Insulin Responses in Equivocal and Definite Diabetes, with Special Reference to Subjects Who Had Mild Glucose Intolerance but Later Developed Definite Diabetes

Kinori Kosaka, M.D., Ryoko Hagura, M.D., and Takeshi Kuzuya, M.D., Tokyo

SUMMARY

Insulin secretory responses during the 100-gm. glucose tolerance test (GTT) were studied in subjects who had or had had glucose intolerance. Patients who had metabolic diseases other than diabetes were excluded. The ratio (ΔIRI/ΔBS) of increments of blood insulin to blood sugar 30 minutes after glucose load was used as the most sensitive index to detect the abnormality of early insulin release in diabetes. In patients with definite diabetes (i.e., those whose fasting blood sugar values (FBS) were or had been higher than 140 mg./100 ml. or who had diabetic retinopathy), ΔIRI/ΔBS ratios were almost invariably subnormal regardless of FBS levels and the types of glucose tolerance at the time of GTT. In the rest of the patients (equivocal diabetics), ΔIRI/ΔBS ratios were either normal or subnormal. The decrease in ΔIRI/ΔBS was a fairly stable characteristic of each individual; in 330 equivocal diabetics, only 28 cases (8.4 per cent) moved between high- and low-insulin-responder groups during the follow-up. In 39 patients who had equivocal diabetes at the initial examination but subsequently developed definite diabetes (20 who began to have FBS above 140 mg./100 ml. and 19 who developed retinopathy), the insulin response were already subnormal at the initial GTT and remained low throughout the follow-up periods, although their glucose tolerance varied between normal, borderline, and diabetic types. Thus, definite diabetes occurred exclusively in the low-insulin-responder group among equivocal diabetics. The decrease in insulin response to glucose seems to be a more inherent, specific, and stable feature of true diabetes than glucose intolerance, because it precedes the occurrence and persists after the remission of derangement of carbohydrate metabolism in definite diabetes.

DIABETES *26*:944-52, October, 1977.

It is now established that insulin secretory response to glucose is generally decreased in moderate or severe diabetes. With respect to insulin response in mild or so-called chemical diabetes, there still exists some controversy. Many investigators have reported that insulin response to glucose load is decreased also in mild diabetics if the influences of higher glycemic stimulus and of coexisting obesity are taken into account.[1-4] There are some reports, however, that insulin response during intravenous or oral glucose tolerance test is not decreased in mild diabetic patients.[5,6] Savage et al.[7] pointed out that the magnitude of insulin response depends on the degree of glucose intolerance in mild diabetes. Conventionally, chemical or mild diabetes without clinical symptoms has been diagnosed on the basis of glucose intolerance. It is possible, however, that these mildly diabetic subjects consist of etiologically and pathophysiologically heterogeneous populations, in view of diverse causes capable of affecting glucose tolerance. Not all of them may progress ultimately to the stage of overt diabetes with the elevation of fasting blood sugar.

From the Third Department of Internal Medicine, University of Tokyo, Hongo, Tokyo, Japan 113.

Present addresses: (Dr. Hagura) Institute for adult diseases, Asahi Life Foundation, Nishishinjuku, Tokyo; Japan 160; (Dr. Kuzuya) Department of Medicine, Jichi Medical School, Minamikawachi-machi, Tochigiken, Japan 329-04.

Accepted for publication May 17, 1977.

In our experience, insulin response is not necessarily subnormal in some patients who have borderline or mildly diabetic glucose tolerance. We were interested in whether their insulin response becomes impaired progressively during the follow-up and whether the development of overt diabetes differs between groups with normal and subnormal insulin responses. In our previous paper[8] we reported that insulin response to glucose of definitely diabetic patients remained low even when their glucose tolerance improved to normal range after treatment. In this study, we attempted to compare equivocal and unequivocal (definite) diabetes patients with reference to their insulin responses. In particular, we analyzed the insulin responses of mildly diabetic patients whose fasting blood sugar became elevated later or who developed diabetic retinopathy during the course of observation.

MATERIALS AND METHODS

The subjects of this study were mainly the patients seen regularly at the diabetic outpatient clinics of University of Tokyo Hospital and Tokyo Women's Medical College. They were treated by diet or oral hypoglycemic agents and examined by the 100-gm. oral glucose tolerance test (GTT) before and at varying intervals after the initiation of the treatment. GTT was carried out after an overnight fast. Medications, including oral hypoglycemic agents, were withheld on the day of examination until the end of GTT. Patients who had other metabolic or endocrine diseases (e.g., liver diseases, thyrotoxicosis) or conditions known to affect glucose tolerance (e.g., gastrectomy, corticosteroid treatment) were excluded from the present analysis. Control subjects were without family history of diabetes and with normal glucose tolerance by 100-gm. GTT according to the criteria described below. Patients and control subjects who were heavier than 110 per cent ideal body weight [i.e., (body height (cm.) − 100) × 0.9 (kg.)] were classified as obese.[9]

