Impact of lack of suppression of glucagon on glucose tolerance in humans

PANKAJ SHAH, ANANDA BASU, RITA BASU, AND ROBERT RIZZA
Endocrine Research Unit, Mayo Clinic, Rochester, Minnesota 55905

Shah, Pankaj, Ananda Basu, Rita Basu, and Robert Rizza. Impact of lack of suppression of glucagon on glucose tolerance in humans. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E283–E290, 1999.—People with type 2 diabetes have defects in both α- and β-cell function. To determine whether lack of suppression of glucagon causes hyperglycemia when insulin secretion is impaired but not when insulin secretion is intact, twenty nondiabetic subjects were studied on two occasions. On both occasions, a “prandial” glucose infusion was given over 5 h while endogenous hormone secretion was inhibited. Insulin was infused so as to mimic either a nondiabetic (n = 10) or diabetic (n = 10) postprandial profile. Glucagon was infused at a rate of 1.25 ng·kg⁻¹·min⁻¹, beginning either at time zero to prevent a fall in glucagon (nonsuppressed study day) or at 2 h to create a transient fall in glucagon (suppressed study day). During the “diabetic” insulin profile, lack of glucagon suppression resulted in a marked increase (P < 0.002) in both the peak glucose concentration (11.9 ± 0.4 vs. 8.9 ± 0.4 mmol/l) and the area above basal of glucose (927 ± 77 vs. 546 ± 112 mmol·l⁻¹·6 h) because of impaired (P < 0.001) suppression of glucose production. In contrast, during the “nondiabetic” insulin profile, lack of suppression of glucagon resulted in only a slight increase (P < 0.02) in the peak glucose concentration (9.1 ± 0.4 vs. 8.4 ± 0.3 mmol/l) and the area above basal of glucose (654 ± 146 vs. 488 ± 118 mmol·l⁻¹·6 h). Of interest, when glucagon was suppressed, glucose concentrations differed only minimally during the nondiabetic and diabetic insulin profiles. These data indicate that lack of suppression of glucagon can cause substantial hyperglycemia when insulin availability is limited, therefore implying that inhibitors of glucagon secretion and/or glucagon action are likely to be useful therapeutic agents in such individuals.

In nondiabetic individuals, insulin concentrations promptly increase and glucagon concentrations promptly decrease after carbohydrate ingestion (1, 6, 21). These hormonal changes act in concert to minimize the postprandial rise in glucose. In contrast, people with type 2 diabetes have defects in both insulin and glucagon secretion (1, 6, 21). As the severity of diabetes increases, insulin secretion decreases, with peak concentrations typically not occurring until 1–2 h after the start of a meal (6, 21). In addition, glucagon concentrations either are not suppressed or, paradoxically, may even increase (6, 21). The relative contribution of these concomitant alterations in insulin and glucagon secretion to postprandial hyperglycemia is currently not known.

Numerous studies have demonstrated that glucagon is a potent stimulus of glucose production, with the magnitude of the effect being dependent on the prevailing insulin concentration (10, 11, 17, 18, 26, 30). We have previously shown that lack of postprandial suppression of glucagon in the presence of relative insulin deficiency can impair glucose tolerance in people with type 1 diabetes (9). On the other hand, we (12) and others (16, 27) also have reported that lack of suppression or even a three- to fourfold increase in glucagon does not alter glucose tolerance in nondiabetic humans under conditions in which insulin secretion is not inhibited. Taken together, these observations suggest that lack of suppression of glucagon is important only in the presence of insulin deficiency, or that the sensitivity to glucagon differs in nondiabetic and type 1 diabetic subjects (23). Because a variety of agents that either inhibit glucagon secretion (8, 25, 32) or antagonize glucagon action (24, 28, 34–36) are currently being developed as therapeutic agents for the treatment of diabetes, it is important to distinguish between these two possibilities. The former explanation would imply that inhibition of glucagon would provide little added benefit if early postprandial insulin secretion also is normalized (e.g., as occurs with glucagon-like peptide-1), whereas the latter explanation would imply that the response to a glucagon antagonist would primarily be determined by the degree of hepatic sensitivity to glucagon.

