

ORIGINAL RESEARCH

Unstimulated Whole Saliva Cortisol Levels during Ramadan in Iranian Muslims

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ABSTRACT

Aims: Ramadan is a great opportunity for scientific research due to its peculiar nature. This study was designed to investigate whether morning saliva cortisol pick can change during and after Ramadan compared to before it.

Materials and methods: A total of 30 healthy volunteer men (aged between 30 and 76 years) were asked to participate in a cross-sectional study between August 12 and September 10, 2010. Saliva was collected 2 weeks before the beginning of Ramadan (BR), during the first week (R1), middle (R2), the last week (R3) of Ramadan and 3 weeks after Ramadan (AR). Cortisol concentration was analyzed by ELISA. Statistical analysis of one-way repeated measures analysis of variance (ANOVA) was used.

Results: The mean unstimulated saliva cortisol concentration and its output were significantly higher in the BR than during and after Ramadan.

Conclusion: Mean saliva cortisol concentration and its output tended to be lower during and 3 weeks after Ramadan.

Clinical significance: Cortisol as a stress hormone seems to be low during Ramadan.

Keywords: Ramadan, Cortisol, Unstimulated saliva.

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INTRODUCTION

Ramadan is the ninth month of the Islamic lunar calendar in which all healthy adult Muslims neither eat nor drink anything during the month, between dawn (sahar) and sunset (iftar) as a religious duty. The experience of fasting teaches

Muslims self-discipline and self-restraint, and reminds them of the feelings of the impoverished. In addition, eyes, ears, tongue, and indeed the whole body, are equally obligated to be restrained.

Cortisol is the primary glucocorticoid in the human body, and follows a circadian rhythm with peak level at about 8 am and its nadir in the late evening. Its secretion also increases markedly in response to stress, whether physical (illness, trauma, surgery, fever, physical exercise, fasting and extreme temperatures) or psychological (clinical depression, anxiety, strain, fear and pain).^{1,2}

Saliva as a diagnostic specimen can give not only the same information as serum testing, but also additional or new information that cannot be obtained from serum.³⁻⁷ Saliva is a clear colorless liquid, whereas serum may become milky when lipemic, red when blood cells are hemolyzed due to trauma, and icteric in the presence of liver disease. Because serum possesses more proteins than saliva, assaying trace amounts of factors may result in a greater risk of nonspecific interference and a greater chance for hydrostatic interactions between the factors and serum proteins. Because of these significant characteristics, finding biomarkers in saliva for the detection of serious systemic illnesses is of great interest for most salivary researchers. Today, salivary cortisol is routinely used as a biomarker of psychological stress and related mental or physical diseases.⁸⁻¹⁰

Ramadan month is a great opportunity for scientific research due to its peculiar nature. Despite the fact that it concerns the Muslim community of more than 1 billion people, the effects of Ramadan on humans have not been adequately investigated. With this in mind, this study was designed to investigate whether morning saliva cortisol pick can change during and after Ramadan compared to before it.

MATERIALS AND METHODS

Participants

The protocol was approved by the ethics committee of Tehran University of Medical Sciences (TUMS), Iran, and all participants gave informed consent before participation in the study.

A total of 30 healthy volunteer men were asked to participate in a cross-sectional study, conducted at the laboratory of basic rehabilitation sciences between August 12, 2010 and

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Table 1: Age range of participants

Age range (yrs)	Frequency
30-39	8
40-49	10
50-59	10
>60	2

September 10, 2010. The participants were aged between 30 and 76 years (Table 1), were not taking any medication either before or during the study. Smokers, obese patients (body mass index (BMI) >30 kg/m²), patients with systemic diseases or any chronic or acute somatic or psychiatric disorder, oral candidiasis, or with a bad oral health condition and periodontal disease were excluded.

Saliva Collection

Unstimulated whole saliva was collected under resting conditions in a quiet room (to reduce any stressful condition which may influence the cortisol secretion), between 8 am and 9 am (near the peak of serum cortisol level), about 5 hours after the last intake of food or drink during Ramadan and about 2 hours before or after Ramadan. Saliva was collected in all participants 2 weeks before the beginning of Ramadan (BR), during the 1 week (R1), middle (R2), the last week (R3) of Ramadan and 3 weeks after Ramadan (AR). The duration of saliva sample collecting was recorded with a stopwatch. Unstimulated whole saliva was collected for about 5 minutes into a preweighed, dry, deionized and sterilized plastic tube. By subtracting the empty tube weight from the saliva filled one, saliva sample weight was determined to calculate the salivary flow rate. The flow rate was calculated in gm/min, which is almost equivalent to ml/min.¹¹ Cortisol output was calculated as its salivary concentration (ng/ml), multiplied by saliva flow rate (ml/min). The samples were clarified by centrifugation (2500 gm, 10 minutes), and immediately stored at -20°C for later determination of cortisol.

At BR, R1, R2, R3 and AR, the weight and standing height of each subject was measured in the morning at the same time of day along with saliva sampled. Body mass index was then calculated as weight (kg) divided by square of height (m).

Analysis of Saliva

Cortisol concentration was analyzed by ELISA technology using commercially available kits [(diagnostics biochem Canada (DBC)].