GTT was performed by the use of 300 ml. Trelan G 50 (Simizu Seiyaku Co., Japan). It has been reported that plasma glucose and insulin responses after drinking of this solution are nearly the same as those after a 100-gm. glucose load.[10-12] Blood sugar was determined with blood samples taken from a cut on the earlobe before and 30, 60, 90, 120, and 180 minutes after the injection of the solution. Samples for serum or plasma insulin assay were taken at the same time from an antecubital vein. Blood sugar was measured first by Hagedorn-Jensen's method[13] and later by Technicon AutoAnalyzer[14] or by a glucose oxidase method (Glucostat, Worthington). As Hagedorn-Jensen's method gave about 10-20-mg./100-ml. higher values than the other methods at hand, 15 mg./100 ml. was subtracted from all the data determined by this method. Serum or plasma insulin was assayed by a double-antibody method.[15]

The types of glucose tolerance curve were classified according to the recommendation of the Japan Diabetic Society;[16] the glucose tolerance curve in which 60-minute and 120-minute values were higher than 180 and 160 mg./100 ml., respectively, was defined as the diabetic type, and the glucose tolerance curve in which 60-minute and 120-minute values were less than 160 and 120 mg./100 ml., respectively, was defined as the normal type. Intermediate curves were all defined as the borderline type.

In the previous paper,[8] we diagnosed patients as having definite or unequivocal diabetes when their fasting blood sugar (FBS) had been above 150 mg./100 ml. by the Hagedorn-Jensen method or when they had clear diabetic retinopathy plus glucose intolerance. The same definition is again adopted, except that the FBS higher than 140 mg./100 ml. was taken as indicative of definite diabetes in this paper, according to the difference in blood sugar determination methods.

RESULTS

Insulin Responses During GTT of Patients with Definite (Unequivocal) and Equivocal Diabetes

Patients with definite diabetes, as defined in the preceding section, may have diabetic, borderline, or normal-type glucose tolerance at the time of GTT because metabolic derangement is not static but varies spontaneously or by treatment. Figure 1 illustrates the mean blood sugar and insulin curves of nonobese and obese definite diabetics. Regardless of their types of glucose tolerance at the time of GTT, the mean insulin curves of definite diabetics were all lower than those of healthy controls of similar degree of obesity.

The insulin responses of definite diabetics with FBS lower than 140 mg./100 ml. were compared with those of equivocal diabetics (i.e., those whose FBS levels had never been proved to be above 140 mg./100 ml. and who had no diabetic retinopathy). As shown in figure 2, mean insulin responses of the definite-diabetes group were lower than those of the equivocal-diabetes group with similar degree of glucose intolerance.

