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*Original Research Article*

**EFFECT OF RAMADAN FASTING ON SERUM PROTEIN  
CONCENTRATIONS IN MALE AND FEMALE UNIVERSITY STUDENTS**

**S.A. Nagra<sup>1</sup>, N. Shaista<sup>1</sup>, M.Z.A. Nomani<sup>2</sup>, and A. Ali<sup>3\*</sup>**

<sup>1</sup>Biochemistry Section, Institute of Chemistry, University of the Punjab, Lahore-54590, Pakistan

<sup>2</sup>Human Nutrition and Foods Discipline, Division of Family Resources, West Virginia University, Morgan Town 26506-6124, USA

<sup>3</sup>Department of Food Science and Nutrition, College of Agricultural and Marine Sciences, Sultan Qaboos University, P.O. Box 34, PC 123, Al-Khoud, Muscat, Oman.

**ABSTRACT**

The present study evaluated the effect of Ramadan fasting on metabolic changes in serum proteins concentration in male and female university students. Thirty healthy university students (15 female and 15 male; average age  $22 \pm 1.8$  years) with average body weights of  $52.4 \pm 2.8$  kg and  $64.7 \pm 5.1$  kg for female and male respectively, residing in separate university student's hostel for males and females but with common dining arrangements, participated in the study. Fasting blood samples were obtained two days before the start of Ramadan fast and on 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day of Ramadan. Daily dietary energy and protein intakes were  $2100 \pm 141$  kcal and  $2378 \pm 145$  kcal and 55.4 g and 58.8 g for females and males respectively. Overall the body weight did not show any significant ( $P > 0.05$ ) variation during or at the end of fasting period. Serum total proteins and albumin levels declined in both the genders with advancing days of fasting. The decline in albumin levels in females was however much sharper and curvilinear ( $r^2 = 0.4$ ,  $P < 0.001$ ) whereas in males it was linear ( $r = -0.69$ ,  $P < 0.001$ ). The minimum level of albumin (4.2 g/dL) reached on 21<sup>st</sup> and 28<sup>th</sup> day of fasting. Although the levels of serum globulin, blood urea nitrogen and serum creatinine increased significantly ( $P < 0.05$ ) with the advancement of fasting days, the values remained within the normal physiological limits. The results suggest that Ramadan fasting has no adverse effects in normal healthy male and female university students.

**Keywords:** Ramadan fasting, serum proteins, body weight, health effects, university students.

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\* **Corresponding Author:** Dr. Amanat Ali, <sup>3</sup>Department of Food Science and Nutrition, College of Agricultural and Marine Sciences, Sultan Qaboos University, P.O. Box 34, PC 123, Al-Khoud, Muscat, Oman. Email: [amanat@squ.edu.om](mailto:amanat@squ.edu.om)

