Insulin Dose-Response Characteristics for Suppression of Glycerol Release and Conversion to Glucose in Humans

N. NURJHAN, P. J. CAMPBELL, F. P. KENNEDY, J. M. MILES, AND J. E. GERICH

SUMMARY

To compare the dose-response characteristics for suppression of lipolysis and suppression of glucose production by insulin, 13 normal nonobese individuals were infused with insulin at rates of 0.1, 0.2, 0.4, 0.8, and 1.6 mU · kg^{-1} · min^{-1} while normoglycemia was maintained with the glucose clamp technique. Glucose appearance and glycerol appearance (taken as index of lipolysis) were measured isotopically with simultaneous infusions of 3-[^{3}H]glucose and U-[^{14}C]glycerol. Baseline glucose and glycerol rates of appearance were 14 ± 0.5 and 1.7 ± 0.2 μmol · kg^{-1} · min^{-1}, respectively. Approximately 3% of plasma glucose originated from glycerol, and this accounted for ~50% of glycerol disposal. During the insulin infusions, arterial insulin (basal, 9.8 ± 0.6 μU/ml) increased to 14 ± 0.5, 20 ± 0.5, 31 ± 1, 58 ± 2, and 104 ± 6 μU/ml; calculated portal venous insulin (basal, 24 ± 2 μU/ml) increased to 26 ± 1, 32 ± 3, 70 ± 4, and 115 ± 6 μU/ml. The rate of glucose appearance was suppressed 100%, whereas the rate of appearance of glycerol was maximally suppressed only 85%. Nevertheless, the insulin concentration that produced half-maximal suppression of glucose appearance was twice as great as that required for half-maximal suppression of glycerol appearance (26 ± 2 vs. 13 ± 2 μU/ml, P < .001). Insulin decreased both the absolute rate of glycerol conversion to plasma glucose and the percent of glycerol disposal appearing in plasma glucose (both P < .001). These results indicate that in normal humans the suppression of lipolysis is more sensitive to insulin than is the suppression of hepatic glucose production and that in addition to reducing glycerol availability, insulin suppresses glycerol incorporation into plasma glucose by another (presumably hepatic) mechanism. DIABETES 1986; 35:1326-31.

The major metabolic actions of insulin include not only its suppression of hepatic glucose production and stimulation of peripheral glucose utilization but also its inhibition of lipolysis in adipose tissue. In the basal (postabsorptive) state, suppression of release of glucose from the liver and suppression of release of free fatty acids (FFA) from adipose tissue are probably the more important actions of insulin because 60–80% of glucose uptake in the postabsorptive state is non–insulin mediated[1,2] and because a slight reduction in circulating insulin levels increases glucose output and plasma FFA concentrations without decreasing glucose utilization.[3,4]

In humans the suppression of hepatic glucose output (HGO) is more sensitive to insulin than is the stimulation of glucose utilization.[5,6] In vitro studies of isolated human adipocytes[7] and studies in human volunteers[3,8,9] indicate that suppression of lipolysis is also more sensitive to insulin than is stimulation of glucose utilization.[3] However, no direct information is presently available concerning the relative insulin sensitivity of hepatic glucose production and adipose tissue lipolysis.

Howard et al.,[8] Zierler and Rabinowitz,[9] and Bakir and Jarret[10] found that increases in plasma insulin to ~20–50 μU/ml suppressed plasma FFA concentrations ~50%. Although none of these studies assessed HGO, these results suggest that suppression of lipolysis and HGO might be comparably sensitive to insulin because the half-maximal effective dose (ED$_{50}$) for suppression of HGO in normal humans is ~30 μU/ml.[5]

Nevertheless, these studies used changes in plasma FFA levels as an index of lipolysis,[8–10] and this could have resulted in an error in estimation of the insulin sensitivity of lipolysis because FFA can be reesterified and the insulin sensitivity of FFA reesterification might differ from that of lipolysis. In contrast, glycerol formed during lipolysis cannot be reesterified to a significant extent in human adipose tissue.[11] Consequently, the rate of appearance of glycerol should be a more precise index of lipolysis.

Therefore, in this study, we compare the dose-response characteristics for insulin-induced suppression of glycerol and glucose appearance rates to assess the relative insulin sensitivity of suppression of lipolysis and glucose production in postabsorptive normal humans. Moreover, because ~50% of the glycerol produced in the postabsorptive state is converted to glucose,[12] we examined the effect of insulin on glycerol incorporation into plasma glucose as a function of glycerol disposal to determine whether insulin inhibited glycerol incorporation into plasma glucose exclusively by decreasing glycerol availability to the liver (inhibition of lipolysis) or whether another mechanism was also involved.

