# Role of the Decrement in Intraislet Insulin for the Glucagon Response to Hypoglycemia in Humans

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**OBJECTIVE** — Animal and in vitro studies indicate that a decrease in β-cell insulin secretion, and thus a decrease in tonic α-cell inhibition by intraislet insulin, may be an important factor for the increase in glucagon secretion during hypoglycemia. However, in humans this role of decreased intraislet insulin is still unclear.

**RESEARCH DESIGN AND METHODS** — We studied glucagon responses to hypoglycemia in 14 nondiabetic subjects on two separate occasions. On both occasions, insulin was infused from 0 to 120 min to induce hypoglycemia. On one occasion, somatostatin was infused from −60 to 60 min to suppress insulin secretion, so that the decrement in intraislet insulin during the final 60 min of hypoglycemia would be reduced. On the other occasion, subjects received an infusion of normal saline instead of the somatostatin.

**RESULTS** — During the 2nd h of the insulin infusion, when somatostatin or saline was no longer being infused, plasma glucose (~2.6 mmol/l) and insulin levels (~570 pmol/l) were comparable in both sets of experiments (both $P > 0.4$). In the saline experiments, insulin secretion remained unchanged from baseline (−90 to −60 min) before insulin infusion and decreased from 1.20 ± 0.12 to 0.16 ± 0.04 pmol · kg$^{-1}$ · min$^{-1}$ during insulin infusion ($P < 0.001$). However, in the somatostatin experiments, insulin secretion decreased from 1.18 ± 0.12 pmol · kg$^{-1}$ · min$^{-1}$ at baseline to 0.25 ± 0.09 pmol · kg$^{-1}$ · min$^{-1}$ before insulin infusion so that it did not decrease further during insulin infusion (−0.12 ± 0.10 pmol · kg$^{-1}$ · min$^{-1}$, $P = 0.26$) indicating the complete lack of a decrement in intraislet insulin during hypoglycemia. This was associated with ~30% lower plasma glucagon concentrations (109 ± 7 vs. 136 ± 9 pg/ml, $P < 0.006$) and increments in plasma glucagon above baseline (41 ± 8 vs. 67 ± 11 pg/ml, $P < 0.008$) during the last 15 min of the hypoglycemic clamp. In contrast, increases in plasma growth hormone were ~70% greater during hypoglycemia after somatostatin infusion ($P < 0.007$), suggesting that to some extent the increases in plasma glucagon might have reflected a rebound in glucagon secretion.

**CONCLUSIONS** — These results provide direct support for the intraislet insulin hypothesis in humans. However, the exact extent to which a decrement in intraislet insulin accounts for the glucagon responses to hypoglycemia remains to be established.

*Diabetes Care* 28:1124–1131, 2005

Defense against hypoglycemia normally involves inhibition of endogenous insulin secretion and increased release of several counterregulatory hormones, of which glucagon is considered to be most important (1,2). Epinephrine responses, although normally not critical for the defense against hypoglycemia, become critical when glucagon secretion is deficient (1,2). In type 1 diabetes, glucagon responses to hypoglycemia are universally lost early in the course of the disease (3). This abnormality therefore plays a key role in the development of defective glucose counterregulation when patients subsequently develop reduced epinephrine responses (3). The clinical importance of this condition is illustrated by the fact that the combined defect in glucagon and epinephrine responses is associated with at least a 25-fold increased risk for severe hypoglycemia (4,5), the major limiting factor for obtaining near-normal glycemic control (6).

Various hypotheses have emerged to explain the loss of glucagon response to hypoglycemia in type 1 diabetes including disruption of islet architecture (7), autonomic neuropathy (8–10), and loss of glucoreception (11,12). Although these factors may contribute, several observations suggest that none of these is a complete explanation. First, glucagon responses to stimuli other than hypoglycemia are largely, if not entirely, intact in type 1 diabetes (11,13), suggesting a signaling rather than a structural abnormality of α-cells. Secondly, counterregulatory glucagon responses were found to be unaltered by autonomic blockade using adrenergic and cholinergic antagonists in humans (14,15) and glucagon secretion by the denervated allografted pancreas increases during hypoglycemia (16), suggesting that autonomic neuropathy may not be the key factor. Finally, isolated viable α-cells from cadaveric pancreas donors have been found not to release glucagon in response to a low glucose medium (17), indicating that in humans the increased glucagon release in

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Received for publication 16 November 2004 and accepted 15 January 2005.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

response to hypoglycemia may not depend solely on α-cell glucoreception.

Because insulin normally inhibits glucagon release, Samols et al. (18) proposed more than 30 years ago that a decrease in β-cell insulin secretion, and thus a decrease in tonic intraislet α-cell inhibition by insulin, may be the signal for increased glucagon secretion during hypoglycemia. According to this concept, absence of a decrease in intraislet insulin due to the lack of β-cells would be a plausible explanation for the blunted glucagon responses to hypoglycemia in type 1 diabetes. This so-called intraislet insulin hypothesis is supported by the observation that the loss of glucagon responses is linked to the loss of β-cell function in patients with type 1 diabetes in most (19,20) but not all (21) studies and by the finding that counterregulatory glucagon responses deteriorate with progression of β-cell failure in patients with type 2 diabetes (22).

