A Study of Diurnal Variation in Serum and Urine Osmolalities and in Serum Cortisol During Ramaḍān Fasting: Evidence Suggesting Increased Intrinsic Water Production

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Abstract

Daily abstinence from food and drink for some 15 hours in Ramaḍān is a unique type of fasting. Morning (0800 hour) and evening (1900 hour, just preceding sunset) estimation of serum and urine osmolalities and of serum cortisol were conducted on 22 healthy volunteers who fasted Ramaḍān of 1407 Hijri in Mosul on days 1, 14 and 28 of the month. A diurnal variation in serum and urine osmolalities that narrowed as Ramadan progressed was observed. The evening serum cortisol was high on days 1 and 14 but decreased on day 28. In four of the 66 occasions comparing morning and evening serum osmolalities the evening values were 2 mOsmol/kg lower than morning values.

Our results were interpreted as unexplainable by vasopressin action on the kidney alone. Evidence that daily fasting triggers increased capacity to synthesize and store glycogen which is dictated by increased glucose needs for the new prolonged intermeal interval is given. We maintain that glycogen synthesis during eating hours incorporates water intracellularly; water is released during daytime glycogenolysis in order to help prevent undue increase in serum osmolality during fasting. Our concept offers explanations for many phenomena that are experienced by people who fast Ramaḍān.

Key words: *Fasting, Ramaḍān, osmolalities, cortisol*

The pattern of daily abstinence from food and drink from dawn to sunset is unique for Muslims who fast the lunar month of Ramaḍān. At Mosul City, it mounts up to 17 hours on the longest days of the summer and down to 11 hours on the shortest days of the winter. Overnight fast and short term (three days) fasting have been reasonably well studied.[1] They, however, differ from the status of Ramaḍān fasting by the repeated daily fasting for 29-30 days, by deprivation from water during fasting time, and by extension of the fast to several hours more than an overnight fast.

It is common knowledge among people who fast Ramaḍān that, sometimes, they experience intense thirst at mid-day but, after rest or sleep in the afternoon, thirst eases or may even subside without drinking any water. The serum osmolality has been

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reported not to show a significant change during Ramaḍān fasting in one report.[2] Elsewhere, it was stated to increase significantly.[3] The above-mentioned observation and the lack of consistent findings would justify further evaluation of osmolality.

As water excretion by the kidney is influenced by glucocorticoid activity and as the daily feeding-fasting and sleeping-waking times change during Ramaḍān fasting in such a manner as to possibly modify the diurnal rhythm of cortisol secretion,[4] estimation of diurnal cortisol levels was thought complementary to the main aim of the study -osmolality.

Materials and methods:

The study included 22 (16 males and 6 females) medical and paramedical apparently healthy volunteers aged 16-50 years with a mean age of 30.2 years. Males fasted the whole month except for a single day of break of fast in two of them because of travel. Each female, however, had to break the fast for 5-6 days during the menstrual period. Morning specimens of blood and urine were collected one week before Ramaḍān and both morning (at 0800 hour) and evening (at 1900 hour shortly before sunset) specimens on days 1, 14, and 28 of fasting from each volunteer. Luckily, all volunteers were fasting on the three days of collection of blood and urine specimens during Ramaḍān.

Aliquots of serum were kept at -20 °C for a cortisol assay. Fresh serum and the urine were tested for osmolality using Humburg 90-Luveburgerstrasse 2 Osmometer from FG Bode and Co. Laboratory Equipment. Cortisol assay was confined to the six groups of blood specimens that were collected during Ramaḍān; each single day group of specimens was tested by the same batch using Amersham Kit, U.K.[5] The analysis of variance technique was applied for analysing the data. Tukey's and Dunnett's procedures[6] were used to compare all means of serum and urine osmolality and of serum cortisol. Controls were compared with the means of serum and urine osmolality. A p value of 0.05 was considered significant.

