Serum Glucose Measurement after Five to Six Hours is Comparable to Eight Hours Overnight Fasting in Ramadan

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Abstract

Objectives: This study was done to evaluate whether a shorter fasting duration of five to six hours can be used as an alternative to the usually recommended eight hours fasting glucose measurement. Methods: An observational, cross-sectional study was conducted during Ramadan (May) 2019, on 200 individuals. Two fasting serum glucose (FSG) venous samples taken, the first after 5-6 hours of predawn meal (suhoor), and the second after 8 hours. Participants were divided into two groups; normal individuals, and those who have type 2 diabetes mellitus (T2DM). Patients with T2DM further subdivided into three groups: those without treatment, those on oral antidiabetic drugs (OAD), and those using insulin and OAD. Results: There was no significant difference between the mean FSG readings in mg/dL (mmol/L) between the first and second samples for healthy individuals, 104.5 ± 21.4 (5.79±1.18) and 104.8 ± 12.6 (5.82±0.7), respectively. Generally, the same is true for T2DM patients with FSG values of 235 ± 107 (13.04±5.94) and 230 ± 105 (12.77±5.83). Untreated T2DM patients have consecutive FSG readings of 194.0 ± 151.5 (10.77±8.41) and 193.9 ± 128.9 (10.76±7.15), respectively, in the two samples without significant difference. Patients using insulin and OAD showed similar pattern of FSG 268 ± 111
(14.87±6.16) and 269 ± 114 (14.93±6.33), respectively. The only significant difference was observed in patients on OAD which have 220 ± 78 (12.21±4.33) and 207 ± 77 (11.49±4.27) for their successive FSG samples. **Conclusions:** The overnight fasting duration of 5-6 hours, can give a comparable measurement of fasting serum glucose as that obtained by 8 hours.

**Keywords:** Glucose; Fasting; Duration; Diabetes Mellitus

**Introduction**

Disruption in the typical cyclical pattern of glucose tolerance is the hallmark of type 2 diabetes mellitus (T2DM), thus understanding the normal control of glucose metabolism hour by hour is critical for achieving optimal diabetes care. The current recommendations emphasize the standardized measurement of fasting plasma glucose (FPG) after overnight fasting for at least eight hours to assess the magnitude of basal glucose abnormalities.\(^1\)-\(^5\) However, the discordance with other diagnostic tests and uncertainty about the duration of fasting reported by some individuals raises the doubt about the applicability of the current diagnostic criteria to diagnose diabetes mellitus.\(^6\),\(^7\)

Although plasma is devoid of red and white blood cells, they still contain platelets, which consume glucose over time. On the other hand, serum would be free from any cells, and hence it would be suitable for blood glucose estimation particularly when there is a time delay in the measurement. If assayed immediately, blood glucose measured in fluoride plasma correlated linearly with blood glucose in the serum, and serum value is lower than the corresponding fluoride plasma value by 1.15%. This may not be physiologically relevant. Hence, serum may be used alternatively for blood glucose determination with an error of 1.15%.\(^8\)

Despite wide variations in glucose flux in feeding and fasting states, blood glucose is maintained in a narrow range and eating late-night meals can interrupt healthy sleep and usually affects glucose control in a patient with DM. However, the circadian rhythm of cortisol is robust and minimally affected.\(^4\),\(^9\),\(^10\)

This study aims to evaluate whether a shorter fasting duration of five to six hours can substitute the standard glucose measurement using eight hours overnight fast.
Methods
This one-month observational, cross-sectional study conducted mostly in May and initial days of June 2019 during the fasting month of Ramadan, on individuals attending Faiha Specialized Diabetes, Endocrine, and Metabolism Center (FDEMC) in Basrah (Southern Iraq) with different demographic characteristics. All individuals share the standard behavior of eating a pre-dawn meal (Suhoor) at around 3:00 am, followed by a complete fast (null by mouth) for many hours. Two fasting serum glucose (FSG) samples were collected from each participant, one after five to six hours and second after eight hours.

The exclusion criteria were: Non-fasting or incomplete fasting state, patients with Type 1 diabetes mellitus, venous blood samples taken before five hours or after eight hours, fasting without a prior pre-dawn meal, individuals who had no real pre-dawn meal, i.e. continue to eat frequent small meals until fasting time, and night-shift workers.