The present experiments therefore were undertaken to test the hypotheses that lack of glucagon suppression impairs glucose tolerance in the presence of relative insulin insufficiency but does not do so in the presence of insulin sufficiency. To test these hypotheses, we studied nondiabetic subjects under conditions in which suppression or lack of suppression of glucagon was produced by means of somatostatin and appropriately timed glucagon infusions. Responses were determined in the presence of systemic insulin concentrations that mimicked those typically observed in either nondiabetic or type 2 diabetic subjects after food ingestion (1, 6, 21). We report that lack of suppression of glucagon has little if any impact on glucose tolerance when accompanied by a prompt rise in insulin (i.e., such as occurs in nondiabetic individuals) but causes substantial hyperglycemia when the rise in insulin concentration is delayed and decreased (i.e., such as occurs in people with type 2 diabetes mellitus).

METHODS

Subjects. After approval from the Mayo Institutional Review Board, twenty healthy nondiabetic subjects gave written
consent to participate in the study. All subjects were in good health, had normal blood pressure, and were at stable weight. None regularly engaged in vigorous exercise or regularly took medications other than oral contraceptive pills, estrogen, or thyroxin replacement. Except for two subjects, who were adopted and therefore could not provide family history, none had a family history of diabetes mellitus. The characteristics of the subjects are shown in Table 1.

Experimental design. All subjects were studied on two occasions separated by ≥5 days. Subjects were admitted to the General Clinical Research Center at 1700 on the evening before each study. After ingestion of a standard 10 kcal/kg meal (50% carbohydrate, 15% protein, 35% fat) between 1730 and 1800, subjects remained fasting (with the exception of occasional sips of water) until the end of the study. At 0600 on the following morning, an 18-gauge catheter was inserted into a forearm vein and used for all infusions. A cannula also was inserted retrogradely into a dorsal vein of the other hand. The hand was then placed in a heated Plexiglas box and maintained at a temperature of ~55°C to allow sampling of arterialized venous blood.

A primed-continuous (110 μCi prime, 1.10 μCi/min continuous) infusion of NaH\(^{14}\)CO\(_3\) (New England Nuclear, Boston, MA) was started at 0600, and a primed-continuous (10–12 μCi prime, 0.10–0.12 μCi/min continuous) infusion of [6-\(^{3}\)H]glucose (New England Nuclear) was started at 0700. Beginning at 1000, the infusion rate of \[^{6}\text{-}^{3}\text{H}\]glucose was varied so as to approximate the anticipated pattern of change of endogenous glucose release (2). In addition, all infused glucose contained \[^{6}\text{-}^{3}\text{H}\]glucose. This resulted in maintenance of plasma glucose specific activity within 15% of basal on all study days, permitting accurate measurement of glucose turnover.

All subjects were studied on two occasions in random order. On both occasions, endogenous hormone secretion was inhibited with somatostatin (60 ng·kg\(^{-1}\)·min\(^{-1}\); Bachem California, Torrance, CA). On one occasion, glucagon (Eli Lilly, Indianapolis, IN) was given at a rate of 1.25 ng·kg\(^{-1}\)·min\(^{-1}\) throughout the study in an effort to maintain portal venous glucose concentrations constant. On the other occasion, no glucagon was given from 0 to 120 min, and then an infusion of glucagon was given at a rate of 1.25 ng·kg\(^{-1}\)·min\(^{-1}\) from 121 to 300 min, thereby permitting glucagon to fall during the first 2 h as normally occurs in non diabetic individuals after carbohydrate ingestion (16, 21, 27). Growth hormone (Genentech, South San Francisco, CA) was infused at a rate of 3.0 ng·kg\(^{-1}\)·min\(^{-1}\) to maintain basal levels.

Infusions of glucose and insulin (Eli Lilly) also were initiated at 1000. All subjects received a variable intravenous glucose infusion designed to mimic the systemic rate of appearance normally observed after ingestion of 50 g of glucose (2). Ten volunteers were infused with insulin in a manner designed to mimic a normal nondiabetic postprandial insulin profile, whereas the other 10 subjects were infused with insulin in a manner designed to mimic a “diabetic” postprandial insulin profile (1). The same amount of insulin was given with both infusions.

Arterialized-venous blood was collected at regular intervals for measurement of glucose and hormone concentrations as well as [\(^{6}\text{-}^{3}\text{H}\)]glucose and \[^{14}\text{C}\]glucose specific activities. Breath was collected to measure the \[^{14}\text{C}\]co2 specific activity, as described previously (20).

