Salivary Insulin in Normal and Type I Diabetic Subjects

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We studied the relationship of salivary insulin to serum insulin concentrations in normal subjects and type I (insulin-dependent) diabetic patients to test the hypothesis that salivary insulin might be a simple measure of insulinemia in diabetes. In 8 nondiabetic subjects, salivary insulin levels increased after an oral glucose load but with a delay in peak concentrations of ~45 min in comparison with serum insulin levels. There was a significant correlation ($r = .810$, $P < .01$) between mean serum insulin and the salivary insulin 30 min later. In 12 type I diabetic patients, day profiles of saliva and serum insulin were obtained during usual insulin treatment, diet, and physical activity. In serum, the mean (±SE) percentage of bound insulin was 58.8 ± 5.2%, and in saliva it was 45 ± 3.5%. The mean ratio of salivary to serum free insulin throughout the day was 1:1.6. Although there was a significant correlation ($r = .913$, $P < .001$) between mean serum free insulin for all patients and the corresponding mean free salivary insulin, several individual profiles showed marked discrepancies between the timing and magnitude of insulin changes in the two compartments. We would not, therefore, recommend salivary insulin concentrations as a reliable index of insulinemia in individuals with type I diabetes. ***Diabetes Care*** **11:489–94, 1988**

Measurement of circulating insulin concentrations throughout the day in diabetic patients is of interest for several reasons. Serum free-insulin profiles, for example, are needed to assess subcutaneous insulin absorption and disposition and the variations thereof with certain patients and with different regimens, species, and formulations of insulin (1). Hyperinsulinemia has been reported in many diabetic patients treated by modern intensified insulin regimens (2) and its magnitude, frequency, and significance (as an atherogenic factor, for example) must be further investigated (3).

It is in population studies that frequent 24-h blood samples for assay of insulin is particularly troublesome. We investigated alternative measures of insulinemia, especially those in which sampling could be easily undertaken by the patient at home. We have shown a highly significant correlation between the daily urinary insulin excretion rate and mean serum free insulin in type I diabetic patients, indicating that urinary insulin may be a useful indicator of insulinemia (4).

In this report, we investigate whether salivary insulin concentrations can also be used as an index of either average levels of serum free insulin over a period of time or at a given moment in type I (insulin-dependent) diabetes. For comparison, we also measured serum and salivary insulin in a group of nondiabetic subjects.

Our study is of interest in view of the recent reports of salivary insulin in type II (non-insulin-dependent) diabetic subjects (5) and nondiabetic subjects (5–7). There is no detailed information on salivary insulin in type I patients.

MATERIALS AND METHODS

Twelve type I diabetic patients volunteered for the study, and their clinical features are shown in Table 1. They were chosen as representing patients with a variety of ages, durations of diabetes, and insulin regimens and

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TABLE 1
Clinical features of patients

Case	Sex	Age (yr)	Duration of diabetes (yr)	Body mass index (kg/m²)	Insulin dosage (U/day)*
1	M	47	30	21.5	AR 13/7 MT 10/9
2	M	52	31	27.5	CSII basal 15 bolus 5/5/5
3	M	49	26	27.1	IT 30/25/10
4	M	46	11	27.4	AR 8/10 MT 30/20
5	M	35	9	23.5	AR 10/3/3 MT 17/0/0
6	M	52	28	23.5	HI 16/12
7	M	37	31	25.2	CSII basal 25 bolus 7/4/6
8	F	54	35	24.7	AR 4/6/8 UT 0/0/30
9	F	32	25	27.4	CSII basal 12 bolus 4/6/4
10	F	24	16	23.3	AR 8/8 MT 12/14
11	F	58	19	31.9	MX 18/15
12	F	37	31	26.3	HS 10/5 UT 0/12
Mean ± SE		43.6 ± 3.0	24 ± 2.5	25.8 ± 0.8	

AR, Actrapid porcine; MT, Monotard porcine; IT, Insulatard; HI, Humulin I; HS, Humulin S; MX, Mixtard; CSII, continuous subcutaneous insulin infusion; UT, Ultratard.
*Two values, morning and evening doses; 3 values, morning, midday, and evening doses. Two types of insulin were used by most patients.

were not receiving drugs other than insulin. The patients were admitted to a metabolic ward and, after an overnight fast, a cannula was inserted in an antecubital vein for blood sampling and was kept patent with heparinized 0.154 M sterile saline.

