# Comparison of Impedance to Insulin-Mediated Glucose Uptake in Normal Subjects and in Subjects with Latent Diabetes

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ABSTRACT A technique was devised for a more accurate measurement than has been heretofore possible of one of the factors repsonsible for hyperglycemia in the complex syndrome of diabetes. This factor is termed impedance and represents the tissues' insensitivity or resistance to insulin-mediated glucose uptake. It was measured by use of steady-state exogenous insulin and glucose infusions during a period of pharmacological suppression of endogenous insulin secretion. Endogenous new glucose production was also inhibited. Impedance as calculated is a direct function of steady-state glucose concentrations, since exogenous insulin concentrations were similar in all studies. Two groups of normal weight subjects were studied. One had maturity onset latent diabetes, and the other (matched for age, weight, and per cent adiposity) was normal. Impedance was closely reproducible in the same individual and remained relatively constant during prolonged infusions. The diabetics had average infusion glucose concentrations (and thus impedance) 68% higher than the normal group, and it is of note that their previously measured glucose intolerance differed by a similar degree; that is, the diabetic's intolerance (as defined by mean weighted plasma glucose response after oral glucose) was 52% greater than that of the normal individuals.

## INTRODUCTION

The finding of Yalow and Berson (1) that many patients with maturity onset diabetes were hyperglycemic (after oral glucose ingestion) in spite of simultaneous elevations of plasma immunoreactive insulin (IRI) cast doubt on the traditional assumption that diabetes mellitus was simply a function of insulin deficiency. Yalow and Berson suggested instead that insulin insensitivity could play an important role in the hyperglycemia of diabetes (1), and considerable evidence has since accumulated in support of their original findings and conclusions (2–13). Interpretation of these studies is difficult and uncertain because of the many uncontrolled variables present in use of either oral and intravenous glucose tolerance tests (1–4, 6, 9, 11) or of intravenous insulin tolerance tests (12, 13). In an effort to avoid some of the problems of "tolerance" tests, prolonged intravenous glucose infusions were used to achieve the advantages of steady-state conditions (7, 10). Since endogenous insulin was present, the apparent insulin insensitivity noted in these glucose infusions studies (7, 10) instead could have been due only to a lesser biological activity of the subjects' insulin. In this circumstance, the explanation for hyperglycemia despite an apparently adequate or increased quantity of insulin response (measured by immunoreactivity) is either that such patients secrete an abnormal form of insulin or that their insulin is complexed to other plasma proteins after secretion, the complex being less active biologically. This subject was critically reviewed by Berson and Yalow in 1966 (14), and they concluded that there was very little to support these alternative views. However, since 1966 two laboratories (15, 16) have published evidence for substances of greater molecular weight than insulin, which react with anti-insulin antibody, but which appear to have a lessened biological activity. In addition, several groups of workers (17–19) disagree on other grounds with the original conclusion of Yalow and Berson. These authors believe that proper data interpretation requires that the

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insulin response to an oral glucose challenge be related to some other variable, i.e., the concomitant plasma glucose concentration (17, 18) or the fasting insulin level (19). When this is done they contend that hyperglycemia in all patients with maturity onset diabetes is caused by failure of pancreatic insulin secretion. Finally, a diminished early response of plasma insulin concentration to oral (20, 21) or intravenous (22) glucose has been clearly shown in juvenile diabetics (20), in supposedly normal relatives of patients with severe diabetes (22), and in patients with mild diabetes (21). This could be called a defect in timing of insulin release.

In light of these proposed defects in timing, quantity, or quality of the insulin response, the pathogenesis of the hyperglycemia of diabetes mellitus remains unclear. We have investigated this issue by a different experimental approach. We chose two groups of subjects who had either normal or decreased glucose tolerance, and because of previously noted positive correlations between age, obesity, and hyperglycemia, the groups were carefully matched for age, weight, and per cent adiposity. They were given a constant 2½ hr intravenous infusion of exogenous crystalline insulin, and endogenous insulin secretion was inhibited by the simultaneous administration of low doses of epinephrine and propranolol. With this technique new glucose production was inhibited, and similar plasma levels of exogenous insulin were reached in all subjects. Under these conditions, the steady-state plasma glucose concentration reached as the result of a constant glucose infusion is a measure of the resistance (or impedance) of tissues to insulin-mediated glucose uptake. Measurements of impedance obtained in this manner should eliminate some of the variables of an intact feedback loop (as in any type of glucose or insulin tolerance test).

