PRELIMINARY COMMUNICATION

Diurnal Pattern of Plasma Insulin Concentration in the Human*

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Summary. Insulin in human plasma was assayed by the radioimmunological procedure of YALOW and BERSON (10). Plasma insulin levels in the course of the day were compared with those found during the night. In the subjects studied, a higher insulin concentration was observed during the night when there is no stimulation brought about by food ingestion than during the day when normal feeding occurred. The differences at midnight and at 8 A.M. were statistically significant. The variation of the plasma insulin levels suggests the existence of a circadian rhythm of the plasma insulin content.

Fluctuation de l'insuline dans le plasma pendant le nyctémère.

Résumé. L'insulinémie a été dosée par la méthode radioimmunologique de YALOW et BERSON (10). Les taux d'insuline observés au cours de la journée ont été comparés avec ceux constatés pendant la nuit. Chez les sujets étudiés la concentration insulínique pendant la nuit sans apport nutritif était plus élevée qu'au cours de la

journée pendant laquelle le régime alimentaire était normal. Les différences des insulinémies constatées à minuit et à 8 heures du matin étaient statistiquement significatives. Cette observation suggère que les insulinémies varient également selon un rythme circadien.

Der Tagesrhythmus des Insulins im Plasma des Menschen.

Zusammenfassung. Insulin wurde im menschlichem Blutplasma mittels der radioimmunologischen Methode nach YALOW und BERSON (10) bestimmt. Der Plasmainsulinspiegel während des Tages wurde mit dem in der Nacht verglichen. In den beobachteten Fällen waren die Plasmainsulinwerte während der Nacht, wenn keine Stimulation durch Nahrungszufuhr stattfand, höher als während des Tages bei normaler Ernährung. Der Unterschied zwischen dem Mitternachtspiegel und dem Blutspiegel von 8 Uhr morgens war statistisch signifikant. Die Veränderungen der Plasmainsulinwerte lassen das Bestehen eines Tag-Nacht-Rhythmus des Plasmainsulinspiegels vermuten.

The development of radioimmunoassay methods [4, 5, 10] has made it possible to make a precise and specific measurement of the variations in the plasma insulin level. In the human, the administration of glucose, tolbutamide or L-leucine (in some cases) appears to be a potent stimulus of insulin secretion [10].

The purpose of this communication is to report our studies concerning the spontaneous variations of plasma insulin levels and to confirm our previous observations [7] showing the existence of a circadian rhythm in some subjects.

The radioimmunoassay method of YALOW and BERSON [10] was employed to measure the plasma insulin levels. Human insulin twice recrystallized¹ was used for standards. Beef or pig insulin, ten times recrystallized² was iodinated with iodine-¹³¹I according to the technique reported by GREENWOOD et al. [3]. The iodinated insulin was purified on a column of cellulose powder [1].

The mean specific activity of insulin-¹³¹I obtained for ten iodination procedures, was 453 mC/mg and contained a maximum of 6-7% of radioactive impurities.

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The same batches of beef or pig insulins were emulsified in complete FREUND's adjuvant and employed to produce antibodies in the guinea-pigs. Incubation of insulin-antigen-antibody mixtures was carried out for 4 days at 4°C. Free (F) and antibody bound (B) insulin-¹³¹I were separated by paper chromatography-electrophoresis on Whatman 3 MC paper at 4°C.

The radioactivity on the paper strips was measured by an automatic scanogram. The concentration of endogenous insulin in the plasma samples was calculated from its B/F ratio by referring to a standard curve. All measurements were made in duplicate. Our experimental studies of this method give a mean recovery of 92-102%. In order to evaluate the precision of the measurement, duplicates and replicates were carried out and mean variation coefficients ($\frac{s}{x}$)

of 10% for the standards concentrations (between 0 μ U/ml and 250 μ U/ml) and of 15% for the plasma samples (concentrations between 0 μ U/ml and 380 μ U/ml) were obtained. Thirteen females and three males, ranging from 17 to 50 years, served as experimental subjects. They were hospitalized and received a normal diet of 2000 calories subdivided in 3 meals served at 8 A.M., 12 A.M. and 6 P.M.