## INTRODUCTION

Ramadan fasting is a religious obligation for every healthy adult Muslim as ordained in Al-Quran (the holy book of Muslims), "O you who believe, fasting is prescribed to you as it was prescribed to those before you so that you can learn "Taqwa" (meaning piety, or abstaining from wrong doings and God fearing)" (Al-Quran 2:183). An exemption from fasting is however allowed under certain conditions e.g., for travelers, sick persons and people with some physiological disabilities, the situations where fasting is expected to be dangerous for the fast observers. One of the mandatory restrictions of fasting in Ramadan (9<sup>th</sup> month of lunar calendar) is to abstain from eating and drinking between the dawn and sunset. The length of the Ramadan fast may vary from 12 to 19 hours depending upon the season of the year and the geographical location of the country. Ramadan fasting differs from the total fasting as eating is imperative at least twice in 24 hours (pre-dawn and after sunset and in between these timings) and there is no restriction on the nature of foods and drinks to be consumed provided it is otherwise permissible (*Halal*) by Islam. Ramadan fasting can however affect the eating patterns, eating frequency and sleep that can change the biorhythm of nutrient intake drastically (Reilly and Waterhouse 2007, Erol *et al.*, 2008, Haouari *et al.*, 2008, Bahammam *et al.*, 2010). Apart from religious and spiritual considerations, Ramadan fasting has been the subject of scientific discussions whether or not Ramadan fasting confers any detrimental physiological and health effects on the body (Azizi, 2010, Maughan *et al.*, 2010). During Ramadan fasting, changes in eating pattern and circadian rhythms occur that affect the chronobiological and behavioural parameters and may alter the important enzymatic and metabolic responses (Nagra and Gilani, 1991, Nagra *et al.*, 1998, Rocky *et al.*, 2004, Ibrahim *et al.*, 2008). A number of studies have been conducted to evaluate the various aspects of Ramadan fasting on different gender groups under different physiological states and in different geographical locations (Gumaa *et al.*, 1978, Mustafa *et al.*, 1978, Nomani *et al.*, 1989, Nagra *et al.*, 1991, Khan and Khatkhat, 2002, Azizi 2010, Maughan *et al.*, 2010). The total quantum of results from these studies is however variable and inconsistent to draw any definite conclusion about adverse effects of Ramadan fasting on human health. Many studies concluded that Ramadan fasting did not induce any harmful effects in young healthy subjects (Rocky *et al.*, 2004, Ibrahim *et al.*, 2008) but on the contrary can produce improvement in carbohydrate and lipid metabolism (Adlouni *et al.*, 1997, Furuncuoglu *et al.*, 2007). Variable results have been reported in the literature on body weight changes as a result of Ramadan fasting (Fedail *et al.* 1982; Hallak and Nomani, 1988; Nomani *et al.*, 1990; El-Ati *et al.*, 1995; Maislos *et al.*, 1998; Aksunger *et al.*, 2005; Ziaee *et al.*, 2006; Al-Hourani and Atoum, 2007; Mansi, 2007). Ramadan fasting affects various biochemical and physiological parameters of blood. Most of the studies regarding the effects of Ramadan fasting on blood lipid profile have shown either no change or some slight decrease in total cholesterol and triglycerides (El-Ati *et al.*, 1995; Adlouni *et al.*, 1997; Aksunger *et al.*, 2005; Ziaee *et al.*, 2006). A few studies have reported an increase in blood lipid levels (Fedail *et al.*, 1982; Gumaa *et al.*, 1978). However, most of the studies have shown some positive effects of Ramadan fasting on the plasma lipids and lipoproteins. Changes in feeding behaviour during Ramadan have been reported to have some beneficial effects on serum apo-lipoprotein metabolism and can lower the risk of coronary artery disease (Adlouni *et al.*, 1998, Bener *et al.*, 2006; Lamri-Senhadji *et al.*, 2009). El-Hazmi *et al.* (1987) reported an increase in serum total proteins and albumin whereas Aksunger *et al.* (2005) and Maislos *et al.* (1998) observed no changes in serum proteins as a result of Ramadan fasting. The variability in results could be attributed to a number factors such as differences in study protocols, choice of sampling, nutritional and socioeconomic status of study subjects,

diurnal variations, dietary habits and customs, geographical location, as well as seasonal and climatic differences in the month of Ramadan. The present study was conducted to evaluate the impact of Ramadan fasting on body weight and serum protein concentrations in adult male and female university students residing in Punjab University hostels under local socio-cultural traditions.

## MATERIALS AND METHODS

### *Study Sample and Time*

The study was conducted on graduate students residing on campus in the student's hostel of the University of the Punjab. Thirty normal adult healthy university students (15 female and 15 male) between the age of 21-24 years (average age  $22 \pm 1.8$  years) participated in the study. The average body weight and body mass index (BMI) of the female and male volunteers were  $52.4 \pm 2.8$  kg  $67.4 \pm 5.1$  kg and  $21.8 \pm 1.3$  and  $22.6 \pm 1.5$  respectively. The physical activity level within both the genders was almost identical. All the volunteers were briefed about the study protocol and an oral consent was obtained for their participation in the study. The plan of the study was approved by the Institutional Research Ethics Committee.

