Insulin Response to Glucagon

The Opposing Effects of Diabetes and Obesity

Peter M. Crockford, M.D., William R. Hazzard, M.D., and Robert H. Williams, M.D., Seattle

SUMMARY

Glucose and immunoreactive insulin responses to glucagon were studied in thirty-seven subjects with varying degrees of relative body weight and glucose tolerance. Glucagon was administered intravenously as an infusion (0.5 mg./30 min.), followed by the acute injection of 1.0 mg. Insulin and glucose levels rose concomitantly during the infusion, but only insulin values increased significantly following the acute injection, providing separation of the glycogenolytic and insulinogenic properties of glucagon. During the 30 min. following the glucagon injection, glucose levels fell to normal in the nondiabetic subjects but remained elevated among the diabetics.

Obesity was associated with elevated basal insulin levels related to the degree of adiposity ($r = .402$, $p < .02$) but independent of glucose tolerance ($r = < .030$). In all subjects, the insulin responses to both glucagon infusion and injection were in turn related to the basal insulin values. The insulin response to glucagon, when expressed as per cent increment above basal in order to correct for the effect of varying degrees of insulin sensitivity as reflected in that basal level, was in all subjects related in a reciprocal manner to the degree of glucose intolerance as reflected by fasting glucose values ($r = 0.891$, $p < .001$). Thus, progressive glucose intolerance was associated with progressively impaired insulin secretion. Direct comparison of subjects classified on the basis of obesity or diabetes confirmed the exaggerating influence of obesity and the limiting effect of diabetes upon the insulin response to glucagon. DIABETES *18*:216-24, April, 1969.

Discovery of the potent insulin secretory effect of glucagon[1-3] has led to further studies of this property in vivo[4-8] and in vitro.[9-12] The possible role of intestinal or pancreatic glucagon in the promotion of insulin secretion[2] has led to the question of an abnormality in the beta cell response to glucagon in the diabetic state. In order to investigate this question, the present studies of the immunoreactive insulin (IRI) response to exogenous glucagon have been carried out. A method which segregates the effects of obesity from those of diabetes has been utilized in the interpretation of the results.

METHODS

Thirty-seven subjects, aged twenty to sixty-seven years, were tested by a method which combined the infusion and acute injection of pharmacological quantities of glucagon (vide infra). All subjects were asymptomatic and well nourished. For three days prior to the study, sulfonylureas and all nonessential medications were discontinued, and the subject's usual diet was supplemented with 250 gm. carbohydrate daily. The test was carried out at bed rest after an overnight fast. Blood samples were taken from an indwelling venous cannula, and plasma (heparin) and serum specimens were stored in the frozen state until analysis.

The test period was divided into three parts. The first was a control interval, blood specimens for basal glucose and immunoreactive insulin being drawn at —20, —10 and 0 min. This was followed by 30 min. of glucagon* infusion (Period 2) into a contralateral antecubital arm vein at a rate of 16.7 μg. per minute (total 0.5 mg.). Blood specimens were collected after 10, 20, and 30 min., at which time the infusion was

From the Department of Medicine, Division of Endocrinology, University of Washington, Seattle, Washington 98105. Dr. P. M. Crockford's present address is Assistant Professor of Medicine, Division of Endocrinology, University of Alberta Hospital, Edmonton, Alberta, Canada. Dr. W. R. Hazzard's present address is Fellow, Research Training Unit, University of Washington School of Medicine (VA Hospital), Seattle.

*Crystalline beef-pork glucagon, Eli Lilly and Company.

terminated and 1.0 mg. glucagon injected acutely into the same vein. Blood specimens during the third (post-injection) period were drawn every minute for 7 min. and at 10, 15, 20, and 30 min.

Plasma samples were analyzed for glucose by the ferricyanide method utilizing the Technicon Auto-Analyzer. Results were plotted as a function of time during the three periods of the study and the areas encompassed by the curves calculated by integration (PDP-8 computer).

Serum immunoreactive insulin (IRI) was determined by a modification of the double antibody technic[13] utilizing I-125-labeled insulin. All specimens were analyzed in duplicate. Serial assays were compared by the inclusion within each of aliquots of normal fasting and postprandial serum pools. Among eighteen consecutive assays the fasting pool mean IRI was 14.0 μU./ml. and the fed 98.0. The within-assay precision among duplicates (s)* was 3.7 for the fasting and 6.4 for the fed pools. The between-assay reproducibility, reflected by the standard deviation of the mean value computed from the individual assay means, was 5.7 for the fasting and 22.6 for the fed pools.

