Glucose clamp technique: a method for quantifying insulin secretion and resistance

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DeFronzo, Ralph A., Jordan D. Tobin, and Reubin Andres. *Glucose clamp technique: a method for quantifying insulin secretion and resistance.* Am. J. Physiol. 237(3): E214–E223, 1979 or Am. J. Physiol.: Endocrinol. Metab. Gastrointest. Physiol. 6(3): E214–E223, 1979.—Methods for the quantification of beta-cell sensitivity to glucose (hyperglycemic clamp technique) and of tissue sensitivity to insulin (euglycemic insulin clamp technique) are described. *Hyperglycemic clamp technique.* The plasma glucose concentration is acutely raised to 125 mg/dl above basal levels by a priming infusion of glucose. The desired hyperglycemic plateau is subsequently maintained by adjustment of a variable glucose infusion, based on the negative feedback principle. Because the plasma glucose concentration is held constant, the glucose infusion rate is an index of glucose metabolism. Under these conditions of constant hyperglycemia, the plasma insulin response is biphasic with an early burst of insulin release during the first 6 min followed by a gradually progressive increase in plasma insulin concentration. *Euglycemic insulin clamp technique.* The plasma insulin concentration is acutely raised and maintained at approximately 100 μU/ml by a prime-continuous infusion of insulin. The plasma glucose concentration is held constant at basal levels by a variable glucose infusion using the negative feedback principle. Under these steady-state conditions of euglycemia, the glucose infusion rate equals glucose uptake by all the tissues in the body and is therefore a measure of tissue sensitivity to exogenous insulin.

tissue sensitivity to insulin; beta-cell response to glucose

A COMPREHENSIVE UNDERSTANDING of the mechanism of disorders of glucose homeostasis in man requires the assessment of multiple physiological variables. Among the primary variables are: *1*) beta-cell response to glucose and *2*) sensitivity of body tissues to insulin. There have been attempts to derive these variables from data obtained on glucose tolerance tests. On the oral version of the test, plasma glucose concentration rises and then falls; mean glucose curves derived from large populations are quite smooth, but curves on individuals are commonly erratic. Plasma insulin concentration curves generally follow a similar but lagging time course. In order to relate the response of the beta-cell (as assessed by changes in plasma insulin concentration) to the ever-changing stimulus of the plasma glucose concentration, the use of an "insulinogenic index" has been proposed and widely used (11, 15). The index is computed from the ratio of the insulin-to-glucose (I/G) concentrations;

at times the absolute I and G concentrations are used, but the increases in these concentrations over basal levels have also been used. The use of this index carries the assumptions that *1*) at any moment the plasma insulin concentration reflects the response to that moment's plasma glucose concentration and *2*) this dose-response relationship is a simple linear one.

The same data (plasma glucose and insulin values) have been used to compute an index of tissue sensitivity to insulin, the G/I ratio. The problem in using such a ratio results from the feedback loop relating these two variables and from the fact that neither variable is held constant. Thus, a rise in plasma glucose concentration stimulates the beta-cell release of insulin; the resultant rise in plasma insulin concentration stimulates the cellular uptake of glucose, and the plasma glucose concentration falls. These processes are, however, not sequential, as described above, but occur simultaneously.

Studies of these two processes in vitro are not subject to these problems. The excised pancreas or isolated islet preparation can be perfused at fixed hyperglycemic levels, and individual tissues can be perfused at fixed insulin concentrations. It was with this type of control in mind that we had developed the glucose clamp technique.[1] The technique places the plasma glucose concentration under the investigator's control and thus breaks the simple glucose-insulin feedback loop. Both beta-cell sensitivity to glucose and tissue sensitivity to insulin can be quantified. We have previously presented a very early version of the clamp technique (2) with some experimental studies using the method (2, 8, 17). This study delineates the technical details for the accomplishment of a square-wave hyperglycemia study and the maintenance of euglycemia during an insulin-infusion study.

METHODS

Hyperglycemic clamp

Subjects. Eleven healthy volunteers (7 males and 4 females) ranging in age from 21–45 (mean, 29 ± 2) yr were studied. All were within 10% of desirable body weight and had a normal oral glucose tolerance test prior to study. Subjects consumed a weight-maintaining diet

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[1] This name was suggested by Dr. Joseph J. Blum of the Department of Physiology, Duke University, who pointed out the analogy to the voltage clamp technique.

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containing at least 200 g of carbohydrate per day for 3 days prior to study. None were taking any medications, and there was no family history of diabetes mellitus. All studies were performed at 8 A.M. after a 12-h overnight fast. A small polyethylene catheter was inserted under local Xylocaine anesthesia into a brachial artery for blood sampling. A second catheter was inserted into an antecubital vein for infusion of 20% dextrose in water. After a stabilization period of 45–60 min, studies were performed as described below. *Subjects 1–6* were restudied 3–4 wk later to evaluate the reproducibility of the hyperglycemic clamp technique. Informed, written voluntary consent was obtained from all subjects prior to their participation in the study.

Glucose infusion. The goal of the hyperglycemic clamp is to raise the plasma glucose concentration acutely to a fixed hyperglycemic plateau and to maintain it at that level for 2 h. This is accomplished by an intravenous glucose infusion consisting of two phases: *1*) a 15-min "priming dose" needed to raise the glucose level in plasma and in extravascular glucose compartments to the desired plateau and (2) a "maintenance dose" that is computed at 5-min intervals throughout the study.

PRIMING DOSE (TABLE 1). The pattern of the priming dose was determined through trial and error and is empirically set as a constant for all subjects. The dose is computed per square meter body surface area. The total glucose priming dose necessary to raise the plasma glucose concentration by 125 mg/dl in the first 14 min is 9,622 mg/m^2. This is equivalent to approximately 240 mg/kg body wt in normal weight individuals or nearly 2 mg/kg for each mg/dl increase in plasma glucose.