### *Food Intake and Meal Plan*

All the volunteers resided in University of The Punjab hostels where a fixed menu was served to each volunteer at *Sahoor* (a predawn meal) and *Iftar* (light breakfast and dinner). There was no special change in the hostel food menu before and after the onset of Ramadan, except that during the month of Ramadan at the time of Iftar (light breakfast at sunset). The predawn meal (*Sahoor*) is usually a little lighter than the main meal served at sunset, at the time of opening the fast. The predawn meal consisted of Pratha (local bread baked with oil/fat), vegetable curry, vegetables mixed with meat curry and tea or some yoghurt drink. The *Iftar* meal (the sunset meal/breakfast at the time of opening the fast) consisted of a glass of juice or yoghurt drink, dates, pakoras (a sort of fried spicy food made from chickpea flour dough and onions) and mixed fruit salad with spicy boiled chickpeas. The main course consisted of Chapati (local wheat flour bread) with meat, chicken and/or mixed vegetables curry. Rice dishes were also offered twice a week which included either boiled rice or Biryani (rice cooked with meat or chicken). Almost the same types of foods were offered on routine basis with slight changes in the menu to break the monotonous offering of the same foods. Standard portion sizes of various foods were offered to all the students. However, there was no restriction on eating of foods and the students could get extra food if they needed. The leftover foods were carefully monitored and were taken into consideration while calculating their daily nutrient and energy intakes.

The average daily consumption of macronutrients (protein, carbohydrate and fat) and the total caloric intake were worked out from the food composition tables (Shah, 1988). The total daily energy intake from the meals was calculated based on the physiological fuel values of 4, 9, and 4 kcal/g for carbohydrate, fat and protein respectively. The amount of protein and energy turned out as 55.4 g and 58.8 g and 2100 kcal and 2378 kcal for female and male students respectively. These estimates are based on the amount of foods offered to the volunteers and do not exclude the leftover foods. It was observed that the amount of leftover food increased (about 5-10%) with the advancement of Ramadan fasting. The percentage contribution of energy from various

macronutrients for females was 11% from protein, 29% from fat and 60% from carbohydrates whereas for males it was 10% from protein, 32% from fat and 58% from carbohydrates. The menstruating women were assumed to have almost the same consumption pattern as the fasting one. This was due to the fact that during the fasting month at daytime none of the hostel canteen or the snack bar is open and they only had the chance to eat at predawn and sunset together with other volunteers. Moreover prior to the onset of study this problem was discussed with the volunteers and they promised not to make a marked depart from the routine during the menstruating period.

### *Blood sampling*

Seven ml of venous blood was drawn from each volunteer after about 11 hours of fasting two days before the onset of holy month of Ramadan (Day 0) and then on 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day of Ramadan. After the extraction of serum, various biochemical tests were performed.

### *Reagents and Procedures*

Serum total proteins and albumin (A) were determined by Biuret method (Reinhold, 1953) and dye binding method (Gowenlock *et al.*, 1988), respectively. Blood urea nitrogen was determined enzymatically (Wilcox *et al.*, 1966) using the diagnostic kit obtained from "Biocon Diagnostik" Germany. Serum creatinine was determined according to the method as described by Heinegård and Tiderström (1973). Serum globulins (G) were calculated by subtracting the serum albumin from total proteins. Albumin to globulin ratio (A/G ratio) was also worked out. Weekly body weights were recorded using an electronic balance reading up to 0.1 kg. All the subjects were weighed in light clothing and without shoes.

### *Statistical Procedures*

Descriptive statistics and one way analysis of variance (ANOVA) was performed and the means were compared by LSD test as described by Snedecor and Cochran (1989). The data obtained on day 0, 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> days was analyzed by using the statistical software package SPSS.