Blood samples for insulin and glucose were withdrawn just before the usual morning insulin injection was given (normally 0830 h), 30 min later when breakfast was started, and then at the time intervals shown in Fig. 1, until 7 h after breakfast. Patients were fully ambulant and ate lunch and snacks at midmorning and midafternoon. All meals were given according to the patients' usually prescribed diet. Smoking was not permitted.

Approximately 3 ml saliva was collected in a sterile container at the same time as blood samples were taken. After centrifugation (3000 rpm for 30 min) the supernatants were stored temporarily at 4°C until addition of polyethylene glycol (PEG).

Eight nondiabetic subjects (4 men, 4 women) were also studied. Their mean age was 33.9 ± 3.4 yr (range 24–55 yr), and mean body mass index 21 ± 0.4 (range 19.2–22.5). After an overnight fast, a cannula was inserted for blood sampling and a 75-g oral glucose tolerance test was performed, with blood taken for glucose and insulin assay at −15 and 0 min (oral glucose given),

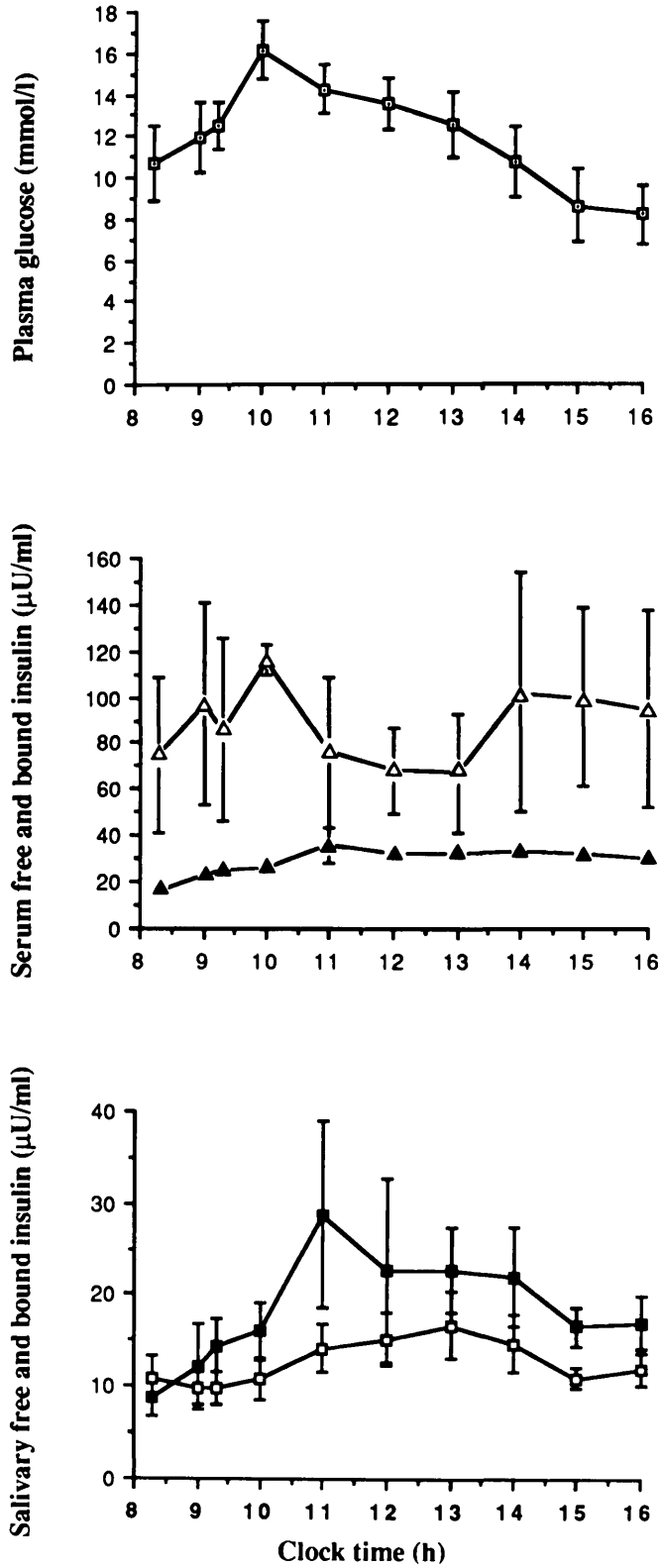

FIG. 1. Day profile for mean ± SE plasma glucose (⊡) in 12 type I diabetic patients receiving their usual insulin treatment (*top*). Corresponding serum free-insulin (▲) and antibody-bound (△) insulin levels (*center*). Salivary free (■) and bound (□) insulin levels (*bottom*). Small error *bars* in ▲ omitted for clarity.

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and subsequently at the intervals shown in Fig. 2. As with the diabetic subjects, saliva was collected at the same time as each blood sample.

On a separate occasion, the effect of increasing salivary flow rate on salivary insulin levels was studied in the normal subjects. In the fasting state, blood samples were taken from an indwelling cannula at 3-min intervals for 15 min for later insulin assay. Saliva samples were collected continuously at 3 min or 1.5 min (after stimulation) intervals for volume measurement and insulin assay. After 6 min, subjects chewed wax to stimulate salivary flow rate. This stimulation increased the flow rate by a mean of 2.6-fold ($P < .001$), but neither the serum nor the salivary insulin concentrations were significantly changed.

