Deranged Insulin-secretory Dynamics in Offspring of Two Diabetic Parents After Double Stimulation with Intravenous Glucose

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SUMMARY

Nine offspring of two diabetic parents and 18 normals were studied with two intravenous glucose loads (0.5 gm./kg. body weight), 60 minutes apart. By thus stressing the beta cell, subtle defects could be identified in the prediabetics: (1) An inverse relationship between insulin peak response and insulin concentration 60 minutes postglucose was seen, a phenomenon exactly the opposite to that seen in normals. (2) Insulin peak response was delayed slightly after the first pulse and significantly after the second. (3) A less effective handling of the glucose load when compared with normals was brought out by the second stimulation. (4) There was a significant reduction in the insulin response per unit change in glucose after the first glucose pulse that was accentuated after the second pulse. This double-stimulation technique amplifies previously detected slight but significant defects in insulin secretion that might help to identify a diabetes-prone population. DIABETES *26*:1184-91, December, 1977.

Considerable effort has been spent to elucidate differences in insulin secretion between the offspring of two diabetic parents ("prediabetics") or identical twins of diabetics with normal glucose tolerance and that of normal controls with no known family history of diabetes. Such differences would not only identify persons with the highest risk of developing overt diabetes, but would also shed light upon the earliest changes of beta-cell dysfunction, thereby providing insight into the defect(s) causing diabetes mellitus. Conflicting results of such investigations are described in a recent review by Johansen et al.[1] and in other publications.[2-4] By employing a double-infusion technique in normals, such characteristics of beta-cell function have been recently noted that are not apparent when a single test dose is administered.[5] It appeared reasonable to apply this double-stimulation procedure to prediabetics to amplify any differences in insulin secretory dynamics that might be characteristic of this group.

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Accepted for publication August 9, 1977.

SUBJECTS AND METHODS

Nine prediabetics (four males, five females) and 18 normals (12 males, 6 females) were employed in the study. The prediabetics were the offspring of two well-documented maturity-onset diabetic parents, with a mean age (±S.E.M.) of 32.3±3.2 years (range 15 to 45), and a mean per cent ideal weight of 103±2 (Metropolitan Life Insurance Tables, 1959). The normals had a mean age of 25.6±1.1 years (range 19 to 36), averaged 99±2 per cent of ideal weight, had no personal or family history of diabetes, and had previously been studied by several intravenous and oral glucose tolerance tests with normal results. The difference in mean age of the two groups was significant ($p<0.05$); that of their per cent ideal weight was not.

None of the subjects were taking any medication, including oral contraceptives. They consumed a high carbohydrate diet (greater than 250 gm. per day) for three days before the test. A needle was inserted in an antecubital vein and kept open with a slow infusion of isotonic saline.

All subjects received an intravenous glucose tolerance test, in a dose of 0.5 gm. per kg. of body weight administered over a three-minute period, followed by a second dose of the same magnitude given exactly 60

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minutes after the end of the first injection (G-60-G). Venous blood was withdrawn before and at 1, 3, 5, 10, 20, 30, 40, 50, and 60 minutes after completion of the injections.

Whole-blood glucose levels were measured by an AutoAnalyzer method[6] and serum immunoreactive insulin by a double-antibody radioimmunoassay.[7] Early insulin response after both the first and second pulses was determined by calculating the area under the 0-10-minute insulin curve above the baseline level, the total insulin response by the area under the 0-60-minute insulin curve. The 60-minute value after the first pulse served as baseline for the calculation of the second early and total insulin responses. The highest insulin concentration after each glucose injection was designated as the peak response and the time when it was detected as the insulin peak time. Thus, peak insulin response and time of peak do not necessarily correspond with the highest mean insulin concentration of the group. Glucose disposal rate (K) was calculated by multiplying the slope of the regression of ln blood glucose on time by -100.

Comparisons were made by Student's paired t-tests. Correlations of glucose and insulin responses between and within pulses were performed by the conventional Pearson method. In addition, analysis of covariance was used to compare insulin and glucose responses, adjusting for age, which differed significantly between the two study groups.

