Relationships Between Fasting Plasma Glucose Levels and Insulin Secretion During Intravenous Glucose Tolerance Tests

JOHN D. BRUNZELL, R. PAUL ROBERTSON, ROGER L. LERNER, WILLIAM R. HAZZARD, JOHN W. ENSINCK, EDWIN L. BIERMAN, AND DANIEL PORTE, JR.

The Department of Medicine, University of Washington School of Medicine, and the Veterans Administration Hospital, Seattle, Washington

ABSTRACT. Insulin secretion and glucose disappearance rate were measured in 66 subjects with a wide range of fasting plasma glucose levels. The acute insulin response was present in subjects with fasting glucose levels below 115 mg/dl but was absent above this level. The glucose disappearance rate related to the relative acute insulin response in subjects with fasting glucose below 115 mg/dl and to total insulin response when fasting glucose levels were above 115 mg/dl. A calculated glucose disappearance rate of 1.06 per cent per minute was found when the acute insulin response was zero. All subjects with fasting glucose levels > 115 mg/dl had glucose disappearance rates < 1.06. These studies support 1) epidemiological data indicating 115 mg/dl as an upper limit of normal for fasting plasma glucose levels and 1.0 per cent per minute as a lower limit of normal for the glucose disappearance rate, and 2) evidence for an important role for the acute insulin response in the determination of glucose disappearance rates during intravenous glucose tolerance tests. (*J Clin Endocrinol Metab* **42:** 222, 1976)

INSULIN secretion following a glucose stimulus is multiphasic both *in vitro* in the perfused pancreas (1) and *in vivo* following intravenous glucose (2,3). The results of previous studies have shown that when varying glucose loads were used in normal subjects, the glucose disappearance rate (Kg) was a linear function of the acute or first phase insulin response (the increase over fasting insulin during the first three to five minutes) with a maximum for both the Kg and acute insulin response at twenty grams of intravenous glucose injected rapidly (4). A decrease in the early or acute phase of insulin release following intravenous glucose in subjects with carbohydrate intolerance has been repeatedly described (2,5–12). We have previously reported preliminary data that selected thin subjects with fasting hyperglycemia had an absent acute or early insulin response (8) when a submaximal glucose pulse was given.

Received February 26, 1975.

Reprints: Dr. Brunzell, Veterans Administration Hospital, 4435 Beacon Avenue South, Seattle, Washington 98108.

In this study we have explored the relationships among insulin secretion, fasting glucose levels, and glucose disappearance rates during a maximal intravenous glucose pulse in a much larger group of subjects with a wide range of fasting plasma glucose levels.

Materials and Methods

The intravenous glucose tolerance tests were all consecutive studies on adult subjects eating a weight-maintaining balanced food or formula diet, these subjects having had a 20 gram intravenous glucose tolerance test performed during 1971 and 1972 in our laboratory. They were not selected for body weight, plasma lipid levels, or family history of diabetes. If several intravenous glucose tolerance tests had been performed, only the first such test was reported. None of the subjects had uremia, liver or thyroid disease, or an acute illness other than untreated non-ketotic hyperglycemia. Subjects treated with insulin, oral sulfonylureas, estrogens, or glucocorticoids were excluded.

The intravenous glucose tolerance test was performed at bed rest on a metabolic ward after an overnight fast of 12 to 14 hours as previously described (3). A 19 gauge scalp vein

needle was inserted into an antecubital vein and kept patent with a slow infusion of normal saline. Blood samples for glucose and insulin measurements were obtained 15, 30, 45, and 60 minutes after insertion of the needle. Twenty grams of 50% glucose were then injected through the scalp vein needle in 0.2 to 0.4 minutes; the time at which one-half of the glucose had been injected was designated as zero time. Post-injection samples for glucose and insulin were taken at 3, 4, 5, 7, 10, 15, 20, 25, 30, 45, 60, 90, and 120 minutes. No extravasation of the injected glucose or subsequent phlebitis occurred in any subject.

The samples for glucose were collected in EDTA (1 mg/ml) and placed in ice until centrifuged at 4 C. The plasma was kept frozen until subsequently analyzed by the autoanalyzer ferricyanide method. Samples for insulin were allowed to clot at room temperature and the serum was frozen and later analyzed by a modification of the method of Morgan and Lazarow (13).

