The effects of continuous infusions of insulin in physiologic doses on glucose kinetics and circulating counterregulatory hormones (epinephrine, norepinephrine, glucagon, cortisol, and growth hormone) were determined in normal subjects and diabetics. The normals received insulin at two dose levels (0.4 and 0.25 mU/kg per min) and the diabetics received the higher dose (0.4 mU/kg per min) only.

In all three groups of studies, continuous infusion of insulin resulted in an initial decline in plasma glucose followed by stabilization after 60-180 min. In the normal subjects, with the higher insulin dose there was a fivefold rise in plasma insulin. Plasma glucose fell at a rate of 0.73±0.12 mg/min for 45 min and then stabilized at 55±3 mg/dl after 60 min. The initial decline in plasma glucose was a result of a rapid, 27% fall in glucose output and a 33% rise in glucose uptake. Subsequent stabilization was a result of a return of glucose output and uptake to basal levels. The rebound increment in glucose output was significant ($P < 0.05$) by 30 min after initiation of the insulin infusion and preceded, by 30-45 min, a significant rise in circulating counterregulatory hormones.

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Influence of Continuous Physiologic Hyperinsulinemia on Glucose Kinetics and Counterregulatory Hormones in Normal and Diabetic Humans

LUIGI SACCÀ, ROBERT SHERWIN, ROSA HENDLER, and PHILIP FELIG, Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut 06510

ABSTRACT The effects of continuous infusions of insulin in physiologic doses on glucose kinetics and circulating counterregulatory hormones (epinephrine, norepinephrine, glucagon, cortisol, and growth hormone) were determined in normal subjects and diabetics. The normals received insulin at two dose levels (0.4 and 0.25 mU/kg per min) and the diabetics received the higher dose (0.4 mU/kg per min) only.

In all three groups of studies, continuous infusion of insulin resulted in an initial decline in plasma glucose followed by stabilization after 60–180 min. In the normal subjects, with the higher insulin dose there was a fivefold rise in plasma insulin. Plasma glucose fell at a rate of 0.73±0.12 mg/min for 45 min and then stabilized at 55±3 mg/dl after 60 min. The initial decline in plasma glucose was a result of a rapid, 27% fall in glucose output and a 33% rise in glucose uptake. Subsequent stabilization was a result of a return of glucose output and uptake to basal levels. The rebound increment in glucose output was significant (P < 0.05) by 30 min after initiation of the insulin infusion and preceded, by 30–45 min, a significant rise in circulating counterregulatory hormones.

With the lower insulin infusion dose, plasma insulin rose two- to threefold, plasma glucose initially fell at a rate of 0.37±0.04 mg/min for 75 min and stabilized at 67±3 mg/dl after 75 min. The changes in plasma glucose were entirely a result of a fall in glucose output and subsequent return to base line, whereas glucose uptake remained unchanged. Plasma levels of counterregulatory hormones showed no change from basal throughout the insulin infusion.

In the diabetic group (plasma glucose levels 227±7 mg/dl in the basal state), the initial rate of decline in plasma glucose (1.01±0.15 mg/dl) and the plateau concentration of plasma glucose (59±5 mg/dl) were comparable to controls receiving the same insulin dose. However, the initial fall in plasma glucose was almost entirely a result of suppression of glucose output, which showed a twofold greater decline (60±6%) than in controls (27±5%, P < 0.01) and remained suppressed throughout the insulin infusion. In contrast, the late stabilization in plasma glucose was a result of a fall in glucose uptake to values 50% below basal (P < 0.001) and 39% below that observed in controls at termination of the insulin infusion (P < 0.01). Plasma norepinephrine and glucagon failed to rise during the insulin infusion, whereas plasma epinephrine, cortisol, and growth hormone rose to values comparable to controls receiving the same insulin dose.

