

The Effect of Ramadan Fasting on Biochemistry of Saliva

Narjes Khaleghifar¹, Reyhaneh Sariri^{1*}, Mahmoodreza Aghamaali¹, Hosein Ghafoori¹

Abstract

Muslims fast every day from dawn to sunset during the holy month of Ramadan. The possible side effect of Ramadan fasting on general health has been widely studied, but oral health may also alter due to one month fast affecting some of the salivary secondary metabolites. The aim of present research was identifying the influence of fasting on saliva of healthy individuals. The subjects were selected from non-smoker male employees of one factory in Rasht. 35 healthy male (aged 30-50 years), who fasted the whole Ramadan entered. Their unstimulated saliva samples were collected before, during and at the end of Ramadan. Concentration of salivary uric acid, the activity of alkaline phosphatase (ALP) and aspartate aminotransferase (AST) were then measured using spectrophotometric methods according to procedure given for serum. The results showed a significant reduction in the concentration of salivary uric acid and AST, while the activity of ALP was significantly increased. It was concluded that some of salivary biochemical markers undergo various fluctuations in response to fasting. However, more studies with a larger population and various biochemicals are required to certainly comment on the matter.

Keywords: Alkaline Phosphatase, Aspartate Aminotransferase, Fasting, Ramadan, Saliva, Uric Acid

1. Department of Biology, University of Guilan, Rasht, Iran

* Corresponding Author

Reyhaneh Sariri
Department of Biology, University of Guilan,
Rasht, Iran
E-mail: sariri@guilan.ac.ir

Submission Date: 7/10/2017

Accepted Date: 10/29/2017

Introduction

Several great religions recommend a period of fasting or abstinence from certain foods. In Islam religion, fasting is mandatory for healthy adults during the whole month of Ramadan. Muslims fast every day from dawn to sunset during this month. They must avoid not only from eating and drinking, but also from consuming oral medications and intravenous nutritional fluids. Although in the healthy subject, Ramadan fasting may not appreciably affect one's health, it could induce some complications in patients with important metabolic disorders such as diabetes [1]. The effect of experimental short-term fasting on carbohydrate metabolism has been extensively studied [2, 3]. We have previously studied alternations of glucose in saliva during fasting in Ramadan and reported that the concentration of glucose in saliva is reduced during the fasting period [4]. Ramadan type fasting has been shown to influence activity of some proteins and enzymes in other body fluids such as tears [5]. However, studies on heart patients have claimed that, among the test population, most patients with stable cardiac disease can fast during Ramadan without significant detrimental effects [6]. It is believed that fasting during Ramadan has a minimal effect on stable patients with chronic heart failure [7]. According to their study, most patients suffering from stable cardiac disease can fast during Ramadan without significant detrimental effects [7]. The possible effect of Ramadan fasting on hemoglobin, glucose and renal function has been studied [8]. They have reported no significant decrease in hemoglobin and glucose and no significant increase in creatinine [8]. Heart rate variability (HRV) is an independent predictor of increased mortality of patients with myocardial infarction

and congestive heart failure [9]. It has been found that HRV parameters have decreased in Ramadan month compared to after Ramadan in hypertensive patients [9].

Although, it can be suggested that dehydration process could aggravate formation of urinary calculi, the effects of fluid and food restriction on calculus formation is not thoroughly studied. However, it is reported that Ramadan fasting could influence total excretion and concentrations of urinary precipitate [10].

Saliva is the first biological fluid to encounter external factors including changes in eating habits and environmental or physical changes. The biochemical composition of saliva, as the first body fluid gating the gastrointestinal tract, is of prime importance. Various salivary markers may influence oral health both through its non-specific physico-chemical properties and specific effects [9]. Saliva is well known for its highly protective functions against deleterious agents such as microorganisms, toxins and various oxidants [11]. The antioxidant capacity and reducing power of saliva is altered due to various factors including age [12] exercise [13-17] dietary supplementation [18, 19], food preservatives [20], internal diseases [21-24], smoking [25] and even passive smoking [26]. It has been shown that in vitro exposure to cigarette smoke could significantly decrease some enzymatic activities, both in plasma and in saliva [18, 27]. Due to the presence of valuable markers, non-invasive and easy sampling, saliva has recently attracted our attention to be used as a diagnostic body fluid [28, 29].