Glucose disappearance rates were calculated from the slope of the logarithm of the glucose level from 10 to 30 minutes. The fasting insulin level was taken as the mean of the four samples before the glucose injection. Total incremental insulin response was calculated by computer as the integrated area above the fasting insulin level from 0 to 120 minutes after the glucose injection. The acute insulin response was calculated as the area above the fasting insulin level from 3 to 5 minutes. The relative insulin response was calculated as the per cent increase above fasting insulin levels (14) both for the total time and for the acute insulin response:

$$\frac{\text{incremental insulin area above basal}}{\text{basal insulin}} \times 100$$

Statistical analysis was performed by Student's t test and the least squares regression analysis. Results are reported as mean ± 1 standard deviation.

Results

The increment in insulin level above fasting in response to intravenous glucose, in particular the acute insulin response, was markedly reduced when the fasting glucose level exceeded 115 mg/dl (Fig. 1). The acute insulin response was present in all subjects with a fasting plasma glucose below 115 mg/dl, whereas there was no acute insulin response in those subjects with fasting glucose levels above 115 mg/dl (Fig. 2). Thus, those subjects with fasting glucose levels above 115 mg/dl were examined separately from those with fasting glucose levels of 115 mg/dl or lower.

Subjects with fasting glucose levels below 115 mg/dl. In the subjects with fasting glucose levels below 115 mg/dl (n = 51), both the acute insulin response above fast-

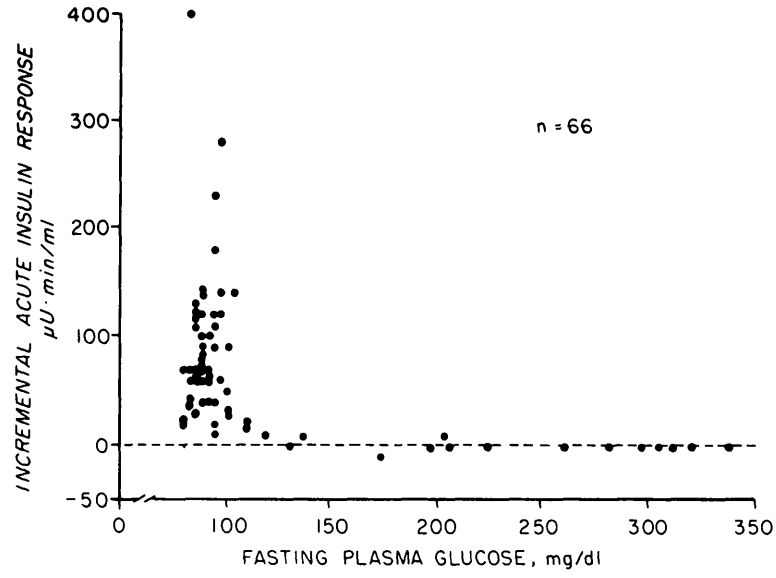

FIG. 1. Relationship between incremental acute (three to five minute) insulin response and fasting glucose level in all subjects.

ing (r = .46, $P < .001$) and the total insulin response above fasting (r = .56, $P < .001$) were correlated with the fasting insulin level. Therefore, the measures of glucose metabolism evaluated in this study were examined in relation in both the absolute and the relative (*i.e.*, per cent of fasting insulin level) incremental insulin secretion (14,15).

In these subjects there was no relationship between the fasting plasma glucose level and the relative acute insulin response (r = .24) or the absolute acute insulin response (r = .06). If the subjects in the present study are divided into subgroups based on fasting glucose levels, those subjects with fasting glucose levels between 100 and 114 mg/dl tended to have a diminished acute insulin response compared with the subjects with fasting glucose levels below 100 mg/dl (Fig. 2, $P < .01$). A histogram of the magnitude of both the absolute and the relative acute insulin response in these subjects appeared to be unimodal, if perhaps skewed to the side of the higher insulin responses. Although there was no significant relationship between the absolute acute insulin response and the glucose disappearance rate (Kg) (r = .26), Kg was a function of the relative acute insulin response (r = .49, $P < .001$, Fig. 3). There was no relationship between the Kg and the absolute total insulin response (r = .02) or the relative total insulin response (r = .08).

