Role of Hyperglucagonemia in Maintenance of Increased Rates of Hepatic Glucose Output in Type II Diabetics

ALAIN D. BARON, LINDA SCHAEFFER, PAUL SHRAGG, AND ORVILLE G. KOLTERMAN

SUMMARY
Elevated rates of basal hepatic glucose output (bHGO) are significantly correlated with the fasting serum glucose (FSG) level in subjects with non-insulin-dependent diabetes mellitus (NIDDM). This observation suggests that bHGO is a major determinant of the severity of the diabetic state in these subjects. In addition, basal glucagon levels (bGL) are higher in these diabetics than in control subjects, despite the concurrent basal hyperglycemia and hyperinsulinemia, two factors known to suppress glucagon secretion. Although bGL is responsible for sustaining two-thirds of bHGO in normal humans, its role in sustaining elevated rates of bHGO in NIDDM has not been previously defined. To this end, we have studied 13 normal and 10 NIDDM subjects; mean FSG levels were 90 ± 2 and 262 ± 21 mg/dl, respectively (P < .001). The mean fasting serum insulin and glucagon levels were higher in the diabetics than in the controls: 17 ± 2 vs. 9 ± 1 μU/ml (P < .01) and 208 ± 37 vs. 104 ± 15 pg/ml (P < .01), respectively. On separate days, HGO was assessed isotopically (with 3-3H]glucose) in the basal state and during infusion of somatostatin (SRIF) (600 μg/h) alone and in conjunction with replacement infusions of glucose and insulin. The results demonstrate that 1) bHGO is higher in diabetics than in controls: 145 ± 12 vs. 89 ± 3 mg m⁻² · min⁻¹, P < .01); 2) during infusion of SRIF alone, HGO was suppressed by 25% (P < .05) and 34% (P < .05) of the basal value in controls and diabetics, respectively; 3) when the studies were repeated with glucose levels held constant at or near the FSG level by the glucose-clamp technique, the pattern and degree of HGO suppression was similar to that obtained by infusion of SRIF alone; 4) during isolated glucagon deficiency (SRIF + insulin, 5 mU · m⁻² · min⁻¹, with serum glucose maintained at basal level), HGO was suppressed by 71 ± 8% of the basal value in controls (P < .001) and by 58 ± 7% in diabetics (P < .001); and 5) when isolated glucagon deficiency with similar hyperglycemia was created in control subjects, HGO was suppressed by 87% of the basal value. We conclude that 1) elevated glucagon levels contribute significantly to the elevated rates of bHGO in NIDDM subjects, 2) basal glucagon levels sustain 71% of basal HGO in control and 58% in NIDDM subjects, and 3) the excess glucagon effect is largely responsible for the apparent hepatic insulin resistance seen in NIDDM subjects. Diabetes 36:274–83, 1987

Elevated rates of basal hepatic glucose output (bHGO) have been documented by numerous investigators in patients with type II [non-insulin-dependent diabetes mellitus (NIDDM)] (1–8). In addition, a strong correlation exists between the degree of fasting hyperglycemia and the rate of bHGO in NIDDM subjects (1–6), suggesting that this parameter is an important determinant of the severity of the diabetic state. In support of this concept, recent reports have indicated that the degree of improvement in the diabetic state (glycemic control) is strongly related to the concomitant reduction in the rate of bHGO that occurs as a result of the therapeutic intervention. This relationship is consistently found when NIDDM patients are managed by caloric restriction and weight reduction (4) or are treated with sulfonylurea agents (5–7) or with intensive insulin therapy (3,8).

Based on these observations, the basal rate of HGO is apparently critically important in the pathogenesis of NIDDM. Therefore, it is important to elucidate the factors that control and sustain hepatic glucose release in NIDDM subjects. Therefore, our study was designed to evaluate the role of glucagon in maintaining the elevated rates of bHGO in NIDDM subjects. Glucagon has been shown to play a significant role in maintaining bHGO in normal humans (9), and elevated basal glucagon levels have been reported in...
NIDDM subjects (10,11). However, the role of glucagon in sustaining the elevated rates of bHGO observed in NIDDM subjects has not been studied previously.

To define this role for glucagon, the interactions between factors known to acutely influence HGO, particularly the elevated rates of HGO in NIDDM subjects.