It is concluded that (a) in normal and diabetic subjects, physiologic hyperinsulinemia results in an initial decline followed by stabilization of plasma glucose despite ongoing infusion of insulin; (b) in the normal subjects, a rebound increase in glucose output is the initial or principal mechanism counteracting the fall in plasma glucose and occurs (with an insulin dose of 0.25 mU/kg per min) in the absence of a rise in circulating counterregulatory hormones; (c) in diabetics, although the changes in plasma glucose are comparable to controls, the initial decline is a result of an exaggerated suppression of glucose output, whereas the stabilization of plasma glucose occurs primarily as a consequence of an exaggerated fall in glucose uptake; and (d) failure of plasma norepinephrine as well as glucagon to rise in the diabetics may contribute to the exaggerated suppression of glucose output.
INTRODUCTION

The counterregulation of insulin-induced hypoglycemia has recently been investigated in studies involving bolus injections of pharmacologic doses of insulin (1–6). Although the secretion of a variety of hormones (epinephrine, norepinephrine, glucagon, cortisol, and growth hormone) is stimulated by insulin hypoglycemia, elevations in circulating catecholamines (1) and increased activity of the adrenergic nervous system (1, 5–7) have been proposed as being of particular importance in counteracting the hypoglycemic action of insulin. In contrast to bolus injections of insulin, the effects of sustained, physiologic elevations in plasma insulin on changes in glucose kinetics have been examined in the dog (8, 9), but such data are not available in man. Furthermore, the relationship of changes in glucose kinetics induced by physiologic hyperinsulinemia to changes in counterregulatory hormone secretion has not been established. Such data are of particular interest because secretion of counterregulatory hormones may depend, in part, upon the rate of decline in plasma glucose and, thus, the dose of insulin employed (10). Furthermore, continuous infusion of insulin in low doses (rather than bolus injections of supraphysiologic doses) has become a common practice in the management of severely decompensated diabetes (11–13).

This study was consequently undertaken to examine the effects of continuous infusion of physiologic doses of insulin on glucose turnover and circulating levels of counterregulatory hormones in normal and diabetic subjects. Particular attention was paid to the relative importance of changes in glucose output vs. glucose uptake in determining the plasma glucose response. In addition, the temporal relationships between changes in glucose kinetics and alterations in plasma hormone levels were examined.

METHODS

Subjects. Two groups of subjects were studied. The normal control group consisted of 11 healthy male subjects, 22–32 yr of age. The diabetic group consisted of five juvenile-onset diabetics, 23–34 yr of age, who had been receiving 36–70 U of intermediate-acting insulin per day for 2–8 yr. All subjects were within 15% of ideal body weight (Metropolitan Life Insurance Tables, 1959). Other than insulin, they were taking no medications. None of the subjects had a history of or evidence of liver or renal disease or of peripheral neuropathy. All subjects gave written, informed consent before their participation.

Procedures. The studies were performed in the morning, after an overnight, 12- to 14-h fast. The diabetic subjects received their last injection of intermediate-acting insulin 24–26 h before the study. An indwelling catheter was placed in an antecubital vein in each arm for infusion of insulin and tritiated glucose as well as for withdrawal of blood samples. During an initial 3-h equilibration period, [3-3H]glucose (New England Nuclear, Boston, Mass.) was administered as a primed-continuous infusion. The ratio of the priming dose:continuous infusion dose was 1:120 in normals and 1:250 in diabetics. The continuous infusion dose was 0.35 μCi/min in both groups.

After the equilibration period, crystalline zinc insulin (Eli Lilly and Co., Indianapolis, Ind.) was administered as a continuous infusion for 150 min in the controls and 220 min in the diabetics. The insulin was infused at two dose levels in the controls, 0.4 mU/kg per min (n = 6) and 0.25 mU/kg per min (n = 5). The diabetics were given insulin at the higher dose level (0.4 mU/kg per min) only.

The infusion of [3-3H]glucose was continued throughout the infusion of insulin. Blood samples were drawn at 10-min intervals for 20 min before initiation of the insulin infusion (baseline samples) and at 15- to 20-min intervals thereafter.

