Dose-response characteristics for effects of insulin on production and utilization of glucose in man

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HIZZA, ROBERT A., LAWRENCE J. MANDARINO, AND JOHN E. GERICH. Dose-response characteristics for effects of insulin on production and utilization of glucose in man. Am. J. Physiol. 240 (Endocrinol. Metab. 3): E630–E639, 1981.—To determine the dose-response characteristics for the effects of insulin on glucose production, glucose utilization, and overall glucose metabolism in normal man, 15 healthy subjects were infused with insulin for 8 h at sequential rates ranging from 0.2 to 5.0 mU· kg⁻¹·min⁻¹; each rate was used for 2 h. Glucose production and utilization were measured isotopically ([3-3H]glucose). Tissue insulin receptor occupancy was estimated from erythrocyte insulin binding. Glucose production was completely suppressed at plasma insulin concentrations of ~60 μU/ml. Maximal glucose utilization (10–11 mg·kg⁻¹·min⁻¹) occurred at insulin concentrations of 200–700 μU/ml. The concentration of insulin causing half-maximal glucose utilization (55 ± 7 μU/ml) was significantly greater than that required for half-maximal suppression of glucose production (29 ± 2 μU/ml, P < 0.01). Maximal effects of insulin on glucose production and utilization occurred at plasma insulin concentrations causing 11 and 49% insulin receptor occupancy, respectively. The above dose-response relationships indicate that in man 1) glucose production is more sensitive to changes in plasma insulin concentration than is glucose utilization; 2) both hepatic and peripheral tissues may contain “spare” insulin receptors; and 3) relatively minor changes in plasma insulin concentration or insulin receptor function can cause appreciable alterations in glucose metabolism.

sequential infusions of glucose; glucose clamp technique; steady-state glucose infusion rates

ALTERATIONS OF INSULIN ACTION may be due to changes in insulin sensitivity and/or insulin responsiveness (30). In the former, there is an alteration in the concentration of insulin required for a half-maximal effect; in the latter, there is an alteration in the maximal response to insulin. Thus, to adequately characterize alterations of insulin action, it is necessary to examine the effect of multiple concentrations of insulin to permit determination of the maximal effect of insulin as well as the concentration of insulin producing a half-maximal effect. The present studies were undertaken, therefore, to define the dose-response characteristics for the effects of insulin on glucose metabolism in normal man.

A variety of techniques have been employed to characterize the appropriateness of insulin action in man. These include glucose (11, 28, 34, 39, 48) and insulin (4, 5, 47) tolerance tests, forearm perfusion studies (10, 12, 53), and simultaneous infusion of glucose and insulin along with pharmacologic agents such as epinephrine (40) or somatostatin (26, 37). Each of these procedures, however, has limitations and potential drawbacks (15, 23, 41, 43, 45). The recent application of the euglycemic insulin clamp technique (15) to evaluate insulin action obviates most of these difficulties. In this technique, plasma insulin is increased to a predetermined constant level by a primed-continuous infusion of exogenous insulin, and plasma glucose is maintained constant at a euglycemic level by means of a variable infusion of exogenous glucose; the amount of exogenous glucose required to maintain euglycemia is the sum of the decrement in glucose production and the increment in glucose utilization caused by insulin and can thus serve as an index of the overall effect of insulin on total-body glucose metabolism. When this technique is used in conjunction with isotopic measurement of glucose turnover, the individual effect of insulin on glucose production and glucose utilization can also be assessed (14, 16, 31).

As currently employed (15, 31), use of the euglycemic clamp technique to determine the insulin dose-response relationships in a given individual would require 3-4 separate days of insulin infusions and would thus preclude evaluation of acute changes in insulin action. In the present studies, therefore, sequential infusions of insulin were used in conjunction with the glucose clamp technique and isotopic ([3-3H]glucose) determination of rates of glucose production and utilization to assess the dose-response characteristics for the individual effects of insulin on glucose production and glucose utilization and for its effect on total-body glucose metabolism in man. In addition, the relationship between insulin receptor binding and the effects of insulin on glucose production and glucose utilization as well as the effects of the insulin infusions on insulin binding to circulating monocytes and erythrocytes were determined.

METHODS

Informed consent was obtained from 15 healthy adult volunteers (7 females, 8 males, aged 34 ± 4 yr, range 18–56). All were within 10% of their ideal body weight (Metropolitan Life Insurance Tables) and had no family history of diabetes mellitus. Subjects were admitted to the outpatient facility of the Mayo Clinic General Clinical Research Center between 7:00 and 8:00 a.m. on the morning of each experiment after having fasted overnight.
INSULIN DOSE-RESPONSE CURVE FOR GLUCOSE METABOLISM IN MAN

(10–12 h). Eleven subjects were studied on two occasions separated by at least 48 h.