Further evidence for the intraislet insulin hypothesis has recently been provided by animal and in vitro studies (23,24), which indicate that both normal isolated human and rat islets and islets from streptozotocin-administered rats (which characteristically do not release glucagon when exposed to a very low glucose concentration) can respond to glucose deprivation by releasing glucagon if they are first provided with increased endogenous or exogenous insulin. These studies also demonstrated that in streptozotocin-induced diabetic rats with near complete β-cell failure, glucagon responses to hypoglycemia, which had been absent, were restored when the decrement in intraislet insulin was reestablished by an infusion of insulin into the superior pancreaticoduodenal artery that was switched off when blood glucose level fell to <60 mg/dl (24). However, whether these animal and in vitro studies are relevant to human physiology is unclear.

Definite evidence for the intraislet insulin hypothesis in humans has yet to be provided. Banarer et al. (25) reported that in normal subjects counterregulatory glucagon responses were blunted when the fall in intraislet insulin during hypoglycemia was prevented by infusion of the insulin secretagogue tolbutamide. However, in these studies intraislet insulin increased above baseline during the tolbutamide infusion and because insulin suppresses glucagon release (26), the blunted glucagon responses might have been merely the result of tolbutamide-induced intraislet hyperinsulinemia.

This study was therefore undertaken to retest the intraislet insulin hypothesis in humans using an experimental design that would obviate previous interpretative difficulties. To this end, we examined counterregulatory plasma glucagon responses of healthy subjects on two separate occasions. On one occasion, the subjects' insulin secretion was suppressed before induction of hypoglycemia by an infusion of somatostatin, which was stopped once hypoglycemia had developed so that the decrement in intraislet insulin during hypoglycemia would be reduced. On the other occasion, subjects received an infusion of normal saline before inducing hypoglycemia so that the decrement in intraislet insulin during hypoglycemia would not be reduced.

## RESEARCH DESIGN AND METHODS

— Informed written consent was obtained from 14 healthy volunteers after the protocol had been approved by the University of Rochester Institutional Review Board. Subjects (4 men and 10 women) were 36.4 ± 3.8 years of age and had BMI of 26.3 ± 1.7 kg/m$^2$. All subjects had normal fasting glucose tolerance according to American Diabetes Association criteria (27) and no family history of diabetes.

All subjects were studied on two separate occasions at least 1 week apart. For each study, subjects were admitted to the University of Rochester General Clinical Research Center between 5:00 and 6:00 P.M. the evening before experiments, received a standard dinner (10 kcal/kg: 50% carbohydrate, 35% fat, and 15% protein) between 6:30 and 7:00 P.M. and fasted thereafter until the experiments were completed.

At ~7:00 A.M. the following morning, a retrograde venous catheter was inserted into a dorsal hand vein and kept in a thermoregulated Plexiglas box at 65°C for sampling arterialized venous blood (28). Approximately 1 h later, two blood samples were collected at 30-min intervals (−90 and −60 min) for measurement of baseline concentrations of plasma glucose, insulin, C-peptide, glucagon, epinephrine, norepinephrine, growth hormone, and cortisol. At −60 min, a 2-h intravenous infusion of somatostatin (500 μg/h) was begun on one occasion to artificially reduce the decrement of intraislet insulin during the subsequent hypoglyce-

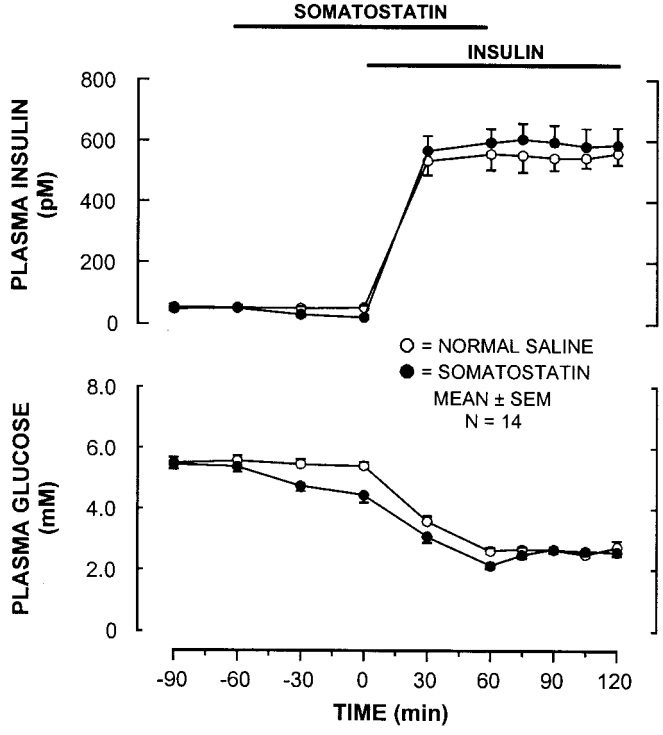


**Figure 1**—*Plasma concentrations of insulin and glucose in 14 volunteers during hypoglycemia after infusion of somatostatin or normal saline.*