Analytical procedures. Plasma glucose was determined on a Beckman glucose analyzer (Beckman Instruments, Inc., Fullerton, Calif.). Plasma insulin and growth hormone were measured by radioimmunoassay (14). Plasma glucagon was determined by radioimmunoassay with Unger 30K antibody (15). Plasma epinephrine and norepinephrine were measured by a radioenzymatic technique (16). With this technique, the interassay variation for plasma epinephrine and norepinephrine is 6.2±1.4%, respectively, and the intra-assay variation for replicate determinations of the same sample is 6.2±1% for both epinephrine and norepinephrine. Plasma cortisol was determined by a fluorimetric assay (17). Plasma [3-3H]glucose radioactivity was determined by deproteinizing plasma samples with Ba(OH)2·ZnSO4, evaporating the supernate to dryness at 70°C to remove tritiated water, dissolving the dry residue in 1 ml of water which was added to 10 ml Aquasol (New England Nuclear), and then counted in a liquid scintillation spectrometer.

Calculations. The rates of glucose output and uptake were calculated in the steady state before insulin administration and during nonsteady-state conditions by the equations of Steele (18) in their derivative form. The value of 0.65 of the initial glucose pool size (pool fraction) was used as the rapidly mixing compartment of the glucose pool to compensate for nonuniform mixing within the glucose pool (19). The metabolic clearance rate of glucose was calculated by dividing the rate of glucose uptake by the plasma glucose concentration.

Statistical analyses were performed with the Student's t test (the paired t test was used when applicable) (20). Data in the text, tables, and figures are presented as the mean±SE.

RESULTS

Effects of insulin infusion at a dose of 0.4 mU/kg per min in normal subjects (Figs. 1 and 2). As shown in Table I, the infusion of insulin at a rate of 0.4 mU/kg per min produced a rapid, fivefold increase in plasma insulin levels at 15 min, which remained stable throughout the 150-min study period. The plasma glucose concentration (88±4 mg/dl in the basal state) fell at a rate of 0.73±0.12 mg/min for the initial 45 min, but thereafter (60–150 min) remained remarkably constant at a concentration of 55±3 mg/dl. Glucose output fell from 1.96±0.13 mg/kg per min in the basal state to 1.42±0.08 mg/kg per min at 15 min (P < 0.001), a decline of 27±5%. However, beyond 15 min, glucose output rapidly rose, reaching values at 45 min which were not significantly different from the basal state. The increment in glucose output between 15 and 30 min was statistically significant (P < 0.05) as were all subsequent values as com-
pared with the 15-min value \((P < 0.02-P < 0.01)\). Glucose uptake increased significantly \((P < 0.05-P < 0.01)\) in the interval between 15 and 60 min, reaching a peak value 33\% above basal at 30 min and then slowly returning to values not statistically different from base line at 75 min. Glucose clearance \((2.25\pm 0.15 \text{ ml/kg/min in the basal state})\) increased twofold in response to insulin at 45–75 min and remained at levels 75–90\% above base line \((P < 0.01-P < 0.001)\) throughout the experimental period.

Fig. 2 shows the changes in plasma concentrations of epinephrine, norepinephrine, glucagon, cortisol, and growth hormone. The plasma levels of these hormones were stable in the basal state and showed no significant change during the first 45 min of insulin infusion. Plasma epinephrine \((25\pm 9 \text{ pg/ml in the basal state})\) increased fivefold at 60 min \((P < 0.05)\) and continued to rise to levels 8- to 12-fold above base line \((P < 0.05-P < 0.01)\) in the latter part of the insulin infusion. Plasma norepinephrine \((250\pm 25 \text{ pg/ml in the basal state})\) increased by 45\% at 75 min \((P < 0.02)\) and thereafter showed only minor fluctuations. Plasma glucagon \((110\pm 26 \text{ pg/ml in}

### Table I

**Plasma Insulin Concentrations during Intravenous Infusion of Insulin in Normal Subjects**

<table>
<thead>
<tr>
<th>Infusion dose</th>
<th>Min</th>
<th>(0^*)</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>150</th>
</tr>
</thead>
<tbody>
<tr>
<td>(0.40 \text{ mU/kg per min})</td>
<td>8±2</td>
<td>39±5</td>
<td>41±4</td>
<td>43±4</td>
<td>42±5</td>
<td>41±5</td>
<td>44±6</td>
<td></td>
</tr>
<tr>
<td>(0.25 \text{ mU/kg per min})</td>
<td>11±1</td>
<td>24±3</td>
<td>25±3</td>
<td>27±4</td>
<td>27±2</td>
<td>26±2</td>
<td>25±2</td>
<td></td>
</tr>
</tbody>
</table>