Plasma glucose concentration was measured by a glucose oxidase method (Yellow Springs, model 23M glucose analyzer, Yellow Springs, OH). Plasma and salivary insulin in nondiabetic subjects was measured by direct radioimmunoassay (Phadeseph kit, Pharmacia, Uppsala, Sweden). Serum free and bound insulin was determined by a PEG precipitation method (8) in which 2 ml whole blood was immediately mixed with 1.2 ml 25% (wt/vol) PEG solution (mol. wt. 8000, Sigma, Poole, UK) in 0.02 M sodium phosphate buffer pH 7.4; 1.5 ml of the PEG/blood mixture was then processed for free and bound insulin radioimmunoassay by centrifugation through Ficoll type 400 (Sigma), as described previously (8).

Salivary free and bound insulin was determined by a method analogous to that used for blood, except that 1 ml centrifuged saliva was mixed with 1 ml PEG solution. The recovery of added insulin in the salivary supernatant was measured by the addition of 0.1 ml ^{125}I-labeled insulin (Phadeseph) to 1 ml saliva. After incubation for 1 h at 37°C, samples were centrifuged and radioactivity was determined in the supernatant and precipitate. The mean recovery in the supernatant was 88 and 94% in normal and diabetic subjects, respectively.

The recovery in the free fraction after PEG addition to nondiabetic saliva and centrifugation over Ficoll was assessed in two ways. First, 1 ml saliva was mixed with 10 μl ^{125}I-insulin (50 μCi/μg) and was incubated for 1 h at 37°C. One milliliter 25% PEG was then added, and the mixture was processed as before. The recovery of counts was 102% in the free fraction. Second, 0.1 ml of insulin solutions at concentrations of 40, 90, 140, and 190 μU/ml was added to nondiabetic saliva and, after incubation for 1 h at 37°C and PEG addition, was treated as before. The recovery in the free fraction was 100 (40 μU/ml), 98 (90 μU/ml), 105 (140 μU/ml), and 96% (190 μU/ml).