## METHODS

*Subjects.* Table I summarizes some clinical features of the two groups of seven subjects studied. The sole criterion for separation into normal and diabetic groups was the subject's plasma glucose response to oral glucose, using criteria for the 1 and 2 hr period established by Fajans and Conn (23). It should be emphasized that the patients termed diabetic have a mild degree of glucose intolerance, and though they all meet one or more of the criteria for "latent" or "chemical" diabetes (24) by the revised criteria of Fajans and Conn (25) none were diabetic by the criteria of the Wilkerson point system (25). As can be seen

TABLE I

*General Characteristics and Glucose Tolerance Test Results of Subjects*

| Subjects | Age | Sex | Height | Weight | Adiposity | Oral glucose tolerance test | | | | | Mean weighted glucose* response | Mean weighted insulin* response |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Fasting | ½ hr | 1 hr | 2 hr | 3 hr | | |
| | | | *cm* | *kg* | *%* | *mg/100 ml plasma* | | | | | *mg/100 ml* | *μU/ml* |
| Diabetics | | | | | | | | | | | | |
| T. S. | 42 | F | 171 | 71.0 | 38.0 | 95 | 167 | 191 | 183 | 166 | 172 | 193 |
| T. W. | 50 | M | 175 | 84.0 | 29.6 | 92 | 228 | 296 | 201 | 110 | 205 | 154 |
| C. H. | 36 | F | 162 | 72.7 | 32.0 | 91 | 146 | 234 | 158 | 74 | 155 | 68 |
| L. K. | 47 | M | 173 | 75.7 | 29.1 | 106 | 200 | 254 | 226 | 110 | 199 | 69 |
| F. R. | 62 | M | 175 | 74.0 | 27.2 | 91 | 154 | 180 | 171 | 108 | 155 | 50 |
| E. H. | 45 | M | 178 | 85.5 | 23.3 | 99 | 160 | 216 | 156 | 86 | 153 | 96 |
| G. R. | 43 | M | 171 | 84.0 | 23.5 | 102 | 204 | 324 | 146 | 105 | 200 | 60 |
| Mean | 46.4 | | 172 | 78.0 | 29.0 | | | | | | 177 | 99 |
| Range | 36–62 | | 162–178 | 71.0–85.5 | 23.3–38.0 | | | | | | 153–205 | 50–193 |
| Normals | | | | | | | | | | | | |
| V. B. | 54 | F | 151 | 76.1 | 42.0 | 98 | 216 | 178 | 82 | 82 | 130 | 63 |
| R. K. | 46 | M | 185 | 84.1 | 30.0 | 102 | 163 | 113 | 111 | 90 | 116 | — |
| H. K. | 57 | M | 169 | 84.8 | 27.1 | 98 | 180 | 147 | 92 | 97 | 122 | 46 |
| W. A. | 55 | M | 177 | 72.6 | 30.0 | 100 | 156 | 151 | 98 | 72 | 117 | 57 |
| H. M. | 38 | M | 170 | 78.9 | 27.8 | 86 | 126 | 136 | 104 | 69 | 108 | 60 |
| D. D. | 46 | M | 177 | 71.7 | 25.1 | 102 | 121 | 153 | 103 | 82 | 115 | 75 |
| G. W. | 40 | M | 177 | 64.9 | 25.6 | 98 | 141 | 133 | 87 | 78 | 107 | 63 |
| Mean | 47.1 | | 172 | 76.1 | 29.7 | | | | | | 116 | 63 |
| Range | 38–57 | | 151–180 | 64.9–84.8 | 25.1–42.0 | | | | | | 107–130 | 46–75 |

* Mean weighted glucose and insulin responses = weighted average of fasting, ½, 1, 2, and 3 hr values of glucose tolerance test. Weighting formula (V = glucose or insulin value in mg/100 ml or μU/ml) = $1/12V_0 + 1/6V_{30} + 1/4V_{60} + 1/3V_{120} + 1/6V_{180}$.

in Fig. 1 there is no overlap between the oral glucose tolerance of the two groups, and the diabetics'[1] glucose levels were 52% higher than those of the normal individuals when compared as their mean weighted glucose response (Table I). Also, the diabetics' mean weighted insulin response exceeded that of the normal subjects by 57%, but considerable overlap was present between groups (Table I). Those with diabetes were selected from our referral patient group to represent a subgroup with mild maturity onset diabetes, they were free from other endocrine disease, and they had never received insulin. A further selection was made to match the diabetics for weight, age, and per cent adiposity with the inherently leaner group of normal subjects. Normal individuals were selected after interviews with a group of volunteers who had recently been discharged from a local minimum security prison. Volunteers responded to a notice asking for assistance in a research project which would furnish their living expenses during a 2 wk hospital stay.

