Salivary Immunoreactive Insulin: a New Entry in Clinical Chemistry?

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The distribution of salivary immunoreactive insulin (S-IRI) and its relation to plasma insulin (P-IRI) and other clinical and metabolic variables were investigated in 93 nondiabetic subjects (60 males, 33 females, ages 11–70 y). S-IRI was measured by RIA, with moniodinated insulin as labeled antigen and with standards consisting of serial dilutions in saliva of known amounts of native insulin. The sensitivity of the method was 12.1 pmol/L. The intra- and between-assay CVs were near 10%, and analytical recovery exceeded 93% for various insulin concentrations added to saliva. S-IRI, measured after overnight fast, showed a nongaussian distribution in males (skewness 1.18, kurtosis 1.41) and females (skewness 1.71, kurtosis 1.18); mean and median values were higher in males (14.3 and 12.1 nmol/min) than in females (12.1 and 7.15 nmol/min), but not significantly so. S-IRI was significantly correlated with P-IRI both in males (r = 0.75, P < 0.0001) and females (r = 0.72, P < 0.0001). These results, particularly the correlation between S-IRI and P-IRI, indicate the possibility of using S-IRI measurement in clinical practice.

Additional Keyphrase: radioimmunoassay

Preliminary reports have demonstrated the presence of salivary immunoreactive insulin (S-IRI) in small groups of normal subjects (1, 2). More recently, studies from our (3, 4) and other laboratories (5–7) showed that S-IRI can also be
found in selected groups of obese nondiabetic and non-obese diabetic patients, is related to their plasma insulin (P-IRI) after oral glucose, and is not a compound produced in situ or some cross-reacting interferent. These findings prompted us to further investigate the significance of S-IRI in a sufficiently wide group of nondiabetic subjects and to evaluate the relationship between S-IRI and P-IRI and other clinical and metabolic variables in these subjects.

The preliminary step before we could address these issues was to develop a radioimmunoassay for measurement of insulin in saliva. Below, we detail this assay and discuss the results we obtained, which suggest that measurement of S-IRI may be useful in clinical chemistry.

Materials and Methods

Subjects and Methods

Ninety-three volunteers (60 males and 33 females—generally, but not all, blood donors—ages 11–70 years) were investigated. No subject had close (first-degree) relatives with diabetes mellitus or was taking any drug.

Subjects were studied after an overnight fast and in 30 cases (16 males and 14 females) also 2 h after breakfast. Blood was sampled from an antecubital vein and plasma was separated for the enzymatic measurement of glucose in a Beckman glucose analyzer (8); aliquots were stored at −20 °C until analysis for insulin. Plasma immunoreactive insulin (P-IRI) was measured with a radioimmunoassay devised in our laboratory (9), with HPLC-purified A14-125I-labeled insulin (10) as labeled antigen.

Radioimmunoassay of Salivary Insulin

Preparation of the tracer. A14-125I-labeled insulin (i.e., insulin selectively moniodinated in the tyrosine located at position 14 of the A-chain) was obtained as previously described (10).

Antibody dilution curves. We incubated, in triplicate, 100 μL of a solution containing the tracer (30 pmol/L in potassium phosphate buffer, 40 mmol/L, pH 7.4, containing bovine serum albumin, 5 g/L) for 23 h with 100 μL of guinea pig antiserum to porcine insulin at serial dilutions (from 1:1250, corresponding to an excess of binding capacity, to 1:1290 × 109) and 200 μL of the albumin-containing potassium phosphate buffer. We then added 1 mL of 21 g/L dextran-coated charcoal in the potassium phosphate buffer, and allowed the mixture to stand for 10 min at 4 °C. After centrifugation (2500 × g, 10 min) the count rate for A14-125I-labeled insulin in 0.7 mL of the supernatant fluid was measured, in triplicate, together with that for a sample containing the total radioactivity in the same volume and medium. We calculated nonspecific absorption of the tracer by dextran-coated charcoal after incubating 100 μL of A14-125I-labeled insulin with 300 μL of the phosphate buffer for 23 h. The mean (n = 3) antibody titer was 1:560 000 (SD 88 000).

Standard curves. Standard solutions of unlabeled porcine insulin were prepared by dissolving crystalline mono-component porcine insulin to give a final concentration of 1430 pmol/L, as confirmed by measuring the absorbance of the solution at 277 nm in a spectrophotometer (PM Q II; Zeiss, Oberkochen, F.R.G.). Reference saliva samples with known amounts of immunoreactive insulin were prepared as follows. We collected 50 mL of saliva from one healthy volunteer during approximately 2 h after an overnight fast and centrifuged (10 000 × g, 15 min, 4 °C). We added 18 g of dextran-coated charcoal and stirred the suspension at 4 °C for 12 h. After another centrifugation (20 000 × g, 60 min, 4 °C), we measured the supernate for immunoreactive insulin by the method described below: measurable concentrations of immunoreactive insulin were not found. We then made serial dilutions of insulin (11.4–1430 pmol/L) in insulin-free saliva samples to construct the inhibition curves.

