Study of the Relationship Between Plasma Insulin Concentration and Efficiency of Glucose Uptake in Normal and Mildly Diabetic Subjects

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SUMMARY

Removal of glucose-U-C-14 from plasma was studied in normal subjects and in patients with maturity-onset diabetes mellitus. All measurements were made after an overnight fast, during a period in which plasma glucose and immunoreactive insulin concentrations were not changing. The mean irreversible loss rate (ILR) of glucose from the plasma of normal subjects was not different from that of patients with diabetes: mean ILR (± the coefficient of variation) of all patients was 1.36 (± 14 per cent) mg. glucose/kg. body weight per min. This narrow range attests to the similarity of ILR in the patients studied. There was no correlation between glucose ILR and plasma glucose concentration, plasma immunoreactive insulin concentration, or ponderal index. In contrast, fractional glucose irreversible loss rate varied considerably from patient to patient, with a coefficient of variation of 35 per cent. Furthermore, there was a strong negative correlation between plasma glucose concentration and fractional glucose irreversible loss rate ($r = -0.82$, $p < .01$). This decrease in efficiency of glucose removal from plasma of hyperglycemic patients was not related to an absolute lack of insulin. It is concluded that net glucose utilization in the fasting state is similar in normal and mildly diabetic patients, and that fasting hyperglycemia is due to a decrease in efficiency of glucose removal which cannot be attributed to absolute insulin deficiency. DIABETES *19*:571-78, August, 1970.

In 1937 Soskin and Levine pointed out that "when one compares the rate of utilization of the normal animal at its usual normal blood sugar level with the rate of utilization of the diabetic animal at the hyperglycemic levels which it ordinarily maintains, it is apparent that the diabetic animal habitually uses as much or more sugar than the normal animal."[1] With assumption of the validity of this conclusion, it follows that fasting hyperglycemia in the diabetic patient results from a decrease in the efficiency with which glucose is removed from plasma. Furthermore, if hyperglycemia is a direct function of insulin lack, the decrease in efficiency of glucose uptake in the diabetic should be accompanied by a concomitant decrease in plasma insulin concentration.

The relationship between hyperglycemia and insulin secretion usually has been studied by measuring the plasma glucose and insulin response to an acute glucose load. The difficulties inherent in interpretation of data in which two variables are undergoing fluctuation under nonsteady state conditions is exemplified by the current controversy over the role of insulin lack in the genesis of diabetes.[2-6] Conclusions drawn from such studies are complicated by the fact that plasma insulin levels only reflect rates of endogenous insulin secretion under steady state conditions.[7] In an effort to avoid these problems, we have previously studied the steady state plasma insulin response to a continuous intravenous glucose infusion.[8] The results obtained supported the thesis that impedance to insulin-mediated glucose transport was a factor in the hyperglycemia of patients with mild maturity-onset diabetes. Since both plasma insulin and glucose were permitted to rise independently to their steady state concentrations, however, interpretation of the results could be obscured by debate over the "appropriateness" of any given insulin response when it is related to the inciting glycemic stimulus.

Previously we had become aware of the fact that fasting plasma insulin concentrations of patients with mild diabetes were comparable to those of subjects with normal glucose tolerance. In order to take experimental advantage of this fortuitous similarity, we have studied the relationship between plasma insulin concentration and both the total amount of glucose irreversibly removed from plasma (ILR) and the efficiency of this

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TABLE 1

Clinical characteristics

Patient	Age	Sex	Ht. (cm.)	Wt. (kg.)	PI*	Glucose tolerance (mg./100 ml.)†			
						0	1	2	3
W.W.	56	M	169	65.7	12.67	71	122	84	68
B.K.	39	F	170	73.1	12.31	73	133	98	74
J.H.	50	M	180	79.8	12.65	88	106	106	92
F.B.	59	M	174	86.9	11.86	104	178	109	85
G.B.	53	M	171	74.4	12.31	73	166	116	66
D.H.	51	F	157	67.6	11.69	76	173	131	110
R.D.	45	M	173	97.3	11.37	136	193	250	224
C.D.	56	M	172	63.5	13.08	152	350	270	174
E.A.	61	F	155	58.7	12.06	123	276	292	246
F.H.	68	M	172	78.9	12.14	148	236	336	244
J.B.	54	M	169	62.2	12.90	150	312	380	368
H.H.	68	M	182	63.1	13.81	182	341	412	384

