Diurnal Change of Plasma Insulin, Free Fatty Acids and Human Growth Hormone in Diabetics during Bonito Insulin Treatment

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TOYOTA, T., SATO, S., OHNEDA, A. and GOTO, Y. *Diurnal Change of Plasma Insulin, Free Fatty Acids and Human Growth Hormone in Diabetics during Bonito Insulin Treatment.* Tohoku J. exp. Med., 1972, 107 (2), 167-172 — The present study was undertaken to see whether the function of the pancreas in diabetics is improved by the exogenous insulin treatment. In seven diabetic patients, bonito insulin was injected every day until the urine sugar became negative, and the diurnal change of plasma insulin was estimated before insulin injection and during the urine sugar-free period. Plasma insulin area, calculated by our formula, increased in six of the patients. As bonito insulin does not show any immunological cross-reaction with pork, beef and human insulin, this increase in plasma insulin area after the bonito insulin treatment may be due to an improvement of the impaired function of the pancreas ——— bonito insulin; insulin treatment; plasma insulin; diurnal rhythm

The radioimmunoassay technique (Yalow and Berson 1960) has enriched our knowledge on blood insulin levels in various conditions and also has given many evidences to support the classical theories on the pathogenesis of diabetes mellitus. In the previous study (Toyota 1969), we reported the diurnal fluctuation of plasma insulin before and after the sulfonylurea treatment and found that the plasma insulin levels of the mild diabetics decreased after the control of blood sugar, and on the contrary, the plasma insulin levels of the moderately severe diabetics increased by the treatment. These observations impelled us to extend our study to see a change of plasma insulin level after the treatment with insulin. The estimation of the endogenous circulating insulin during insulin treatment is possible if bonito insulin is used, because it has no immunological cross-reaction with pork, beef or human insulin (Yamagata *et al.* 1969).

MATERIALS AND METHODS

Seven primary diabetic inpatients were selected for this study. Age of the cases ranged from 36 to 52 years old. Five of them were male and two female (Table 1). The

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diet of 1,600 kcal or 1,800 kcal was given to the patients during the study. Breakfast was served at 8:00 a.m., lunch at noon and supper at 4:30 p.m. No other foods were given during this experiment. Blood samples for blood glucose, plasma insulin, free fatty acids (FFA) and growth hormone (GH) analyses were obtained by antecubital venipuncture with a heparinized syringe at 7:00 a.m., 11:00 a.m., 1:00 p.m., 4:00 p.m., 6:00 p.m., 12:00 midnight and 4:00 a.m. and 7:00 a.m. of the following morning. Daily excretion of urine sugar was measured during the hospitalization. The initial determination of diurnal rhythm of blood glucose, plasma insulin, FFA and GH was performed before the start of the bonito insulin therapy and the second determination was made after 8 to 33 days of the therapy, when the 24-hour urinary sugar excretion became zero. Unmodified bonito insulin was injected subcutaneously thirty minutes before each meal, *i.e.* at 7:30, at 11:30 a.m. and 4:30 p.m. Total dosage of bonito insulin ranged 294 to 1,144 units in the series (Table 1).

Blood glucose was determined by the glucose oxidase method of Teller (1956). The plasma insulin was extracted by the modified method of Jephcott (1931) and was measured by the double antibody method of Morgan and Lazarow (1962). FFA was estimated by the method of Itaya-Ui (1965) and GH was measured by double antibody method of Utiger (1964). Antibody to human growth hormone and ^{131}I -GH used here were prepared by Dainabot Radioisotope Laboratory. Bonito insulin was supplied by Shimizu Pharmaceutical Co.

The area of blood glucose, plasma insulin, FFA and GH were calculated by the formula described in the previous paper (Toyota 1969).

TABLE 1. *Seven diabetic patients and dosage of bonito insulin*

No.	Sex	Age	Height (cm)	Weight (kg)	Daily excretion of urine sugar (g/day)		Bonito insulin (units)	Duration of treatment (days)
					Before *	After		
S.T.	M	52	154	60	20	0	948	33
H.T.	F	34	160	44	42	0	1144	26
S.C.	M	43	160	53	135	0	480	8
S.I.	F	54	152	50	34	0	670	16
N.S.	M	36	160	47	65	0	550	22
S.K.	M	40	168	77	64	0	360	12
E.S.	M	37	166	53	16	0	294	16

RESULTS

The results are shown in Tables 2 and 3. The blood glucose levels decreased significantly after the bonito insulin treatment in all patients. This was clearly shown in the changes of blood glucose area; namely, it decreased from $6,020 \pm 535$ to $2,666 \pm 236$ mg/100 ml /24 hrs ($P < 0.01$). The plasma insulin area, however, increased in six of the seven patients and decreased in one case after the treatment. The mean value of the insulin area changed from 794 ± 129 to $1,008 \pm 249$ $\mu\text{U}/\text{ml}$ /24 hrs after the bonito insulin therapy ($0.1 < P < 0.2$).

