Glucagon Increases Glutamine Uptake Without Affecting Glutamine Release in Humans

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Glucagon causes transient hyperglycemia and persistent hypoaminoacidemia, but the mechanisms of this action are unclear. To address this question, the present study measured the effects of glucagon on glucose, leucine, phenylalanine, and glutamine kinetics. Seven healthy subjects each underwent three pancreatic clamp studies (octreotide 30 ng/kg/min, insulin 0.15 mU/kg/min, and glucagon 1.4 ng/kg/min) lasting 7 hours. During the last 3.5 hours of the studies, glucagon infusion was either unchanged (study 0) or increased to 4 and 7 ng/kg/min (studies 1 and 2). The higher glucagon infusion rates increased the glucagon concentration by 50% and 100%, respectively. [6,6-$^2H_2$]glucose, [2-^{15}N]glutamine, $^2H_5$-phenylalanine, and $^2H_3$-leucine were infused to quantify the respective fluxes. Glucagon transiently increased glucose concentrations by stimulating glucose production, which peaked in 15 minutes to 3.82 ± 0.36 and 4.21 ± 0.33 mg/kg/min in studies 1 and 2 and then returned to the postabsorptive levels. Glucagon decreased the glutamine concentration (−10% ± 2% and −22% ± 2% in studies 1 and 2 *v* study 0, $P < .05$), because glutamine uptake became greater than glutamine release (balance from −1.9 ± 0.9 in study 0 to − 8.1 ± 1.1 and −13.6 ± 1.0 μmol/kg/h in studies 1 and 2, $P < .01$). Glucagon decreased the leucine concentration (−11% ± 3% in study 2 *v* study 0, $P < .02$) and caused a small increment in proteolysis (+6% in study 2 *v* study 0, $P < .01$) that was related to the decrement in glutamine concentrations. Phenylalanine kinetics were not significantly affected. These results show that glucagon promotes the uptake of gluconeogenic substrates but does not increase their release, suggesting that glucagon-induced hyperglycemia is short-lived because glucagon fails to provide more fuel for gluconeogenesis. The small increase in proteolysis and the depletion of circulating glutamine prove that physiologic hyperglucagonemia can contribute to protein catabolism.

WITHIN MINUTES, glucagon can induce profound changes in glucose metabolism. The mechanisms of glucagon-induced hyperglycemia have been ascribed to an initial increase in glycogenolysis and a subsequent increase in gluconeogenesis.[1-3] Within hours, glucagon-induced hyperglycemia dissipates, but a profound hypoaminoacidemia persists.[4,5] Hyperglucagonemia is an important feature of catabolic conditions like stress, trauma, burns, sepsis, uncompensated diabetes, and glucagonoma. However, neither the mechanisms relating increased glucagon and protein catabolism nor the reason why glucagon induces temporary hyperglycemia but persistent hypoaminoacidemia have been explained.

Despite the fact that glucagon's putative catabolic role has been shown in rat tissues,[6,7] many studies in humans failed to show that glucagon increases whole-body proteolysis,[5,8-10] except under conditions when insulin is also deficient.[11] In contrast, increased amino acid oxidation by glucagon has been demonstrated in various conditions.[8-11] Evidence for a catabolic role of glucagon during an amino acid load have been presented by Boden et al,[12] who demonstrated an increased amino nitrogen conversion to urea, and more recently by Charlton et al,[13] who found a decreased stimulation of protein synthesis. Consistent with these findings, glucagon also enhances hepatic nitrogen clearance for urea synthesis.[14] Altogether, these data suggest that glucagon does not affect amino acid release from proteins but stimulates their catabolism, diverting their fate from protein synthesis.

Within 2 to 3 hours, glucagon decreases the levels of amino acids that can be converted to glucose, eg, glutamine and alanine.[4,5,11,15] In the dog, glucagon increases glutamine utilization by the gut.[16] Glutamine is a key amino acid that is important in a variety of processes. Glutamine is produced in muscle in large amounts, and most of the muscle-derived glutamine is transported to the gut.[17] The gut uses both glutamine and glucose as energy sources. Increasing glutamine uptake could be a means of reducing gut glucose utilization, which could have a glucose-sparing effect in favor of other tissues. Glutamine is also a prominent gluconeogenic amino acid. From the quantitative standpoint, glutamine is the most important carrier of gluconeogenic carbons from the peripheral tissues to the liver and kidney.[18,19] Glutamine depletion is a common feature of most catabolic conditions.[17] In the muscle, glutamine levels are related to protein catabolism.[20-22] Other counterregulatory hormones such as cortisol exert catabolic actions through an acceleration of glutamine turnover.[23]

The effects of glucagon on glutamine kinetics have not been assessed in humans. The hypothesis of the present study is that glucagon promotes the disposal of glutamine for gluconeogenesis but does not stimulate the rate of glutamine appearance. Without accelerated release of glutamine from muscle, the glucagon-induced increase in glutamine uptake causes a reduction in plasma levels of glutamine. The reduction in plasma glutamine, in turn, may provide an explanation as to why the hyperglycemic effects of glucagon are transient. To support this hypothesis, the present study evaluated the effect of hyperglucagonemia in the physiologic range on the kinetics of glucose and glutamine, and of two essential amino acids, phenylalanine and leucine.