Individual IRI values were similarly plotted as a function of time and the areas encompassed during each of the test periods calculated by integration (μU./ml.-min.). Values were obtained for both the basal and glucagon-stimulated periods and have been expressed for the latter either as the absolute or per cent increment above basal levels.

The data were analyzed by standard statistical technics with the aid of the PDP-8 computer. Regression analyses were performed by the method of least squares. Direct comparisons between subgroups, obese and nonobese, diabetic and nondiabetic, were made by analysis of variance and the F-test.

All thirty-seven subjects were included in the regression analyses of the relationships between basal insulin levels and relative body weight, between basal insulin levels and IRI response to glucagon, and between basal plasma glucose and per cent incremental IRI response to glucagon. In addition, so as to define more clearly the characteristics of the obese and nonobese and diabetic and nondiabetic states, the subjects were further categorized. Obese subjects were defined as those whose weight exceeded 125 per cent of ideal based on Metropolitan Life Insurance Company tables; nonobese subjects were those weighing less than 125 per cent of ideal.*

Each individual's state of glucose tolerance was defined with reference to the mean basal glucose level among twelve of the thirty-seven subjects who were nonobese and had negative personal and family histories of diabetes and cardiovascular disease. The mean fasting plasma glucose concentration of this group was 96 mg. per 100 ml. Five of the thirty-seven with values lying between 1 and 2 S.D. above this mean (103-110 mg. per 100 ml.) were considered borderline glucose intolerant and were excluded only from analyses directly comparing the diabetic and nondiabetic populations. Those individuals exceeding 2 S.D. above the mean were classified as diabetic. Utilizing these criteria, there were nineteen nondiabetics (mean age 37.5 yrs., thirteen nonobese, six obese) and thirteen diabetics (mean age 45.1 yrs., six nonobese, seven obese). In all but one instance the diabetics exceeded the normal mean basal plasma glucose by >3 S.D. (117 mg. per 100 ml.).

RESULTS

I. *Plasma glucose response to glucagon*

All subjects exhibited a hyperglycemic response to glucagon of a magnitude related to their basal glucose level. There was a correlation of high statistical significance ($p < .001$) between basal plasma glucose concentration and the total area encompassed by the glucose response curve both during glucagon infusion ($r = 0.966$) and after injection ($r = 0.920$), as well as during the entire period of observation ($r = 0.967$).

There was a similar, though less striking, correlation between the basal glucose level and the glucose increment above basal during infusion ($r = 0.353$, $p < .05$); after glucagon injection the correlation was more significant ($r = 0.737$, $p < .001$), as was the association between basal glucose and glucose increment during both periods of glucagon stimulation ($r = 0.616$, $p < .01$). The delayed return to basal glucose levels characteristic of the diabetic response to glucose was also seen in the diabetic subjects after glucagon (figure 1). The injection of 1 mg. of glucagon after 30 min. of infusion sustained the diabetics' hyperglycemia at a constant or slightly increasing level for 30 min., where-

*$s = \frac{\Sigma d^2}{2n}$ where d is the difference between the values observed for members of a pair of duplicate determinations and $2n$ is the number of such pairs.[14]

*For determination of relative body weight, all subjects were assumed to be of medium frame. The upper limit allowable for that height and frame was defined as 100 per cent of ideal.

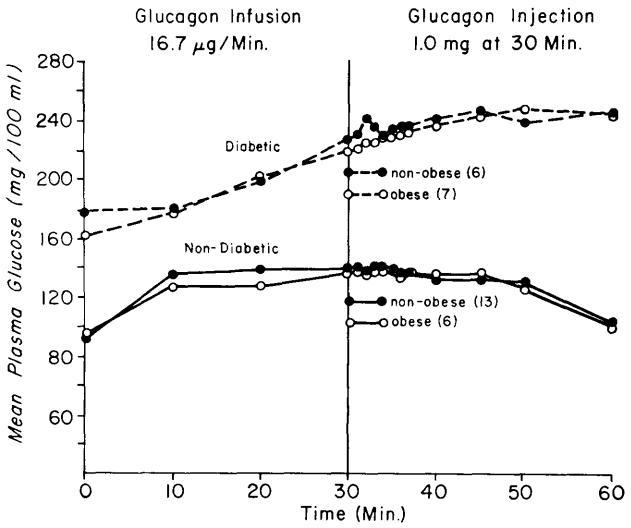

FIG. 1. Mean plasma glucose responses to glucagon infusion and injection in diabetic and nondiabetic subjects.

as the normal subjects returned to near fasting (basal) glucose concentrations by the end of the same interval.

There was no relationship between relative body weight and basal glucose ($r = .094$) or glucose response to glucagon, as reflected in the nearly superimposable curves for nonobese and obese subjects in both the diabetic and nondiabetic groups depicted in figure 1.