MAINTENANCE DOSE. The computation for the periodic adjustments in the glucose infusion is made every 5 min and is based on the negative feedback principle: if the actual glucose concentration is higher than the goal, the infusion is decreased and vice versa. The computation has two components that, for convenience, have been designated as a "volume" component and a "metabolic" component. Consider the situation during the study in which the actual level falls to 10 mg/dl below the goal. The uptake of glucose by tissues thus was greater than the glucose infusion preceding the blood collection, and an increase in the glucose infusion rate is called for. In addition to this increase in metabolic need, however, a quantum of glucose, the volume component, is required in order to bring the glucose in the total glucose compartment up to the desired goal. The requirements of the formula to carry out the needed adjustment are that *1*) the increase not be so large as to cause a serious overshoot because such a formula would lead, in turn, to subsequent undershoot with the development of increasing harmonic deviations and *2*) the increase not be so small that the formula does not allow a rapid enough adjustment in infusion rate to keep up with changing metabolic requirements.

The formula for computing the periodically adjusted infusion rate is

$$S_i = SV_i + SM_i$$

where S_i is the setting of the infusion pump at time i; SV_i, that portion of the setting needed for the volume component; and SM_i, that portion of the setting needed for the metabolic component.

In order to accomplish these periodic adjustments as rapidly as possible, the infusion rate is computed in terms of the actual dial setting on the infusion apparatus. The computations are based on the use of a 20% dextrose in water infusate via a calibrated infusion pump (Harvard Instrument Co., Millis, MA). The factor for the conversion of the infusion rate (ml/min) to a dial setting is designated as PF, the infusion pump factor.

1) Volume component, SV_i.

$$SV_i = \frac{(G_d - G_i) \times 10 \times (0.19 \times \text{body wt})}{G_{\text{inf}} \times 15} \times PF$$

The individual components of this formula are: $(G_d - G_i)$ $\times 10 \times (0.19 \times \text{body wt})$ equals the total body glucose deficit or excess in milligrams where G_d is the desired plasma glucose concentration (mg/dl); G_i, the actual plasma glucose concentration at any time, i; the multiplication by 10 converts plasma glucose concentration from milligrams per deciliter to milligrams per liter; 0.19 $\times$ body wt (in kg) is the glucose space in liters; G_{inf} is the glucose concentration in the infusate in milligrams per milliliter and converts the glucose dose from milligrams to milliliters of infusate.

The division by 15 is based on a simple empiric decision to carry out the correction for the volume component over a 15-min period because the intravenously infused glucose required time for distribution in the total glucose space. The division by 15 converts the computed infusion rate from milliliters to milliliters per minute. Note that the volume correction is never entirely carried out because the computed glucose infusion rate will only be used for a 5-min period; in 5 min a new plasma glucose concentration becomes available, and a revised computation replaces the previous one.

The multiplication by PF, the infusion pump factor, converts the infusion rate in milliliters per minute to that portion of the final dial setting needed for the volume component.

The volume factor will be negative if the actual glucose concentration is greater than the desired level, that is, when $G_d - G_i$ is negative. Such a negative volume factor has the effect of allowing a portion of the glucose required for metabolic needs to be removed from the glucose space so that the actual glucose concentration will fall toward the desired goal.

2) Metabolic component, SM_i. The setting for the metabolic component of the infusion rate is calculated as an iterative procedure.

$$SM_i = SM_{i-2} \times FM_i \times FM_{i-1}$$

where SM_{i-2} is the metabolic component calculated two iterations (10 min) previously, and

$$FM_i = (G_d - G_b)/(G_i - G_b)$$

where G_b is the basal glucose concentration; G_d, the desired glucose concentration; G_i, the glucose concentration at any time, i. FM_i is a dimensionless correction factor that compensates for the "error" in the plasma glucose concentration (the distance from the goal). FM_{i-1}

is the FM_i calculated one iteration (5 min) previously. The need for the use of FM_{i-1} factor is empiric. In the hyperglycemic clamp, as will be shown in RESULTS, despite the creation of a square wave of hyperglycemia, there is a continuously increasing glucose infusion requirement during the 2-h study period. In many subjects, in whom this increasing glucose requirement was large, the formula did not permit a rapid enough escalation of the glucose infusion rate unless FM_{i-1} was introduced as an additional multiplier. Consider, for example, the situation in which $G_b = 90$, $G_d = 215$, and $G_i = 205$. The FM_i would be 1.087. If, in fact, the requirement for glucose were increasing at a rate greater than 8.7% in 5 min, then despite the adjustment in the infusion rate, the G_i would fall progressively farther from the goal. The introduction of FM_{i-1} provides for these trends.

The rationale for the use of SM_{i-2} can be appreciated by considering the following example. Suppose that the glucose concentration in the 50-min sample is read out to the investigator at 53 min. Although the 50-min concentration is the resultant of the entire previous 50-min "history" of the study, the dominant infusion rate for this sample is the rate that had been set at 43 min (in response to the readout of the 40-min glucose concentration) and that ran from 43 to 48 min when a new rate was begun. Thus, the computation of the metabolic component for the 50-min sample, SM_i, is based on the metabolic component computed two iterations previously (SM_{i-2}), the 40-min sample.