* Control values represent the mean of three observations on each subject preceding insulin administration.
the basal state) increased by 55% \((P < 0.05)\) at 60 min and remained at levels 60–100% above base line \((P < 0.05–P < 0.02)\) for the rest of the experimental period. Plasma cortisol \((12±2\ \mu g/dl\ \text{in the basal state})\) showed a significant increase at 75 min \((P < 0.05)\) and thereafter persisted at levels 75–85% above base line \((P < 0.05)\). Plasma growth hormone \((1.6±0.24\ \text{ng/ml (in the basal state)})\) rose ninefold \((P < 0.05)\) at 75 min and remained stable for the rest of the experimental period. Thus, initiation of the reversal in insulin-induced inhibition of glucose output, which was apparent by 30 min (Fig. 1), preceeded a significant rise in counterregulatory hormones by 30–45 min.

Effects of insulin infusion at a dose of 0.25 mU/kg per min in normal subjects (Figs. 3 and 4). The infusion of insulin at the rate of 0.25 mU/kg per min produced a two- to threefold rise in plasma insulin, which remained stable throughout the experiment (Table I). Plasma glucose levels \((90±2\ \text{mg/dl (in the basal state)})\) declined at a rate of 0.37±0.04 mg/min for 60 min and stabilized at 67–68 mg/dl at 75–90 min. Glucose output \((2.02±0.1\ \text{mg/kg per min (in the basal state)})\) fell by 18% to 1.65±0.1 mg/kg per min at 15 min \((P < 0.01)\) and then progressively rose, reaching values not significantly different from base line at 60–75 min. The rise in glucose output between 15 and 30 min was statistically significant \((P < 0.05)\). In contrast to the elevation in glucose uptake caused by the higher dose of insulin, no significant increment in this parameter occurred in this group of experiments. Similarly, glucose clearance \((2.25±0.1\ \text{ml/kg per min (in the basal state)})\) increased by only 40–50% \((P < 0.05)\) (Fig. 3), as compared with the 75–100% increments observed with insulin infusion at a rate of 0.4 mU/kg per min (Fig. 1).

![Figure 3](image_url)

**Figure 3** Effect of the lower dose insulin infusion \((0.25\ \text{mU/kg per min})\) in normal subjects on plasma glucose and glucose kinetics.

![Figure 4](image_url)

**Figure 4** Effect of the lower dose insulin infusion \((0.25\ \text{mU/kg per min})\) in normal subjects on plasma epinephrine, norepinephrine, glucagon, cortisol, and growth hormone. No significant changes from basal \((P > 0.1)\) were observed in any of the hormones during the course of the insulin infusion.

Effects of insulin infusion \((0.4\ \text{mU/kg per min})\) in diabetic patients (Figs. 5 and 6). Before the infusion of insulin, plasma glucose concentration \((222±7\ \text{mg/dl})\) was stable at values 100–150 mg/dl above those in normal controls \((P < 0.001)\). During the infusion of insulin,
plasma glucose fell initially at a constant rate (1.01±0.15 mg/min) that was not significantly different from that observed in normal controls receiving the same insulin dose (Fig. 1) (P > 0.1). The plasma glucose concentration stabilized at 180–220 min at values (59±5 mg/dl) that were also identical to those observed in controls (Fig. 1). Glucose output (2.48±0.15 mg/kg per min, pre-infusion), which was increased by 25% (P < 0.05) in the diabetics as compared with controls, declined progressively for the initial 60–80 min, reaching a nadir of 0.97±0.07 mg/kg per min, 60±6% below base line (P < 0.005). As compared with normal subjects infused with the same dose of insulin (Fig. 1), both the relative decline in glucose output (60±6 vs. 27±5%, P < 0.01) and the absolute fall (1.53±0.21 vs. 0.54±0.10 mg/kg per min, P < 0.01) were greater in the diabetic group. Beyond 80 min, a small, progressive rise in glucose output was observed (the increment between 80 and 120 min was significant, P < 0.05). However, the values observed at 220 min (1.60±0.15 mg/kg per min) remained 30% below base line (P < 0.05) and 25% below those observed in normal subjects at termination of the insulin infusion (Fig. 1) (P < 0.05). Glucose uptake (2.48±0.15 mg/kg per min in the basal state) showed a transient, 22% rise at 20 min (P < 0.05) and rapidly returned to basal levels for the ensuing 100 min. Thereafter (beyond 120 min), there was a progressive decline in glucose uptake to values 30% below base line at 180 min (P < 0.02) and 50% below base line at 220 min (P < 0.001). The rate of glucose uptake at termination of the insulin infusion (1.30±0.10 mg/kg per min) was 39% below that observed in the normal subjects (2.14 ±0.11 mg/kg per min) at the end of the infusion of insulin at the same dose (Fig. 1) (P < 0.01). Glucose clearance (1.11±0.05 ml/kg per min, pre-infusion) began to rise only after 80 min, reaching values which were three times the basal rate at 140–160 min (P < 0.01). The peak value for glucose clearance (3.15±0.20 ml/kg per min) was, however, 28% below that observed in the normal subjects receiving the same dose of insulin (4.35±0.20 ml/kg per min, P < 0.01).

