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P Felig, ... , J Wahren, R Hendler

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### Research Article

To evaluate the effects of physiologic hyperglucagonemia on splanchnic glucose output, glucagon was infused in a dose of 3 ng/kg per min to healthy subjects in the basal state and after splanchnic glucose output had been inhibited by an infusion of glucose (2 mg/kg per min). In the basal state, infusion of glucagon causing a 309 +/- 25 pg/ml rise in plasma concentration was accompanied by a rapid increase in splanchnic glucose output to values two to three times basal by 7-15 min. The rise in arterial blood glucose (0.5-1.5 mM) correlated directly with the increment in splanchnic glucose output. Despite continued glucagon infusion, and in the face of stable insulin levels, splanchnic glucose output declined after 22 min, returning to basal levels by 30-45 min. In the subjects initially receiving the glucose infusion, arterial insulin concentration rose by 5-12 muU/ml, while splanchnic glucose output fell by 85-100%. Infusion of glucagon causing an increment in plasma glucagon concentration of 272 +/- 30 pg/ml reversed the inhibition in splanchnic glucose production within 5 min. Splanchnic glucose output reached a peak increment 60% above basal levels at 10 min, and subsequently declined to levels 20-25% below basal at 30-45 min. These findings provide direct evidence that physiologic increments in plasma glucagon stimulate splanchnic glucose output in the basal state and reverse insulin-mediated [...]

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## Influence of Physiologic Hyperglucagonemia on Basal and Insulin-Inhibited Splanchnic Glucose Output in Normal Man

PHILIP FELIG, JOHN WAHREN, and ROSA HENDLER

*From the Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut 06510, and the Department of Clinical Physiology, Karolinska Institute and Serafimer Hospital, Stockholm, Sweden*

**ABSTRACT** To evaluate the effects of physiologic hyperglucagonemia on splanchnic glucose output, glucagon was infused in a dose of 3 ng/kg per min to healthy subjects in the basal state and after splanchnic glucose output had been inhibited by an infusion of glucose (2 mg/kg per min).

In the basal state, infusion of glucagon causing a  $309 \pm 25$  pg/ml rise in plasma concentration was accompanied by a rapid increase in splanchnic glucose output to values two to three times basal by 7–15 min. The rise in arterial blood glucose (0.5–1.5 mM) correlated directly with the increment in splanchnic glucose output. Despite continued glucagon infusion, and in the face of stable insulin levels, splanchnic glucose output declined after 22 min, returning to basal levels by 30–45 min.

In the subjects initially receiving the glucose infusion, arterial insulin concentration rose by 5–12  $\mu$ U/ml, while splanchnic glucose output fell by 85–100%. Infusion of glucagon causing an increment in plasma glucagon concentration of  $272 \pm 30$  pg/ml reversed the inhibition in splanchnic glucose production within 5 min. Splanchnic glucose output reached a peak increment 60% above basal levels at 10 min, and subsequently declined to levels 20–25% below basal at 30–45 min.

These findings provide direct evidence that physiologic increments in plasma glucagon stimulate splanchnic glucose output in the basal state and reverse insulin-mediated inhibition of splanchnic glucose production in normal man. The transient nature of the stimulatory effect of glucagon on splanchnic glucose output suggests the rapid development of inhibition or reversal of glucagon action. This inhibition does not appear to depend on increased insulin secretion.

### INTRODUCTION

Increased plasma levels of glucagon have been demonstrated during fasting (1, 2), after protein feeding (3, 4), and in a variety of disease states (5, 6). The physiologic significance of these changes derives from the presumed ability of increments in plasma glucagon to stimulate hepatic glycogenolysis. Direct evidence of a stimulatory effect of glucagon on splanchnic glucose production and gluconeogenesis in intact man has been reported with pharmacologic doses of glucagon, achieving circulating plasma levels of 5,000 pg/ml (7, 8). Data on the effects of physiologic increments in plasma glucagon (100–300 pg/ml) on splanchnic glucose output have not, however, been reported. Of particular interest is whether physiologic hyperglucagonemia can reverse the inhibition in splanchnic glucose output induced by small increments (10–20  $\mu$ U/ml) of insulin

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(9). This counter-insulin effect of glucagon has been suggested as the mechanism whereby euglycemia is maintained in the face of the hyperinsulinemia accompanying a protein meal (3, 10). The current study was consequently undertaken to examine the effects of physiologic increments in plasma glucagon on splanchnic glucose production in the basal state in normal man. In addition, we determined whether such elevations in glucagon can reverse insulin-mediated inhibition of splanchnic glucose output induced by infusion of glucose.