The percentage of total body weight as adipose tissue (per cent adiposity) was calculated by the method of Steinkamp and coworkers (26, 27) which they found superior to other estimates of adiposity based on measures such as single skinfold thickness, relative weight, or ponderal index. Multiple series of anthropometric measurements were made by one observer, whose repeat data agreed within a ±5% coefficient of variation. The means of these pooled data were then entered into a computer program which uses regression equations to give estimates of total body fat which compared closely to those obtained by direct methods (27). For our male subjects we chose the four-variable equation applied by Steinkamp and his coworkers to white males, 35–44 yr of age (27). For our female subjects we used the three-variable equation for white females, 35–44 yr of age (27). Eight of our subjects were over 45 yr of age, whereas the method had been applied only to subjects up to 44 yr of age. We assumed that these equations could also be applied to our older subjects. Table I shows that there were approximately equal ranges of body weight and per cent adiposity in both groups. The average adiposity of our mixed group was 29.4%, and this value is intermediate to that reported in a comparable normal United States population of 24.7 and 33.2% for males and females, respectively (27). This of course does not pass judgment on whether "obesity" is present in our two groups or in the "normal" population.

*Experimental protocol.* Subjects were hospitalized but ambulatory, and they were fed liquid formula diets in amounts designed for weight maintenance. The detailed compositions of these diets have been previously described (28). On entry, they consumed a liquid diet similar to an ad libitum diet, then after 1 or 2 days, a glucose tolerance test was done. In this test, 7 oz of a commercial carbohydrate beverage[2] was given, and blood was obtained for measurement of glucose and insulin concentrations before and ½, 1, 2, and 3 hr later. Subjects were then given a high carbohydrate diet which contained 85% of its calories from a partially hydrolyzed starch.[3] 4 days of ingestion of this diet were sufficient to adapt the body to the large amounts of carbohydrate in the experimental infusions and thereby

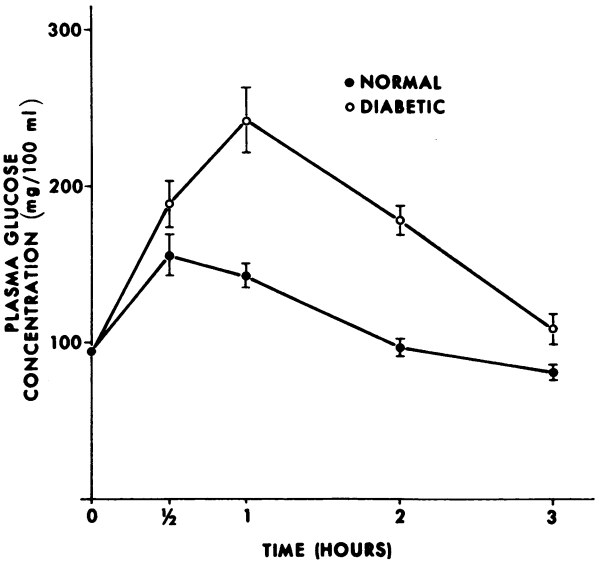


FIGURE 1 Results of oral glucose tolerance tests in normal and diabetic subjects (Mean ±SE).

to allow the subjects to reach a steady state of plasma glucose concentration during the infusion conditions described below. So, after 4 or more days of the diet, infusion studies were done as follows: after an overnight fast, 5 mg of propranolol was given intravenously; 5 min later an infusion mixture of glucose (6 mg/kg of body weight per min), epinephrine (6 μg/min), propranolol (0.08 mg/min), and pork insulin[4] (50 mU/min) was steadily infused via a constant infusion pump for 150 min. We had earlier found that 90 min was required to reach a steady state which persisted for at least 60 additional min. Accordingly, samples were drawn every 10 min during the last hour of the 150 min infusion. This protocol was followed on all 14 subjects, and in two it was repeated on two further occasions, at least 4 days elapsing between studies. Also, other studies were done with slight variations (see Results) to determine the effects of prolongation of infusion time and to find whether both endogenous insulin and endogenous hepatic glucose output were suppressed during the standard infusions. During the studies, pulse, ECG, and systemic blood pressure were monitored. Patients remained comfortable throughout. Pulse rates were slowed in all subjects, usually by 10–15 beats per min, and a rise of systolic blood pressure averaging 20 mm Hg (range 10–30 mm Hg) occurred in all subjects. No change, other than slowing of the pulse, was noted on ECG.

*Analytical procedures.* Plasma was separated in a refrigerated centrifuge and quickly frozen in acetone–dry ice. Plasma glucose was measured by a Technicon AutoAnalyzer with a ferricyanide reagent (29). Plasma immunoreactive insulin was measured in triplicate on two separate occasions by the double antibody immunoprecipitation technique of Hales and Randle (30), using insulin$^{125}$I and insulin binding reagent obtained from the Radiochemical Centre, Amersham, England. For the first nine studies, plasma insulins were measured on each of the six 10-min samples as described above (six measurements per sample). It was found that a

[1] Hereafter the word latent is generally omitted in referring to the group of patients with latent diabetes.