Fig. 6 shows the changes in counterregulatory hormones. Before insulin administration, base-line levels of epinephrine, norepinephrine, glucagon, cortisol, and growth hormone in the diabetic subjects were not significantly different from normal controls. After insulin

**FIGURE 5** Effect of insulin infusion (0.40 mU/kg per min) in diabetic subjects on plasma glucose and glucose kinetics.

**FIGURE 6** Effect of insulin infusion (0.40 mU/kg per min) in diabetic subjects on plasma epinephrine, norepinephrine, glucagon, cortisol, and growth hormone. The asterisks indicate the first determination that was significantly greater than the basal, preinfusion values (P < 0.05). In the case of norepinephrine and glucagon, no significant rise was observed at any time during the insulin infusion.
was infused, plasma epinephrine rose significantly at 180 min \( (P < 0.05) \), reaching values 18-fold above basal at 220 min, \( (436±109 \text{ pg/ml}, P < 0.01) \). These values were not significantly different from the peak concentrations observed in normal controls (Fig. 2) \( (312±100 \text{ pg/ml}, P < 0.1) \). Plasma cortisol rose 70% at 180–220 min \( (P < 0.05) \) to values comparable to controls (Fig. 2). Plasma growth hormone increased significantly at 180 min \( (P < 0.05) \), reaching values at 200 min \( (43.4±12.0 \text{ ng/ml}) \) that were not significantly greater than the peak concentrations observed in normal subjects \( (17.6±5.6 \text{ ng/ml}) \) infused with the same dose of insulin \( (P < 0.05) \). In contrast, neither plasma norepinephrine \( (269±46 \text{ pg/ml}) \) nor plasma glucagon \( (101±29 \text{ pg/ml}) \) concentration demonstrated a consistent rise above base-line values during the insulin infusion.

DISCUSSION

In this study we examined the effect of continuous physiologic, hyperinsulinemia on glucose kinetics and circulating levels of counterregulatory hormones in normal and diabetic subjects. Two physiologic dose levels of insulin were employed in normal subjects, and the higher dose level was used in the diabetic patients. In all studies, infusion of insulin produced an initial decline in plasma glucose concentration, which later stabilized with remarkable constancy despite ongoing infusion of insulin. Despite the similarities in the plasma glucose curves (an initial fall and subsequent stabilization), the responses in glucose kinetics demonstrated different characteristics depending upon the dose of insulin (high vs. low) and the subject group (normal vs. diabetic) studied (Table II).

In normal subjects receiving the higher physiologic dose of insulin \( (0.4 \text{ mU/kg per min}) \), in whom plasma insulin rose fivefold, the fall in plasma glucose was caused by a prompt decrease in glucose output as well as an increase in glucose uptake. Subsequently, a rapid rebound increase in glucose production to basal rates and the return of glucose uptake to basal values provided for the stabilization of plasma glucose levels in the face of ongoing hyperinsulinemia. Thus, the changes in plasma glucose accompanying a fivefold increment in plasma insulin occur as a consequence of oppositely directed changes in the rates of glucose output as well as glucose uptake.