**MATERIALS AND METHODS**

**MATERIALS**

Porcine monocomponent insulin was generously supplied by Dr. Ronald Chance of Eli Lilly (Indianapolis, IN), and purified pork insulin was obtained from Squibb-Nov0 (Wil10n, CN); 125 I-labeled insulin, 125 I-labeled glucagon, and 3-

**SUBJECTS**

The study group consisted of 13 control subjects and 10 subjects with NIDDM, as defined by the criteria of the National Diabetes Data Group (12). The clinical and metabolic characteristics of the subjects are summarized in Table 1.

**TABLE 1**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>N</th>
<th>Age (yr)</th>
<th>RBW</th>
<th>BMI</th>
<th>FS glucose (mg/dl)</th>
<th>FS insulin (µU/ml)</th>
<th>FS glucagon (pg/ml)</th>
<th>FS C-peptide (pmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13</td>
<td>36 ± 2</td>
<td>1.00 ± 0.03</td>
<td>23 ± 1</td>
<td>90 ± 2</td>
<td>9 ± 1</td>
<td>104 ± 15</td>
<td>0.82 ± 0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(25–45)</td>
<td>(0.77–1.16)</td>
<td>(19–28)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIDDM</td>
<td>10</td>
<td>52 ± 4*</td>
<td>1.10 ± 0.04*</td>
<td>26 ± 1†</td>
<td>262 ± 21‡</td>
<td>17 ± 2†</td>
<td>208 ± 37‡</td>
<td>1.38 ± 0.18‡</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(25–69)</td>
<td>(0.91–1.26)</td>
<td>(23–31)</td>
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</tr>
</tbody>
</table>

Data are means ± SE. Values in parentheses are ranges. RBW, relative body wt; BMI, body mass index; FS, fasting serum.

*P < .05.
†P < .01.
‡P < .001.

**DIET**

All subjects were placed on a liquid formula diet (32 cal/kg), designed to maintain their body weight, given three feedings containing 20, 40, and 20% of the total daily calories at 0800, 1200, and 1700 h, respectively. The diet contained 45% carbohydrate, 40% fat, and 15% protein. All subjects were on the study diet for at least 48 h before any measurements were performed and remained on the diet throughout the study period.

**HEPATIC GLUCOSE OUTPUT**

The rate of glucose appearance (Ra) and overall glucose disappearance (Rd) were quantitated in the basal state and during each of the infusion protocols by infusing 3-[3H]glucose in a primed continuous manner (13). With this technique, 50 µCi of tracer is injected as a bolus followed by a continuous infusion at the rate of 0.50 µCi/min. After an equilibration period of 60 min, blood samples were obtained at 20-min intervals for the determination of both the concentration and specific activity of serum glucose. Ra and Rd were then calculated from the Steele equations in their modified derivative form (13,14) because the tracer exhibits non-steady-state kinetics under these conditions. When exogenous glucose is not being administered, Rd represents the rate of HGO. When exogenous glucose is being administered, Rd represents the sum of the infusion rate of exogenous glucose (Ig) and HGO, and therefore HGO = Rd – Ig.

**STUDY CONDITIONS**

Five different study conditions were used to allow the independent effects of glucagon, insulin, and glucose on HGO to be assessed. Each study period was of 200 min duration, and each condition was assessed on a separate day with patients in the postabsorptive state. It was not feasible to assess all conditions in each of the subjects.

1. **Basal study.** On this day, HGO was assessed isotopically after an overnight fast. Available data indicates that bHGO remains relatively constant from day to day providing the fasting glucose level remains constant. Therefore, any subject whose fasting serum glucose level subsequently varied at San Diego and remained active to approximate their prehospital exercise levels. Therapy with oral hypoglycemic agents was discontinued for at least 3 wk before and exogenous insulin therapy was withdrawn for at least 10 days before the study. All subjects were euthyroid, and no subject had a concurrent disease or was ingesting pharmacologic agents known to affect carbohydrate metabolism.
by >10% from one day to another was excluded from the study. In normal subjects, the fasting glucose levels and basal rates of HGO are known to be quite stable over time (9). Therefore, in each control subject, basal Rb was measured over three 20-min intervals after a 60-min period of tracer equilibration. The mean of these values was used to represent the mean bHGO. In each diabetic subject, basal Rb was measured during 10 consecutive 20-min intervals after a 60-min equilibration period, and the mean of these 10 values was used as the representative bHGO for the subject.

2. Somatostatin alone. On this day, SRIF (600 μg/h) was infused for 200 min to assess the effects of combined insulin and glucagon deficiency. It was necessary to characterize this condition because it serves as the baseline for the next three study conditions. An SRIF infusion rate of 600 μg/h has been shown to provide significant suppression of the glucoregulatory hormones without inducing major alterations in hepatic blood flow (9).

