Effects of Fasting on Saliva Composition

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Abstract

The concentrations of calcium, phosphate, protein and nitrite in whole unstimulated saliva, and the salivary flow rate under fasting conditions (saliva collected at least after 6 h without food and water) were compared with those under control conditions (saliva collected within 30 min to 1 h after food). The flow rate of fasting saliva was half that of control (0.098 ml/min vs 0.208 ml/min) and no significant differences in the flow rate were observed between sexes. The concentration of nitrite under fasting conditions was 50% higher than that in control saliva (p < 0.05). The protein concentration was decreased, but not significantly, under fasting conditions. The composition of fasting saliva with regard to calcium and phosphate concentrations was comparable to that of the control. No significant variations in these components between sexes were observed under either condition.

Introduction

Saliva collection, unlike blood collection, is non-invasive. Extensive studies over the last 35 years have shown that the composition of saliva varies with salivary flow rate¹⁻⁴ and may vary with time of day in any one individual⁵⁻⁹, while age and possibly sex may be sources of variation between individuals¹⁰⁻¹². Some reports have indicated the possibility of using saliva for the diagnosis and monitoring of disease¹²,¹³. If saliva were to be used in diagnosis, samples would have to be collected, and the method of collection is important. The true “resting” saliva to be considered as the control is difficult to obtain, since salivary flow is always influenced by some form of stimulation¹¹⁻¹²,¹⁴. It has been reported that a short period of fasting reduces the salivary flow rate¹⁵, and that under this condition, subjects are subjected to the influence of psychological and physiological reactions to starvation, involving stress and behavioral changes¹⁵.

The objective of the present study was to examine the effect of fasting on certain salivary constituents such as calcium, phosphate, nitrite and protein. Since future work will involve measurements of these components, it is pertinent to choose them among other salivary constituents. Differences in the constituents between sexes were also determined.

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Materials and Methods

Chemicals All the chemical reagents used were of analar grade except for the following: Folin-Ciocalteau reagent and Coomassie brilliant blue G-250 were obtained from Sigma Chemical Co.

Methods

Collection and preparation of saliva samples

Whole unstimulated saliva collected from subjects at least 30 min to 1 h after food intake was used as control saliva. Whole unstimulated saliva from subjects who had abstained from food and water for at least 6 h prior to collection was regarded as fasting saliva. Whole saliva was used because it reflects the actual situation of the oral environment.

Twenty-two fasting (10 females and 12 males) and 19 control (9 females and 10 males) subjects with fair to good oral hygiene were used in the study. Whole saliva was collected into clean, prechilled containers by expectoration without stimulation for a period of one hour. The collection was done between 9 a.m. and 11 a.m. Saliva collection was carried out at the same time of day to minimize any variations in saliva composition due to the time of day. The subjects used in the study were aged between 23 and 61 years. Both the control and fasting subjects were of Malay race and the experiment was carried out during the Muslim fasting month, i.e. Ramadan.

The collected saliva samples were centrifuged at 5,000 x g for 10 min at 4°C. Supernatants were used immediately for analysis of protein, calcium, phosphate and nitrite contents.

Determination of protein concentration

The proteins in the saliva were quantified using the Bradford method. Fifty-microliter saliva samples were diluted to 100 μl with deionized distilled water and used for the assay.

Determination of calcium concentration

Two tenths of a milliliter of saliva was mixed with 1 ml of 1.25 N KOH and 0.1 ml calcon indicator (0.2 g calcon in 100 ml methanol). The mixture was then titrated with EDTA (0.45 g per liter water) until the blue color appeared. After addition of the first 0.25 ml, the EDTA was added in fractions of 0.02 ml to avoid overshooting the end-point of the titration. The titration was carried out within 3 min, since the indicator is not stable. The calcium determination was repeated for standard calcium (10 mg calcium per 100 ml water).

Estimation of phosphate

One-milliliter saliva samples were precipitated with 6.5 ml of 2% trichloroacetic acid to remove the protein. After centrifugation for 5 min, the clear supernatant was used for the assay. To 1 ml of the supernatant, 2.8 ml of water, 0.5 ml of sodium molybdate (7.5% molybdate mixed with 5 N sulfuric acid (1:1, v/v)) and 0.2 ml of ANSA reagent were added. The mixture was left at room temperature for 10 min before reading the absorbance at 680 nm.

Determination of nitrite
Nitrite was estimated according to the method described by Phizackerley and Al-Dabbagh[17].

**Results**

Table 1 shows the concentrations of protein, calcium, phosphate and nitrite and the salivary flow rate for whole unstimulated fasting and control saliva. The salivary flow rate decreased by almost 50% on fasting (0.208±0.020 ml/min vs 0.098±0.023 ml/min). There was no significant decrease in the protein content, but the level of nitrite increased by almost 50% (p<0.05). No significant changes in the concentrations of calcium and phosphate were observed under the two conditions.