In all experiments, the subjects were placed at bed rest and maintained supine thereafter. Contralateral arm veins were cannulated with 18-gauge catheters (Jelco Laboratories, Raritan, NJ); one, a wrist vein, was used for continuous blood withdrawal by a Biostator (Life Science Instruments, Miles Laboratories, Elkhart, IN), which was used solely for constant glucose monitoring (42); the other, an antecubital vein, was used for infusion of glucose and insulin via separate Harvard pumps (Harvard Instruments, model 600–00, Boston, MA). In addition, a hand vein was cannulated retrogradely with a no. 19 butterfly needle (Surflo, Terumo, Tokyo, Japan); both the wrist vein (Biostator) and the hand vein were maintained at 55°C in a thermoregulated Plexiglas box. The hand vein was used for intermittent sampling of arterialized venous blood (35) for determination of plasma glucose concentrations; these values were used to recalibrate the Biostator at 10-min intervals to permit clamping at arterial glucose concentrations. This was done using the Biostator pump ratio adjustments knob. These adjustments, when necessary, were small (1–3 mg/dl) probably due to the fact that the Biostator was partially sampling arterialized venous blood and also due to the fact that the changes in insulin action were gradual. In experiments in which rates of glucose production and utilization were determined, a primed (22 μCi) continuous (0.22 μCi/min) infusion of [3-3H]glucose (New England Nuclear, sp gr 17.54 Ci/mm) was begun for isotopic determination of glucose appearance and disappearance rates. A 2 h equilibration period was allowed prior to initiation of experiments.

Subjects were infused with insulin at increasing rates of either 0.2, 0.5, 2.0, and 5.0 mIU·kg⁻¹·min⁻¹ (n = 6); 0.9, 0.5, 1.0, and 2.0 mIU·kg⁻¹·min⁻¹ (n = 5), or 0.5, 1.0, 2.0, and 5.0 mIU·kg⁻¹·min⁻¹ (n = 4); insulin was infused at each rate for 2 h. Prior to the initial insulin infusion and each subsequent increase in the insulin infusion rate, a priming intravenous bolus of insulin was given over 10 s according to the formula: insulin bolus (μg) = distribution volume (100 ml/kg × body wt in kg) × desired increment in plasma insulin (μU/ml) × 1,000. These amounts of insulin were chosen on the basis of previously published studies of insulin compartmental analysis (49) to increase plasma insulin concentrations acutely to 30, 60, 100, 200, and 500 μU/ml for the 0.2, 0.5, 1.0, 2.0, and 5.0 mIU·kg⁻¹·min⁻¹ insulin infusion rates, respectively; a basal plasma insulin concentration of 10 μU/ml and an insulin distribution volume of 10% body weight was assumed. Concurrent with the insulin infusion, variable amounts of glucose were infused with a Harvard pump to “clamp” the plasma glucose level at the same concentration that had been present in the basal state. The necessary adjustments in the glucose infusion rate were made empirically by altering the percent dial of the Harvard pump depending on changes in the plasma glucose concentrations observed during continuous glucose monitoring as previously described (42).

To examine the potential influence of antecedent insulin infusions on responses to subsequent insulin infusions and to assess the reproducibility of responses to a given insulin infusion rate, 11 subjects were restudied on another occasion with single 4-h infusions of insulin at rates of either 0.5 (n = 3), 2.0 (n = 4), or 5.0 (n = 4) mIU·kg⁻¹·min⁻¹. This longer duration for the insulin infusion was chosen to determine whether maximal responses to a given rate of insulin infusion had been achieved during the 120-min sequential insulin infusions. In these experiments, determinations of plasma insulin were made at 30 min intervals, and plasma glucose was again “clamped” at basal concentrations as described above.

To examine the potential effect of the insulin infusion on insulin receptor function, insulin binding to monocytes (n = 6) and erythrocytes (n = 13) was determined before and at the end of the sequential insulin infusions in these experiments. Blood (100 ml) was sampled immediately prior to the first and following the last insulin infusion. Mononuclear cells were isolated from heparinized blood by a modification of the Ficoll-Hypaque technique (8). An aliquot of erythrocytes was separated from mononuclear cells and granulocytes by the same method. The extensive washing steps of these procedures have been shown to dissociate insulin bound to the cells prior to their isolation (44). Mononuclear cells and erythrocytes were counted with a Coulter counter (Coulter Electronics, Hialeah, FL). The proportion of monocytes in each mononuclear cell preparation was determined by nonspecific esterase staining (51) and was found not to differ before (18.8 ± 1.5%) and after (21.2 ± 2.5%) the insulin infusion. Insulin binding to mononuclear cells was determined at 18°C in a buffer consisting of (in mM) HEPES, 100; NaCl, 120; KCl, 5; MgSO₄, 1.2; sodium acetate, 15; glucose, 10; KCl, 1; and 0.1% bovine serum albumin (pH 8.0) by the method of Gambhir et al. (22). Insulin was labeled with ¹²⁵I to a specific activity of 150–200 μCi/μg by the stoichiometric method of Kahn and Goldfine as outlined by DeMeys (17). Binding data were further analyzed by the method of Scatchard (46) and the average affinity method of DeMeys and Roth (18). Erythrocyte insulin receptor occupancy was calculated as previously described by Gambhir et al. (22).