## **RESULTS AND DISCUSSION**

Overall no significant ( $P < 0.05$ ) differences were observed in body weight of both genders either during or at the end of the fasting period (Fig 1). Variable results have been reported in the literature on body weight changes as a result of Ramadan fasting. Some studies have shown a decrease in body weight (Fedail *et al.*, 1982; Hallak and Nomani, 1988; Ziaee *et al.*, 2006; Al-Hourani and Atoum, 2007; Mansi, 2007; Khalid and Belbraouet, 2009) whereas the others (El-Ati *et al.*, 1995, Maislos *et al.*, 1998; Aksunger *et al.*, 2005) did not observe any difference in body weight as a result of Ramadan fasting. Our results are in line with these findings. The variability in the results could be attributed not only to differences in the study design but also to many other factors such as environmental, geographical region, socio-cultural and lifestyle.

The results on the effects of Ramadan fasting on the total serum protein levels are given in Table 1. Significant ( $P < 0.05$ ) differences were observed in the total serum proteins level as a result of Ramadan fasting. Irrespective of the gender, a general increase in serum total proteins level was observed after the first week of fasting, which showed a declining trend after the 2<sup>nd</sup> week of

fasting but became almost stable during the rest of fasting period in case of females. However, in case of males it touched the lowest levels at the end of 4<sup>th</sup> week of fasting. The net decrease in the total serum proteins level was 0.17 g and 0.25 g/dL in case of female and male volunteers, respectively. Apparently the loss of total protein was greater in case of males as compared to females. Serum total proteins are comprised of various fractions, mainly pre-albumin, albumin and globulins. The existence of a small amount of protein reserve in the liver that can readily leave the liver as a result of fasting or due to an abrupt dietary change from higher to lower protein intake is termed as storage or labile protein. It has been shown that with the increase in fasting period, the proteins leaving the liver increases. This protein store is considered to constitute about 3 per cent of body protein in well-nourished human beings. This amount appears insignificant in terms of total body nitrogen economy but is important for understanding the adoptive response (Munro, 1964). The nutritional disturbances affect the regulatory control of hepatic protein synthesis (Sidransky, 1976). Inconsistent results have however been reported in the literature on the serum protein level as a result of Ramadan fasting (El-Hazmi *et al.*, 1987; Maislos *et al.*, 1998; Aksunger *et al.*, 2005). Aksunger *et al.* (2005) and Ibrahim *et al.* (2008) did not observe any change in serum protein and albumin levels as well as in biomarkers of oxidative stress whereas El-Hazmi *et al.* (1987) reported an increase in the values. The changes in serum protein levels observed in this study indicate the changing dietary patterns/intakes during the Ramadan fasting.

**Table 1 Effect of Ramadan fasting on serum total proteins level (g/dL)\***

Days of Fasting	Total Protein			
	Female	Variation from day 0	Male	Variation from day 0
Day 0	7.61 ± 0.55 <sup>a</sup>		7.48 ± 0.48 <sup>a</sup>	
Day 7	7.70 ± 0.27 <sup>a</sup>	+ 0.09	7.68 ± 0.28 <sup>a</sup>	+0.20
Day 14	7.47 ± 0.33 <sup>b</sup>	- 0.14	7.59 ± 0.37 <sup>a</sup>	+0.11
Day 21	7.41 ± 0.25 <sup>b</sup>	- 0.20	7.48 ± 0.28 <sup>b</sup>	0.00
Day 28	7.44 ± 0.16 <sup>b</sup>	- 0.17	7.23 ± 0.17 <sup>c</sup>	-0.25

\*= Different alphabets in the same column means significant at 5%.

**Table 2 Changes in the serum albumin level during Ramadan fasting (g/dL)\***

Days of Fasting	Albumin			
	Female	Variation from day 0	Male	Variation from day 0
Day 0	5.04 ± 0.49 <sup>a</sup>		4.98 ± 0.35 <sup>a</sup>	
Day 7	4.59 ± 0.37 <sup>b</sup>	-0.45	4.87 ± 0.31 <sup>a</sup>	-0.11
Day 14	4.32 ± 0.33 <sup>c</sup>	-0.72	4.63 ± 0.39 <sup>b</sup>	-0.35
Day 21	4.22 ± 0.31 <sup>c</sup>	-0.82	4.35 ± 0.30 <sup>c</sup>	-0.63
Day 28	4.27 ± 0.37 <sup>c</sup>	-0.77	4.18 ± 0.26 <sup>c</sup>	-0.80

\*= Different alphabets in the same column means significant at 5%.

