Evidence that Glucose Load Is an Important Determinant of Plasma Insulin Response in Normal Subjects

Daniel T. Peterson, B.A., and Gerald M. Reaven, M.D., Palo Alto

SUMMARY

Two consecutive oral glucose tolerance tests were performed on twelve healthy, male medical students, in which each subject received 20 and 40 gm. of glucose per square meter body surface area. The tests were conducted one week apart, and the sequence in which the two glucose loads was given was randomized. The larger glucose load elicited an increase in both plasma glucose and immunoreactive insulin response. However, the increase in the plasma insulin response was much greater than the increase in plasma glucose response. Thus, the insulinogenic index, defined as the plasma insulin level divided by the plasma glucose level, significantly increased as the glucose load increased. These results indicate that glucose load is an important determinant of plasma insulin response, and that in any individual the plasma insulin response is not a simple linear function of coexisting plasma glucose concentration. DIABETES *20*:729-33, November, 1971.

It is now more than a decade since Yalow and Berson[1] first suggested insulin deficiency could not be considered the sole cause of hyperglycemia in patients with maturity-onset diabetes, since many of these patients had greater-than-normal levels of circulating insulin. Considerable controversy still exists, however, as to the role of insulin deficiency in the genesis of hyperglycemia in these patients.[2-6] The major area of conflict has revolved around the question as to what actually constitutes a "greater than normal" level of circulating insulin. It has been suggested that one cannot simply compare the plasma insulin response of various subjects, but that the insulin response must be related to the coexisting plasma glucose level.[3,4] When this is done, for example, by dividing the plasma insulin concentration by the concomitant glucose concentration, it has been concluded that the "greater than normal" absolute insulin response of the patient with chemical diabetes is actually a "less than normal" response.[3,4,7] Furthermore, it has been shown that comparable levels of hyperglycemia, achieved by infusing different glucose loads, elicit greater insulin responses in normal subjects than in patients with chemical diabetes.[8] This observation has also been considered to prove that hyperglycemia in patients with chemical diabetes is due to insulin lack. Obviously, these conclusions are based upon the assumption that the primary factor controlling pancreatic insulin secretion (and subsequent levels of circulating insulin) is plasma glucose concentration, and that plasma insulin response is a linear function of plasma glucose level. There is no particular reason to believe, however, that this is the case, and the studies of Hales and Randle[9] suggest that glucose load might be a more important determinant of insulin response than glucose concentration. They administered 50 and 100 gm. glucose loads to the same normal individuals on different occasions. The plasma glucose response was the same irrespective of the amount of the glucose challenge. On the other hand, the plasma insulin response was considerably greater with the 100 gm. load. Obviously, in these patients insulin response was not a linear function of coexisting plasma glucose concentration, and the load of glucose administered had a profound effect on insulin response. But this point was not the major goal of their studies—the two oral glucose tolerance tests were only carried out on five subjects, and it was not clear if the differences were statistically significant. For these reasons we felt it necessary to see if we could confirm the results of Hales and Randle. Our studies differ in that we enlarged the patient group, the order in which the two tests were given was randomized, and oral glucose loads of 20 and 40 gm. of glucose per

From the Department of Medicine, Stanford University School of Medicine and Palo Alto Veterans Administration Hospital, Palo Alto, California.

Reprint requests should be addressed to Gerald M. Reaven, M.D., Veterans Administration Hospital, Department of Medicine, 3801 Miranda Avenue, Palo Alto, California 94304.

square meter body surface area were administered.[10] The results to be presented confirm the earlier findings of Hales and Randle, and indicate that the amount of glucose administered is a very important determinant of insulin response.

METHODS

1. *Subjects.* Twelve male medical students, aged twenty-one to twenty-four, volunteered for these studies. All subjects were in good general health, free of any disease known to influence glucose tolerance, without a family history of diabetes mellitus, and within the ideal weight limits for their height as judged by Metropolitan Life Insurance Company tables.