Our previous research activities were mainly based on the importance of saliva as a non-invasive biological fluid and its antioxidant alternations at a wide range of biological

conditions. Therefore, the main aim of present research was to investigate alternations of some important non-antioxidant enzymes as well as the major salivary water soluble antioxidant, uric acid, in the oral fluid of subjects before, during and after the month of Ramadan fasting.

Materials and Methods

Materials

The level of uric acid in saliva was measured using an enzymatic method based on oxidation of uric acid by uricase followed by determination of resulting H_2O_2 in the presence of peroxidase. The enzymatic uric acid assay kit was purchased from Pars AzmoonTM.

Salivary ALP was measured using a kinetic method in the presence of *p*-nitrophenyl phosphate as substrate. Salivary AST was also measured by a kinetic method based on oxidation of NADH to NAD^+ by malate dehydrogenase. NADH oxidation rate is proportional to the activity of AST samples. The appropriate kits for assay of AST and ALP were also purchased from Pars AzmoonTM.

Design

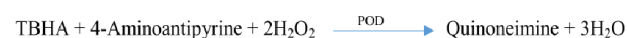
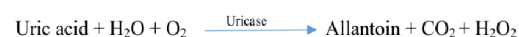
The subjects were selected from non-smoker male employees of one factory in Rasht. 35 healthy male (aged 30-50 years), who fasted the whole Ramadan agreed to participate in our study. The aim of research was explained to all of them and a precise signed consent was obtained from each individual. They had healthy teeth and mouth, without any oral or internal disease and exceptions were excluded from the research.

Saliva sampling

After gagging their mouth with about 5.0 ml of distilled water for about one minutes, their un-stimulated whole saliva samples (about 3ml) were collected in clean, dry and sterile pre-weighted tubes. They were asked to donate their saliva samples the day before Ramadan, day 15 and the last day of the month at noon (after about 8 hours fasting). Their first saliva sample, i.e. before Ramadan fasting, was used as control. All saliva samples were immediately centrifuged at 800 g for 10 min at 4°C to remove squamous cells and cell debris. The resulting supernatant was stored at -18°C until used for determination of uric acid concentration and activity of AST and ALP. They were analyzed within 48-72 hours of collection. Each assay was repeated three times and data obtained were expressed as mean \pm SD of the three measurements.

Salivary uric acid

Uric acid concentration was determined in supernatant of saliva samples collected from volunteers. The level of uric acid in saliva was measured using an enzymatic method based on oxidation of uric acid by uricase followed by quantitation of resulting H_2O_2 in the presence of peroxidase (POD).



The procedure was followed as recommended by the manufacturer. In practice, the two reagents of the kit were mixed at a ratio of four to one. 1000 μ l of the mixed solution was added to 20 μ l of each saliva sample. The mixture

was incubated in 37°C water bath and the intensity of the resulting color was measured spectrophotometrically at 546 nm against a blank containing 1000 μ l reagent and 20 μ l distilled water. The concentration of uric acid (C_U) was then calculated using the Beer-Lambert's equation from absorbencies of standard solution (A_S), sample (A_T) and concentration of standard (C_S).

$$C_U \text{ (mg/ml)} = A_T/A_S \times C_S \text{ (mg/dl)}$$

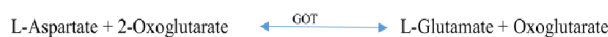
Assays of alkaline phosphatase (ALP) and aspartate aminotransferase (AST)

The activity of ALP was determined in supernatant of saliva samples collected from volunteers.

The level of ALP in saliva was measured using a Pars Azmoon kitTM based on a kinetic method in the presence of *p*-nitrophenylphosphate as substrate. The product of reaction, *p*-nitrophenol, was calculated by measuring its absorbance at 405 nm. The increase in absorption at this wavelength is proportional to the activity of ALP.