Analytic techniques. Arterialized plasma samples were placed on ice, centrifuged at 4°C, separated, and stored at −20°C until assay. Plasma C-peptide and glucagon concentrations were measured by radioimmunoassay by use of reagents purchased from Linco Research (St. Louis, MO). Plasma insulin and growth hormone concentrations were measured using reagents provided by Beckman Laboratories (Chaska, MN). Plasma \[^{14}\text{C}\]glucose and [\(^{6}\text{-}^{3}\text{H}\)]glucose specific activities were determined as previously described (3, 20). Body composition was measured by dual-energy X-ray absorptiometry (DEXA scanner; Hologic, Waltham, MA). Glucose and lactate concentrations were measured using a Yellow Springs glucose and lactate analyzer (Yellow Springs Instruments, Yellow Springs, OH).

Calculations. Glucose specific activities were smoothed using the method of Bradley et al. (4). Glucose appearance and disposal were calculated using the non-steady-state equations of Steele et al. (29), in which the actual tracer infusion rate for each interval was used in the calculation. The volume of distribution of glucose was assumed to equal 200 ml/kg, and the pool correction factor was assumed to be 0.65 (5). Endogenous glucose production was determined by subtracting the glucose infusion rate from the tracer-determined rate of glucose appearance. All rates of infusion and turnover were expressed per kilogram of lean body mass. The fraction of glucose derived from \[^{14}\text{CO}_2\] was calculated by dividing the plasma \[^{14}\text{C}\]glucose specific activity by breath \[^{14}\text{CO}_2\] specific activity (20).

Statistical analysis. Data in Table 1, Figs. 1–7, and the text are expressed as means ± SE. Values observed from -30 to 0 min on each study day were averaged for each individual and considered as basal. Area above or below basal was calculated using the trapezoidal rule. If a study was terminated due to hypoglycemia (defined as a glucose concentration of <3.3 mmol/l), the data up to that time were used for calculation of area under the curve on both study days. Two-tailed paired and nonpaired Student’s t-tests were used to test for within- and between-group differences. A P value of <0.05 was considered to be statistically significant.

RESULTS

Plasma insulin concentrations. Insulin concentrations were comparable both before and during the insulin infusions on the glucagon-suppressed and glucagon-nonsuppressed study days (Fig. 1). Insulin concentrations peaked earlier (33 ± 6 vs. 125 ± 5 min) during the nondiabetic than during the diabetic insulin profile. Plasma glucagon concentrations. Glucagon concentrations did not differ during the baseline period (i.e., from

<p>| Table 1. Subject characteristics |</p>
<table>
<thead>
<tr>
<th>n</th>
<th>Age, yr</th>
<th>Gender, F/M</th>
<th>TBW, kg</th>
<th>BMI, kg/m²</th>
<th>LBM, kg</th>
<th>%BF</th>
<th>GlycoHb, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic insulin profile</td>
<td>10</td>
<td>25.40 ± 3.60</td>
<td>5/5</td>
<td>69.17 ± 4.45</td>
<td>23.61 ± 1.53</td>
<td>47.10 ± 3.25</td>
<td>24.14 ± 3.82</td>
</tr>
<tr>
<td>Nondiabetic insulin profile</td>
<td>10</td>
<td>26.70 ± 1.65</td>
<td>5/5</td>
<td>74.15 ± 4.30</td>
<td>24.56 ± 0.96</td>
<td>51.41 ± 3.81</td>
<td>23.87 ± 3.28</td>
</tr>
</tbody>
</table>

Values are means ± SE. TBW, total body weight; BMI, body mass index; LBM, lean body mass; %BF, percent body fat; GlycoHb, glycosylated hemoglobin.
Plasma C-peptide and growth hormone concentrations. Plasma C-peptide concentrations did not differ during either insulin infusion on either study day, with somatostatin producing prompt and equal suppression of insulin secretion on all occasions (Fig. 3). Growth hormone concentrations also did not differ on the suppressed and nonsuppressed study days.

Plasma glucose concentrations. Fasting plasma glucose concentrations were comparable in both groups on both study days (Fig. 4). Plasma glucose concentrations rose during nondiabetic insulin and prandial glucose infusions, reaching a peak at \( \sim 30 \) min. Both the peak glucose concentration (9.1 \pm 0.4 vs. 8.4 \pm 0.3 mmol/l) and the area above basal of glucose (654 \pm 146 vs. 488 \pm 118 mmol \cdot l^{-1} \cdot 6 h) were slightly, but significantly \((P < 0.05)\) greater on the glucagon-nonsuppressed than the glucagon-suppressed study days. To avoid the confounding effects of glucose counterregulation, the study was terminated before 300 min if glucose concentration fell below 3.3 mmol/l. Glucose fell below 3.3 mmol/l in three subjects on the glucagon-nonsuppressed study day (one at 135 min and two at 210 min) but in no subjects on the glucagon-suppressed study day.