Statistical Analysis

For statistical analysis, the data are presented as a mean ± standard error of mean (SEM). Data were analyzed by

one-way repeated measures analysis of variance (ANOVA). $p < 0.05$ was considered statistically significant.

RESULTS

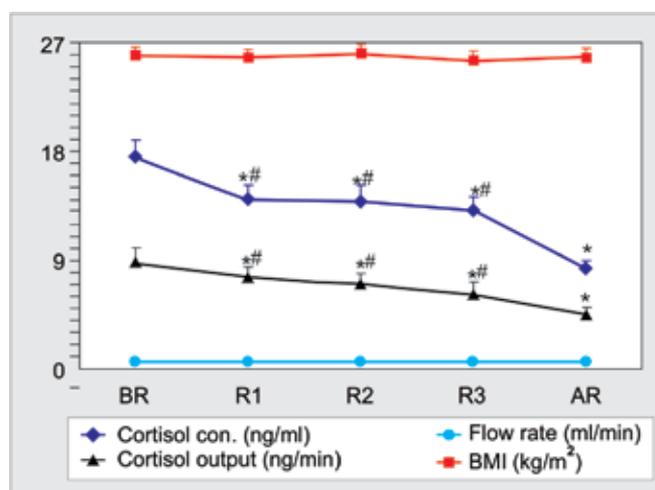
One-way repeated measures of ANOVA showed that mean BMI and unstimulated whole saliva flow rate were unchanged during Ramadan and 3 weeks after it (Graph 1). There were significant differences in mean saliva cortisol concentration and its output among the BR, R1, R2, R3 and AR (see Graph 1). They were higher in the pre-Ramadan than during and after Ramadan. Mean saliva cortisol concentration and its output tended to be lower during Ramadan and were significantly lower 3 weeks after Ramadan.

DISCUSSION

The aim of this study was to determine whether the Ramadan would affect the unstimulated saliva cortisol morning pick of healthy Iranian Muslims. To the best of our knowledge this is the first study to examine the effect of Ramadan on saliva cortisol in men. The main result emerged from this study was significant decrease in saliva cortisol during and 3 weeks after Ramadan in comparison to pre-Ramadan. However, BMI and saliva flow rate were unchanged.

Our data showed that BMI was not influenced by Ramadan in men. The finding of the present study was consistent with the results of other investigators.¹²⁻¹⁵ It has been shown that physical activity decreases in this month.¹⁶ Therefore, it seems that there is a balance between decrease in energy intake and activity, so the BMI unchanged during Ramadan.

Salivary cortisol, as representative of circulating free cortisol, has been recommended as an index for stress and its use has also been adopted, because this avoids the stress



Graph 1: Body mass index (BMI), unstimulated whole saliva flow rate, cortisol concentration and output before Ramadan (BR), in the first week (R1), middle (R2), the last week (R3) of Ramadan and 3 weeks after Ramadan (AR) (Data are expressed as mean ± SEM). *different from BR; #different from AR; $p < 0.05$

caused by venopuncture and reflects the free plasma concentration and bioactive component of steroid hormones.¹⁷⁻²⁰

Saliva cortisol concentration and its output decreased at R1 and remained lower than BR even 3 weeks after ending the Ramadan. This is consistent with studies by other investigators on serum cortisol.²¹⁻²⁴

It may be supposed that differences in saliva cortisol level between 2 weeks before Ramadan and during Ramadan relates to difference in interval between the last food intake and the saliva collection time. However, decrease in saliva cortisol level lasted 3 weeks after Ramadan despite the interval was the same (two hours) in both before and after Ramadan. Therefore, it seems that decrease in saliva cortisol during Ramadan is unrelated to interval between the last food intake and the saliva collection time.

It has been shown that saliva flow rate decreases and cortisol increases in stress condition.^{1,25,26} As saliva flow rate unchanged and saliva cortisol decreased during Ramadan in this study; it seems that Ramadan alleviates stressor effects. This result could be due to the emphasis on inner control (taghva) and abstinence in religion. In this month, it is recommended to pay more attention to good order, discipline and punctuality, respect familial and interpersonal relationships and economical structure of personal and social life. During Ramadan, every part of the body must be restrained. The tongue must be restrained from backbiting and gossip. The eyes must restrain themselves from looking at unlawful things. The hand must not touch or take anything that does not belong to it. The ears must refrain from listening to idle talk or obscene words. The feet must refrain from going to sinful places. In such a way, every part of the body observes the fast. Therefore, fasting is not merely physical, but is rather the total commitment of the person's body and soul to the spirit of the fast. Ramadan is a time to practice self-restraint; a time to cleanse the body and soul from impurities and refocus one's self on the worship of God.

There was a limitation to this study. We were not able to measure the evening levels of cortisol in the study population that might allow us to comment more on the effects of Ramadan fasting on the circadian rhythm of cortisol secretion.

CONCLUSION

Mean saliva cortisol concentration and its output tended to be lower during and 3 weeks after Ramadan. It seems that Ramadan may decrease stress hormones, such as cortisol.

CLINICAL SIGNIFICANCE

Cortisol as a stress hormone seems to be low during Ramadan.

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