Subjects with fasting glucose levels above 115 mg/dl. Since the acute insulin response was absent in subjects with fasting glucose levels above 115 mg/dl, no correlation between glucose levels and the acute insulin

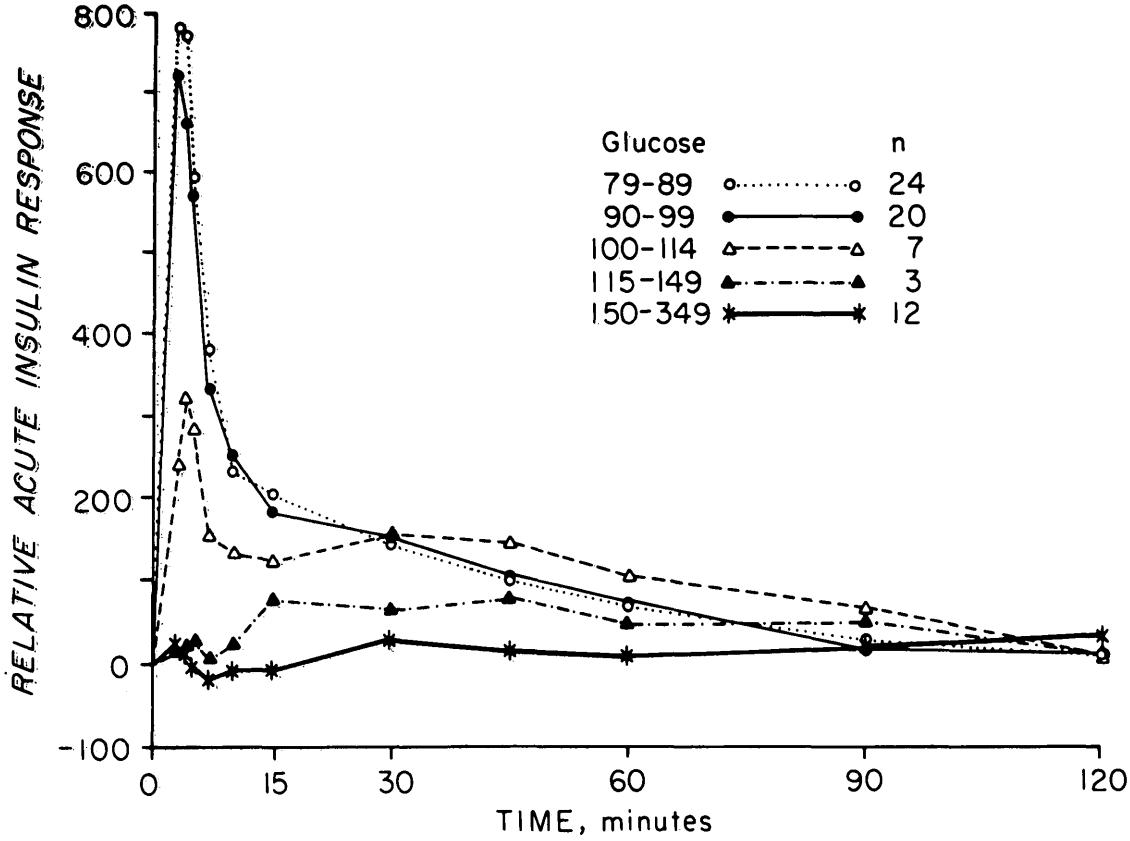

FIG. 2. Mean relative incremental insulin levels following intravenous glucose in arbitrarily divided subgroups of subjects based on fasting glucose levels. Note presence of acute insulin response in subjects with fasting glucose levels below 115 mg/dl, and absence of response above 115 mg/dl.