2. *Test procedure.* The oral glucose loads administered were 20 gm. and 40 gm./M.^{2} of body surface area, calculated on the basis of each subject's height and weight.[10] The two tests were performed seven days apart, and the order was randomized by coin toss. Dietary histories were taken and dietary instruction provided in order to insure that each subject consumed approximately 250 gm. of carbohydrate daily for three days before each glucose tolerance test. The oral glucose load was given at 8 a.m. following a twelve-hour fast, and was administered as 300 ml. of a cold, lemon extract-flavored drink. Nausea was not felt by any subject. Subjects spent the two-hour blood-drawing period resting in the lounge of the Clinical Research Center.

3. *Analytical procedures.* Venous blood was withdrawn before and 30, 60, and 120 min. after glucose administration, placed into tubes containing EDTA, and immediately centrifuged. The plasma was separated into aliquots, and immediately frozen in an acetone-dry ice bath. Plasma glucose concentration was determined with the Technicon AutoAnalyzer by a ferricyanide reduction method,[11] and plasma immunoreactive insulin concentration by the method of Hales and Randle,[12] using I-125 insulin and insulin-binding reagent obtained from Schwarz Bioresearch, Orangeburg, N.J. In order to maximize measurement accuracy, plasma glucose and insulin concentrations from both studies (for each subject) were determined on the same day. Pooled plasma standards were included in each glucose and insulin assay, and the day-to-day coefficient of variation was 2.5 per cent for glucose and 7 per cent for insulin.

4. *Data analysis.* Concentration of plasma glucose and immunoreactive insulin for both studies at each time point represent the basic data. In addition, an insulinogenic index was calculated by dividing the insulin concentration at each time point after the glucose load by the coexisting plasma glucose concentration (I/G ratio) as suggested by Perley and Kipnis.[3] The statistical significance of the differences in mean plasma glucose concentration, plasma insulin concentration, and I/G ratio that resulted from varying the glucose challenge were evaluated by use of the sign test. This approach was used because we did not feel that we could assume that the subjects were a random sample, nor that the insulin and glucose data were normally distributed. Under these conditions a nonparametric statistic seemed most reasonable, and the sign test appeared to represent the most appropriate choice.[13]

RESULTS

The effect of doubling the oral glucose challenge on the subsequent plasma glucose and insulin responses is seen in figure 1. Although mean plasma glucose levels increased somewhat as the glucose load increased, the

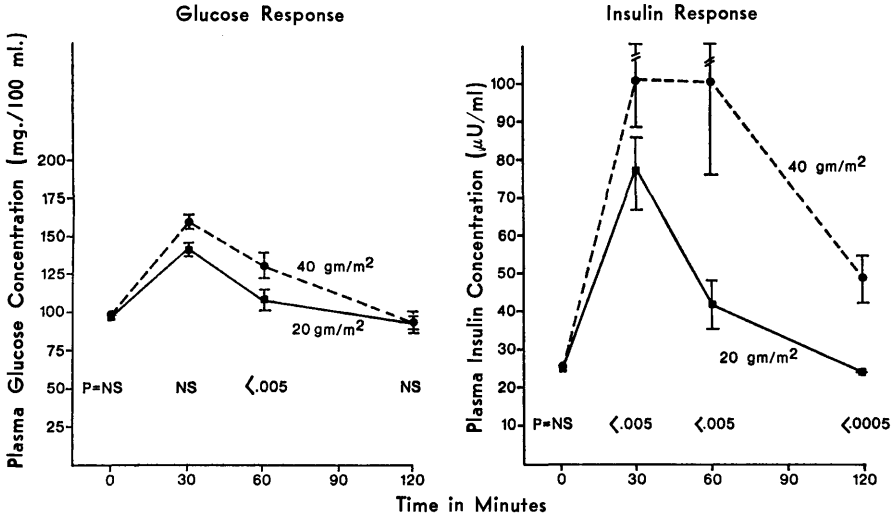

FIG. 1.

The mean (± SE) plasma glucose and immunoreactive insulin responses of twelve normal subjects to oral glucose loads of 20 and 40 gm. of glucose per square meter body surface area. The order of the two tests was randomized, and they were performed one week apart. The sign test was used to evaluate the level of statistical significance.

differences were not great; they only reached statistical significance at the sixty-minute time point, and by two hours the plasma glucose levels were identical.