Results:

The mean urine and serum osmolalities and their standard deviations are shown in Tables 1 and 2. The diurnal difference in urine osmolality (Table 1, and Figure 1) gradually decreased as Ramaḍān advanced between 28 April and 27 May, 1987 despite the associated rise of ambient temperature. Mean ambient temperatures for April, May, and June at Mosul are 17.5, 24.1 and 30.4 °C respectively.[7] The highest urine osmolality (1320 mOsmol/kg) was obtained on the first day of fasting at 1900 hour. Figure 2 and Table 2, likewise, show a gradual decrease of diurnal difference in serum osmolality as daily fasting progressed. The marginal increase in morning serum osmolality with the progress of fasting could reflect the change in the starting time of the fast. On the first day of Ramaḍān fasting began at 0430 hour whereas it began at 0357 hour on its last day.

Analysis of variance of the data failed to show a significant change in serum osmolality. By applying Dunnett's method to urine values, the only significant difference was between mean control and first day mean evening value. Tukey's method, however, showed a significant difference between the mean of day 1 and the means of days 14 and 28, but not between 14 and 28.

The mean and standard deviation of serum cortisol for Ramaḍān specimens are shown in Table 3 and Figure 3. It is noted that morning-evening variation was narrowest on day 11, remained almost the same on day 14, but had became much wider, by day 28. In other words, the morning-evening variation was approaching normality. Day 28 serum cortisol evening value was significantly lower than those of days 11 and 14.

Discussion

Unexpectedly, our results revealed a decrease in the diurnal difference of both urine and serum osmolalities with the continuation of daily fasting. We stated "unexpectedly" because of the associated increase in ambient temperatures and prolongation of fasting time as Ramaḍān progressed; high ambient temperatures increase body water losses.[8]

Although our serum osmolality results did not reach a statistically significant level, we could not ignore them because of encountering very similar results in the first four weeks of fasting in the single analogous study that we came across in the literature;[2a] three results[2a] were left without comment.[2] Moreover, Table 4 shows a less striking narrowing in diurnal variation of serum osmolality among females, who had to break their fast during menstruation, than among males who fasted the whole month almost completely. This suggests that continuation of daily fasting helps to decrease the diurnal fluctuation already mentioned.

If arginine vasopressin (AVP) action on the kidney, in the absence of water drinking to allay thirst, were the single mechanism to maintain the "constancy" of serum osmolality, ever-rising morning-evening differences in urine and serum osmolalities on days 14 and 28 had to be expected with the associated rise in ambient temperatures and lengthening of fasting time. Moreover, in four (2 on day 14 and 2 on day 28) of the 66 occasions comparing morning-evening osmolalities in the same volunteer for the same fasting day, the evening serum osmolality was 2 mOsmol/kg less than it was in the same morning. Although theoretically not impossible, we are not aware of any mention in the literature that endogenous AVP secretion in response to in-

Table 1. Mean ± S.D. of Urine osmolality (in mOsmol/kg) for the seven groups of 22 samples each.

Sampling time	Dates of collection of urine samples			
	Before Ramaḍān	Day 1 of fasting	Day 14 of fasting	Day 28 of fasting
Morning (0800 hour)	890 ± 14	904 ± 176	848 ± 145	895 ± 153
Evening (1900 hour)	-	1115 ± 142	949 ± 115	964 ±143
Morning-evening differences	-	211	102	69

Table 2. Mean ± S.D. of Serum osmolality values (in mOsmol/kg) for the seven groups of 22 samples each.

Sampling time	Dates of collection of blood samples			
	Before Ramaḍān	Day 1 of fasting	Day 14 of fasting	Day 28 of fasting
Morning (0800 hour)	276 ± 9	276 ± 10	278 ± 8	280 ± 7
Evening (1900 hour)		283 ± 9	282 ± 8	282 ± 7
Morning-evening differences	-	7	4	3