When insulin was infused at the lower rate of 0.25 mU/kg per min and plasma insulin was raised two- to threefold, the glycemic response with regard to both the initial fall and the later stabilization of plasma glucose was primarily determined by changes in glucose output. An initial decline in glucose output was followed by a rebound increment to basal levels. The rise in glucose clearance that accompanied the lower dose insulin infusion contributed to the initial fall in plasma glucose and accounted for the maintenance of glucose uptake at base-line values despite a decline in plasma glucose levels. However, the absolute rate of glucose uptake remained unchanged throughout these studies. The latter observation is in keeping with earlier studies demonstrating that twofold increments in plasma insulin fail to stimulate forearm \( (21) \) or total body glucose uptake \( (22) \), yet consistently inhibit hepatic glucose output \( (22) \). The varying patterns of glucose kinetics observed with the two dose levels of insulin are summarized in Table II.

To gain more insight into the mechanism governing the glucoregulatory response to physiologic hyperinsulinemia in the normal subjects, the circulating levels of the various counterregulatory hormones were determined and temporally related to changes in glucose kinetics. In the studies involving the higher insulin infusion \( (0.4 \text{ mU/kg per min}) \), plasma epinephrine and glucagon increased significantly at 60 min after the start of the insulin infusion. A significant increase in norepinephrine, cortisol, and growth hormone was observed at 75 min. However, the reversal of insulin-induced suppression of hepatic glucose production was evident by 30 min, thus preceding a consistent rise in counterregulatory hormones by 30–45 min. These observations imply that in the normal subjects, factors other than the circulating levels of counterregulatory hormones initiated the reversal of insulin-induced inhibition of glucose output. Recent studies, however, have shown a synergistic interaction among counterregulatory hormones in stimulating glucose production \( (23) \). Thus, the possibility cannot be excluded that the small, although not significant, changes in counterregulatory hormones contributed to the rebound in glucose production observed at 30–60 min.

### Table II

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Initial fall of plasma glucose</th>
<th>Late stabilization of plasma glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal subjects (insulin, 0.4 mU/kg per min)</td>
<td>↓↓</td>
<td>↑↑</td>
</tr>
<tr>
<td>Glucose output</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose uptake</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Normal subjects (insulin, 0.25 mU/kg per min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose output</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Glucose uptake</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Diabetic subjects (insulin, 0.4 mU/kg per min)</td>
<td>↓↓↓</td>
<td>↓</td>
</tr>
<tr>
<td>Glucose output</td>
<td>↑</td>
<td>↓↓↓</td>
</tr>
<tr>
<td>Glucose uptake</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Changes in glucose kinetics are indicated by: ↑, increase; ↓, decrease; and –, no change. The relative magnitude of the changes is indicated by the number of arrows.

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L. Saccà, R. Sherwin, R. Hendler, and P. Felig
Evidence of reversal of insulin-induced suppression of glucose output independent of counterregulatory hormonal intervention is even more apparent in the experiments with the lower dose of insulin (0.25 mU/kg per min). In these studies, glucose output, which fell by 20% at 15 min, returned to basal levels in the absence of any appreciable increment in plasma counterregulatory hormones at any point during the course of the insulin infusion. These data thus provide evidence that stabilization of plasma glucose in the face of sustained, small elevations in plasma insulin (10-15 μU/ml) can occur in normal man without the intervention of a significant increase in circulating anti-insulin hormones.