Anti-insulin antibody levels were determined by the method of Kurtz et al. (9). Ten microliters of plasma were mixed with 150 μl assay buffer (0.04 M phosphate buffer pH 7.4 containing 9.0 g/L bovine γ-globulin) and 10 μl ^{125}I-porcine insulin (3.6×10^6 counts per minute/ml; Amersham, Amersham, UK). The mixture was incubated at 4°C for 24 h, then 1.5 ml PEG (200 g/L

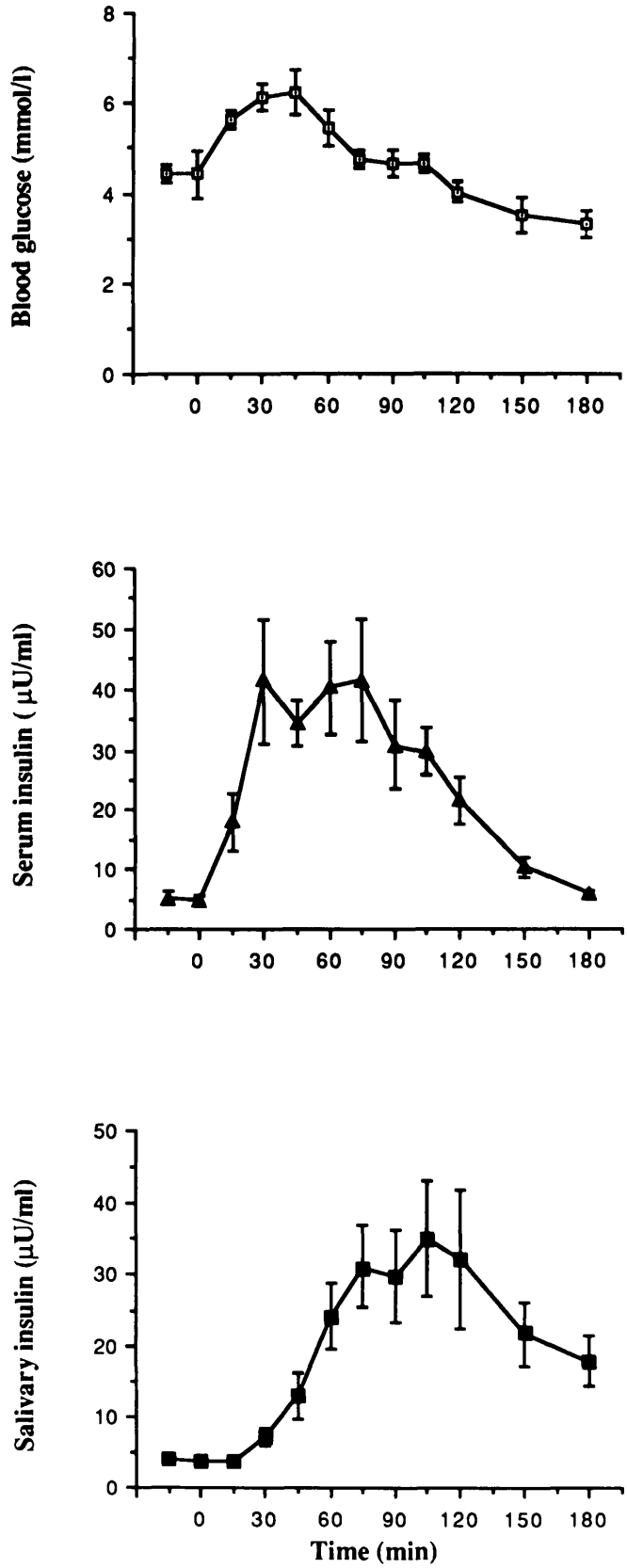

FIG. 2. Mean ± SE blood glucose (⊡), serum (▲), and salivary (■) insulin concentrations in 8 nondiabetic subjects after 75-g oral glucose load given at time 0.

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containing 1.0 g/L Tween 20 in 0.05 M Veronal buffer pH 8.6) was added. After centrifugation for 60 min at 4°C, the precipitate was counted as a measure of bound insulin. Normal subjects bound 4.1 ± 0.1% (mean ± SE). Statistical comparison of groups was by *t* test.