As the absolute increase in blood insulin depends

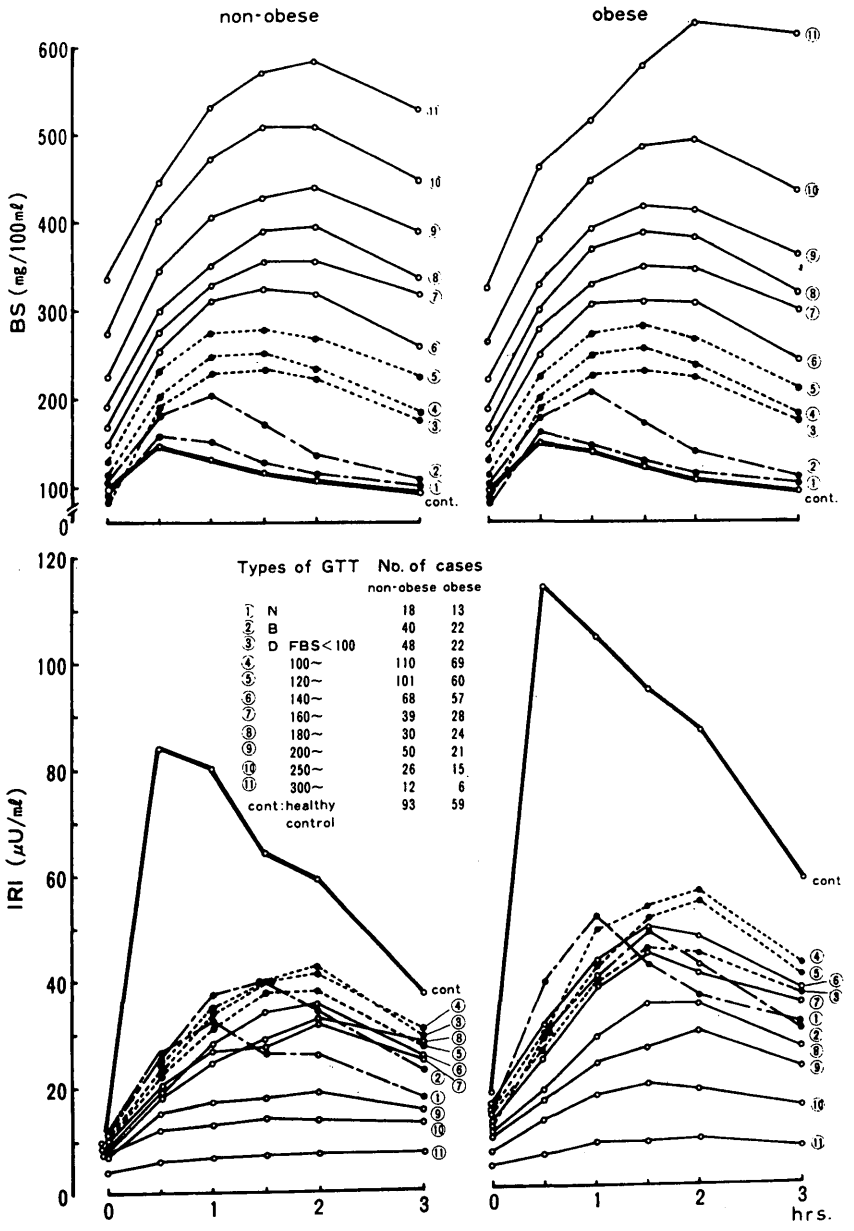

FIGURE 1

Insulin responses during 100-gm. GTT of definitely diabetic patients with varying degrees of glucose intolerance. Only mean curves of the different groups are shown. Type of GTT: N=normal, B=borderline, D=diabetic.

on the magnitude of glycemic stimulus,[1,2] the ratios (ΔIRI/ΔBS) of increment of insulin (ΔIRI in μU./ml.) to that of blood sugar (ΔBS in mg./100 ml.) 30 minutes after glucose load were calculated (figure 3). The ΔIRI/ΔBS values of healthy controls scattered considerably, but most (97.6 per cent) were higher than 0.5. In patients who had FBS higher than 140 mg./100 ml., ΔIRI/ΔBS values were invariably lower than 0.5 whether or not they were obese. As groups, patients with normal or borderline GTT had ΔIRI/ΔBS lower than normal values, but there were considerable overlaps with those in the healthy control group. The majority of patients with diabetic GTT and with FBS lower than 140 mg./100 ml. had ΔIRI/ΔBS values lower than 0.5, but there were still some overlaps of ΔIRI/ΔBS ratios with those of control subjects. When each group is analyzed in more detail by subdividing definite and equivocal diabetic patients, it becomes evident that ΔIRI/ΔBS values exceeding 0.5 were almost invariably derived from the equivocal-diabetes group. However, the reverse is not true. Many equivocal diabetics had ΔIRI/ΔBS ratios lower than 0.5. It is noted that virtually all patients with definite diabetes had ΔIRI/ΔBS values below 0.5 whether their glucose tolerance was normal, borderline, or diabetic.