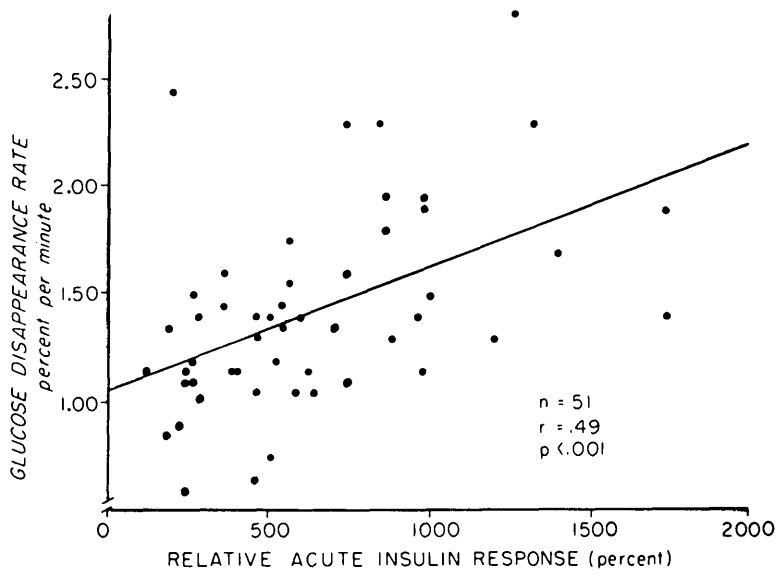

FIG. 3. Relationship between glucose disappearance rate and relative acute insulin response in subjects with fasting glucose levels below 115 mg/dl.

response could be examined. In these subjects the fasting glucose was inversely related to the total insulin response (n = 15, r = −.69, $P < .01$; Fig. 4a) and to the Kg (r = −.65, $P < .01$; Fig. 4b). Thus, the Kg was a function of the total insulin response (r = .72, $P < .005$, Fig. 4c) in subjects who do not have an acute insulin response. There was also a correlation between the Kg and the relative total insulin response (r = .49, $P < .05$).

Glucose disappearance rate. In the subjects with fasting plasma glucose levels below 115 mg/dl, the Kg was 1.42 ± 0.46 per cent per minute. This was significantly greater than that found in the subjects with fasting plasma glucose levels above 115 mg per cent (0.63 ± 0.22 per cent, $P < .001$, Fig. 5).

Five, of fifty-one, of the subjects with fasting glucose levels below 115 mg per cent had Kgs below 1.0 per cent per minute, a commonly accepted lower limit for normal (2), while all but one of the subjects with fasting glucose levels above 115 mg per cent were below this level (Fig. 5). The subjects with fasting glucose levels below 115 mg/dl who had a Kg below 1.0 per cent per minute (Fig. 3) had a lower relative acute insulin response (314 ± 129 per cent) than those with a Kg above 1.0 per cent per minute (665 ± 393 per cent, $P < .001$). In subjects with fasting glucose levels below 115 mg/dl the regression of the Kg against the relative acute insulin response (Fig. 3) intersects the zero acute insulin response axis at 1.06 per cent per minute.

Discussion

This study demonstrates that the acute insulin response is consistently absent when fasting plasma glucose levels exceed 115 mg/dl. Furthermore, the total insulin response to intravenous glucose appears to be markedly diminished in subjects with fasting glucose levels above 150 mg/dl. It is of interest, therefore, that in community surveys the upper normal limit (mean + 2 standard deviations) for fasting glucose levels in normal middle-aged populations has been found to be 113 mg/dl,* 118 mg/dl (16), and 125 mg/dl (17) for plasma measurements, and 100 mg/dl (18) to 118 mg/dl (19) for whole blood.

The pathophysiologic meaning of the loss of the acute insulin response at a distinct level of circulating glucose is not clear.

* Personal communication, Dr. Ruben Andres, Baltimore, Maryland.