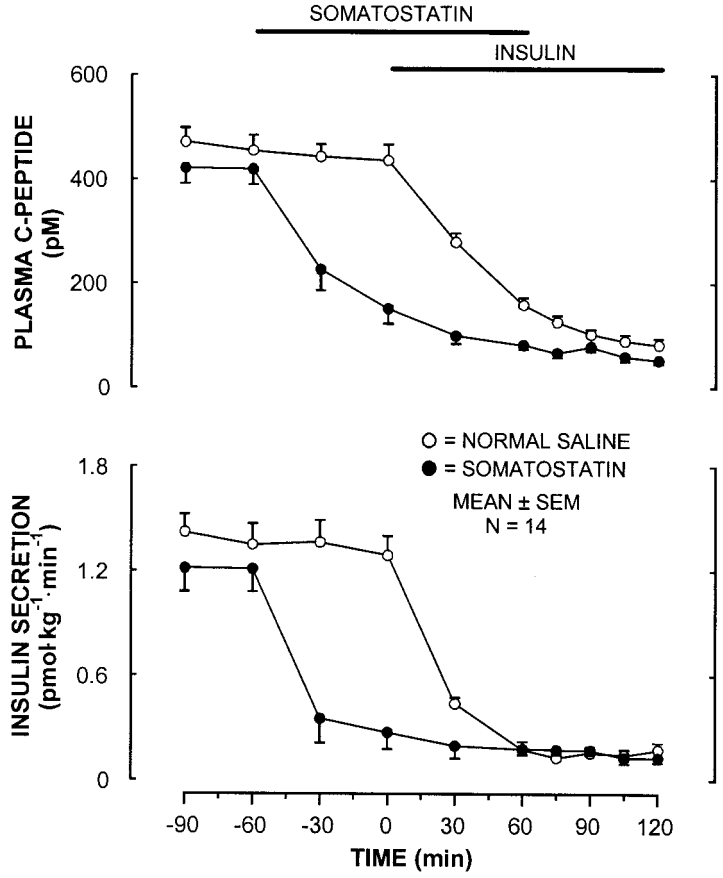


**Figure 2—***Plasma C-peptide concentrations and rates of insulin secretion in 14 volunteers during hypoglycemia after infusion of somatostatin or normal saline.*

mic clamp; on the other occasion, an infusion of normal saline was given instead. Infusions were given in a single-blinded randomized fashion. During the 1st h of the infusions, plasma glucose concentrations were maintained at baseline levels. At 0 min, a continuous infusion of insulin (1 mU · $kg^{-1}$ · $min^{-1}$) was begun, and plasma glucose concentrations were allowed to decrease to 45–50 mg/dl (2.5–2.8 mmol/l) during the following 60 min. At the end of this period (60 min), the infusion of somatostatin or normal saline was stopped, and plasma glucose concentrations were maintained at 45–50 mg/dl until 120 min using the glucose clamp technique (29). Blood samples were collected as described above at −30, 0, 30, 60, 75, 90, 105, and 120 min.

### Analytical procedures

Blood samples were collected for plasma insulin, C-peptide, glucagon, cortisol, and growth hormone in EDTA tubes containing a protease inhibitor and for plasma catecholamines in EGTA tubes. Plasma glucose was immediately determined in duplicate with a glucose analyzer (Yellow Springs Instrument). For other determinations, samples were placed immediately in a 4°C ice bath, and plasma was subsequently separated by centrifugation at 4°C. Plasma samples of both experiments of a given subject were analyzed in the same assay. Plasma insulin, C-peptide, glucagon, growth hormone, and cortisol concentrations were determined by standard radioimmunoassays. Plasma glucagon was determined by a double antibody radioimmunoassay (Linco Research, St. Charles, MO) with an intra-assay coefficient of variation of 5.9% and a sensitivity of 20–400 ng/l. Plasma epinephrine and norepinephrine concentrations were measured by a radioenzymatic method as previously described (30).

### Calculations

Rates of insulin secretion were calculated by deconvolution analysis of plasma C-peptide using an open two-compartmental model (31,32) and population-based transition coefficients (33) as described by Hovorka and Jones (34). The software (ISEC Version 2) was kindly provided by Dr. R. Hovorka, Center for Measurement and Information in Medicine, City University, London, U.K.

### Statistical analyses

Unless stated otherwise, data are expressed as means ± SEM. Paired two-tailed Student's *t* tests were used to compare corresponding data for both sets of experiments. Because secretion of glucagon and growth hormone is suppressed by somatostatin, means of plasma glucagon and growth hormone concentrations at 105 and 120 min and the increments of these means above baseline (mean of −90 and −60 min) were used for comparisons. Thus, at least 45 min were allowed for achievement of maximum plasma glucagon and growth hormone levels in response to hypoglycemia after the somatostatin infusion had been discontinued (60 min). This 45-min interval was chosen because, at a constant glucagon secretion, steady-state plasma glucagon levels would be achieved within 30 min (i.e., at least 5 × the 3–6 min $t_{1/2}$ of plasma glucagon) (35).

## RESULTS

### Plasma glucose, insulin, C-peptide, and glucagon

At baseline plasma glucose, insulin, C-peptide, and glucagon concentrations as well as rates of insulin secretion were comparable in both sets of experiments (Figs. 1–3). Plasma glucose, insulin, C-peptide, and glucagon concentrations and rates of insulin secretion remained unchanged from baseline during infusion of saline until the insulin infusion was begun. Over the comparable period during which somatostatin was infused, plasma glucose decreased to levels that were slightly lower than those in the saline experiment (4.4 ± 0.2 vs. 5.4 ± 0.1 mmol/l immediately before the start of the insulin infusion, $P < 0.01$); plasma concentrations of insulin, C-peptide, and glucagon and rates of insulin secretion decreased to levels that were significantly lower than those in the saline experiment (21 ± 3 vs. 53 ± 7 pmol/l, 151 ± 29 vs. 436 ± 31 pmol/l, 50 ± 4 vs. 65 ± 3 ng/l, 0.25 ± 0.09 vs. 1.20 ± 0.12 pmol · $kg^{-1}$ · $min^{-1}$, respectively; all $P < 0.001$). During the