[2] Glucola; Ames Co., Div. Miles Labs, Inc., Elkhart, Ind.

[3] Dexin; Burroughs Wellcome and Co., Tuckahoe, N. Y.

[4] Crystalline Insulin, U-40, lot number 1CE16C; Eli Lilly & Co., Indianapolis, Ind.

satisfactory steady state was indeed achieved (the average coefficient of variation for these nine studies was ±6.2%), so in subsequent experiments the following insulin data were obtained. An equal aliquot of each 10 min plasma sample was pooled, and the pooled sample was measured in triplicate on three separate occasions for a total of nine measurements per experiment. The mean of these was used in subsequent data analysis. The reason for such replication lies in the relatively unstable nature of the insulin method. In our hands, a replicability of a pooled plasma standard (as a coefficient of variation) of ±5% was found for a single day and of ±10% for separate days. The relatively small numbers of experiments in this study, coupled with a desire to decrease the requisite volume of plasma, made it helpful to increase the precision of any one analysis as described. Plasma glucose specific activity was determined in the experiment designed to measure hepatic glucose production rate. Labeled glucose[5] specific activity in plasma samples was measured by the method of Nikkilä and Ojala (31) with the following modification of the initial extraction steps: 19 ml of chloroform-methanol (2:1) was added to 1 ml of plasma; the mixture was heated to approximately 56°C with tap water to coagulate the protein and then filtered through Sharkskin paper.[6] 3 ml of water was added to 15 ml of the ensuing filtrate; the mixture was shaken carefully and was centrifuged for 10 min at 2000 rpm, and 4.8 ml of the resultant upper phase was removed (equivalent to 0.5 ml of the original plasma). The extract was then carried through the separation steps outlined by Nikkilä and Ojala (31) following evaporation to near dryness under nitrogen.

*Calculations.* The theory behind the impedance measurement stems from the following premises: if glucose infusion rate is constant (and therefore equal to tissue uptake rate), and if plasma insulin levels are the same, then higher steady-state plasma glucose levels are caused by impedance to glucose uptake, most likely at the level of transport through the cell membrane but possibly including intracellular utilization as well. The word impedance is used as a synonym for hindrance, insensitivity, or resistance, but it is preferred to the latter to avoid confusion with the special use of resistance in diabetics who have acquired antibodies to exogenous insulin. The equation describing impedance is $V = k_u G$, where V is the glucose uptake rate in milligrams per kilogram of body weight per minute (equal to infusion rate—see Results for the evidence that no endogenous glucose enters plasma in these experiments); $k_u$ is an uptake constant analogous to a diffusion constant in an equation for simple diffusion; and G is plasma glucose concentration in mg/100 ml. Under the experimental conditions used, impedance equals $1/k_u$ and should be a constant for any given individual through the narrow experimental range of V. Since the experimental design seeks equivalent insulin values for all, the levels of plasma insulin do not significantly affect the difference in results between normal and diabetic and, accordingly, the role of insulin was ignored entirely in considering the relative impedance between normal and diabetic. However, use of an insulin term could allow one to achieve impedance values which reflect the probable modifying role of insulin and to correct for major variations in insulin values produced experimentally. We are currently doing such studies. As written, the equation assumes that glucose is taken up as a linear function of its concentration in plasma, whereas in rat hearts (32), in erythrocytes (33), or in adipose tissue (34) uptake is known to be saturable. However, without further experiments it is difficult to kiow what corrections are needed in the linearity assumption. Such experiments are in progress, and we have proposed tentative values for parameters of a carrier-mediated uptake system in man (35). Meanwhile the impedance calculation should represent a satisfactory index of the degree of inefficiency of glucose removal.

## RESULTS

*Standard infusion studies and impedance calculations.* Fig. 2 and Table II show that the mean steady-state insulin values were almost identical in the two groups, whereas the comparable glucose values differed, the diabetic to normal glucose ratio being 1.68. This clearly less efficient uptake of glucose in the face of similar

[5] Glucose-U-$^{14}$C, 3.3 μCi/μmole; New England Nuclear Corp., Boston, Mass.

[6] Schleicher and Schuell, Inc., Keene, N. H.