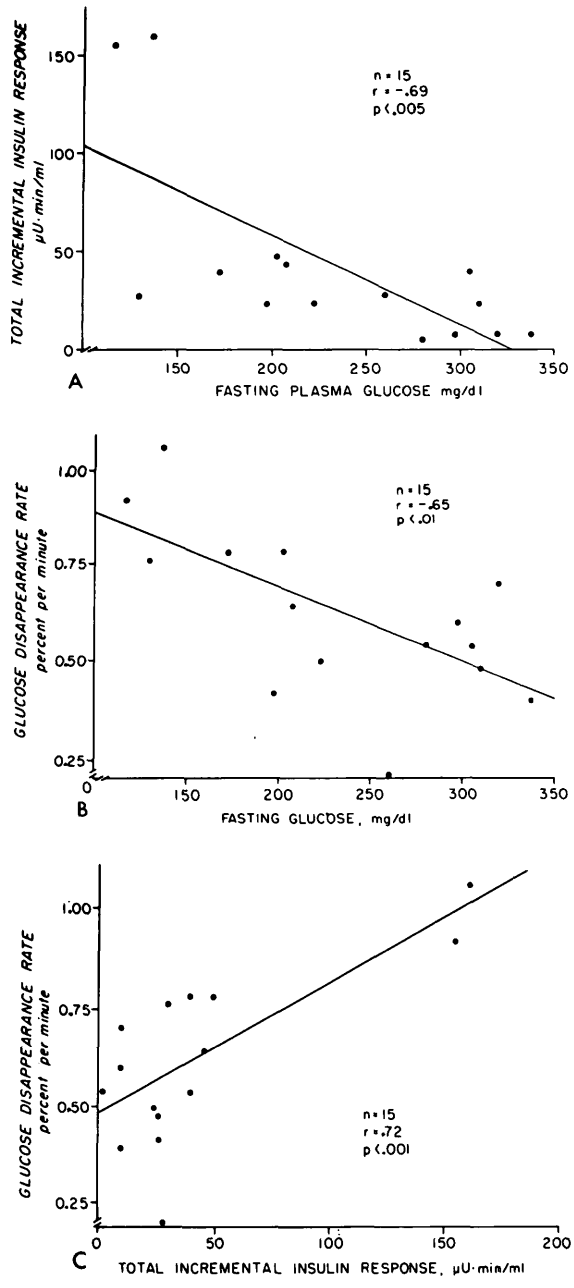

FIG. 4. Interrelationship between fasting glucose levels, total incremental insulin response and glucose disappearance rate in subjects with fasting glucose levels above 115 mg/dl. A) fasting glucose and total incremental insulin response. Correlation for those subjects with glucose levels above 150 mg/dl was $r = -.64$, $P < .02$ ($y = -0.178 \times +71.2$). B) fasting glucose and glucose disappearance rate. C) total incremental insulin response and glucose disappearance rate.

It may be that fasting hyperglycemia develops as a direct effect of the loss of acute insulin secretion. Alternatively, the beta cell response may have a threshold for stimulation by glucose and this may be made refractory by persistent hyperglycemia. In support of this latter concept, Cerasi *et al.* (20) have demonstrated in glucose dose-response studies a glucose threshold for insulin secretion of between 100 and 125 mg/dl. A threshold also has been demonstrated in the perfused pancreas or pancreatic islets where no insulin secretion is found until the perfusion glucose concentration is elevated to above 100 mg/dl (21,22). Furthermore, subjects with glucose intolerance and normal fasting plasma glucose levels have previously been demonstrated to have a diminished acute insulin response when rechallenged with a glucose pulse at elevated glucose levels maintained by a prolonged glucose infusion (8).

Normal (4,20) and "prediabetic" (20) subjects with normal fasting glucose levels respond to increasing glucose loads with increasing incremental acute insulin responses. A small number of subjects with fasting glucose levels greater than 115 mg/dl have been demonstrated to have a lack of an acute insulin response to 5 grams of intravenous glucose in a previous study (8) and now a larger group has shown the same lack to 20 grams of intravenous glucose in this study. In studies by others (12), no acute insulin response was found in diabetic subjects given doses of glucose over three times greater than that used in this study (12). Therefore, it appears that the acute insulin response is absent with fasting plasma glucose levels greater than 115 mg/dl regardless of the amount of glucose given.

The disappearance of the acute insulin response with fasting hyperglycemia appears to be specific for the response stimulated by glucose. A similar phenomenon does not appear to occur with isoproterenol (23), secretin (24,25), tolbutamide (26,27), glucagon (28), or amino acids (25,29). The

uniqueness of this abnormality to glucose-stimulated insulin secretion may relate to a postulated defect in glucose "receptors" in the diabetic pancreatic islet (22,23).

