoglobin, blood glucose, lipid profile, alanine amino transferase (ALT), aspartate amino transferase (AST),

alkaline phosphatase (ALP), total serum bilirubin, creatinine, urea, serum electrolytes, vitamin D, testosterone, and C-reactive protein. Each volunteer after 9 hours of fasting on the first and 28th day of Ramadan provided a 5 ml blood venous sample with minimal stasis from an antecubital vein into a plain vacutainer tube. An aliquot of blood was immediately removed and mixed with EDTA as an anticoagulant. This blood sample was used for determination of the haematocrit with Wintrobe hematocrit tubes, haemoglobin by Sahli's method [17]. Which were measured on the same day the blood was collected. The remainder of the blood was allowed to clot and then was centrifuged at 3000rpm for 10 min, and then serum was separated and stored at  $-20^{\circ}\text{C}$  until subsequent analysis. Total glucose, urea, creatinine, total cholesterol, HDL, triglycerides, AST, ALT, ALP, total bilirubin were determined in blood serum by enzymatic methods using commercial kits obtained from Solarbio Science & Technology Co., Beijing, China, while VLDL concentration calculated by dividing the triglyceride concentration by 5 and then mathematically deriving the LDL cholesterol concentration [18]. Serum electrolytes (sodium, potassium, and chloride) were determined by potentiometry. The sera used

for testing by enzyme linked immunosorbent assay (ELISA) to determine human vitamin-D total (MBS495106-KAP1971, My BioSource), human testosterone (ab178655, UK) and CRP human (KA0238 Abnova, USA).

### Statistical Analysis

Statistical analysis was performed using the SPSS Version 18.0. All data in the text and tables were expressed as means and standard deviations. Comparison between the means was made using the student's paired t-test.  $P < 0.05$  was considered significant.

### Results

Ramadan fasting share in fact to check increasing or decreasing risk of serum factors, this will give effect on Ramadan fasting to lipid metabolism by following the changes with time of total cholesterol, triglycerides, HDL-C, LDL-C before and during Ramadan in 28th day of fasting.

Table (1) summarizes the anthropometric parameters measured during the two phases of the study; according to this table observed change in body weight and BMI both significantly affected by fasting ( $P = 0.0001$ ). Body mass index reduced from  $(27.179 \pm 3.50)$  for 1st day of fasting while  $(26.639 \pm 3.31)$  at 28th day for all 25 participants.

**Table (1):** Anthropometric measurements of healthy male participants.

Parameters	1 <sup>st</sup> day of Ramadan (Mean $\pm$ SD)	28 <sup>th</sup> day of Ramadan (Mean $\pm$ SD)	p-value
Body-Weight (kg)	80.026 $\pm$ 12.11	78.404 $\pm$ 11.71	0.00001
BMI (kg/m <sup>2</sup> )	27.179 $\pm$ 3.50	26.639 $\pm$ 3.31	0.00001

Changes in biochemical parameters are reported in table (2). Serum glucose decreased significantly during the estimated days of fasting ( $P < 0.001$ ). With regard to changes in lipid profile, LDL-cholesterol decreased significantly during the estimated days of fasting ( $P < 0.001$ ) and the blood HDL-cholesterol level showed a significant ( $P = 0.0041$ ) rise on day 28th ( $33.443 \pm 8.86$ ) as compared to day 1 ( $31.609 \pm 7.39$ ). Blood

total cholesterol level showed a slight decrease during the month of fasting but the decrease was non-significant. Whereas, both VLDL-cholesterol and triglycerides increased insignificantly during Ramadan fasting.

In compare to 1st day of fasting values the rates of total serum bilirubin reduced that made a significant change ( $0.892 \pm 0.38$ ,  $0.646 \pm 0.25$ ,  $P < 0.001$ ). Alanine amino transferase (ALT)

values showed significant reduce ( $P < 0.001$ ) while aspartate amino transferase (AST) values showed a mild decrease but showed no significance also for alkaline phosphatase enzyme (ALP) value there was mild increase without any significant value. Compare to 1st day of fasting, the recorded values of serum creatinine concentration and blood urea showed significant decrease table (2).

No significant change in serum potassium and chloride level was observed throughout the study period. There was significant increase in the level of serum sodium by 28th day of Ramadan  $P < 0.0214$  Table (2).

