Effects of Low-Dose and High-Dose Glucagon on Glucose Production and Gluconeogenesis in Humans

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The analysis of mass isotopomers in blood glucose and lactate can be used to estimate gluconeogenesis (Gneo), glucose production (GP), and, by subtraction, nongluconeogenic glucose release by the liver. At 6 AM, 18 normal subjects received a 7-hour primed constant infusion of [U-$^{13}C_6$] glucose. After a 3-hour baseline period (12 hours of fasting), somatostatin, insulin, hydrocortisone, growth hormone (GH), and glucagon were infused for 4 hours. Glucagon was infused at a low-dose (n = 6) or high-dose (n = 6) concentration for 4 hours and was compared with fasting alone (n = 6). Low-dose glucagon infusion increased plasma glucagon (64 ± 3 *v* 44 ± 7 ng/L, low glucagon *v* baseline). GP increased above baseline (15.5 ± 0.5 *v* 13.8 ± 0.5 μmol/kg/min, $P < .05$), which was also greater than fasting alone (11.5 ± 0.6 μmol/kg/min, $P < .05$). The elevation in GP was due to a near doubling of nongluconeogenic glucose release compared with fasting alone (8.3 ± 0.6 *v* 4.7 ± 0.5 μmol/kg/min, $P < .01$). High-dose glucagon infusion (125 ± 25 ng/L) increased GP above baseline (15.8 ± 0.6 *v* 13.5 ± 0.5 μmol/kg/min, $P < .05$), which was also greater than fasting alone (11.5 ± 0.6 μmol/kg/min, $P < .05$). The increase in GP was due to an increase in Gneo (8.5 ± 0.5 *v* 6.8 ± 0.7 μmol/kg/min, $P < .05$) and nongluconeogenic glucose release (7.4 ± 0.5 *v* 4.7 ± 0.4 μmol/kg/min, $P < .05$) compared with fasting. Low-dose glucagon increases GP only by stimulation of nongluconeogenic glucose release. High-dose glucagon increases GP by an increase in both Gneo and nongluconeogenic glucose release.

IN RECENT YEARS, several methods have been developed to estimate the contribution of glycogenolysis and gluconeogenesis (Gneo) to glucose production (GP) in vivo.[1-3] We have used mass isotopomer analysis with [U-^{13}C] glucose to estimate a number of parameters of glucose metabolism in humans.[1,4] We estimated the contribution of Gneo to GP in individuals with diabetes and cancer and, more recently, its contribution to GP in 12-, 20-, and 40-hour fasted subjects.[5] In the postprandial state, glucose is produced from lactate, amino acids, and glycerol and by hepatic glycogenolysis. We show here that glucagon affects both glycogenolysis and Gneo.

Prior investigations have demonstrated that glucagon administration increases GP but the initial increase is not sustained after 120 to 240 minutes.[6-9] The early effect of glucagon is attributable to an increase in glycogenolysis, while its later effect involves gluconeogenic stimulation. In dogs, when hydrocortisone is not coadministered with glucagon, GP returns to normal after 2 to 4 hours.[6,7] When hydrocortisone is coadministered, GP is 35% to 40% above baseline and remains elevated for 5 hours.[7] Similar to the animal data, when subjects are not coinfused with hydrocortisone, GP decreases to normal after 100 to 160 minutes.[8,9]

Infusion of hydrocortisone alone increases overnight fasting blood glucose concentrations and fasting GP in humans.[10] Under hyperinsulinemic-euglycemic conditions, hydrocortisone infusion increases endogenous glucose production (10.5 ± 0.7 *v* 5.0 ± 0.8 μmol/kg/min, mean ± SEM, $P < .005$). These data would suggest that hydrocortisone administration influences GP in humans. Since cortisol decreases after the early morning hours (when many infusion studies begin), the failure to sustain serum cortisol concentrations may be responsible, in part, for the return to normal rates of GP observed in the 2 published human studies.[8,9]

In this study, we maintained critical hormone concentrations by infusing the hormones to maintain the values at baseline concentrations. Unlike prior studies,[8,9] hydrocortisone was coadministered to prevent the decrease in serum cortisol observed after the morning hours. The preliminary aim of this study was to determine if low- or high-dose glucagon administration increases Gneo. A secondary aim was to test whether GP returns to normal during a high-dose glucagon infusion combined with somatostatin and replacement insulin, growth hormone (GH), and cortisol administration. Several mechanisms may be responsible for the decrease in GP after the initial increase, such as (1) a failure to activate gluconeogenic enzymes and Gneo, (2) a reduction in the supply of gluconeogenic amino acids, (3) a failure to sustain glycogenolysis, or (4) an inhibition of GP by an elevated blood glucose concentration. We indirectly tested the first 3 possibilities for the waning effect of glucagon on GP. We estimated gluconeogenic activity by measuring Gneo, and gluconeogenic substrate concentrations by measuring plasma amino acid profiles. We estimated glycogenolysis by subtracting Gneo from GP. A pilot study demonstrated that despite a 2-fold increase in plasma glucose during high-glucagon pituitary-pancreatic (P-P) infusion that maintained serum cortisol and insulin at baseline concentrations, both estimates of Gneo and nongluconeogenic glucose release were greater than fasting alone.[5] The rationale to administer hydrocortisone to maintain a normal (200 to 390 nmol/L) 9 AM blood cortisol concentration was to minimize the decrease in amino acids during the glucagon infusion. The 9 AM blood cortisol concentration was our target, where we coinfused hydrocortisone to maintain plasma cortisol to minimize any effect of the diurnal decrease in cortisol's ability to mobilize amino acids.