The acute insulin response has been shown to correlate well with the glucose disappearance rate (Kg) (4,30,31). In the present study of subjects with widely varying fasting insulin levels, confirmation of this finding with intravenous glucose was only observed after elimination of the effects of variations in basal insulin level on the insulin response by calculating the relative insulin response. This method of expressing the insulin response to glucose was used because the fasting insulin levels were found to correlate with the magnitude of the acute insulin response to intravenous glucose in a fashion similar to that shown previously for the insulin response to oral glucose (14). When the Kg was examined in terms of the relative insulin response, there was a significant correlation between Kg and relative insulin response in the subjects with fasting glucose levels below 115 mg/dl. In no study has a correlation higher than 0.7 been demonstrated, indicating that no more than fifty per cent of the variance in Kg is due to the acute release of insulin. Since the Kg is 1% per minute in the absence of an acute insulin response, the Kg is clearly dependent upon other factors in addition to the acute insulin secretory response (31). The finding that the Kg is even lower when the insulin response to glucose totally disappears suggests a physiological role for the insulin secreted during the second phase. Clearly, then, any insulin secreted may be physiologically "effective," no matter when it it secreted, but the major difference between normal and hyperglycemic subjects is the presence of an insulin response at the early time periods after intravenous glucose.

"Normal" values for glucose disappearance rates during intravenous glucose tolerance tests have been developed from epidemiological studies. Results in this study suggest that a Kg below which a gross defect in insulin secretion can be expected, can be approximated from knowledge of the fasting plasma glucose level on a constant diet. Those subjects with fasting glucose levels above 115 mg/dl had no acute insulin response, and all but one had a Kg below 1.0 per cent per minute, while 46 of 51 subjects with fasting plasma glucose below 115 mg/dl had Kg values above 1.0. The regression of the KG against the relative acute insulin response (Fig. 3) in subjects with fasting glucose levels below 115 mg/dl intersects the zero acute insulin response at a Kg of 1.06%/min which lends physiological meaning to the Kg of 1.0 per cent per minute determined as the lower limit of normal in other studies (2).

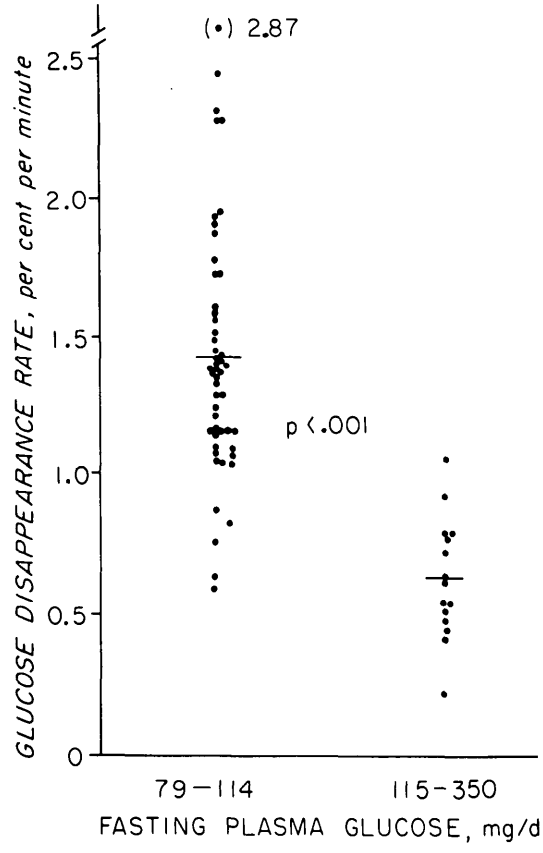

FIG. 5. Comparison of glucose disappearance rates in subjects with fasting glucose levels above and below 115 mg/dl.