The insulin area of Case 6 alone, in which the initial fasting plasma insulin was abnormally high, decreased from 1,353 to 963 $\mu\text{U}/\text{ml}/24$ hrs by the treatment (Table 3). This result suggested that the endogenous insulin secretion increased when the blood glucose closed to normal range by the insulin therapy, unless the initial plasma insulin level was abnormally high.

Case 3 was an insulin-deficient diabetic and his plasma insulin area was 310 $\mu\text{U}/$

ml/24 hrs and the blood glucose area was 8,956 mg/100 ml/24 hrs (mean value of normal plasma insulin area and blood glucose area are $628 \mu\text{U/ml/24 hrs}$ and $1,746 \text{ mg/100 ml/24 hrs}$, respectively). After injection of 480 units of bonito insulin for 8 days, his insulin area increased and the blood glucose area decreased. Case 2 was an moderately severe diabetic. His plasma insulin area was $615 \mu\text{U/ml/24 hrs}$ before the treatment. In this case 1,144 units of bonito insulin was used for 25 days until the urinary sugar disappeared. These results indicated that the sensitivity to the insulin was variable among the patients (Table 1).

FFA and GH levels became within the normal range after the bonito insulin therapy (Table 2); namely, FFA area changed from 14.78 ± 4.4 to $9.27 \pm 1.7 \text{ mEq/L/24 hrs}$ by the insulin treatment ($0.1 < P < 0.2$), and growth hormone area changed from 128 ± 46 to $99.5 \pm 27 \text{ m}\mu\text{g/ml/24 hrs}$ after the bonito insulin treatment ($0.1 < P < 0.2$).

DISCUSSION

It is reasonable to expect that the plasma insulin levels may become normal when the blood glucose fluctuates within nearly normal range with the management of diabetes, unless the insulin antagonists still remain and the islet-cells of the pancreas are destroyed.

Lukens and Dohan (1942) found that the hydropic-degenerated β -cells of the experimental diabetic cat pancreas showed a regeneration, when the hyperglycemic condition was managed with insulin. On the basis of this experiment, it was surmised that the plasma insulin level of diabetes increased after the exogenous insulin treatment because of the amelioration of islet-cells function. It is easy to analogize this experiment in clinical phenomenon, *i.e.* the remission of diabetic state observed in juvenile diabetics.

Our result showed that the plasma insulin area in six of seven patients increased after the bonito insulin treatment. This suggests that the injection of bonito insulin improves the function of pancreatic islet-cells.

On the other hand, two different results are reported concerning the effect of the exogenous insulin on the insulin secretion from the pancreas. Grodsky and his co-workers (1968) found no suppressive effect of the bonito insulin on the insulin secretion from rat pancreas. However, an inhibition of the insulin release from the pancreas by insulin *in vitro* was demonstrated by Sodoyez *et al.* (1969), Hahn and Michael (1970), and Iversen (1971). Since in our study the plasma insulin area increased by the treatment with bonito insulin, it seems unlikely that bonito insulin suppresses the secretion of insulin from the pancreas.

The previous study (Toyota 1969) showed that the insulin area of the mild diabetic decreased significantly with the sulfonylurea treatment and we speculated that it was due to an increase of the sensitivity of the tissues to insulin. If the insulin antagonists are present in diabetics, the insulin may be secreted in a large amount from the pancreas. FFA and GH, which are thought to be insulin antagonists, did not increase after the bonito insulin therapy in this study.

