Steady State Plasma Insulin Response to Continuous Glucose Infusion in Normal and Diabetic Subjects

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SUMMARY

Normal subjects and diabetic patients with various degrees of hyperglycemia were given continuous intravenous glucose infusions for three hours in order to achieve constant plasma levels of glucose and immunoreactive insulin. During the last ninety minutes of this infusion period, at which time steady state conditions were observed, blood was obtained for measurement of plasma glucose and immunoreactive insulin concentration. Absolute levels of plasma insulin produced by the stimulus of the glucose infusions were extremely variable from patient to patient and were relatively independent of the degree of hyperglycemia. Patients with diabetes did not have lower steady state plasma insulin concentrations as a group, and this was true whether the insulin response was considered in absolute or relative terms. It is concluded that significant differences do exist in individual sensitivity to endogenous insulin, and that hyperglycemia in patients with maturity onset diabetes is not simply a function of lack of insulin. DIABETES *18*:273-79, May, 1969.

In 1960 Yalow and Berson[1] expressed the view that hyperglycemia in patients with maturity onset diabetes could not be entirely attributed to an absolute deficiency of insulin, and suggested that the "tissues of the maturity onset diabetic do not respond to his insulin as well as the tissues of the nondiabetic respond to his insulin." These same authors have subsequently confirmed and extended their original findings.[2] Recent reports from several laboratories[3-5] disagree with this concept of the pathogenesis of maturity onset diabetes and state that hyperglycemia in these patients is, indeed, due to an inadequate insulin response. This divergence of opinion does not seem related to differences in patient selection, nor can it entirely be attributed to variations in experimental design. Instead, the controversy seems to rest largely on the manner in which the experimental results are interpreted. Yalow and Berson[1,2] simply compare the absolute plasma immunoreactive insulin responses of various groups of patients to an oral glucose load, while the other three groups[3-5] judge the adequacy of the insulin response to oral glucose ingestion by various transformations of the basic data. Since, at this time, there are no convincing reasons to favor either of these approaches, a quandary exists as to whether maturity onset diabetes can result from "insulin resistance" or whether it is always due solely to insulin lack.

One way out of the dilemma might be to try a different experimental approach. For example, previous studies have emphasized measurements of the plasma insulin response to acute glucose loads, either oral or intravenous. There are inherent problems in studying nonsteady state events, and it seemed possible that measurement of the insulin response during steady state conditions might provide results which were consistent regardless of the method of interpretation. With this thought in mind, we studied steady state insulin responses during a continuous glucose infusion. The results indicate that patients with mild maturity onset diabetes are hyperglycemic in spite of an adequate insulin response, and this response was adequate in terms of several previously described methods of evaluation.

MATERIAL AND METHODS

Subjects

Patients were selected on the basis of their oral glucose tolerance in order to study a population which varied from the most normal to patients with severe

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nonketotic diabetes. The patients were classified as either *normal, mild diabetes* or *severe diabetes* (see Results). The youngest patient was twenty-seven years of age, the oldest sixty-eight; the mean (± S.D.) age of the twenty-one patients was 48 ± 12 yrs. Fifteen subjects were male, six were female. None of the patients had ever received insulin. They ranged rather widely in body weight, but only three patients were greater than 15 per cent above ideal weight; two of these were in the severe diabetic group and one was in the mild diabetic group. There were no significant differences in either the age or the ponderal indices (a measure of obesity) of the three groups. Four had been treated with sulfonylurea compounds in the past, but had not received these agents within four months of these studies. Some of the patients were extremely hyperglycemic, but they gave no history of ketoacidosis, and their urines were free of ketone bodies during the experimental period. Therefore, all patients in the two diabetic groups were classified as having maturity onset diabetes.