In marked contrast is the plasma insulin response to increasing the oral glucose challenge from 20 to 40 gm./M.^{2} body surface area. The greater load elicited an increased insulin response, and this difference was statistically significant at every time point. It is particularly interesting to note that the plasma insulin concentration two hours after the test began was still twice as great following the 40 gm./M.^{2} load, at a time when the plasma glucose levels were identical.

Since the plasma insulin response increased more than the plasma glucose response, it is obvious that the I/G ratio must also have increased when the oral glucose challenge was doubled. These results are summarized in figure 2, and do indicate that the insulinogenic index (I/G ratio) of the same group of subjects was significantly altered by simply varying the glucose challenge. The relative speed with which plasma glucose levels fell prevented us from carrying out a complete analysis of the effect of glucose load on another insulinogenic index,[4] in which the increment in plasma insulin is divided by the concomitant rise in plasma glucose ($\Delta I/\Delta G$). For example, seven of the twelve subjects receiving the smaller glucose load had glucose levels equal to or less than their baseline values by sixty minutes. However, we could carry out this analysis at the thirty-minute interval, and at this time point the mean $\Delta I/\Delta G$ ratio was significantly greater ($p < .05$) in subjects receiving the larger glucose load (1.4 vs 1.1).

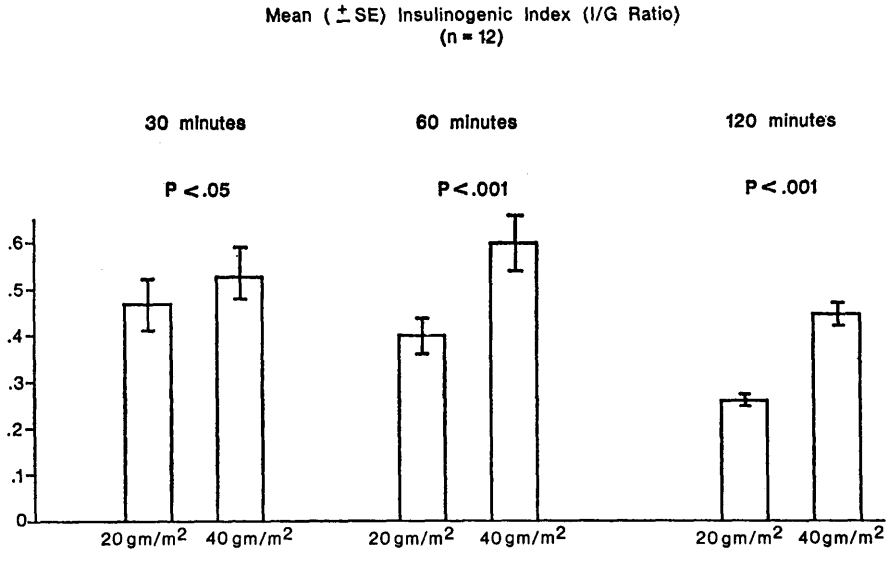

FIG. 2.

Differences in the insulinogenic index, derived by dividing plasma immunoreactive insulin concentration by plasma glucose concentration, 30, 60, and 120 min. after receiving 20 and 40 gm. of oral glucose per square meter body surface area.

DISCUSSION

In these experiments we have attempted to confirm the earlier observations of Hales and Randle[9] on the effect of varying oral glucose loads on plasma glucose and insulin by extending their studies to a larger, more clearly defined population, in which the sequence of tests was explicitly randomized. The two protocols seem to differ primarily in that their patients received 50 and 100 gm. glucose loads, whereas we complied with the suggestion of the American Diabetes Association and administered oral glucose on the basis of body surface area.[10] However, the actual amounts of glucose received by our subjects, an average of 41 and 82 gm. of glucose, seem reasonably analogous to the 50 and 100 gm. challenges utilized by Hales and Randle. The results of the two studies are also similar, and indicate that a greater glucose load augments the plasma insulin response much more than the plasma glucose response. Indeed, Hales and Randle could show no effect on plasma glucose concentration when the glucose challenge was doubled, and we could only demonstrate a change at the sixty-minute time point. This effect of increments in glucose load is further supported by the results of a similar study carried out by Castro and associates,[14] and published while this manuscript was in preparation. Although they analyzed their data quite differently, their conclusion "that the relative change in insulin response was greater than the relative change in glucose response as the glucose load was increased" is similar to ours. Thus, in all three studies it is apparent that the insulinogenic index (I/G ratio) of normal

subjects increased significantly as the glucose load was increased. It is therefore apparent that the I/G ratio is not a physiological constant for any individual, and that the insulin response of normal subjects is not a simple linear function of coexisting plasma glucose concentration. In light of these findings it would seem reasonable to urge caution in the manner in which I/G ratios are used to interpret physiological data.