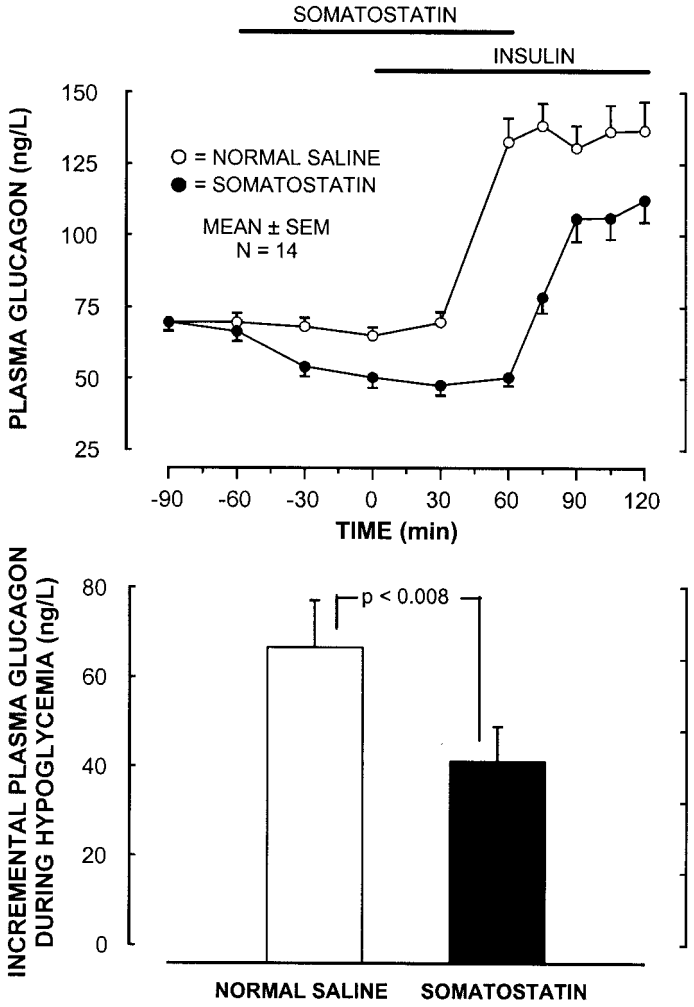


**Figure 3**—*Plasma glucagon concentrations and increments of plasma glucagon above baseline in 14 volunteers during hypoglycemia after infusion of somatostatin or normal saline.*

2-h insulin infusion, plasma insulin levels were comparable in the saline and somatostatin experiments (548 ± 41 and 588 ± 52 pmol/l, respectively, $P = 0.11$).

Over the initial 60 min of the insulin infusion while somatostatin or saline was being infused, plasma glucose decreased to slightly lower levels in the somatostatin experiments (2.2 ± 0.1 vs. 2.6 ± 0.1 mmol/l, $P < 0.001$). However, during the final hour of the insulin infusion when somatostatin or saline was no longer being infused, plasma glucose levels were virtually identical in both sets of experiments (2.6 ± 0.1 vs. 2.6 ± 0.1 mmol/l, $P = 0.68$).

Plasma C-peptide decreased to significantly lower levels in the somatostatin experiments during the initial 60 min of the insulin infusion (82 ± 8 vs. 159 ± 13 pmol/l, $P < 0.001$) and the final hour of the insulin infusion when somatostatin or saline was no longer being infused (52 ± 6 vs. 82 ± 12 pmol/l, $P < 0.004$). However, because plasma C-peptide had been suppressed before the insulin infusion in the somatostatin experiments, decrements of plasma C-peptide during the entire insulin infusion period (99 ± 28 vs. 354 ± 32 pmol/l, $P < 0.001$) and the last hour of the insulin infusion (29 ± 6 vs. 77 ± 14 pmol/l, $P < 0.009$) were markedly reduced compared with the saline experiments.

In the saline experiments, rates of insulin secretion, calculated using deconvolution analysis of plasma C-peptide, decreased to 1.01 ± 0.12 pmol · kg$^{-1}$ · min$^{-1}$ ($P < 0.001$) during the first hour of the insulin infusion but did not decrease further during the final hour of the insulin infusion (−0.03 ± 0.04 pmol · kg$^{-1}$ · min$^{-1}$, $P = 0.45$). In the somatostatin experiments, because insulin secretion had been suppressed ~80% before the insulin infusion, insulin secretion did not decrease during the 1st h of the insulin infusion (−0.09 ± 0.11 pmol · kg$^{-1}$ · min$^{-1}$, $P = 0.41$), the final hour of the insulin infusion (−0.03 ± 0.02 pmol · kg$^{-1}$ · min$^{-1}$, $P = 0.21$), or the entire insulin infusion period (−0.12 ± 0.10 pmol · kg$^{-1}$ · min$^{-1}$, $P = 0.26$) indicating the complete lack of a decrement in intraislet insulin during hypoglycemia.

Compared with values before insulin infusion, plasma glucagon increased approximately twofold in saline experiments during the initial 60 min of the insulin infusion (65 ± 3 vs. 133 ± 8 ng/l, $P < 0.001$) but remained unchanged in the somatostatin experiments (50 ± 3 vs. 50 ± 4 ng/l; NS). After discontinuation of the somatostatin infusion, plasma glucagon increased within 15 min and plateaued during the last three sampling times, indicating that sufficient time was allowed for maximum plasma glucagon levels to occur in response to hypoglycemia. During the last 15 min of the insulin infusion, both the absolute values (109 ± 7 vs. 136 ± 9 ng/l, $P < 0.006$) and the increments above baseline of plasma glucagon (41 ± 8 vs. 67 ± 11 ng/l, $P < 0.008$) were significantly reduced in the somatostatin experiments.