All reagents were prepared on the morning of each experiment. Insulin (crystalline insulin U100, Eli Lilly, Indianapolis, IN) was dissolved in 0.9% NaCl containing 1% human serum albumin (Cutter Laboratories, Berkeley, CA) to a final concentration of 26 μU·kg⁻¹·min⁻¹. Glucose was administered at a 50% solution. Both were given along with a carrier infusion of 0.9% NaCl (0.5 ml/min), containing 0.22 meq KCl/ml to prevent hypokalemia. Initial plasma potassium concentrations averaged 4.2 ± 0.1 meq/liter and plasma potassium concentrations at the end of the insulin infusions averaged 3.7 ± 0.1 meq/liter.

All glucose and insulin concentrations in text and figures were determined on arterialized venous samples. Blood samples for glucose and insulin specific activity were collected at 10-min intervals in NaF-oxalate tubes (Kimble-Terumo, Elkton, MD) and immediately centri-
fuged; an aliquot of the resultant plasma was used for determination of glucose concentration in duplicate using a glucose oxidase method (Yellow Springs Instrument, Yellow Springs, OH). The remainder was aliquoted in triplicate (0.6 ml) and deproteinized by addition of 0.1 ml chilled perchloric acid (3M) for subsequent glucose radioactivity as previously described (41). Samples for plasma insulin were collected at 10-min intervals during the final 40 min of each insulin infusion rate in EDTA-containing tubes (Sigma Chemical, St. Louis, MO) and centrifuged immediately after each experiment. The resultant plasma was stored at $-20^\circ$C until radioimmunoassay as previously described (27).

The amount of glucose infused to maintain euglycemia was calculated at 10-min intervals throughout all experiments and expressed as the amount infused per minute per kilogram. Because insulin action on tissues is thought to be dependent on insulin concentrations in a slowly equilibrating extravascular compartment (49) and because it has been demonstrated that equilibration between plasma insulin concentrations and the concentrations of insulin in this extravascular compartment takes at least 90 min (49), only the glucose infusion rates over the last 40 min of each insulin infusion were used for generation of the insulin dose-response curve. As demonstrated in the text, during this interval, glucose infusion rates had reached a stable plateau; for this reason and because of the steady-state conditions with respect to the plasma glucose and insulin concentrations under which they are determined, these rates have been referred to as steady-state glucose infusion rates (SSGIR). Because under these steady-state conditions the amount of glucose infused to maintain euglycemia is the sum of the insulin-induced increase in glucose utilization and the insulin-induced suppression of glucose production, the steady-state glucose infusion rate is a measure of the overall effect of insulin on total-body glucose metabolism.

Rates of glucose turnover were determined in nine subjects prior to infusion of insulin and during the last 40 min of each insulin infusion: during 0.2, 0.5, 2.0, and 5.0 mU·kg$^{-1}$·min$^{-1}$ insulin infusion in five subjects; during 0.2, 0.5, 2.0, and 5.0 mU·kg$^{-1}$·min$^{-1}$ insulin infusions in one subject; and during 0.5, 1.0, 2.0, and 5.0 mU·kg$^{-1}$·min$^{-1}$ insulin infusions in three subjects. Rates of total glucose appearance (endogenously produced and exogenously infused) and glucose disappearance were calculated by employing the equations of Steele et al. (50) as modified by DeBodo (13). Endogenous glucose production was obtained by subtracting the amount of glucose infused from the isotopically determined total glucose appearance rate as previously described (31, 42).

Data in text and figures are given as means ± standard errors (SE). Statistical analysis was performed using two-tailed paired and, when appropriate, unpaired $t$ tests (52); a $P$ value less than 0.05 was considered to be statistically significant.