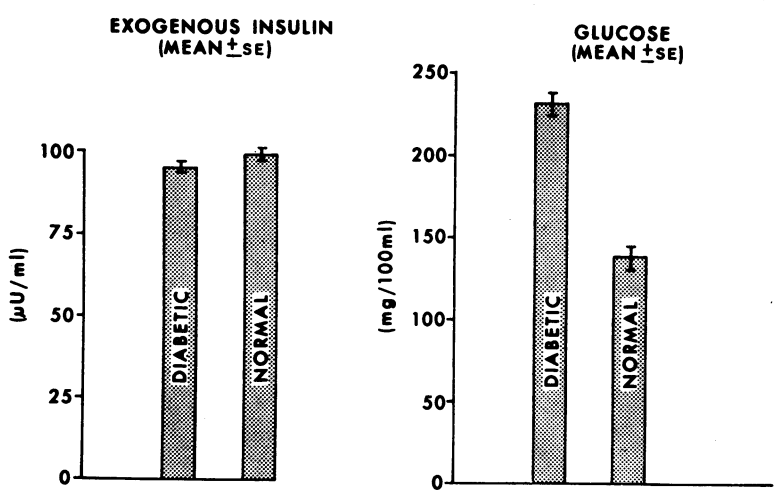


FIGURE 2 Steady-state plasma glucose and insulin concentrations of standard infusion studies in normal and diabetic subjects.

exogenous insulin concentrations demonstrates the greater impedance in the diabetic group. Table II also shows the individual values of plasma insulin, and it is clear that the range was comparable in the two groups. For purposes of simplicity of interpretation we had attempted to achieve identical values for V and for plasma insulin for all subjects; however, small variations occurred due to technical reasons (Table II). The impedance equation (see Methods) automatically corrects for the variations in V. The average impedance value of the diabetic group was 38.1 (range 33.5–44.7), and that of the normal group was 22.9 (range 16.5–26.8).

*Reproducibility of standard infusion test data.* In addition to the constancy of the plasma glucose and insulin levels during a single infusion, it would be desirable if this response were also reproducible in the same subject. To estimate the reproducibility, one subject from each group received the standard infusion on 3 different days. These results are seen in Table III, and they show a close repeatability of steady-state responses in these two subjects.

*Correlations between impedance and insulin and glucose responses to oral glucose.* Table IV shows that individual impedance values were significantly correlated with all but the fasting and 30-min plasma glucose samples of the various times of the glucose tolerance test and to the mean weighted glucose response. Correlations were highest with the latter ($r = 0.92$, $P < 0.001$) and with the 1 hr glucose value ($r = 0.87$, $P < 0.001$) and were lowest with the fasting ($r = 0.03$, N.S.) and with the 30 min glucose ($r = 0.52$, N.S.). In contrast, only the 2 hr insulin value had a significant correlation with impedance ($r = 0.58$, $P < 0.05$) (Table IV).

*Effect of prolonged infusions on impedance.* To determine whether the differences between normal and diabetic subjects persists during longer infusions, the continuous infusion was prolonged to 8 hr from the usual 2½ hr in one subject from each group. These results are seen in Fig. 3. Plasma glucose concentrations were maintained at the same level for the first 4 hr and then they declined slightly over the last 4 hr of the infusion. However, the difference between the two subjects remained constant during the entire 8 hr. If hyperglycemia

TABLE II
*Results of Standard Infusion Studies*

| Subjects | Glucose infusion rate (V) | Plasma glucose* | Plasma insulin* | Impedance‖ |
|---|---|---|---|---|
| | *mg/kg per min* | *mg/100 ml* | *μU/ml* | |
| Diabetics | | | | |
| T. S. | 5.9 | 241 ±4 | 91 ±4 | 40.8 |
| T. W. | 6.4 | 236 ±6 | 107§ | 36.9 |
| C. H. | 6.2 | 211 ±3 | 86 ±5 | 34.0 |
| L. K. | 6.2 | 236 ±5 | 94 ±11 | 38.1 |
| F. R. | 6.0 | 201 ±6 | 90 ±3 | 33.5 |
| E. H. | 6.0 | 231 ±6 | 86 ±5 | 38.5 |
| G. R. | 6.0 | 268 ±11 | 95§ | 44.7 |
| Mean | 6.1 | 232 | 93 | 38.1 |
| Range | 5.9–6.4 | 201–268 | 86–107 | 33.5–44.7 |
| Normals | | | | |
| V. B. | 5.9 | 154 ±4 | 96 ±5 | 26.1 |
| R. K. | 6.0 | 118 ±7 | 112§ | 19.7 |
| H. K. | 5.9 | 158 ±4 | 93§ | 26.8 |
| W. A. | 5.9 | 140 ±6 | 94 ±4 | 23.7 |
| H. M. | 6.2 | 145 ±4 | 90 ±6 | 23.4 |
| D. D. | 6.1 | 149 ±3 | 87§ | 24.4 |
| G. W. | 6.0 | 99 ±4 | 110 ±6 | 16.5 |
| Mean | 6.0 | 138 | 97 | 22.9 |
| Range | 5.9–6.1 | 99–158 | 87–112 | 16.5–26.8 |

* Mean ±SD.
‖ See Methods.
§ Values obtained from pools of individual samples. See Methods.