*Ponderal index $= \frac{\text{height (inches)}}{\sqrt[3]{\text{weight (pounds)}}}$

†Plasma glucose concentration; patients arranged in order of increasing two-hour glucose values.

process (fractional ILR) in normal and diabetic subjects after an overnight fast. No significant differences were observed in the amount of glucose irreversibly lost from plasma when normal subjects were compared to patients with maturity-onset diabetes. As predicted by Soskin and Levine[1] the efficiency of this process (glucose fractional ILR) was decreased in diabetic subjects, and the more hyperglycemic, the lower the fractional removal rate of glucose. Moreover, patients with hyperglycemia (and decreased fractional rates of glucose removal) did not have insulin levels lower than normal, indicating the hyperglycemia in these patients could not be attributed to insulin lack.

MATERIAL AND METHODS

Experimental protocol

Patients were selected in order to provide a population with a relatively continuous spectrum of fasting plasma glucose concentrations ranging from approximately 75 to 175 mg./100 ml. None had ever received insulin or oral antihyperglycemic therapy, and they were all in good general health. The age, sex, height, weight, and an estimate of relative weight (ponderal index) of each patient are given in table 1.

All patients were hospitalized for the entire study. Upon admission they were placed on an isocaloric, liquid formula diet (control diet) in which 15 per cent of calories were derived from protein, 43 per cent from carbohydrates, and 42 per cent from fat.[8] After three days of hospitalization, the plasma glucose response to seven ounces of a synthetic carbohydrate beverage* was determined in each patient. The results of these oral glucose tolerance tests are seen in table 1, and indicate that the experimental population could be considered to consist of six normal subjects and six patients with maturity-onset diabetes mellitus.[9]

Disappearance rates of glucose-C-14 were determined in each patient after at least one week of hospitalization. These studies were started after an overnight fast of fifteen hours. Patients received 25 μc. of glucose-U-C-14† by rapid intravenous injection, and arterial blood was withdrawn through a polyethylene cannula (placed in the brachial artery the night before) 2, 4, 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 210 and 240 minutes after injection. In two diabetic subjects (C. D. and H. H.) glycosuria invalidated determination of plasma glucose-C-14 disappearance when studied on the control diet. In these, and certain other patients, measurement of disappearance rate was repeated after three weeks of either an isocaloric high fat diet (per cent of calories = 68 per cent fat:17 per cent carbohydrate:15 per cent protein) or high carbohydrate diet (per cent of calories = 85 per cent of carbohydrate:15 per cent protein). In three patients studies were performed after all the test diets.

Analytical procedures

Blood was withdrawn into tubes containing EDTA. Plasma, separated in a refrigerated centrifuge, was divided into appropriate aliquots for measurement of plasma glucose concentration, plasma insulin concentration, and determination of glucose specific activity. These aliquots were immediately frozen in acetone-dry

*Glucola, Ames Co., Inc., Elkart, Indiana.

†Glucose-U-C-14, 3.3 mc./mmole, New England Nuclear Corp., Boston, Mass.

ice, and stored at —20° C. until analyzed. Plasma glucose was measured by means of a Technicon Auto-Analyzer[10] on all blood samples obtained, while plasma immunoreactive insulin was determined by the method of Hales and Randle[11] on the fasting, 60, 120, 180 and 240-minute specimens. Samples were prepared for determination of glucose specific activity by the method of Nikkilä and Ojala[12] with a modification of the initial extraction steps as follows: 19 ml. of chloroform:-methanol (2:1) were added to 1 ml. of plasma, the mixture was heated with tap water to coagulate the protein, then filtered through Sharkskin paper. Three milliliters of water was added to 15 ml. of the ensuing filtrate, the mixture shaken carefully, centrifuged for ten minutes at 2,000 RPM, and 4.8 ml. of the resultant upper phase removed (equivalent of 0.5 ml. of original plasma). The extract was then carried through the separation steps outlined by Nikkilä and Ojala. One aliquot was taken for measurement of glucose concentration, while another was dissolved in Bray's solution and counted in a Packard 4,000 liquid scintillation counter.