**RESULTS**

*Plasma glucose and insulin concentrations and glucose infusion rates during sequential infusions of insulin (Fig. 1).* Plasma glucose concentrations during the 8-h glucose clamp (95.0 ± 1.2 mg/dl) did not differ significantly from basal plasma glucose concentrations (96.9 ± 1.7 mg/dl). The coefficient of variation of the plasma glucose concentration during the clamps, calculated based on 10-min sampling intervals, was 4.5 ± 0.2%

When insulin was infused sequentially at rates of 0.2, 0.5, 2.0, and 5.0 mU·kg$^{-1}$·min$^{-1}$, steady-state plasma insulin concentrations averaged 26 ± 2, 58 ± 5, 201 ± 13, and 652 ± 34 μU/ml, respectively. Basal plasma insulin concentrations averaged 11 ± 1 μU/ml. The exogenous glucose infusion rates over the last 40 min (SSGIR) during the 0.2, 0.5, 2.0, and 5.0 mU·kg$^{-1}$·min$^{-1}$ insulin infusions were 2.0 ± 0.3, 5.2 ± 0.8, 9.4 ± 0.8, and 10.7 ± 0.6 mg·kg$^{-1}$·min$^{-1}$, respectively.

When insulin was infused sequentially at rates of 0.2, 0.5, 1.0, and 2.0 mU·kg$^{-1}$·min$^{-1}$, steady-state plasma insulin concentrations averaged 30 ± 3, 53 ± 3, 103 ± 5, and 192 ± 5 μU/ml, respectively; basal plasma insulin concentrations averaged 12 ± 1 μU/ml. The SSGIR during the 0.2, 0.5, 1.0, and 2.0 mU·kg$^{-1}$·min$^{-1}$ insulin infusions were 1.5 ± 0.4, 5.1 ± 0.7, 9.6 ± 1.2, and 10.5 ± 0.8 mg·kg$^{-1}$·min$^{-1}$, respectively.

When insulin was infused sequentially at rates of 0.5, 1.0, 2.0, and 5.0 mU·kg$^{-1}$·min$^{-1}$, steady-state plasma insulin concentrations averaged 53 ± 3, 95 ± 5, 231 ± 12, and 722 ± 42 μU/ml, respectively. Basal plasma insulin concentrations averaged 13 ± 3 μU/ml. The SSGIR during the 0.5, 1.0, 2.0, and 5.0 mU·kg$^{-1}$·min$^{-1}$ insulin infusions were 4.4 ± 0.5, 7.2 ± 0.3, 8.8 ± 0.4, and 9.6 ± 0.2 mg·kg$^{-1}$·min$^{-1}$, respectively.
The SSGIR were not significantly different when the 0.5 mU·kg⁻¹·min⁻¹ insulin infusion was the first or second infusion, when the 1.0 mU·kg⁻¹·min⁻¹ insulin infusion was the second or third infusion, or when 2.0 mU·kg⁻¹·min⁻¹ insulin infusion was the third or fourth infusion.

Dose-response characteristics for overall effect of insulin on total-body glucose metabolism (Fig. 2). To determine the dose-response characteristics for the overall effect of insulin on total-body glucose metabolism, the SSGIR of each sequential insulin infusion were plotted semilogarithmically versus the corresponding steady-state plasma insulin concentrations present during that insulin infusion (Fig. 2). The resultant dose-response curve is sigmoidal with SSGIR increasing steeply from 1.8 ± 0.4 mg·kg⁻¹·min⁻¹ at insulin concentrations of 28 ± 2 μU/ml to 8.3 ± 0.6 mg·kg⁻¹·min⁻¹ at insulin concentrations of 101 ± 4 μU/ml. With further increases in insulin concentrations to 225 ± 7 and 679 ± 27 μU/ml, SSGIR increased only to 9.9 ± 0.5 and 10.3 ± 0.4 mg·kg⁻¹·min⁻¹, respectively, which were not significantly different from one another. Thus, the maximal effect of insulin occurred at insulin concentrations between 200 and 700 μU/ml and averaged approximately 10-11 mg·kg⁻¹·min⁻¹. When the dose-response curve of each subject was analyzed individually, the half-maximal effect of insulin was found to occur at an insulin concentration of 58 ± 3 μU/ml (range: 42-89 μU/ml).

Dose-response characteristics for effects of insulin on glucose production and utilization (Figs. 3 and 4). The overall effect of insulin on total-body glucose metabolism is the sum of the effects of insulin on suspension of glucose production and stimulation of glucose utilization. The dose-response characteristics for the effects of insulin on these components of total-body glucose metabolism are shown in Fig. 2 for the nine subjects in whom rates of glucose production and utilization were determined isotopically during sequential infusion of insulin. Glucose production was suppressed from a basal rate of 2.0 ± 0.1 to 0.8 ± 0.1 mg·kg⁻¹·min⁻¹ with the lowest insulin infusion (which increased plasma insulin concentrations from 11 ± 2 to 28 ± 2 μU/ml) and was completely suppressed at insulin concentrations of 57 ± 3 μU/ml. Glucose utilization increased from a basal rate of 2.0 ± 0.1 mg·kg⁻¹·min⁻¹ to a maximum of 9.6 ± 0.7 mg·kg⁻¹·min⁻¹ at insulin concentrations of 678 ± 17 μU/ml. Based on analysis of individual dose-response curves, the concentrations of insulin required for half-maximal stimulation of glucose utilization (55 ± 7 μU/ml) was signifi-
cantly greater than that required for half-maximal suppression of glucose production (29 ± 2 µU/ml, P < 0.001). Because glucose production was completely suppressed at insulin concentrations of approximately 60 µU/ml, at and above these insulin concentrations the steady-state glucose infusion rates should equal rates of glucose utilization. As shown in Fig. 4, the SSGIR were strongly correlated (r = 0.95, P < 0.001) with the rates of glucose utilization during each of the four insulin infusions in the nine subjects even when the values for the insulin infusion rate producing insulin concentrations less than 50 µU/ml were included.