Glucose concentrations rose during the diabetic insulin and prandial glucose infusions, reaching a peak at \( \sim 75 \) min. Both the peak (11.9 \pm 0.4 vs. 8.9 \pm 0.4 mmol/l) and the area above basal (927 \pm 77 vs. 546 \pm 112 mmol \cdot l^{-1} \cdot 6 h) were greater \((P < 0.002)\) on the glucagon-nonsuppressed than glucagon-suppressed study days. As anticipated, the difference in glucose concentration (623 \pm 39 vs. 350 \pm 41 mmol \cdot l^{-1} \cdot 2 h) was most marked \((P < 0.001)\) during the first 2 h, when glucagon concentrations also differed. Glucose concentrations fell below 3.3 mmol/l in 5 subjects on the glucagon-suppressed study day (from 180 to 300 min) and in 3 subjects on the glucagon-nonsuppressed study day (from 210 to 300 min).

Glucose disappearance. Plasma glucose concentration is determined by the balance between the rates of glucose appearance and disappearance. The increase in glucose disappearance above basal did not differ during either the nondiabetic (3.27 \pm 0.38 vs. 3.10 \pm 0.27 mmol \cdot kg^{-1} \cdot 6 h) or the diabetic (3.31 \pm 0.29 vs. 2.79 \pm 0.29 mmol \cdot kg^{-1} \cdot 6 h) insulin profiles on the glucagon-nonsuppressed and glucagon-suppressed study days (Fig. 5). Rates of glucose disappearance during the first 2 h of study, if anything, tended to be greater on the glucagon-nonsuppressed than the glucagon-suppressed study days, with the difference being more evident during the diabetic (2.33 \pm 0.13 vs. 1.97 \pm 0.16 mmol \cdot kg^{-1} \cdot 2 h) than the nondiabetic (2.40 \pm 0.22 vs. 2.23 \pm 0.01 mmol \cdot kg^{-1} \cdot 2 h) insulin profile, indicating that the higher prevailing glucose concentrations did not result from lower rates of disappearance. Although glucose disappearance during the first 2 h of the nondiabetic and diabetic insulin profiles was similar on
the nonsuppressed study days, this occurred in the presence of markedly different plasma glucose concentrations. This suggests that the rapid increase in insulin was primarily responsible for the increase in glucose clearance during the first 2 h of the nondiabetic study day, whereas the higher glucose concentrations were primarily responsible for the increase in glucose clearance on the diabetic study day.

Endogenous glucose production. The combined effect of the nondiabetic insulin profile and the concomitant rise in glucose concentrations resulted in prompt inhibition of endogenous glucose production, followed by a subsequent rise on both the glucagon-nonsuppressed and glucagon-suppressed study days (Fig. 6). Although total integrated rates over the entire period of study did not differ on the glucagon-nonsuppressed and glucagon-suppressed study days (2.98 ± 0.36 vs. 2.89 ± 0.26 mmol·kg⁻¹·h⁻¹), endogenous glucose production was less inhibited (P < 0.01) during the first 2 h (i.e., when glucagon concentrations differed) of the glucagon-nonsuppressed than the glucagon-suppressed study days (−0.57 ± 0.15 vs. −1.08 ± 0.06 mmol·kg⁻¹·h⁻¹).

Whereas the combined effect of the diabetic insulin profile and the rise in glucose concentration resulted in a prompt decrease in endogenous glucose production on the glucagon-suppressed study day, glucose production transiently increased on the glucagon-nonsuppressed study day. This resulted in substantially less (P < 0.001) inhibition of glucose production during the first 2 h of the glucagon-nonsuppressed than the glucagon-
suppressed study days (0.19 ± 0.12 vs. −0.90 ± 0.11 mmol·kg\(^{-1}\)·2 h), resulting in greater (P < 0.01) overall rates of production on the nonsuppressed than suppressed study days (3.32 ± 0.26 vs. 2.31 ± 0.31 mmol·kg\(^{-1}\)·6 h).