RESULTS

Following the first glucose pulse, the mean insulin concentrations in the prediabetics were below those of normals from 1 to 20 minutes and above them from 40 to 60 minutes (figure 1). Mean early and total insulin responses of prediabetics were also below that of normals (table 1). The peak insulin response occurred at 6.9+5.4 minutes in prediabetics and at 3.3+2.2 minutes in normals. None of these differ-

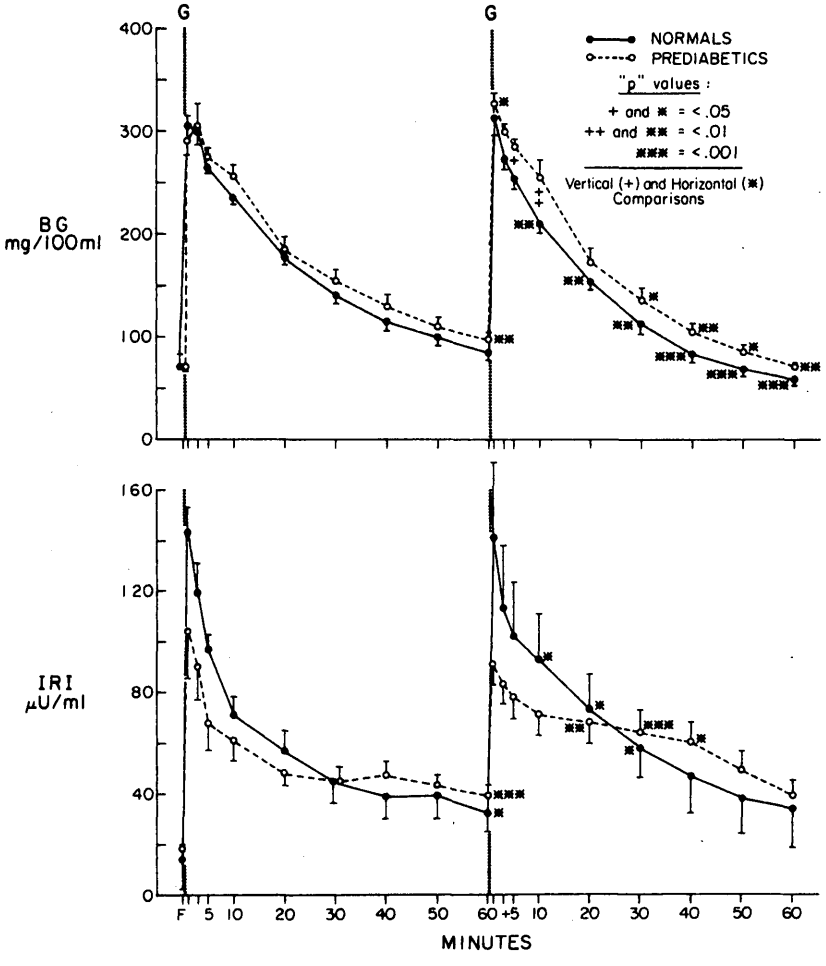

FIGURE 1

Levels of blood glucose (BG) and serum immunoreactive insulin in normals (n = 18) and prediabetics (n = 9) after intravenous injection of glucose followed 60 minutes later by a second glucose injection. Shown are means ± (S.E.M.).

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TABLE 1

Comparison of various derived glucose and insulin functions between normals and prediabetics for the first and second responses (mean ± S.E.M.)

	First response			Second response		
Function	Normals	p	Prediabetics	Normals	p	Prediabetics
BG-area*	1,891	—	1,966	1,615	<.05	1,810
0-10 min.	±72		±104	±53		±41
BG-area*	5,275	—	5,958	3,279	<.05	3,861
0-60 min.	±388		±405	±158		±179
K-rate	2.46	—	1.97	3.11	—	2.54
% per min.	±0.23		±0.16	±0.20		±0.13
IRI-Peak	144	—	106	145	—	97
μU./ml.	±28		±17	±30		±8
IRI-Peak	3.3	—	6.9	2.3	<.05	12.2
time (min.)	±2.2		±5.4	±0.7		±5.6
IRI-area†	837	—	549	728	—	384
0-10 min.	±166		±113	±175		±72
IRI-area‡	2,382	—	1,961	1,975	—	1,448
0-60 min.	±478		±245	±385		±248

*mg. per 100 ml. × min.
†μU. per ml. × min.

ences were statistically significant. Following the second pulse, the prediabetics showed a somewhat different insulin-secretory pattern from that of the normals. While normals had a rapid rise in mean insulin levels comparable to that following the first response, the insulin levels of the prediabetics were again below that of the normals initially, with a significantly delayed peak insulin response (2.3+0.7 vs. 12.2+5.7 min.). The total insulin response was smaller after the second pulse than after the first in both normals and prediabetics as expressed by the areas under the 0-60-minute insulin curves (table 2). However, insulin secretion was more sustained after the second pulse than after the first in both groups, as judged by significantly greater insulin concentrations from 60+10 to 60+30 minutes in normals and from 60+20 to 60+40 minutes in prediabetics (figure 1 and table 3).