With respect to the mechanism underlying the reversal of glucose output independent of elevations in counterregulatory hormones, three possibilities can be postulated. First, hypoglycemia per se may be the stimulus whereby the liver increases glucose output in the face of hyperinsulinemia. This interpretation is consistent with previous studies showing that glucose output from the isolated perfused liver increases in response to a reduction in glucose concentration of the perfusate (24, 25). This possibility is also consistent with in vitro studies which demonstrate the ability of glucose per se to regulate the activity of the enzymes involved in the hepatic synthesis and breakdown of glycerogen (26). A second possibility is that hypoglycemia triggers a neurogenic signal, which in turn leads to intrahepatic release of norepinephrine. This possibility is not inconsistent with the unchanged plasma levels of norepinephrine during the first 75 min of the higher insulin infusion (Fig. 2) and throughout the lower dose infusion (Fig. 4), because rapid, local inactivation and re-uptake of norepinephrine (27) could have prevented the catecholamine from being released into the bloodstream. A third possibility is that a fall in endogenous insulin secretion contributed to the rebound in glucose production by resulting in a decline in portal insulin concentration from peak levels achieved early in the infusion despite ongoing administration of insulin. Inhibition of endogenous insulin secretion is likely to have been only partial because plasma glucose remained above 65 mg/dl, and plasma insulin was only modestly increased (two- to threefold) in the low dose insulin infusion. Furthermore, hyperinsulinemia per se in the range of 200 μU/ml (four- to eightfold the levels achieved in this study) has been shown to result in less than a one-third decline in endogenous insulin secretion (28). Nevertheless, a small decline in portal insulin may contribute to changes in glucose output and yet not be reflected in peripheral insulin levels in view of the normal portal-peripheral insulin gradient of 2.5-3.1 (29).

Whatever the explanation, our data in normal subjects indicate that a rebound increase in glucose output is a principal mechanism counteracting the fall in plasma glucose induced by physiologic hyperinsulinemia, and that this rebound in glucose output may occur in the absence of a rise in circulating counterregulatory hormones.

As noted above, with the higher insulin infusion dose, an increment in each of the counterregulatory hormones was observed in the normal subjects at 60 min or later (Fig. 2). Thus, whereas reversal of insulin-induced inhibition of glucose output may have occurred independently of a rise in counterregulatory hormones, the various counterregulatory hormones are likely to have augmented and/or sustained this response. In previous studies that employed bolus injections of supraphysiologic doses of insulin, which cause more rapid declines in plasma glucose than observed in this study, a rise in plasma catecholamines was observed to precede or coincide with reversal of inhibition of glucose output (1). It is conceivable that the rate of fall in plasma glucose and/or the dose of insulin employed may determine whether hypoglycemia per se or a change in circulating counterregulatory hormones initiates the reversal of insulin-induced inhibition of glucose output (10).

The rise in epinephrine may also be responsible, at least in part, for the decline in glucose uptake observed after 60 min of the higher dose insulin infusion (Fig. 1). A decrease in the rate of fractional glucose utilization has been observed with in vivo (30, 31) as well as in vitro (32) administration of epinephrine. In addition, recent studies indicate that the infusion of epinephrine blunts the stimulatory effect of exogenous insulin (physiologic doses) on glucose uptake in the dog. Furthermore, in the low dose insulin infusion, in which no change in circulating catecholamines or other hormones was observed, glucose uptake was unaltered throughout the experimental period (Fig. 3).

With respect to the observations in the diabetic group, during the insulin infusion the initial rate of decline in plasma glucose (Fig. 5) was comparable to controls receiving the same dose of insulin (Fig. 1). Furthermore, the concentration at which stabilization of plasma glucose was achieved (55-60 mg/dl), was identical in the two groups. Nevertheless, the changes in glucose kinetics induced by the insulin infusion in the diabetics differed markedly from those observed in normal subjects with respect to both the initial decline and the subsequent stabilization of plasma glucose (Table II).

In the diabetic subjects, the initial decline in plasma glucose was almost entirely a result of a fall in glucose output because only a very transient increase in glucose uptake was observed. In addition, the maximal suppression of glucose output in the diabetics was greater (60 vs. 27%) and lasted longer (100 vs. 15 min) than in control subjects. Furthermore, glucose output remained below that observed in controls even at termination of the insulin infusion. The greater suppressive effect of the insulin infusion on glucose output during the initial

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1 Saccà, L., and R. S. Sherwin. Unpublished observations.
100–120 min may be a consequence of the higher ambient glucose levels in the diabetics. Recent studies employing isotopic tracer techniques (33) as well as the hepatic venous catheter technique (34) have shown that hyperglycemia per se has an inhibitory effect on glucose production so long as basal insulin levels are available.