These studies involve 4-hour infusions of either low- or high-dose glucagon to document glucagon's effect on Gneo and GP. We have intentionally kept the insulin replacement at a physiological concentration similar to that observed with fasting, to prevent the effects of an elevated insulin concentration

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on hepatic and peripheral glucose metabolism. This was based on recent data demonstrating that insulin at a higher concentration has a profound effect on glycogenolysis.[11] We found that both low- and high-dose glucagon administration for 4 hours elevate GP and sustain blood glucose concentrations. A high-dose glucagon infusion, but not a low-dose infusion, can also increase Gneo.

SUBJECTS AND METHODS

Inpatient Clinical Research Center Schedule

Eighteen normal subjects provided informed consent and were enrolled onto an inpatient Clinical Research Center protocol approved by the Institutional Review Board. The mean age of the subjects was 41 ± 4 years (weight, 70 ± 3 kg), and 3 of the 18 were females. On day 1, the subjects were placed on a balanced diet with calories at 1.25 times the estimated basal energy expenditure, 1 g/kg protein intake per day, and a minimum of 300 g carbohydrate intake per day. Daily food intake was recorded. The last food (snack) was provided at 9 PM on day 2. All patients also had a 24-hour dietary recall for determination of recent dietary intake. On day 3, all subjects underwent an infusion study to measure Gneo, GP, nongluconeogenic glucose release (GP minus Gneo), and blood glucose.

Hormone Infusion Protocol

Three groups of 6 normal subjects each (fasting, low glucagon, and high glucagon) were studied on day 3. We used the modified P-P clamp technique to control critical hormone levels.[12] Metyrapone was not administered due to its effect on Gneo.[13] Hormone infusion studies began at 9 AM, or 3 hours after the start of the isotope infusion (Fig 1). Six patients received a low-dose glucagon infusion (0.7 ng/kg/min) for 4 hours and 6 received a 4-hour high-dose glucagon infusion (2.8 ng/kg/min). During the 4-hour infusion period, somatostatin (0.1 μg/kg/min), insulin (140 μU/kg/min), hydrocortisone (0.6 μg/kg/min), and human GH (7 ng/kg/min) were administered to maintain the 9 AM concentrations. For only the low-dose glucagon group, the 4-hour low-dose glucagon P-P infusion period was followed by a 4-hour high-glucagon P-P infusion (2.8 ng/kg/min). The third group of 6 subjects did not receive the P-P infusion but fasted over the same period.

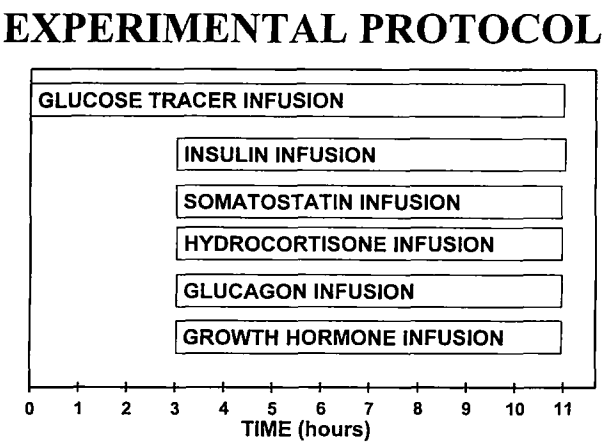

Fig 1. Infusion protocol for a 7-hour and 11-hour isotope infusion period. The 3-hour baseline [U-^{13}C] glucose infusion period started at 6 AM. The P-P infusion study started after 3 hours of a baseline stable glucose infusion, or 9 AM (12 hours of fasting). At 9 AM, glucagon was infused at a low dose (0.7 ng/kg/min) or high dose (2.8 ng/kg/min) for a 4-hour infusion period (9 AM-1 PM). The 6 subjects who received the low-dose glucagon infusion first subsequently received the high-dose glucagon infusion between hours 7 and 11 (1 PM-5 PM). The dose of all other hormones (insulin, hydrocortisone, GH, and somatostatin) was equal during the 4- to 8-hour hormone infusion period. Six subjects were infused with glucose tracer only and acted as 12-hour, 16-hour, and 20-hour fasting-alone controls. There were 6 subjects in each group (low/high glucagon, high glucagon, and fasting alone).

Stable-Isotope Infusion Protocol

At 6 AM, all patients received a primed continuous 7-hour infusion of 0.17 to 0.28 μmol/kg/min [U-$^{13}C_6$] glucose. The priming dose was 16.8 μmol/kg. Baseline measurements of GP and an estimate of Gneo were obtained between 8 and 9 AM (12 hours of fasting) in all 3 groups. Six subjects (controls) were studied during a progressive fast (12, 16, and 20 hours). The 12- and 20-hour fasting data were reported previously.[5] Six subjects received a 4-hour high-dose glucagon infusion. Six others received a 4-hour low-dose glucagon infusion first, followed by a 4-hour high-dose glucagon infusion. This was a square-wave infusion with only the glucagon hormone increased (0.7 to 2.8 ng/kg/min). The 20-hour high-glucagon data were compared with the 20-hour fasting-alone data.[5]

In all patients, indirect calorimetry was performed over 30-minute periods between 8 and 9 AM and between 12 noon and 1 PM using a Delta track (Sensor Medics, Yorba Linda, CA) to measure the respiratory quotient (RQ). Both fat and glucose oxidation were determined by the equations of Weir. These data were also obtained for the 12 subjects who received either extended 20-hour fasting or high-dose glucagon infusion after the low-dose glucagon infusion.