The subjects with fasting glucose levels below 115 mg/dl, Kgs below 1.0 per cent per minute, and reduced relative acute insulin responses may be comparable to those subjects characterized as "prediabetic" by Cerasi and Luft. Their "prediabetic" subjects were defined as those who had low ten-minute insulin responses (2). They also had significantly lower glucose disappearance rates (12,32), and some were thinner than control populations (33), which, because of the associated lower fasting insulin levels, may account for smaller early insulin responses. Indeed, many of their different groups of "prediabetics" with low insulin responses were noted to have increased insulin sensitivity (2), and thus they may have counter-regulated to low insulin responses entirely on this basis. The acute insulin response in the "prediabetic" subjects of Cerasi and Luft suggested a mode distinct from normal (2), a finding which could not be confirmed in the present study. Whether the subjects with low Kgs and fasting glucose levels below 115 mg/dl, the subjects with fasting glucose levels between 100 and 114 mg/dl, or the "prediabetic" subjects of Cerasi and Luft will eventually develop hyperglycemia, and are thus truly prediabetic can only be determined by prospective studies (32).

Acknowledgments

The authors would like to thank Ms. Martha Kimura, Susan Coates, Martha Pleasant, Shirley Corey, and Mr. Howard Beiter for their technical assistance. Part of this work was performed at the Clinical Research Centers of the University Hospital and Harborview Medical Center (NIH grants FR-37 and RR-133) and the VA Hospital Metabolic Ward. This study was supported in part by National Institutes of Health grants AM 06670, AM 05498, and AM 12829.

References

1. Curry, D. L., L. L. Bennett, and G. M. Grodsky, Dynamics of insulin secretion by the perfused rat pancreas, *Endocrinology* **83:** 572, 1968.
2. Cerasi, E., and R. Luft, The plasma insulin response to glucose infusion in healthy subjects and in diabetes mellitus, *Acta Endocrinol (Kbh)* **55:** 278, 1967.
3. Porte, D., Jr., and A. A. Pupo, Insulin responses to glucose: Evidence for a two-pool system in man, *J Clin Invest* **48:** 2309, 1969.
4. Lerner, R. L., and D. Porte, Jr., Relationships between intravenous glucose loads, insulin responses, and glucose disappearance rate, *J Clin Endocrinol Metab* **33:** 409, 1971.
5. Seltzer, H. S., E. W. Allen, A. L. Herron, Jr., and M. T. Brennan, Insulin secretion in response to glycemic stimulus: Relation of delayed initial release to carbohydrate intolerance in mild diabetes mellitus, *J Clin Invest* **46:** 323, 1967.
6. Simpson, R. G., A. Benedetti, G. M. Grodsky, J. H. Karam, and P. H. Forsham, Early phase of insulin release, *Diabetes* **17:** 684, 1968.
7. Boden, G., J. S. Soeldner, R. E. Gleason, and A. Marble, Elevated serum human growth hormone and decreased serum insulin in prediabetic males after intravenous tolbutamide and glucose, *J Clin Invest* **47:** 729, 1968.
8. Lerner, R. L., and D. Porte, Jr., Acute and steady-state insulin responses to glucose in nonobese diabetic subjects, *J Clin Invest* **51:** 1624, 1972.
9. Johansen, K., Mild diabetes in young subjects, *Acta Med Scand* **193:** 23, 1973.
10. Linde, J., and T. Deckert, Increase of insulin concentration in maturity-onset diabetics by phentolamine (Regitine) infusion, *Horm Metab Res* **5:** 391, 1973.
11. Hecht, A., H. Gershberg, and M. Hulse, Effect of chlorpropamide treatment on insulin secretion in diabetics: Its relationship to the hypoglycemic effect, *Metabolism* **22:** 723, 1973.
12. Efendic, S., E. Cerasi, and R. Luft, Quantitative study on the potentiating effect of arginine on glucose-induced insulin response in healthy, prediabetic, and diabetic subjects, *Diabetes* **23:** 161, 1974.
13. Morgan, C. R., and A. Lazarow, Immunoassay of insulin: Two antibody system, *Diabetes* **13:** 115, 1963.
14. Bagdade, J. D., E. L. Bierman, and D. Porte, Jr., The significance of basal insulin levels in the evaluation of the insulin response to glucose in diabetic and nondiabetic subjects, *J Clin Invest* **46:** 1549, 1967.
15. Porte, D., Jr., and J. D. Bagdade, Human insulin secretion: An integrated approach, *Ann Rev Med* **21:** 219, 1970.
16. Gibson, T. C., and E. B. Whorton, The prevalence of hyperlipidemia in a natural community, *J Chronic Dis* **26:** 227, 1973.
17. Rosselin, G. E., J. R. Claude, E. P. Eschwege, *et al.*, Diabetes Survey: Plasma insulin during 0–2 h oral glucose tolerance test systematically

carried out in a professional group, *Diabetologia* **7:** 34, 1971.