This group comprised three normals, seven obese subjects, two latent diabetics and four subjects with various endocrine diseases. The weight of obese subjects was 20% in excess of the normal weight expressed in kg as: height (in cm) - 100. The subjects having two

Table 1. Circadian rhythm of plasma insulin levels ($\mu\text{U}/\text{ml}$) in obese females

Number of the case	7.30 A.M.	12 A.M. ²	4 P.M.	6 P.M. ²	8 P.M.	12 P.M.	4 A.M.	D ₁ ¹	D ₂ ¹
1	51.0	56.0	24.0	112.5	—	156.0	445.5	+105.0	+394.5
2	69.0	78.0	90.0	—	90.0	183.0	267.0	+114.0	+198.0
3	7.0	4.0	46.0	—	107.5	129.0	87.0	+122.0	+80.0
4	30.0	330.0	—	234.3	—	79.2	122.1	+49.2	+92.1
5	6.3	47.5	—	40.0	—	138.6	250.0	+132.3	+243.7
6	80.0	107.5	—	140.0	—	225.0	150.0	+145.0	+70.0
7	42.9	59.4	—	72.6	—	12.5	—	—30.4	—
Mean	40.9	97.5	53.3	119.9	98.8	131.9 ³	220.3 ³	+91.0	+122.6

¹ D₁ = Differences in plasma insulin levels between 12 P.M. and 7.30 A.M.

D₂ = Differences in plasma insulin between 4 A.M. and 7.30 A.M.

² = Precise time relation between blood samplings and meals are unknown.

³ If gaussian populations are assumed, statistical differences are found ($p < 0.01$) between night values (12 P.M. or 4 A.M.) and 7.30 A.M. value.

Table 2. Circadian rhythm of plasma insulin levels ($\mu\text{U}/\text{ml}$) in three normal females

Number of the case	7.30 A.M.	12 A.M. ²	4 P.M.	6 P.M. ²	8 P.M.	12 P.M.	4 A.M.	D ₁ ¹	D ₂ ¹
8	15.5	17.5	—	—	18.8	442.5	16.3	+427.0	+0.8
9	50.0	57.5	—	80.0	—	32.5	110.0	—17.5	+60.0
10	18.8	178.2	79.2	—	—	89.1	56.1	+70.3	+37.3
Mean	28.1	84.4				188.0	60.7	+159.9	+32.6

¹ and ²: See foot note Table 1.

or more of the following characteristics were considered as latent diabetics:

- 1) diabetes in the parents or the siblings.
- 2) one or more children weighing more than 4 kg at birth.
- 3) a glucose tolerance test (100 g by mouth) of the latent diabetic type¹ with or without cortisone sensibilisation [2].

An indwelling needle was placed into the antecubital vein and blood samples were obtained on EDTA in the morning fasting, at 12 A.M., 4 P.M., 6 P.M., 8 P.M., 12 P.M. and 4 A.M. Blood was immediately centrifuged and the plasma was stored at -10°C till the moment of assay. All determinations on the plasma samples of the same subject were done simultaneously. The high plasma insulin values (above $125 \mu\text{U}/\text{ml}$) were obtained by further adequate dilution.

The plasma insulin levels of the obese females studied are presented in Table I. In six patients we have observed during the night, without any exogenous stimulation, higher plasma insulin levels than those observed at 7.30 A.M. The means of the plasma insulin levels during the second part of the night are higher than those observed during the day at mealtimes. Three normal

subjects and four out of six patients with miscellaneous endocrine disorders showed also a nocturnal increase of the plasma insulin levels (Tables 2 and 3). Our experimental studies for precision and reproductibility of the assay method employed suggest that the wide spread of the insulin levels have to be attributed to individual biological variations. The variations of the insulin levels during the day when food is ingested may be related with the fluctuations of the levels of blood glucose. However, in our experiments, food intake has not been allowed during the night. Moreover glycemia measured in cases no. 2 and 8 only, throughout remained within normal range (55 to 86 mg/100 ml) and none of the patients presented any signs or symptoms of hypoglycemia during the test period. This is in accordance with the nocturnal normoglycemia observed in normal subjects when continuous recording is performed [8].