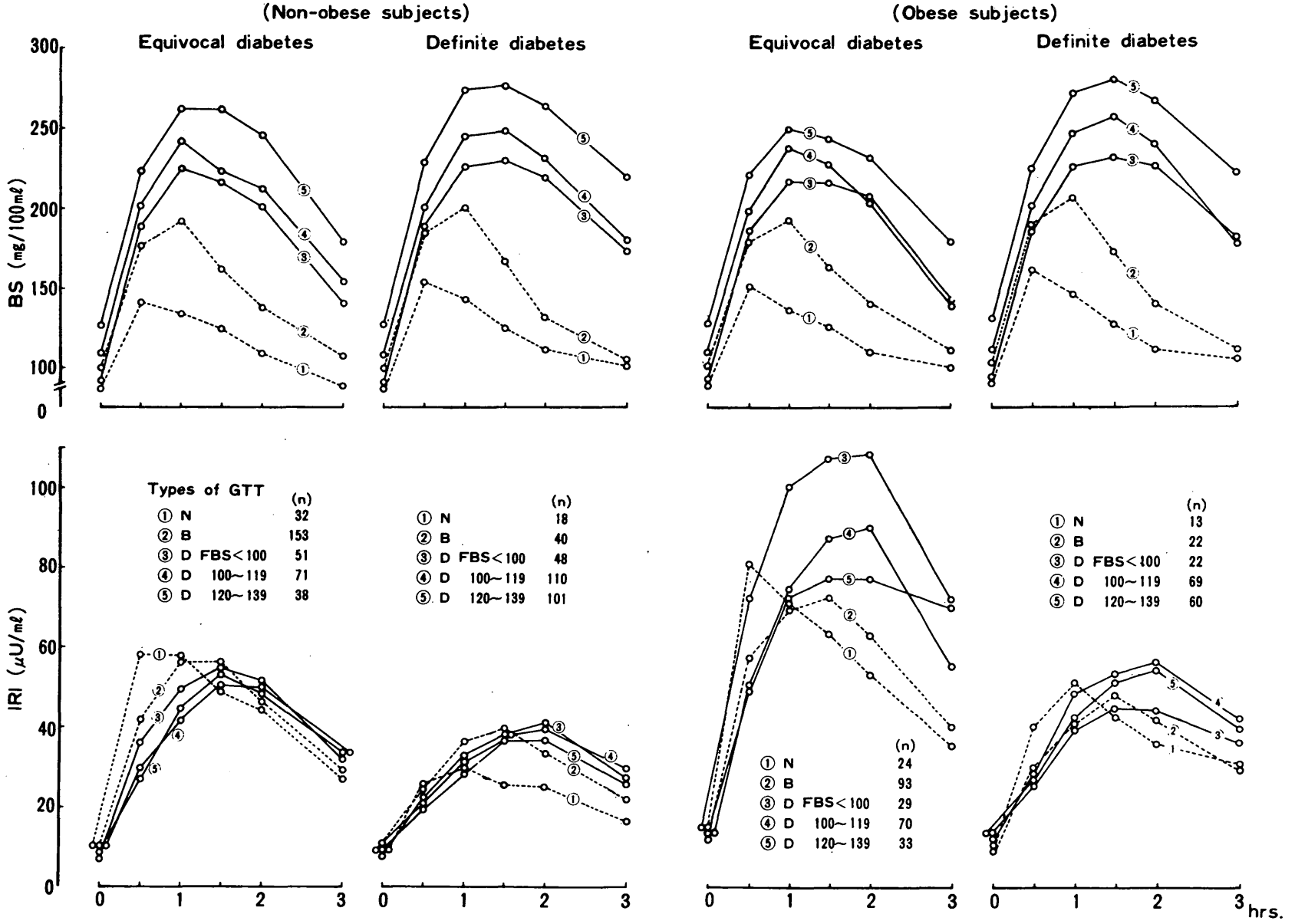

FIG. 2. Insulin responses during 100-gm. GTT of patients with equivocal diabetes and definite diabetes, with varying degree of glucose intolerance. Type of GTT: N=normal, B=borderline, D=diabetic.

Stability of ΔIRI/ΔBS Ratios During Follow-up

Table 1 summarizes the data on ΔIRI/ΔBS of patients attending the diabetic outpatient clinic in whom insulin responses were repeatedly examined. Of 1,250 GTTs performed in 446 patients with definite diabetes, only 15 tests (1.2 per cent) in 13 patients (2.9 per cent) gave ΔIRI/ΔBS values higher than 0.5. In 330 patients with other than definite diabetes, 934 GTTs were performed. In these cases, ΔIRI/ΔBS ratios were more variable on repeated tests than in the definite-diabetes group. Particularly, ΔIRI/ΔBS values exceeding 0.5 varied considerably during the follow-up of the same individual. Accordingly, the ΔIRI/ΔBS values were classified into two zones above and below 0.5, and the frequency of movements between these two zones was analyzed. In 302 cases (91.5 per cent), ΔIRI/ΔBS fell in the same zone throughout repeated tests, ΔIRI/ΔBS being consistently lower than 0.5 in 234 cases and higher than 0.5 in 68 cases.

The Insulin Responses During 100-gm. GTT of Subjects Who Had Equivocal Diabetes Initially but Later Developed Definite Diabetes

During the follow-up of equivocal diabetics (i.e., those with FBS levels lower than 140 mg./100 ml. and without retinopathy) for three months to eight years, about 40 patients became definitely diabetic. In 20 patients, their FBS levels were elevated and eventually exceeded 140 mg./100 ml. Their clinical features are presented in table 2. For example, the first patient in table 2 was first seen at the age of 53, when she had borderline-type glucose tolerance and ΔIRI/ΔBS of 0.19. Her FBS became elevated to 160 mg./100 ml. after seven years and eight months. Three glucose tolerance tests were performed during the follow-up period, resulting in two borderline and one diabetic-type curves, and the respective ΔIRI/ΔBS ratios were 0.28, 0.11, and 0.21. Table 3 shows similarly the clinical features of 19 patients who had equivocal diabetes initially but later developed diabetic retinopathy. About half of these patients had a family history of diabetes. Their initial glucose tolerance curves were either diabetic, borderline, or normal. It is noteworthy that the initial ΔIRI/ΔBS ratios were all less than 0.5 in both of these groups, irrespective of their types of glucose tolerance. With only a few exceptions, ΔIRI/ΔBS values remained consistently low during the entire follow-up periods regardless of changes in types of glucose tolerance.

DISCUSSION

In agreement with previous studies, our present data demonstrated that insulin responses during GTT were consistently subnormal in diabetic patients with FBS levels higher than 140 mg./100 ml. Insulin responses of patients who were suspected of having mild diabetes but who had an FBS lower than 140 mg./100 ml. were either subnormal or normal. As shown in figure 3, insulin responses in some patients who had or had had a diabetic GTT were not necessarily decreased even if the effects of obesity and glycemic stimulus were taken into account. With regard to the arguments whether mild diabetics have lower or higher insulin responses than normal, Savage et al.[7] reported that the mean insulin response was not decreased in Pima Indians if their two-hour blood sugar during GTT was below 200 mg./100 ml., and the insulin response progressively decreased with the

TABLE 1

Stability of ΔIRI/ΔBS on repeated GTTs during follow-up

Definite Diabetes			Equivocal Diabetes				
				Values of ΔIRI/ΔBS			
Frequency of GTTs	Number of patients	Cases with high insulin response*	Frequency of GTTs	Number of patients	Consistent† low	Consistent† high	Inconsistent‡ (low—high)
2	239	5	2	170	116	38	16
3	119	4	3	94	68	20	6
4	48	2	4	35	25	6	4
5	22	1	5	18	15	2	1
6	18	1	6	13	10	2	1
Total	446	13 (2.9%)		330	302 (91.5%)		28 (8.5%)
Total number of GTTs	1,250	15 (1.2%)		934			

*Number of patients who had ΔIRI/ΔBS higher than 0.5 on at least one GTT.