Of interest, despite marked differences in plasma insulin concentrations during the first 2 h of the nondiabetic and diabetic insulin profiles, the degree of inhibition of endogenous glucose production did not differ on the two glucagon-suppressed study days (−1.07 ± 0.06 vs. −0.90 ± 0.11 mmol·kg\(^{-1}\)·2 h; P = 0.18). These findings indicate that suppression of glucagon may in itself be sufficient to restore inhibition of endogenous glucose production and reduction in prandial glucose excursion in people with type 2 diabetes mellitus who have impaired insulin secretion. On the glucagon-suppressed study days, the rates of endogenous glucose production during the last 4 h of the diabetic insulin profiles were slightly (but significantly) lower (P < 0.05) than those observed during nondiabetic insulin profiles (area above baseline: −0.48 ± 0.22 vs. 0.13 ± 0.13 mmol·kg\(^{-1}\)·4 h).

Incorporation of \(^{14}\)CO\(_2\) into glucose. The percentage of glucose derived from \(^{14}\)CO\(_2\) decreased to a comparable extent during the first 2 h of the nondiabetic and diabetic insulin profiles on both the glucagon-nonsuppressed and glucagon-suppressed study days (Fig. 7), implying similar suppression of gluconeogenesis. Thereafter, incorporation of \(^{14}\)CO\(_2\) into glucose increased, with the magnitude of the increase being equivalent during the latter portion of the diabetic insulin profile but greater (P < 0.05) during the latter portion of the nondiabetic insulin profile on the glucagon-nonsuppressed than the glucagon-suppressed day (−13.2 ± 1.4 vs. −17.6 ± 1.7% during the last 2 h).

Plasma lactate concentrations. Plasma lactate concentrations did not differ before or during the nondiabetic and diabetic insulin profiles on either day (data not shown).

**DISCUSSION**

People with type 2 diabetes have abnormalities in both \(\alpha\)- and \(\beta\)-cell function (1, 6, 21). Carbohydrate ingestion either fails to suppress or causes a paradoxical increase in plasma glucagon concentrations (1, 6, 21). In addition, insulin secretion decreases with increasing severity of diabetes, with peak postprandial insulin concentrations becoming progressively decreased and delayed (1, 6, 21). The present studies demonstrate that in the absence of an appropriately timed increase in insulin (i.e., during the diabetic insulin profile), lack of suppression of glucagon can cause substantial hyperglycemia. Lack of suppression of glucagon resulted in an almost twofold increase in the integrated glycemic response and a 3.0 mmol/l (~50 mg/dl) increase in the peak glucose concentration. Lack of suppression of glucagon caused hyperglycemia by impairing glucose- and insulin-induced inhibition of endogenous glucose production. In contrast, lack of suppression of glucagon only had a minimal effect on glucose tolerance when insulin concentrations rapidly increased (i.e., during the nondiabetic insulin profile). Of particular interest, suppression of glucagon in itself was sufficient to restore glucose concentrations observed during the diabetic insulin profile to almost the same levels as were observed during the nondiabetic insulin profile. These data suggest that lack of suppression of glucagon, by impairing suppression of endogenous glucose production, potentially can contribute to postprandial hyperglycemia in people with limited...
insulin secretory reserve (e.g., people with type 2 diabetes). They also imply that inhibitors of glucagon secretion and/or glucagon action are likely to be useful therapeutic agents in such individuals.

Numerous in vitro and in vivo studies have emphasized the importance of the portal venous glucagon-to-insulin ratio in the regulation of hepatic glucose production (10, 17, 30). The observation that lack of suppression of glucagon caused greater hyperglycemia when the increase in insulin concentrations was delayed (i.e., the diabetic insulin profile) than when there was a prompt increase in insulin (i.e., the nondiabetic insulin profile) is consistent with this concept. As discussed earlier (9), the peripheral venous glucagon concentrations of ~150–160 ng/l present during the replacement glucagon infusions (i.e., from 0 to 300 min on the nonsuppressed days and from 121 to 300 min on the suppressed days) likely represented either no change or a slight increase in portal glucagon concentrations relative to those present before the somatostatin infusion. In this instance, it is highly probable that the tenfold rise in circulating insulin concentrations that occurred during the first 30 min of the nondiabetic insulin profile resulted in a substantial decrease in the portal venous glucagon-to-insulin ratio, regardless of whether or not glucagon was suppressed. Conversely, during the first 30 min of the diabetic insulin profile, peripheral insulin concentrations only increased from ~20 to ~60 pmol/l, and the C-peptide concentrations decreased threefold. This suggests that the portal venous insulin, and hence, the glucagon-to-insulin ratio, either stayed the same or increased slightly, depending on whether portal venous glucagon concentrations stayed the same or increased slightly. The transient increase in endogenous glucose production followed by a subsequent delay in suppression suggests the latter. This pattern is remarkably similar to that previously observed in people with type 2 diabetes when the dual-isotope technique was used to trace endogenous glucose production after ingestion of a meal (6, 20). Although in the past we have been concerned that the apparent paradoxical increase in glucose production was an artifact of tracer non steadiness, the present studies suggest that this may not be the case. Instead, it may be due to a brief paradoxical increase in glucagon (21), resulting in a transient increase in the portal venous glucagon-to-insulin ratio. If so, then agents that inhibit glucagon secretion or antagonize glucagon action may be particularly effective in restoring the rate of suppression of postprandial glucose production in people with type 2 diabetes toward that observed in nondiabetic individuals.