Plasma glucose and lactate enrichment and plasma insulin, C-peptide, glucose, glucagon, GH, cortisol, catecholamines, free fatty acids (FFAs), and amino acids were determined every 20 minutes over the final 60 minutes of each infusion period (hour 2 to 3 or 8 to 9 AM baseline, 12 to 1 PM, and 4 to 5 PM). All hormones and substrates except glucagon were measured as previously described.[14] Glucagon was assayed by radioimmunoassay (Linco Research, St. Charles, MO) with an antibody that detects much lower glucagon concentrations than those previously reported using the Unger method.[1,7,8] Portal insulin was calculated by the method of DeFeo.[12] Hepatic insulin was calculated assuming a 72% vascularization of the liver by the portal vein and 28% by the hepatic artery between 12 and 20 hours of fasting. FFAs were determined by a calorimetric method.[15] Plasma amino acids were measured using a Beckman Gold amino acid analyzer (Beckman Instruments, Fullerton, CA).

Calculations

The equations for the Cori cycle, Gneo, and GP were recently published.[4] M_1, M_2, and M_3 are the enrichment in glucose, and m_1, m_2, and m_3 in lactate. M_0 is the fraction of glucose containing no labeled carbon, and M_6 contains ^{13}C in all 6 carbons. Sigma M 1 to 3 (Σ_1^3 M) is the fraction of glucose with 1 to 3 carbons, and Σ_1^6 M is the fraction of glucose containing 1 to 6 carbons. The molar enrichments of these fractions are similar to the specific activity, and are Σ_1^3 Mn and Σ_1^6 Mn, respectively. Similarly, Σ_1^3 m is the molar enrichment of lactate. We assume equal pyruvate cycling under all conditions and that arterial lactate accurately reflects hepatic (and renal) pyruvate. Briefly, we estimate Gneo as the product of the Cori cycle and the dilution of hepatic pyruvate. The method overestimates Gneo due to $^{13}CO_2$ fixation (5% to 10%):

$$\text{GP} = \text{infused dose (μmol/kg/min)}/\text{M}_6, \quad (1)$$

$$\text{recycling of carbon} = \sum_1^3 \text{Mn}/\sum_1^6 \text{Mn}, \quad (2)$$

$$\%\ \text{Cori cycle} = \sum_1^3 \text{M}/\sum_1^6 \text{M}, \quad (3)$$

$$\text{dilution of hepatic pyruvate (fold)} = \sum_{1}^{6} \text{Mn}/2\text{x} \sum_{1}^{3} \text{mn}, \quad (4)$$

$$\text{and \% contribution of Gneo} = \text{Eq 3} \times \text{Eq 4} = \frac{\sum_{1}^{3} \text{M} \times \sum_{1}^{6} \text{Mn}}{\sum_{1}^{6} \text{M} \times 2 \sum_{1}^{3} \text{mn}}. \quad (5)$$

Data Analysis

Data were compared by ANOVA between fasting and the 2 glucagon-treated groups (high and low glucagon). In addition, repeated-measures ANOVA (12, 16, and 20 hours) was used for data analysis within the 2 groups (low/high and fasting alone) who received the 11-hour stable-isotope infusion. Simple linear and multiple-step linear regression analysis was performed by the method of least squares. Significance was defined as a P level less than .05. The data are presented as the mean ± SEM.

RESULTS

Low-dose glucagon administration under P-P conditions increased plasma glucose by 70% after 1 hour, and this declined to a 50% increase by 4 hours. In comparison, high-dose glucagon increased plasma glucose by 120%, and this was still 90% above fasting values after 4 hours (Fig 2). Fasting alone resulted in a 12% decrease in plasma glucose. Insulin concentrations were similar at all time points during the P-P protocol (Table 1). The slight increase was greater during the high-glucagon infusion versus the 20-hour fast alone, but this was not greater versus the low-dose glucagon period (16-h Fasting) or the baseline insulin concentration. The estimated hepatic insulin concentrations were also similar at each period. However, 16-hour hepatic insulin was lower versus baseline for both somatostatin infusion protocols. C-peptide concentrations decreased over time and were similar in the 3 groups. Plasma glucagon did not change in response to fasting alone, but it increased by 50% during the low-glucagon infusion period and by 2- to 3-fold during both high-glucagon infusion periods (Table 1).

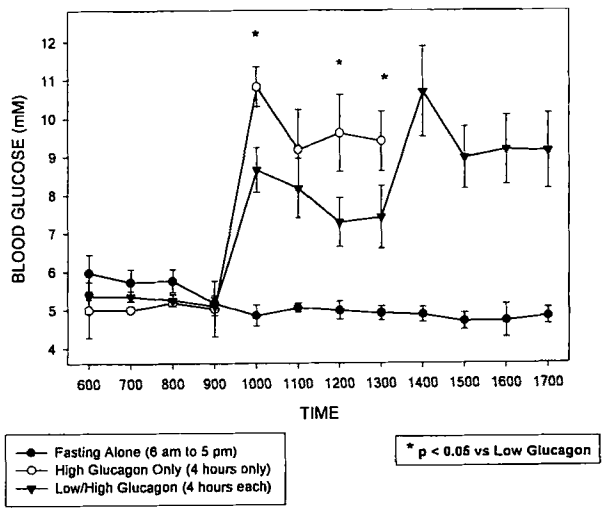

Fig 2. Change in blood glucose over the 7- to 11-hour study period. High-dose glucagon administration under P-P conditions increases blood glucose to a greater extent than low-dose glucagon administration (*$P < .05$). High-dose glucagon infusion increases blood glucose significantly greater than fasting alone ($P < .05$; time 1000 to 1700 [10:00 AM-5:00 PM]).