METHODS

Subjects. Informed consent was obtained from 13 normal male volunteers (aged 27 ± 1 yr). They weighed 77 ± 3 kg, all were within 10% of their ideal body weight (Metropolitan Life Insurance Company Tables, 1985), and none had a family history of diabetes mellitus. Subjects consumed a weight-maintenance diet (~30 kcal/kg) containing 200–300 g carbohydrate for at least 3 days before the experiments. On the evening before the experiments, subjects were admitted to the Mayo Clinic General Clinical Research Center after dinner and subsequently fasted until completion of the studies.

Between 0600 and 0800 h, contralateral arm veins were cannulated with 18-gauge catheters (Jelco, Raritan, NJ). One, a wrist vein, was used for continuous blood withdrawal by a Biostator (Life Science, Miles, Elkart, IN), which was used solely for constant glucose monitoring[5]; 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, Terukmo, 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[13] 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 with the Biostator-pump ratio-adjustments knob. These adjustments, when necessary, were small (1–3 mg/dl).

Three hours before initiating the insulin dose-response experiments, a primed continuous infusion of 3-[³H]D-glucose (25 μCi, 0.25 μCi/min; New England Nuclear, Boston, MA) and U-[¹⁴C]glycerol (6 μCi, 0.3 μCi/min; Research Products International, Mount Prospect, IL) was started for the determination of glucose and glycerol fluxes. After a 2-h isotope-equilibration period, insulin was infused at either 0.1, 0.2, 0.8, and 1.6 mU · kg⁻¹ · min⁻¹ (N = 7) or 0.2, 0.4, 0.8, and 1.6 mU · kg⁻¹ · min⁻¹ (N = 6) for 2 h. Euglycemia was maintained throughout the experiments with the glucose clamp technique described previously.[5]

Blood samples for plasma glucose and glycerol concentrations, [³H]glucose and [¹⁴C]glucose sp act, [¹⁴C]glycerol sp act, and C-peptide and insulin levels were taken before and at 10-min intervals during the last 30 min of each insulin infusion.

Analytical procedures. Plasma glucose was measured with a YSI glucose analyzer (Yellow Springs, Yellow Springs, OH).

Plasma glycerol was determined by an enzymatic micro-fluorometric method[14] with a detection limit (95% confidence interval) of 10 μmol/L and with an inter- and intra-assay variability of <5%. Plasma insulin[15] and C-peptide[16] concentrations were determined by standard radioimmunoassays.

For determination of their specific activities, glucose and glycerol were isolated from plasma by ion-exchange chromatography: 4 ml of plasma were deproteinized by addition of an equal volume of chilled 7% (wt/vol) perchloric acid. After centrifugation, the supernatant was decanted into a separate tube, the pellet was washed once with 1 ml of distilled water, and the wash was added to the previously decanted supernatant. After neutralization to a pH between 6.7 and 7.2 with 4 N KOH, the sample was transferred to three stacked (1 × 7-cm) polypropylene columns (BioRad, Richmond, CA). The top column contained 9 ml of AG1 (formate form) anion-exchange resin (100–200 mesh, Bio-Rad); the middle column contained 6 ml of AG50 (hydrogen form) cation-exchange resin (100–200 mesh, Bio-Rad). Glycerol, glucose, and other neutral compounds were eluted off the AG1 and AG50 columns with two washes of 20 ml distilled water,[17] and the wash was passed through the bottom column containing 6 ml of AG1 (borate form). The borate columns were prepared by first converting an AG1, chloride form (100–200 mesh, Bio-Rad) to the hydroxide form with six 20-ml washes of 2 N NaOH and then converting it to the borate form by 3–4 20-ml washes of 0.5 M boric acid until the pH reached 4.2–4.3.[18] Glycerol was eluted from the borate column with 20 mM K-tetraboric acid in three fractions of 20 ml each. An additional wash with 20 ml of 20 mM K-tetraboric acid was performed before eluting the glucose with 0.5 M acetic acid in two fractions of 20 ml each. The eluates were evaporated under vacuum (Speedvac Concentrator, Savant, Hicksville, NY) and resuspended in 1 ml
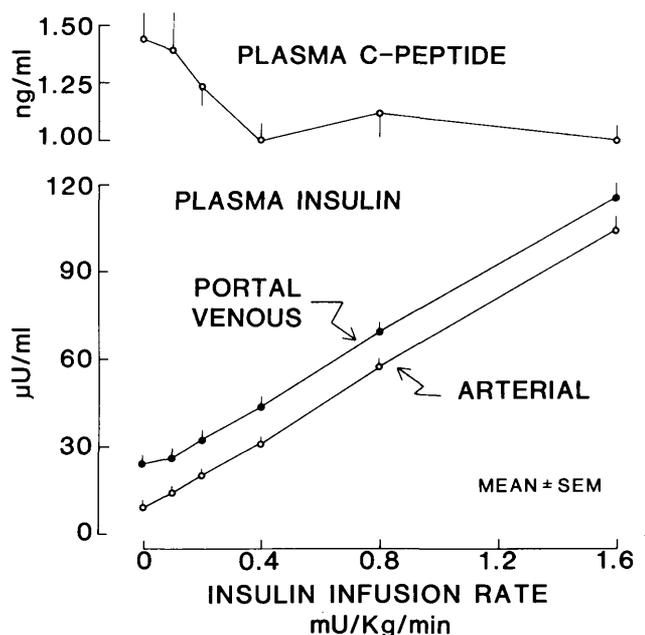