†Values of ΔIRI/ΔBS on repeated GTTs were consistently low (<0.5) or high (≥0.5).

‡Values of ΔIRI/ΔBS varied below and above 0.5 on repeated GTTs.

TABLE 2

The insulin responses during 100-gm. GTT of subjects who had equivocal diabetes initially but later came to have an FBS above 140 mg./100 ml.

Age	Sex	Family history of diabetes	Initial GTT FBS	2-hr. BS	Type*	ΔIRI/ΔBS	Types* of GTT and ΔIRI/ΔBS during the follow-up					Duration	FBS†
53	F	Mother, aunt	117	143	B	0.19	B 0.28	B 0.11	D 0.21			7y 8m	160
32	F	Parents, sister, brother	90	204	D	0.22	D 0.16	D 0.34				6y 8m	173
43	M	(—)	116	244	D	0.33	D 0.13	D 0.13				6y 3m	150
21	F	Mother, sister, uncle	115	190	D	0.46	B 0.42	D 0.38	D 0.06			6y	195
49	M	(—)	104	264	D	0.04	D 0.03	D 0.01				6y 0m	149
26	F	(—)	103	191	D	0.16	D 0.20	N 0.26	D 0.09			5y 3m	149
53	F	Daughter	116	148	B	0.36	D 0.56	D 0.32	D 0.16			4y 8m	154
49	M	(—)	114	169	D	0.30	D 0.32	D 0.09	D 0.3			3y 11m	142
65	M	Father	70	150	B	0.14	B 0.40	D 0.32	B 0.38	D 0.16		3y 10m	160
62	M	Father	110	148	B	0.22	B 0.22	D 0.02				3y 10m	242
37	M	(—)	105	224	D	0.01	D 0.32	D 0.09	D 0.03			3y 9m	213
39	M	(—)	118	129	B	0.21	D 0.04	D 0.05	B 0.18	D 0.05		2y 11m	180
66	F	(—)	111	158	B	0.36	D 0.32	D 0.24				1y 10m	152
24	F	Sister	103	119	N	0.11	B 0.37	B 0.30	B 0.21	D 0.06	D 0.02	1y 8m	170
50	F	(—)	104	188	D	0.35	D 0.12	D 0.22	B 0.54	N 0.36	D 0.39	1y 3m	145
35	M	Mother, brother	111	204	D	0.07	D 0.17	D 0.12				1y 3m	157
65	M	(—)	105	114	B	0.16	D 0.05	D 0.24				1y 2m	160
17	F	Parents, sister	92	152	B	0.32	D 0.42	D 0.01				0y 6m	212
42	M	Mother, sister	105	137	D	0.42	D 0.21	D 0.08				0y 5m	199
55	F	(—)	108	205	D	0.11	D 0.23					0y 3m	160

*Type of GTT: D =diabetic, B=borderline, N=normal.
†FBS after the development of definite diabetes.

TABLE 3

The insulin responses during 100-gm. GTT of subjects who had equivocal diabetes initially but later developed diabetic retinopathy

Age	Sex	Family history of diabetes	Initial GTT FBS	2-hr.-BS	type*	ΔIRI/ΔBS	Types* of GTT and ΔIRI/ΔBS during the follow-up					Duration	Diabetic retinopathy†
57	M	(—)	115	222	D	0.11	D 0.05	D 0.08				6y 1m	I
33	M	Parents, brother	118	237	D	0.13	D 0.03					6y 1m	III
45	M	(—)	105	296	D	0.05	D 0.08	D 0.12	B 0.25	B 0.26	D 0.09	5y 0m	I
39	M	(—)	119	206	D	0.08	D 0.02	D 0.03				5y 0m	I
62	F	Cousin	81	150	B	0.15	D 0.05	D 0.11	D 0.12			4y 8m	I
47	F	Parents	78	186	B	0.11	N 0.36	B 0.28	D 0.20	D 0.06		4y 7m	I - II
48	M	(—)	104	233	D	0.23	B 0.48	D 0.23				4y 6m	I
40	M	Parents, brother	92	189	D	0.14	D 0.15	B 0.01	D 0.14	D 0.11	B 0.08	4y 2m	I
50	F	(—)	85	190	D	0.25	D 0.13					4y 0m	I
50	F	(—)	88	134	B	0.21	B 0.26	N 0.36	D 0.16			3y 3m	I
58	F	Mother	113	280	D	0.26	D 0.26	D 0.20	D 0.12			3y 2m	I - III
63	M	(-)	116	248	D	0.10	D 0.25					2y 7m	I
55	M	(—)	70	178	B	0.15	N 0.34	D 0.10				1y 9m	I
53	F	(—)	100	136	B	0.20	D 0.32	B 0.28				1y 3m	I - II
64	M	Brother	88	214	D	0.04	D 0.08					1y 2m	I - II
49	M	(—)	90	133	B	0.18	B 0.21	B 0.17	B 0.27	B 0.02		1y 1m	I - II
51	M	(—)	110	209	D	0.08	D 0.08	D 0.06	D 0.01			0y 11m	II
47	M	(—)	100	145	B	0.35	D 0.25					0y 10m	I
55	M	(—)	114	133	B	0.15	B 0.40	B 0.15	B 0.28			0y 10m	I