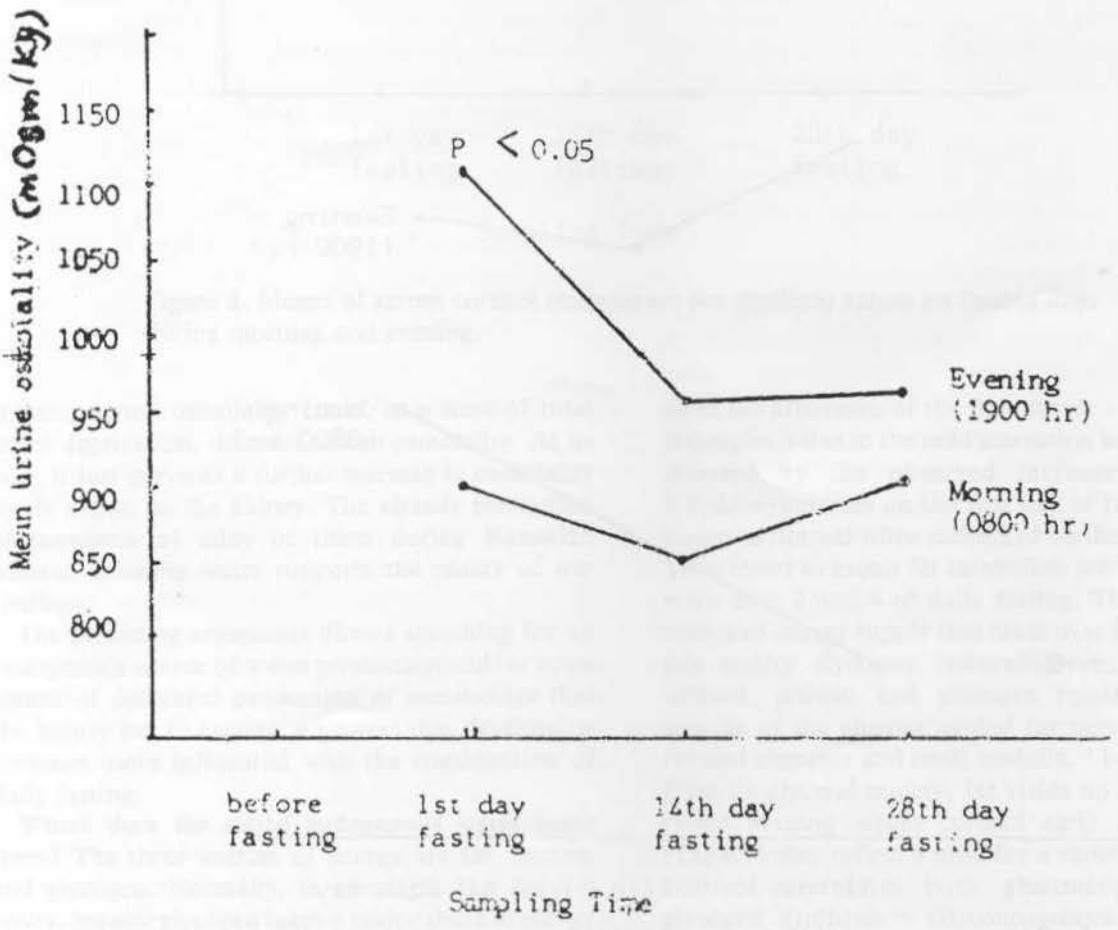

Figure 1. Mean urine osmolality values for control (before fasting) and fasting days during morning and evening.

Table 3. Mean ± S.D. of Serum cortisol values (ug/dl) with evening/morning percentages during Ramaḍān fast for the 22 volunteers.

Sampling time	Dates of collection of blood samples in Ramaḍān		
	Day 1 of fasting	Day 14 of fasting	Day 28 of fasting
Morning (0800 hour)	19.2 ± 2.7	19.0 ± 2.1	18.6 ± 2.4
Evening (1900 hour)	16.7 ± 2.4	15.5 ± 2.1	13.3 ± 2.2
Evening/morning ratio	86.8%	81.7%	71.5%

Table 4. Mean diurnal differences in serum osmolality (in mOsmol/kg) during Ramaḍān fasting in 16 males and 6 females.