**Table (2):** Changes in serum biochemical parameters in healthy male participants during Ramadan fasting.

Parameters	1 <sup>st</sup> day of Ramadan (Mean $\pm$ SD)	28 <sup>th</sup> day of Ramadan (Mean $\pm$ SD)	p-value
Blood glucose (mg/dl)	88.595 $\pm$ 7.21	84.181 $\pm$ 6.57	0.0002
Total Cholesterol (mg/dl)	147.297 $\pm$ 27.31	144.619 $\pm$ 24.85	NS
HDL-Cholesterol (mg/dl)	31.609 $\pm$ 7.39	33.443 $\pm$ 8.86	0.0041
LDL-Cholesterol (mg/dl)	97.052 $\pm$ 22.91	90.764 $\pm$ 20.55	0.005
VLDL-Cholesterol (mg/dl)	22.644 $\pm$ 11.17	23.652 $\pm$ 9.77	NS
Triglycerides (mg/dl)	113.236 $\pm$ 55.89	118.284 $\pm$ 48.89	NS
AST (U/L)	22.629 $\pm$ 14.39	19.329 $\pm$ 4.00	NS
ALT (U/L)	25.519 $\pm$ 17.91	15.889 $\pm$ 7.06	0.0095
ALP (U/L)	72.704 $\pm$ 22.72	74.113 $\pm$ 30.82	NS
Total Bilirubin (mg/dl)	0.892 $\pm$ 0.38	0.646 $\pm$ 0.25	0.00004
Serum creatinine (mg/dl)	0.853 $\pm$ 0.13	0.793 $\pm$ 0.11	0.0134
Blood urea (mg/dl)	26.594 $\pm$ 6.69	23.232 $\pm$ 3.75	0.0074
Serum-Na (mmol/l)	140.04 $\pm$ 3.58	142.32 $\pm$ 2.67	0.0214
Serum-K (mmol/L)	4.49 $\pm$ 0.15	4.46 $\pm$ 0.34	NS
Serum-Cl (mmol/L)	101.34 $\pm$ 2.94	100.28 $\pm$ 3.22	NS

Hemoglobin and hematocrit values showed a slight decrease during the month of fasting but the decrease was non-significant ( $P > 0.05$ )

compared to 1st day of Ramadan as illustrated in table (3).

**Table (3):** Values of hematological indices in participants during Ramadan fasting (n= 25).

Parameters	1 <sup>st</sup> day of Ramadan (Mean $\pm$ SD)	28 <sup>th</sup> day of Ramadan (Mean $\pm$ SD)	p-value
Hemoglobin conc. (g/dl)	15.77 $\pm$ 1.06	15.68 $\pm$ 0.88	NS
Hematocrit (%)	46.32 $\pm$ 3.15	46.04 $\pm$ 2.60	NS

The levels of vitamin-D and CRP decreased significantly during Ramadan fasting compared to 1st day of Ramadan ( $P = 0.0109$  and  $P = 0.0132$ , respectively).

However, testosterone concentration were decreased insignificantly during Ramadan compared to 1st day of Ramadan ( $P > 0.05$ ) as shown in table (4).

**Table (4):** Effect of fasting during the month of Ramadan on serum levels of vitamin D, testosterone and CRP in healthy male subjects (n = 25).

Parameters	1 <sup>st</sup> day of Ramadan (Mean $\pm$ SD)	28 <sup>th</sup> day of Ramadan (Mean $\pm$ SD)	p-value
Vitamin D (ng/ml)	12.641 $\pm$ 4.54	10.262 $\pm$ 5.37	0.0109
Testosterone (ng/ml)	2.371 $\pm$ 1.50	2.011 $\pm$ 1.24	NS
C-Reactive Protein	2.336 $\pm$ 2.158	1.904 $\pm$ 2.041	0.0132

## Discussion

Fasting should be done during the month of Ramadan which is around 29-30 days according to Lunar Islamic calendar. The residual interval fasted Muslim individuals hold on eating from sunrise to grass widower with limitation in dietary intake. Many researchers in their studies determined modifications in immune-bio-chemical markers before and after fasting month. Merely, outcomes are struggling because of fluctuation in intake. Impact of Ramadan fasting on immune-bio-chemical markers measured in this study among 25 healthy adult males.