18. Boyns, D. R., J. N. Crossley, M. E. Abrams, R. J. Jarrett, and H. Keen, Oral glucose tolerance and related factors in a normal population sample. I. Blood sugar, plasma insulin, glyceride, and cholesterol measurements and the effects of age and sex, *Br Med J* **1:** 595, 1969.
19. Nilsson, S. E., H. Lindholdm, S. Bülow, N. Forstberg, T. Emilsson, and G. Stenkula, The Kristianstad survey, *Acta Med Scand* [*Suppl*] **428:** 5, 1964.
20. Cerasi, E., R. Luft, and S. Efendic, Decreased sensitivity of the pancreatic beta cells to glucose in prediabetic and diabetic subjects, *Diabetes* **21:** 224, 1972.
21. Ashcroft, S. H. J., J. M. Bassett, and P. J. Randle, Insulin secretion mechanisms and glucose metabolism in isolated islets, *Diabetes* **21:** *Suppl* **2:** 538, 1972.
22. Matschinsky, F. M., R. Landgraf, J. Ellerman, and J. Kotler-Byajtburg, Glucoreceptor mechanisms in islets of Langerhans, *Diabetes* (Suppl) **2:** 555, 1972.
23. Robertson, R. O., and D. Porte, Jr., The glucose receptor: A defective mechanism in diabetes mellitus distinct from the beta adrenergic receptor, *J Clin Invest* **52:** 870, 1973.
24. Lerner, R. L., and D. Porte, Jr., Secretin and glucose: Stimulation of rapid insulin response from separate functional pools, *Clin Res* **19:** 478, 1971.
25. Kalk, W. J., A. I. Vini, S. Bank, P. Keller, and W. P. U. Jackson, Selective loss of beta cell response to glucose in chronic pancreatitis, *Horm Metab Res* **6:** 95, 1974.
26. Perley, M., and D. M. Kipnis, Plasma insulin responses to glucose and tolbutamide of normal weight and obese diabetic and nondiabetic subjects, *Diabetes* **15:** 867, 1966.
27. Bagdade, J. K., E. L. Bierman, and D. Porte, Jr., Basal and stimulated insulin levels: Comparison of insulinogenic effects of oral glucose and intravenous tolbutamide in nondiabetic and diabetic subjects, *Metabolism* **20:** 1000, 1971.
28. Crockford, P. M., W. R. Hazzard, and R. H. Williams, Insulin response to glucagon: The opposing effects of diabetes and obesity, *Diabetes* **18:** 216, 1969.
29. Unger, R. H., E. Aguilar-Parada, W. A. Muller, and A. M. Eisentraut, Studies of pancreatic alpha cell function in normal and diabetic subjects, *J Clin Invest* **49:** 837, 1970.
30. Bengtsson, C., E. Blohme, and J. Waldenstrom, Diabetes mellitus, carbohydrate tolerance and early insulin response to an intravenous glucose injection in a population sample of women and in women with ischaemic heart disease, *Acta Med Scand* [*Suppl*] **549:** 65, 1973.
31. Reaven, G. M., and J. M. Olefsky, Relationship between insulin response during the intravenous glucose tolerance test, rate of fractional glucose removal and the degree of insulin resistance in normal adults, *Diabetes* **23:** 454, 1974.
32. Cerasi, E., and R. Luft, Pathogenesis of diabetes in man, *Isr J Med Sci* **8:** 207, 1972.
33. Edström, K., E. Cerasi, and R. Luft, Insulin response to glucose infusion during pregnancy. A prospective study of high ahd low insulin responders with normal carbohydrate tolerance, *Acta Endocrinol (Kbh)* **75:** 87, 1974.