II. *Serum insulin response to glucagon*

Infusion of 0.5 mg. of glucagon over 30 min. resulted in progressive elevation of serum IRI levels concomitant with the rise in plasma glucose (figure 2). The subsequent acute injection of 1 mg. of glucagon, while causing no appreciable additional hyperglycemia, resulted in a rapid increase in serum IRI which reached a peak at 4 to 7 min. and declined thereafter to near basal levels by 30 min. There was no apparent difference between diabetic and nondiabetic groups in the time at which the maximum IRI was achieved or the rate at which it subsequently declined (figure 2).

A. *The relationship between obesity and basal insulin levels.* Relative adiposity correlated with basal insulin levels among all subjects, increasing degrees of

TABLE 1

Serum immunoreactive insulin (IRI μU./ml.) and plasma glucose (mg. per 100 ml.) concentrations during glucagon stimulation (mean values ± S.E.M.)*

	Nondiabetic				Borderline diabetic (5)		Diabetic			
	Nonobese (13)		Obese (6)				Nonobese (6)		Obese (7)	
Age	38.1±4.3		36.3± 3.5		44.4±4.7		48.2±2.4		42.4±3.2	
Weight kg.	72.8±3.5		106±10.5		78.2±2.8		68.8±3.8		105±6.3	
Per cent ideal	103±2.8		152±10.3		111±3.6		106±8.2		162±9.0	
	IRI	Glucose	IRI	Glucose	IRI	Glucose	IRI	Glucose	IRI	Glucose
Basal period (minutes)	9.9± 2.6	93.8±1.5	27± 8.7	93.5± 2.3	16± 2.7	108± 1.7	15± 3.7	179±24	21± 5.2	162±16
Glucagon infusions (16.7 mg./min.)										
10	37± 7.7	115±2.8	81± 12	108± 2.1	47± 5.2	127± 5.4	30± 2.2	189±26	31± 8.6	179±14
20	72±12	136±5.2	125± 44	128± 6.4	77± 8.2	152± 4.5	39± 7.4	208±25	34± 9.0	201±15
30	94±16	139±6.8	234± 80	137±10	91±10	179±20	41± 6.6	227±25	44±13	226±18
Postglucagon injection (1.0 mg. at 30 min.)										
31	103±13	140±7.1	220± 71	137±11	92±10	177±22	54±15	231±26	42± 8.5	221±14
32	142±23	138±7.2	346±114	136±11	156±31	176±23	83±22	246±25	110±52	224±15
33	194±30	141±7.8	410±123	139±11	192±32	183±27	85±27	236±25	122±48	226±16
34	206±30	141±8.1	452±117	138±10	197±38	184±24	69±21	231±24	132±50	230±15
35	201±29	139±7.9	443±123	139±12	209±27	177±25	84±23	234±24	118±40	229±16
36	232±42	137±8.3	451±110	134±11	213±26	179±25	97±32	236±25	124±42	231±15
37	181±31	136±7.8	442± 98	118±13	208±29	161±13	98±39	237±23	123±47	233±15
40	153±22	133±8.4	323± 83	136±12	161±29	161±13	85±39	241±23	83±27	237±16
45	100±19	130±9.6	197± 47	134±12	85±21	157±16	51±19	246±21	38± 6.5	242±19
50	62± 9.7	124±9.1	108± 22	129±11	62± 9.5	151±20	28± 8.2	238±25	33± 6.1	248±18
60	32± 3.5	104±10	71± 16	118±11	45± 8.7	138±24	26± 8.1	245±23	41±11	243±15

*Complete tables of individual data are available from the authors upon request

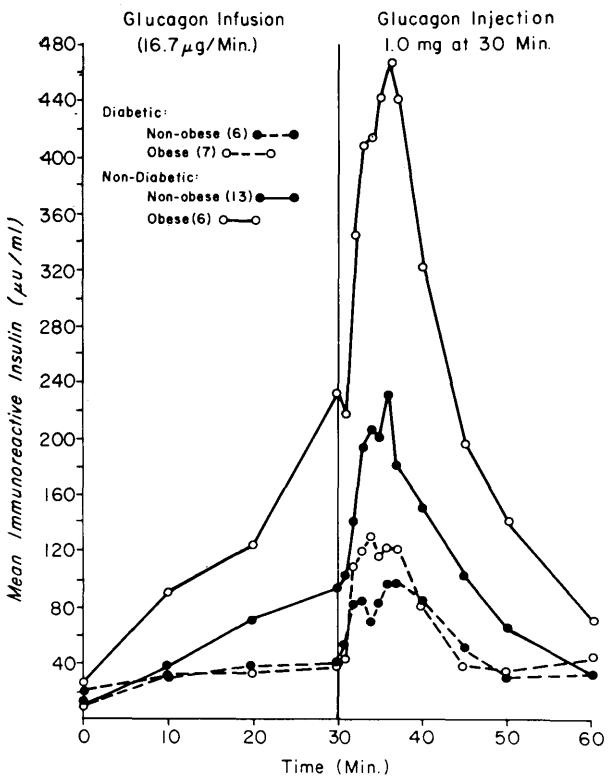

FIG. 2. Mean absolute immunoreactive insulin responses to glucagon infusion and injection in diabetic and nondiabetic subjects.