**Table 3 Variations in serum globulin levels with Ramadan fasting (g/dL)\***

Days of Fasting	Globulin			
	Female	Variation from day 0	Male	Variation from day 0
Day 0	2.57 ± 0.17 <sup>b</sup>		2.48 ± 0.20 <sup>b</sup>	
Day 7	3.11 ± 0.31 <sup>a</sup>	+ 0.54	2.81 ± 0.21 <sup>a</sup>	+ 0.33
Day 14	3.15 ± 0.32 <sup>a</sup>	+ 0.58	2.95 ± 0.34 <sup>a</sup>	+ 0.47
Day 21	3.19 ± 0.28 <sup>a</sup>	+ 0.62	3.11 ± 0.30 <sup>a</sup>	+ 0.63
Day 28	3.17 ± 0.38 <sup>a</sup>	+ 0.60	3.05 ± 0.27 <sup>a</sup>	+ 0.57

\*= Different alphabets in the same column means significant at 5%.

**Table 4 Variations in A/G ratio with Ramadan fasting**

Days of Fasting	Albumin/Globulin ratio			
	Female	Variation from day 0	Male	Variation from day 0
Day 0	1.96 <sup>a</sup>		2.00 <sup>a</sup>	
Day 7	1.48 <sup>b</sup>	-0.48	1.73 <sup>b</sup>	-0.27
Day 14	1.37 <sup>bc</sup>	-0.59	1.57 <sup>c</sup>	-0.43
Day 21	1.32 <sup>c</sup>	-0.64	1.40 <sup>d</sup>	-0.60
Day 28	1.35 <sup>c</sup>	-0.61	1.37 <sup>d</sup>	-0.63

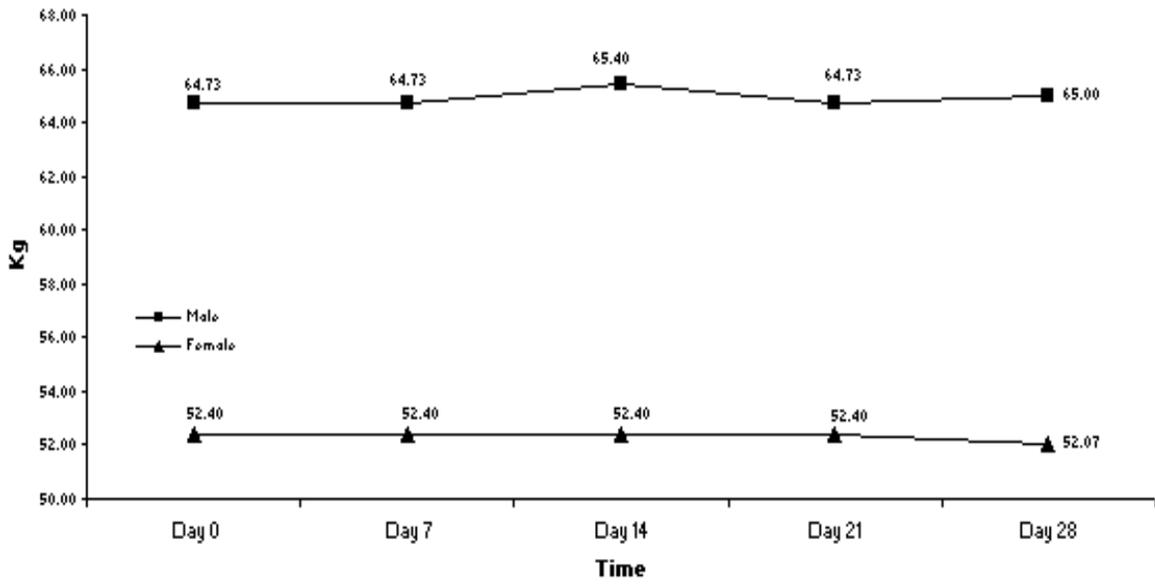
\*= Different alphabets in the same column means significant at 5%.