Radioimmunoassay. We incubated 100 μL of each standard solution and saliva sample (with unknown content of insulin) in duplicate with 100 μL of tracer, 100 μL of antiserum, and 100 μL of the potassium phosphate buffer for 23 h at 4 °C. After absorption of the free hormone with 1 mL of dextran-coated charcoal and centrifugation, we counted the radioactivity of 0.7 mL of the supernate, together with that of a sample for total radioactivity in the same volume and medium. Nonspecific absorption of the tracer by dextran-coated charcoal was calculated after incubation for 23 h of 100 μL of labeled insulin with 300 μL of the potassium phosphate buffer.

Analysis of data. A computer algorithm was used for the analysis of the dose–response curve, both for the standard samples and for the samples with unknown amounts of S-IRI (11). The proper measure of S-IRI was expressed in concentration per flow rate (nmol/L per L/min), which reduces to nmol/min.

The results are expressed as mean ± SD. Differences between means were compared by Student's t-test for paired or unpaired data. Univariate regression analysis and Spearman's rank correlation coefficient were used to evaluate correlations between parameters, as appropriate.

Results and Discussion

The main clinical and metabolic characteristics of subjects are reported in Table 1. The mean values of body mass index (BMI) were in the normal range both in males and in females; however, 12 subjects (seven males and five females) had BMI higher than 30 kg/m²; mean fasting P-IRI was 77.2 pmol/L in males and 70.1 pmol/L in females. Mean salivary flow was 276 μL/min (range 52–1026 μL/min); in 13 subjects, salivary flow measured on two additional occasions, under the same experimental conditions, remained fairly constant (the observed variations were less than 20%).

The precision profile of the radioimmunological method for measuring S-IRI is depicted in Figure 1. The range in which the dose could be read with the best precision attainable by the method was approximately 71.5–715 pmol/L. The sensitivity of the method was 12.1 pmol/L (lowest value different from zero, confidence limits 2 SD).

The intra-assay CV for salivary insulin, computed for five replicates of concentrations of 50, 107, 322, and 572 pmol/L, was near or less than 10%. Mean interassay CVs, assessed by assaying two different saliva samples, were 8.2% and 9.8% at insulin concentrations of 71.5 and 393 pmol/L, respectively.

The analytical recovery of porcine insulin added to saliva

<p>| Table 1. Clinical and Metabolic Characteristics (Mean and SD) of Subjects Subdivided According to Sex |
|---------------------------------|---|---|---|---|</p>
<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Age, y</th>
<th>BMI, kg/m²</th>
<th>FPG, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>60</td>
<td>38.6 (13.5)</td>
<td>25 (3.9)</td>
<td>785 (92)</td>
</tr>
<tr>
<td>Females</td>
<td>33</td>
<td>37.0 (16.2)</td>
<td>26 (2.9)</td>
<td>749 (84)</td>
</tr>
</tbody>
</table>

BMI, body mass index; FPG, fasting plasma glucose.
samples exceeded 93% at added insulin concentrations of 357, 715, and 1430 pmol/L.

An unimodal nongaussian distribution of S-IRI was observed both in males (mean 14.3 pmol/min, median 12.1 nmol/min) and in females (mean 12.1 pmol/min, median 7.1 nmol/min); sex-related differences were not significant (P > 0.1). Skewness and kurtosis values were, respectively, 1.18 and 1.41 in males and 1.71 and 1.18 in females. The values of S-IRI did not differ significantly by age group. Both in males (r = 0.75, P < 0.0001) and in females (r = 0.72, P < 0.0001) S-IRI was significantly correlated with P-IRI (Figure 2). The after-breakfast values were significantly increased from fasting values: for P-IRI, 202 ± 33 vs 85 ± 21 pmol/L (P < 0.01); for S-IRI, 27 ± 6.4 vs 13.5 ± 4.2 nmol/min (P < 0.02).

For the first time, information on the distribution of S-IRI (insulin directly measured in saliva samples) and its relation to other clinical and metabolic parameters in a wide group of non-diabetic subjects is reported. Like other biological parameters S-IRI has an unimodal, nongaussian distribution both in males and in females; the distribution is slightly skewed toward lower values in females. However, there are no significant quantitative differences between sexes and between different age groups. Finally, P-IRI and S-IRI are significantly correlated, both in males and females.

Taken together, these results indicate the possibility of using measurements of salivary insulin in clinical chemistry, especially for subjects in whom sampling venous blood may be troublesome.

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References