Relationship between insulin receptor occupancy and effects of insulin on glucose production and utilization (Table 1). Erythrocyte insulin receptor occupancy at which insulin caused half-maximal and maximal suppression of glucose production and stimulation of glucose utilization was determined in the seven subjects in whom both erythrocyte insulin binding and isotopic glucose turnover studies were performed (Table 1). The insulin receptor occupancy at half-maximal and maximal stimulation of glucose utilization were approximately 2 and 4 times greater, respectively, than those for half-maximal and maximal suppression of glucose production. Maximal effects of insulin on both glucose production and glucose utilization occurred at an insulin receptor occupancy of less than 50%.

Comparison of SSGIR during single infusions and sequential infusions of insulin (Figs. 5 and 6). To determine whether antecedent infusion of insulin had altered the response to subsequent infusion of insulin and to assess the reproducibility of responses to insulin, 11 subjects were restudied on a separate occasion during a single 4-h infusion of insulin at either 0.5, 2.0, or 5.0 mU·kg⁻¹·min⁻¹ during which plasma glucose again was clamped at basal concentrations. Plasma glucose concentrations during the 4-h glucose clamps (94.8 ± 1.8 mg/dl) did not differ significantly from basal plasma glucose concentrations (93.2 ± 1.9 mg/dl). The coefficient of variation of the plasma glucose concentration during the clamp, calculated based on 10-min sampling intervals, averaged 3.9 ± 0.4%. Steady-state plasma insulin concentrations were achieved within 30 min and averaged 58 ± 4, 195 ± 9, and 570 ± 17 µU/ml for insulin infusion rates of 0.5, 2.0, or 5.0 mU·kg⁻¹·min⁻¹, respectively; these values as well as the basal plasma insulin concentrations (15 ± 2, 12 ± 1, and 12 ± 1 µU/ml) were not different from those observed when the same individuals were infused with insulin at the same rate during the sequential insulin infusion study.

As shown in Fig. 5, the amount of glucose required to maintain euglycemia during the 0.5 and 2.0 mU·kg⁻¹·min⁻¹ insulin infusions did not reach a stable plateau before 150–180 min. Although glucose infusion rates reached a stable plateau somewhat earlier during the 5.0 mU·kg⁻¹·min⁻¹ insulin infusions, the time required for

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<th>Fractional Receptor Occupancy, %</th>
<th>Half-maximal</th>
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<td>Suppression of glucose production</td>
<td>6.7 ± 1.1</td>
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<td>Stimulation of glucose utilization</td>
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Values are means ± SE, n = 7 subjects. Occupancy at half-maximal effects were determined from individual dose-response curves. For determination of occupancy at maximal effects, individual plasma insulin concentrations during the 0.5 mU·kg⁻¹·min⁻¹ insulin infusion were used for suppression of glucose production; for stimulation of glucose utilization, individual plasma insulin concentrations during insulin infusions at which the greatest utilization occurred were used.

![FIG 5. Plasma glucose concentrations and glucose infusion rates necessary to maintain euglycemia during single insulin infusions.](http://ajpendo.physiology.org/)

![FIG 6. Correlation between steady-state glucose infusion rates (SSGIR) necessary to maintain euglycemia during sequential and single infusions of insulin in same subjects.](http://ajpendo.physiology.org/)
the glucose infusions to reach a stable plateau during the single insulin infusions was generally greater than that observed when insulin was infused at the same rate as part of the sequential insulin infusion study (Fig. 1). Nevertheless, as was observed with the sequential insulin infusions, the SSIGR during the 2.0 mU·kg⁻¹·min⁻¹ insulin infusion (10.8 ± 0.9 mg·kg⁻¹·min⁻¹) and the 5.0 mU·kg⁻¹·min⁻¹ infusion (10.9 ± 1.7 mg·kg⁻¹·min⁻¹) were not significantly different.