RESULTS

Normal subjects. Fig. 2 shows the mean ± SE plasma glucose and the serum and salivary insulin concentrations in 8 normal subjects in the fasting state and after a 75-g oral glucose load. There were similar concentrations of insulin in serum and saliva throughout the test, except that the increase in salivary insulin was delayed, the peak in mean salivary insulin occurring ~45 min after that in the serum (range 0–90 min). The best correlation was between the mean serum insulin values at a given time point and the mean salivary insulin levels 30 min later ($r = .810$, $P < .01$; Fig. 3).

The correlation between mean serum insulin in the different patients over the period of the test and the corresponding mean salivary insulin concentration did not reach significance ($r = .437$, $P > .05$).

Diabetic subjects. Fig. 1 shows profiles for plasma glucose and free- and bound-insulin concentrations in serum and saliva in 12 type I diabetic patients during the pre-breakfast-to-preevening meal period and while they were receiving their usual insulin treatment.

In diabetic patients the mean ± SE percentage of serum insulin in the bound fraction after the addition of PEG solution was 58.8 ± 5.2% and that bound in the saliva was 45.2 ± 3.5% ($P < .05$). The salivary binding was not significantly related to either the percentage binding

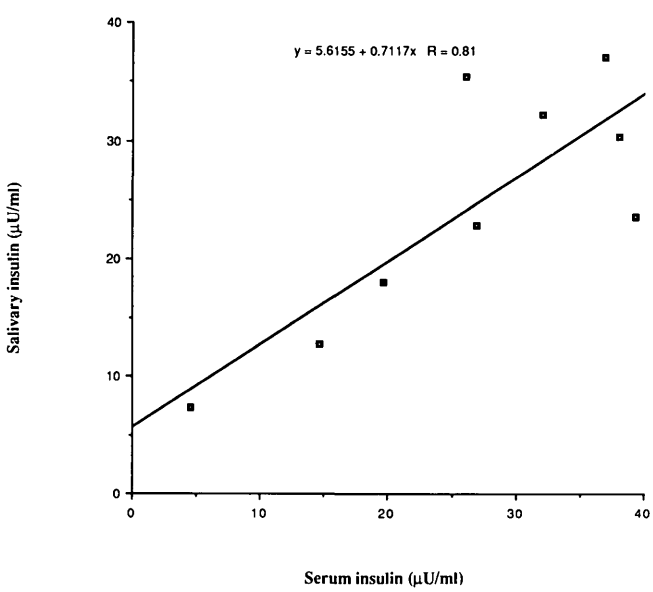

FIG. 3. Correlation between mean serum insulin and salivary insulin concentration 30 min later, after an oral glucose load in 8 normal subjects.

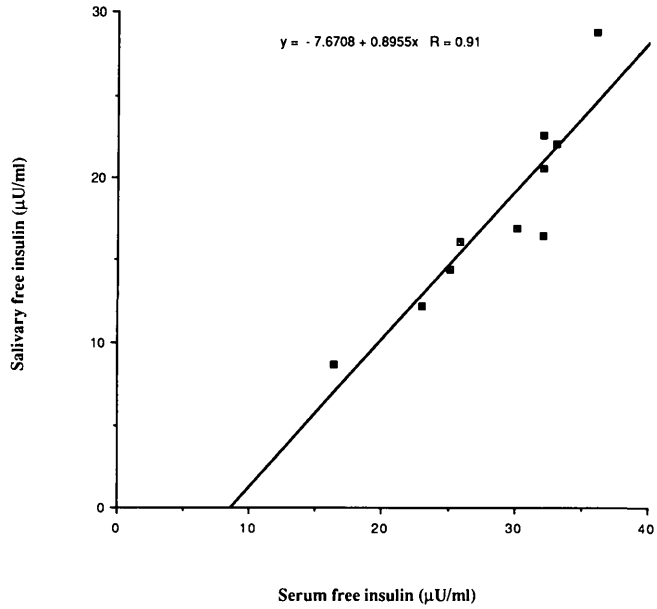

FIG. 4. Correlation between mean serum free insulin and mean salivary free insulin in 12 type I diabetic subjects.

in serum ($r = .424$, $P > .05$) or to the insulin antibody level ($r = .413$, $P > .05$).