Sex	Mean diurnal differences in serum osmolality		
	Day 1	Day 14	Day 28
Males	7.75	3.31	2.25
Females	4.7	5.0	4.3

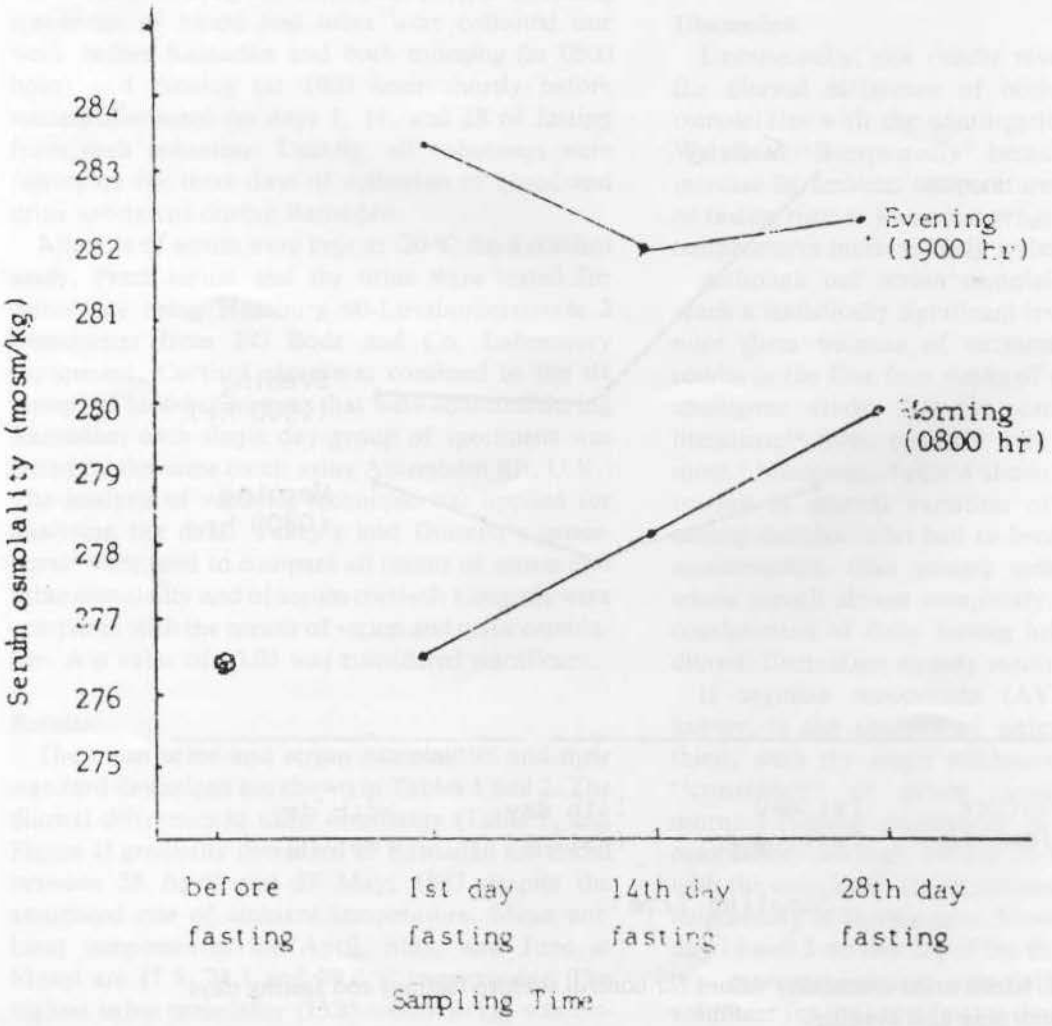

Figure 2. Mean serum osmolality values for control (before fasting) and fasting days during morning and evening.