The final formula for computing the adjusted infusion rate is

$$S_i = \frac{(G_d - G_i) \times 10 \times (0.19 \times \text{body wt})}{G_{inf} \times 15}$$
$$\times PF + \frac{(SM_{i-2}) \times (G_d - G_b)}{(G_i - G_b)} \times (FM_{i-1})$$

Of importance in this formula is that for each succeeding blood sample, every parameter of the formula is known except for the variable G_i, the awaited glucose concentration. With the use of a programmable portable or desk-top calculator, all factors can be preloaded while awaiting the plasma glucose concentration readout and the computation of the dial setting, S_i, requires only a few seconds. Note that the programming of the formula should be devised to provide S_i as the initial readout; the FM_i and SM_i should then be separately read out because they will be used for the sequential computations of subsequent dial settings: FM_i becomes FM_{i-1} for the next sample and SM_{i-2} for the sample after that.[2]

The priming dose, as noted, is empirically set and is the same for all subjects. The first adjustment of the glucose infusion rate is made when the glucose concentration of the blood sample drawn at 10 min becomes available. In carrying out the initial computations, values for SM_{i-2} and FM_{i-1} must be empirically assigned: the SM_{i-2} is the glucose infusion rate (mg/(kg·min)) that would be set at 13 min if no adjustment were necessary (Table 1). This value is also assigned as the SM_{i-2} for the

TABLE 1. *Glucose prime during hyperglycemic clamp*

Time, min	Glucose Infusion Rate, mg/m² Surface Area·min
0–1	1,768
1–2	1,428
2–3	1,156
3–4	918
4–5	782
5–6	646
6–7	544
7–8	442
8–9	408
9–10	374
10–11	340
11–12	306
12–13	272
13–14	238
Total 9,622 mg/m²	

Priming infusion of glucose was calculated to raise the plasma glucose concentration by 125 mg/dl during the hyperglycemic clamp. The priming infusion can be adjusted upward or downward on a proportional basis to achieve any level of plasma glucose concentration.

computation of the glucose infusion rate based on the 15-min blood sample. At 20 min and thereafter, the previously computed SM values are available. Thus, the SM_{i-2} used for the 20-min blood sample is the SM computed for the 10-min sample, etc.

The FM_{i-1} used for the computation of the infusion rate for the 10-min plasma glucose sample is assigned a value of 1.00; the FM used for the 15-min blood sample is the FM computed for the 10-min sample, etc.

Euglycemic Clamp

Subjects. The same 11 subjects who were studied with the hyperglycemic clamp were restudied with the euglycemic insulin clamp at 3- to 4-wk intervals. Subject preparation was identical to that described for the hyperglycemic clamp study. Insulin was infused via the antecubital catheter along with the glucose infusate. *Subjects 1, 2, 3,* and *8* were restudied 3 to 4 wk later to evaluate the reproducibility of the euglycemic insulin clamp technique.

Insulin infusion. The goal of the euglycemic insulin clamp is to raise the plasma insulin concentration acutely to a new plateau and to maintain it at that level. In the studies reported in this study, the plasma insulin concentration was raised to approximately 100 $\mu U/ml$ above basal and maintained at this level for 120 min. This level would result in profound, rapidly developing hypoglycemia if the plasma glucose concentration were not maintained at its euglycemic level. Thus, the study consists of an insulin infusion of predetermined fixed dosage and a variable rate glucose infusion.

The insulin infusate is prepared in isotonic saline to which 2 ml of the subject's blood per 50 ml infusate is added in order to prevent absorption of insulin to glassware and plastic surfaces. Insulin recovery in the infusates averaged 102 ± 3%. Crystalline porcine insulin (Eli Lilly Co., Indianapolis, IN) is diluted to a concentration of 300 mU/ml. A 10-min priming infusion (Table 2) is followed by a constant infusion of 40 mU/m² surface area per minute for 110 min. The priming dose was set before

TABLE 2. *Insulin prime during euglycemic insulin clamp*

Time, min	Insulin Infusion Rate, mU/m² Surface Area· min
0–1	127.6
1–2	113.6
2–3	101.2
3–4	90.2
4–5	80.2
5–6	71.4
6–7	63.6
7–8	56.8
8–9	50.4
9–10	45.0
10–120	40.0

Priming infusion of insulin was calculated to raise the plasma insulin concentration 100 μU/ml during the euglycemic insulin clamp. The priming infusion can be adjusted upward or downward on a proportional basis to achieve any desired level of plasma insulin concentration.

accurate kinetic compartmental analysis of unlabeled insulin was available (17); the priming dose that we have used results in an initial overshoot in plasma insulin concentration that lasts for approximately 20 min.

Glucose infusion. Hypoglycemia with its attendant counterregulatory neuroendocrine responses is prevented by application of the glucose clamp technique in order to maintain each subject at his own basal arterial plasma glucose concentration. Modifications in the formulas given above for the hyperglycemic clamp studies are needed because in the euglycemic studies the desired glucose goal (G_d) equals the basal glucose level (G_b). Thus the FM_i is simply G_b/G_i, and the final formula is

$$S_i = \frac{(G_d - G_i) \times 10 \times (0.19 \times \text{body wt})}{G_{\text{inf}} \times 15}$$

$$\times PF + [(SM_{i-2}) \times (G_b/G_i) \times (FM_{i-1})]$$

The glucose infusion is not begun until 4 min after the initiation of insulin infusion and it is empirically set at 2.0 mg/(kg·min). It is increased at 10 min to 2.5 mg/(kg·min). The servocorrection formula to modify the infusion rate is first used when the 10-min plasma glucose concentration becomes available. For the computation of the infusion rates based upon the 10- and 15-min samples, the assigned SM_{i-2} value is 4.0 mg/(kg·min). For the 20-min and subsequent samples, the computed SM_{i-2} values become available and are used. The FM_{i-1} value for the 10-min plasma glucose computation is assumed to be 1.00; after that time the computed FM_{i-1} is used.

Alteration in glucose load

To examine what effect an increase or a decrease in the glucose infusion rate above or below that predicted by the glucose clamp formula would have on the plasma glucose concentration, two subjects received repeat hyperglycemic (*subjects 5* and *8*) and insulin clamp (*subjects 1* and *2*) studies. During the 1st h, both the hyperglycemic and insulin clamp studies were performed as previously described. At 60 min, however, the glucose infusion rate was either increased or decreased by 10% from the value calculated using the glucose clamp formula, and the resultant plasma glucose and insulin re-

sponse were compared to those obtained in the same subject when the clamp study was performed according to the glucose clamp formula.