TABLE III
*Reproducibility of Standard Infusion Studies in Two Subjects*

| Subject | Infusion study | Glucose infusion rate (V) | Insulin* | Glucose* | Impedance‡ |
|---|---|---|---|---|---|
| | | *mg/kg per min* | *μU/ml* | *mg/100 ml* | |
| T. W. | 1 | 5.8 | 90 ±3 | 229 ±8 | 39.5 |
| (Diabetic) | 2 | 6.4 | 107 ±2 | 236 ±6 | 37.0 |
| | 3 | 5.9 | 85 ±2 | 226 ±4 | 38.4 |
| D. D. | 1 | 6.1 | 87 ±4 | 149 ±3 | 24.4 |
| (Normal) | 2 | 6.1 | 96 ±11 | 126 ±3 | 20.6 |
| | 3 | 6.3 | 79 ±10 | 131 ±4 | 20.8 |

* Mean ±SD.
‡ See Methods (Calculations).

TABLE IV

*Correlations between Impedance and Glucose and Insulin Responses to Oral Glucose*

| | Glucose tolerance test times | | | | | Mean weighted glucose response |
|---|---|---|---|---|---|---|
| | Fasting | 30 min | 60 min | 120 min | 180 min | |
| *r* | 0.03 | 0.52 | 0.87 | 0.80 | 0.63 | 0.92 |
| *P* | N.S.* | N.S.* | <0.001 | <0.001 | <0.02 | <0.001 |
| | | | | | | Mean weighted insulin response |
| *r* | 0.16 | 0.04 | 0.21 | 0.58 | 0.52 | 0.48 |
| *P* | N.S.* | N.S.* | N.S.* | <0.05 | N.S.* | N.S.* |

* Not significant at the 5% level.

in patients with maturity onset diabetes is due to previous insulin deficiency, it is apparent that this impairment of glucose uptake was not restored by 8 hr of continuous insulin administration.

*Suppression of endogenous insulin secretion.* The observation that mean steady-state insulin concentrations were virtually identical in the two groups of subjects provides presumptive evidence that endogenous insulin secretion had been inhibited during the infusions. However, to demonstrate the probable extent of inhibition of secretion, the following procedure was performed on one subject from each group. For the first 60 min they were infused with only epinephrine (6 μg/min) and propranolol (0.08 mg/min) in 0.85% saline. This was followed by 120 min of a similar infusion, to which glucose had been added (6 mg/kg of body weight per min). This, in turn, was followed by another 120 min infusion of the usual combination of epinephrine (6 μg/min), propranolol (0.08 mg/min), glucose (6 mg/kg of body weight per min), and insulin (50 mU/min). These results are seen in Fig. 4, and it is apparent that when epinephrine and propranolol were infused simultaneously, immunoreactive insulin concentration fell to less than 50% of the fasting value, confirming the data presented by Porte (36). When glucose was added to the infusion the steady-state insulin concentrations remained low (9 μU/ml and 12 μU/ml) in the two subjects. At the end of this time period corresponding plasma glucose concentrations were 316 mg/100 ml and 398 mg/100 ml, respectively. Therefore, despite extreme hyperglycemia the blockade of insulin release remained effective. When exogenous insulin was added to the infusion fluid (which lowered plasma glucose), the final steady-state insulin concentrations were 105 μU/ml and 115 μU/ml, of which at most 9 μU/ml and 12 μU/ml, respectively, represented endogenous insulin.

*Inhibition of hepatic new glucose production.* Use of plasma glucose concentration as a direct measure of the