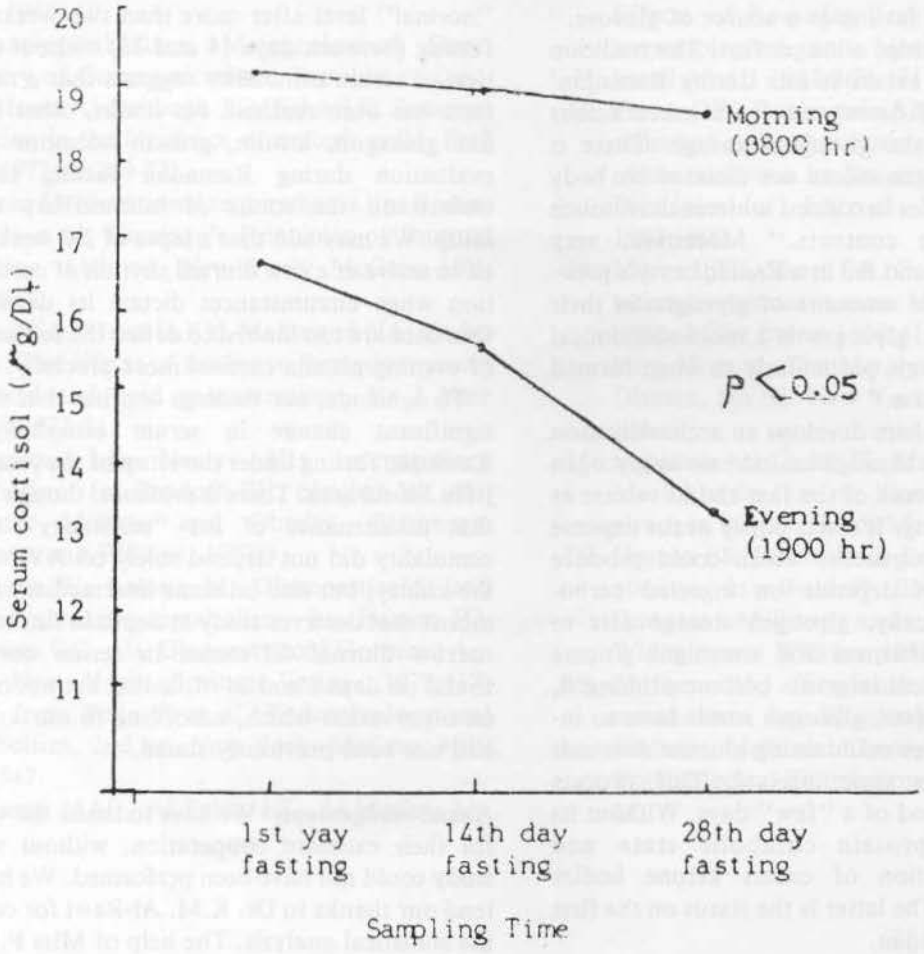

Figure 3. Means of serum cortisol (microgram per decilitre) values on fasting days during morning and evening.

creasing serum osmolality could, in a state of total water deprivation, decrease serum osmolality. At its best, it just prevents a further increase in osmolality by its action on the kidney. The already mentioned phenomenon of allay of thirst during Ramaḍān without drinking water supports the reality of our findings.

The preceding arguments dictate searching for an endogenous source of water production and/or some means of decreased production of metabolites that the kidney has to excrete; a process that develops or becomes more influential with the continuation of daily fasting.

Where does the stated endogenous water come from? The three sources of energy are fat, protein and glycogen. Normally, in overnight fast for 7-9 hours, hepatic glycogen takes a major share in energy supply (75%).[1] On the third day of continuous fasting, glycogenolysis contributes just about 9% to energy production;[1] this glycogen is probably an outcome of gluconeogenesis. The duration of fasting in Ramaḍān is longer than overnight fast and, being at day time, it demands higher grades of energy production than in overnight sleep. The headache experienced in the afternoon of the first day or two of fasting Ramaḍān is due to the mild starvation ketosis. This is attested by the observed increase in plasma 3-hydroxybutyrate on the first day of fasting and its return to normal when rechecked on the fourth day.[9] Thus resort to excess fat catabolism settles down between days 2 and 4 of daily fasting. The alternative source of energy supply that takes over has to be protein and/or glycogen. Naturally, even when fat is utilized, protein and glycogen remain the main sources of the glucose needed for nerve cell, blood formed elements and renal medulla,[10] because, apart from its glycerol moiety, fat yields no glucose. The raised evening serum cortisol early in Ramaḍān (Table 3) may reflect a need for a supply of glucose; cortisol stimulates both gluconeogenesis and glycogen synthesis.[11] Glyconeogenesis from body protein is dictated by the reported decrease in total serum proteins, including albumin, in the first week of Ramaḍān fasting.[12] The return of serum proteins to prefasting levels in the second week of Ramaḍān onwards[12] despite continuous need for glucose and the lack of evidence of frank fat catabolism justifies concluding that glucogen stores, which normally