Computations

1) Under steady-state plasma glucose conditions, the glucose infused must equal the glucose being translocated out of the glucose space (i.e., glucose metabolized, M) provided that endogenous glucose production is completely suppressed. The glucose infusion, in practice, must however be modified by two factors before it can be equated to the quantity of M. In the hyperglycemic studies, a small correction for urinary glucose loss is generally necessary; it averages 0.2 mg/(kg·min). This small urinary glucose correction is made by collecting urine immediately after the end of the study and measuring urine glucose concentration. It is assumed that, because a square wave of hyperglycemia has been created, urinary glucose losses are distributed equally over the 120-min experimental period. In both the hyperglycemic and the euglycemic clamp studies, the plasma glucose concentration is of course not maintained perfectly and a correction for this must be carried out. This "space correction" adjusts for glucose that has either been added to or removed from the glucose space. Thus, if M is being computed for the time period of 20–40 min, the plasma glucose concentrations at the beginning and end of that time period are considered. Suppose the glucose concentration at 20 min is 215 and at 40 min is 210 mg/dl. It is assumed that 5 mg of glucose were removed from each deciliter of glucose space in a 20-min period. The computation for the space correction (SC) in terms of mg/(kg· min) is

$$SC = \frac{(G_2 - G_1) \times 10 \times (0.19 \times \text{body wt})}{20 \times \text{body wt}}$$

where: *1*) G_2 and G_1 are the glucose concentrations in milligrams per deciliter at the end and at the beginning of the time period; *2*) $10 \times (0.19 \times \text{body wt})$ (kg) $\times$ ($G_2 - G_1$) computes the glucose (mg) removed from or added to the glucose space in the 20-min period; *3*) the final terms 20 and body wt convert the dimensions to mg/(kg·min). The formula reduces to

$$SC = (G_2 - G_1) \times 0.095$$

The space correction may be a factor of considerable magnitude if plasma glucose levels are relatively unstable. Thus if the level changes by 10 mg/dl, the space correction is 0.95 mg/(kg·min). The simple correction proposed here is adequate when plasma glucose concentration changes are small. However, in the early phase of the hyperglycemic clamp, when glucose concentration increases by 125 mg/dl, the correction formula noted here is inadequate and values for glucose metabolized in the first 20 min of the study are not reported in this study. More accurate but much more complex correction for the space factor can be made by using the information available from kinetic compartmental analysis of glucose distribution in the body (8). This information will be reported in a subsequent communication.

The computation of M in this paper is made for 20-min study intervals according to the equation

$$M = INF - UC - SC$$

where INF is the glucose infusion rate, UC is the correction for urinary loss of glucose, and SC is the space correction, and all values are computed in dimensions of mg/(kg·min). The total M is calculated from the means of the five 20-min periods from 20 to 120 min of the study.

In the hyperglycemic clamp studies, M is essentially a measure of glucose tolerance. In the usual glucose tolerance test, the dose of glucose is fixed, and the measure of tolerance is the plasma glucose concentration. In the clamp studies, the plasma glucose concentration is fixed and the glucose administered becomes the measure of tolerance.

2) The plasma insulin response (I) to fixed hyperglycemia is a measure of the beta-cell response to glucose. During the hyperglycemic clamp studies, the plasma insulin concentration was determined every 2 min for the first 10 min and every 10 min thereafter to determine both the early and late phases of insulin release.

3) The ratio M/I is a measure of the quantity of glucose metabolized per unit of plasma insulin concentration and is thus a reasonable index of tissue sensitivity to insulin (17). For convenience of data expression we have multiplied the M/I ratio by 100.

4) The metabolic clearance rate (MCR) for insulin is computed from data on the euglycemic clamp as previously reported (17)

$$MCR = \frac{\text{insulin infusion rate}}{\text{increase in plasma insulin concn above basal}}$$

The MCR is expressed in milliliters per square meter surface area per minute when the infusion rate is in microunits per square meter per minute, and the increase in insulin concentration (μU/ml) is the difference between the mean concentration in the 40- to 120-min time period and the basal concentration. This computation for the MCR$_I$ is based on the assumption that basal insulin secretion is unchanged by the insulin infusion. Because we have previously demonstrated that elevation of the plasma insulin concentration by approximately 100 μU/ml leads to a 60% reduction in basal insulin secretion as determined by the fall in plasma C-peptide concentration (unpublished observations), the absolute value for MCR$_I$ may be overestimated by 5–10%.

5) The endogenous systemic (or posthepatic) delivery rate of insulin (SDR) during the hyperglycemic clamp can be approximated from the product of the MCR derived from the euglycemic clamp and the mean plasma insulin concentration during the hyperglycemic clamp. This calculation of the SDR of insulin assumes that the MCR of insulin is constant in any given subject. Estimation of the minute-to-minute posthepatic insulin secretion can be computed by the more complex method of "deconvolution" of the kinetic compartmental model for insulin (17). This computation will be reported in a subsequent communication.

Analytical Methods

Plasma glucose concentration was measured by the glucose oxidase method (Glucostat, Beckman Instruments Corp., Fullerton, CA, 92634). Plasma insulin concentration was measured by radioimmunoassay using talc to separate bound from free insulin (18). Values are means ± SE throughout.

RESULTS

Hyperglycemic Clamp (Table 3)

The fasting plasma glucose concentration for the 11 subjects averaged 92 ± 2 mg/dl. During the period of sustained hyperglycemia (10–120 min), the mean glucose concentration for the 11 subjects was 213 ± 2 mg/dl (Fig. 1). This was 98 ± 1% of the desired goal. The stability of the plasma glucose concentration during the period of sustained hyperglycemia is reflected by the coefficients of variation of the individual studies, which averaged 3.7 ± 0.1%. Following the priming infusion of glucose, the plasma glucose concentration at 15 min averaged 220 ± 3 mg/dl, which was 1 ± 1% above the desired goal. The amount of M during the 20- to 120-min time period was 8.03 ± 0.68 mg/(kg·min). The time course of the glucose infusion is shown in Fig. 1. From 20 to 60 min, M increased only slightly. From 60 to 120 min, however, M increased approximately twofold.