Table 1. Hormone Concentrations During P-P Glucagon Infusion Study (n = 6, mean ± SEM)

Parameter	Baseline (12-h fasting)	16-h Fasting	20-h Fasting
Insulin (pmol/L)			
Fasting alone	56 ± 19	34 ± 5	23 ± 4*
Low/high glucagon	46 ± 8	55 ± 16	62 ± 11†
High glucagon only	35 ± 9	39 ± 24	
Hepatic insulin (pmol/L)			
Fasting alone	98 ± 25	75 ± 7	56 ± 12
Low/high glucagon	107 ± 7	59 ± 13*	81 ± 17
High glucagon only	107 ± 6	81 ± 11*	
C-peptide (μg/L)			
Fasting alone	0.7 ± 0.09	0.5 ± 0.03*	0.5 ± 0.10*
Low/high glucagon	0.9 ± 0.19	0.3 ± 0.09*	0.5 ± 0.14*
High glucagon only	0.7 ± 0.04	0.4 ± 0.09*	
Glucagon (ng/L)			
Fasting alone	50 ± 9	44 ± 5	53 ± 10
Low/high glucagon	44 ± 7	64 ± 3*	166 ± 9*†
High glucagon only	44 ± 3	125 ± 25*†	
Cortisol (nmol/L)			
Fasting alone	250 ± 25	188 ± 20	124 ± 25*
Low/high glucagon	200 ± 22	380 ± 42*†	396 ± 38*†
High glucagon only	302 ± 82	290 ± 85	
GH (μg/L)			
Fasting alone	1.0 ± 0.3	2.0 ± 0.6	2.0 ± 0.3*
Low/high glucagon	1.0 ± 0.2	2.5 ± 0.4*	2.6 ± 0.3*
High glucagon only	1.0 ± 0.3	1.6 ± 0.3	
FFA (mmol/L)			
Fasting alone	0.54 ± 0.09	0.63 ± 0.09	0.85 ± 0.11*
Low/high glucagon‡	0.55 ± 0.05	0.99 ± 0.17*†	1.06 ± 0.12*
High glucagon only‡	0.47 ± 0.08	0.80 ± 0.04*†	

*$P < .05$ *v* baseline.
†$P < .05$ *v* fasting.
‡n = 5.

Serum cortisol decreased in the fasting group over time due to normal diurnal variation (Table 1). Cortisol concentrations were similar at baseline in all groups. Serum cortisol was greater versus both baseline and 16-hour fasting alone in the low/high-glucagon group. Serum cortisol was not increased in the high-glucagon group compared with baseline or 16-hour fasting alone. Plasma GH concentrations were similar in the fasting group and the glucagon groups, and both increased above baseline. Serum catecholamine concentrations were similar in all groups (data not shown). FFAs were increased at the end of both the 4-hour low-dose and 4-hour high-dose glucagon infusions (Table 1).

As expected, the RQ decreased in the 12-, 16-, and 20-hour fasting group (0.87 ± 0.03, 0.82 ± 0.02, and 0.80 ± 0.02, $P < .05$ *v* baseline). The RQ remained unchanged in the low (0.86 ± 0.02 and 0.86 ± 0.04)- and high (0.88 ± 0.03 and 0.83 ± 0.03)-glucagon groups. Fat oxidation increased with time in the 12-, 16-, and 20-hour fasting group (0.92 ± 0.11, 1.08 ± 0.13, and 1.28 ± 0.16 mg/kg/min). Fat oxidation did not increase in the low- and high-glucagon groups. Carbohydrate oxidation decreased with time in the 12-, 16-, and 20-hour

fasting group (2.11 ± 0.13, 1.69 ± 0.30, and 1.37 ± 0.33 mg/kg/min). Carbohydrate oxidation did not decrease in the low-glucagon (2.12 ± 0.33 and 2.05 ± 0.45) or high-glucagon (2.39 ± 0.38 *v* 1.51 ± 0.42 mg/kg/min) groups.

Table 2 details the change in amino acid concentrations in the 3 groups. At 20 hours of fasting, plasma leucine was increased above baseline (12-hour fasting). High-dose glucagon significantly reduced alanine, glycine, serine, and total amino acids at 20 hours by approximately 20%. A similar 20% decrease was found for alanine, glycine, serine, and total amino acids in the 6 subjects given high-glucagon first (hour 16; Table 2). Low-dose glucagon administration reduced serine but otherwise had no effect on amino acid levels.

Baseline Measurements

GP was similar in all 3 groups (13.5 ± 0.2, 13.8 ± 0.5, and 13.0 ± 0.6 μmol/kg/min, high-glucagon, low/high-glucagon, and fasting alone, respectively). In addition, the estimated rates for the Cori cycle (19% ± 1%, 19% ± 1%, 18% ± 1%), nongluconeogenic glucose release (7.8 ± 0.4, 8.7 ± 0.5, and 7.7 ± 0.3 μmol/kg/min), and Gneo (5.8 ± 0.4, 5.0 ± 0.2, and 5.3 ± 0.3 μmol/kg/min) were also similar in the 3 groups.

Effect of Low-Dose Glucagon on Gneo

Low-dose glucagon P-P infusion increased GP compared with baseline values (13.8 ± 0.5 *v* 15.5 ± 0.5 μmol/kg/min, 12 hours (9 AM) *v* 16 hours (1 PM), $P < .05$; Fig 3). Low-dose glucagon also increased GP compared with the rate in individuals who fasted for the same length of time (Fig 3 and Table 3). Estimates of nongluconeogenic glucose release decreased between 12 and 16 hours of fasting (7.7 ± 0.3 to 4.9 ± 0.5 μmol/kg/min, 9 AM to 1 PM, $P < .01$). Low-dose glucagon infusion prevented the decline in nongluconeogenic glucose release (8.7 ± 0.5 to 8.3 ± 0.6 μmol/kg/min, 9 AM to 1 PM; Table 3). Low-dose glucagon nearly doubled nongluconeogenic glucose release versus 16 hours of fasting alone (Table 3). The 4-hour low-dose glucagon infusion had no effect on the estimated rates of Gneo.