FIG. 1. Arterial plasma C-peptide and insulin concentrations and calculated portal venous insulin concentrations during sequential insulin infusions.

of distilled water; the fractions of glycerol and glucose were separately pooled. Ten milliliters of liquid scintillation cocktail (Safety-Solv, Research Products International, Mount Prospect, IL) was added to the pooled eluates for liquid scintillation counting. Correction was made for recoveries of glycerol and glucose from plasma (~60 and ~80%, respectively) by adding [³H]glycerol as an internal standard and [¹⁴C]glucose as an external standard before deproteinization of plasma. With the above isolation technique, <0.6% of 20,000 dpm [³H]glycerol were found in the glucose fraction, and no counts from 20,000 dpm [¹⁴C]glucose were found in the glycerol peak.

Calculations. Rates of glucose and glycerol appearance (Ra) and disappearance (Rd) were calculated from the Steele equations modified for non-steady-state conditions, as described by DeBodo et al.[19] For both substrates, a fractional pool size of 0.5 was used; distribution spaces of 200 and 650 ml/kg were used for glucose[19] and glycerol,[20] respectively. During the glucose clamp experiments, rates of exogenously infused glucose were subtracted from overall rates of glucose appearance to obtain rates of endogenous glucose appearance.[5] Values obtained during the last 30 min of each insulin infusion were averaged and used for dose-response calculations.

The percent of plasma glucose derived from glycerol was determined from the ratio of plasma [¹⁴C]glucose and plasma [¹⁴C]glycerol sp act, as described by Kreisberg et al.[21] The rate of glycerol conversion to glucose was calculated by multiplying the percent of glucose derived from glycerol by glucose Ra. The percent of glycerol disposal incorporated into plasma glucose was calculated by dividing the rate of glycerol conversion to glucose by glycerol Rd. Portal venous insulin concentrations were calculated from changes in arterial plasma insulin and C-peptide concentrations with the non-steady-state equations of DeFeo et al.[22]

The plasma insulin ED_{50} values for suppression of glucose and glycerol appearance were determined in individual subjects from standard log-logit plots.[23] $Log[Y/(100 - Y)]$ is plotted versus log(plasma insulin concentration), where Y is the percent of maximal inhibition. The apparent ED_{50} is the x-intercept, which was determined by least-squares linear regression. The ED_{50} for suppression of hepatic glucose production was determined from calculated portal venous insulin concentrations. Arterial insulin concentrations were used to estimate the ED_{50} for suppression of lipolysis.

Statistical analysis. Data in the text and figures are given as means ± SE. Statistical evaluation was performed with the paired two-tailed Student's t test. A P value <.05 was considered to be statistically significant.

RESULTS

Plasma insulin and C-peptide concentrations. Basal arterial plasma insulin and C-peptide concentrations were 9.8 ± 0.6 μU/ml and 1.4 ± 0.1 ng/ml, respectively. During infusions of insulin, arterial plasma insulin increased to 14 ± 0.5, 20 ± 0.5, 31 ± 1, 58 ± 2, and 104 ± 6 μU/ml during the 0.1-, 0.2-, 0.4-, 0.8-, and 1.6-mU · kg⁻¹ · min⁻¹ infusions, respectively; plasma C-peptide decreased progressively to ~70% of basal values (1.0 ± 0.1 ng/ml) by the 0.4-mU · kg⁻¹ · min⁻¹ insulin infusion and did not decrease further. Calculated portal venous insulin increased from a basal
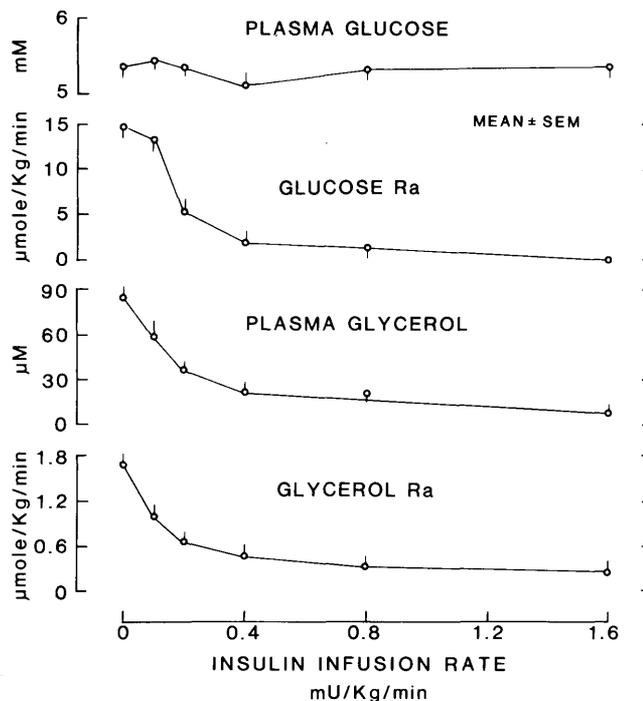