In nondiabetic individuals, ingestion of carbohydrate results in a rise in glucose and insulin that in turn suppresses glucagon secretion (37). This is followed several hours later by an increase in glucagon secretion that accompanies the fall in glucose and insulin concentrations back toward basal levels. Inhibition of this late compensatory increase in glucagon secretion can cause late postprandial hypoglycemia (33). A similar phenomenon appears to have occurred in the present experiments. Infusion of the prandial glucose and nondiabetic insulin profiles on the glucagon-suppressed study day resulted in a nearly normal glycemic excursion, followed by a smooth return of glucose to basal levels. In contrast, although glucose concentrations peaked slightly higher on the glucagon-nonsuppressed day, the study had to be stopped early (i.e., 135–210 min) in three individuals because of a fall in glucose to a level below 3.3 mmol/l. As is evident in Fig. 6, this presumably resulted from the fact that the late compensatory increase in glucose production was blunted by the lack of a concomitant late increase in glucagon. This observation is consistent with previous studies showing that although the hepatic response to constant glucagon wanes with time, it continues to respond to a change in glucagon concentration regardless of when it occurs (10, 11, 26).

Persistent and inappropriately elevated insulin concentrations also can cause hypoglycemia. Late hypoglycemia was commonly observed with the diabetic insulin profile, with the study being stopped in three subjects on the glucagon-nonsuppressed and in five subjects on the glucagon-suppressed study day. This likely reflects the fact that the diabetic insulin profile resulted in slightly but persistently higher insulin concentrations during the latter part of the study. The higher insulin concentrations presumably blunted the compensatory increase in glucose production that accompanied the increase in glucagon concentration on the glucagon-suppressed day. Glucose production was even more blunted on the glucagon-nonsuppressed day. However, fewer individuals developed hypoglycemia on the glucagon-nonsuppressed day because their glucose concentration was falling from a far higher peak. These data highlight a potential problem that may occur with the use of glucagon antagonists to treat diabetes. The onset of action of these agents will need to coincide with the period of time during which glucose concentrations are rising, and the duration of action will need to be carefully matched to insulin availability. If not, lower peak postprandial glucose concentrations may be achieved at the expense of a greater frequency of hypoglycemia occurring 4–5 h after meal ingestion.

The present experiments only measured the rate of glucose release into the systemic circulation. They therefore cannot determine the site of origin of the glucose. However, recent studies using the renal vein catheterization technique indicate that glucagon has little if any effect on glucose production by the kidney, suggesting that glucagon primarily acts by modulating hepatic glucose release (31). Glucose released by the liver originates from either glycogenolysis or gluconeogenesis. As discussed in detail elsewhere (20), incorporation of 14CO2 into glucose measures flux through the pyruvate carboxylase pathway. It therefore primarily traces new glucose synthesis from pyruvate, lactate, and alanine. Plasma lactate concentrations did not differ, and the percentage of glucose derived from 14CO2 was equally inhibited during the initial portion of the nondiabetic and diabetic insulin profiles on both the glucagon-suppressed and glucagon-nonsuppressed
study days. This implies comparable inhibition of gluconeogenesis on all occasions and that the observed difference in endogenous glucose production on the suppressed and nonsuppressed study days was primarily due to differences in glycogenolysis. Of note, the percent incorporation of $^{14}$CO$_2$ into glucose appeared to increase more rapidly during the latter portion of the nondiabetic insulin profile on the nonsuppressed study day, implying that glucagon exerted a late stimulatory effect on gluconeogenesis. This pattern was not observed during the diabetic insulin profile, presumably because of the higher plasma insulin concentrations that were present over this interval. These observations are consistent with reports by Cherrington et al. (7) that glucagon causes a time-dependent increase in gluconeogenesis that is more evident in the presence of low compared with high insulin concentrations. However, this post hoc finding needs to be interpreted with caution, because incorporation of $^{14}$CO$_2$ into glucose only provides a qualitative estimate of gluconeogenesis and because the specific activity within the hepatic oxaloacetate pool is not known. Furthermore, the contribution of glycerol to gluconeogenesis is not measured with this technique (20). However, because the dilution of the oxaloacetate pool appears to remain relatively constant over a wide range of metabolic conditions (14), and because glucagon does not alter either lipolysis (13) or intrahepatic glycerol extraction (19), any error is likely to be small.