The plasma insulin response (Fig. 1) to the sustained hyperglycemia was biphasic, with an early burst of insulin release followed by a phase of gradually increasing insulin concentration that lasted until the end of the study. The early (0–10 min) and late (10–120 min) I concentrations averaged 47 ± 6 and 64 ± 3 μU/ml, respectively (Table 3). The 0- to 120-min insulin response was 62 ± 3 μU/ml, and from this value the mean SDR of insulin was calculated to be 24.8 ± 2.0 mU/(min·m^2). The M/I ratio, a measure of tissue sensitivity to endogenously secreted insulin, was 13.08 ± 1.17 mg/(kg·min) per μU/ml × 100.

Subjects 1–6 received a repeat hyperglycemic clamp study 3–4 wk after the initial study (Table 3). In these repeat studies the fasting plasma glucose concentration was 87 ± 3 mg/dl and was maintained at 208 ± 3 mg/dl during the period of sustained hyperglycemia. This was 98 ± 1% of the desired goal. The stability of the plasma glucose concentration during the period of hyperglycemia was again reflected by the coefficient of variation, 3.8 ± 0.3%. The amount of M averaged 8.70 ± 0.85 mg/(kg·min) compared to 9.17 ± 0.89 mg/(kg·min) in the same subjects during the first study (Table 3). The plasma insulin concentration (65 ± 8 μU/ml) and the M/I ratio (14.67 ± 2.47 mg/(kg·min) per μU/ml × 100) were also similar to the first study when they were 61 ± 3 μU/ml and 15.25 ± 1.62 mg/(kg·min) per μU/ml × 100, respectively.

Euglycemic Insulin Clamp (Table 4)

The basal plasma insulin concentration was 14 ± 1 μU/ml. Following the prime-continuous infusion of insulin a steady-state plateau of hyperinsulinemia (40–120 min) was achieved, which averaged 105 ± 5 μU/ml (Fig.
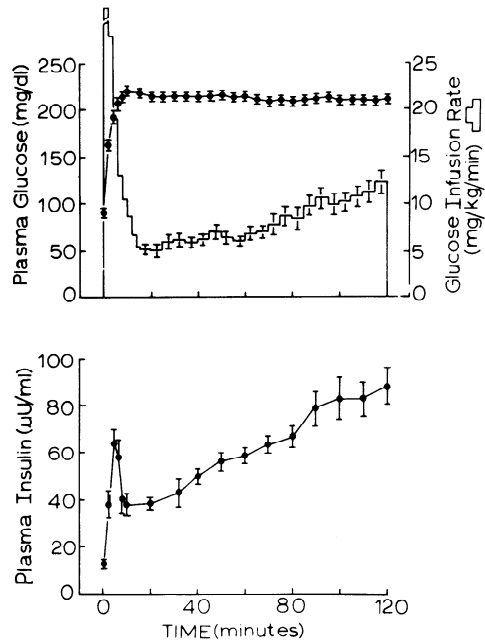
FIG. 1. Summary of plasma insulin response, steady-state plasma glucose concentration, and glucose infusion rate during hyperglycemic clamp. All values represent means ± SE. For convenience of plotting, glucose infusions during priming period are shown for 2-min intervals.

TABLE 3. *Hyperglycemic clamp*

Subject	Fasting Plasma Insulin Concn, μU/ml	Early Insulin Response (0-10 min), μU/ml	Late Insulin Response (10-120 min), μU/ml	Total Insulin Response (0-120 min), μU/ml	Glucose Metabolism (20-120 min), mg/(kg·min)	Insulin Sensitivity M/I Ratio, mg/(kg·min) per μU/ml × 100	Insulin Systemic Delivery Rate, mU/(min·m²)
Study 1							
1	11	41	58	57	8.60	15.09	24.5
2	14	39	73	70	11.89	16.99	42.4
3	14	38	48	47	9.96	21.19	21.9
4	15	54	60	59	5.37	9.09	38.1
5	10	34	70	67	9.14	13.64	28.5
6	15	66	65	65	10.10	15.53	22.8
7	16	38	70	67	8.26	12.33	26.5
8	16	96	76	78	8.41	10.78	35.0
9	18	48	70	68	5.67	8.34	33.2
10	13	34	63	61	5.86	9.61	23.0
11	17	33	46	45	5.09	11.31	17.3
Mean	14	47	64	62	8.03	13.08	24.8
±SE	1	6	3	3	0.68	1.17	2.0
Study 2							
1	14	43	61	60	8.45	14.08	25.8
2	16	52	93	90	12.02	13.36	54.5
3	11	30	37	36	8.84	24.57	16.7
4	16	61	71	70	5.51	7.87	45.2
5	11	38	87	83	8.19	9.86	35.4
6	14	53	50	50	9.19	18.38	17.6
Mean	14	46	66	65	8.70	14.67	32.5
±SE	1	5	9	3	0.85	2.47	6.2

Summary of the plasma insulin response, glucose metabolism, and tissue sensitivity to insulin (M/I ratio). No significant differences in any parameter were observed in *subjects 1-6* during the repeat study.