Blood glucose levels in both groups were comparable after the first pulse. In normals, mean blood glucose concentrations returned to the baseline level by 60 minutes, but in the prediabetics it remained slightly but significantly above the fasting value. Glucose disposal as expressed by the K-rate was slightly but not significantly slower in the prediabetics than in the normals (figure 1 and table 1). Following the second pulse the normals showed lower mean glucose concentrations than after the first between 60+10 and 60+60 minutes (figure 1 and table 2). In

TABLE 2

Comparison of various derived glucose and insulin functions between first and second responses in normals and prediabetics during G-60-G (Mean ± S.E.M.)

	Normals (N = 18)			Prediabetics (N = 9)		
Function	First	p	Second	First	p	Second
BG-area*	1,891	<0.01	1,615	1,966	—	1,810
0-10 min.	±72		±52	±104		±41
BG-area*	5,275	<0.001	3,279	5,958	<0.001	3,861
0-60 min.	±388		±158	±404		±179
K-rate	2.46	<0.01	3.11	1.97	<0.05	2.54
% per min.	±0.23		±0.20	±0.16		±0.13
IRI-peak	144	—	145	106	—	97
μU./ml.	±27		±30	±17		±8
IRI-peak	3.3	—	2.3	6.9	—	12.2
time (min.)	±2.2		±0.7	±5.4		±5.6
IRI-area†	837	—	728	549	—	384
0-10 min.	±166		±175	±113		±72
IRI-area†	2,382	< .05	1,975	1,961	< .01	1,448
0-60 min.	±478		±385	±245		±248

*mg. per 100 ml. × min.
†μU. per ml. × min.

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TABLE 3

Individual glucose (BG-mg./100 ml.) and insulin (IRI-μU./ml.) levels, sex, age, and per cent ideal weight of the nine prediabetic subjects

Patient	Sex	Age (yrs.)	Ideal wt. (%)		Time (min.) F	1	3	5	10	20	30	40	50	60	+1	+3	+5	+10	+20	+30	+40	+50	+60
R.B.	M	15	100	BG	70	320	480	302	288	115	70	54	54	56	380	264	252	218	96	66	54	53	53
				IRI	12	160	124	64	44	28	18	16	14	10	70	60	52	30	28	16	18	14	12
C.B.	F	24	106	BG	70	339	309	303	249	162	134	116	97	91	303	276	282	261	183	151	108	96	86
				IRI	18	210	162	144	116	62	48	48	30	28	126	114	92	92	68	68	56	54	36
B.H.	F	25	106	BG	74	276	274	272	232	204	180	158	136	116	316	290	308	312	189	154	120	91	72
				IRI	30	56	42	38	42	40	40	54	56	56	74	64	64	62	72	70	68	60	40
M.H.	F	27	103	BG	72	280	292	268	324	218	192	170	152	138	342	324	320	352	200	158	122	92	74
				IRI	14	38	34	32	36	36	36	38	42	42	56	54	54	52	56	60	68	60	50
J.D.	F	36	111	BG	70	291	276	264	255	197	172	136	119	110	318	318	312	276	214	172	136	122	94
				IRI	16	86	62	48	56	56	60	58	48	48	80	80	82	80	86	84	96	94	76
R.S.	M	36	109	BG	76	360	321	309	264	230	174	154	125	97	375	318	306	255	198	152	110	88	61
				IRI	22	86	88	60	62	64	70	66	56	48	116	112	122	112	98	100	82	48	36
H.C.	F	39	105	BG	77	285	270	246	240	182	155	131	105	95	300	312	264	228	169	130	120	91	74
				IRI	16	100	86	72	62	58	56	58	50	44	72	74	72	92	94	78	74	50	50
R.F.	M	44	104	BG	70	249	276	282	240	181	157	131	101	96	312	324	270	213	175	141	106	95	82
				IRI	16	68	84	60	50	30	30	28	36	32	96	78	56	48	36	38	36	26	26
R.L.	M	45	86	BG	68	219	234	228	222	172	150	116	94	78	300	270	264	201	135	105	69	59	55
				IRI	22	128	124	92	80	62	52	56	52	42	126	110	108	98	74	66	46	38	28
	Total		Mean BG		72	291	303	275	257	184	154	129	109	97	327	300	286	257	173	146	105	87	72
			(± S.E.M.)		1	14	24	9	10	11	12	11	9	8	10	8	8	17	12	11	9	7	5
			Mean IRI		18	104	90	68	61	48	46	47	43	39	91	83	78	71	68	64	60	49	39
			(± S.E.M.)		2	18	14	11	8	5	5	5	5	5	9	8	8	9	8	8	8	8	6