Ultimately, we enrolled 200 individuals, 57.5% (n=115) of them have T2DM, on different modalities of treatment. The presence of T2DM was the base to divide the candidates into two groups; (individuals with T2DM) and (individuals without T2DM). Individuals with T2DM were subdivided according to the modality of treatment into: Patients using only oral antidiabetic medications (OAD), patients using insulin with OAD, and patients with no treatment. We assess the enrolled individuals for demographic characteristics, like gender, age, and body mass index (BMI), timing of meals, fasting pattern, and the type of treatment for patients with T2DM.

After ensuring the fasting state for five to six hours, a venous blood sample of two to three milliliters collected into a plain test tube. The samples were immediately transferred to the laboratory, and allowed to be clotted then centrifuged prior to the estimation of FSG by (COBAS, INTEGRA 400 PLUS, Roche Company, Switzerland). Similar steps were done after eight hours of fasting for each individual.

Data were entered and matched via Microsoft Excel and then analyzed on IBM SPSS Statistics for Windows, Version 23.0 (Armonk, NY: IBM Corp.) Software Statistical Packages for Social Sciences (IBM-SPSS-23) for analysis of different variables. The study used the (mean± standard deviation) or frequency (%) for data expression. We used the paired (t) test to compare the difference between the mean FSG readings in the two occasions, and considered a (p-value) of less than <0.05 to be statistically significant. The
study designed to show the non-inferiority of fasting for only 5-6 hours to give valid fasting glucose measurement as compared to the standard 8 hours fasting. If the mean difference between the two samples was less than 6 mg/dL, they would be considered equivalent.

After a detailed illustration of the study with the possible participants, written consent was signed by each participant before the examination day. The FDEMC ethical committee approved the study.

**Results**

The study recruited 200 individuals with a mean age of 45.6 ± 14.4 years. Men constituted 56.5% of the cohort (n=113). One hundred fifteen participants (57.5%) had T2DM, which were further subdivided according to their therapy into; individuals on OAD (n=54), individuals on insulin and OAD (n=45), and individuals without medication for their T2DM (n=16) (Table 1).

The mean FSG after five to six hours for healthy individuals was 104.5 ± 21.4 mg/dL (5.79 ± 1.18 mmol/L), as compared to an eight-hours sample of 104.8 ± 12.6 mg/dL (5.82 ± 0.7 mmol/L), the mean difference was insignificant -0.2 ± 15.9 mg/dL (0.01 ± 0.88 mmol/L). While for T2DM patients in general, the mean FSG was 235 ± 107 mg/dL (13.04 ± 5.94 mmol/L) after five to six hours and 230 ± 105 mg/dL (12.77 ± 5.83 mmol/L) after eight hours overnight fasting, with a mean insignificant difference of 5.6 ± 45.6 mg/dL (0.31 ± 2.53 mmol/L) (Table 2). According to the management model of T2DM, the mean FSG values for untreated patients were 194.0 ± 151.5 mg/dL (10.77 ± 8.41 mmol/L) after five to six hours overnight fasting and 193.9 ± 128.9 mg/dL (10.76 ± 7.15 mmol/L) after eight hours. Among T2DM patients treated by OAD, the FSG mean values were 220.0 ± 78 mg/dL (12.21 ± 4.33 mmol/L) after five to six hours and 207 ± 77 mg/dL (11.49 ± 4.27 mmol/L) after eight hours of overnight fasting, with a mean significant difference of 13.1 ± 37.8 mg/ dL (0.73 ± 2.10 mmol/L), while for patients on insulin with OAD, the FSG mean values were 268 ± 111 mg/dL (14.87 ± 6.16 mmol/L) and 269 ± 114 mg/dL (14.93 ± 6.33 mmol/L) respectively with a mean insignificant difference of -1.4 ± 54.7 mg/dL (0.08 ± 3.04 mmol/L) (Table 2 and Figure 1)
Discussion

Overnight FPG measurement is a core component in glycemic regulation in T2DM and related disorders. However, the definition of fasting state is not clear or missing, i.e., World Health Organization (WHO) recommends an 8–14 hours fast, while the American Diabetes Association (ADA) defines fasting as no caloric consumption for at least eight hours.