2). The stability of the plasma insulin concentration during this period of time is reflected by the coefficients of variation in the individual studies, which averaged 7.6

TABLE 4. *Euglycemic insulin clamp*

Subject	Fasting Plasma Insulin Concn, μU/ml	Steady-State (40-120 min) Plasma Insulin Concn, μU/ml	Metabolic Clearance Rate of Insulin,* ml/(min·m²)	Glucose Metabolized (20-120 min), mg/(kg·min)
Study 1				
1	16	109	430	8.63
2	16	82	606	9.93
3	15	101	465	8.94
4	13	75	645	4.78
5	10	104	426	8.73
6	13	127	351	6.66
7	15	116	396	6.32
8	16	105	449	6.23
9	13	95	488	5.78
10	13	119	377	5.73
11	16	120	385	5.33
Mean	14	105	456	7.00
±SE	1	5	28	0.52
Study 2				
1	13	111	408	9.05
2	13	80	597	9.43
3	14	105	439	9.40
8	17	117	400	6.23
Mean	14	103	461	8.53
±SE	1	8	46	0.77

Summary of plasma insulin concentration, metabolic clearance rate of insulin, and glucose metabolism. No significant differences in any parameter were observed in the 4 subjects who were restudied with the same experimental protocol. * Metabolic clearance rate = insulin infusion rate (40 mU/m²·min) ÷ steady-state (40-120) plasma insulin concn — basal plasma insulin concn.
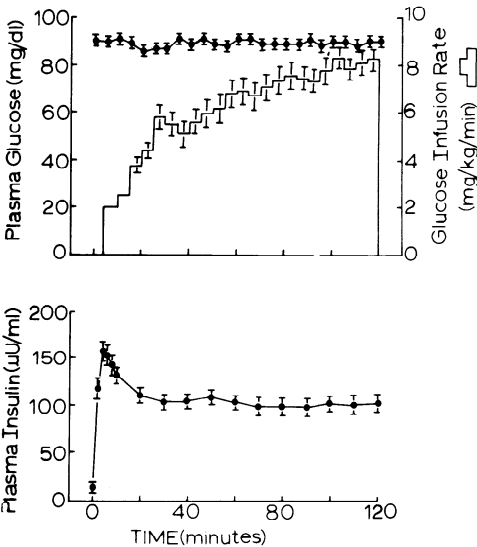

FIG. 2. Summary of steady-state plasma insulin and glucose concentrations and glucose infusion rate during euglycemic insulin clamp. All values represent means ± SE.

± 0.7%. During the initial 20 min of the insulin infusion, a moderate overshoot in the plasma insulin concentration occurred (see METHODS). By 20 min, however, the plasma insulin concentration had reached a steady state and neither the 20- or 30-min insulin values were statistically different from the 40-min or subsequent values. The MCR of insulin averaged 456 ± 28 ml/(min·m²).

The fasting plasma glucose concentration averaged 92

± 1 mg/dl (Fig. 2). During the period of hyperinsulinemia, the glucose concentration was maintained at 90 ± 2 mg/dl. The coefficient of variation of the glucose concentration during this period (0–120 min) averaged 5.0 ± 0.1%. The amount of M provides an index of tissue sensitivity to exogenous insulin and averaged 7.00 ± 0.52 mg/(kg·min) in the 11 subjects. The time course of the glucose infusion is shown in Fig. 2.

Subjects 1, 2, 3, and *8* received a repeat euglycemic insulin clamp 3–4 wk after the initial study. During the repeat study, the steady-state plasma insulin concentration (40–120 min) averaged 103 ± 8 μU/ml with a coefficient of variation of 7.2 ± 1.1%. These values are similar to those obtained on these four subjects during the first study (99 ± 6 μU/ml and 6.3 ± 0.9%, respectively). The amount of M was 8.53 ± 0.77 mg/(kg·min) compared to 8.43 ± 0.78 during the initial study.

Alteration in Glucose Load

The effect of increasing or decreasing the glucose infusion rate above or below that predicted by the glucose clamp formula during the hyperglycemic clamp is shown in Fig. 3. Both subjects (5 and 8) displayed similar responses, and only the data from *subject 8* are shown. At 60 min the formula called for the glucose infusion rate to be set at 6.56 mg/(kg·min). Instead, the infusion rate was increased by an additional 10% to 7.22 mg/(kg·min). This was associated with a rise in plasma glucose concentration from 217 to 230 mg/dl. These values were significantly higher than those during the preceding 20-min time interval (i.e., 40–60 min) ($P < 0.01$; unpaired t test). At 80 min the glucose infusion rate was increased by an additional 10% from 7.22 to 7.94 mg/(kg·min). From 80 to 120 min the plasma glucose concentration again increased slightly (232–237 mg/dl). These values were also significantly higher than during the 40- to 60-min period ($P < 0.001$; unpaired t test). The glucose

values during the 60- to 120-min time period of the repeat study were significantly higher than during the initial control study ($P < 0.001$; paired t test). The plasma insulin response was also significantly higher than in the control study during the 60- to 120-min time period ($P < 0.001$; paired t test).

During the third study the glucose infusion rate at 60 min was calculated to be 6.02 mg/(kg·min) by the glucose clamp formula. Instead the rate was decreased by 10% to 5.42 mg/(kg·min). During the subsequent 60 min, the plasma glucose concentration fell progressively compared to the 40- to 60-min time interval ($P < 0.001$) and compared to the same time interval during the initial control study ($P < 0.001$). The plasma insulin response also fell and was significantly less than in the control study during the 90- to 120-min time interval ($P < 0.05$).

The effect of increasing or decreasing the glucose infusion rate above that predicted by the glucose clamp formula during the euglycemic insulin clamp is shown in Fig. 4. Both subjects (1 and 2) displayed similar responses and only data from *subject 1* are shown in Fig. 4. At 60 min the formula called for the glucose infusion rate to be set at 8.58 mg/(kg·min). Instead the infusion rate was increased by an additional 10% to 9.44 mg/(kg·min). This increase was associated with a rise in plasma glucose concentration from 92 to 107 mg/dl at 90 min. These glucose values were significantly higher than during the preceding 20-min period ($P < 0.001$) and during the 60- to 90-min time interval in the same subject during the initial control study ($P < 0.001$). The plasma insulin concentration was similar to the control study at all times.