Anthropometric parameters, liver functions, lipid profile, renal function, serum electrolytes, haematological parameters, vitamin-D, testosterone and C-reactive protein tests showed variations in their levels during 1st and 28th day of fasting. Previous studies have described that Ramadan fasting decreases body weight and body fat percentage. It appears that the observed decrease in body weight may be due – at least in part – to dehydration as proposed by Bouhlel (2006) and Chaouachi (2008). These decreases may also be partly a function of increased utilization of stored body fat. Such a finding has been reported in previous investigations [21][22].

Total cholesterol, VLDL-C, TGs, AST, serum-ALP, serum electrolytes (K, Cl), haemoglobin concentrations, haematocrit, and testosterone levels showed changes meanwhile, with no statistically significant. This study also showed cholesterol increase in subjects during fasting Ramadan and there was no significance change [23][24]. But some studies have reported there are decreases in total cholesterol during fasting Ramadan [25]. The change are occurred maybe due to the change of amount and type of fat intake by subjects. Statistically significance reduced tendency was observed

in tested serum glucose, LDL-C, ALT, TSB, creatinine and blood urea ( $P < 0.05$  and  $P < 0.0001$ ). But there statistically significant tendency increases in serum HDL-C and Na. An increase of HDL-cholesterol also associated with the weight loss was also observed in these studies and others. In study done by Adlouni (1997) showed the HDL cholesterol marked increase during Ramadan and this increase of HDL-cholesterol due to loss of body weight and change of meal frequency[25].

Excluding measuring dietary restriction in our study registered, generally in Ramadan the individuals' most probably increases carbohydrate intake than portentous. Non-significant alterations in total-cholesterol, VLDL-C, TGs, AST, ALP, serum electrolytes (K, Cl), Hb, haematocrit, and testosterone that observed in agreement with various previous reports [26][27][28]. The levels of enzymes ALT that is protein in nature chemically and total serum bilirubin observed significant decreases which also reported by many workers [14][29].

In the presence study there were significant decrease in BMI and body weight ( $P < 0.0001$ ). Mostly investments reported similar results [11, 30]. Despite the fact that, after Ramadan some others reported no-significant changes in body weight [31] and BMI [31][32].

A reduction in haemoglobin concentration and haematocrit might be due to the incomplete dehydration period which amplified by stop drinking and nutritional habits during the holy Ramadan [33].

Vitamin D is an essential fat-soluble vitamin with multiple functions, the main source of vitamin D in humans is exposure of the skin to sunlight [34]. However, there is a limited researches about impact of fasting on levels of vitamin D, references shows that measuring of vitamin D should be done from September to March for healthy individuals.

According to our data there is a significant decreases in vitamin D levels for healthy adult males ( $p < 0.01$ ) and investigations showed that there were a correlation between vitamin D deficiency and lipid profile/dyslipidemia as far Karhapää et al reports serum vitamin D levels are negatively correlated with TC, TG and LDL-C in middle-aged Finnish males [35]. As well as the findings from Sun's study of 136 Japanese males aged 20-79 years indicate that vitamin D is inversely correlated with TG and LDL-C:HDL-C [36]. Dietary intake and sunlight duration besides fasting could have good prognoses on lowering of vitamin D deficiency in the month of Ramadan.

As Mesbahzadah (2005) reported in his study is the sex hormone in healthy adult males affected by fasting become reduced however, it is not reached to significant level [28].

Previous studies showed that abstinence from eating and drinking during Ramadan fast, which is accompanied by variations in the sleeping and waking pattern, and the psychological effects of fasting may bring about rhythmic changes in the secretion of most body's hormones [37][38].

The present study found decrease in level of CRP after 28 day, this result in agreement with Aksungar (2007) reported there was significant low level of CRP ( $P < 0.001$ ) among fasting subjects when it compared to basal values (one week before Ramadan) [39]. Systemic inflammation can be measured by CRP which is made by liver [40]. As well as investigations have shown that in young, apparently healthy men, serum leptin but not BMI, was freely related to CRP, which indicate that the amount of body fat may be the most significant predictor of CRP [41].

In conclusion, consuming two meals per a day during month of Ramadan has a balancing effect on biochemical, enzymes and CRP in fasted men, dietary restrictions

should be hold during fasting month.

Lowering of serum vitamin D as it was marked in our study can predict the effect of this vitamin on lipid profile and risk of cardiovascular diseases in future. C-reactive protein observed to be statistically significant which foretell as an indicator for systemic inflammatory markers.

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