**Table 5 Variations in blood urea nitrogen with Ramadan fasting (mg/dL)\***

Days of Fasting	Blood Urea			
	Female	Variation from day 0	Male	Variation from day 0
Day 0	9.11 ± 1.77 <sup>d</sup>		9.94 ± 1.89 <sup>d</sup>	
Day 7	12.80 ± 2.08 <sup>c</sup>	+ 3.69	14.16 ± 2.80 <sup>bc</sup>	+ 4.22
Day 14	13.43 ± 1.85 <sup>bc</sup>	+ 4.32	14.58 ± 2.05 <sup>b</sup>	+ 4.64
Day 21	15.33 ± 2.77 <sup>ab</sup>	+ 6.22	16.02 ± 1.58 <sup>ab</sup>	+ 6.08
Day 28	16.61 ± 2.26 <sup>a</sup>	+ 7.50	17.42 ± 1.73 <sup>a</sup>	+ 7.48

\*= Different alphabets in the same column means significant at 5%.

**Fig 1: Body Weight at different stages of Ramadan fasting**



**Fig 2: Serum Albumin at different stages of Ramadan fasting**

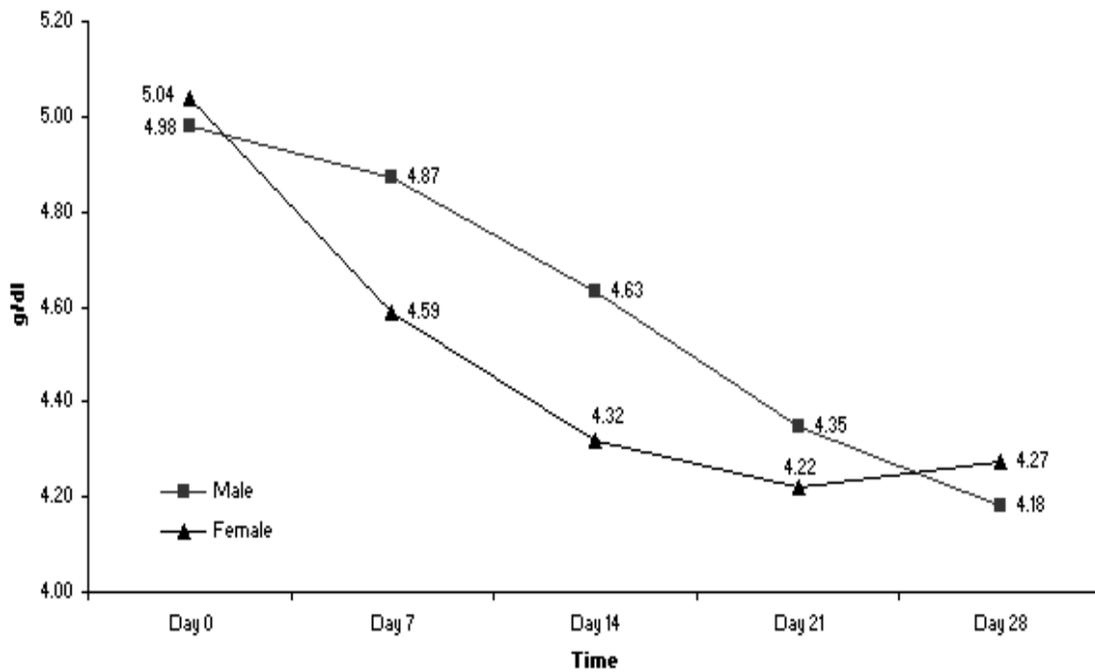
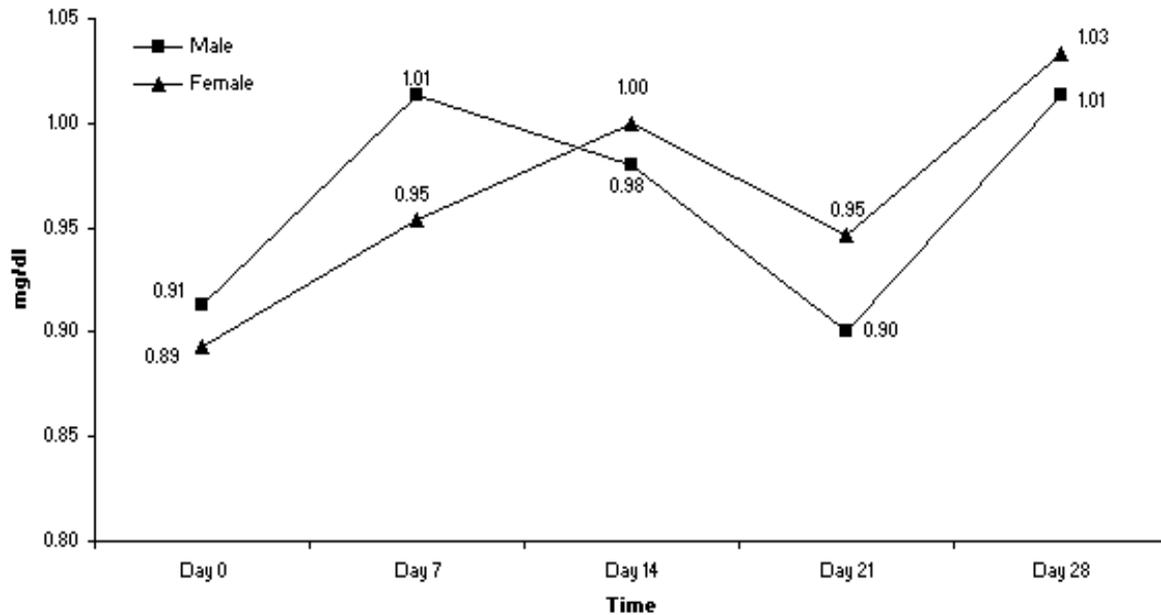