Table 2. Selected Plasma Amino Acid Concentrations (μmol/L) During a 20-Hour Fast and During a P-P Glucagon Infusion Study

Amino Acid	Baseline (12-h fasting)	16-h Fasting	20-h Fasting
Alanine			
Fasting alone	406 ± 31	345 ± 34	349 ± 38
Low/high glucagon	432 ± 30	359 ± 25	308 ± 42*
High glucagon only	400 ± 14	301 ± 20*	
Glycine			
Fasting alone	310 ± 55	283 ± 45	276 ± 27
Low/high glucagon	291 ± 40	248 ± 32	198 ± 37*†
High glucagon only	285 ± 16	206 ± 23	
Serine			
Fasting alone	147 ± 14	149 ± 16	174 ± 20
Low/high glucagon	175 ± 24	132 ± 12*	130 ± 13*†
High glucagon only	160 ± 11	125 ± 13*	
Leucine			
Fasting alone	144 ± 12	141 ± 13	172 ± 17*
Low/high glucagon	156 ± 17	132 ± 14	139 ± 7†
High glucagon only	132 ± 6	121 ± 7*	
Total amino acids			
Fasting alone	2,523 ± 159	2,448 ± 189	2,690 ± 182
Low/high glucagon	2,657 ± 274	2,282 ± 216	2,106 ± 237*†
High glucagon only	2,436 ± 87	1,942 ± 132*	

NOTE. Results are the mean ± SEM (n = 6 for fasting alone; n = 5 for all others).

*$P < .05$ *v* baseline.

†$P < .05$ *v* fasting.

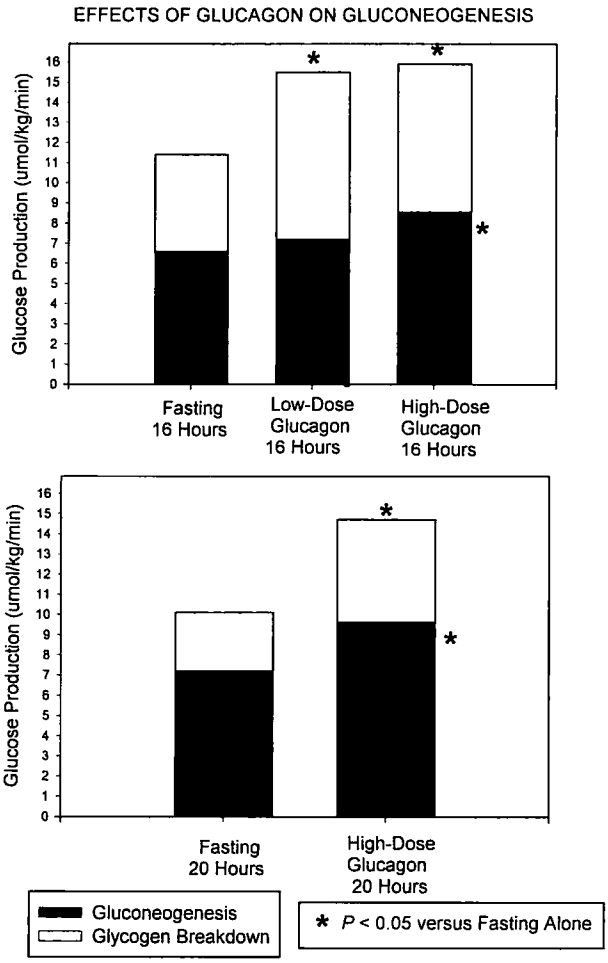

Fig 3. The bars represent net GP with the solid black area representing the amount of gluconeogenesis and the white area representing nongluconeogenic glucose release (estimate of glycogen breakdown). After 12 hours fasting, the groups received a 4-hour hormone infusion study and were compared to fasting alone (16-hours fasting). Low-dose glucagon administration had no effect on gluconeogenesis, but significantly increased glycogen breakdown. High-dose glucagon administration increased gluconeogenesis, glycogen breakdown, and net GP at 16 hours fasting and also at 20 hours fasting. The SEMs are presented in Tables 3 and 4.

Effect of High-Dose Glucagon on Gneo

High-dose glucagon administration under P-P hormonal conditions increased GP significantly above baseline (15.8 ± 0.6 *v* 13.5 ± 0.2 μmol/kg/min, $P < .05$), which was even greater versus 16 hours of fasting alone (11.5 ± 0.6 μmol/kg/min, $P < .01$; Table 3 and Fig 3). The 4.3-μmol/kg/min increase in GP above 16-hour fasted controls was due to a 2.6-μmol/kg/min increase in nongluconeogenic glucose release and a 1.9-μmol/kg/min increase in Gneo. When we compared the effects of

Table 3. Effects of P-P Study and Low- and High-Dose Glucagon Versus Fasting Alone (16 h) on Gneo and Other Parameters

Condition	GP (μmol/kg/m)	Carbon Recycling (%)	Cori Cycle (%)	Dilution of Pyruvate (fold)	Gneo % of GP	Gneo μmol/kg/m	Nongluconeogenic Glucose Release (μmol/kg/m)
16-h fasting							
Mean	11.5 ± 0.6	14 ± 1	31 ± 3	1.9 ± 0.05	57 ± 5	6.6 ± 0.6	4.8 ± 0.5
Range	8.8-12.7	9-19	21-42	1.7-2.0	38-78	3.4-8.3	2.3-5.5
Low glucagon							
Mean	15.5 ± 0.5*	13 ± 1	28 ± 3	1.63 ± 0.10	46 ± 3*	7.2 ± 0.3	8.3 ± 0.6*
Range	13.3-18.3	9-14	27-32	1.3-1.9	37-52	5.6-8.1	6.5-10.6
High glucagon							
Mean	15.8 ± 0.6*	14 ± 1	31 ± 1	1.73 ± 0.09	54 ± 3*	8.5 ± 0.5*	7.4 ± 0.5*
Range	14.0-17.0	11-16	26-34	1.3-2.0	28-60	3.5-10.1	5.7-10.0

NOTE. Results are the Mean ± SEM.
*$P < .01$.

high-glucagon following 4 hours of low-glucagon administration, a similar effect on GP was observed (Table 3). GP was significantly increased (14.7 ± 0.7 *v* 10.1 ± 0.6, $P < .05$). The 4.6-μmol/kg/min increase in GP above the 20-hour fasted controls was due to a 2.1-μmol/kg/min increase in nongluconeogenic glucose release and a 2.5-μmol/kg/min increase in Gneo (Table 4).