Experimental protocol

Although hospitalized, patients remained ambulatory throughout the study. Calories were supplied in four equicaloric feedings of a liquid formula diet given at 8 and 11 a.m., and 2 and 5 p.m. Each formula was ingested over a 30-min. period. Two diets were employed in these studies. One, a control formula diet, consisted of 15 per cent protein, 42 per cent fat, and 43 per cent carbohydrate, and was an attempt to approximate the average American diet. The other diet, a high carbohydrate formula, consisted of 15 per cent protein and 85 per cent carbohydrate. All the protein and 17 per cent of the calories from carbohydrates came from Dexin.* The carbohydrates of Dexin are obtained by partial hydrolysis of starch, yield entirely glucose on complete hydrolysis, and consist of the following components: 62 per cent dextrins, 18 per cent maltodextrins, and 19 per cent maltose. The fat in the control diet was obtained from a mixture of egg yolk, lard and butter. Body weight was closely maintained to within ± 0.5 kg. during each dietary period. Patients were maintained for the first week on the control formula diet. At the end of this time, an oral glucose tolerance test was performed, using 7 ounces of a synthetic carbohydrate beverage† as the standard challenge. All patients were changed to the high carbohydrate diet after the glucose tolerance tests and maintained on this program for two weeks. At the end of this period, they received a continuous infusion of 20 per cent glucose, calculated to deliver 6 mg./kg. body weight of glucose per minute. All studies were started at 8 a.m. after an overnight fast of fifteen hours. Glucose was given by a constant infusion pump (Harvard) into an antecubital vein, and blood was withdrawn for measurement of plasma glucose and immunoreactive insulin concentration from the other arm. The infusion lasted for 180 minutes. Blood was drawn just before the infusion was started, and again 90 min. later. From this time, blood was drawn every 15 min. for the next 90 min.

Analytical procedures

All blood was drawn free-flowing into tubes containing EDTA. Plasma was obtained after separation in a refrigerated centrifuge and frozen quickly in acetone-dry ice. Plasma glucose concentrations were determined in duplicate at every time point with an AutoAnalyzer.[6] Plasma insulin concentrations were measured in triplicate on two separate analyses by a modification of the method of Hales and Randle,[7] using Insulin I-125 and insulin binding reagent obtained from the Radiochemical Centre, Amersham, England. (Thus, each insulin value represents the average of six measurements.)

Analysis of data

Fasting plasma glucose and insulin concentration were based on the average of five samples. These included the specimen obtained the day of the experiment as well as from additional specimens drawn twice weekly during the preceding two weeks of the high carbohydrate diet. The seven values for glucose and insulin concentrations obtained during the last 90 min. of the infusion period were averaged, and this figure used as the measurement of response to the continuous glucose infusion. In one instance steady state conditions were not maintained during the 90-min. experimental period, and this study was repeated.

RESULTS

A. *Oral glucose tolerance tests*

Seven patients were classified as having normal glucose tolerance on the basis of plasma glucose concentrations less than 180 mg./100 ml., one hour, and less than 140 mg./100 ml. two hours after the oral glucose load. These criteria are similar to those suggested by Fajans and Conn,[3] taking into consideration the difference between blood and plasma glucose concentration.[9] Seven patients had fasting plasma glucose concentrations

*Dexin, Burroughs Wellcome and Co., Tuckahoe, New York.
†Glucola, Ames Co., Elkhart, Indiana.

TABLE 1

Steady state plasma glucose concentrations (mg./100 ml.)

Patients	Time (minutes after starting infusion)							
Normal	90	105	120	135	150	165	180	Mean±S.D.
F.B.	148	146	154	151	148	154	152	150±3.1
R.B.	172	175	169	173	176	175	176	174±2.6
W.C.	131	135	134	143	140	135	138	137±4.0
L.K.	166	169	170	164	166	162	164	166±2.8
G.L.	154	153	152	156	155	148	150	153±2.8
C.P.	165	167	170	169	173	173	170	170±2.9
W.W.	166	165	167	168	160	162	158	164±3.8
Mild diabetes								
D.C.	204	191	206	193	193	204	198	198±6.2
E.C.	298	310	314	288	294	295	298	300±9.2
W.G.	238	245	238	228	244	240	235	238±5.7
F.H.	194	184	192	188	195	199	203	193±6.4
W.K.	225	197	231	226	208	225	231	220±12.9
C.L.	193	188	186	175	184	189	191	187±5.9
D.W.	201	214	227	220	218	221	215	217±8.1
Severe diabetes								
E.D.	325	334	336	332	328	332	323	330±4.8
J.E.	332	339	331	321	327	309	307	324±12.0
S.E.	355	379	366	372	392	383	405	379±16.6
E.K.	437	412	424	432	407	434	436	426±12.1
P.M.	348	375	371	381	365	378	372	370±11.0
G.R.	294	283	271	288	281	299	289	286±9.2
A.W.	461	498	480	480	492	487	507	486±14.8

TABLE 2

Steady state plasma insulin concentrations (μU./ml.)