### Plasma epinephrine, cortisol, and growth hormone

Baseline plasma epinephrine, cortisol, and growth hormone levels were comparable in saline and somatostatin experiments (Fig. 4). During the infusion of insulin, plasma epinephrine increased earlier in the somatostatin experiments, probably because of lower plasma glucose levels. However, during the last hour of the insulin infusion, increments above baseline of epinephrine in saline and somatostatin experiments were not significantly different (4,110 ± 567 vs. 3,840 ± 508 pmol/l, $P = 0.40$). Increments of plasma norepinephrine during the same interval were also not significantly different in saline and somatostatin experiments (902 ± 197 vs. 920 ± 204 pmol/l, $P = 0.87$). Similarly, increments in plasma cortisol during this interval were comparable in saline and somatostatin experiments (268 ± 28 vs. 299 ± 33 nmol/l, $P = 0.35$).

Before the infusion of insulin, plasma growth hormone did not change during the infusion of saline but decreased about

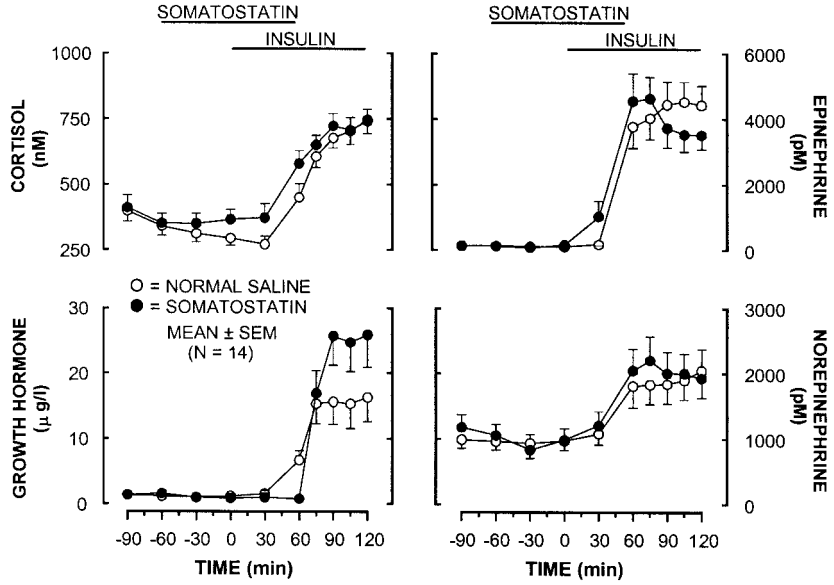


**Figure 4**—*Plasma concentrations of epinephrine, norepinephrine, cortisol, and growth hormone in 14 volunteers during hypoglycemia after infusion of somatostatin or normal saline.*

40% during the infusion of somatostatin ($P < 0.01$). However, increments of plasma growth hormone above baseline values during the last 15 min of the insulin infusion were significantly greater in the somatostatin than in the saline experiments (23.8 ± 4.8 vs. 14.2 ± 3.8 μg/l, $P < 0.007$).

**CONCLUSIONS** — This study was designed to examine the intraislet insulin hypothesis in humans using an approach that would simulate the conditions in type 1 diabetes patients, who lack the fall in intraislet insulin during hypoglycemia because of the complete loss of β-cell function. We infused somatostatin to suppress insulin release before hypoglycemia so that the decrement in insulin secretion during hypoglycemia would be minimized. As indicated by deconvolution analysis of plasma C-peptide, insulin secretion was suppressed ~80% by somatostatin before the infusion of insulin so that insulin secretion did not further decrease during hypoglycemia, including the period when somatostatin was no longer being infused and glucagon secretion was no longer being inhibited. This absent decrement in insulin secretion—and thus intraislet insulin—was associated with ~30% lower absolute and incremental plasma glucagon concentrations during hypoglycemia.

Because glucagon secretion was suppressed by the somatostatin infusion, it may be argued that glucagon responses to hypoglycemia should have been assessed using increments in plasma glucagon after the somatostatin infusion rather than using absolute plasma concentrations or increments above baseline. It is of note, however, that because of the short $t_{1/2}$ of plasma glucagon, plasma glucagon concentrations reach a steady state within ~30 min of a constant glucagon delivery into the systemic circulation (36,37). Consequently, despite the fact that plasma glucagon had been suppressed by somatostatin, plasma glucagon concentrations would be expected to be similar at the end of the hypoglycemic clamp in both experiments if glucagon secretion were comparable. We found, however, that after the somatostatin infusion had been stopped, plasma glucagon increased rapidly and plateaued after 30 min at levels that were ~30% lower than in the saline experiments. Moreover, plasma growth hormone, which had also been suppressed by somatostatin, was actually increased to a greater extent at the end of the hypoglycemic period than in the saline experiments. These observations therefore indicate that the reduced glucagon responses in the somatostatin experiments were neither the consequence of our approach to analyze the data nor due to residual effects of somatostatin inhibiting the release of glucagon into the circulation.

In the present studies, plasma glucose concentrations were slightly lower during the somatostatin infusion than during the saline infusion until these infusions were stopped. It may thus also be argued that the reduced glucagon responses in the somatostatin experiments were the result of lower antecedent glycemia rather than the reduced decrement in intraislet insulin. However, the following considerations cast doubt on this notion. First, in the somatostatin experiments plasma glucose concentrations were at all times well above the glycemic threshold for stimulation of glucagon secretion (~3.8 mmol/l) (38) before the insulin infusion and were only 0.4 mmol/l lower than in the saline experiments at the end of the 1st h of the insulin infusion. During the final hour of the insulin infusion, during which somatostatin or normal saline was no longer being infused, plasma glucose concentrations were virtually identical on both occasions. Second, if the lower antecedent glycemia was responsible for the reduced glucagon responses in the somatostatin experiments, one would expect gradually decreasing glucagon responses during prolonged hypoglycemia. This has, however, not been observed in several previous studies (39–41). Third, the slightly lower plasma glucose concentrations during the 1st h of the insulin infusion in the somatostatin experiments would be expected, if anything, to have caused increased accumulation of glucagon in the α-cells. This would have been available for release after somatostatin was stopped and resulted in greater but not lower glucagon responses.