For assay of AST, the level of glutamate oxaloacetate transaminase (GOT) was first measured in saliva by a kinetic method. GOT catalyzes the transfer reaction of an amino group of L-aspartate to 2-oxoglutarate with formation of L-glutamate and oxoglutarate. The resulting oxoglutarate then reacts with NADH by the action of malate dehydrogenase to produce NAD^+ . The oxidation rate of NADH to NAD^+ is proportional to the GOT activity in sample.



Statistical analysis

Each assay was repeated triplicate and the results were presented as mean \pm SD values. Statistical difference between groups was compared by paired t-test, *p* values less than 0.5 were taken as significant.

Results

Figure 1 shows mean content of uric acid in saliva of all volunteers during fasting and non fasting period. In Fig. 2 the average activity of ALP in saliva of volunteers during fasting and non fasting period has been compared. The average activity of AST is shown in Fig. 3.

The data presented in these figures are the mean values obtained from examining saliva samples of 35 volunteers.

Discussion

According to the rules of the Islam, it is mandatory for every healthy adult to fast one month each year. In holy month of Ramadan it is allowed to eat and drink two times a day, one before dawn and one after sunset. The period of fasting differs each year and sometimes Ramadan occurs in summer. Some biochemical composition of saliva may change due to fast, leading to metabolic disorders. The results of this study showed the effects of fasting during

Ramadan on some biochemical salivary compounds. Only a few studies have reported the effects of fasting during this month on biochemical composition of saliva. For example, no significant change in urea and uric acid concentrations have been reported [30]. The mentioned study was conducted during November (temperature about 15°C) in order to measure the influence of fasting on serum urea and uric acid in serum. They have found no significant differences in the level of both uric acid and urea before and after fast. Uric acid, the product of purine metabolism, is an important antioxidant in most body fluids.

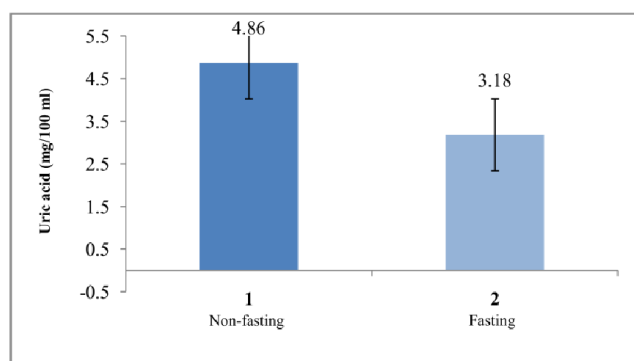


Figure 1. Significant differences ($p \leq 0.05$) between mean concentration of uric acid in saliva of non-fasting (control) and fasting group.

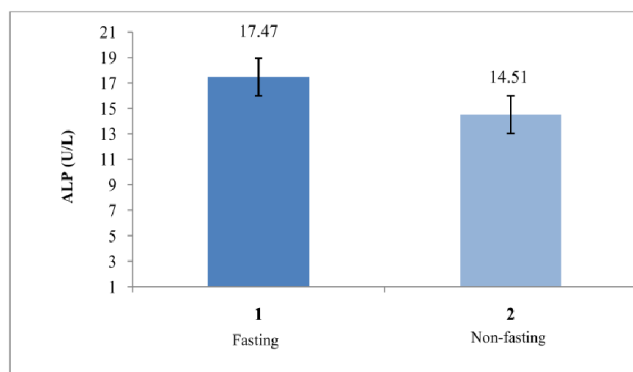


Figure 2. Significant differences ($p \leq 0.05$) between mean activity of ALP in saliva of non-fasting (control) and fasting group.

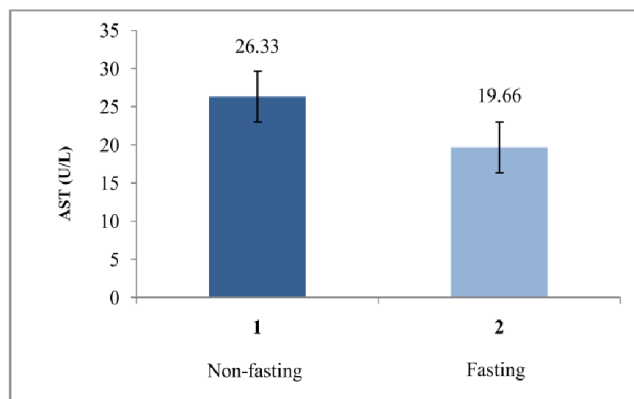


Figure 3. Significant differences ($p \leq 0.05$) between mean activity of AST in saliva of non-fasting (control) and fasting group.