Patients	Time (minutes after starting infusion)							
Normal	90	105	120	135	150	165	180	Mean±S.D.
F.B.	55	50	49	58	57	51	54	53±3.5
R.B.	49	48	51	42	43	46	43	46±3.5
W.C.	65	69	75	70	74	77	73	72±4.1
L.K.	64	57	54	58	60	56	53	57±3.7
G.L.	48	42	53	51	52	44	49	48±4.1
C.P.	65	60	54	59	57	61	52	58±4.4
W.W.	47	49	56	50	55	46	52	51±3.8
Mild diabetes								
D.C.	80	68	75	70	73	72	69	72±4.1
E.C.	248	259	270	264	268	279	257	264±10.0
W.G.	109	101	95	107	101	104	99	102±4.8
F.H.	58	67	61	60	70	65	66	64±4.3
W.K.	155	168	162	170	174	159	154	163±7.7
C.L.	127	145	142	147	140	132	136	138±7.2
D.W.	81	75	68	78	72	73	80	75±4.7
Severe diabetes								
E.D.	43	36	47	40	45	43	39	42±3.8
J.E.	115	110	112	103	106	115	118	111±5.3
S.E.	47	35	39	46	51	42	50	44±5.9
E.K.	66	60	68	58	53	60	64	61±5.1
P.M.	84	93	98	91	86	81	99	90±6.9
G.R.	84	74	85	83	78	87	89	83±5.2
A.W.	45	40	39	50	51	49	46	46±4.7

in excess of 150 mg./100 ml., and were considered to have *severe maturity onset diabetes.* The remaining seven had values between these two extremes, and were classified as having *mild maturity onset diabetes.* The mean responses of the three groups are seen in figure 1.

Plasma glucose concentration of patients with *mild diabetes* was significantly greater than that of normal subjects both before and at all time intervals after the glucose challenge ($p < 0.005$). Correspondingly, plasma glucose concentration of patients with *severe*

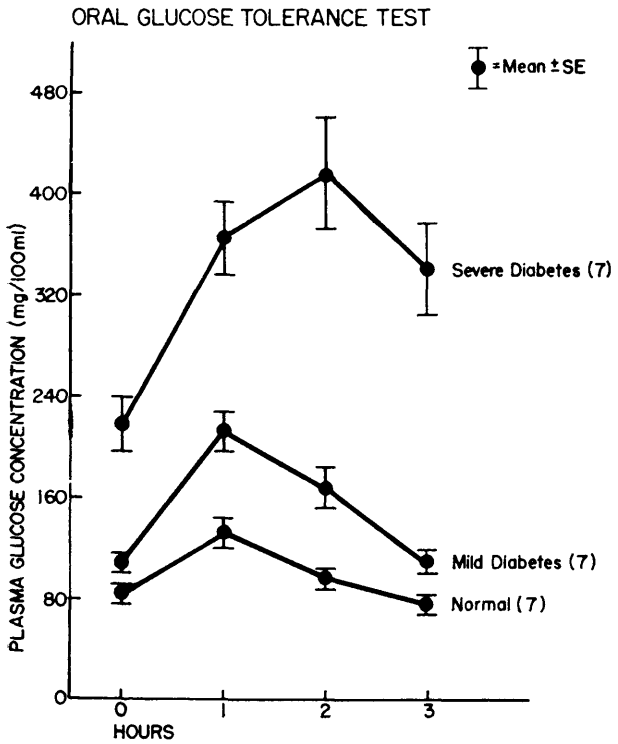

FIG. 1. Mean plasma glucose responses to an acute oral glucose challenge in seven normal subjects and fourteen patients with diabetes. On the basis of these results the diabetic group was further subdivided into seven patients with mild and seven patients with severe maturity onset diabetes.

diabetes was greater than that of *mild diabetics* at these same times.