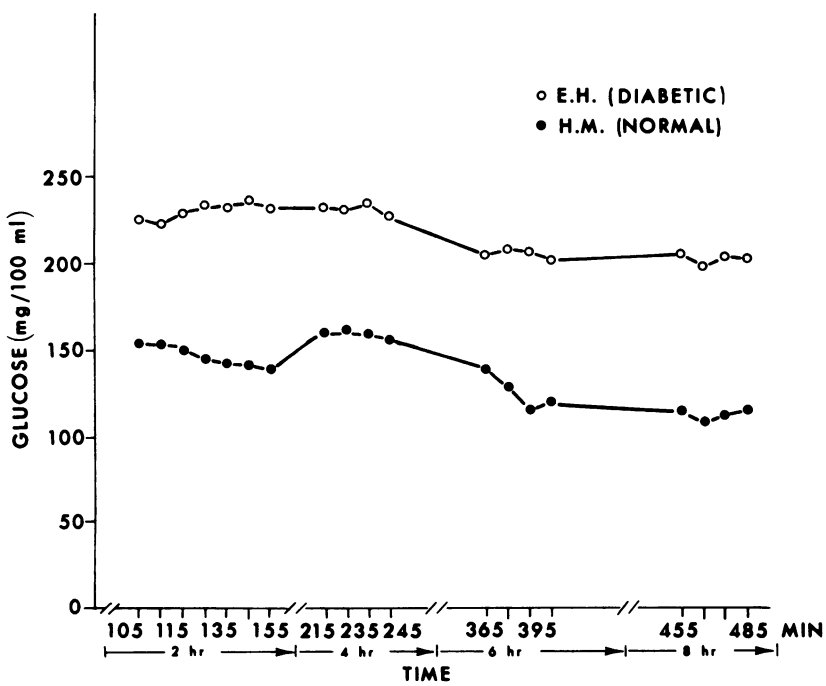


FIGURE 3 Plasma glucose concentrations observed during prolonged infusion studies in one subject from each group.

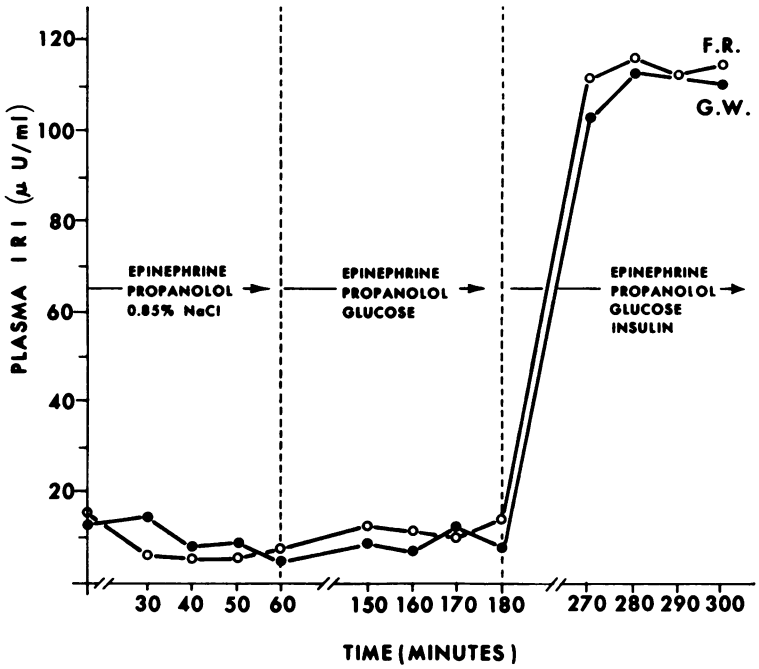


FIGURE 4 Response of plasma insulin in two subjects to different infusion fluids as a test of suppressibility of endogenous insulin. IRI = immunoreactive insulin.

effectiveness of insulin to promote glucose uptake is based on the assumption that the liver (or other potential glucose producing organs, such as the kidney) does not contribute glucose to the plasma during the duration of the experiment. Previous studies in dogs (37) indicate that the combined infusion of glucose and insulin should inhibit hepatic glucose output, but it seemed desirable to confirm this directly especially since epinephrine's action could well overcome the inhibition. In order to do so, the following experiment was performed on one subject from each group. Epinephrine, propranolol, glucose, and insulin were infused as previously described, but in this instance they also received a priming dose of uniformly labeled glucose-$^{14}$C followed by a constant infusion of this radioactive glucose. Data from this experiment permits two independent means of calculating the rate at which glucose is irreversibly removed from the plasma. In the simplest manner, since there was a steady state of plasma glucose concentration, glucose uptake is equal to glucose infusion rate. In a more complicated fashion, by determining the specific activity of the administered tracer glucose, as well as the specific activity of the plasma glucose, irreversible loss rate (uptake rate) of glucose (38) can be calculated by the following formula (39):

$$\frac{\text{ml infused/min} \times \text{dpm/ml}}{\text{dpm/mg of glucose}}$$

The results of calculating glucose irreversible loss rate by these two methods are seen in Table V, and they can be seen to give comparable results. If hepatic glucose output was making a significant contribution to plasma glucose concentration, glucose irreversible loss rate calculated by the constant infusion of isotope would have resulted in a greater value for loss rate. The similarity supports our assumption that hepatic glucose output was inhibited during these experiments and supports the assumption that glucose infusion rate is equal to glucose uptake rate (see Calculations in Methods).