Figure 6 compares the steady-state glucose infusion rates required to maintain euglycemia over the final 40 min of these experiments with those for the same individuals when insulin was infused at the same rate as part of the sequential insulin infusion study. The SSIGR on both occasions were virtually identical (r = 0.95, P < 0.001). Thus, although antecedent infusion of insulin shortened the time required for the amount of glucose infused to maintain euglycemia to reach a stable plateau, it did not affect responses to subsequent infusion of insulin assessed by the SSIGR; a given insulin infusion rate reproducibly caused a given SSIGR whether or not it was preceded by another insulin infusion.

**Effects of insulin infusion on binding of insulin to circulating monocytes and erythrocytes (Table 2, Fig. 7).** Insulin binding to circulating monocytes (n = 6) and erythrocytes (n = 13) was determined before and after sequential infusions of insulin. Insulin binding curves and Scatchard plots for monocytes and erythrocytes are shown in Table 2; individual data are given in Table 2. Maximum insulin binding to monocytes decreased significantly from 15.8 ± 1.3 to 9.7 ± 1.1% (P < 0.01). The number of insulin binding sites per monocyte decreased significantly from 22,816 ± 3,227 to 15,000 ± 2,300 (P < 0.01). Average empty site affinity decreased from 5.5 ± 0.8 to 4.6 ± 0.6 M⁻¹ but this decrease was not statistically significant. In contrast, erythrocyte maximum insulin binding, the number of insulin binding sites per erythrocyte, and average empty site affinity of erythrocytes were unaltered.

**DISCUSSION**

Insulin resistance has been reported to occur in a variety of disease states (6, 20, 21). Although methods for assessing insulin resistance have varied considerably (4, 5, 10–12, 26, 28, 34, 37, 39, 40, 47, 48, 53), all have generally evaluated insulin action at only one insulin dose or concentration. Such an approach does not permit characterization of the mechanisms responsible for insulin resistance and could potentially underestimate or fail to demonstrate the presence of insulin resistance. A decreased biologic effect of insulin at a given insulin concentration may be due to decreased sensitivity to insulin and/or to decreased responsiveness to insulin. In the former, there is an increase in the concentration of insulin necessary to produce a half-maximal effect with a normal maximal effect of insulin being observed (shift to the right of the insulin dose-response curve); thus, if a maximally effective dose of insulin were employed to assess insulin resistance, a normal response would be observed, and the presence of insulin resistance would be missed. With decreased responsiveness to insulin, all responses to insulin are reduced including maximal responses, but the concentration of insulin causing a half-maximal effect is normal; thus, if only a submaximal dose of insulin were employed to assess insulin resistance, one could not distinguish between the presence of decreased sensitivity or decreased responsiveness to insulin.

From the above considerations, it can be readily seen that, to adequately characterize insulin resistance and to estimate the relative contributions to it of alterations in insulin sensitivity and insulin responsivity, it is necessary to examine the effects of multiple concentrations of insulin to permit determination of the maximal effect of insulin as well as the concentration of insulin producing half-maximal effect. The same considerations hold for evaluation of conditions associated with enhanced insulin action. Current methods using the glucose clamp technique require 3–4 days of separate experiments to acquire such insulin dose-response data (15, 31) and thus preclude precise assessment of acute (24–48 h) changes in insulin action. Therefore, in the present studies, sequential infusions of insulin were used to generate insulin dose response curves for the overall effect of insulin on total-body glucose metabolism as well as on its components (suppression of glucose production and stimulation of glucose utilization by insulin). The exogenous glucose infusion rates required to maintain euglycemia at steady

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<th>Table 2. Insulin binding to circulating monocytes and erythrocytes before and after insulin infusion</th>
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*P < 0.01 versus before insulin infusion values. Infusions in mU·kg⁻¹·min⁻¹; †0.2, 0.5, 1.0, 2.0; ‡0.5, 1.0, 2.0, 6.0; §0.2, 0.5, 2.0, 5.0 mU·kg⁻¹·min⁻¹.
state were taken as a measure of the effect of insulin on total-body glucose metabolism because under the experimental conditions employed, these infusion rates are equal to the sum of the decrement in glucose production and the increment in glucose utilization caused by insulin.

As shown in Fig. 1, the glucose infusion rates necessary to maintain euglycemia during each of the sequential insulin infusions increased gradually and did not usually reach a stable plateau until approximately 80 min into each insulin infusion. For this reason, only glucose infusion rates during this plateau from 80 to 120 min were used to quantitate insulin action and were called the steady-state glucose infusion rates (SSGIR). As shown in Fig. 2, when the SSGIR during each insulin infusion was plotted against the logarithm of plasma insulin concentration achieved during the insulin infusions, a sigmoidal dose-response curve was obtained. Maximal SSGIR were observed at plasma insulin concentrations of between 200 and 700 μU/ml and a half-maximal effect of insulin occurred at a plasma insulin concentration of approximately 60 μU/ml.