In this study, the mean FSG value after five or six hours was similar to the value obtained after eight hours.

Moebus et al. measured the hourly decrease in FPG since the last nightly meal in healthy individuals. They found non-significant decrease (0.16–0.43 mg/dL) for each additional fasting hour. Unexpectedly, they found that three hours of fasting is sufficient to give a comparable FPG result as that of eight hours. The results of whom were ascertained by the British Regional Heart Study.

Glucose homeostasis is never a constant. The human body can tightly maintain normal blood glucose levels by modifying insulin response to stimulate glucose disposal under the tightly-controlled circadian pattern; however, the relative contribution of the circadian versus the behavioral cycle is unclear. Generally, the endogenous hormonal control of glucose homeostasis precedes the influential role of the suprachiasmatic nucleus (SCN), which occurs later on throughout the day.

The FPG and plasma insulin maintain a nadir overnight in healthy individuals, with a modest, transient elevation in insulin secretion rate (ISR) at pre-dawn to restrain endogenous glucose production (EGP) and prevent hyperglycemia. The increased insulin requirement at dawn had been attributed to either increased insulin clearance that may increase about two folds or decreased insulin action and hepatic insulin resistance.

Morris et al. showed a nonsignificant change in FPG, insulin, ISR, and growth hormone (GH) after five and eight hours overnight fasting. Healthy humans have a time-of-day variation in glucose tolerance, being highest in the early hours of the day and a trough at night, unlike patients with diabetes, which show marked fluctuations.
In our study, which has been done in the early day-time hours, individuals without diabetes had FSG readings in the normal upper range after eight hours overnight fast. This was maintained the same for the last two to three hours, and this matches the cycle of internal glucose metabolism. On the other hand, patients with T2DM had fasting hyperglycemia according to the diagnostic guidelines.

The concept of (dawn phenomenon) could explain the early morning hyperglycemia in T2DM patients. This event had been observed in patients with diabetes and in (some) individuals without diabetes where glucose concentration peaked before the onset of daily activity. When plasma glucose is high before the start of the activity, humans seem to be more glucose-tolerant, with a preserved glucose homeostasis during the day. Nocturnal elevations in GH and early morning increases in cortisol secretion may contribute to this phenomenon by stimulating EGP through gluconeogenesis and glycogenolysis.

Van Cauter et al. evaluated individuals after the ingestion of two low caloric mixed meals in the morning and evening. They found no difference in FPG levels between five and eight hours, while cortisol level peaks after five hours, which is slightly more than that after eight hours, yet the difference is insignificant. Van Cauter et al. suggest the possible contribution of the cortisol in carbohydrates tolerance, given the failure of insulin secretion to increase in proportion to changes in post meal glucose responses.

The changes in cortisol concentration occurred following the changes in EGP for the control group, while for patient with diabetes, EGP increased three hours before cortisol rise. Moreover, the later on decline in cortisol does not accompany the EGP fall, and this might be due to defective SCN activity in patients with diabetes and/or nonlinear personal behavior.

Many studies found that individuals without diabetes have a steady-state in glucose utilization, insulin concentration, EGP, and glucagon, while those with diabetes have a slight increase in glucose utilization after fasting for eight hours as compared to five hours, which reflects the rise in the EGP during that time.

A recent Danish study had explained the nonlinear inverse relationship between insulin sensitivity and glucagon response and described high fasting glucagon level versus lower insulin sensitivity and inadequate glucagon suppression after meals.
Monnier et al. found that OAD does not adequately control the early morning hyperglycemia that occurs between 600 and 800, even when they are given in combination. Sulphonylureas increase the risk of developing afternoon or evening hypoglycemia after dose adjustment to counteract the early morning hyperglycemia.\textsuperscript{28,29} Oral incretin-based therapies may improve blood glucose in postprandial, but not the fasting period.\textsuperscript{30} On the contrary, the evening administration of basal insulin is effective in abolishing the dawn phenomenon by restraining EGP and lipolysis.\textsuperscript{17}

The present study shows no significant difference in the first and second FSG readings in patients with diabetes, as well as in individuals without diabetes. The only exception was for those using only OAD, who showed a significant difference between the two fasting readings.