TABLE V
*Comparison of Total Glucose Uptake Calculated by Two Different Methods*

| Subject | Glucose uptake rate | |
|---|---|---|
| | Glucose-$^{14}$C disappearance* | Glucose infusion rate‡ |
| | *mg/min* | *mg/min* |
| H. M. | 428 | 429 |
| F. R. | 419 | 444 |

* Obtained from the mean specific activity of plasma glucose from the six samples of blood drawn every 10 min during the steady-state period of the study.
‡ Obtained from the measured "cold" glucose infusion rate.

## DISCUSSION

This newly developed technique of estimating impedance has allowed us to detect a marked difference in glucose uptake efficiency between normal and mildly diabetic subjects. The following two issues deserve discussion: the validity and meaning of the technique, and the physiological and clinical meaning of the differences in measurement between the two experimental groups. The technique is elaborate, yet safe and reproducible, and it allows use of a human as a "bioassay preparation" where many known variables are eliminated and input rates of hormone and substrate are fixed at known steady-state levels with endogenous sources seemingly nil. This therefore allows the differences in glucose concentration reached (in the face of closely comparable plasma insulin concentrations) to be considered a direct function of differing efficiencies of glucose uptake at the particular insulin concentration employed. Of course, the higher glucose concentration indicates a lower efficiency since uptake rates are equal, and direct inspection allows one to judge the relative efficiencies, for example, as a ratio of any two glucose concentrations. However, ease of comparison is enhanced and understanding of the physiological meaning increased if "efficiencies" are transformed into absolute numbers, accordingly our use of impedance calculated by the method outlined (see Methods). The high correlation of impedance with the mean glucose response and with the 2 hr insulin response suggests that the oral glucose tolerance test (GTT) may be used to obtain an estimate of impedance. It is also of interest that the correlation of impedance was least with the fasting and 30-min glucose samples than of all time periods of the oral GTT, suggesting that the early time periods may not be as representative of the tissue factors responsible for glucose intolerance as are the later times of an oral GTT.

One must be concerned about the possibility that the pharmacologic intervention of the infusion (epinephrine, propranolol, insulin, and glucose) introduces more unknown variables than it eliminates. We believe that this seemingly elaborate array of infusands was essential to the research design for the following reasons.

First, the primary goal of these studies was to diminish the importance of endogenous insulin and to consider only a constant level of exogenous insulin in any comparison of glucose uptake efficiency. In prior attempts to study this question by use of prolonged glucose infusions (7, 10), one still had to consider the uncertainty that endogenous insulin could vary in biological effectiveness. Also, during these earlier infusion studies plasma insulins sought their own level, and these levels varied (10). Thus, a second variable was present. However, the propranolol-epinephrine method of endogenous insulin inhibition devised by Porte (36) allowed us to achieve our primary goal (Figs. 2 and 4).

Secondly, we wished glucose uptake rate to equal glucose infusion rate. This was achieved by inhibiting endogenous new glucose production (Table IV) and by achieving a steady state of glucose concentration (Figs. 2 and 3). Exogenous insulin (37), exogenous glucose (40), and possibly propranolol inhibited endogenous hepatic glucose output. The use of propranolol for this purpose could be exerted through its beta-adrenergic effects which would prevent the anticipated hepatic glycogenolytic effect of epinephrine (41).

Thirdly, we wished to achieve glucose and insulin concentrations that mimicked those commonly found in the postprandial state (3). This was also achieved (Fig. 2). Other questions remain; for example, are there important cardiovascular effects? The patients had cardiac slowing and slightly raised systolic blood pressures. Therefore, it is possible that altered regional blood flow in skin, muscle, and splanchnic areas resulted from the net action of these two agents, though it is unlikely that splanchnic adipose tissue was appreciably deprived of its blood supply since Porte reported no effect of identical doses of epinephrine on splanchnic blood flow (36). Our final defense rests on our view that all the changes were systematic and were shared by both patient groups, and even if due to some subtle difference in their response to the high carbohydrate diet or to one of the infusands, this elusive difference will in itself be worthy of study.

It remains then to consider the possible physiological and clinical implications of our work. There now exists a spirited yet confusing set of arguments about the cause of diabetes. These arguments or disagreements seem to induce polarized views of the cause of hyperglycemia in conditions where plasma insulin is present in varying amounts. According to our results, hyperglycemia (at least in some "latent" diabetics) cannot be entirely caused by insulin deficiency, and therefore, these results support the idea that the tissues of these patients resist glucose uptake. We also give a quantitative estimate of the degree of the factor (impedance) which we believe is an important cause of these latent diabetics' hyperglycemia. Perhaps use of this method will shed more light on other unanswered questions. For example, does the juvenile insulin-dependent diabetic also have a high impedance? Or, is impedance increased by weight gain, or reduced by weight loss, and is it correlated with the degree of glucose intolerance in more severely diabetic individuals? Some of these questions are now under study.

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