Only a single diabetic and nondiabetic insulin profile was evaluated. Although insulin secretion varies considerably in diabetic individuals, the pattern of insulin concentration that we chose to create (i.e., decreased and delayed) closely mimics that commonly observed after food ingestion in people with moderately severe type 2 diabetes mellitus (1, 6, 21). On the other hand, people with a lesser impairment in β-cell function frequently have postprandial insulin concentrations that are intermediate between those produced by the nondiabetic and diabetic insulin profiles (6). We anticipate that effects of lack of suppression of glucagon in such individuals also would be intermediate between those observed in the present experiments with the two insulin profiles. We studied lean, healthy, nondiabetic subjects. We did so because we wished to evaluate whether glucagon’s effect on the liver was dependent on the time course of change of plasma insulin concentrations. We chose not to study individuals who were markedly insulin resistant (e.g., severely obese type 2 diabetic subjects), because the impact of differences in insulin availability may be moot under conditions in which insulin has little if any biological effect. Furthermore, we have previously shown that hepatic sensitivity to glucagon does not differ in nondiabetic and type 2 diabetic subjects (22). Nevertheless, future studies in people with type 2 diabetes with varying degrees of insulin resistance will be of considerable interest, because these are the individuals in whom glucagon antagonists are likely to be used. Finally, because these experiments were performed in humans, insulin and glucagon were infused into the systemic rather than the portal venous circulation. We doubt whether this substantially influenced the outcome, since glucagon is not known to have any effects on peripheral tissues and because portal and peripheral insulin infusion appears to have comparable effects on glucose production under conditions in which glucagon secretion is controlled (15). Perhaps more importantly, from the perspective of the present experiments, endogenous insulin secretion was equally inhibited by somatostatin, insuring equivalent portal venous insulin concentrations on the glucagon-suppressed and glucagon-nonsuppressed study days.

In summary, the present experiments demonstrate that lack of suppression of glucagon causes marked hyperglycemia under conditions of relative insulin insufficiency. Conversely, suppression of glucagon substantially improves glucose tolerance, even though the pattern of insulin availability remains abnormal (i.e., mimicking that observed in people with type 2 diabetes). Lack of suppression of glucagon minimally alters glucose tolerance when insulin concentrations rapidly rise, once again confirming the concept that an increase in the portal venous insulin-to-glucagon ratio inhibits endogenous glucose production regardless of the absolute glucagon concentration. Although these experiments suggest that agents that either inhibit glucagon secretion or antagonize glucagon action may result in a considerable improvement in glycemic control in people with diabetes, they also introduce a note of caution, because they lend further support to results in the report of Tse et al. (33) that prolonged suppression of glucagon may increase the risk of late postprandial hypoglycemia. Therefore, it is likely that ability to appropriately craft the rapidity of onset and offset of such agents will determine whether their benefits will outweigh their risks.

We thank C. Ettet, T. Madson, and B. Dickie for technical assistance; M. Davis for assistance in the preparation of the manuscript; and the staff of the Mayo General Clinical Research Center for assistance in performing the studies. This study was supported by the National Institutes of Health (DK-29953 and RR-00858) and the Mayo Foundation. Dr. P. Shah was supported in part by a research grant from Novo-Nordisk.

Address for correspondence and reprint requests: R. A. Rizza, Endocrine Research Unit, Mayo Clinic, 200 First St. S.W., Rochester, MN 55905.

Received 19 January 1999; accepted in final form 15 April 1999.

REFERENCES