Effect of Underreplacement of Hydrocortisone on GP

In 4 additional subjects, we accidentally underinfused hydrocortisone and glucagon during the 4-hour low-dose glucagon infusion period (dosing error). Glucagon concentrations were similar versus the low-glucagon group (59 ± 13 *v* 64 ± 3 ng/L). Serum cortisol concentrations decreased (176 ± 49 *v* 220 ± 38 nmol/L, 16-hour *v* baseline, NS), and it was similar to that found with 16-hour fasting alone (188 ± 20 nmol/L). In these 4 subjects, GP failed to increase above baseline (12.6 ± 1.2 *v* 12.7 ± 1.4, hour 16 *v* baseline hour 12), nor was GP increased compared with 16-hour fasting alone (11.5 ± 0.6 μmol/kg/min). In addition, blood glucose was not significantly increased above baseline (6.8 ± 2.1 *v* 5.4 ± 0.8 mmol/L) at the end of the 4-hour infusion. These data, while preliminary, would suggest that the maintenance of serum cortisol concentrations is important during P-P infusion studies to evaluate the effects of glucagon on hepatic glucose metabolism.

Hormonal and Substrate Associations With Cori Cycle and Gneo

Plasma glucose and glucagon concentrations were directly correlated with GP ($r = .623$, $P < .01$ and $r = .446$, $P < .05$, respectively). Plasma glucose, glucagon, and FFA concentrations were directly correlated with Cori cycle activity ($r = .344$, $P < .05$, $r = .451$, $P < .05$; and $r = .647$, $P < .01$, respectively). As expected, the Cori cycle was correlated with Gneo ($r = .883$, $P < .01$). Glucose, glucagon, and FFAs were also directly correlated with Gneo ($r = .568$, $P < .01$, $r = .625$, $P < .01$, and $r = .407$, $P < .05$, respectively). We have obtained unpublished data that FFA is also directly correlated with Gneo in patients with type 2 diabetes, cancer patients, and normal subjects (see Discussion). Neither insulin, GH, nor catecholamines were correlated with Gneo. Multiple-step regression analysis demonstrated that both glucagon and FFA are correlated with Gneo (combined $R^2 = .693$, $P < .01$). In our prior study, there was no correlation between glucagon and Gneo, which may have been due to the use of the Unger antibody, which overestimates the actual glucagon concentration.[1,4]

DISCUSSION

This study demonstrates that a 4-hour infusion of low-dose glucagon under P-P conditions increases blood glucose acutely and sustains an elevation in blood glucose and GP by increasing nongluconeogenic glucose release. The effect on blood glucose was noted as early as 10 minutes after the start of glucagon infusion and persisted during the 4-hour infusion. Low-dose glucagon increased blood glucose by approximately 2.5 mmol/L, and high-dose glucagon increased blood glucose by approximately 4.5 mmol/L. The initial increase followed by the waning effect may be due to glucose's ability to suppress nongluconeogenic glucose release.[6] Gneo was not increased by low-dose glucagon. Only high-dose glucagon administration increased Gneo. The inability of low-dose glucagon to increase Gneo may have been due to the increase blood glucose, which is known to inhibit gluconeogenic enzymes.[16] Higher-dose glucagon has

Table 4. Effects of P-P Study and High-Dose Glucagon Versus Fasting Alone (20 h) on Gneo and Other Parameters

Condition	GP (μmol/kg/m)	Carbon Recycling (%)	Cori Cycle (%)	Dilution of Pyruvate (fold)	Gneo %	Gneo μmol/kg/m	Nongluconeogenic Glucose Release (μmol/kg/m)
20-h fasting							
Mean	10.1 ± 0.6	15 ± 2	35 ± 3	2.0 ± 0.15	70 ± 4	7.2 ± 0.6	2.9 ± 0.4
Range	7.7-11.7	10-20	21-43	1.7-3.0	56-81	4.5-8.2	1.9-3.7
High glucagon							
Mean	14.7 ± 0.7*	17 ± 3	41 ± 4	1.63 ± 0.17	66 ± 4	9.7 ± 0.7*	5.0 ± 0.5*
Range	12.2-17.2	13-23	33-48	1.3-2.1	56-79	7.1-12.4	3.3-7.6

NOTE. Results are the mean ± SEM.
*$P < .01$.

been shown to have a transient effect on glycogenolysis, while its stimulation of Gneo is more persistent.[6]

Although glucagon can increase the conversion of ^{14}C alanine to glucose and the gluconeogenic efficiency of the liver, it has little or no effect on the supply of gluconeogenic precursors reaching the liver. Without cortisol's effect on proteolysis, any hepatic effect of glucagon alone would soon result in a depletion of the circulating gluconeogenic precursors, and the amount of glucose produced via Gneo would return to control rates despite an activation in the liver.[11] High-dose glucagon administration without hydrocortisone reduces gluconeogenic amino acids by approximately 35% in humans.[17] High-dose glucagon with hydrocortisone in our study only reduced total amino acids by 20%. Fifty percent of the micromole decrease in amino acid concentration was due to the reduction in alanine, glycine, and serine (Table 2). It is unclear what effect this decrease in these gluconeogenic amino acids had on the rate of GP and Gneo in these subjects. One would expect that the lower gluconeogenic precursors may have limited the increase of Gneo found with the high-dose glucagon infusion. Studies that administer amino acids to sustain the plasma (and hepatic) concentrations would clarify the influence of the amino acid concentration on Gneo.