TABLE 2. *Mean value of diurnal change of blood glucose, plasma*

		7 a.m.	9 a.m.	11 a.m.	1 p.m.
Blood glucose mg/100ml	Before	185.0 ±12.2	308.9 ±21.4	276.2 ±31.0	320.9 ±40.3
	After	105.1 ±5.4	138.7 ±6.7	107.1 ±9.4	91.3 ±15.9
Plasma insulin μU/ml	Before	26.1 ±5.5	34.0 ±5.8	38.3 ±6.2	35.9 ±2.7
	After	42.4 ±8.6	48.9 ±12.6	51.4 ±11.6	49.7 ±13.6
FFA mEq/L	Before	0.62 ±0.09	0.51 ±0.16	0.39 ±0.08	0.48 ±0.11
	After	0.58 ±0.06	0.45 ±0.11	0.31 ±0.05	0.32 ±0.08
HGH mμg/ml	Before	4.1 ±0.7	3.7 ±1.8	6.2 ±2.2	7.7 ±5.1
	After	4.0 ±0.43	2.3 ±0.5	4.4 ±1.3	3.7 ±1.4

Mean±s.e. N=7

TABLE 3. *Diurnal change of blood glucose and plasma insulin*

Case		Time									Blood glucose area (mg/100 ml/ 24 hours)
		7 a.m.	9 a.m.	11 a.m.	1 p.m.	4 p.m.	6 p.m.	Midnight 0	4 a.m.	7 a.m.	
		Blood glucose (mg/100 ml)									
1	Before	238	238	230	240	209	266	254	209	236	5,713
	After	116	180	139	103	153	109	98	94	116	2,764
2	Before	222	316	276	273	247	305	242	216	195	6,143
	After	152	202	161	164	98	121	151	130	141	3,435
3	Before	201	417	438	556	396	443	333	236	215	8,956
	After	94	124	89	64	83	44	60	80	89	1,729
4	Before	173	322	327	283	301	494	238	169	154	6,797
	After	126	130	151	65	126	89	122	110	119	2,673
5	Before	164	258	257	360	202	263	232	197	209	5,864
	After	89	132	100	185	141	189	156	119	146	3,550
6	Before	152	268	254	210	173	201	136	121	152	4,253
	After	115	135	99	85	146	131	84	103	76	2,536
7	Before	151	343	152	324	239	266	159	104	108	4,778
	After	87	128	82	91	69	180	60	75	78	1,977

insulin, FFA and GH after bonito insulin treatment

Time						
	4 p.m.	6 p.m.	12 midnight	4 a.m.	7 a.m.	Area
	265.7 ±25.2	319.7 ±37.5	227.7 ±22.7	178.9 ±17.5	181.3 ±15.7	6020 ±535
	113.1 ±12.8	104.0 ±16.9	94.3 ±12.2	94.6 ±5.9	103.1 ±8.9	2666 ±263
	35.9 ±2.8	37.1 ±6.4	31.9 ±6.3	28.1 ±4.2	25.0 ±4.7	794 ±129
	39.4 ±5.5	44.6 ±10.2	38.0 ±5.7	43.1 ±10.3	38.9 ±8.7	1008 ±249
	0.48 ±.09	0.46 ±0.09	0.54 ±0.09	0.51 ±0.10	0.65 ±0.13	14.78 ±4.4
	0.34 ±0.08	0.34 ±0.10	0.36 ±0.09	0.52 ±0.09	0.55 ±0.08	9.27 ±1.7
	3.4 ±1.0	5.2 ±1.7	8.3 ±2.9	4.3 ±2.2	2.7 ±0.5	128.1 ±460
	3.7 ±0.8	3.1 ±0.9	7.1 ±2.2	3.3 ±0.7	7.0 ±3.7	99.5 ±27.1

in seven diabetic patients after bonito insulin treatment

Time									Insulin Area (μ U/ml/ 24 hours)
7 a.m.	9 a.m.	11 a.m.	1 p.m.	4 p.m.	6 p.m.	Midnight 0	4 a.m.	7 a.m.	
Plasma insulin (μ U/ml)									572 781
10 38	32 8	14 30	28 26	20 34	32 44	26 40	26 26	8 20	
16 10	28 24	58 24	38 40	20 18	18 30	18 20	20 20	20 22	615 957
14 8	10 24	16 24	24 18	10 16	14 18	10 8	8 2	10 10	310 320
26 90	58 110	50 68	38 38	56 38	46 38	44 56	40 58	38 52	1,064 1,292
35 35	28 38	40 38	35 38	38 28	40 32	26 28	26 96	26 82	783 1,049
56 40	54 30	54 42	48 56	66 60	68 36	64 36	42 34	40 18	1,353 963
26 40	28 60	36 82	40 82	36 82	42 124	35 40	35 52	28 42	861 1,697

Therefore, the increase of the plasma insulin area after the treatment is probably due to an improvement of the islet function and not related with an increase of antagonist in the plasma.

In conclusion, the dysfunction of the islet cells of the pancreas in diabetics is improved by the bonito insulin therapy. Further investigation is necessary to see the effect of a long-term insulin therapy on the endogenous insulin secretion of the pancreas.

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