3. Somatostatin with glucose held constant. On this day, the SRIF infusion was administered as described above. Because HGO falls more rapidly than Rb during the infusion of SRIF, the serum glucose level falls initially and returns toward baseline during continued SRIF infusion, i.e., beyond 2 h. To ensure that this late rise in the glucose level was not secondary to an initial hypoglycemic stimulus and to evaluate the continued suppressive effects of the basal glucose level during combined insulin and glucagon deficiency, the serum glucose level was maintained at the basal level by means of an exogenous infusion of 20% dextrose by the glucose-clamp technique (17). During these studies, the glucose infusion was discontinued if the serum glucose level rose above the basal level.

4. Somatostatin with insulin replacement and glucose held constant (glucagon deficiency). On this day, SRIF was infused and the glucose level was held constant at the basal level, as previously described, during a concomitant insulin infusion (5 mU·m⁻²·min⁻¹), which created a condition of isolated glucagon deficiency. The insulin infusion rate was chosen to achieve basal peripheral insulin levels similar to those found in the control subjects (10 μU/ml). This condition was designed to assess the effect of isolated glucagon deficiency on bHGO.

5. Somatostatin with insulin replacement and hyperglycemia. This condition was designed to permit direct comparison of HGO suppression under similar conditions in control and NIDDM subjects. To achieve this, four control subjects were infused with SRIF and replacement insulin (5 mU·m⁻²·min⁻¹) and their serum glucose levels were raised, via an exogenous glucose infusion, and held at a level of 250 mg/dl. Thus, parameters similar to those of the diabetics studied under condition 4 were reproduced.

**ANALYTICAL METHODS**

Blood for serum glucose determination was drawn, placed in untreated polypropylene tubes, and spun in a Beckman microfuge (Spinco, Palo Alto, CA). The glucose concentration of the supernatant was then measured immediately by the glucose oxidase method in a YSI glucose analyzer (Yellow Springs, OH).

Blood for determination of serum insulin level and plasma glucose specific activity was collected in untreated and NaF-treated tubes, respectively. Insulin samples were allowed to clot, and samples for glucose specific activity were placed on ice immediately. The specimens were spun, and the serum and plasma were removed and stored at −20°C until the determinations were made. Serum insulin levels were measured by double-antibody radioimmunoassay (18). In the NIDDM subjects previously treated with insulin, free insulin levels were determined according to the method of Kuzuya et al. (19). Blood for determination of plasma glucagon and C-peptide levels were collected in tubes containing Trasylol (500 μU/ml) and EDTA and Trasylol, respectively. C-peptide samples were placed on ice. Glucagon and C-peptide samples were processed in a manner similar to that of insulin samples and were analyzed by the appropriate radioimmunoassay (19,20). The C-peptide measurements were performed in Dr. A. H. Rubenstein's laboratory at the University of Chicago.

**Data analysis.** All calculations were performed on the CLINFO system operational on the digital PDP 11-24 computer of the Clinical Research Center at the University of California at San Diego Medical Center.

Statistical analysis was done by two-tailed Student's t test for paired and unpaired data as indicated. Data represent means ± SE unless otherwise stated.

**RESULTS**

**Basal serum glucose and hormone levels.** As shown in Table 1, the FSG levels were significantly higher in NIDDM than in control subjects: 262 ± 21 vs. 90 ± 2 mg/dl (P < .001). Similarly, basal serum insulin and C-peptide levels were higher in NIDDM than in control subjects: 17 ± 2 vs. 9 ± 1 μU/ml (P < .01) and 1.38 ± 0.18 vs. 0.82 ± 0.08 pmol/ml (P < .001), respectively. Basal glucagon levels showed large individual variation, ranging from 68 to 178 pmol/l.

![Fig. 1. Basal rates of HGO in control (N = 13) and NIDDM (N = 10) subjects. Data are means ± SE.](http://diabetesjournals.org/diabetes/article-pdf/36/3/274/355487/36-3-274.pdf)
pg/ml in control subjects and from 113 to 450 pg/ml in NIDDM subjects. However, mean levels were significantly higher in the diabetic than in the control subjects: 208 ± 37 vs. 104 ± 15 pg/ml (P < .001).