*Type of GTT: D=diabetic, B=borderline, N=normal.
†The degree of retinopathy was graded according to Miki et al.[27]

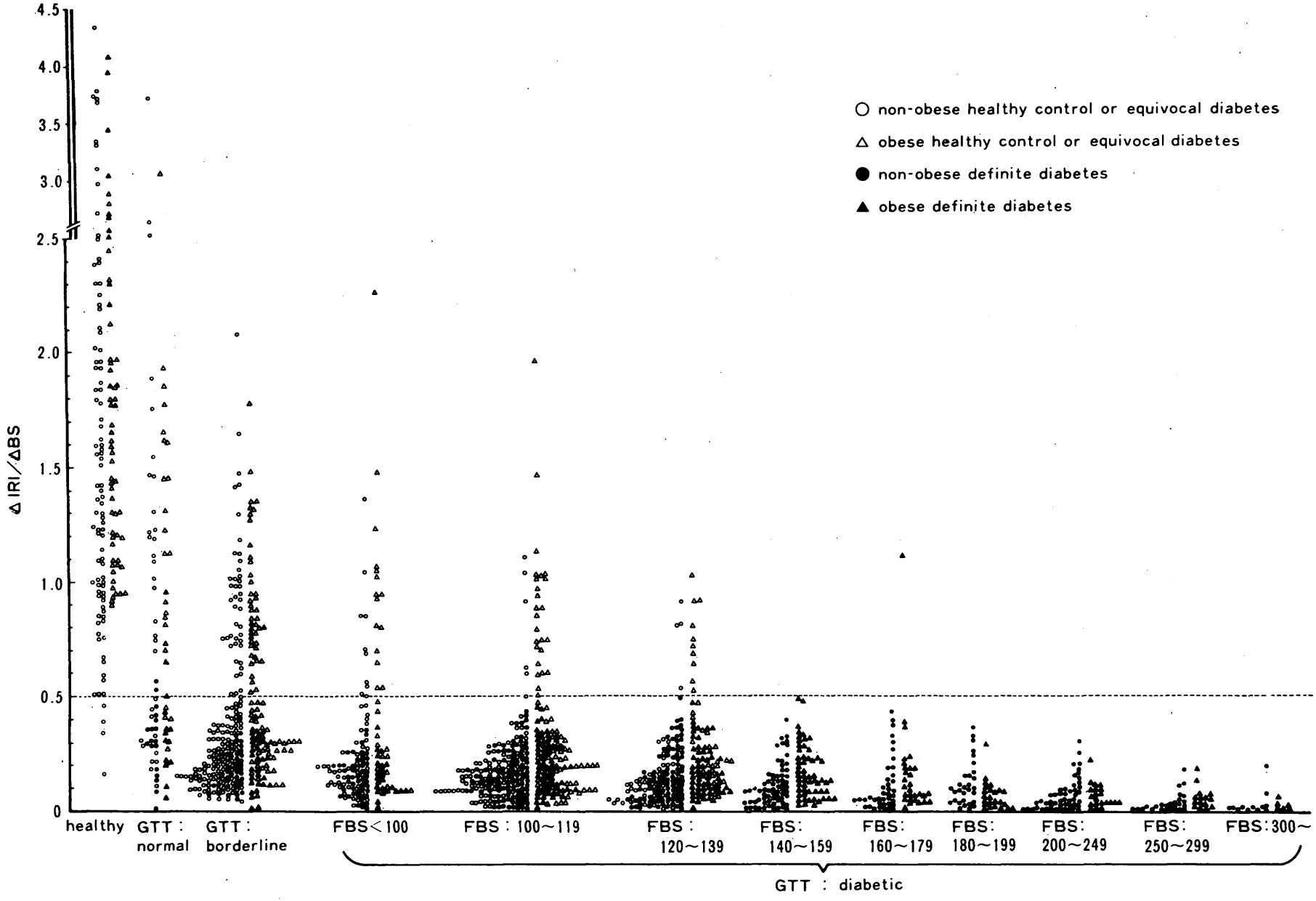

FIG. 3. The ratios (ΔIRI/ΔBS) of the increment of insulin (ΔIRI in μU./ml.) to that of blood sugar (ΔBS in mg./100 ml.) 30 minutes after oral administration of 100 gm. glucose.

further elevation of the two-hour blood sugar. Their data are not readily comparable to ours, but they are in agreement that insulin responses in so-called mild diabetics are variable from normal to subnormal, according to their degree of glucose intolerance.