The final consideration, and perhaps the most compelling, is that in a subanalysis using the data of only half of our subjects in whom plasma glucose concentrations of both experiments were well matched, similar results were obtained for glucagon responses. In these subjects, except for slightly lower levels from −30 to 0 min in the somatostatin experiments (5.0 ± 0.2 vs. 5.4 ± 0.1 mmol/l, $P < 0.02$), plasma glucose concentrations were virtually identical on both occasions (2.5 ± 0.1 vs. 2.6 ± 0.1 mmol/l at 60 min, $P = 0.48$; 2.6 ± 0.1 vs. 2.6 ± 0.1 mmol/l during the last hour of the insulin infusion, $P = 0.84$). As in all 14 subjects, plasma glucagon concentrations (116 ± 6 vs. 137 ± 6 ng/l, $P < 0.01$) and increments of plasma glucagon above baseline (48 ± 6 vs. 69 ± 6 ng/l, $P < 0.02$) during the last 15 min of the hypo-

glycemic clamp were significantly reduced when the decrement in intraislet insulin was prevented by the antecedent somatostatin infusion. These observations not only indicate that differences in the antecedent glycemia had very little if any effect on the counterregulatory glucagon responses but also that our findings are quite robust.

The finding that glucagon responses were only 30% lower in the somatostatin than in the saline experiments suggests that a decrement in intraislet insulin may not be essential for increased glucagon secretion in response to hypoglycemia and that factors other than the lack of the decrement of intraislet insulin may be involved in the blunted glucagon responses in type 1 diabetes patients. It is of note, however, that our results might underestimate the importance of the decrement in intraislet insulin for two reasons. First, the observed glucagon response during hypoglycemia in the somatostatin experiments might have reflected a rebound of glucagon secretion following discontinuation of the somatostatin infusion owing to accumulated α-cell hormone rather than an actual response to hypoglycemia. This concept would be consistent with the finding of Gerich et al. (42) that, despite mild hyperglycemia, plasma glucagon increased rapidly to levels that were approximately threefold greater than baseline after prolonged somatostatin infusion in type 1 diabetes subjects. Moreover, it is supported by the finding of the present study that plasma growth hormone responses were ~70% greater after the infusion of somatostatin than normal saline. Second, we cannot rule out the possibility that in the somatostatin experiments, the decrement in intraislet insulin before hypoglycemia provided a signal for glucagon secretion once hypoglycemia was established and somatostatin was no longer being infused. In other words, a glucagon response, albeit diminished, might have occurred because a β-cell signal (decreased insulin secretion) was provided in advance of hypoglycemia when it would normally happen during hypoglycemia.

In the present studies, glucagon responses to hypoglycemia were assessed during the last 15 min of a fixed 2-h insulin infusion, which resulted in physiologic and similar hyperinsulinemia in both sets of experiments. Moreover, most previous studies have found that in humans counterregulatory glucagon responses are not affected by the degree of peripheral hyperinsulinemia within the physiologic range (43–47), indicating that our findings were not the result of differences in peripheral plasma insulin levels. Mellman et al. (46) found ~25% reduced glucagon responses after prolonged (3.5 h) compared with short (30 min) peripheral hyperinsulinemia, suggesting that the duration of antecedent hyperinsulinemia may have an influence. It is thus possible that the magnitude of glucagon responses might have been different in the present study if we had used a different duration of the insulin infusion. Nevertheless, we know of no evidence suggesting that the relative importance of the decrement in intraislet insulin for counterregulatory glucagon responses may vary with different durations of antecedent hyperinsulinemia.

In summary, the present study indicates that a decrement in intraislet insulin is an important factor for increased glucagon secretion during hypoglycemia in humans consistent with the intraislet insulin hypothesis. However, it remains unclear whether the decrement in intraislet insulin is an essential signal for the increased glucagon secretion and to what extent the lack of a decrement in intraislet insulin accounts for the blunted glucagon responses in patients with type 1 diabetes.

**Acknowledgments**— This study was supported in part by the Division of Research Resources–GCRC Grants 5M01 RR-00044 (University of Rochester) and 5M01 RR00036 (University of Washington School of Medicine), the National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-20411, and an American Diabetes Association Career Development Award to C.M.

The authors thank Becky Miller for her excellent editorial assistance and the nursing and laboratory staff of the General Clinical Research Center for their superb help.

## Note added in proof

After the submission of this article, Raju and Cryer (48) reported findings consistent with those of the present study using a similar study design. In that study, oral diazoxide was given prior to inducing hypoglycemia, so that the decrement in plasma C-peptide during hypoglycemia was ~50% reduced compared with control experiments. This was associated with an ~50% reduction in counterregulatory glucagon responses, further supporting the intraislet hypothesis in humans.