Analysis of data

The disappearance curves of glucose-C-14 from plasma were all curvilinear when plotted on semilog paper (figure 1), indicating that a multicompartmental system exists for removal of glucose from plasma. Curvilinearity results because radioactive material, before it is irreversibly lost from plasma, enters one or more extraplasma compartments, which exchange with the plasma pool. These nonplasma compartments are unlabeled at the onset of the experiment. Gradually they become labeled resulting in recycling of some of the radioactive material back into the plasma. Therefore, the resulting disappearance curves cannot be analyzed by methods based on the assumption that labeled glucose is removed from a single well mixed pool by a first order process.[7] Using procedures developed previously,[13] we found that a minimum of three exponentials was necessary to describe the curves. The mathematical description is given by the following formula:

Glucose specific activity (DPM/mg. glucose) $= k_1 e^{-g_1 t} + k_2 e^{-g_2 t} + k_3 e^{-g_3 t}$. The k_i and g_i ($i = 1, 2, 3$) were obtained by the method of peeling and these values were optimized by a nonlinear least square procedure.[13] The data were normalized into units of fraction of dose by dividing each data point by $k_1 + k_2 + k_3$, and the resulting curves are described by:

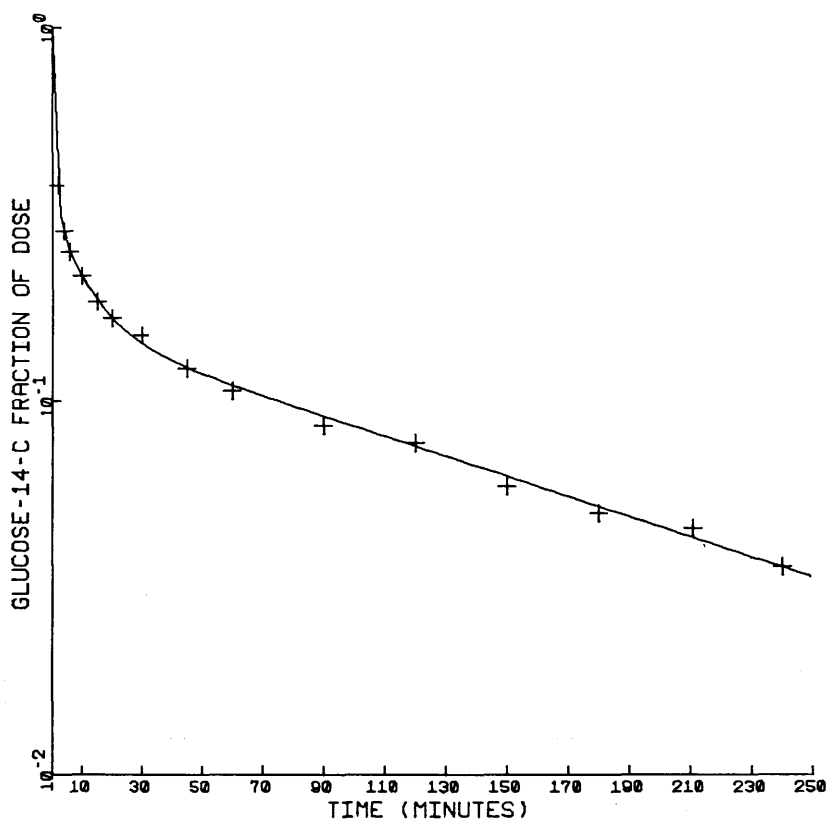

FIG. 1. A typical disappearance curve of glucose-C-14 from plasma. The +'s are the data points, and the continuous curve is the calculated curve describing the data. This is a photograph of the data and curve as displayed on a cathode ray tube.

Fraction of dose $= h_1e^{-g_1t} + h_2e^{-g_2t} + h_3e^{-g_3t}$ where $h_i = \frac{k_i}{k_1 + k_2 + k_3}$.