It has low solubility in water and its increased concentration may result in urinary urate stones. Although increased uric acid concentration is expected due to dehydration that occurs in the last few hours of a fasting day, but we found that uric acid in saliva is decreased. This result is important when one considers its antioxidant role in saliva. The effect of Ramadan fasting on many biochemical and health factors are studied to various extents. For example the impact of Ramadan fasting in glucose level and diabetes control [1, 2], heart failure patients [7, 8], blood pressure [9], renal function [10], urinary risk factor [30-32], lipids in blood [33], apolipoproteins [34], even voice of male [35] and female [36] have been investigated and reported. However, according to our literature survey, possible alternations of uric acid levels in serum or saliva during Ramadan fasting has not been investigated yet. In the present research, we found a significant decrease in salivary uric acid during Ramadan fasting. It is suggested that decrease in uric acid level could be related to lower intake of purine bases during Ramadan fasting and reduced purine catabolism.

We also found a significant increase in the activity of alkaline phosphatase of saliva in fasting conditions as compared to before Ramadan. It has been shown that ALP in maternal plasma increased significantly during Ramadan, but no difference ($P > 0.05$) was observed between ALP at the end of Ramadan and 2 weeks after Ramadan [32]. The increase in maternal ALP during pregnancy is due to ALP of placental origin leading to a sharp increase in the rate of bone metabolism. We suggest that the observed increased activity of salivary ALP in fasting state may be the result of major changes in metabolic interactions among the organs that produce this enzyme. Therefore, higher levels of ALP may be released into the blood and saliva during starvation and illness. Although salivary ALP is not investigated widely, it is reported that liver alkaline phosphatase has significantly increased due to a Ramadan type fasting in rats [37]. The mentioned study has also shown that many important enzymes and non enzymatic factor are altered as a result of a Ramadan type fasting [37].

The result of our study also indicated that activity of AST in fasting volunteers decreased significantly during Ramadan. In 2011, Choudhury *et al.*, observed that feeding of semi cooked fresh oyster mushroom at the iftar table during Ramadan fasting decreased the serum levels of AST ($P < 0.05$) of male subjects indicating oyster mushroom is able to ameliorates liver functions [38]. The increase can be explained by the fact that fasting reduces the metabolism of body tissues cells including oral cavity cells, leading to reduced rate of saliva during fasting.

Conclusion

Normal salivary function is a critical factor for maintenance of healthy oral mucosa. Oral fluid provides an easily available non-invasive biological sample to be considered for use as a diagnostic novel body fluid for a wide range of diseases and clinical situations. The laboratory findings reported in this research indicate that the concentration of uric acid, the important water soluble salivary antioxidant, and the activity of AST in saliva is decreased as a result of

Ramadan fasting, but the biological activity of ALP is significantly increased. Based on these results, it could be concluded that some of salivary biochemicals undergo various fluctuations in response to fasting. However, more studies with a larger population and various testing factors are required to certainly conclude the matter.

Acknowledgements

The authors would like to express their thanks to University of Guilan.