B. *Glucose and insulin responses to continuous infusion*

Individual values for plasma glucose and insulin concentrations during the last 90 min. of the continuous infusion are listed in tables 1 and 2 and attest the constancy of the steady state. The mean plasma glucose and insulin responses for each patient are illustrated in figure 2, and group means are seen in table 3, in which both fasting and steady state responses appear. Mean fasting plasma glucose concentration of patients with *mild diabetes* was significantly higher than that of *normal* subjects ($p < 0.005$) but lower than that of patients with severe diabetes ($p < 0.005$). The steady state plasma glucose concentrations of the three groups were equally different. Fasting plasma insulin concentra-

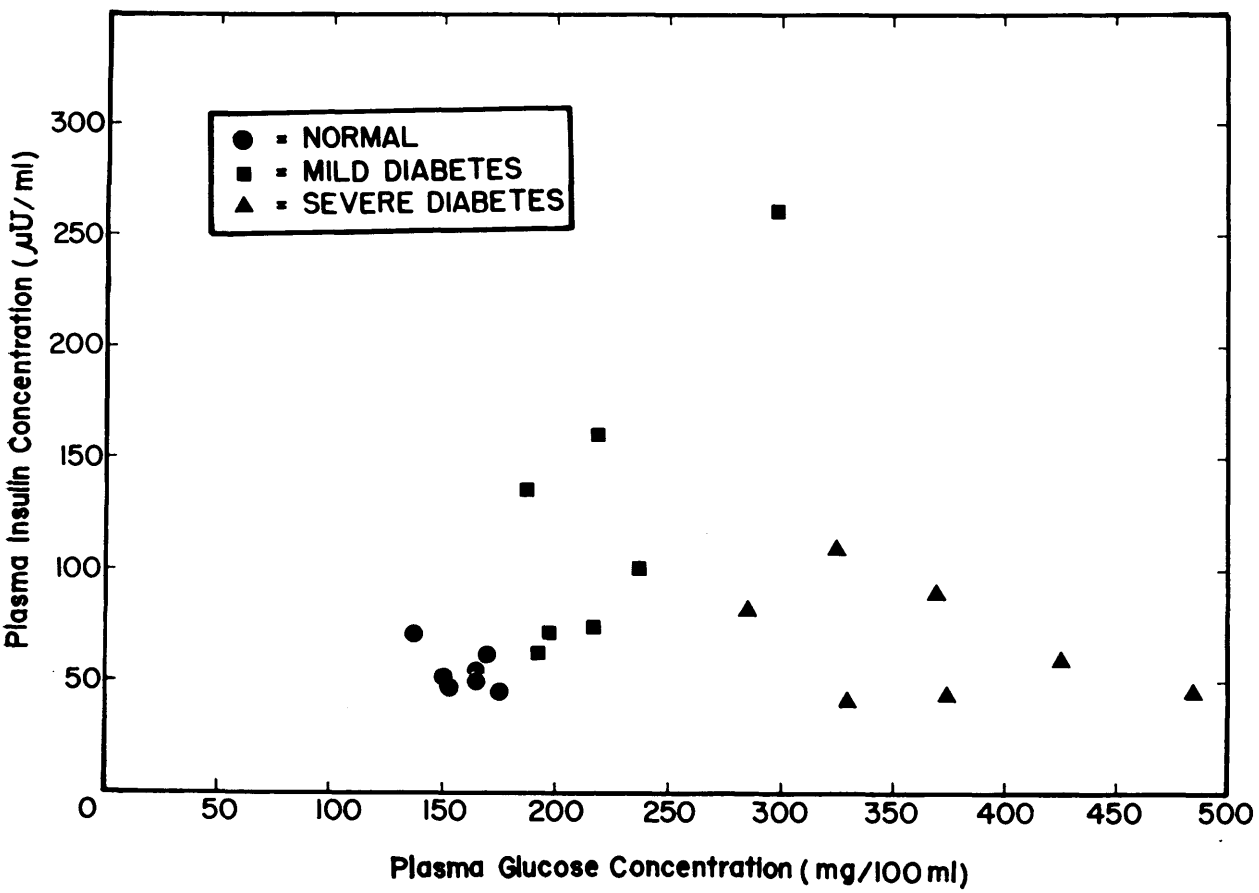

FIG. 2. Steady state plasma glucose and immunoreactive insulin response for each patient. Data obtained during glucose infusion study.