**Basal hepatic glucose output.** In NIDDM subjects, bHGO tended to progressively fall over time, but the value quantitated during the first hour was not statistically different from the value for the last hour of measurement: 160 ± 14 vs. 128 ± 15 mg · m⁻² · min⁻¹. Similarly, the mean serum glucose level (262 ± 21 mg/dl) fell -12% (to 231 ± 20 mg/dl) over the 200-min period. Thus, bHGO was taken as the mean value obtained over the entire 200-min study period, which followed the initial 60-min equilibration period in the NIDDM subjects. This value was 66% higher than the corresponding value for control subjects: 145 ± 12 vs. 89 ± 3 mg · m⁻² · min⁻¹ (P < .01) (Fig. 1). As indicated previously, the glucose level was clamped at the initial level determined each morning in each subject, providing it was within 10% of the initial value obtained during the basal measurement.

Consistent with previous reports (1,3), we found a positive and significant correlation between the basal rate of HGO and the FSG level in NIDDM subjects (r = .76, P < .01). Thus, the NIDDM subjects with the most elevated bHGO levels had the highest FSG levels. No significant relationship could be demonstrated between bHGO and either the basal insulin or glucagon levels in these subjects: r = .27 (P not significant) and r = .36 (P not significant), respectively.

**Somatostatin alone.** Figure 2 illustrates mean data obtained during the infusion of SRIF (600 μg/h) in eight control and seven NIDDM subjects. As can be seen in the top panels, glucagon fell by 41 ± 3% in control and by 44 ± 3% in NIDDM subjects to mean levels of 73 ± 1 and 119 ± 26 pg/ml, respectively. Similarly, insulin levels dropped to mean levels at or below the detectable limit of the assay (4 μU/ml) in both groups. Paralleling insulin levels, the C-peptide levels were suppressed by >92% in both groups, achieving steady-state levels of 0.04 ± 0.01 pmol/ml in control and 0.13 ± 0.03 pmol/ml in NIDDM subjects (data not shown). The suppression of endogenous hormone secretion obtained in this study was sustained throughout the experimental period; furthermore, this degree of suppression was obtained reliably during SRIF infusion under each of the subsequent study conditions.

These hormonal changes were accompanied by a rapid and significant initial fall in HGO in both groups (Fig. 2B). In the 1st h, HGO fell by 35% in control and by 33% in NIDDM subjects. This is probably due to the rapid offset of glucagon action to sustain HGO relative to the slow offset of insulin action to suppress HGO. Because Rₐ falls relatively slowly during this time, an imbalance between HGO and Piₐ is established in which Rₐ exceeds HGO, leading to a fall in the serum glucose level (Fig. 2C). The sustained rate of Rₐ in the face of insulin deficiency and a fall in the serum glucose level probably represent the long half-time (~40–50 min) for the decay of insulin action to stimulate glucose disposal (21,22). By the 2nd h, HGO spontaneously returns toward baseline, despite ongoing glucagon and insulin suppression, while Rₐ continues to fall because of the dissipation of...
HEPATIC GLUCOSE OUTPUT IN TYPE II DIABETES

Somatostatin + Glucose

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E ~

150

100

50

20

10

0

250

150

50

co

ro

E

160

140

120

100

80

-. 110

90

300

250

200

irnTTTTTTTTi:

60

120 180

129

Minutes

Controls

Diabetics

FIG. 3. Effects of somatostatin with serum glucose held at basal level in control (N = 11) and NIDDM (N = 7) subjects on serum insulin and glucagon levels (A), HGO (B), and serum glucose (C). First data point (time 0) is basal value for each parameter; each subsequent data point is for a 20-min interval. Data are means ± SE.

Peripheral insulin action and the concomitant fall in the serum glucose level. Thus, by the 3rd h, HGO exceeds Rg, inducing a slight rise in the serum glucose level. However, HGO remains significantly below baseline in both control and NIDDM subjects during the 3rd h of SRIF infusion: 65 ± 7 and 96 ± 12 mg • m⁻² • min⁻¹ (P < .05), respectively (P < .01 between values).

Because the biological effect of insulin to suppress HGO and stimulate glucose disposal decays slowly after the induction of insulin deficiency, we have chosen to use data obtained between 120 and 200 min in each study in all subjects for the calculations used in subsequent analysis. This ensures that residual insulin action is not operative and makes all calculations and comparisons uniform. For the purpose of clarity and brevity, only HGO data are presented for the subsequent studies.