## METHODS

The subjects were 12 healthy nonobese adults, 21-37 yr of age. All were within 10% of ideal body weight (based on Metropolitan Life Insurance Co. Tables, 1959). The nature, purpose, and possible risks involved in the study were carefully explained to all the subjects before their voluntary, written consent to participate was obtained.

The subjects were studied in the postabsorptive state after an overnight, 12-14-h fast. Hepatic venous and brachial arterial catheterization were performed as described previously (9-11). In addition, an indwelling catheter was placed in an antecubital vein for infusion of glucagon and/or glucose. After placement of the catheters, base-line blood samples were obtained at 10-min intervals over 30-min.

Two types of studies were conducted. In one group of studies (six subjects), crystalline glucagon (Eli Lilly and Company, Indianapolis, Ind.) was infused intravenously at a rate of 3 ng/kg per min for 45 min. The procedures em-

ployed in the preparation and administration of the glucagon infusate were as described previously (2, 6, 12), except that human serum albumin rather than whole blood was added to the infusate (in a final concentration of 500 mg/100 ml of infusate) to prevent glucagon adsorption to tubing or glassware. In a second group of studies (six subjects), glucose was continuously infused at a rate of 2 mg/kg per min for 45 min. This rate of glucose infusion was chosen since it is known to inhibit splanchnic glucose output without stimulating peripheral glucose utilization (9, 11). After 45 min, an infusion of crystalline glucagon was started at a rate of 3 ng/kg per min. The simultaneous infusions of glucagon and glucose were then continued for an additional 45 min.

Hepatic blood flow was estimated by the continuous infusion technique (13) with indocyanine green dye (14). Glucose was measured in whole blood by the glucose oxidase reaction (15). Plasma glucagon was analyzed by radioimmunoassay with Unger antibody 30K (16). Insulin was determined by radioimmunoassay with talc to separate bound from free insulin (17).

Data in the text, tables, and figures are given as the mean  $\pm$  SE. Standard statistical methods have been employed (18), with the paired *t* test when applicable.

## RESULTS

*Response to glucagon infusion in the basal state.* In Table I, the effects of the infusion of glucagon in the basal state on arterial levels of glucagon, glucose, and insulin are shown. During the infusion of glucagon,

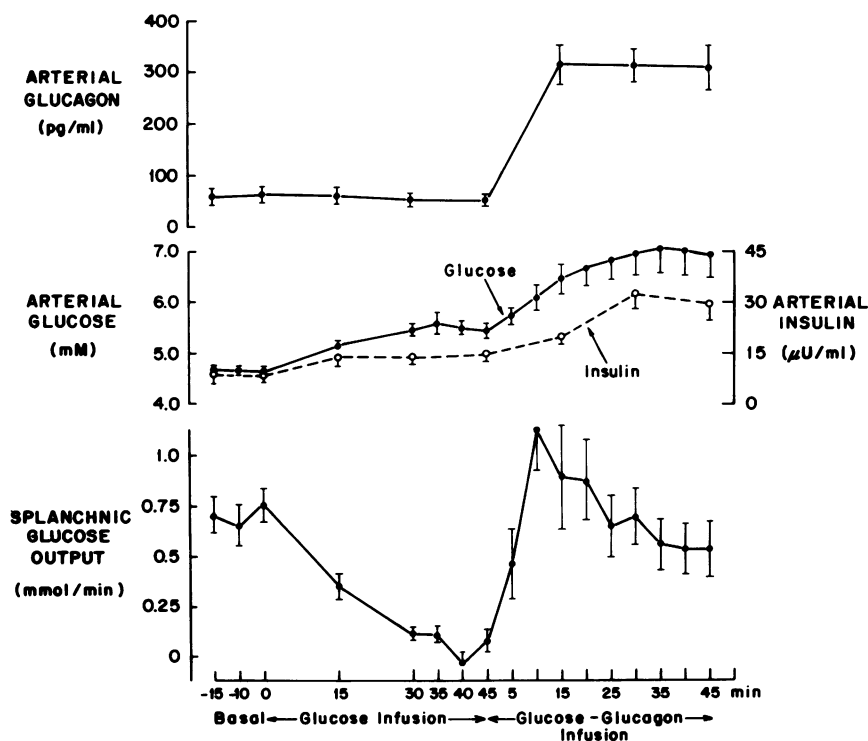


FIGURE 1 Arterial plasma glucagon, glucose, and insulin concentrations, and splanchnic glucose output in the basal state and in response to infusion of glucose (2 mg/kg per min), followed by infusion of glucose plus glucagon (3 ng/kg/min).