TABLE 3

Mean (±S.E.) steady state glucose and insulin responses

Group	Plasma glucose (mg./100 ml.) Fasting	Plasma glucose (mg./100 ml.) Steady state	Plasma insulin (μU./ml.) Fasting	Plasma insulin (μU./ml.) Steady state
Normal	81±2	159±5	19±3	55±3
Mild diabetes	100±5	222±15	34±8	125±27
Severe diabetes	201±24	372±26	35±4	68±10

tions of both groups with diabetes were also greater than those of *normal* subjects (*mild diabetics vs normals,* $p < 0.05$, *severe diabetics* vs *normals,* $p < 0.005$). Patients with mild diabetes had the greatest insulin response to the continuous glucose infusion, and this response was significantly greater than that of both *normal* subjects ($p < 0.01$) and patients with *severe diabetes* ($p < 0.05$). Although the mean response of patients with *severe diabetes* was greater than that of *normal* subjects, this difference was not of statistical significance ($p < 0.25$, $p > 0.1$). Since the three groups could not be differentiated on the basis of age or of obesity, these differences cannot be due *only* to the effect of these variables on insulin response.

C. *"Adequacy" of insulin response*

In table 4 an attempt has been made to judge the "appropriateness" of the insulin response by four methods. The first is to simply compare the three groups on the basis of the measured insulin response to a standard glucose challenge, while the other three methods involve various transformations of these results. Perley and Kipnis[3] have suggested dividing the insulin response by the glucose response (I/G), Seltzer and associates[4] prefer dividing the increment in insulin response over fasting by the same increment in glucose response ($\triangle I/\triangle G$), while Bagdade et al.[5] think it essential that the insulin response be related to fasting insulin concentration (infusion insulin concentration/fasting insulin concentration). Irrespective of the method used, the data in table 4 clearly establish the adequacy of the insulin response of patients with *mild diabetes.* With two methods of interpretation, the insulin response is actually significantly greater than *normal,* while with the other two methods, although greater, the differences just miss levels of statistical significance. The situation is more complicated when patients with *severe maturity onset diabetes* are compared with subjects with normal glucose tolerance. Although patients with *severe diabetes* do not have a complete or total deficiency of insulin, the other methods that have been used to judge adequacy of insulin response indicate that they have a deficient response when compared with *normal subjects.* However, before insulin deficiency is accepted as being the sole cause of hyperglycemia in patients with *severe maturity onset diabetes,* it should be pointed out that there is no a priori reason to assume that these methods of interpretation have any physiological significance. In any case, independent of these considerations, the insulin response of patients with *mild maturity onset diabetes* provides clear support of the original contention of Yalow and Berson[1,2] that at least some patients with maturity onset diabetes are hyperglycemic in the face of an adequate insulin response.

DISCUSSION

In this study glucose and insulin responses of normal and diabetic subjects have been compared during the last 90 min. of a three-hour continuous glucose infusion, at which time steady state plasma glucose and insulin concentrations had been achieved. The glucose infusion study was preceded by a fourteen-day period of a very high carbohydrate diet (85 per cent of daily calories), a step needed to condition the subjects to glucose and avoid the unsteady glucose levels that will

TABLE 4

"Adequacy" of steady state insulin response

Group	Insulin (μU./ml.)*	Insulin (μU./ml.)* / Glucose (mg./100 ml.)	△ Insulin (μU./ml.)* / △ Glucose (mg./100 ml.)	Infusion insulin (μU./ml.)* / Fasting insulin (μU./ml.)
Normal (N)	55±3	0.34±0.03	0.45±0.07	3.27±0.37
Mild Diabetes	125±27 > N, $p < 0.025$	0.56±0.13 = N	0.75±0.13 > N, $p < 0.05$	3.94±0.47 = N
Severe diabetes	68±10 = N	0.18±0.03 < N, $p < 0.005$	0.19±0.05 < N, $p < 0.005$	1.94±0.20 < N, $p < 0.025$

*Mean ± S.E.