The results of these studies indicate that the amount of oral glucose administered plays an important role in determining plasma insulin response, and this conclusion is not unreasonable in view of studies which suggest that insulin secretion is a function of the amount of glucose taken up and consumed by the pancreas.[15-17] If one assumes that the load of intravenous glucose also plays a role in control of insulin secretion, conclusions based upon measurements of plasma insulin response in normal and diabetic subjects under conditions of "equivalent" glycemic stimuli must be reevaluated. For example, Perley and Kipnis[8] assume that similar concentrations of plasma glucose in normal and diabetic subjects are "equivalent" glycemic stimuli, and they have shown that the insulin response of diabetic patients is less than normal under these conditions. However, in order to achieve similar plasma glucose levels in normal and diabetic subjects they had to administer a considerably greater load of intravenous glucose to the normal subjects. Were the glycemic stimuli equivalent in the two groups? Was the decreased insulin response of the diabetic group due to a defect in insulin secretion or simply a function of the fact that they received less glucose? Obviously, it is impossible to answer these questions, since one cannot equalize *both* plasma glucose level and load of administered glucose when evaluating insulin response to a glucose challenge in normal and diabetic subjects. However, since there seems to be little reason to assume that plasma glucose level is a more important determinant of insulin response than the glucose load, it is not necessary to accept the conclusion of Perley and Kipnis[8] that hyperglycemia in diabetes is entirely due to lack of insulin, when they have ignored the variable of glucose load. In this regard, it is of interest to point out when the "equivalent" glycemic stimuli is amount of glucose being utilized, either in the fasting state[18] or during continuous glucose infusion,[19] that patients with maturity-onset diabetes have insulin levels that are equal to or greater than normal. Thus, depending upon choice of "equivalent" glycemic stimuli, the cause of hyperglycemia in the diabetic patient can be due to either lack of insulin or resistance to insulin-mediated glucose uptake. This dilemma simply highlights the need for greater understanding of factors controlling insulin secretion, distribution and degradation, and in this regard it is hoped that this paper will be of help in underscoring the influence that glucose load has on insulin response.

ACKNOWLEDGMENT

The study was supported in part by the Medical Scientist Training Program Grant GM 1922-03 from the National Institute for General Medical Science, by Grant HE 08506, National Heart Institute, Grant FR-70, General Clinical Research Centers Branch, National Institutes of Health, and from the Charles Pfizer Laboratories, Inc.

The work was performed while Mr. Peterson was an M.S.T.P. Trainee.

REFERENCES

[1] Yalow, R. S., and Berson, S. A.: Immunoassay of endogenous plasma insulin in man. J. Clin. Invest. *39*:1157-75, 1960.

[2] Berson, S. A., and Yalow, R. S.: Some current controversies in diabetes research. Diabetes *14*:547-72, 1965.

[3] Perley, M., and Kipnis, D. M.: Plasma insulin responses to glucose and tolbutamide of normal weight and obese diabetic and nondiabetic subjects. Diabetes *15*:867-74, 1966.

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

[5] Reaven, G., and Miller, R.: Study of the relationship between glucose and insulin responses to an oral glucose load in man. Diabetes *17*:560-69, 1968.

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

[7] Chiles, R., and Tzagournis, M.: Excessive serum insulin response to oral glucose in obesity and mild diabetes. Diabetes *19*:458-64, 1970.

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

[9] Hales, C. N., and Randle, P. J.: Effects of low carbohydrate diet and diabetes mellitus on plasma concentrations of glucose, non-esterified fatty acid and insulin during oral glucose tolerance test. Lancet *I*:790-94, 1963.