References

- Lessan, N., Hannoun, Z., Hasan, H., Barakat, M.T., Glucose excursions and glycaemic control during Ramadan fasting in diabetic patients: Insights from continuous glucose monitoring (CGM). *Diabetes Metab*, 2015, Vol. 41(1), pp. 28-36.
- Cahill, G.F., Starvation in men, *N Engl J Med*, 1970, Vol. 282, pp. 668-675.
- Herber, D. Endocrine response to starvation, malnutrition and illness. In: *DeGroot, Ly*, Ed. *Endocrinol*. 3rd Ed., Philadelphia: Saunders, 1995, pp. 2663-2678.
- Sariri, R., Varasteh, A., Erfani, A., Alternations in salivary glucose during Ramadan fasting. *Health*, 2010, Vol. 2(7), pp. 769-772.
- Sariri, R., Varasteh, A., Sajedi, R.H. Effect of Ramadan fasting on tear proteins. *Acta Medica (Hradec Králové)*, 2010, Vol. 53(3) pp. 147-151.
- Chamsi-Pasha, H., Waqar, H., Khaled, A., Al-Shaibi, F., The cardiac patient during Ramadan and Hajj, *J Saudi Heart Assoc*, 2014, Vol. 26(4), pp. 212-215.
- Haloui, F., Elazaoui, N., Habbal, R., The impact of diurnal fasting during Ramadan on patients with chronic heart failure. *Arch Cardiovasc Dis*, 2015, Vol. 7(2), pp. 175-176.
- Haloui, F., Elazaoui, N., Habbal, R., Does Ramadan fasting has any effects on hemoglobin, glucose and renal function in patients with chronic heart failure?. *Arch Cardiovasc Dis*, 2015, Vol. 7(2), pp. 172-181.
- Bensahi, I., Houari, C., Elfhal, A., Habbal, R., Does fasting in Ramadan affect right ventricular diastolic function in hypertensive patients? A moroccan prospective observational study. *Arch Cardiovasc Dis*, 2015, Vol. 7(2), pp. 185-190.
- Einollahi, B., Lessan-Pezeshki, M., Simforoosh, N., Nafar, M., Pour-Reza-Gholi, F., Firouzan, A., Khatami, M.R., Nourbala, M.H., Pourfarzini, V., Impact of Ramadan fasting on renal allograft function. *Transplant Proc*, 2005, Vol. 37(7), pp. 3004-3005.
- Dodds, M.W.J., Johnson, D.A. Yeh, C.K., Health benefits of saliva. *J Dent*, 2015, Vol. 33(3), pp. 223-233.
- Kohen, R., Tirosh, O., Kopolovich, K., The reductive capacity index of saliva obtained from donors of various ages. *Exp Gerontol*, 1992; Vol. 27(2), pp. 161-168.
- Damirchi, A., Sariri, R., Kiani, M., Jafarian, V., Response of salivary peroxidase to exercise intensity. *Eur J Appl Physiol*, 2010, Vol. 108, pp. 1233-1237.
- Nazari, Y., Damirchi, A., Sariri, R., Tahery, M., Response of salivary antioxidants to intense exercise in non-athlete men. *J Exerc Physiol online*, 2012, Vol. 15(3), pp. 1-9.
- Erfani Karimzadeh, A., Rezaei, A., Sariri, R., Salivary antioxidant enzymes in young exercised women. *Medicina Sportiva*, 2013, Vol. 9(3), pp. 2166-2170.
- Rahmati, S., Arazi, H., Sariri, R., Resistance exercise loading pattern and antioxidant capacity of saliva. *Medicina Sportiva*, 2013, Vol. 36(4), pp. 2221-2227.
- Sariri, R., Damirchi, A., Nazari, Y., Salivary antioxidant variations in athletes after intense exercise. *Medicina Sportiva*, 2013, Vol. 9(1), pp. 2043-2050.
- Amani, S., Erfani Karimzadeh Toosi. A., Sariri, R., Razmi, N., Beneficial effect of aerobic exercise and green tea on serum and saliva antioxidants. *Medicina Sportiva*, 2013, Vol. 9(3), pp. 2198-2202.
- Damirchi, A., Saati, A.R., Sariri, R., Salivary antioxidants of male athletes after aerobic exercise and garlic supplementation on: A randomized, double blind, placebo-controlled study. *J Oral Biol Craniofac Res*, 2015, Vol. 5, pp. 146-152.