The mean ratio of salivary to serum free-insulin concentration throughout the study was 1:1.6.

There was a significant correlation between the mean serum free insulin for all patients and the mean salivary insulin concentration at each time point ($r = .913$, $P < .001$, Fig. 4) but, as in nondiabetic subjects, the relationship between the mean insulin level in serum and saliva over the entire time of study in each patient was not significant ($r = .02$, $P > .05$), nor was the correlation with total salivary insulin levels significant ($r = .299$, $P > .05$).

As examples of the poor correlation between serum free insulin and salivary free and bound insulin in a particular patient, Fig. 5 shows four typical profiles where clinical decisions about circulating insulin would be difficult if based only on salivary values. In case 5, the peak in serum free insulin was some hours after the peak in salivary insulin (the opposite of the pattern in normal subjects). In case 6, two clear peaks of serum free insulin occurred, in the morning and in the afternoon, compared with a single sharp peak of salivary free insulin. In cases 7 and 12, a single large peak of serum free insulin in the morning was associated with little change in salivary insulin throughout the day.

DISCUSSION

This study confirms previous reports that immunoreactive insulin is present in the saliva of normal subjects (5–7) and that the concentrations increase during an oral glucose tolerance test with a delay in the peak salivary insulin of ~45 min. Unlike

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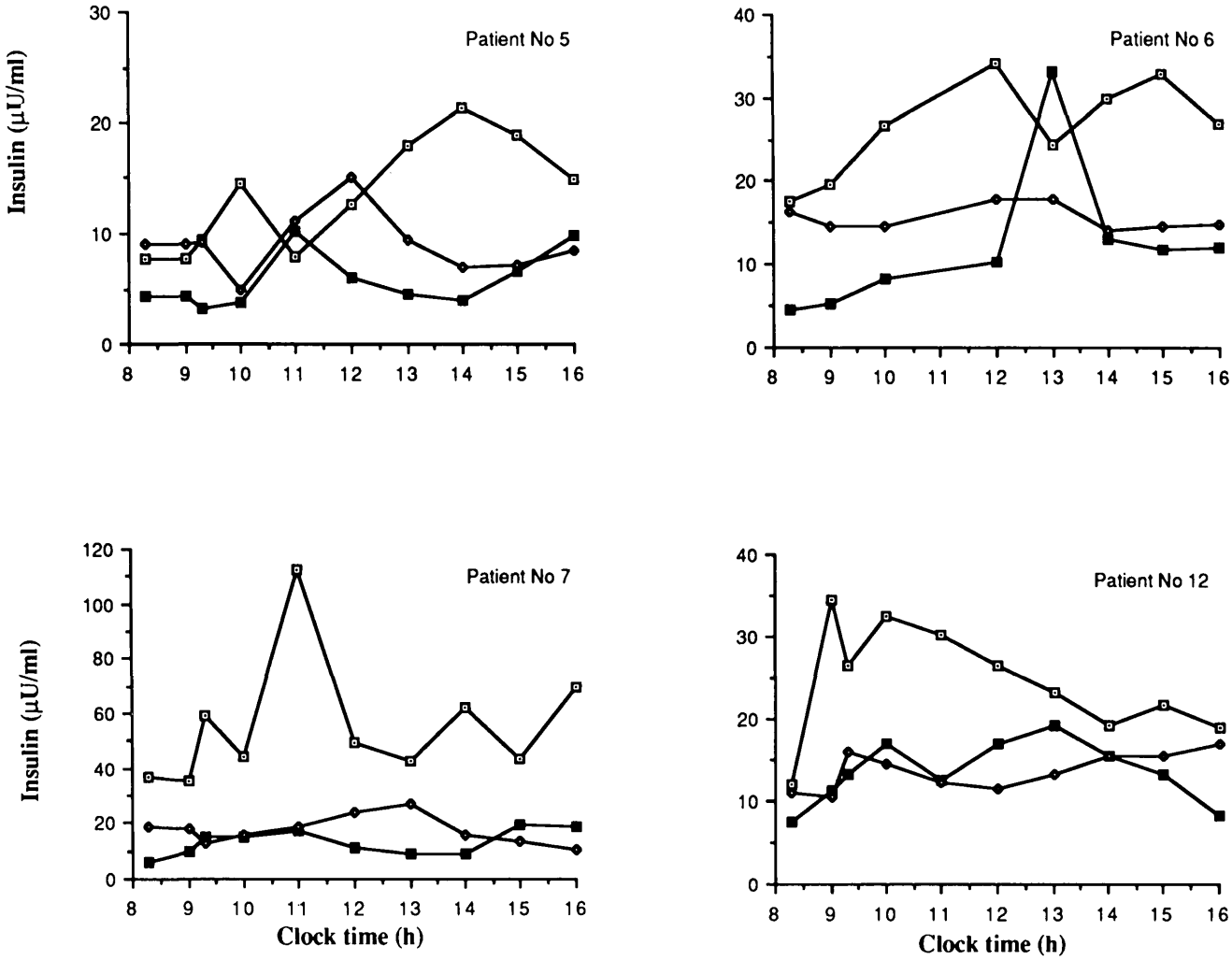