**Somatostatin with serum glucose clamped at FSG levels.**

During infusion of SRIF alone, we noted a rapid initial fall in HGO accompanied by a fall in the serum glucose level followed by a spontaneous rise in both HGO and the serum glucose levels during the 3rd h of SRIF infusion. To assure that this recovery of HGO was not due to a “perceived” hypoglycemic stimulus, we clamped the serum glucose level at the FSG level by variable-rate exogenous glucose infusion in 11 control and 7 NIDDM subjects. As shown in Fig. 3C, the serum glucose level was not allowed to fall below the basal level in either group. The pattern and degree of insulin and glucagon suppression were similar to those obtained during infusion of SRIF alone (Fig. 3A). The resulting rates of HGO are shown in Fig. 3B: HGO fell rapidly in both groups, reaching a lower nadir than during infusion of SRIF alone. Within the 1st h, HGO was suppressed by 45% in control and by 41% in NIDDM subjects compared with respective values of 35 and 33% during infusion of SRIF alone. During the 2nd h, HGO began to return toward baseline in a manner similar to that observed during infusion of SRIF alone, albeit more slowly. By the 3rd h, HGO had returned to values seen during infusion of SRIF alone but remained significantly below basal values: 35% reduced in the control group (P < .05) and 40% reduced in the NIDDM group (P < .01).

These data indicate that the spontaneous recovery of HGO toward baseline 2 h after the infusion of SRIF is not secondary to a hypoglycemic stimulus. However, the maintenance of the basal glucose level does not appear to significantly enhance the suppression of HGO observed during combined insulin and glucagon suppression.

**Somatostatin with insulin and glucose clamped.** In this condition, a state of isolated glucagon deficiency was created by the concomitant infusion of SRIF and insulin (5 mU • m⁻² • min⁻¹), with serum glucose maintained at the basal level by an exogenous glucose infusion, in 10 control and 8 NIDDM subjects. Figure 4A depicts the steady-state serum insulin levels achieved. The mean peripheral serum insulin level was 10 ± 1 μU/ml in control values, which is analogous to the basal level of 10 ± 1 μU/ml. In NIDDM subjects, the steady-state insulin level was 12 ± 1 μU/ml,
which is similar to the level obtained in the control group but
dsignificantly lower than the basal value of 16 ± 1 μU/ml for
this group of NIDDM subjects (P < .02). Thus, under these
conditions, NIDDM subjects experienced relative peripheral
basal insulinopenia. Furthermore, because portal and pe-
ripheral insulin levels are similar under these conditions, both
groups experienced marked portal insulinopenia relative to
basal levels if one assumes a 3:1 portal-to-peripheral ratio
for insulin in the basal state. Glucagon levels under this
condition exhibited the same rapid and sustained fall from
basal levels seen in previous conditions. Figure 4C shows
the serum glucose level clamped at the basal level in both
groups. The course of HGO in both groups during isolated
glucagon deficiency is depicted in Fig. 4B. Both groups
exhibited a rapid fall in HGO similar to that obtained during
condition 3 (SRIF + serum glucose clamped). However, in
this hormonal milieu, HGO suppression was sustained
throughout the experimental period and exhibited no return
toward baseline; steady-state HGO was 26 ± 6 and 63 ± 10
mg · m⁻² · min⁻¹ in control and NIDDM subjects, respec-
tively (P < .05). Thus, HGO fell by an average 71 ± 8% from
basal in control subjects (P < .001) and by 58 ± 7% in
NIDDM subjects (P < .001). Because absolute basal HGO
was higher in NIDDM subjects, absolute HGO suppression
was greater in NIDDM subjects than in controls: 82 ± 11 vs.
61 ± 8 mg · m⁻² · min⁻¹ (P < .05). However, the percent
HGO suppression from basal in control (71 ± 8%) and
NIDDM (58 ± 7%) subjects was not significantly different
between the two groups (P not significant). Thus, these data
demonstrate an important role for glucagon in maintaining
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Somatostatin with insulin and glucose clamped at hy-
perglycemia. In this study, four control subjects were
studied under conditions of isolated glucagon deficiency with
the serum glucose level raised and held at ~250 mg/dl via
an exogenous glucose infusion to allow direct comparison
of the combined effects of insulin and hyperglycemia on
HGO in control and diabetic subjects. The basal and steady-
state insulin levels were 8 ± 1 and 7 ± 1 μU/ml, respec-
tively, in this group (Fig. 5). As in the other conditions, glu-
cagon fell by 40% of the basal value, reaching steady-state
levels of ~70 pg/ml. The basal C-peptide level was 0.62
pmol/ml and was suppressed to steady-state levels of 0.12
pmol/ml (>80% suppression). The serum glucose level was
clamped at ~250 mg/dl, with a coefficient of variation of
5%. Under these conditions, HGO was rapidly suppressed
by >80% in the 1st h and remained suppressed by 87%
during the last 60 min of the study.