Tables 2 and 3 compare the salivary compositions between males and females under fasting and control conditions. There were no significant differences in the levels of protein, calcium, phosphate and nitrite between sexes under both conditions.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Mean calcium, phosphate, protein and nitrite levels in whole unstimulated saliva (control) and in whole unstimulated fasting saliva (fasting), and the salivary flow rate under both conditions</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein, mg/ml</td>
<td>Control</td>
<td>Fasting</td>
</tr>
<tr>
<td>n</td>
<td>( \bar{x} )</td>
<td>SD</td>
</tr>
<tr>
<td>19</td>
<td>1.450 ± 0.542</td>
<td></td>
</tr>
<tr>
<td>Ca, mg/ml</td>
<td>19</td>
<td>0.099 ± 0.018</td>
</tr>
<tr>
<td>P, mg/ml</td>
<td>19</td>
<td>0.770 ± 0.304</td>
</tr>
<tr>
<td>Nitrite, ( \mu )M</td>
<td>19</td>
<td>102.010 ± 72.630</td>
</tr>
<tr>
<td>Flow rate, ml/min</td>
<td>19</td>
<td>0.208 ± 0.020</td>
</tr>
</tbody>
</table>

*\( p<0.05 \)  n = no. of samples  \( \bar{x} \) = mean  SD = standard deviation

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Comparison of mean calcium, phosphate, protein and nitrite levels in whole unstimulated (fasting) saliva between male and female subjects</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Protein, mg/ml</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>n</td>
<td>( \bar{x} )</td>
<td>SD</td>
</tr>
<tr>
<td>14</td>
<td>1.304 ± 0.732</td>
<td></td>
</tr>
<tr>
<td>Ca, mg/ml</td>
<td>14</td>
<td>0.107 ± 0.022</td>
</tr>
<tr>
<td>P, mg/ml</td>
<td>14</td>
<td>0.854 ± 0.274</td>
</tr>
<tr>
<td>Nitrite, ( \mu )M</td>
<td>14</td>
<td>170.580 ± 95.850</td>
</tr>
<tr>
<td>Flow rate, ml/min</td>
<td>14</td>
<td>0.097 ± 0.021</td>
</tr>
</tbody>
</table>

*\( p<0.05 \)  n = no. of samples  \( \bar{x} \) = mean  SD = standard deviation
Discussion

In this study, the effects of fasting and differences in sex on salivary flow rate and salivary calcium, phosphate, protein and nitrite concentrations were determined.

The flow rate of whole unstimulated saliva (control) was 0.208 ml/min whereas the values that have been reported previously for normal resting saliva have been as low as less than 0.1 to as high as 0.35 ml/min. Thus the control value reported in the present study was within this range. However, under fasting conditions the flow rate was decreased to almost 50% of the control flow rate, which was less than 0.1 ml/min (0.098 ml/min). The data also show that fasting decreased the flow rate in both sexes, without any significant difference.

It has been reported that the mean levels of calcium and phosphorus in submandibular and sublingual saliva for caries-free children are higher than for caries-susceptible children, although only the phosphorus level is significantly different \[19\]. A low salivary flow rate like that in children after treatment with antidepressive drugs \[20\] and in cases of xerostomia \[21\] causes a marked increase in caries activity, although no mention has been made of the relationship with calcium and phosphate levels. We were interested to know the levels of calcium and phosphate under conditions of decreased salivary flow rate shown to be induced by fasting. Our data showed that fasting, although decreasing the salivary flow rate, does not affect the calcium and phosphate concentrations in whole unstimulated saliva.

High salivary flow rates are said to increase protein levels \[11\]. The present data showed that the reduced flow rate induced by fasting caused a decrease in protein concentration, though not to a significant extent. This suggests that flow rate has some influence on protein concentration.

It has been reported that a high nitrite level may have some relationship with oral cancer \[22\]. The normal level of salivary nitrite varies greatly, and is within the range 30-210 µM \[23\]. In the present study, it was shown that fasting caused an increase of 30-50% in the nitrite level. The levels of nitrite ranged from 72.62-102.01 µM in the control, and 100.55-162.33 µM in the fasting saliva. The low salivary flow rate under fasting conditions could stimulate bacterial activity, which would then act on available nitrate, converting it to nitrite, thereby increasing the level

<table>
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<tr>
<th>Table 3 Comparisons of mean calcium, phosphate, protein and nitrite levels in whole unstimulated (control) saliva between male and female subjects</th>
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<td></td>
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<tr>
<td>----------------------------------------------------------------------------------------------------------</td>
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<tr>
<td>Protein, mg/ml</td>
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<tr>
<td>Ca, mg/ml</td>
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<tr>
<td>P, mg/ml</td>
</tr>
<tr>
<td>Nitrite, µM</td>
</tr>
<tr>
<td>Flow rate ml/min</td>
</tr>
</tbody>
</table>

*p < 0.05  n = no. of samples  x = mean  SD = standard deviation
of nitrite. If salivary nitrite is to be used for diagnosis, it would be important to have a standardized control method of saliva collection.

**Conclusion**

The present study revealed that the concentrations of calcium and phosphate in whole unstimulated saliva collected at the same time of day were not affected by fasting. However, the salivary flow rate and protein concentration were found to be reduced, although only the flow rate was significantly reduced, while the nitrite concentration was increased under fasting conditions.

**References**