## References

1. Rizza R, Cryer P, Gerich J: Role of glucagon, catecholamines, and growth hormone in human glucose counterregulation. Effects of somatostatin and combined α- and β-adrenergic blockade on plasma glucose recover and glucose flux rates after insulin-induced hypoglycemia. *J Clin Invest* 64:62–71, 1979
2. Rosen S, Clutter W, Birk M, Shah S, Cryer P: Epinephrine supports the postabsorptive plasma glucose concentration and prevents hypoglycemia when glucagon secretion is deficient in man. *J Clin Invest* 73:405–411, 1984
3. Bolli G, DeFeo P, Compagnucci P, Cartechini M, Angeletti G, Santeusanio F, Brunetti P, Gerich J: Abnormal glucose counterregulation in insulin-dependent diabetes mellitus: interaction of anti-insulin antibodies and impaired glucagon and epinephrine secretion. *Diabetes* 32:134–141, 1983
4. White N, Skor D, Cryer P, Bier D, Levandoski L, Santiago J: Identification of type I diabetic patients at increased risk for hypoglycemia during intensive therapy. *N Engl J Med* 308:485–491, 1983
5. Bolli G, DeFeo P, DeCosmo S, Perriello G, Ventura M, Benedetti M, Santeusanio F, Gerich J, Brunetti P: A reliable and reproducible test for adequate glucose counterregulation in type I diabetes mellitus. *Diabetes* 33:732–737, 1984
6. Cryer P: Banting Lecture: Hypoglycemia, the limiting factor in the management of IDDM. *Diabetes* 43:1378–1389, 1994
7. Unger R: Insulin-glucagon relationships in the defense against hypoglycemia. *Diabetes* 32:575–583, 1983
8. Maher T, Tanenberg R, Greenberg B, Hoffman J, Doe R, Goetz F: Lack of glucagon response to hypoglycemia in diabetic autonomic neuropathy. *Diabetes* 26: 196–200, 1977
9. Hilsted J, Masbad S, Krarup T, Sesloft L, Christensen J, Tromier B, Galbo H: Hormonal, metabolic and cardiovascular responses to hypoglycemia in diabetic autonomic neuropathy. *Diabetes* 30:626–633, 1981
10. Taborsky GJ Jr, Ahren B, Mundinger TO, Mei Q, Havel PJ: Autonomic mechanism and defects in the glucagon response to insulin-induced hypoglycaemia. *Diabetes Nutr Metab* 15:318–322, 2002
11. Gerich J, Langlois M, Noacco C, Karam J, Forsham P: Lack of glucagon response to hypoglycemia in diabetes: evidence for an intrinsic pancreatic alpha-cell defect. *Sci-*