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contrast, the prediabetics had a significantly higher mean glucose concentration one minute after the second pulse than after the first. Prediabetics also showed lower mean glucose concentrations after the second pulse (between 60+30 and 60+60 minutes) than after the first, but the mean glucose levels remained significantly above those of the normals at 60+5 and 60+10 min. Relationships between glucose responses to the first and second pulses were also reflected in the calculated areas under the glucose curves. Early glucose response (0-10-minute area) was significantly lower after the second pulse than after the first in normals but not in prediabetics (table 2). Total glucose response (0-60-minute area) was significantly lower in both groups following the second pulse. The difference between the second responses of normals and prediabetics was significant, indicating a greater glucose response both in the early and late phase in the prediabetics. The K-rate also improved significantly in both groups following the second pulse, but in the prediabetics it remained below that of normals, albeit not significantly.

In normals the first peak insulin response correlated positively ($p<0.001$) with insulin concentrations from three to 60 minutes—i.e., lower peaks were followed by lower insulin levels. Quite the opposite was noted in the prediabetics, in whom a negative correlation was found between the peak and the 60-minute insulin concentration ($p<0.05$)—i.e., the lower the insulin peak response, the more sustained the insulin secretion in the late phase (table 3). In addition, the 50- and 60-minute insulin concentrations in prediabetics correlated negatively with the glucose disposal rate, a phenomenon not observed in normals. These relationships were observed only in response to the first stimulation.

Group comparisons of insulin and glucose responses adjusted for age (analysis of covariance) indicated that observed differences were not due to an age influence.

The amount of change that occurred in the insulin concentration for each unit of change in the glucose concentration (b) was estimated after the first and after the second pulses in both normals and prediabetics by computing pooled regressions from all paired glucose and insulin measurements. The regression lines (plotted in figure 2) for both pulses showed a significantly lower slope (b when $y = a + bx$) after both pulses in prediabetics than in normals. In the normals, the slope did not change after the second pulse; however, the prediabetics showed a further significant decrease in their already lower slope from the first ($b = 0.25$) to the second ($b = 0.17$) stimulation ($p<0.01$).

DISCUSSION

Reviewing the literature, Johansen et al.[1] came to the conclusion that there is no consistent pattern in regard to insulin secretion in prediabetes. To this may be added: after a single stimulation, since other types of studies were not done in prediabetics, with one notable exception in which a small group of well-documented prediabetics was studied with a glucose infusion, preceded by a glucose pulse.[8] Metz et al. have studied normals and mild diabetics with three consecutive intravenous glucose pulses,[9] and Cerasi has recently reported experiments with sequential glucose infusions in a group of "low insulin responders."[10]

Previous studies were mainly concerned with quantitative differences in the secreted insulin. However, the defect in prediabetics—if it exists—is in its very early stage, and only subtle changes may be expected. This is probably the reason why differences between normals and prediabetics are extremely hard to demonstrate. Qualitative changes in insulin secretion might be detected earlier than quantitative differences. It is also debatable whether a single stimulation following an overnight fast with an unphysiologic amount of glucose is the proper method to uncover subtle changes in insulin-secretory patterns. Such changes may become manifest only in the course of repeated physiologic challenges represented by the repeated food intake during the day. It appeared logical, therefore, to apply sequential glucose stimulations in studying insulin secretion. With this experimental design the following defects were identified in the prediabetics: (1) An inverse relationship between peak insulin response and insulin concentration during the late phase of the secretory response after the first stimulation. (2) A delayed postglucose insulin peak response amplified by the second stimulation. (3) Greater glucose response with higher glucose concentrations than in normals following the second pulse. (4) A decreased serum insulin–blood glucose relationship apparent during the first stimulation but greatly amplified by the second pulse, suggesting a defective beta-cell sensitivity to rapid glycemic stimuli.