Our previous study[8] disclosed that insulin responses during GTT were consistently low in definitely diabetic patients, even after their glucose tolerance was imporved to normal range. This implies that insulin response to glucose is related not only to the degree of glucose intolerance at the time of GTT but also to the past history of diabetes in each patient. The present data indicate again that the presence of overt diabetes in the past and the existence of diabetic microangiopathy are strongly associated with the decrease in insulin response to glucose.

Insulin responses to glucose are not decreased in many patients with secondary diabetes,[8,17] in which the glucose intolerance seems to be attributable to other known diseases. The patients with equivocal diabetes and with normal ΔIRI/ΔBS in our series apparently do not have other diseases or conditions that would affect glucose tolerance. The possibility cannot be ruled out, however, that a part of these patients with normal insulin response are similar to the secondary diabetes group in pathogenesis of their glucose intolerance, in the sense that it is caused by some extrapancreatic, as yet unidentified, diabetogenic factor(s). Mild diabetes is often diagnosed solely on the basis of glucose intolerance. Such diagnosis is rather arbitrary, since GTT is not sufficiently reproducible on repeated testing,[18,19] and there are several different criteria of GTT by different investigators.[16,20] It is evident that ordinary examinations and the simple GTT cannot discriminate or classify the possible heterogeneous populations consisting of equivocal diabetics. Of diabetic patients thus diagnosed, therefore, some may progress to overt diabetes, but some may remain mild or borderline or regress to normal GTT.

The most interesting finding of this study is that, in all patients who had equivocal diabetes at the time of initial examination but later came to have either FBS above 140 mg./100 ml. or diabetic retinopathy, the ΔIRI/ΔBS ratios were already lower than normal at the initial GTT and remained consistently low on repeated tests. These data suggest that the impaired insulin release during GTT is a feature of true diabetes appearing during the stage of equivocal diabetes, preceding the elevation of FBS or the retinopathy. There is a hypothesis by Cerasi and Luft[21] that true diabetes develops only from low insulin responders. Our patients cannot be called prediabetics, since most already had abnormal glucose tolerance when they were seen initially. These data simply indicate that definite diabetes develops preferentially from low insulin responders among the equivocal-diabetes group. It is not clear, however, whether low and high insulin responders comprise essentially and distinctly different groups. The distribution of ΔIRI/ΔBS ratios is continuous rather than bimodal. Repeated GTTs revealed that the types of insulin response were more consistent than the types of glucose tolerance in equivocal diabetics, but there were some (8.4 per cent) who moved from low- to high- or high- to low-responder groups during the follow-up. Although low and high insulin responses occur as early as childhood,[22] there are reports that diabetes developed in subjects who had previously been high insulin responders.[23,24] Therefore, it is possible that a part of high insulin responders lose insulin secretory capacity gradually during the course of aging and disease and ultimately reach the stage of definite diabetes if follow-up is continued long enough. Even in apparently normal populations, there is some tendency to a gradual decrease in insulin response with older age.[25]

The systematic survey of Pima Indians revealed that the two-hour blood sugar after glucose load had bimodal distribution, and the occurrence of retinopathy was largely confined to the second, or hyperglycemic, component in the population, in which two-hour postload palsma glucose was higher than 200 mg./100 ml.[26] According to the same group of investigators,[7] this hyperglycemic component in Pima Indians is the very group that has significantly lower insulin response during GTT. They suggested that only the second hyperglycemic component may represent true diabetes in Pima Indians. Our observations are in agreement with their data, in that retinopathy occurs preferentially in low responders. The association of retinopathy with low insulin response poses a question whether hyperglycemia or subnormal insulin response is a stronger contributory factor to the development of microangiopathy. The previous and present observations support the view that decreased insulin response to glucose is a more inherent, stable, and specific characteristic of true diabetes than the impairment of glucose tolerance. It not only is an almost invariable feature of established definite diabetes but precedes the occurrence and persists after the improvement of carbohydrate metabolism of definite diabetes.

REFERENCES

[1]Seltzer, H. S., Allen, E. W., Herron, A. L., Jr., and Brennan, M. T.: Insulin secretion in response to glycemic stimulus; Relation of delayed initial release to carbohydrate intolerance in mild diabetes mellitus. J. Clin. Invest. *46*:323-35, 1967.

[2]Perley, M. J., and Kipnis, D. M.: Plasma insulin responses to oral and intravenous glucose—studies in normal and diabetic subjects. J. Clin. Invest. *46*:1954-62, 1967.

[3]Bagdade, J. D., Bierman, E. L., and Porte, D., Jr.: The significance of basal insulin levels in the evaluation of the insulin response to glucose in diabetic and non-diabetic subjects. J. Clin. Invest. *46*:1549-57, 1967.

[4]Fujita, Y., Herron, A. L., Jr., and Seltzer, H. S.: Confirmation of impaired early insulin response to glycemic stimulus in nonobese mild diabetics. Diabetes *24*:17-27, 1975.

[5]Reaven, G. M., Shen, S. W., Silvers, A., and Farquhar, J. W.: Is there a delay in the plasma insulin response of patients with chemical diabetes mellitus? Diabetes *20*:416-23, 1971. Abstract.