[10] Report of the Committee on Statistics of the American Diabetes Association: Standardization of the oral glucose tolerance test. Diabetes *18*:299-307, 1969.

[11] Plasma glucose procedure N-9. *In* AutoAnalyzer Manual. Chauncey, N.Y., Technicon Instruments Corporation, 1964.

[12] Hales, C. N., and Randle, P. J.: Immunoassay of insulin with insulin-antibody precipitate. Biochem. J. *88*:137-46, 1963.

[13] Savage, R.: Nonparametric statistics. *In* Statistics in Endocrinology. McArthur, J. W., and Colton, T., Eds. Cam-

bridge, Mass., M.I.T. Press, 1970, p. 215.

[14] Castro, A., Scott, J. P., Grettie, D. P., McFarlane, D., and Bailey, R. E.: Plasma insulin and glucose responses of healthy subjects to varying glucose loads during three-hour oral glucose tolerance tests. Diabetes *19*:842-51, 1970.

[15] Grodsky, G. M., Betts, A. A., Bennett, L. L., Vcella, G., McWilliams, N. B., and Smith, D. F.: Effects of carbohydrates on secretion of insulin from isolated rat pancreas. Amer. J. Physiol. *205*:638-44, 1963.

[16] Coore, H. G., and Randle, P. J.: Regulation of insulin secretion studied with pieces of rabbit pancreas incubated in vitro. Biochem. J. *93*:66-78, 1964.

[17] Malaisse, W. J., Malaisse-Lagae, F., and Mayhew, D.: A possible role for the adenylcyclase system in insulin secretion. J. Clin. Invest. *46*:1724-34, 1967.

[18] Reaven, G. M., Silvers, A., and Farquhar, J. W.: Study of the relationship between plasma insulin concentration and efficiency of glucose uptake in normal and mildly diabetic subjects. Diabetes *19*:571-78, 1970.

[19] Reaven, G. M., and Farquhar, J. W.: Steady state plasma insulin response to continuous glucose infusion in normal and diabetic subjects. Diabetes *18*:273-79, 1969.

Effects of Dietary Changes on Kidney Metabolism

(*Continued from page* 728)

pyruvate carboxylase. Because of the relationship between gluconeogenesis and ammoniagenesis, renal glucose-6-phosphatase and glutaminase were measured. Glucose-6-phosphatase did not change, but glutaminase was reduced in the trained rats when fasted for forty-eight hours.

Urine excretion and water intake were measured in relation to the changes in the feeding patterns. In general, whenever the food intake pattern was changed total water intake was reduced and the relationship between food and water altered.

Thus it would appear that repeated periods of starvation lead to an adaptive increase in gluconeogenic capacity of the kidney and an increase in activity of two key gluconeogenic enzymes—pyruvate carboxylase and PEPCK. For the former repeated starvation is necessary, but for the latter enzyme there was no difference in activity between the rats who were trained and those subjected to only a single forty-eight-hour fast.

Perhaps future studies will show the significance of the difference in behavior in the kidney and liver of the hexosemonophosphate shunt dehydrogenases and the NADP malic enzyme. The function of the HMP shunt in the kidney is still obscure, but W. D. Lotspeich and his colleagues (Science *155*:1066, 1967) have shown that it is activated by such conditions as sodium depletion and metabolic acidosis.

The decrease in activity of renal glutaminase in the fasted rats is of interest if we assume that part of the mechanism of the increased renal gluconeogenesis and increased PEPCK activity is through development of a metabolic acidosis, since it has been shown convincingly that metabolic acidosis leads to an increase in ammonia production and glutaminase activity (B. M. A. Davies and J. Yudkin, Biochem. J. *52*:407, 1952). The present study would indicate either that in the control or trained rats, even when fasted, there is no acidosis or that in these circumstances the relationship between gluconeogenesis and ammonia production does not obtain. The studies on water excretion are of interest but their relationship to the renal enzymatic and metabolic profiles is not clear.

It is hoped that there will be additional studies to delineate the effects of dietary changes on renal metabolism, especially on the enzyme activities specifically related to fluxes through metabolic pathways and utilization of substrates.

From *Nutrition Reviews,* Vol. 29,
No. 4, April 1971, pp. 96-97