While some investigators have found no difference in insulin secretion between normals and prediabetics, others have reported a lower response in the latter, especially after an intravenous glucose challenge.[1] These differences, however, were small and difficult to

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demonstrate. It is not surprising, then, in the present study with a relatively small number of prediabetics, that no significant differences were found between the insulin concentrations of the two groups (figure 1). Several authors, however, have observed a significant elevation of insulin levels in the late phase of the secretory response in prediabetics, at 60 minutes following IVGTT,[11,12] at 60 and 120 minutes with the OGTT,[13] and after a glucose infusion,[8] indicating a more sustained insulin secretion in this group. Markedly sustained late-phase insulin secretion was found by Cerasi[10] after two infusions with same dose of glucose in the "low insulin responders" as compared with "normals," and this was also present in mild diabetics after two glucose pulses.[9] The observation of this current study—that lower insulin peaks are followed by higher insulin levels at 60 minutes in the prediabetics—is in line with the previously found sustained late-phase secretion and seems to be a characteristic of the prediabetic beta cell. This is underscored by the observation that this relationship is exactly the opposite in normals. That sustained insulin secretion may indeed be a manifestation of an over-all progressive defect of the beta cell is further supported by the observation that in the prediabetics, decreasing K-rates, albeit in the normal range, were nevertheless associated with increasingly higher insulin levels at 50 and 60 minutes.

In several studies a high incidence of delayed peak insulin was observed in prediabetics.[8,14-18] In this present study, the mean time to reach peak insulin concentration was only slightly longer in prediabetics than in normals after the first pulse. However, the second pulse, 60 minutes later, caused a significant delay in the mean peak response time in the prediabetics (tables 1 and 2).

The low and delayed early insulin response as well as the sustained insulin secretion in the late phase, following the second pulse in prediabetics, bears strong resemblance to the insulin response in normals when the second glucose pulse follows only 30 or 46 minutes after the first.[5,19] On the basis of these studies it could be postulated that in normals a glucose sensor or a signal relay mechanism for square-wave stimulation becomes depolarized or saturated for 30-45 minutes after a glucose pulse. This mechanism must have recovered 60 minutes after the pulse, however, because a second glucose load given at that time resulted in a rapid and unimpaired insulin peak comparable to that after the first in the same normal subjects. It was found[8] that prediabetics responded with a significantly delayed peak also to a slow-rise glucose infusion when compared with normals. Repeated glucose stimulation resulted in blunted early insulin responses and markedly delayed insulin peaks in both "low insulin responders"[10] and mild diabetics.[9] Furthermore, when glucose was abruptly stopped,[8] prediabetics continued to secrete insulin for a significantly longer period of time, indicating that there might be a delayed transmission of the "off signal" as well. This was the case also in the "low insulin responders" of Cerasi,[10] whose insulin secretion, in contrast to that of "normals," continued to increase after stopping the first glucose infusion. It is conceivable, therefore, that one of the earliest manifestations of beta-cell impairment in prediabetics is a decrease in the responsiveness of a glucose sensor or relay mechanism to both increasing and decreasing glucose levels, resulting in a slightly lower initial insulin response and a delay in the peak response as well as in a more sustained insulin secretion. Since the total insulin output is not appreciably diminished (table 1) it appears that because of the lag in signal relay, a shift in insulin secretion occurs from the initial phase to the late phase in prediabetics, regulated by a mechanism not operative in the normal beta cell. Following glucose stimulation this postulated sensor or relay mechanism remains partially depolarized or saturated in the prediabetic for a much longer period than in normals (>60 minutes vs. 30-45 minutes), resulting in a significant delay of insulin peak response, with increased shift of the insulin secretion to the late phase and a decreased over-all sensitivity of the beta cell to the subsequent glucose stimulation.