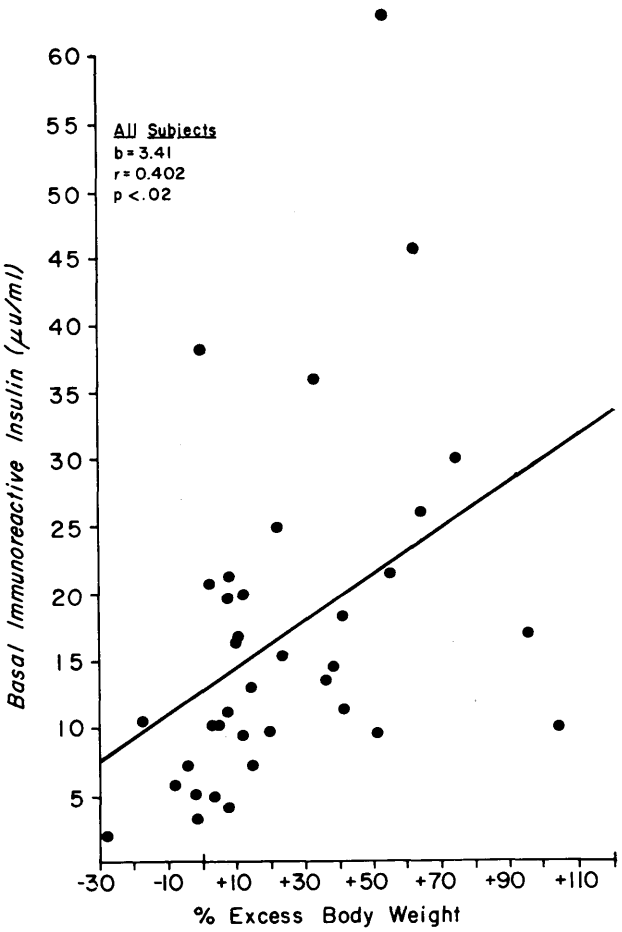

FIG. 3. Relationship between basal immunoreactive insulin and per cent excess body weight in all subjects.

obesity being associated with increasingly elevated fasting IRI concentrations ($r = .402$; $p < .02$) (figure 3). Thus, all obese subjects showed significantly ($p < .01$) elevated basal IRI levels (487 ± 319 area units) in comparison with all nonobese (256 ± 182). There was no relationship, however, between basal levels of IRI and plasma glucose ($r = 0.030$).

B. *The effect of obesity upon the absolute insulin response to glucagon.* Among nondiabetics obesity was associated with a significantly magnified *absolute* IRI response to glucagon, whether measured during infusion, after injection, or expressed as the total response during both periods (table 2, and figure 4, top; and figure 2). Among diabetics the absolute IRI responses during each period of glucagon stimulation or the combination of the two did not differ significantly between obese and nonobese subgroups (table 2, and figure 4, top; and figure 2).

C. *The relationship of basal insulin levels to the incremental IRI response to glucagon.* The magnitude of the absolute IRI response to glucagon correlated with the basal IRI level among all subjects for both the infusion ($r = 0.452$, $p < .01$) and postinjection ($r = 0.413$, $p < .02$) periods, as well as for the entire period of observation ($r = 0.425$, $p < .01$).

A similar relationship has been reported between basal IRI levels and the IRI response to oral glucose.[15] In that study expression of the IRI responses in relative terms as the per cent increment above basal corrected for individual variation in tissue insulin sensitivity as reflected in basal IRI values and thereby permitted assessment of the IRI response to glucose independent of relative body weight. Therefore, IRI responses in the current study have been expressed not only as *absolute* (table 2, and figure 4, top; and figure 2) but also *relative* (per cent incremental) values (table 2, and figure 4, bottom; and figures 5 and 6).

D. *The relationship between glucose tolerance and the IRI response to glucagon: The effect of diabetes.* Expression of IRI responses in *relative* terms as per

TABLE 2

The absolute and relative (per cent) incremental IRI response to glucagon among obese and nonobese diabetics and nondiabetics (means ± S.D.)