FIG. 2. Arterial plasma glucose and glycerol concentrations and rates of appearance (Ra) of glucose and glycerol during sequential insulin infusions.

concentration of 24 ± 2 to 26 ± 1, 32 ± 1, 43 ± 2, 70 ± 2, and 115 ± 6 μU/ml during the ascending insulin infusions (Fig. 1).

Plasma glucose and glycerol concentrations and Ra values. Basal plasma glucose was 5.3 ± 0.1 mM; during the insulin infusions, it was maintained between 5.1 and 5.4 mM with the glucose clamp technique (coefficient of variation, <5%). Plasma glycerol decreased from a basal concentration of 86 ± 5 μM to 14 ± 2 μM during the highest insulin infusion ($P < .01$) (Fig. 2).

Basal glucose and glycerol Ra values were 14 ± 0.5 and 1.7 ± 0.2 μmol · kg⁻¹ · min⁻¹, respectively. Both decreased during the insulin infusions. The first significant decrease in glucose Ra was observed during the second (0.2 mU · kg⁻¹ · min⁻¹) insulin infusion (5.4 ± 1.1 μmol · kg⁻¹ · min⁻¹ $P < .001$), and it was totally suppressed during the highest insulin infusion. Glycerol Ra decreased >40% to 1.0 ± 0.1 μmol · kg⁻¹ · min⁻¹ during the first (0.1 mU · kg⁻¹ · min⁻¹) insulin infusion ($P < .01$), and it was suppressed ~85% (to 0.3 ± 0.04 μmol · kg⁻¹ · min⁻¹) during the highest insulin infusion. Because this rate was not significantly lower than that observed during the preceding insulin infusion, it was considered to represent maximal suppression (Fig. 2).

Dose-response characteristics for insulin-induced suppression of glucose and glycerol Ra values. The liver is primarily exposed to and presumably predominantly influenced by portal venous insulin concentrations, whereas adipose tissue is exposed to arterial insulin concentrations. Consequently, the dose-response characteristics for insulin-induced suppression of glucose Ra were examined in terms of calculated portal venous insulin concentrations, whereas those for glycerol Ra were examined in terms of arterial in-
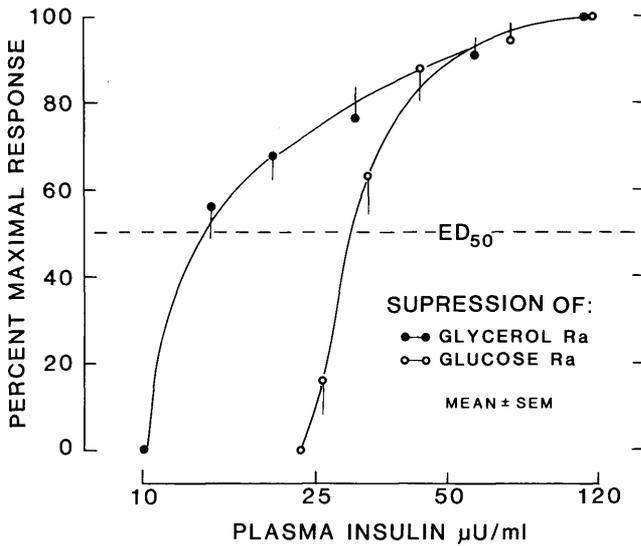
FIG. 3. Insulin dose-response curves for suppression of glycerol and glucose rates of appearance (Ra). Glycerol Ra was plotted against arterial insulin concentrations, and glucose Ra was plotted against calculated portal venous insulin concentrations.

sulin concentrations (Fig. 3). Maximal suppression of glucose and glycerol Ra values occurred at approximately the same plasma insulin concentrations ($\sim$60–80 μU/ml). In contrast, half-maximal suppression of glycerol and glucose Ra differed significantly ($P < .001$). Based on log-logit analyses of data from individual subjects, the ED_{50} for suppression of glucose Ra (25.6 $\pm$ 2.1 μU/ml) was about twice as great as the ED_{50} for suppression of glycerol appearance (13.4 $\pm$ 1.6 μU/ml).