Fig 3: Serum Creatinine at different stages of Ramadan fasting



The results on the effects of Ramadan fasting on serum albumin and globulin levels are given in Tables 2 and 3. An overall decrease in serum albumin level in both the genders was almost identical i.e. 0.77 and 0.80 g/dL for female and male respectively. However, the pattern of change was distinctly different in case of females. Significant ( $P < 0.05$ ) differences in the serum albumin levels were observed as a result of Ramadan fasting. Albumin makes up approximately 60% of the total serum proteins and its major function in the blood is to maintain the colloidal osmotic pressure. As albumin is synthesized within the liver, it is an important measure of hepatic function. Medically a generous concentration of albumin in the blood stream is regarded as a measure of quality of life. Both serum albumin and globulin are the main measures of the nutritional status. The albumin fraction of total protein can however be fictitiously elevated in dehydrated persons. Comparing the data on the level of serum total proteins, albumin and globulin indicated that the changes in serum proteins were mainly due to the changes in serum albumin. The rate of decrease in serum albumin was rapid and showed a different pattern in female than in male students. The results are in line with the earlier findings who either reported a decrease (Mohtasham *et al.*, 2001) or no changes (Kaboudi *et al.*, 2001) in the serum albumin levels as a result of Ramadan fasting. Shirpoor *et al.* (2001) observed an increase in serum albumin levels as a result of Ramadan fasting. El-Hazmi *et al.* (1987) reported an increase in serum total proteins and albumin whereas Aksunger *et al.* (2005) and Maislos *et al.* (1998) observed no changes in serum proteins as a result of Ramadan fasting. These variations may be attributed to the differences in geographical location, season of the year, duration of day light, dietary, lifestyle and socio-cultural factors. Socio-economic, dietary and cultural changes have been reported to affect the serum albumin level in healthy populations. However, no gender differences were observed (Adewoye and Fawibe, 1978). A mild increase in total proteins during the first week of Ramadan fasting may be attributed to the consumption of all the food offered to volunteers with minimum left over food. It was generally observed that during the initial days of Ramadan, the volunteers consumed more to overcome the feeling of hunger during the day.