The conclusion that a maximal effect of insulin is approached at plasma concentrations of 200-300 μU/ml is supported by the fact that in the present studies the SSGIR at this insulin concentration (9.9 ± 0.5 mg·kg⁻¹·min⁻¹) was not significantly different from that observed at plasma insulin concentration in excess of 600 mU/ml (10.3 ± 0.4 mg·kg⁻¹·min⁻¹); moreover, similar glucose infusion rates have been previously reported to be necessary to maintain euglycemia in the presence of plasma insulin concentrations ranging from 300 to 10,000 μU/ml (1, 31). Finally our estimate of the maximally effective plasma insulin concentration is in close agreement with results of human forearm perfusion studies (12) in which maximal glucose uptake is also found to occur at plasma insulin concentrations of 200–300 μU/ml.

Because total-body glucose metabolism is a function of the effect of insulin on both glucose production and glucose utilization, the dose-response characteristics for suppression of glucose production and stimulation of glucose utilization by insulin were determined in 9 of the 15 subjects during sequential infusions of insulin using [3-3H]glucose to estimate these parameters. Complete suppression of glucose production occurred at insulin concentrations of 50–60 μU/ml, whereas maximal stimulation of glucose utilization did not occur until insulin concentrations exceeded 200 μU/ml. The insulin concentration required for half-maximal suppression of glucose production (29 ± 2 μU/ml) was significantly less than that required for half-maximal stimulation of glucose utilization (55 ± 7 μU/ml, P < 0.01).

Although portal venous insulin concentrations were probably somewhat greater than the arterial concentrations to which peripheral tissues were exposed in this study, our results indicate that hepatic glucose production is more sensitive to changes in insulin concentration than is peripheral tissue glucose utilization. Thus, a 10–20 μU/ml increment in insulin concentration was sufficient to cause half-maximal suppression of glucose production, whereas a 40–50 μU/ml increment was required to cause half-maximal stimulation of glucose utilization. Previous studies in man (19, 31) have suggested that
hepatic glucose production might be more sensitive than peripheral glucose utilization to insulin. In one study (19), infusion of glucose that resulted in a 10 μU/ml increment in circulating arterial insulin concentrations was associated with an 85% suppression of splanchnic glucose output in the absence of a change in estimated glucose utilization; however, because plasma glucose concentrations also increased, it is not possible to attribute the suppression of glucose production solely to the increment in circulating insulin concentration because hyperglycemia per se can suppress glucose production (9, 42). In the other study, Kolterman et al. (31) reported that the insulin concentration necessary to stimulate glucose utilization half-maximally (130 ± 10 μU/ml) was greater than that required for half-maximal suppression of glucose production (less than 100 μU/ml); however, the latter could not be precisely determined because the effects of insulin concentrations less than 100 μU/ml were not examined.

The insulin concentration found by Kolterman et al. (31) for half-maximal stimulation of glucose utilization is twofold greater than that found in this study (55 ± 7 μU/ml). A partial explanation for this discrepancy may be that Kolterman et al. subtracted approximately 70% of basal glucose utilization in their dose-response calculations based on the assumption that this amount of glucose utilization in the postabsorptive state is not due to insulin. Such a maneuver would have shifted their dose-response curve for glucose utilization to the right and resulted in a greater plasma insulin concentration for half-maximal stimulation. In these studies, we did not subtract an estimate of noninsulin-mediated basal glucose utilization from our dose-response calculations because a precise estimate for this value cannot be determined in man and we thus considered that such a maneuver would introduce an arbitrary variable. However, it can be appreciated from examination of the dose-response curve in Fig. 3 that, even if it is assumed that all glucose utilization in the postabsorptive state were noninsulin-mediated, our estimate of the insulin concentration for half-maximal stimulation of glucose utilization would increase only 15-25 μU/ml (to approximately 70-80 μU/ml) and would still be considerably less than found by Kolterman et al. (31).

It should be pointed out that this discrepancy does not appear to be due to the fact that sequential infusions of insulin were used in these studies, whereas Kolterman et al. (31) used separate infusions of insulin. When the potential effect of antecedent infusion of insulin on the responses to subsequent infusion of insulin were examined in these experiments by restudying each subject who had received a sequential infusion of insulin with a single infusion of insulin on a separate occasion, the steady-state glucose infusion rates on both occasions were virtually identical (r = 0.95, P < 0.001). Because glucose production is completely suppressed at insulin concentrations of approximately 60 μU/ml, the steady-state glucose infusion rates are equal to rates of glucose utilization at insulin concentrations above 60 μU/ml. Thus it can be inferred that the dose-response characteristics for insulin-stimulated glucose utilization are also virtually identical whether insulin is infused sequentially or as multiple individual infusions.