cover 8-10 hours of fasting as a source of glucose,[13] have increased to bridge a longer fast. The tradition of consumption of excess sweets during Ramaḍān[2] (between sunset and dawn) may be a subconscious means to increase the glycogen storage. There is evidence that glycogen stores are dictated by body needs: muscle biopsies in trained athletes show much increased glycogen contents.[14] Moreover, very recently, rats fasted and fed in a Ramaḍān style proved to store increased amounts of glycogen in their livers.[15] Caloriewise, glycogen is a most economical form of storing energy, particularly so when formed from absorbed glucose.[16]

We propose that there develops an acclimatization to the Ramaḍān fast through an increase in glycogen storage during the break of the fast and its release as glucose during fasting. It starts partly at the expense of proteins gluconeogenesis, which could produce glycogen, but soon depends on ingested carbohydrates. Teleologically, glycogen storage acts to bridge the usual intermeal and overnight glucose needs. When intermeal intervals become prolonged, as in the Ramaḍān fast, glycogen stores have to increase so as to suffice maintaining glucose demands for the new wider time interval. This process develops over a period of a "few" days. Without its development, a protein catabolic state and undesirable production of excess ketone bodies become inevitable. The latter is the status on the first day of fasting Ramaḍān.

Glycogen incorporates with it 2-4 times its weight of water.[10] When metabolized in daytime, glycogenolysis releases the water without adding an osmolar load on the kidney as happens with protein catabolism. The high urine osmolality on the evening of day 1 of fasting (Table 1) has to be ascribed partly to the mentioned early protein catabolic state. Fat catabolism, although it does not burden the kidney with an osmolar load, is, caloriewise, a much poorer source of endogenous water production than glycogen.[10] Interestingly, AVP is a recognized stimulator of glycogenolysis,[17] as to suggest an additional mode of AVP action in controlling serum osmolality apart from its main action on the kidney.

Quantitatively speaking, production of 75 Calories per one hour[16] from glycogenolysis during 4 hours rest in the afternoon in a 70 kg human with 40 litres of total body water means release of up to 300 (75 x 4) ml of water as 1 gm of glycogen releases 4 Calories and up to 4 ml of water.[10] Subtracting an imperceptible water loss of 84 (500/24 x 4) ml during the same time, the remaining 216 ml effect reduction in serum osmolality of about 1.5 mOsmol/kg. If we add the water produced from glucose breakdown into carbon dioxide and water, which originally came from glycogenolysis, a fall of 2 mOsmol/kg in serum osmolality would be quite reasonable.

The settlement of the evening serum cortisol at "normal" level after more than two weeks of daily fasting (between days 14 and 28) without deterioration of serum osmolality suggests that a new steady state has been reached. No doubt, other hormones like glucagon, insulin, growth hormone etc. need evaluation during Ramaḍān fasting to further understand the status of intermediary metabolic setup. We may add that a lapse of 2-3 weeks is needed to arrive at a new diurnal rhythm of cortisol secretion when circumstances dictate its development.[4] Our data are too limited to define the settlement time of evening plasma cortisol more precisely.

To conclude, our findings confirm that there is no significant change in serum osmolality during Ramaḍān fasting under the setup of the year 1407 Hijri in Mosul area. There is evidence, though tentative, that maintenance of the "constancy" of serum osmolality did not depend solely on AVP action on the kidney, but also on some intermediary metabolic means that deserves study at depth in future work. A narrow diurnal difference in serum cortisol was found on days 1 and 14 of fasting but not on day 28; an observation which, according to our knowledge, had not been previously stated.

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