DISCUSSION
Type II diabetes is characterized by abnormalities in insulin
action (1,3,24,25,28) and insulin secretion (4,26) and by
alterations in hepatic glucose metabolism (27,28). Whereas
the resistance of peripheral and hepatic tissues to the action
of insulin and the altered pattern and/or deficiency of β-cell
secretion have been extensively studied, the contribution of
the liver to the defect in glucose homeostasis in type II dia-
abetes has received attention only recently (27). We and

FIG. 4. Effects of somatostatin with insulin re-
placed and serum glucose held constant at fast-
ing level (i.e., isolated glucagon deficiency)  in
control (N = 10) and NIDDM (N = 8) subjects on
serum insulin and glucagon levels (A), HGO (B),
and serum glucose level (C). First data point
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HEPATIC GLUCOSE OUTPUT IN TYPE II DIABETES

SOMATOSTATIN

Insulin + Hyperglycemia

FIG. 5. Effects of somatostatin with insulin replaced (5 mU · m⁻² · min⁻¹) (A and B) and with serum glucose level clamped at 250 mg/dl (C) on serum insulin and glucagon levels (A) and HGO (B) in 4 control subjects. First data point (time 0) is basal value for each parameter; each subsequent data point is for a 20-min interval. Data are means ± SE.

The role of counterregulatory hormones in maintaining hepatic glucose production has not been studied in any detail in subjects with NIDDM. Glucagon may play a central role in this regard based on the elegant studies of Cherrington et al. (25,30) and Liljenquist et al. (9), which demonstrated that glucagon accounts for at least two-thirds of bHGO in both dogs and normal humans. Thus, in our study, we have attempted to elucidate the role of glucagon by creating isolated glucagon deficiency in both control and NIDDM subjects utilizing SRIF to suppress pancreatic hormone secretion while replacing insulin and glucose to maintain the remainder of the hormonal and glycemic milieu. The results of our study confirm the findings of Liljenquist et al. in normal humans and demonstrate an essential role for glucagon in maintaining the elevated rates of bHGO observed in NIDDM subjects.

To assess the independent role of glucagon in maintaining HGO in the basal state, it was necessary to control the other known factors that also modulate HGO. We accomplish this by first assessing the effect of SRIF alone (insulin and glucagon deficiency) on HGO. These studies confirmed previous reports describing a transient fall in HGO followed by a spontaneous recovery of HGO toward baseline (15,16). When these studies were repeated and the serum glucose level was not allowed to fall below baseline to eliminate any potential hypoglycemic stimulus for HGO recovery, we found only a small delay but no significant effect on the degree of HGO recovery. These findings support the notion that HGO is not critically dependent on glucagon when severe insu-
linopenia is present. In addition, although the serum glucose level per se is known to inhibit hepatic glucose production (31,32), these results suggest that in control and NIDDM subjects the basal serum glucose level exerts a minimal suppressive effect on HGO in the presence of insulinopenia because under these study conditions bHGO was suppressed 45% in control and 41% in NIDDM subjects in the 1st h of study and 35 and 36%, respectively, in the last hour of study. The cause for the recovery of HGO toward baseline in the 2nd h of combined insulin and glucagon deficiency is unknown. However, the initial delay in HGO recovery after the onset of SRIF infusion is probably due to the rapid activation of glucagon's action to stimulate HGO relative to the slow deactivation of insulin's action to suppress HGO. Note that catecholamine levels during these studies were stable and remained at baseline levels (data not shown) and therefore probably do not account for the phenomenon.