Effect of insulin on incorporation of glycerol into plasma glucose. In the postabsorptive (basal) state, glycerol was incorporated into plasma glucose at a rate of 0.78 $\pm$ 0.1 μmol $\cdot$ kg^{-1} $\cdot$ min^{-1}; this represented 45 $\pm$ 3% of glycerol turnover and accounted for 2.7 $\pm$ 0.2% of the plasma glucose Ra (Fig. 4).

Both the rate of conversion of glycerol into plasma glucose and the percent of plasma glucose derived from glycerol decreased during the initial insulin infusion (calculated portal venous insulin, $\sim$26 μU/ml) (both $P < .05$) and were completely suppressed at calculated portal plasma insulin concentrations of $\sim$45 μU/ml during the 0.4-mU $\cdot$ kg^{-1} $\cdot$ min^{-1} insulin infusion ($P < .001$). Theoretically, both of these changes could have been due to reduced availability of glycerol to the liver as a consequence of insulin-induced suppression of lipolysis. However, the percent of glycerol disappearance converted to plasma glucose also decreased during infusions of insulin (from 45 $\pm$ 3 to 0.2 $\pm$ 0.08% during the 0.4-mU $\cdot$ kg^{-1} $\cdot$ min^{-1} insulin infusion, $P < .001$). This therefore suggests that some other, presumably hepatic, mechanism was also involved.

DISCUSSION

Our studies were undertaken to assess the relative insulin sensitivity of suppression of lipolysis and suppression of glucose production in postabsorptive humans. Using arterial plasma insulin concentrations to assess the dose-response characteristics for suppression of lipolysis and calculated

portal venous insulin concentrations to assess the dose-response characteristics for suppression of hepatic glucose production, we found that the ED_{50} for suppression of lipolysis, as reflected by glucose Ra, was about half the ED_{50} for suppression of glucose production (i.e., $\sim$13 vs. 26 μU/ml). The difference in ED_{50} values for these two processes would still have been statistically significant if we had merely used arterial plasma insulin concentrations in our calculations (i.e., 13 $\pm$ 1 μU/ml for lipolysis vs. 19 $\pm$ 1 μU/ml for glucose production, $P < .05$). Thus, these results indicate that suppression of lipolysis in adipose tissue is more sensitive to insulin than is suppression of hepatic glucose production.

Our results are consistent with those of previous investigators,[8-10] who found a 50% decrease in plasma FFA release with plasma insulin concentrations of $\sim$20–50 μU/ml in normal volunteers, although glucose production was not assessed in these studies. We used glycerol Ra as an index of lipolysis because, in contrast to FFA, glycerol cannot be reesterified in adipose tissue. Although the ED_{50} for suppres-
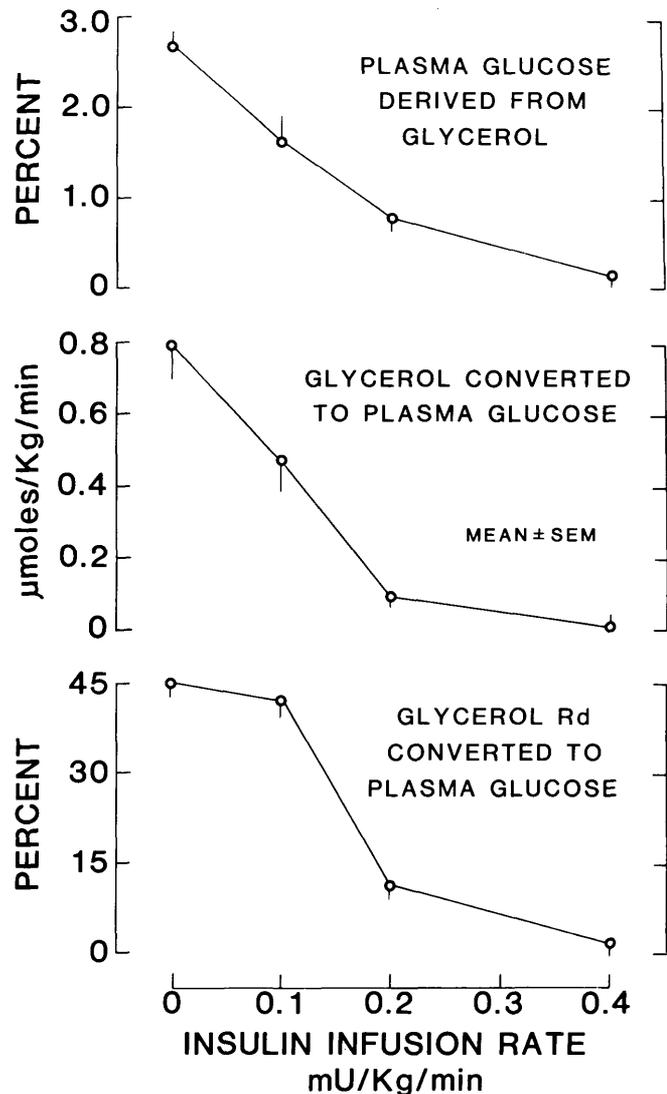