		Glucagon infusion	p	Glucagon injection	p	Glucagon infusion† injection	p
Absolute IRI Response (area units)	Nonobese:						
	Nondiabetic (13)	1,166±616	< .02	2,919±1,402	< .05	4,084±1,905	< .02
	Diabetic (5)	454±227		1,291±1,139		1,744±1,339	
	Obese:						
	Nondiabetic (6)	2,325±1,455	< .01	5,986±2,991	< .01	8,313±4,362	< .01
	Diabetic (7)	278±273		1,425±966		1,703±1,162	
	All nondiabetic:	Nonobese vs. obese	< .05	Nonobese vs. obese	< .01	Nonobese vs. obese	< .01
	All diabetic:	Nonobese vs. obese	NS*	Nonobese vs. obese	NS*	Nonobese vs. obese	NS*
Relative IRI Response (per cent above basal)	Nonobese:						
	Nondiabetic (13)	716±475	< .02	1,925±1,414	< .05	1,352±952	< .025
	Diabetic (5)	117±69		414±301		270±116	
	Obese:						
	Nondiabetic (6)	520±437	< .02	1,293±881	< .025	898±649	< .02
	Diabetic (7)	71±51		370±237		220±130	
	All nondiabetic:	Nonobese vs. obese	NS*	Nonobese vs. obese	NS*	Nonobese vs. obese	NS*
	All diabetic:	Nonobese vs. obese	NS*	Nonobese vs. obese	NS*	Nonobese vs. obese	NS*
	All nondiabetic (19):	654±461	< .001	1,725±1,325	< .005	1,209±876	< .001
	All diabetic (12):	90±59		388±264		241±124	

*NS = not significant ($p > .05$)

cent increase above basal nullified the positive correlation between basal IRI and the absolute IRI response to glucagon. This allowed evaluation of the relationship between glucose tolerance and the IRI response to glucagon independent of relative body weight and other factors affecting both basal and glucagon-stimulated IRI levels. When the IRI per cent incremental response was plotted for all subjects as a function of basal plasma glucose levels, inspection of the graph (figure 7) suggested a hyperbolic function of the form: $xy = 1$ or $x = \frac{1}{y}$.

Consequently, the data were re-expressed as the reciprocal of the total per cent incremental response versus basal plasma glucose (figure 8). A linear, highly significant relationship resulted ($r = 0.891$, $p < .001$), indicating that progressive fasting hyperglycemia was associated with progressive insulin deficiency in response to glucagon.

Thus, diabetes was associated with a restriction in the insulin response to glucagon. This was evident though not striking ($p < .01$ — $< .05$) in the *absolute* IRI responses of diabetic versus nondiabetic subjects. Comparisons of absolute responses were limited to those between subgroups of comparable relative body weight (e.g. nonobese nondiabetic versus nonobese diabetic) because of the significant, magnifying effect of obesity in the nondiabetic subjects. However, expression of the IRI response in relative terms reduced the difference between obese and nonobese nondiabetics to a level of statistical nonsignificance ($p > .25$) without affecting the nonsignificant degree of difference ($p > .25$) between obese and nonobese diabetics