There are limitations to this study. First, we could not determine whether the FSG changes after fasting for five to six and eight hours reflected the effect of the duration of prior fasting or real circadian modulation. Second, we could not assess the quality and patterns of night sleep that impact FSG levels. Third, we could not determine the (exact) timing and amount of the last caloric intake because this was self-reported, and therefore subject to error and potential bias. Although we have approximate recordings of the timing of eating, we cannot identify which ingestions were considered by the subjects to be meals. Fourth, exercise effect not estimated. Fifth, we did not measure the responses of the counter-regulatory hormones and melatonin during and after six and eight hours of fasting, and this may be a prospect for future research.

**Conclusion**

There was no significant difference in FSG after five to six and eight hours fasting in individuals with or without T2DM. The minimal overnight fasting duration to assess FSG can be shortened to five to six hours and no need to fasting an extra two to three hours. Our findings support the recommendation that the standardization of fasting duration is essential in clinical studies that deal with glycemic control. Further studies are needed to evaluate the appropriate timing and duration of fasting in non-Ramadan fasters, as well as evaluation of the effect of sleep quality and the circadian rhythm on the glycemic pattern.
Conflict of Interest
The authors declare no conflicts of interest.

Funding
No funding was received for this study.

References


Table 1: The general demographic characteristics of the enrolled individuals (n=200)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>45.6 ± 14.4</td>
<td></td>
</tr>
<tr>
<td>Body mass index (BMI)</td>
<td>31.3 ± 6.4</td>
<td></td>
</tr>
<tr>
<td>Male gender</td>
<td></td>
<td>113 (56.5)</td>
</tr>
<tr>
<td>Patients with diabetes mellitus</td>
<td></td>
<td>115 (57.5)</td>
</tr>
<tr>
<td>Type of therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No therapy</td>
<td></td>
<td>16 (13.9)</td>
</tr>
<tr>
<td>Oral antidiabetic medications (OAD)</td>
<td></td>
<td>54 (47)</td>
</tr>
<tr>
<td>Insulin with OAD</td>
<td></td>
<td>45 (39.1)</td>
</tr>
</tbody>
</table>

BMI, Body Mass Index; OAD, oral antidiabetic medications; SD, standard deviation.

Table 2: Comparison of fasting serum glucose measurements in mg/dL between 5 to 6 hours and 8 hours.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean ± SD</th>
<th>95% CI for mean difference</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5-6 hours FSG</td>
<td>8 hours FSG</td>
<td>Difference</td>
<td></td>
</tr>
<tr>
<td>Non-T2DM</td>
<td>104.5 ± 21.4</td>
<td>104.8 ± 12.6</td>
<td>-0.2 ± 15.9</td>
<td>-0.3.7 – 3.1</td>
</tr>
<tr>
<td>T2DM</td>
<td>235± 107</td>
<td>230± 105</td>
<td>5.6 ± 45.6</td>
<td>-2.8 – 14.0</td>
</tr>
<tr>
<td>No therapy</td>
<td>194.0 ± 151.5</td>
<td>193.9 ± 128.9</td>
<td>0.06 ± 40.1</td>
<td>-21.3 – 0.9</td>
</tr>
<tr>
<td>OAD</td>
<td>220.0 ± 78</td>
<td>207± 77</td>
<td>13.1 ± 37.8</td>
<td>2.8 – 23.4</td>
</tr>
<tr>
<td>Insulin with OAD</td>
<td>268± 111</td>
<td>269± 114</td>
<td>-1.4 ± 54.7</td>
<td>-17.8 – 15.0</td>
</tr>
</tbody>
</table>

CI, confidence interval; FSG, fasting serum glucose; OAD, oral antidiabetic medications; SD, standard deviation; t, paired t-test value; T2DM, type 2 diabetes mellitus.
Figure 1: Boxplots of serum glucose measurements after 5-6 hours and 8 hours among the study group.

OAD, oral antidiabetic medications; T2DM; type 2 diabetes mellitus