FIG. 4. Effect of insulin on percent of plasma glucose derived from glycerol, rate of glycerol conversion to glucose, and percent of glycerol disappearance (Rd) converted into plasma glucose.

sion of glycerol Ra was less than that for suppression of glucose production, insulin maximally suppressed glycerol Ra only 85%, whereas it inhibited glucose Ra 100%. In in vitro studies of basal as well as stimulated lipolysis, insulin concentrations as great as 1000 μU/ml also do not completely suppress release of glycerol from adipocytes.[23,24] These observations suggest that there may be a non-insulin-suppressible lipolytic pathway. Alternatively, the residual glycerol appearance could arise from conversion of glucose to glycerol rather than from lipolysis. Although negligible amounts of plasma glucose are said to be directly converted to plasma glycerol,[25] the conversion of 0.3% of the glucose turnover to glycerol during the high-insulin infusions could readily account for the residual glycerol Ra.

Note that several recent studies have demonstrated that administration of glucose may acutely alter tissue insulin sensitivity and insulin receptor binding.[26-28] In theory, this phenomenon could have influenced our results because glucose was infused to maintain euglycemia during our insulin infusions. However, Bolinder et al.[28] have shown that this phenomenon occurs with oral but not intravenous glucose administration.

Another aspect of our study deserving comment is the possible effect of its long duration (10 h). During fasting, lipolysis increases and hepatic glucose production decreases. Thus, we may have underestimated the relative sensitivity of lipolysis to insulin compared with that of glucose production.

The findings of our study have implications regarding alterations in the flux of substrates in conditions associated with insulin resistance. Because suppression of lipolysis is more sensitive to insulin than are suppression of glucose production and stimulation of glucose utilization by insulin, one would expect that lipolysis would be the last or the least-affected process during the development of insulin resistance. Although there have as yet been no direct comparisons of rates of lipolysis and glucose production in insulin-resistant conditions, Arner et al.[24] did not find an increase in the ED_{50} for suppression of lipolysis in adipocytes from obese individuals, whereas such adipocytes generally have an increased ED_{50} for glucose uptake.[7,29] Moreover, these findings and those of this study suggest that acceleration of the Randle glucose–fatty acid cycle[30] would be a late event in insulin-resistant states because appreciable defects in glucose uptake should occur before there was increased availability of FFA to compete with glucose for utilization in peripheral tissues. Of course this assumes that reesterification of FFA has a sensitivity to insulin similar to that of lipolysis. The studies by Taskinen et al.[31] support this concept because no increase in FFA turnover was found in insulin-resistant patients with type II diabetes mellitus, who are known to have impaired insulin-mediated glucose disposal.[32]

The other major action of insulin observed in our study was its effect on glycerol incorporation into plasma glucose. We found that in the postabsorptive state, ~3% of glucose production originated from glycerol and that this represented ~45% of glycerol disposal. These results are consistent with previous estimates in humans.[12,20,33,34] Insulin decreased both the percent of glucose derived from glycerol and the rate of glycerol conversion to plasma glucose in our studies.

It has been suggested that the major factor regulating glycerol conversion to glucose is the supply of glycerol originating from lipolysis[35] and that this is the major site at which insulin influences gluconeogenesis from glycerol.[35] The findings by Keller et al.[36] that hepatic uptake of glycerol paralleled arterial glycerol concentrations and was independent of changes in plasma insulin and glucagon support this concept. Nevertheless, to further assess this concept, we examined glycerol incorporation into plasma glucose as a percent of glycerol Rd because plasma glycerol, and hence its availability, decreased due to inhibition of lipolysis during the insulin infusions. If insulin influenced glycerol conversion to plasma glucose merely by affecting the supply of glycerol, the rate of incorporation of glycerol into plasma glucose should have remained a constant percentage of glycerol Rd. However, we found that this percentage also decreased during insulin infusion. Thus, these results indicate that insulin affects glycerol incorporation into plasma glucose by another mechanism in addition to its limiting the supply of glycerol from the periphery.