Recently, Landau et al[18] have "corrected" our equations for Gneo. This "corrected" equation yields estimates of Gneo that are exactly half of our values. We present elsewhere[19] a lengthy analysis of the recycling and vigorously derive our equations for the Cori cycle and Gneo. It is admitted by Landau et al[18] that their estimates of Gneo are too low and physiologically untenable. As shown by Katz and Tayek,[5] we are in close agreement with values in the literature, including the D_2O method of Landau et al.[3] Their corrected equations yield values for Gneo of 20% for overnight-fasted humans and 40% for prolonged fasting. Our estimates are 40% and 80% to 100%, respectively,[5] the same as obtained with the D_2O method.[3]

Our method includes the contribution of glycerol. A very close agreement for results obtained with our original equations using [U-^{13}C]glucose[1] and the mass isotopomer distribution analysis (MIDA) method of Hellerstein using [2-^{13}C] glycerol was found by Sunehag et al[20] in a study with premature infants.

Regardless of this dispute, Landau et al and others agree that stable-isotope enrichment of uniformly enriched glucose (M_6) accurately measures GP. Setting aside our estimates of Gneo, these data demonstrate that high-dose glucagon infusion increases GP by 37% when administered for 4 hours (16 hours of fasting) and by 47% when administered for 4 hours after low-dose glucagon (20 hours of fasting). Low-dose glucagon increased GP by a similar amount (35%). When 4 additional subjects were studied with an underreplacement of hydrocortisone, there was no increase in GP when measured after the 4-hour infusion. However, GP did not significantly decrease as found in the fasting-alone group. Blood glucose in these 4 subjects initially increased but returned to normal between hours 3 and 4. This is similar to prior data when hydrocortisone was not coadministered in dogs and humans.[6-9,11]

Hyperglycemia is known to inhibit glycogenolysis, but it is unlikely that glucose alone would normalize GP. The peak increase in glucose and glycogenolysis at 15 minutes and the waning effect at 2 hours of glucagon[9] also has been found by others.[11] High-dose glucagon reduced alanine, glycine, serine, and total amino acids by approximately 35%.[17] Amino acids were not reported in these studies,[9,11] but a significant reduction in alanine, glycine, serine, and other amino acids also may have been partially responsible for the decrease in GP. This may be due to a reduced availability of amino acids for Gneo.

We introduce the hypothesis that hydrocortisone is needed to sustain amino acid mobilization and/or to increase gluconeogenic stimulation. The practice of not coadministering hydrocortisone most likely resulted in the failure to sustain an increase in GP in many prior reports.[6-9,11] In only 1 animal study was this carefully demonstrated by removal of hydrocortisone from the mixture of hormones infused.[7] A similar study in humans has not been published. Recently, glucagon was administered in the early morning with insulin and somatostatin but without hydrocortisone.[9] After an initial increase in glycogenolysis and GP, the rate of GP returned to normal after 100 minutes.[9] The reason for this is unclear since the liver had abundant glycogen stores by nuclear magnetic resonance, but the rate of GP and glycogen breakdown decreased to baseline at 120 minutes even though liver glycogen concentrations were high and plasma glucagon remained elevated. These data would argue that the reduction in GP was due to a decrease in glycogenolysis and/or a failure to increase Gneo.

In our fasting subjects, plasma cortisol decreased from 250 nmol/L to 124 nmol/L over an 8-hour period. This is part of the normal morning (diurnal) decrease in plasma cortisol. Cortisol was significantly increased compared with baseline in the low/high-glucagon group (Table 1). This was most likely due to the pulsatile nature of cortisol, with the baseline concentration for that group being the lowest of the 3 groups. Gneo was similar in the high-glucagon 20-hour fasting compared with the high-glucagon 16-hour fasting group, despite the fact that serum cortisol was increased at 20 hours.

Glucocorticoid administration increases muscle proteolysis, which could supply gluconeogenic precursors to the liver.[21] The decrease in serum cortisol could result in a failure to mobilize amino acids and promptly reduce the gluconeogenic amino acid[16] supply available for Gneo. Using metyrapone to reduce the plasma cortisol concentration reduces the availability of amino acids during exercise compared with exercise with normal cortisol concentrations.[22] Our current study suggests that while hydrocortisone may increase proteolysis, the amount of proteolysis may be insufficient to sustain amino acid concentrations in the face of an increase in Gneo.

During P-P infusion study, plasma amino acids decreased by 35% when hydrocortisone was not administered[16] and by only 20% in our study when hydrocortisone was administered. An increase in gluconeogenic demand may be one reason for the reduced amino acid concentration. In the previous study,[7] Gneo was likely greater when glucagon was administered with hydrocortisone, since it is known that cortisol induces gluconeogenic enzymes.[23] Administration of hydrocortisone to maintain serum cortisol concentrations without other known proteolytic factors (interleukin-1, interleukin-6, tumor necrosis factor, alpha, etc) may have induced gluconeogenic enzymes to a greater extent than proteolysis, which would explain the

decrease in gluconeogenic amino acids. Despite the earlier data in animals and our observation in humans, prospective testing of cortisol's direct effect on Gneo and glycogenolysis is needed. Earlier investigation in humans demonstrated that a 3-ng/kg/min dose of glucagon increases Gneo from lactate by 60 minutes and that it remains elevated for a 6-hour interval.[24] The investigators infused hydrocortisone to maintain a high-normal serum cortisol concentration (380 nmol/L).