During the third study the glucose infusion rate at 60 min was calculated to be 8.35 mg/(kg·min) by the glucose clamp formula. Instead the infusion rate was decreased by 10% to 7.62 mg/(kg·min). A progressive decline in the plasma glucose concentration ensued, reaching 69 mg/dl at 90 min, at which time the study was stopped to avoid
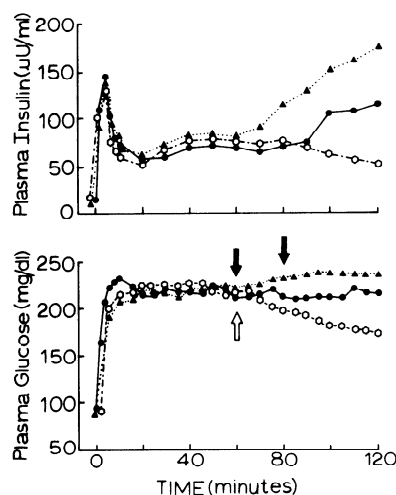

FIG. 3. Effect of varying glucose infusion rate on steady-state plasma glucose concentration and plasma insulin response during hyperglycemic clamp in *subject 8*. Control study is shown by solid circles. During first repeat study (solid triangles), glucose infusion rate was increased by 10% (see solid arrows) above rate calculated by glucose clamp formula at 60 min and again at 80 min. During second repeat study (open hexagons), glucose infusion rate was decreased by 10% (see open arrow) below rate calculated by glucose clamp formula at 60 min.
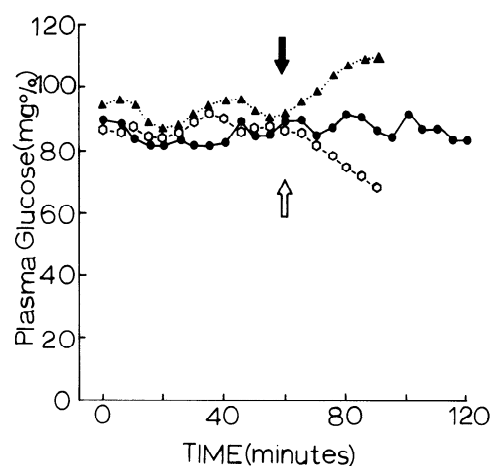
FIG. 4. Effect of varying glucose infusion rate on steady-state plasma glucose concentration during euglycemic insulin clamp in *subject 1*. Control study is shown by solid circles . During first repeat study (solid triangles), glucose infusion rate was increased by 10% (see solid arrow) above rate calculated by glucose clamp formula at 60 min. During second repeat study (open hexagons), glucose infusion rate was decreased by 10% (see open arrow) below rate calculated by glucose clamp formula at 60 min.

hypoglycemic symptoms. The plasma glucose concentration during the 60- to 90-min period was significantly below that during the 40- to 60-min period ($P < 0.001$) and during the same time interval during the initial control study ($P < 0.001$).

DISCUSSION

The hyperglycemic clamp technique affords a highly reproducible method of assessing beta-cell sensitivity to glucose as well as of quantifying the amount of glucose metabolized by the body following a controlled hyperglycemic stimulus. The plasma glucose concentration can be acutely raised and maintained at a chosen hyperglycemic plateau within narrow limits. Because the stimulus to the beta-cell is controlled, studies of beta-cell sensitivity among different subject populations can be reliably compared. Furthermore, the maintenance of the same steady-state plasma glucose concentration in all subjects obviates the need for using the insulin/glucose ratio and allows the plasma insulin response to be directly assessed. The hyperglycemic clamp technique also has a major advantage in that, unlike the oral glucose tolerance test, the time course of the amount of glucose metabolized by the body can be quantified.

The hyperglycemic clamp technique allows for the assessment of three important physiological variables. In addition to providing a measure of the total amount of glucose metabolized, the response of the beta-cell can be quantified and the early and late phases of insulin secretion (3, 12) examined independently. This is particularly important because it has been suggested that loss of the initial phase of insulin secretion is the earliest detectable abnormality in diabetes mellitus (14–16). Because both the amount of M and I are known, the M/I ratio provides a measure of tissue sensitivity to endogenously secreted insulin. To examine the validity of the M/I ratio as a measure of tissue sensitive to insulin, the results obtained from the hyperglycemic clamp were compared to those from the euglycemic insulin clamp. In one sense the euglycemic clamp provides a more reliable measure of tissue sensitivity to insulin because all of the infused insulin is known to be biologically active, whereas a small percentage of the insulin secreted during the hyperglycemic clamp is proinsulin, which is biologically less potent than insulin. A highly statistically significant correlation was observed when M/I from the hyperglycemic clamp is plotted versus M ($r = 0.816$, $P < 0.01$) or M/I ($r = 0.630$, $P < 0.025$) from the euglycemic insulin clamp (Fig. 5). Before utilizing the M/I ratio from the hyperglycemic clamp to assess tissue sensitivity to insulin in pathological states, it should be validated against the euglycemic insulin clamp. Although a high degree of correlation between the measures of tissue sensitivity to insulin derived from the hyperglycemic and insulin clamp techniques has been observed in uremia (6) and metabolic acidosis (4), such a correlation may not exist in other disease states. Use of the glucose clamp technique to assess insulin sensitivity in disease states also assumes that noninsulin-dependent glucose uptake is unaffected by the pathological state. The use of the M/I ratio as a measure of tissue sensitivity to insulin during the hyperglycemic clamp also assumes that hyperglycemia per se
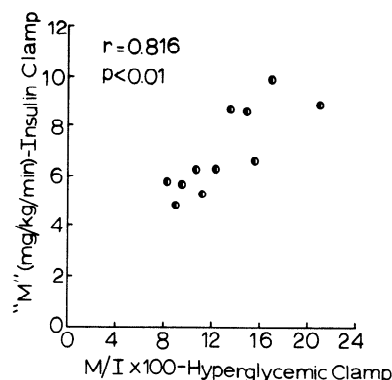

FIG. 5. Relationship between tissue sensitivity to insulin as determined by hyperglycemic clamp (M/I × 100) and euglycemic insulin clamp (M) techniques.

does not enhance glucose uptake. To the extent that this occurs, M will overestimate the amount of insulin-mediated glucose uptake.