*ence* 182:171–173, 1973
12. Gerich J, Langlois M, Noacco C, Lorenzi M, Karam J, Forsham P: Comparison of suppressive effects of hyperglycemia and evaluation of plasma free fatty acid levels on glucagon secretion in normal and insulin-dependent diabetic subjects: evidence for selective alpha-cell insensitivity to glucose in diabetes mellitus. *J Clin Invest* 58:320–325, 1976
13. Cryer PE, Davis SN, Shamoon H: Hypoglycemia in diabetes. *Diabetes Care* 26: 1902–1912, 2003
14. Towler D, Havlin C, Craft S, Cryer P: Mechanism of awareness of hypoglycemia: perception of neurogenic (predominantly cholinergic) rather than neuroglycopenic symptoms. *Diabetes* 42: 1791–1798, 1993
15. Hilsted J, Frandsen H, Holst JJ, Christensen NJ, Nielsen SL: Plasma glucagon and glucose recovery after hypoglycemia: the effect of total autonomic blockade. *Acta Endocrinol* 125:466–469, 1991
16. Diem P, Redman J, Abid M, Moran A, Sutherland D, Halter J, Robertson R: Glucagon, catecholamine, and pancreatic polypeptide secretion in type I diabetic recipients of pancreas allografts. *J Clin Invest* 86:2008–2013, 1990
17. Pipeleers DG, Schuit FC, Van Schravendijk CF, Van de Winkel M: Interplay of nutrients and hormones in the regulation of glucagon release. *Endocrinology* 117: 817–823, 1985
18. Samols E, Tyler J, Marks V: Glucagon-insulin interrelationships. In *Glucagon: Molecular Physiology, Clinical and Therapeutic Implications* Lefebvre P, Unger RH, Eds. Elmsford, NY, Pergamon Press, 1972
19. Fukuda M, Tanaka A, Tahara Y, Ikegami H, Yamamoto Y, Kumahara Y, Shima K: Correlation between minimal secretory capacity of pancreatic β-cells and stability of diabetic control. *Diabetes* 37:81–88, 1988
20. Madsbad S, Hilsted J, Krarup T, Sestof L, Christensen N, Faber O, Tronier B: Hormonal, metabolic, and cardiovascular responses to hypoglycemia in type I (insulin-dependent) diabetes with and without residual B-cell function. *Diabetologia* 23:499–503, 1982
21. Sjoberg S, Ahren B, Bolinder J: Residual insulin secretion is not coupled to a maintained glucagon response to hypoglycaemia in long-term type 1 diabetes. *J Intern Med* 252:342–351, 2002
22. Segel SA, Paramore DS, Cryer PE: Hypoglycemia-associated autonomic failure in advanced type 2 diabetes. *Diabetes* 51: 724–733, 2002
23. Hope KM, Tran PO, Zhou H, Oseid E, Leroy E, Robertson RP, Yang S, Zhang T: Regulation of alpha-cell function by the beta-cell in isolated human and rat islets deprived of glucose: the "switch-off" hypothesis. *Diabetes* 53:1488–1495, 2004
24. Zhou H, Tran PO, Yang S, Zhang T, LeRoy E, Oseid E, Robertson RP: Regulation of alpha-cell function by the beta-cell during hypoglycemia in Wistar rats: the "switch-off" hypothesis. *Diabetes* 53:1482–1487, 2004
25. Banarer S, McGregor VP, Cryer PE: Intraislet hyperinsulinemia prevents the glucagon response to hypoglycemia despite an intact autonomic response. *Diabetes* 51:958–965, 2002
26. Gerich J, Charles M, Grodsky G: Regulation of pancreatic insulin and glucagon secretion. *Annu Rev Physiol* 38:353–388, 1976
27. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Follow-up report on the diagnosis of diabetes mellitus. *Diabetes Care* 26:3160–3167, 2003
28. Abumrad N, Rabin D, Diamond M, Lacy W: Use of a heated superficial hand vein as an alternate site for the measurement of amino acid concentrations and for the study of glucose and alanine kinetics in man. *Metabolism* 30:936–940, 1981
29. DeFronzo R, Tobin J, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 237:E214–E223, 1979
30. Shah S, Clutter W, Cryer P: External and internal standards in the single isotope derivative (radioenzymatic) assay of plasma norepinephrine and epinephrine in normal humans and persons with diabetes on chronic renal failure. *J Lab Clin Med* 106: 624–629, 1985
31. Eaton R, Allen R, Schade D, Erickson K, Standefer J: Prehepatic insulin production in man: kinetic analysis using peripheral connecting peptide behavior. *J Clin Endocrinol Metab* 51:520–528, 1980
32. Polonsky KS, Licinio-Paixao J, Given BD, Pugh W, Rue P, Galloway J, Karrison T, Frank B: Use of biosynthetic human C-peptide in the measurement of insulin secretion rates in normal volunteers and type I diabetic patients. *J Clin Invest* 77: 98–105, 1986
33. Van Cauter E, Mestrez F, Sturis J, Polonsky KS: Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes* 41: 368–377, 1992
34. Hovorka R, Jones RH: How to measure insulin secretion. *Diabete Metab Rev* 10: 91–117, 1994
35. Smith T, Gerich J: Regulation of glucagon secretion. In *Encyclopedia of Hormones.* Henry HL, Norman AW, Eds. San Diego, CA, Academic Press, 2003, p. 74–82
36. Stumvoll M, Meyer C, Kreider M, Perriello G, Gerich J: Effects of glucagon on renal and hepatic glutamine gluconeogenesis in normal postabsorptive humans. *Metabolism* 47:1227–1232, 1998
37. Roden M, Perseghin G, Petersen KF, Hwang JH, Cline GW, Gerow K, Rothman DL, Shulman GI: The roles of insulin and glucagon in the regulation of hepatic glycogen synthesis and turnover in humans. *J Clin Invest* 97:642–648, 1996
38. Mitrakou A, Ryan C, Veneman T, Mokan M, Jenssen T, Kiss I, Durrant J, Cryer P, Gerich J: Hierarchy of glycemic thresholds for counterregulatory hormone secretion, symptoms, and cerebral dysfunction. *Am J Physiol* 260:E67–E74, 1991
39. Fanelli C, Calderone S, Epifano L, DeVincenzo A, Modarelli F, Perriello G, DeFeo P, Brunetti P, Gerich J, Bolli G: Demonstration of critical role for FFA in mediating counterregulatory stimulation of gluconeogenesis and suppression of glucose utilization in man. *J Clin Invest* 92: 1617–1622, 1993
40. Capaldo B, Napoli R, Guida R, Di Bonito P, Antoniello S, Auletta M, Pardo F, Rendina V, Sacca L: Forearm muscle insulin resistance during hypoglycemia: role of adrenergic mechanisms and hypoglycemia per se. *Am J Physiol* 268:E248–E254, 1995
41. DeFeo P, Perriello G, DeCosmo S, Ventura M, Campbell P, Brunetti P, Gerich J, Bolli G: Comparison of glucose counterregulation during short-term and prolonged hypoglycemia in normal humans. *Diabetes* 35:563–569, 1986
42. Gerich J, Lorenzi M, Bier D, Schneider V, Tsalikian E, Karam J, Forsham P: Prevention of human diabetic ketoacidosis by somatostatin: evidence for an essential role of glucagon. *N Engl J Med* 292:985–989, 1975
43. Bolli G, DeFeo P, Perriello G, DeCosmo S, Compagnucci P, Santeusanio F, Brunetti P, Unger R: Mechanisms of glucagon secretion during insulin-induced hypoglycemia in man: role of beta-cell and arterial hyperinsulinemia. *J Clin Invest* 73:917–922, 1983
44. Davis S, Goldstein R, Jacobs J, Price L, Wolfe R, Cherrington A: The effects of differing insulin levels on the hormonal and metabolic response to equivalent hypoglycemia in normal humans. *Diabetes* 42: 263–272, 1993
45. Davis S, Cherrington A, Goldstein R, Jacobs J, Price L: Effects of insulin on the counterregulatory response to equivalent hypoglycemia in normal females. *Am J Physiol* 265:E680–E689, 1993
46. Mellman MJ, Davis MR, Shamoon H: Effect of physiological hyperinsulinemia on counterregulatory hormone responses during hypoglycemia in humans. *J Clin Endocrinol Metab* 75:1293–1297, 1992

47. Liu D, Moberg E, Kolind M, Lins P-E, Adamson U: A high concentration of circulating insulin suppresses the glucagon response to hypoglycemia in normal man. *J Clin Endocrinol Metab* 73:1123–1128, 1991
48. Raju B, Cryer PE: Loss of the decrement in intraislet insulin plausibly explains loss of the glucagon response to hypoglycemia in insulin-deficient diabetes: documentation of the intraislet insulin hypothesis in humans. *Diabetes* 54:757–764, 2005