FIG. 5. Day profiles from cases 5, 6, 7, and 12 show corresponding changes in serum free (⊡) and salivary free (■) and bound (♦) insulin concentrations.

Sweeney et al. (6), we did not demonstrate any clear dependence of salivary insulin concentration on flow rate.

Although it is known that a similar pattern of salivary insulin increases occurs in type II diabetes (5), the relationship between serum and salivary insulin in type I patients during routine insulin treatment is unclear. Particular clinical value is attached to this information because simple measures of insulinemia are needed to study the associations of elevated circulating insulin levels in large-scale studies and, in individual patients, to assist in the choice and adjustment of insulin regimens by study of circulating insulin variations throughout the day.

Our results show that in type I diabetes the salivary insulin and serum insulin contains free and bound fractions, although the degree of binding in the saliva did not correlate with that in serum in this relatively small sample of patients.

Presumably, binding of salivary insulin is caused by the presence of anti-insulin antibodies in the saliva, although this remains to be conclusively established. Normal subjects, who had the expected very low PEG–precipitable insulin-binding in the serum, also had very low bound insulin in the saliva (after incubation with saliva from normal subjects, the recovery of radioactive insulin in the free, non–PEG precipitable fraction was 96–105%; see MATERIALS AND METHODS).

In the whole group of type I diabetic patients the mean salivary insulin at various times during the day was correlated with the corresponding mean serum-insulin concentrations. However, examination of individual profiles of salivary and serum insulin show many major discrepancies in timing and magnitude between the two. In our opinion, it would be unwise to use salivary insulin in studies of single patients.

Vallejo et al. (7) have provided evidence that most of the salivary insulin is derived from the blood, at least when blood insulin is artificially raised by a hyperinsulinemic clamp. The chromatographic and electropho-

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retic properties of salivary insulin are also identical to pancreatic insulin standards (5,7,10). Nevertheless, the parotid and submandibular salivary glands of rats, mice, and other animals contain cells with insulin-like immunostaining (11,12), salivary glands synthesize insulin-like material in vitro (10), and an insulin-like mRNA transcript that is strongly homologous to rat proinsulin mRNA is detectable in mouse salivary glands (13). In streptozocin-induced diabetes (12) and the BB model of diabetes in the rat (14), salivary gland insulin is decreased but not absent. The proportion of insulin in the saliva of type I diabetic subjects that originates in the salivary gland needs further study, especially because we did not find a close serum-to-saliva relationship in all subjects, and the possibility remains that erratic in situ insulin production may contribute to this variation.

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