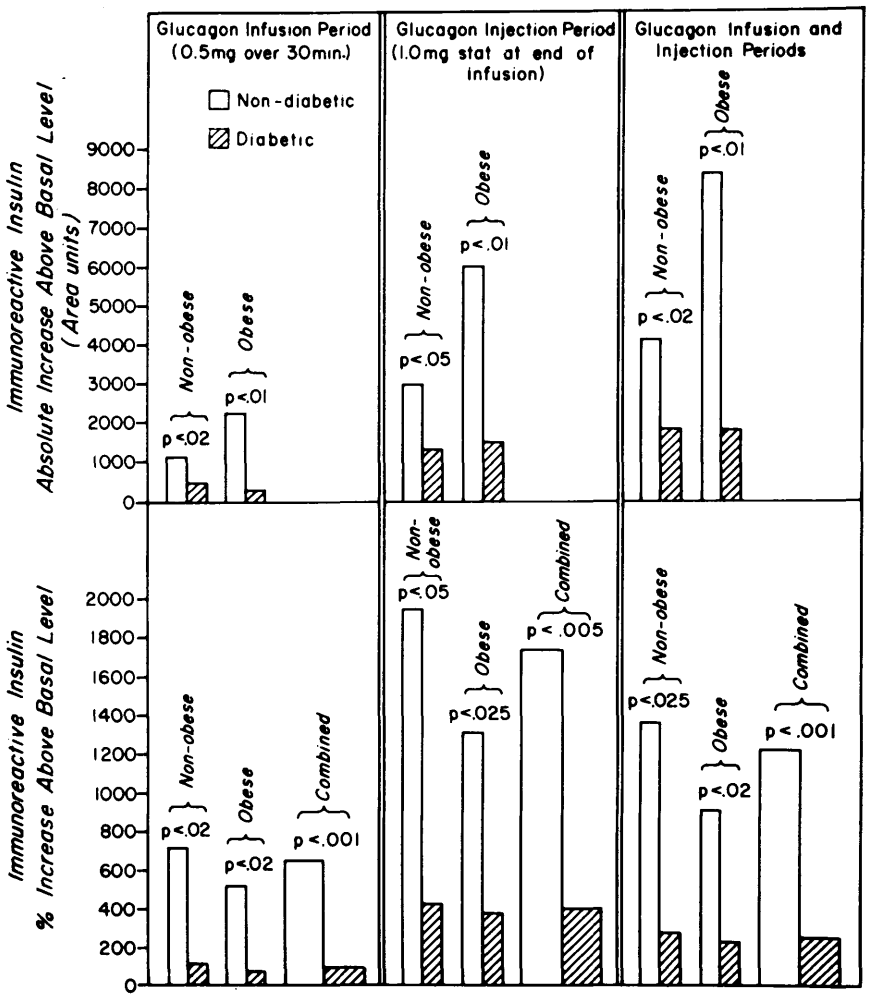

FIG. 4.

Integrated absolute (top) and per cent (bottom) immunoreactive insulin increases above basal with glucagon administration in nondiabetic and diabetic subjects.

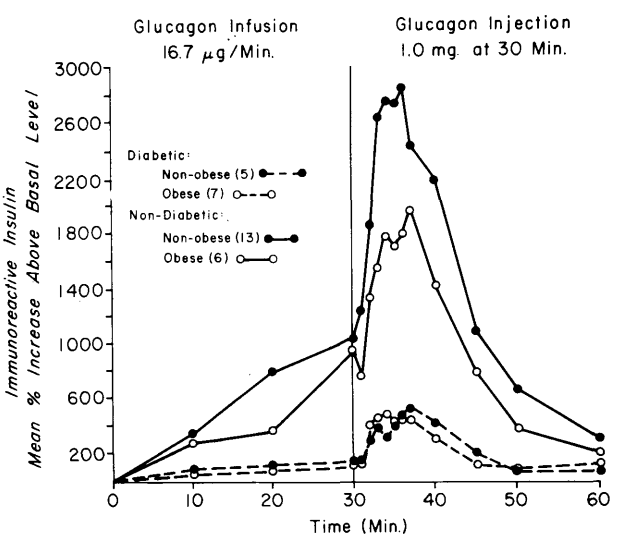

FIG. 5. Mean per cent increase of immunoreactive insulin above basal with glucagon administration in nonobese and obese nondiabetics and nonobese and obese diabetics.

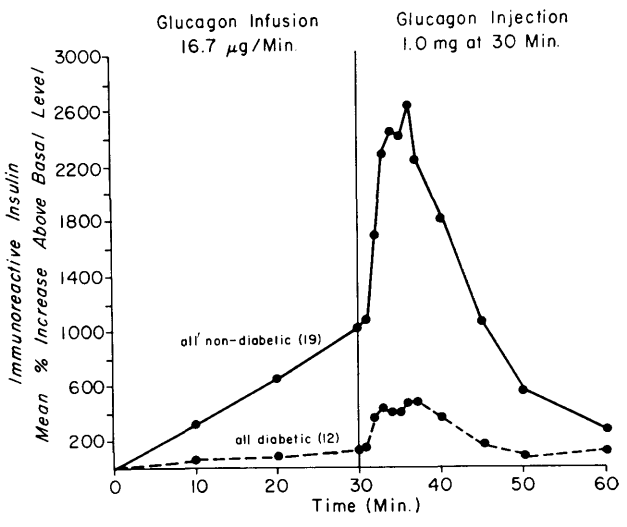

FIG. 6. Mean per cent increase of immunoreactive insulin above basal with glucagon administration in diabetic and nondiabetic subjects (obese and nonobese subgroups combined in both categories).