The impaired responsiveness of the prediabetic beta cell is also revealed by calculating the linear regression of the insulin concentration on blood glucose (figure 2), and again it appears that the second stimulation finds the prediabetics in a more decreased state of responsiveness, indicating that they had not yet recovered full sensitivity after the first stimulus. In contrast, there was little if any change in the insulin-glucose relationship from the first to the second response in normals.

The impaired initial insulin release in prediabetics is most likely responsible for the higher initial blood glucose concentration following the second pulse. The over-all glucose disposal, although slightly slower than in normals, was still significantly faster after the second than after the first pulse. This indicates that the "Staub-Traugot effect" is not a sensitive indicator of early beta-cell dysfunction.

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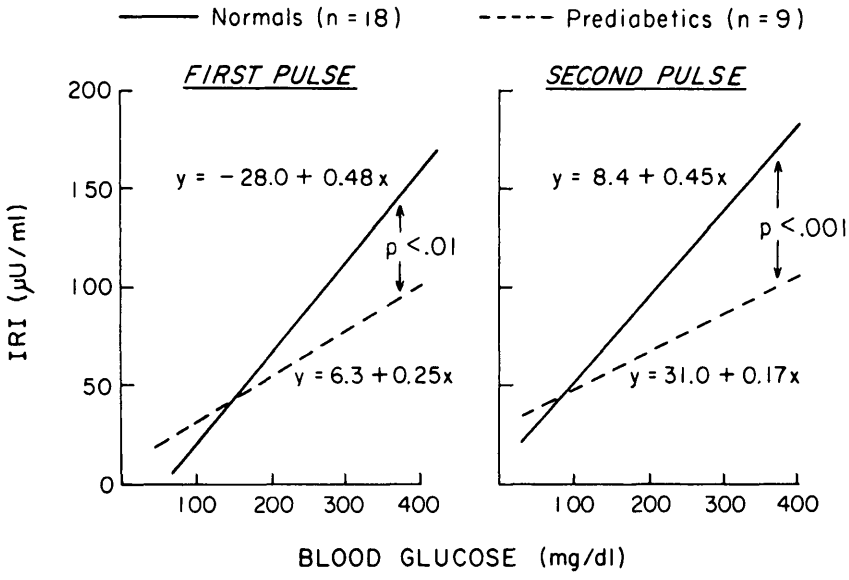

FIGURE 2

Regression lines and equations for serum insulin upon blood glucose. Data shown for normals and prediabetics during first and second pulses. The p-values refer to the degree of significance between slopes (b).

Cerasi[10] found that pretreatment with glucose increased the dose-response relationship between blood glucose and plasma insulin following a second glucose infusion in both "low" and "normal" insulin responders. While many features of the secretory dynamics in these "low responders" are reminiscent of the prediabetics in the present study, as discussed above, the retrospective selection of a group on the basis of their low insulin response remains highly controversial.[1] Thus, comparison of the "low responders" with prospectively selected offspring of two diabetic parents in the present study is problematic. In addition, many differences in the experimental design (infusion vs. pulse, varying doses and time intervals, and method of analysis) exist between the two studies. This is also the case with the small group of retrospectively diagnosed diabetics studied by Metz et al.,[9] who differ from the prediabetics not only in respect to their diabetic glucose tolerance tests but also to their age and the glucose dose they received. Although areas under the insulin curve, insulin-glucose relationships, and correlations were not calculated by Metz et al., their mild diabetics exhibited many of the defects postulated for prediabetics in the present study, albeit quantitatively at a different level, which might indicate a more advanced type of the derangement.

These experiments indicate that qualitative differences between insulin secretion of prediabetics and normals can be demonstrated if all aspects of insulin-secretory dynamics and glucose response are carefully scrutinized. This holds true for a single stimulation; however, a second challenge amplifies both the derangement of the secretory mechanism and the decreased sensitivity of the prediabetic beta cell. Some of the observations made in this study may have significant diagnostic implications.

ACKNOWLEDGMENTS

This work was supported by NIH grants EY-01421, AM-09748, AM-05116, and AM-05077, the Massachusetts Lions Eye Research Fund; the Upjohn Company, Kalamazoo, Michigan; and the Joslin Diabetes Foundation, Inc., Boston, Massachusetts. L. K. was the recipient of a Research and Development Award of the American Diabetes Association.

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