TABLE I  
Influence of Glucagon Infusion on Splanchnic Glucose Output in the Basal State in Normal Subjects

Time, min	Basal			Glucagon infusion					
	-15	-7.5	0	7.5	15	22.5	30	37.5	45
Arterial glucagon, pg/ml	88 ±20	—	102 ±25	—	403* ±26	—	430* ±42	—	378* ±29
Arterial glucose, mM	4.84 ±0.14	4.83 ±0.12	4.92 ±0.12	5.36* ±0.18	5.97* ±0.21	6.15* ±0.25	6.14‡ ±0.32	6.03* ±0.27	6.01‡ ±0.28
Arterial insulin, μU/ml	14 ±2	—	14 ±2	—	18§ ±1	—	22§ ±2	—	18§ ±2
Splanchnic glucose output, mmol/min	0.86 ±0.10	0.98 ±0.15	0.75 ±0.11	2.22‡ ±0.43	2.06§ ±0.58	2.05§ ±0.67	1.10 ±0.12	1.06 ±0.22	0.98 ±0.09
Estimated hepatic blood flow, ml/min	1,387 ±127	1,317 ±107	1,325 ±103	1,327 ±82	1,232 ±43	1,182 ±53	1,336 ±58	1,243 ±100	1,228 ±57

\* Significantly different from basal state,  $P < 0.001$  (paired  $t$  test).

‡ Significantly different from basal state,  $P < 0.01$  (paired  $t$  test).

§ Significantly different from basal state,  $P < 0.05$  (paired  $t$  test).

plasma glucagon levels rose to 380–430 pg/ml, reaching a plateau within 15 min. The mean increment in glucagon was  $309 \pm 25$  pg/ml. Arterial glucose concentration rose by 0.5–1.5 mmol/liter (9–27 mg/100 ml), reaching a peak increment at 22–30 min and then declining slightly at 45 min. Plasma insulin levels rose by 5–12 μU/ml.

Splanchnic glucose production and blood flow are also shown in Table I. During the infusion of glucagon, splanchnic glucose output rose rapidly, reaching levels two or three times basal within 7.5 min. In five of the six subjects, the peak increment was observed at 7.5 min. A direct linear correlation was observed between the maximal increment in splanchnic glucose output and the peak rise in arterial blood glucose ( $r = 0.76$ ,  $P < 0.05$ ). Splanchnic glucose output remained elevated at 15–22 min but returned to basal levels by 30 min. The mean values at 30, 37.5, and 45 min thus were not significantly different from the basal, pre-infusion control period. Splanchnic blood flow was stable during the glucagon infusion.

*Response to glucagon infusion in subjects receiving glucose.* In Fig. 1 the effects of glucagon in subjects in whom splanchnic glucose output had been inhibited by prior infusion of glucose are shown. In accord with previous studies (9, 11), during the initial 45 min of the infusion of glucose, arterial glucose rose by 0.8–0.9 mmol/liter, insulin levels increased by 5–12 μU/ml, ( $P < 0.05$ , paired  $t$  test), and plasma glucagon concentration remained unchanged. Splanchnic glucose output fell progressively and was inhibited by 85–100% at 40–45 min ( $P < 0.01$ ). When the glucagon infusion was then added, arterial glucagon rapidly rose, reaching mean levels of 310–320 pg/ml. The mean increment in glucagon was  $272 \pm 30$  pg/ml. Arterial glucose increased by an additional 1.5 mmol/liter, while plasma insulin rose by 10–20 μU/ml. Despite ongoing glucose adminis-

tration, splanchnic glucose output increased rapidly during the glucagon infusion, rising fivefold within 5 min ( $P < 0.05$ ), and reaching a peak level at 10 min ( $1.12 \pm 0.19$  mmol/min) which was 60% above the basal (before glucose infusion) output ( $P < 0.05$ ). Thereafter, splanchnic glucose output progressively fell, reaching levels 22% below basal ( $P < 0.05$ ) and 57% below the peak increment ( $P < 0.01$ ) at 35–45 min. Splanchnic blood flow ( $1,146 \pm 60$  ml/min in the basal state) remained stable throughout the observations.