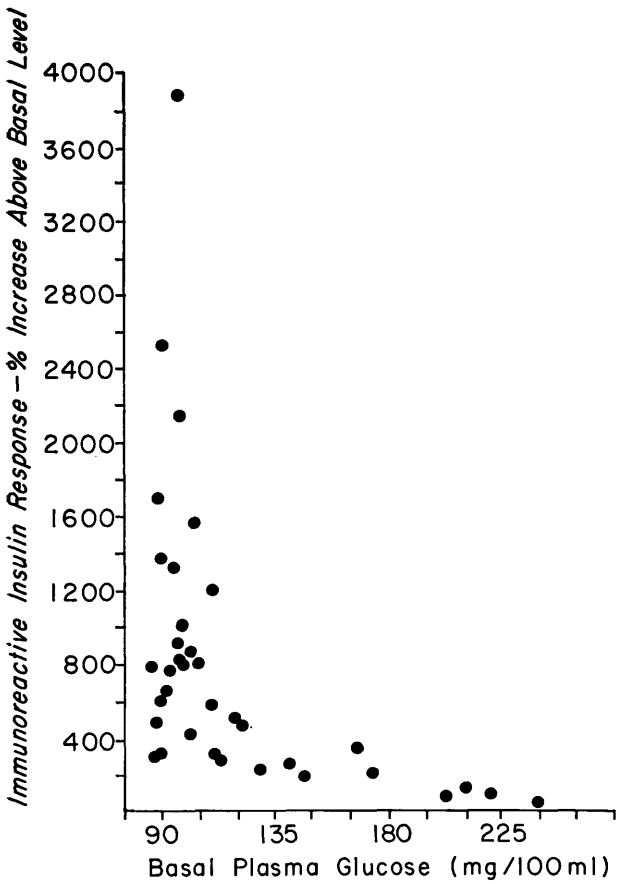

FIG. 7. Relationship between basal plasma glucose and integrated per cent immunoreactive insulin increase above basal in all subjects.

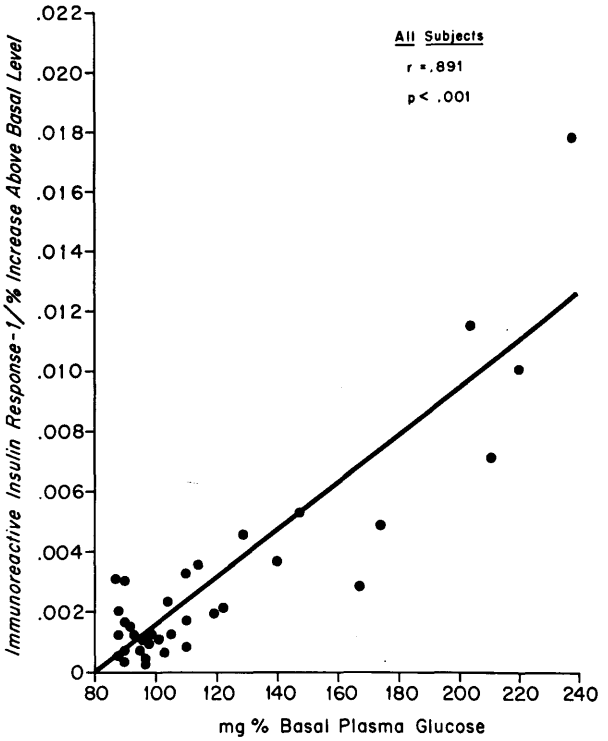

FIG. 8. Relationship between basal plasma glucose and the reciprocal of the integrated per cent insulin increase above basal in all subjects.

(table 2). Moreover, it resulted in a closer approximation of the mean IRI responses of obese and nonobese nondiabetics and a clearer separation of the mean response of nonobese nondiabetics from that of obese diabetics (figure 5). Therefore, the relative responses of obese and nonobese nondiabetics were combined and compared to the response of both diabetic subgroups (figure 6). When the groups were so compared, the limiting effect of diabetes upon the IRI response to glucagon was clear (table 2, and figure 4, bottom).

DISCUSSION

The present study was designed to allow assessment of the insulin response to glucagon under conditions both of association and dissociation between its hyperglycemic and insulinogenic (insulin-secretory) properties. Previous investigation in normal subjects[3] had revealed diminishing IRI responses during a prolonged (3 hr.) infusion of glucagon, concomitant with symptoms of sympathetic discharge and a rise in urinary vanillylmandelic acid (VMA). Therefore, in the current study the glucagon pulse was delivered after just 30 min. of infusion, prior to signs of catecholamine release and while IRI levels were still ascending. The acute administration of glucagon at this time was followed promptly by substantial increases in serum insulin levels without significant further hyperglycemia, separating the insulinogenic from the glycogenolytic effects of glucagon at this point. Therefore, this technic was deemed appropriate for the evaluation of the effects of obesity and diabetes upon insulin secretion in response to glucagon independent of changes in plasma glucose.