- Heidari, Z., Sariri, R., Jafarian, V., Responce of salivary peroxidase to food preservatives. *Pharmacol online*, 2010, Vol. 2, pp. 186-194.
- Motamedinia, M., Masourghanaei, F., Sariri, R., Vesal, M., Salivary enzymes in peptic ulcer disease. *J Oral Biol Craniofac Res*, 2013, Vol. 3, pp. 83-87.
- Boghoori M, Aghamaali MR, Sariri R, Mohamadpour F, Ghafoori H. Salivary enzymes and flow rate: Markers of peptic ulcer. *J Oral Biol Craniofac Res*, 2014, Vol. 4(1), pp. 24-29.
- Zaeni, S.H., Derakhshan, Z., Sariri, R., Alternations of salivary antioxidant enzymes in systemic lupus erythematosus. *Lupus*, 2015, Vol. 24(13), pp. 1400-1405.
- Moori, M., Ghafoori, G., Sariri, R., Nonenzymatic antioxidants in saliva of patients with systemic lupus erythematosus. *Lupus*, 2015, Vol. 24(14), pp. 1-7.
- Sariri, R., Varaste, A., Erfani, A., Heidari, Z., Inhibition of salivary peroxidase by cigarette smoke. *Health*, 2010, Vol. 2(4), pp. 347-351.
- Rezaei, A., Sariri, R., Periodontal status, salivary enzymes and flow rate in passive smokers. *Pharmacol online*, 2011, Vol. 3, pp. 462-467.
- Onyesom, I., Osioma, E., Ifie, E.J., Oweh, O.T., Activity of alpha amylase in saliva and serum of some Nigerian cigarette smokers. *Adv Life Sci*, 2012, Vol. 2(1), pp. 28-30.
- Sariri, R., Damirchi, A., Alternations in salivary amylase due to exercise intensity. *Pharmacol online*, 2010, Vol. 2, pp. 2632-2668.
- Rezaei, A., Sariri, R., Salivary diagnosis of periodontal status, A Review. *Pharmacol online*, 2011, Vol. 1, pp. 428-444.
- Miladipour, A.H., Shakhssalim, N., Parvi, M., Azadvari, M., Effect of Ramadan Fasting on Urinary Risk Factors for Calculus Formation. *Iran J Kidney Dis*, 2012, Vol. 6, pp. 33-38.
- Alhadramy, M.S., Seasonal variations of urinary stone colic in Arabia, *J Pac Med Assoc* 1997, Vol. 47, Vol. 281-284.
- Khoshdel, A., Kheiri, S., Nasiri, J., Mobasheri, M., The effect of Ramadan fasting on biochemical substances relating to the renal and bone function of fasting pregnant women, (2011-2012). *J Fasting Health*, 2013, Vol. 1(2), pp. 79-84.
- Hallak, M.H., Noman, M.Z.A., Body weight loss and changes in blood lipid levels in normal men on hypocaloric diets during Ramadan fasting. *Am J Clin Nutr*, 1988, Vol. 48, pp. 1197-1210.
- Sarrafzadegan, N., Zarei, M., Naderi, G.H., Asgari, S., Samarian, H., Karimi, A., Fatehifar, R., The effect of fasting during the month of holy Ramadan on apolipoproteins and coagulation system. *Atherosclerosis*, 1999, Vol. 144, pp. 174-179.
- Hamdan, A.L., Ashkar, J., Sibai, A., Oubari, D., Hussein, S.T., Effect of fasting on voice in males. *Am J Otolaryngol*, 2011, Vol. 32(2), pp. 124-129.
- Hamdan, A.L., Sibai, A., Rameh, Ch., Effect of fasting on voice in women. *J Voice*, 2007, Vol. 21(4), pp. 495-501.
- Furuncuoglu, Y., Karaca, E., Aras, S., Yonem, A., Metabolic, biochemical and psychiatric alterations in healthy subjects during Ramadan. *J Nutr*, 2007, Vol. 6(3), pp. 209-211.
- Choudhury, B.K., Hassan, M., Mowsumi, F.R., Sarker, N.C., Hosein, Md.S., Choudhury, M.S.K., Oyster mushroom ameliorates liver function during Ramadan fast reflected by reducing the serum level of alanine aminotransferase and aspartate aminotransferase of male volunteers. *Int J Nutr Metab*, 2011, Vol. 3(10), pp. 123-127.