otherwise be encountered during a three-hour glucose infusion.[10] The experimental protocol followed in these experiments seemed to provide certain distinct advantages. In the first place, patients were hospitalized throughout the entire study, and their dietary intake rigidly controlled both in terms of amount and kind of nutrition. Secondly, the effect of the immediate insulin response to an acute glucose challenge was minimized. Thirdly, the possible variable effect of gastrointestinal hormones[11-13] on the insulin response was lessened. Finally, since we had previously shown that insulin concentration during steady state conditions is directly related to the delivery of insulin into the general circulation,[14] the measured steady state insulin concentration can be readily interpreted as a direct reflection of insulin secretion rate. In this manner, by focusing on the steady state situation, it was possible to examine a more chronic relationship between insulin and glucose responses to a standard challenge. The results indicate, that under these conditions, patients with mild maturity onset diabetes secrete insulin in sufficient quantities to achieve plasma concentrations equal to or greater than normal subjects, and this was the case whether the insulin response was considered in absolute or relative terms.

These results provide support for the original contention[1] and its subsequent confirmation[2] of Yalow and Berson that hyperglycemia in patients with maturity onset diabetes cannot be *entirely* attributed to insulin deficiency. As such, these results seem to conflict with those of Perley and Kipnis,[3] Seltzer et al.,[4] and Bagdade et al.,[5] even when we analyzed our data in their terms. The reason(s) for this difference is not clear, and it is possible that the conflict is more apparent than real; we studied the more chronic steady state insulin response to a glucose infusion, while the other groups emphasized the acute response to a sudden glucose challenge. It is possible that these represent two separate facets of the diabetic state, and that future definition of glucose intolerance will necessarily include study of both these aspects of the insulin response to glucose.

If the quantification of insulin response is not limited to comparisons between normal and diabetic patient groups, a generalization of possibly more fundamental importance might be developed from our data. All patients received glucose infusions of approximately 6 mg./kg. body weight/min. It seems reasonable to assume, at least in subjects with normal or moderately impaired carbohydrate intolerance, that hepatic glucose output was markedly reduced during the period of glucose infusion.[15] Since plasma glucose concentration was stable during these studies, glucose uptake therefore can be assumed to be equal to the rate of glucose infusion, i.e., 6 mg./kg. body weight/min. Thus, these patients were disposing of identical glucose loads, but at many different combinations of plasma glucose and insulin concentration. If attention is directed to patients with relatively similar steady state plasma glucose concentrations (figure 2), it is clear that great variations in insulin output were present in the face of identical rates of glucose uptake and at comparable plasma glucose concentrations. This observation is true irrespective of patient groups or of method of data analysis, and is presented as additional support (from steady state experiments) for the contention that wide differences do exist in individual sensitivity to endogenous insulin.[1,2] It seems reasonable to suggest that these differences in sensitivity to insulin be considered as an important factor in discussion concerning the etiology of impaired carbohydrate tolerance in the syndrome of diabetes mellitus.

Finally, it should be pointed out that our conclusions are based upon the assumption that the radioimmunoassay measures the same kind of insulin in all patients and that the biological activity is directly related to the immunoreactivity. This assumption has recently been challenged by the description of "big insulin"[16] and proinsulin,[17] substances (or a substance) of greater molecular weight than insulin, which react with anti-insulin antibody. Thus, it is possible that previous estimates of plasma insulin concentration might be misleading, and they could reflect the combined immunoreactivity of various substances in plasma, present in varying concentrations in different individuals, and conceivably differing widely in their biological activities. In light of these alternatives it seems clear that our conclusions, as well as any others based upon measurement of immunoreactive insulin concentrations, must be accepted with some reservation. However, in preliminary experiments,[18] in which endogenous insulin secretion was suppressed with epinephrine, we have demonstrated that diabetic patients do not respond so well as normal subjects to the same plasma level of exogenous insulin. (These initial studies have been confirmed, and will be reported shortly.) Since only very small amounts of "big" insulin and proinsulin are present in crystalline exogenous insulin, these observations support our conclusion that hyperglycemia in patients with diabetes cannot be attributed solely to

insulin lack, whether the inadequacy of insulin secretion is considered in quantitative or qualitative terms.

ACKNOWLEDGMENT

This study was supported in part by U.S. Public Health Service Grant 08506, by Grant HE 6003 from the Career Development Awards Section, National Institutes of Health, and Grant FR-70 from the General Clinical Research Centers Branch, N.I.H., and from the Nutrition Foundation.

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