In confirmation of several recent studies[15-19] obesity was associated with elevated basal insulin levels, whereas no correlation existed between glucose tolerance per se, as reflected by basal glucose concentrations, and the fasting insulin values. This would suggest that excess adiposity, regardless of the state of glucose tolerance, is associated with fasting hyperinsulinism related to the degree of obesity and insulin insensitivity.[20,21]

Among all subjects, the basal insulin level could be

directly related to the IRI response to glucagon whether in conditions of association (infusion) or dissociation (injection after infusion) between its hyperglycemic and insulinogenic properties. Thus, obesity among normoglycemic individuals was also associated with increased absolute insulin secretion in response to glucagon in amounts consistent with the increased requirements imposed by the associated insulin insensitivity. In the diabetic group this effect was not seen, presumably because of the presence within that group of subjects with glucose intolerance of a degree severe enough to obscure the opposing effect of obesity. Viewed in comparison with the nonobese state, obese subjects with normal glucose tolerance appear to exhibit "hyperinsulinism" in response to a number of insulinogenic stimuli, including glucagon,[22,23] tolbutamide,[16,17] arginine[24] and oral[15-18,25,26] and intravenous[19,25,26] glucose. However, when viewed in light of their degree of insulin-insensitivity as reflected by their elevated basal insulin level, the IRI response to these stimuli would appear to be appropriate and probably responsible for the preservation of their normal glucose tolerance.

The association between basal IRI levels and the magnitude of the IRI response to glucagon suggests that expression of the response as a per cent of the basal may correct for individual variation in insulin sensitivity as reflected in the basal level and allow the assessment of the IRI response as a function solely of glucose tolerance. The validity of this concept was demonstrated by Bagdade et al.[15] in their study of the insulin response to oral glucose. Expression of the present data in this fashion eliminated the absolute difference between obese and nonobese nondiabetic subjects and was felt to justify the combination of these subgroups in the direct comparison of all nondiabetic with all diabetic subjects.

However, classification of the subjects as "nondiabetic" or "diabetic" and "obese" or "nonobese" was arbitrary and performed only to allow more graphic characterization of the states of obesity and/or diabetes. Conclusions have been drawn only from the more encompassing regression analyses among all subjects, including those lying in the area of borderline glucose intolerance (between 1 and 2 S.D. above the mean normal glucose level). The basal (fasting) glucose value was chosen as the index of glucose tolerance because of the known close correlation between fasting glucose levels and the response to oral glucose.[15] Of interest was the equally close correlation between basal glucose levels and the glucose response to glucagon during both periods of stimulation in the present study.

The selection of diabetics of relatively severe degree (twelve of thirteen exceeded the mean normal basal glucose value by > 3 S.D.) assured a contrast between the normal and diabetic states. Thus, when directly compared, the diabetics clearly exhibited an impaired IRI response to glucagon, confirming the report of Simpson et al.[27] for nonobese and of Melani et al.[23] for obese diabetics. The latter noted equivalent absolute IRI levels after intravenous glucagon among nonobese nondiabetics and obese diabetics, a finding not inconsistent with the present study in that no correction was applied for insulin levels in the obese subjects, and the two groups were therefore not directly comparable. In the current study obese diabetics might have similarly exhibited equal or even greater absolute IRI responses than nonobese nondiabetics had a larger number of obese subjects with mild glucose intolerance been studied. In one such obese, mild diabetic, for example, her absolute IRI level reached 390 μU./ml., but her relative response was well below that of any member of the nondiabetic group.

The limiting effect of diabetes upon the IRI response to glucagon is consistent with similarly impaired insulin secretion reported in diabetics in response to a variety of stimuli, including oral glucose,[15,17,24,25,28] intravenous glucose,[24,25,29] intravenous tolbutamide[17] and arginine or a mixture of amino acids.[30] Regression analysis among all subjects in the present study and that of Bagdade et al.[15] eliminated the arbitrary classification of subjects as "diabetic" or "nondiabetic." These studies suggest a continuum of progressive insulin insufficiency in response to either glucose or glucagon in association with progressive deterioration in glucose tolerance.

ACKNOWLEDGMENT

This work was supported in part by U.S. Public Health Service grants AM-02456 and 5-Ti AM-5020. A portion of this work was conducted through the Clinical Research facility, University of Washington (National Institute of Health Grant F-37).

The authors are indebted to Daniel Porte, Jr., M.D., Edwin L. Bierman, M.D., and John D. Bagdade, M.D., for invaluable advice and criticism in the analysis of data and preparation of the manuscript. Ronald Harbeck, B.S. and Barbara Hickernell, B.S. rendered skilled technical assistance. Ramon Reiser, B.A. and Karen Hashimoto aided in the statistical analysis.

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