There is considerable evidence that insulin may regulate its own receptors (2, 3, 7, 24, 32, 36, 44), and that hyperinsulinemia can decrease both insulin receptor number and affinity (2, 3). Therefore, it seemed of interest to determine whether insulin receptor function was altered during the 8-h sequential insulin infusions. For this purpose, maximal insulin binding, the number of insulin binding sites per cell, and their average empty site affinity were determined before and after the 8-h infusions using both circulating monocytes (n = 6) and erythrocytes (n = 13). Maximal insulin binding to monocytes decreased approximately 40% (P < 0.01), whereas no change in erythrocyte insulin binding was observed. Scatchard analysis indicated a decrease in the number of monocyte insulin binding sites in all six subjects. No change in the number of erythrocyte insulin binding sites or their affinity was observed.

Recently, Insel et al. (29) reported that, following euglycemic hyperinsulinemia in an individual subject, monocyte insulin binding was reduced due to a decrease in insulin receptor affinity with no change in the number of insulin binding sites. The decreases in both the number of monocyte binding sites and receptor affinity observed in these studies may have been due to more prolonged and greater hyperinsulinemia. The experiment of Insel et al. (29) was carried out for 5 h in the presence of insulin concentrations of approximately 100 μU/ml; in this study, euglycemic clamps were carried out for 8 h in the presence of insulin concentrations up to 7 times as great. It should be pointed out that, in contrast to these findings, Insel et al. (29) found decreased insulin binding to erythrocytes. We have no explanation for this discrepancy other than the possibility that changes in erythrocyte insulin binding might be transient, and thus the decreased binding observed at 5 h could have been restored to normal by 8 h.

The significance of the changes in insulin binding to circulating monocytes on the shape of the insulin dose-response curves generated during the prolonged infusions of insulin is unclear. It is generally assumed that insulin binding to circulating monocytes may reflect insulin binding to insulin target tissues (liver, muscle, adipocytes). If these changes in monocyte insulin binding had reflected concomitant changes in insulin binding to insulin target tissues, one would anticipate a shift to the right of the insulin dose-response curve (30). Although the present data cannot unequivocally exclude this possibility, it seems unlikely to have occurred because, during all insulin infusions (both sequential and individual), the glucose infusion rates required to maintain euglycemia did not decrease with increasing duration of the insulin infusion as would be predicted if insulin target tissues had become less sensitive to insulin. Furthermore, the SSGIR during the 4-h individual insulin infusions were not different from those observed during the 8-h sequential insulin infusions. Because erythrocyte insulin binding did not change in these studies, the above considerations raise the question as to whether erythrocyte or monocyte insulin binding more accurately reflects insulin binding to insulin target tissues.

In these studies, maximal suppression of glucose pro-
duction and stimulation of glucose utilization occurred at erythrocyte insulin receptor occupancies of approximately 11 and 49%, respectively. These findings support the concept of "spare" insulin receptors proposed on the basis of in vitro studies of animal tissues (25, 33, 38) and suggest that this phenomenon occurs in both hepatic and peripheral tissues in man. It is of interest to note that our estimates for insulin receptor occupancy required for half-maximal (11%) and maximal (49%) stimulation of glucose utilization using the erythrocyte receptor model are in close agreement with those derived using the human adipocyte receptor model (11 and 49%, respectively) (31). Whether insulin binding in these models accurately reflects insulin binding to receptors in liver and skeletal muscle remains to be determined.

In summary, these studies have defined the dose-response characteristics for the effects of insulin on glucose production, glucose utilization, and its overall effect on total body glucose metabolism in man using sequential infusions of insulin in conjunction with the euglycemic insulin clamp technique and isotopic determination of rates of glucose production and utilization. Maximal insulin-stimulated glucose utilization averaged approximately 10–11 mg·kg⁻¹·min⁻¹ and occurred at plasma insulin concentrations between 200 and 700 μU/ml; half-maximal stimulation of glucose utilization occurred at plasma insulin concentrations of approximately 60 μU/ml. Glucose production was completely suppressed at plasma insulin concentrations of approximately 60 μU/ml with half-maximal suppression occurring at approximately 30 μU/ml. These observations provide evidence for the presence of spare insulin receptors in man because maximal effects of insulin on glucose production and glucose utilization occurred at 11 and 49% insulin receptor occupancy, respectively, and suggest that relatively minor changes in plasma insulin concentration or insulin receptor function can cause appreciable alterations in glucose metabolism. Because the SSGIR appear to be a reproducible index for the overall effect of insulin on total body glucose metabolism, sequential infusions of insulin in conjunction with the euglycemic insulin clamp technique offer the potential to assess acute changes in insulin action in many by permitting generation of an insulin dose-response curve in a single day.

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