The fractional irreversible loss rate (λ_e) was obtained from the formula:

$$\lambda_e = \frac{1}{\frac{h_1}{g_1} + \frac{h_2}{g_2} + \frac{h_3}{g_3}} \quad 14.$$

The irreversible loss rate (ILR) of glucose from plasma per minute was calculated by multiplying the plasma pool size (Q) of glucose, calculated on the basis that plasma volume is equal to 4.5 per cent of body weight, by λ_e. The term irreversible loss rate was initially introduced by Baker, Shipley, Clark and Incefy[14] and we have chosen to continue its use as we feel it is the most explicit designation for the phenomenon being studied.

Spearman rank correlation coefficients (r) and regression lines were obtained from a standard computer program, and used to evaluate the statistical significance of the relationship between measures of glucose loss rate and other variables known to modify glucose uptake. Values for estimation of the coefficient of variation, as well as p values for the *t* test, were also obtained from standard computer programs.

RESULTS

1. *Plasma glucose and insulin concentration*

Mean plasma steady state glucose and insulin values for each study are listed in table 2, and the constancy of these levels for any patient during the four hours of the experiment is attested to by the small standard deviations of the individual means. Plasma glucose concentrations ranged from 72 to 147 mg./100 ml., while plasma immunoreactive insulin levels varied from 13 to 26 μU./ml. The mean (± S.E.M.) of the steady state plasma glucose concentrations of the six patients with normal oral glucose tolerance was 92 ± 4 mg./100 ml., whereas the mean (± S.E.M.) steady state glucose level was 117 ± 7 mg./100 ml. for the diabetic subjects. The means for the two patient groups were significantly different at the 5 per cent level. In contrast, there was no significant difference between the mean (± S.E.M.) fasting plasma insulin levels of normal (19 ± 2 μU./ml.) and diabetic patients (20 ± 1 μU./ml.). (In patients studied on more than one occasion the average of the values for glucose and insulin concentration from all studies were used for the above comparisons.)

2. *Fractional irreversible loss rate* (λ_e)

The mean value of λ_e for all studies (table 2) was

TABLE 2

Results of steady state measurements

Patient	Diet*	Plasma glucose Mean±S.D. mg./100 ml.	Plasma insulin Mean±S.D. μU./ml.	$\lambda_e \times 10^3$†	ILR‡
W.W.	CF	72± 1.6	13±1.3	.805	1.72
	HF	75± 2.2	13±1.7	.648	1.42
	HC	72± 4.3	14±1.8	.672	1.30
B.K.	CF	100± 4.8	25±2.3	.458	1.51
J.H.	CF	101± 2.5	16±1.5	.319	1.16
F.B.	CF	95± 1.8	22±5.1	.428	1.57
G.B.	CF	85± 1.4	15±1.6	.559	1.58
	CF	96± 5.9	17±1.5	.358	1.15
	CF	94± 2.2	20±4.8	.361	1.22
D.H.	CF	88± 3.2	19±1.3	.475	1.26
R.D.	CF	116± 8.9	26±3.7	.218	1.11
	HF	108± 4.1	23±5.5	.350	1.66
	HC	87± 2.5	24±4.0	.377	1.42
C.D.	HF	98± 4.8	17±1.5	.446	1.25
E.A.	CF	107± 3.7	20±1.9	.504	1.43
F.H.	CF	115± 5.6	25±3.7	.318	1.30
	HF	120±10.6	21±0.8	.271	1.20
	HC	121± 7.0	20±1.5	.385	1.65
J.B.	CF	144±10.8	18±2.4	.308	1.24
	HC	147± 3.7	21±4.6	.295	1.26
H.H.	HF	127± 9.4	18±1.6	.308	1.11

*Diet: CF = control formula, HF = high fat, HC = high carbohydrate.

†Units of λ_e = (1/min.) per kg. body weight.

‡Units of ILR = mg. of glucose/kg. body weight per minute.