Because insulin does not appear to influence glycerol incorporation into plasma glucose by altering hepatic uptake of glycerol,[36] it must act at some subsequent intrahepatic step. This could involve direct inhibition of gluconeogenesis from glycerol, shunting of glycerol into oxidative or lipogenic pathways, or diversion of glucose derived from glycerol into glycogen rather than to plasma. The studies by Friedman et al.[37] demonstrating that injection of insulin into alloxan-induced diabetic rats increased glycerol incorporation into glycogen without appreciably affecting overall glucose formation from glycerol support the latter explanation.

In conclusion, our studies demonstrate that in postabsorptive normal humans, the suppression of lipolysis is more sensitive to insulin than is the suppression of glucose production. Furthermore, insulin suppressed incorporation of glycerol into plasma glucose not only by decreasing delivery of glycerol to the liver but also by altering intrahepatic glycerol disposition.

ACKNOWLEDGMENTS

REFERENCES

[1] Gottesman, I., Mandarino, L., and Gerich, J.: Estimation and kinetic analysis on insulin-independent glucose uptake in human subjects. Am. J. Physiol. 1983; 244:E632–35.

[2] Baron, A., Kolterman, O., Bell, J., Mandarino, L., and Olefsky, J.: Rates of noninsulin-mediated glucose uptake are elevated in type II diabetic subjects. J. Clin. Invest. 1985; 76:1782–88.

[3] Miles, J., Rizza, R., Haymond, M., and Gerich, J.: Effects of acute insulin deficiency on glucose and ketone body turnover in man. Diabetes 1980; 29:926–30.

[4] Ward, W., Best, J., Halter, J., and Porte, D.: Prolonged infusion of somatostatin with glucagon replacement increases plasma glucose and glucose turnover in man. J. Clin. Endocrinol. Metab. 1984; 58:449–53.

[5] Rizza, R., Mandarino, L., and Gerich, J.: Dose-response characteristics for effects of insulin on production and utilization of glucose in man. Am. J. Physiol. 1981; 240:E630–39.

[6] Kolterman, O., Insel, J., Saekow, M., and Olefsky, J.: Mechanisms of insulin resistance in human obesity. J. Clin. Invest. 1980; 65:1272–84.

[7] Pedersen, O., Hjollund, E., and Schwartz, N.: Insulin receptor binding and insulin action in human fat cells: effects of obesity and fasting. Metabolism 1982; 31:884–95.

[8] Howard, B., Klimes, I., Vasquez, B., Brady, D., Nagulesparan, M., and Unger, R.: The antilipolytic action of insulin in obese subjects with resistance to its glucoregulatory action. J. Clin. Endocrinol. Metab. 1984; 58:544–48.

[9] Zierler, K., and Rabinowitz, D.: Effect of very small concentrations of insulin on forearm metabolism. Persistence of its action on potassium and free fatty acids without its effect on glucose. J. Clin. Invest. 1964; 43:950–62.

[10] Bakir, S., and Jarrett, R.: The effects of low dose intravenous insulin infusion upon plasma glucose and non-esterified fatty acid levels in very obese and non-obese human subjects. Diabetologia 1981; 20:592–95.

[11] Shapiro, B., Chowers, P., and Rose, G.: Fatty acid uptake and reesterification in adipose tissue. Biochim. Biophys. Acta 1957; 23:115–20.

[12] Bortz, W., Pavle, P., Haff, A., and Holmes, W.: Glycerol turnover and oxidation in man. J. Clin. Invest. 1972; 51:1537–46.

[13] McGuire, E., Helderman, J., Tofin, J., Andres, R., and Berman, N.: Effects of arterial versus venous sampling on analysis of glucose kinetics in man. J. Appl. Physiol. 1976; 41:565–73.

[14] Garland, P., and Randle, P.: A rapid enzymatic assay for glycerol. Nature (Lond.) 1962; 196:987–88.

[15] Herbert, V., Lau, K., Gottlieb, C., and Bleicher, S.: Coated charcoal immunoassay of insulin. J. Clin. Endocrinol. Metab. 1965; 25:1375–84.

[16] Faber, O., Binder, C., Markussen, J., Heding, L., Naithani, V., Kuzuya, H., Blix, P., Horwitz, D., and Rubenstein, A.: Characterization of seven C-peptide antisera. Diabetes 1978; 27:170–77.