The failure to observe a rebound increment in glucose output to basal levels in the diabetics, as was observed in controls receiving the same insulin dose, may reflect the absence of a rise in plasma norepinephrine and (or) glucagon in the diabetics (Fig. 6). Previous studies in humans have demonstrated that the counterregulatory response to intracellular glucopenia is suppressed by autonomic denervation (7). The importance of combined autonomic and alpha cell function is suggested by a recent report which indicates that the counterregulation of insulin-induced hypoglycemia in man is impaired by simultaneous adrenergic blockage and somatostatin infusion (6). A diminished alpha-cell response to hypoglycemia has previously been observed in diabetics (35). On the other hand, a normal rise in plasma norepinephrine has been noted in diabetics after pharmacologic injections of insulin (36) or after more rapid reductions in blood glucose concentration than were produced in this study (10). Thus, our failure to observe an elevation in circulating norepinephrine may be a consequence of the more gradual reduction in blood glucose concentration produced by the continuous insulin infusion dose. Nevertheless, these data indicate that as compared with controls, in whom plasma glucose fell at a similar rate, the diabetics demonstrate a secretory defect involving norepinephrine as well as glucagon, which may contribute to an exaggerated suppression of glucose output during physiologic hyperinsulinemia.

In contrast to the exaggerated suppressive effects of the insulin infusion on glucose output, the diabetics failed to demonstrate the persistent rise in glucose uptake observed early in the insulin infusion in the control subjects. Because the method employed in determining glucose uptake includes urinary losses of glucose, the failure to observe an early rise in glucose uptake in the diabetics may reflect amelioration of glycosuria by the insulin infusion. On the other hand, the absolute decline in glucose uptake observed beyond 120 min occurred at a time when plasma glucose (<100 mg/dl) was well below the renal threshold (Fig. 5). In this regard, it should be emphasized that glucose uptake in the diabetics fell to levels 39% below those observed in controls at termination of the insulin infusion. In addition, the late rise in glucose clearance in the diabetics was 28% lower than in controls.

Concerning the mechanism of the decreased glucose uptake in the diabetics as compared with controls at termination of the infusion, because these subjects had received prior treatment with insulin, plasma insulin concentrations were not determined in this group. However, inasmuch as insulin degradation has not been shown to be affected by the diabetic state (37, 38), it is likely that total plasma insulin levels were similar to those observed in the normal subjects receiving the same insulin dose (Table I). Nevertheless, the proportion of the circulating insulin which may have been bound to antibody cannot be determined from this study. It should also be noted that the increments in counterregulatory hormones observed in the diabetics (Fig. 6) were no greater than in normal subjects (Fig. 2). Thus, the differences between the normal and diabetic subjects in glucose uptake may reflect altered availability of biologically active insulin, altered peripheral sensitivity to insulin, and (or) altered tissue sensitivity to counterregulatory hormones. Regardless of the mechanism involved, these data indicate that as compared with normal subjects, in diabetics stabilization of plasma glucose in the face of physiologic hyperinsulinemia occurs primarily as a consequence of an exaggerated decline in glucose uptake and blunted stimulation of glucose clearance in the face of only a minimal reversal of insulin-induced inhibition of glucose output (Table II).

Finally, our data are in keeping with previous studies in the dog, in which larger, albeit physiologic, doses of insulin were employed (8, 39). In those studies, continuous insulin infusion (1 mU/kg per min) resulted in a transient decline in glucose output in normal dogs (8) and a pronounced reduction in glucose output in alloxan-streptozotocin diabetic animals, which entirely accounted for the blood glucose-lowering effect of insulin (39). These observations extend these findings by (a) examining the effect of physiologic hyperinsulinemia on glucose kinetics in humans; (b) comparing the kinetic response in normal and diabetic subjects; (c) demonstrating the importance of alterations in glucose uptake in preventing hypoglycemia during low dose insulin administration in diabetes; and (d) determining the relationship of changes in glucose kinetics induced by physiologic hyperinsulinemia to changes in counterregulatory hormone secretion.

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