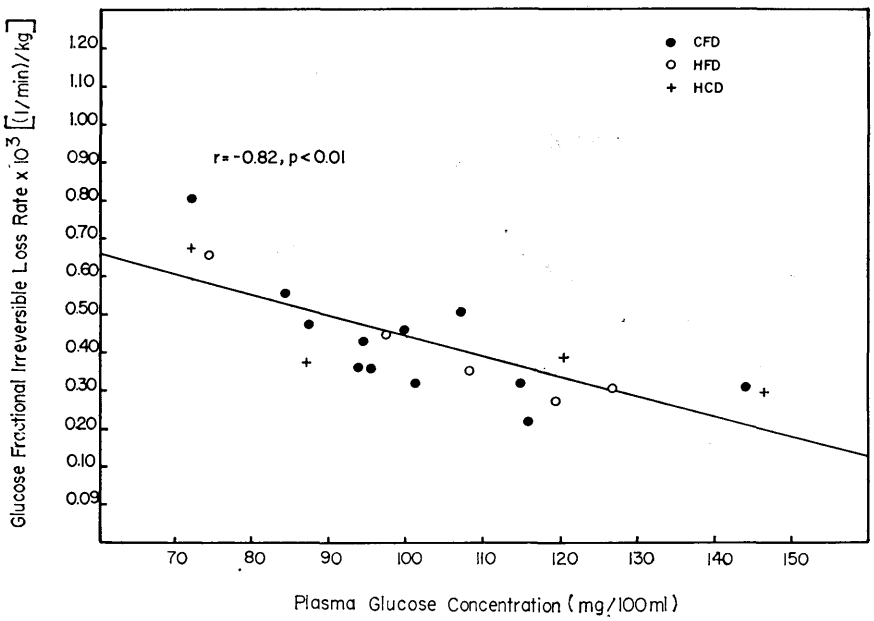

FIG. 2. Relationship between plasma glucose concentration and the efficiency with which glucose is removed from plasma (glucose fractional irreversible loss rate). The best fit line, correlation coefficient and value for p were obtained from standard computer programs. The initials CFD, HFD and HCD refer to antecedent diet, e.g., control formula, high fat and high carbohydrate diet respectively.

0.422×10^{-3}, with a 35 per cent coefficient of variation. The relationship between plasma glucose concentration and fractional rate of glucose removal from plasma is seen in figure 2, in which plasma glucose concentration and λ_e are shown to have a significant negative correlation ($r = -0.82$, $p < 0.01$). In other words, the degree of hyperglycemia is directly proportional to the decrease in the efficiency with which glucose is removed from plasma. A negative correlation was also found between plasma immunoreactive insulin concentration and λ_e ($r = -0.53$), and this relationship is illustrated in figure 3. This correlation just reached statistical significance ($p < 0.05$), and, in view of both the narrow range of insulin concentrations and the limited number of observations, we are reluctant to conclude that a decrease in efficiency of glucose uptake implies an *increase* in plasma insulin concentration. However, these data do seem to point out unequivocally that impaired efficiency of plasma glucose removal in diabetic subjects cannot be attributed to a *decrease*