[6]Jackson, W. P. U., van Mieghem, W., and Keller, P.: Insulin excess as the initial lesion in diabetes. Lancet *1*:1040-44, 1972.

[7]Savage, P. J., Dippe, S. E., Bennett, P. H., Gorden, P., Roth, J., Rushforth, N. B., and Miller, M.: Hyperinsulinemia and hypoinsulinemia. Insulin responses to oral carbohydrate over a wide spectrum of glucose tolerance. Diabetes *24*:362-68, 1975.

[8]Kosaka, K., Hagura, R., Kuzuya, T., and Kuzuya, N.: Insulin secretory response of diabetics during the period of improvement of glucose tolerance to normal range. Diabetologia *10*:775-82, 1974.

[9]Katsura, E.: Theory and Practice of Diet Therapy, 4th edit. (in Japanese). Iwatsuru, R., Ed. Tokyo, Nanzando, 1965, p. 483.

[10]Ikeda, Y., Saito, H., Anzawa, R., Sano, T., Yaginuma, N., and Shimizu, N.: A new carbohydrate solution, SDT-25 for testing oral glucose tolerance. J. Jap. Diab. Soc. *11*:207-10, 1968 (in Japanese).

[11]Hirata, Y., Nakamura, Y., Inuzuka, S., and Katsuki, S.: Side effects during the standard glucose tolerance test and an improved new solution for the test. J. Jap. Diab. Soc. *11*:319-23, 1968 (in Japanese).

[12]Nakao, M., Ito, M., Sakon, J., and Kondo, S.: Changes of blood sugar, insulin and non-esterified fatty acid after Trelan G 50 and glucose loading. A comparative study. J. Jap. Diab. Soc. *15*:314-15, 1972 (in Japanese).

[13]Hagedorn, H. C., and Jensen, B. N.: Zur Mikrobestimmung des Blutzuckers mittels Ferricyanid. Biochem. Z. *135*:46-58, 1923.

[14]Hoffman, W. S.: A rapid photoelectric method for determination of glucose in blood and urine. J. Biol. Chem. *120*:51-55, 1937.

[15]Kanazawa, Y., Kuzuya, T., Ide, T., and Kosaka, K.: Plasma insulin responses to glucose in femoral, hepatic and pancreatic veins in dogs. Am. J. Physiol. *211*:442-48, 1966.

[16]Kuzuya, N., et al.: Report of the committee on the diagnostic criteria of the oral glucose tolerance test for diabetes mellitus. Recommendations on the evaluation of the oral glucose tolerance test for the diagnosis of diabetes mellitus. J. Jap. Diab. Soc. *13*:1-8, 1970 (in Japanese).

[17]Seino, Y., Ikeda, M., Yawata, M., and Imura, H.: The insulinogenic index in secondary diabetes. Horm. Metab. Res. *7*:107-15, 1975.

[18]Kosaka, K., Mizuno, Y., and Kuzuya, T.: Reproducibility of the oral glucose tolerance test and the rice-meal test in mild diabetics. Diabetes *15*:901-04, 1966.

[19]McDonald, G. W., Fisher, G. F., and Burnham, C.: Reproducibility of the oral glucose tolerance test. Diabetes *14*:473-80, 1965.

[20]Seltzer, H. S.: Oral glucose tolerance tests. *In* Diabetes Mellitus: Diagnosis and Treatment, Vol. 3. Fajans, S. S., and Sussman, K. E., Eds. New York, American Diabetes Association, 1971, p. 101-06.

[21]Cerasi, E., and Luft, R.: "What is inherited—what is added" hypothesis for the pathogensis of diabetes mellitus. Diabetes *16*:615-27, 1967.

[22]Cerasi, E., and Luft, R.: The occurrence of low insulin response to glucose infusion in children. Diabetologia *6*:85-89, 1970.

[23]Savage, P. J., Bennett, P. H., Gorden, P., and Miller, M.: Insulin responses to oral carbohydrate in true prediabetics and matched controls. Lancet *1*:300-02, 1975.

[24]Danowski, T. S., Lombards, Y. B., Mendelsohn, L. V., Corredor, D. G., Morgan, C. R., and Sabeh, G.: Insulin patterns prior to and after onset of diabetes. Metabolism *18*:731-40, 1969.

[25]Hagura, R.: Studies on the pathogenesis of diabetes mellitus with special reference to the disturbance of glucose metabolism in the elderly subjects. J. Tokyo Women's Med. Coll. *39*:418-27, 1969 (in Japanese).

[26]Dorf, A., Ballintine, E. J., Bennett, P. H., and Miller, M.: Retinopathy in Pima Indians. Relationships to glucose level, duration of diabetes, age at diagnosis of diabetes, and age at examination in a population with a high prevalence of diabetes mellitus. Diabetes *25*:554-60, 1976.

[27]Miki, E., Fukuda, M., Kuzuya, T., Kosaka, K., and Nakao, K.: Relation of the course of retinopathy to control of diabetes, age, and therapeutic agents in diabetic Japanese patients. Diabetes *18*:773-80, 1969.