When isolated glucagon deficiency was created, we observed a large relative (58%) and absolute (82 mg·m⁻²·min⁻¹) fall in HGO from basal level in NIDDM subjects. Although the relative fall was smaller than in the control group, the absolute fall in HGO was greater in the NIDDM group because the basal HGO was greater. Thus, when a small amount (10 μU/ml) of insulin was added to the ambient basal serum glucose level in the absence of glucagon, the liver was quite sensitive to the combined effects of insulin and glucose to suppress HGO. Because we have demonstrated only a modest suppressive effect of the elevated serum glucose level per se (insulin and glucagon deficiency) on HGO in NIDDM subjects, either the small amount of insulin or its synergism with the ambient glucose level is responsible for the suppression of HGO under isolated glucagon deficiency. This is particularly impressive when one considers that the portal levels of insulin under these conditions were presumably similar to the peripheral level (10 μU/ml) because SRIF abolishes the portal-peripheral gradient for insulin when the replacement insulin is infused peripherally. If it is assumed a 3:1 portal-to-peripheral ratio for insulin in the basal state, the NIDDM subjects were underreplaced at the portal level by at least 30–40 μU/ml. Thus, the data strongly suggest that the hepatic resistance to insulin's action to suppress HGO is largely attributable to the powerful glucagon stimulus to drive HGO. This notion is supported by the data of Révers et al. (34), demonstrating that peripheral serum insulin levels >40 μU/ml were required to achieve a 50% reduction in HGO in NIDDM subjects studied under hyperglycemic conditions without suppression of endogenous glucagon secretion. In conjunction with the results of our study, the data indicate that in NIDDM subjects, a 50% reduction in HGO requires a serum insulin level at least 4 times higher in the presence of basal glucagon than in the glucagon-deficient state. In addition, the data also suggest that NIDDM subjects exhibit significant hepatic resistance to the combined effects of insulin and glucose independent of glucagon's effect to antagonize insulin's action to suppress HGO because the addition of 10 μU/ml insulin in condition 3 (isolated glucagon deficiency) caused an equivalent further drop in HGO in both groups from the levels obtained in condition 2 (SRIF + glucose), 30 ± 7 (53%) vs. 38 ± 10 mg·m⁻²·min⁻¹ (59%), in the face of a prevailing serum glucose level that is 2.5 times higher in the NIDDM group than in controls (254 vs. 90 ± 2 mg/dl). Thus, the combined effects of insulin and glucose to suppress HGO are significantly diminished in NIDDM subjects.

On the other hand, one must note that absolute HGO suppression under condition 5 (matched insulin and glucose level with hypoglucaegenemia) was similar in NIDDM and control subjects: 82 ± 13 and 77 ± 4 mg·m⁻²·min⁻¹, respectively (P not significant). Viewed in this manner the data suggest that NIDDM subjects exhibit normal sensitivity to the suppressive effect of insulin and glucose per se on HGO. This is contrary to the above discussion, and therefore it is apparent that our data are insufficient to resolve whether NIDDM subjects are normally sensitive or resistant to the suppressive effects of glucose and insulin per se on HGO. Regardless, it is clear that residual HGO in condition 5 was virtually absent in the control group but remained significant in the NIDDM group (63 mg·m⁻²·min⁻¹), indicating that factors other than glucagon serve to maintain HGO in NIDDM subjects. These factors might include intrinsic hepatic mechanisms, neural factors, and/or other counterregulatory hormones. In addition, alterations in the flux of gluconeogenic substrates to the liver in NIDDM subjects may serve as a driving force for the elevated rates of HGO observed. Therefore, although the data do not resolve whether NIDDM subjects are normally sensitive to the combined effects of insulin and glucose to suppress HGO in the absence of glucagon, they nevertheless confirm numerous reports (31,32,35–37) demonstrating a powerful effect of hyperglycemia to suppress HGO in the presence of basal insulin levels in normal humans and dogs and indicate that glucagon is largely responsible for the hepatic insulin resistance observed in NIDDM subjects.

As previously noted, SRIF inhibits the release of other hormones, one of which, growth hormone (GH), may play an important role in glucose homeostasis. Although we did not replace GH in this study, GH deficiency probably was not responsible for the suppression of HGO during isolated glucagon deficiency. Evidence to support this comes from work by Gerich et al. (33), who replaced basal GH during an SRIF infusion in normal humans and noted no change in the serum glucose excursions from that observed during infusion of SRIF alone. In addition, no data support a role for the basal level of GH as an acute modulator of HGO; rather, the data support the notion that the basal GH level acts as a counterregulator with a slow onset and a long offset of action (33,38). If the latter is true, small amounts of insulin in the absence of glucagon are apparently able to counteract the GH action to stimulate HGO. More important, numerous reports have confirmed that in acutely somatostatinized humans and dogs, basal rates of HGO can be reestablished by replacement infusions of insulin and glucagon (39,40), suggesting that GH replacement is not necessary in this setting. In addition, although the effect of glucagon to stimulate HGO above basal levels is transient (40,42–43), HGO does not fall below the basal level providing the glucagon infusion is sustained. Thus, despite the effect of SRIF to suppress the secretion of several hormones, the changes in HGO described in this study clearly appear to be related to changes in the glucagon level.
The validity of the data depends on the assumption that SRIF induced a profound if not complete inhibition of endogenous insulin secretion. In this regard, peripheral serum insulin levels were below the detectable limits of the assay, and C-peptide suppression from basal levels was >92% during those study conditions in which the fasting serum glucose level was held constant. However, because we did not have access to the portal circulation, the prehepatic insulin levels might have been higher than those of the peripheral circulation as a result of hepatic insulin extraction. Assuming hepatic C-peptide extraction to be negligible (44), the C-peptide level can be considered to reflect insulin secretory rates. Actual peripheral serum insulin levels can then be estimated even when they fall below the detectable limits of the assay; i.e., the actual serum insulin level = basal insulin level− (%C-peptide suppression/100) × basal insulin level. In addition, if a 3:1 portal-to-peripheral ratio for serum insulin levels is assumed, portal insulin concentrations can be estimated. With this approach, we estimated peripheral insulin levels during SRIF infusions to <1 μU/ml in control subjects and <1.5 μU/ml in NIDDM subjects. Portal insulin levels were estimated to be <3 and 5 μU/ml in control and NIDDM subjects, respectively, when exogenous insulin was not administered, and 13 and 17 μU/ml, respectively, when insulin (5 mU × 2 × min⁻¹) was infused. Under condition 5, portal-level estimates were in the same range as those found under euglycemic conditions in control subjects (12 vs. 13 μU/ml, respectively) but slightly lower than the portal insulin level estimate for the NIDDM group studied at their respective prevailing fasting serum level. Thus, suppression of endogenous insulin secretion was profound but not complete. However, portal and peripheral insulin levels were roughly comparable in control and NIDDM subjects under all experimental conditions.