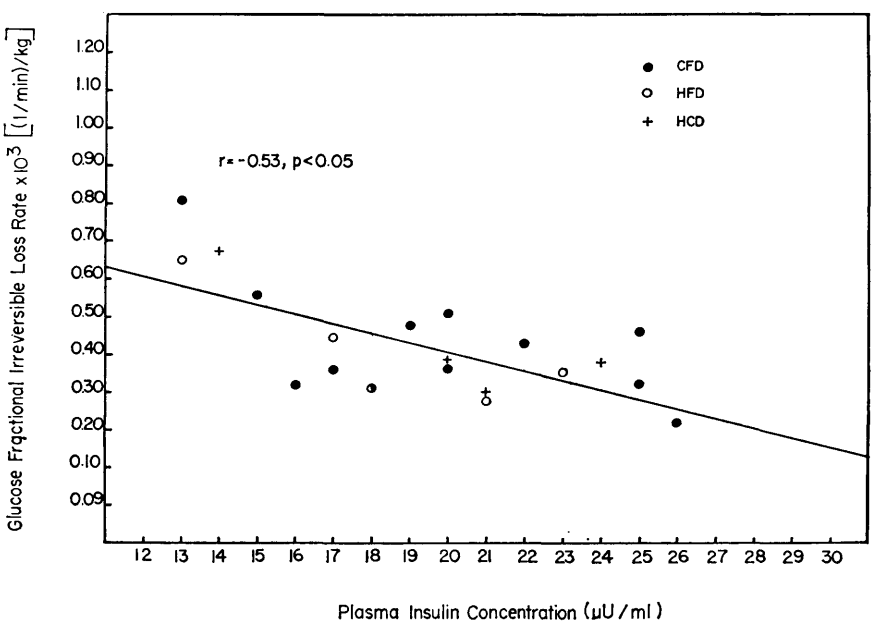

FIG. 3. Relationship between plasma immunoreactive insulin concentration and glucose fractional irreversible loss rate. The best fit line, correlation coefficient and value for p were obtained from standard computer programs. The initials CFD, HFD and HCD refer to antecedent diet, e.g., control formula, high fat and high carbohydrate diet respectively.

in plasma insulin concentration. Finally, no significant correlation was observed between λ_e and ponderal index ($r = 0.13$).

3. *Irreversible loss rate (ILR)*

Estimates of plasma glucose ILR are obtained by multiplying λ_e by the size of the plasma glucose pool, and these data also appear in table 2. Since λ_e was found to be inversely proportional to plasma glucose concentration, ILR should be essentially similar for all patients. Mean ILR for all studies was 1.36 mg. of glucose removed from plasma/kg. body weight per minute, with a 14 per cent coefficient of variation. The relatively small degree of variability from patient to patient supports the view that net glucose uptake is very similar in normal and nonketotic diabetic patients, and provides additional experimental support for a similar conclusion reached by Shreeve[15] in a recent review article. The values for plasma glucose ILR, which are listed in table 2, are somewhat lower than previous[16-18] estimates of "glucose turnover rate" derived from investigation of similar patients. This difference is most likely related to the fact that earlier studies were analyzed on the assumption of a single well-mixed glucose pool, a method of analysis which would tend to overestimate removal rate.[7] The relationship between plasma glucose concentration and ILR illustrated in figure 4 demonstrates the lack of significant correlation ($r = -0.35$) between these variables, and also suggests that antecedent diet does not affect ILR. Similarly, no significant correlation was found between ILR and either plasma insulin level ($r = 0.02$) or ponderal index ($r = 0.16$). However, it should be noted that ponderal index only varied within a small range, and the lack of correlation of ponderal index with λ_e and ILR does not really test the effect of obesity on these estimates of glucose uptake.

DISCUSSION

The experimental results provide two distinct ways of comparing the ability of various subjects to remove glucose from plasma. The first is to consider only the total amount of glucose irreversibly removed from plasma during the four-hour experimental period. These experiments were carried out in a steady state, and since patients with glycosuria were excluded, the irreversible loss rate of glucose from plasma must be equal to the amount of glucose irreversibly taken up by all tissues utilizing glucose. The results indicate that irreversible tissue uptake of glucose occurred at a relatively similar rate in all patients studied, and that this rate was independent of plasma glucose and immunoreactive insulin concentrations. As such, these results support the original contention of Soskin and Levine[1] that glucose utilization is similar in normal and diabetic subjects.

A second way to view glucose removal from plasma is to consider the efficiency of this process, and the fractional irreversible loss rate of glucose from plasma is such a measure. In contrast to the above, there was