However, as Ramadan fasting progressed, the new eating pattern affected the hunger and dietary consumption pattern at *Sahoor* and *Iftar*. The food intake was reduced with a subsequent increase in the quantity of leftovers. Although there was no change in the quality of food, a modified hunger and eating patterns with the advancement of Ramadan fasting lowered the food consumption, in particular the intake of protein rich foods that consequently might have affected the labile proteins. The quality of amino acid pool is determined by the quality of dietary protein (Waterlow, 1981). There was no much difference in the albumin to globulin (A/G) ratio in the male and females (Table 4). However with the advancement of fasting, the maximum decrease took place on the 14<sup>th</sup> day of fasting. Although the reduction trend in serum albumin was similar in either gender, a faster and rapid rate of decrease was observed in case of females (Fig 2). It is expected that the loss in labile protein as observed in this study does not continue and ceased after about a fortnight of fasting and may be attributed to physiological adaptation. Since the body weight of the volunteers (Fig. 2) did not show a significant ( $P > 0.05$ ) variation, it may be postulated that depletion of labile protein did not affect the muscular mass. Our results are in line with the findings of Ibrahim *et al.* (2008) who observed no significant differences in the body weight and body composition as well as in total serum proteins and albumin levels. They reported that Ramadan fasting did not alter oxidative stress parameters or biochemical markers of cellular damage in healthy subjects but reduced the serum glucose and triglycerides.

The results on the effects of Ramadan fasting on blood urea nitrogen (BUN) are shown in Table 5. A progressive increase in the level of BUN was observed with the advancement of fasting period. However, the values remained within the normal ranges as considered for healthy people. The changes in serum creatinine levels are shown in Fig 3. The reduction in serum protein and increase in BUN and serum creatinine values could be due to dehydration, excessive break down of nucleic acids (RNA) in tissues and restriction in energy intake. Our results are in line with the findings of Khan and Khattak (2002) who observed similar changes. Although no significant gender differences were observed in BUN values, the female students showed slightly higher BUN values as compared to males, which may be due to lower water intake by females during Ramadan as women usually show a stronger reactivity to external food-related stimuli to eating disorders (Uher *et al.*, 2006). No detrimental effects on health have been attributed due to negative water balance at the levels that may be produced during Ramadan (Leiper *et al.*, 2003). Changes in fluid and food intake during Ramadan indicated some degree of preparation for fasting before sunrise and a marked “recuperation” from fasting after sunset (Waterhouse *et al.*, 2008). Only small insignificant changes in serum urea, creatinine and uric acid levels were observed during Ramadan fasting (El-Hazmi, *et al.*, 1987; Sliman *et al.*, 1988; Nomani *et al.*, 1989). The average calculated daily intake of protein for female and male students in this study was 55.4 g and 58.8 g respectively. The percentage of energy contribution from protein to the total daily energy intake was only 11 and 10% respectively for female and male students. Healthy individuals consuming the recommended protein intake of 0.75 g/kg/day (FAO/WHO/UNU, 1985) can maintain the nitrogen balance, however the rate of turnover of nutrient transport proteins is slower as compared to those consuming their habitual diet (Afolabi *et al.*, 2004). Hence, they may be less able to mount an adequate metabolic response to a stressful stimulus during fasting. Our results are in line with these findings.

## CONCLUSION

Ramadan fasting in general did not ( $P > 0.05$ ) show any adverse effects on the serum proteins and body weight. Although the daily amount of energy and protein provided by the hostel menu is considered to be sufficient enough to meet the recommended nutrient requirements, the results of this study suggest that the university hostel menus should contribute slightly higher amounts of protein during Ramadan fasting to compensate the depletion of labile proteins due to changing food consumption patterns. The nutritional awareness in the management of appropriate and balanced eating habits may be helpful to build up better protein reserves to withstand the fasting stress in Ramadan. Overall the results suggest that Ramadan fasting has no adverse effects in normal healthy male and female university students.

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