[17] Kreisberg, R., Siegal, A., and Owen, W.: Alanine and gluconeogenesis in man: effect of ethanol. J. Clin. Endocrinol. Metab. 1972; 34:876–83.

[18] Khym, J.: Analytical ion-exchange procedures. In Chemistry and Biology. Englewood Cliffs, NJ, Prentice-Hall, 1974:154–58.

[19] DeBodo, R., Steele, R., Altszuler, N., Dunn, A., and Bishop, J.: On the hormonal regulation of carbohydrate metabolism: studies with C[14] glucose. Recent Prog. Horm. Res. 1963; 19:445–88.

[20] McCulloch, A., Johnston, D., Baylis, P., Kendall-Taylor, P., Clark, F., Young, E., and Alberti, K.: Evidence that thyroid hormones regulate gluconeogenesis from glycerol in man. Clin. Endocrinol. 1983; 19:67–76.

[21] Kreisberg, R., Siegal, A., and Crawford-Owen, W.: Glucose-lactate interrelationships: effects of ethanol. J. Clin. Invest. 1971; 50:175–85.

[22] DeFeo, P., Pierrello, G., DeCosmo, S., Ventura, M., Campbell, P., Brunetti, P., Gerich, J., and Bolli, G.: Comparison of glucose counterregulation during short-term and prolonged hypoglycemia in normal man. Diabetes 1986; 35:563–69.

[23] Arner, P., Bolinder, J., Engfeldt, P., and Ostman, J.: The antilipolytic effect of insulin in human adipose tissue in obesity, diabetes mellitus, hyperinsulinemia, and starvation. Metabolism 1981; 30:753–60.

[24] Arner, P., Bolinder, J., Engfeldt, P., Hellmer, J., and Ostman, J.: Influence of obesity on the antilipolytic effect of insulin in isolated human fat cells obtained before and after glucose ingestion. J. Clin. Invest. 1984; 73:673–80.

[25] Winkler, B., Steele, R., and Altszuler, N.: Relationship of glycerol uptake to plasma glycerol concentration in the normal dog. Am. J. Physiol. 1969; 216:191–96.

[26] Livingston, J., and Moxley, R.: Glucose ingestion mediates a rapid increase in the insulin responsiveness of rat adipocytes. Endocrinology 1982; 111:1749–51.

[27] Arner, P., Bolinder, J., and Ostman, J.: Marked increase in insulin sensitivity of human fat cells one hour after glucose ingestion. J. Clin. Invest. 1983; 71:709–14.

[28] Bolinder, J., Ostman, J., and Arner, P.: Effects of intravenous and oral glucose administration on insulin action in human fat cells. Diabetes 1985; 34:884–90.

[29] Ciaraldi, T., Kolterman, O., and Olefsky, J.: Mechanism of the postreceptor defect in insulin action in human obesity. J. Clin. Invest. 1981; 68:875–80.

[30] Randle, P., Garland, P., Hales, C., and Newsholme, E.: The glucose fatty acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 1963; 1:785–89.

[31] Taskinen, M., Bogardus, C., Kennedy, A., and Howard, B.: Multiple disturbances of free fatty acid metabolism in noninsulin-dependent diabetes. J. Clin. Invest. 1985; 76:637–44.

[32] Bogardus, C., Lillioja, S., Howard, B., Reaven, G., and Mott, D.: Relationships between insulin secretion, insulin action, and fasting plasma glucose concentration in nondiabetic and noninsulin-dependent diabetic subjects. J. Clin. Invest. 1984; 74:1238–46.

[33] Bougneres, P., Karl, I., Hillman, L., and Bier, D.: Lipid transport in the human newborn. J. Clin. Invest. 1982; 70:262–70.

[34] Malmendier, C., Delcroix, C., and Berman, M.: Interrelations in the oxidative metabolism of free fatty acids, glucose, and glycerol in normal and hyperlipemic patients. J. Clin. Invest. 1974; 54:461–76.

[35] Shaw, W., Issekutz, T., and Issekutz, B.: Gluconeogenesis from glycerol at rest and during exercise in normal, diabetic and methylprednisolone-treated dogs. Metabolism 1976; 25:329–39.

[36] Keller, U., Chiasson, J., Liljenquist, J., Cherrington, A., Jennings, A., and Crofford, O.: The roles of insulin, glucagon, and free fatty acids in the regulation of ketogenesis in dogs. Diabetes 1977; 26:1040–51.

[37] Friedman, B., Goodman, E., and Weinhouse, S.: Dietary and hormonal effects on gluconeogenesis and glycogenesis from pyruvate 3-^{14}C, fructose U-^{14}C, and glycerol 2-^{14}C in the rat. Endocrinology 1970; 86:1264–71.