## DISCUSSION

The current findings provide direct evidence that physiologic increments in plasma glucagon produced by infusion of exogenous hormone cause a prompt 2–3-fold rise in splanchnic glucose output in normal man in the basal state. The increments in glucagon achieved in the present study (250–350 pg/ml) are comparable to those reported after a protein meal (3, 4, 10), during starvation (1, 2), with prolonged exercise (19), and in response to acute hypoglycemia (20). These findings thus suggest that in each of these conditions the observed increments in glucagon contribute to glucose homeostasis by stimulating hepatic glucose output. The relative contributions of glycogenolysis and gluconeogenesis to this effect of glucagon cannot be determined from the present data. The rapidity of the response (peak increment within 7.5 min) suggests that the major stimulatory effect of glucagon is on glycogenolysis. However, an effect on gluconeogenesis has also been observed with plasma glucagon increments of 500 pg/ml in the dog (21).

In addition to its effects in the basal state, infusion of glucagon in physiologic amounts resulted in a rapid reversal of insulin-mediated inhibition of splanchnic glucose output induced by glucose infusion (Fig. 1). Splanchnic glucose output, inhibited by 85–100% during

the glucose infusion, rose to levels 60% above the basal within 10 min of initiation of the glucagon infusion. It should be noted that the plasma insulin rise accompanying the glucose infusion was comparable to that observed during a protein meal (3, 4, 10). The increase in glucagon secretion produced by protein feeding has been suggested as the factor responsible for the maintenance of euglycemia in this circumstance (3) by permitting basal rates of splanchnic glucose output to continue in the face of hyperinsulinemia (10). The current findings provide direct evidence that (a) increments in insulin of the magnitude observed with a protein meal can inhibit splanchnic glucose output; and (b) this effect can be overcome by a simultaneous, physiologic rise in plasma glucagon. These findings thus lend strong support to the conclusion previously proposed by Unger et al., that hyperglucagonemia is an essential factor in the maintenance of glucose homeostasis after protein feeding (3).

Of particular interest in the present study was the demonstration that the stimulatory effect of physiologic increments in plasma glucagon on splanchnic glucose output in the basal state persisted for less than 30 min (Table I). Despite ongoing hyperglucagonemia, by 30–45 min splanchnic glucose output had returned to levels not significantly different from the basal state (Table I). (The failure to observe an accompanying decline in arterial glucose is surprising. This may be explained, in part, by the relatively small rise in insulin, which would not be expected to stimulate peripheral glucose utilization [9, 11].) A less than persistent response was also observed with regard to the reversal of insulin-induced inhibition of splanchnic glucose output by glucagon; within 45 min, splanchnic glucose output had fallen to levels 20–25% below basal and 60% below the peak increment (Fig. 1). This rapid loss of hepatic responsiveness to physiologic elevations in plasma glucagon clearly differs from the liver's ongoing response to pharmacologic doses of glucagon (50 ng/kg per min), in which circumstance a sustained rise in splanchnic glucose output is observed that persists throughout a 3-h glucagon infusion (7). The transient effects of physiologic hyperglucagonemia on splanchnic glucose balance also contrast with the sustained action of physiologic increments of insulin. Suppression of hepatic glucose production by hyperinsulinemia has been reported to persist throughout a 2–3-h infusion of insulin (22).

Depletion of liver glycogen does not explain the mechanism of the transient nature of the splanchnic response to glucagon. The total glucose output during the initial 30 min of the glucagon infusion was approximately 10 g (Table I); total liver glycogen stores in postabsorptive man have been shown to be 70–90 g (23). Hyperinsulinemia is also unlikely to account for

this phenomenon. In the subjects studied in the basal state, insulin levels after 45 min of the glucagon infusion were no different than at 15 min, yet splanchnic glucose output had fallen to base line during that interval (Table I). Furthermore, in insulin-dependent diabetic subjects, a similar return of splanchnic glucose output to basal levels has been observed within 30–60 min, despite ongoing infusion of glucagon.<sup>1</sup> Moreover, Cherrington and Vranic observed a waning with time of glucagon's effect on hepatic glucose production in depancreatized dogs (24). These findings thus suggest the rapid development of reversal or inhibition of glucagon action, which may not depend on increased insulin secretion. A similar response to endogenous glucagon is suggested by the transient rise in splanchnic glucose output observed after protein feeding in diabetic patients despite persistence of hyperglucagonemia (10). Regardless of the mechanism involved, the current demonstration of the transience of the stimulatory action of glucagon on glucose production in intact man may explain, in part, the failure of 3–6-h infusions of glucagon to produce glucose intolerance in normal subjects (12), and the failure of continuous hyperglucagonemia induced by 2–3-day infusions of this hormone to produce deterioration of diabetic control (12). These findings may also account for the lack of a correlation between plasma glucose and glucagon levels in diabetic subjects (25).

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