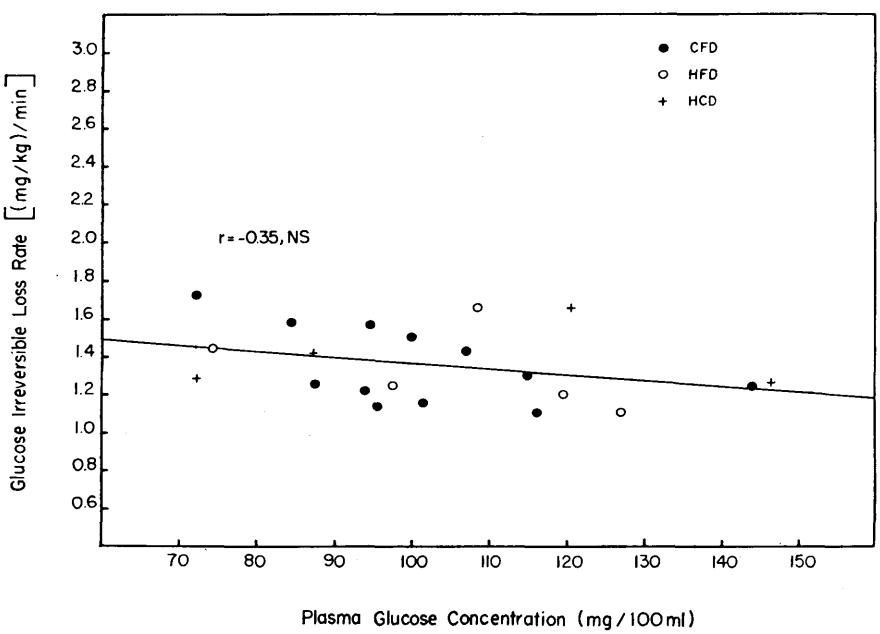

FIG. 4. Relationship between plasma glucose concentration and glucose irreversible loss rate. The best fit line, correlation coefficient and value for p were obtained from standard computer programs. The initials CFD, HFD and HCD refer to antecedent diet, e.g., control formula, high fat and high carbohydrate diet respectively.

a highly significant correlation between fractional irreversible loss rate of glucose from plasma and degree of hyperglycemia. The correlation was a negative one, indicating that decreases in efficiency of glucose uptake were associated with commensurate increases in fasting plasma glucose concentration. Since fractional irreversible loss rates of glucose from plasma were not correlated with insulin lack, these results indicate that hyperglycemia results from a decrease in relative efficiency of insulin-mediated glucose transport.

These results bring certain questions into focus concerning glucose homeostasis. Diabetic and normal subjects maintained comparable rates of irreversible glucose removal, in spite of the diabetic's decreased efficiency of glucose removal. This rate was maintained at the expense of fasting hyperglycemia, and the apparent paradox of patients who can increase insulin secretion but do not respond to the challenge of fasting hyperglycemia has also been recently commented upon by another group of investigators utilizing an entirely different experimental approach.[19] It appears as if preservation of fasting euglycemia is assigned a relatively low priority; the organism seems more concerned about preserving a certain rate of glucose utilization, and it prefers to accomplish this by permitting plasma glucose concentration to rise instead of increasing the rate of insulin secretion. Alternatively, the rate of insulin secretion might be dependent upon the rate of pancreatic glucose uptake and utilization[20-22] and the defect in efficiency of glucose removal in diabetes shared by the pancreas. Consequently, an initial decrease in efficiency of glucose uptake could result in a decrease in rate of insulin secretion. As plasma glucose concentration increased, pancreatic glucose utilization and insulin secretion would also rise. Eventually, plasma glucose and insulin concentrations would stabilize at levels that permit the desired rate of net glucose utilization. These issues are clearly not answered by the current experiments, and are only raised as a means of underlining the current lack of knowledge of factors controlling glucose and insulin homeostasis.

These results have obvious relevance to the current controversy over the role of insulin lack in the etiology of maturity-onset diabetes. The fasting plasma glucose concentrations of the patients studied ranged from 72 mg./100 ml. to 147 mg./100 ml. and the degree of fasting hyperglycemia was highly correlated with concomitant decreases in the efficiency with which glucose was removed from plasma. Decreases in glucose fractional irreversible loss rate were not associated with lower plasma immunoreactive insulin concentrations, and this observation makes it difficult to attribute the hyperglycemia in the patients studied to an absolute lack of insulin. This conclusion is based upon the premise that plasma immunoreactive insulin levels were qualitatively, as well as quantitatively, similar in all patients studied. The validity of this assumption must be questioned following the identification[23,24] of a substance (or substances) which reacts with anti-insulin antibody, but which appears to have limited biological activity. Thus, it is possible that the decrease in glucose fractional irreversible loss rates seen in patients with hyperglycemia might be due to the secretion of an abnormal form of insulin. However, other data from our laboratory,[25] in which endogenous insulin secretion was inhibited by epinephrine, indicate that patients with mild maturity-onset diabetes are more resistant than normal subjects to the effects of similar levels of exogenous insulin on efficiency of in vivo glucose uptake. These results seem to question the significance of biologically ineffectual insulin in the pathogenesis of diabetes but it is clear that this possibility cannot be excluded at present and requires further clarification.

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