Insulin

Our data are the first to show that a 4-hour high-dose glucagon infusion increases Gneo under P-P conditions. This occurred after 16 hours of fasting and after 20 hours of fasting. Under both conditions, blood glucose was elevated and sustained over the 4-hour study period. A greater increase in glucose, GP, and nongluconeogenic release may have been observed over the final 4 hours of the low/high-glucagon P-P infusion study (20 hours) if plasma insulin was kept between 35 and 46 pmol/L. Plasma insulin, while not statistically increased, was one and one-half the baseline value (Table 1). However, estimated hepatic insulin concentrations were not significantly increased in the 20-hour period between the 2 groups (Table 1). This is important because both Petersen et al[25] and Sindelar et al[26] have shown that small increases in plasma insulin can inhibit glycogenolysis. Portal and systemic insulin concentrations have important roles in the regulation of GP in the normal individual.

New evidence would support the effect of peripheral insulin acting in the portal vein via FFA (or a second factor) on the regulation of hepatic glucose metabolism. It has been estimated that the peripheral effect of insulin on FFA accounts for approximately 50% of the effect of an increase in arterial insulin on hepatic glucose metabolism.[27] In the present study, serum insulin concentrations were similar during the 4-hour low-dose and 4-hour high-dose glucagon periods and the 16-hour fasted controls. In the 20-hour high-dose glucagon period, the insulin concentration was 50% greater than that found at 20 hours of fasting alone. However, the insulin concentration in the 20-hour high-glucagon group was not significantly greater than baseline ($P = .10$). Nor was the estimated hepatic insulin concentration greater in the high-glucagon group compared with fasting alone. If there was a higher hepatic insulin, this may have minimized glucagon's effect on Gneo and/or glycogenolysis. The similar C-peptide concentrations in these 2 groups (Table 1) also support the notion that hepatic insulin concentrations were similar, since approximately 72% of the hepatic insulin concentration is attributed to the portal insulin concentration.

We intentionally infused a dose of insulin to prevent portal insulinopenia compared with fasting, since underreplacement of insulin using P-P conditions can increase Gneo and nongluconeogenic glucose release (Tayek, unpublished data, September 1999). We also chose low-dose insulin administration to inhibit any flux of label into glycogen. Recently, Petersen et al[25] reported no glycogen synthase flux at 5 mmol/L glucose and 40 pmol/L insulin and negligible glycogen synthase activity at 10 mmol/L glucose. Not knowing the portal insulin concentrations limits our interpretation of the data, but the near-fasting concentrations of peripheral insulin (and the similar C-peptide concentrations) minimize the loss of label into glycogen.

FFA

Serum FFA increased with both low- and high-dose 4-hour glucagon infusion compared with 16 hours of fasting alone. FFA also significantly increased over time from the 12-hour to 20-hour fasting period. At the end of the low/high-glucagon infusion, FFA increased by 25% but failed to reach significance ($P = .09$). Combining all data, there was a weak correlation of FFA with the rate of Gneo ($r = .477$, $P < .05$). Unpublished observations demonstrate a significant correlation between FFA and Gneo in overnight-fasted normal subjects ($r = .665$, $P < .05$, $N = 14^{1,4}$), type 2 diabetics ($r = .616$, $P < .05$, $N = 9^{1}$), and cancer patients ($r = .599$, $P < .05$, $N = 13^{4}$). These data suggest a weak but significant association between FFA and Gneo. This has also recently been demonstrated by Chen et al.[28]

Rebrin et al[27] have demonstrated a strong correlation between FFA and GP, and showed that this relationship is independent of portal insulin and glucagon concentrations. FFA administration with an increase in peripheral insulin and without glucagon administration maintains GP, which suggests that part of insulins' suppression of GP is not direct. Sindelar et al[26] maintained a normal peripheral glucagon concentration and demonstrated that insulin's peripheral effect on FFA levels accounts for approximately 50% of the observed reduction in GP. Recent evidence suggests that FFA upregulates glucose-6-phosphatase expression in the liver, which may account for the observed effect of FFA on GP.[29]

GH

A small but significant elevation was noted in plasma GH in the 20-hour fasted group. In addition, GH was elevated in the 16-hour fasted low-glucagon treatment group compared with baseline but was not different versus fasting alone (Table 2). GH is believed to be responsible for an increase in the rate of GP in acromegaly. However, we have recent data to suggest that GH administration does not increase GP or Gneo. Under P-P conditions, we provided 4 hours of high-dose GH that increased the GH concentration to 20 μg/L. GH administration to 5 normal subjects increased blood glucose to 9.3 mmol/L, but GH failed to stimulate GP, Gneo, or nongluconeogenic glucose release.[30] It is therefore unlikely that the small increase in GH at hour 16 in the high-dose glucagon group had a physiological effect on hepatic glucose metabolism.

Conclusion

In summary, we have demonstrated that when using a P-P protocol, low-dose (50% above basal) glucagon administration with hydrocortisone for 3 to 4 hours increases GP, which is mostly due to nongluconeogenic glucose release. High-dose glucagon administration (3-fold above normal) increases GP by 40% by increasing both Gneo and nongluconeogenic glucose release. Fifty percent of the increase in GP is due to Gneo and 50% to nongluconeogenic glucose release. Under P-P study conditions, the maintenance of serum cortisol concentrations may play an important role in the evaluation of glucagon's effect on hepatic glucose metabolism. More than a 50% increase in plasma glucagon is required during a 4-hour period to stimulate Gneo to a greater extent versus fasting alone.

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