In the hyperglycemic clamp, the fact that insulin levels vary makes it essential to "correct" the value of M in some manner to take this variance into account. The simplest technique is to compute the ratio of M to the I, that is to compute M/I. The underlying mathematical assumptions of such a computation are that M is linearly related to I and that there is no intercept of M on I. If either of these two assumptions is incorrect, then a more complex formulation would be required. For example, computation of an M/log I ratio might be justified. Detailed information covering the range of plasma insulin concentrations pertaining to this point are not available at this time, and we have therefore chosen to correct for insulin concentrations by the simplest technique, M/I. Most important, the use of such a ratio has been shown empirically to correlate closely with the measure of tissue sensitivity to insulin derived from the euglycemic insulin clamp.

The euglycemic insulin clamp technique offers significant advantages over the commonly used technique for assessing insulin sensitivity, the insulin tolerance test. By maintaining the basal glucose level after insulin administration, not only is the discomfort and potential hazard of hypoglycemic reactions prevented, but the complex neuroendocrine response to hypoglycemia does not occur. The test thus provides a more reliable estimate of tissue sensitivity to insulin. The establishment of a steady-state level of plasma insulin concentration also allows the calculation of the metabolic clearance rate of insulin. Because the rate of glucose metabolism can be determined at 5-min intervals, the time course of change in tissue sensitivity to insulin can be followed. Such information might provide insight into the mechanism of insulin resistance in certain disease states. Thus, if M were diminished during the 1st h of study but rose to control values during the 2nd h, an abnormality in the rate of distribution of insulin from plasma compartment to tissues might be suggested. As described in a previous publication (17), this theory could be tested by subjecting the data to kinetic analysis (9).

Reaven et al. (7, 13) have recently described an ingenious technique that involves the quadruple infusion of epinephrine, propranolol, insulin, and glucose to evaluate

tissue sensitivity to insulin. Both the quadruple infusion and insulin clamp techniques involve the infusion of glucose and insulin, but the clamp technique obviates the need to infuse epinephrine and propranolol, which may have direct effects on sensitivity of tissues to insulin. Another problem with the quadruple infusion method is that it does not permit evaluation of the role of endogenous glucose production in normal or abnormal states of glucose metabolism because the infusion of epinephrine and propranolol automatically disturbs glucose homeostatic mechanisms. The clamp technique, on the other hand, does allow quantification of endogenous glucose production when it is combined with the administration of labeled glucose.

In both the hyperglycemic and euglycemic clamp studies, the use of M as a measure of total body glucose metabolism assumes that basal hepatic glucose production is suppressed by the infusion of glucose and insulin. To the extent that hepatic glucose production is not shut off, M will underestimate the total amount of glucose metabolized. Using [3-^{3}H]glucose we have recently demonstrated that the liver of normal subjects is very sensitive to small increments in plasma insulin concentration and that a 100 μU/ml increment in plasma insulin will decrease hepatic glucose production to less than 10–15% (0.2–0.4 mg/(kg·min)) of basal levels (5). However, it is likely that in states of insulin deficiency or insulin resistance hepatic glucose production may not be completely suppressed by insulin. In these circumstances the use of labeled glucose to follow hepatic glucose production is essential because M will underestimate glucose metabolism.

In a recent study performed on anesthetized dogs (10), it was concluded that there might be a glucose-load sensor in the body and that the beta-cell responds more to glucose load (the rate of glucose infusion) than to the glucose concentration in blood perfusing the pancreas. Our experiences over a period of years with the clamp technique (1, 2, 8, 17) suggested to us that these dog results would not be replicable in unanesthetized man. Thus, on those occasions when errors are made in setting the glucose infusion rate, the plasma glucose concentration deviates from the goal in the predictable direction. For example, if the glucose concentration is incorrectly read out or if there is a keystroke error on the calculator or an incorrect setting of the dial on the infusion apparatus, then the results of the error will be readily apparent in subsequent blood samples. We therefore predicted that if purposeful deviation from the servocontrol formula were introduced, prompt loss of glucose steady-state control would ensue. To test the glucose-load hypothesis, triplicate studies were done on each of four subjects on separate days. In one study the servocontrol formulas for the hyperglycemic and euglycemic clamps dictated the glucose infusion pattern strictly. In the second and third studies of each set, the 1st h of the clamps was performed in the standard way. However, beginning at 60 min, the computed glucose infusion was either decreased or increased by 10%. In all permutations, the plateau glucose concentration established during the 1st h was lost in the predictable direction. Similarly, during the hyperglycemic clamp studies, the plasma insulin concentration either fell or increased in concert with the change in plasma glucose concentration. Whatever the reason for the discrepancies between our human studies and the previously reported dog studies (10), the clamp studies do not support the concept of a glucose load sensor. They further suggest that the computed glucose infusion rate truly reflects the physiological rate of glucose utilization under the controlled conditions of the clamp study. It is a unique value; efforts to deviate from the computed rate cause disruption of the plateau and glucose levels become unclamped.

In summary, the hyperglycemic and euglycemic clamp techniques offer a highly reproducible, physiological method of quantifying both beta-cell sensitivity to glucose and tissue sensitivity to insulin. They allow the calculation of the metabolic clearance rate as well as the basal systemic delivery rate of insulin. When used in conjunction with labeled glucose, the site of insulin resistance (i.e., liver vs. periphery) can also be defined.

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