If glucagon is important in maintaining the elevated rate of HGO in NIDDM subjects, basal glucagon levels in these patients should be positively correlated with the basal rate of HGO. We found no significant relationship between basal glucagon levels and basal rates of HGO and no significant relationship between the insulin-to-glucagon ratio and bHGO in NIDDM subjects, which suggests that the relationship between glucagon and HGO is critical but not closely linked. However, on closer inspection this relationship is quite complex. As shown in Fig. 2, SRIF induced a reduction of insulin levels to below the detectable limits of the assay (4 μU/ml), whereas glucagon levels remained at a mean level of ~80 pg/ml. We now know that this residual glucagon level largely represents immunoreactivity of the 30K antibody with heterogenous forms of glucagon (45–47). This cross-reactivity of the 30K antibody with forms of glucagon of molecular weight other than 3500 is well described (46), and all available data point to the 3500-Mr glucagon species to be the “true” biologically active glucagon (45,48). Thus, peripheral glucagon levels represent a conglomerate of different glucagon species of which only one is truly biologically active. Therefore, correlating rates of HGO with glucagon levels is only valid if one can correlate HGO with the level of the biologically active glucagon species (i.e., 3500 Mr). Another level of complexity is added when one considers that the activity of glucagon is known to be correlated with its extraction by the liver (45). Thus, when one measures peripheral glucagon levels, only a fraction of the 3500-Mr species secreted by the pancreas is measured. In addition, one cannot correlate glucagon levels with its biological effectiveness without taking into account insulin levels, because insulin is the major antagonist to glucagon’s action on the liver. Here again, insulin’s biological activity at the level of the liver is related to its extraction by the liver. Thus, without the knowledge of portal levels of 3500-Mr glucagon and insulin, it is not surprising that we cannot establish a correlation between the prevailing glucagon level and its effectiveness in stimulating HGO.

Despite these difficulties and the lack of a significant correlation between bHGO and basal glucagon levels, we were able to demonstrate during conditions of isolated glucagon deficiency that for an equivalent proportional fall in glucagon (40%), control and NIDDM subjects exhibited comparable proportional decrements in bHGO (71 vs. 58%; P not significant). On the other hand, because NIDDM subjects have higher basal levels of glucagon and higher rates of HGO than controls, it follows that the higher basal glucagon levels in these subjects support a greater absolute amount of bHGO than do those in control subjects (82 ± 11 vs. 61 ± 8 mg · m⁻² · min⁻¹, P < 0.05). Thus, in proportionate terms, basal glucagon accounts for equivalent portions of bHGO in control and NIDDM subjects. However, because basal glucagon levels are elevated in NIDDM subjects, they maintain bHGO levels that are higher than those of control subjects.

In conclusion, the results of our study support the notion that in the presence of basal insulin, glucagon is vital in maintaining basal rates of HGO and that glucagon is responsible for maintaining 71% of bHGO in control subjects and at least 58% in NIDDM subjects. In NIDDM subjects, elevated glucagon levels are largely responsible for the hepatic insulin resistance and elevated rates of bHGO.

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