It thus appears that the marked elevation of insulin levels recorded at night cannot be explained as a response to increased blood glucose. A possible resistance to insulin action may be inferred from these data.

It is of interest to relate these findings to recently published reports showing a major increase of growth hormone [6] and ACTH [9] in the plasma at night.

However, further investigation is required to unravel the regulatory relationship between the secretion of these different hormones and to detect the factors other than food intake which control the secretion of insulin.

¹ GTT of the latent diabetic type present at least two blood-sugar values higher than the upper limit of normal, provided that fasting levels remain below 130 mg/100 ml.

Normal GTT: values not exceeding 100, 180, 140, 110 and 110 mg/100 ml at times: 0, 45, 90, 135 and 180 minutes after glucose loading respectively.

Table 3. Circadian rhythm of plasma insulin levels ($\mu\text{U}/\text{ml}$) in some endocrine diseases

Number of the case	Sex.	Clinical state	7.30 A.M.	12 A.M. ²	4 P.M.	6 P.M. ²	8 P.M.	12 P. M.	4 A.M.	D ₁ ¹	D ₂ ¹
11	Male	Latent-diabetes	—	—	35.0	—	95.0	225.0	142.5	—	—
12	Fem.	Latent-diabetes	40.0	142.5	—	97.5	—	32.5	20.0	—	7.5 — 20.0
13	Male	Cushing syndrome	12.0	27.0	0	51.0	—	600.0	—	+	588.0 —
14	Male	Pituitary tumor + obesity + gonadal insufficiency	25.0	84.0	38.0	—	31.0	165.0	99.0	+	140.0 + 74.0
15	Fem.	Acromegaly + untreated diabetes	25.0	45.0	—	18.8	—	25.0	17.5	0	— 7.5
16	Fem.	Pituitary dwarfism	25.0	24.5	35.5	—	—	82.5	87.5	+	57.5 + 62.5

¹ and ² See foot note Table 1.

References

- [1] BERSON S.A., and R.S. YALOW: Preparation and purification of human insulin-¹³¹I. Binding to human insulin-binding antibodies. *J. clin. Invest.* **40**, 1803—1808 (1961).
- [2] FAJANS, S.S., and J.W. CONN: An approach to the prediction of diabetes mellitus by modifications of the glucose tolerance test with cortisone. *Diabetes* **3**, 296—304 (1954).
- [3] GREENWOOD, F.C., W.M. HUNTER and J.S. GLOVER: The preparation of ¹³¹I labelled human growth hormone of high specific radioactivity. *Biochem. J.* **89**, 114—123 (1963).
- [4] GRODSKY, G.M., and P.H. FORSHAM: An immunochemical assay of total extractable insulin in man. *J. clin. Invest.* **39**, 1070—1079 (1960).
- [5] HALES, C.N., and P.J. RANDLE: Immunoassay of insulin with insulin-antibody precipitate. *Biochem. J.* **88**, 137—146 (1963).
- [6] HUNTER, W.M., and W.M. RIGAL: Plasma growth-hormone in children at night and following a glucose load. *Acta endocr. (Kbh) Suppl.* **100**, 121 (1965).
- [7] LAMBERT, A.E., J.J. HOET, E. EKKA and R. DE HERTOOGH: The influence of circadian rhythm and glucose intake on insulin concentration. Abstract no. 98 of the First Annual Meeting of the European Association for the Study of Diabetes. Montecatini Terme (1965).
- [8] MIROUZE, J.: L'enregistrement glycémique continu par automation en pratique diabétologique. *Rev. roum. Endocrin.* **2**, 321—330 (1965).
- [9] SORGE, F., H.E. MENTSEL, G. SCHUMANN, A. BRENDDEL und E.F. PFEIFFER: Sekretionsrhythmik von ACTH und Cortisol im Blute von Stoffwechselgesunden, Prädiabetikern und Fettsüchtigen. Abstract no. 119 of the First Meeting of the European Association for the Study of Diabetes. Montecatini Terme (1965).
- [10] YALOW, R.S., and S.A. BERSON: Immunoassay of endogenous plasma insulin in man. *J. clin. Invest.* **39**, 1157—1175 (1960).

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