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SUBJECTS AND METHODS

Subjects

Seven healthy non-obese men (age, 26 ± 2 years; weight, 81 ± 4 kg; body mass index, 24 ± 1 kg/m^2) were admitted to the Brigham and Women's Hospital-Harvard Medical School General Clinical Research Center. A medical history, physical examination, and biochemical laboratory screening tests were obtained to verify that each subject was free of chemically evident metabolic, gastrointestinal, cardiovascular, neurologic, or infectious disorders. The subjects were instructed on the purpose, benefits, and risks of the study and provided written consent in accordance with protocols approved by the Brigham and Women's Hospital Human Research Committee.

Materials

Glucagon (1 mg/mL) and human insulin (Humulin R, 100 IU/mL) were obtained from Eli Lilly (Indianapolis, IN). Octreotide (Sandostatin, 1 mg/mL) was a gift from Sandoz (Basel, Switzerland). Glucagon solution was reconstituted in its accompanying sterile diluent. A weighed amount of this solution was further diluted with 0.45% saline to a final volume of 100 mL in a sterile plastic bag on the morning of the study. At the same time, weighed amounts of insulin and octreotide were diluted in separate plastic bags with 0.45% saline and 5 mL of the patient's own blood to a final volume of 150 mL. The infusion lines were extensively primed with the solutions before infusion in the subjects to prevent an initial adsorption of the hormones to the tubing. L-[2-^{15}N]glutamine (99% amino-^{15}N) and D-[6,6-$^2H_2$]glucose (98% ^{2}H, abbreviated d_2-glucose) were purchased from Tracer Technologies (Somerville, MA; presently MassTrace, Woburn, MA); L-[*phenyl*-$^2H_5$]phenylalanine (93% $^2H_3$, abbreviated d_5-phenylalanine) and L-[5,5,5-$^2H_3$]leucine (98% ^{2}H, abbreviated d_3-leucine) were obtained from MSD Isotopes (formerly St Louis, MO; no longer in business). Optical (absence of the D-stereoisomer), chemical, and isotopic purity of the tracers were determined by gas chromatography–mass spectrometry (GCMS). Before every infusion study, sterile solutions of the labeled tracers were prepared using aseptic technique. Accurately weighed amounts of the labeled compounds were dissolved in weighed volumes of sterile, pyrogen-free saline and filtered through a 0.22-μm Millipore (Milford, MA) filter before use. An aliquot of the sterile solution was verified to be pyrogen-free before administration to human subjects. Solutions were prepared no more than 24 hours before use and were kept at 4°C prior to administration.

Infusion Protocol

Each subject participated in three consecutive infusion studies 2 to 4 days apart. Before admission, the subjects met with a nutritionist and were placed on an adequate energy and protein (1.2 g/kg/d) diet until the last study was completed. The diet was required to reduce intersubject variability induced by the different subjects' dietary practices. The format for each of the infusion studies was similar, and differed only in the amount of glucagon infused. The sequence of the studies was randomized among subjects. On the evening before each infusion study, subjects consumed their evening meal by 8:30 PM and then drank only water until completion of the study the next day at 2:30 PM. At 7:00 AM on the infusion day, a catheter was placed in the subject's arm for infusion of octreotide, insulin, glucagon, and the amino acid and glucose tracers. A second catheter was placed retrogradely in a hand vein, and the hand was placed in a warming box to obtain "arterialized" venous blood samples.

Octreotide was infused (30 ng/kg/h) for 7 hours beginning at 7:30 AM to block pancreatic glucagon and insulin secretion. Insulin and glucagon were replaced by infusion at rates of 0.15 mU/kg/min and 1.4 ng/kg/min, respectively. Since the liver is the major target for glucagon and since the portal glucagon concentration is higher than the peripheral concentration, with the present glucagon infusion dose, we chose to slightly increase (20%) the peripheral glucagon concentrations pre-clamp. During the early equilibration phase of the pancreatic clamp, some subjects transiently required small amounts of dextrose or minor increments in the insulin infusion rate to prevent hypoglycemia or hyperglycemia, respectively. By 1.5 hours of the equilibration period, all subjects were euglycemic. However, two of the subjects required exogenous dextrose to prevent hypoglycemia at the end of the 7-hour period in the study in which glucagon was maintained at the basal level. Priming doses of [2-^{15}N]glutamine (4.5 μmol/kg), d_2-glucose (12.0 μmol/kg) d_5-phenylalanine (1.2 μmol/kg), and d_3-leucine (3.6 μmol/kg) were administered intravenously at 0.5 hours of the pancreatic clamp, immediately followed by continuous infusion of the same tracers (7.5, 13.3, 1.5, and 3.6 μmol/kg/h, respectively) throughout the study. After the first 3.5 hours (basal period), the glucagon infusion rate was either maintained at basal (study 0) or increased to obtain a +50% (study 1) and +100% (study 3) increase in peripheral glucagon concentrations for 3.5 hours (4 and 7 ng/kg/min). These glucagon infusions produced glucagon concentrations that are generally observed during stress.

Arterialized blood samples were collected before the start of each infusion, every 15 minutes during the last hour of the basal period, and throughout the 3.5 hours of the study period in which the glucagon dose was either unchanged or increased. Plasma glucose concentrations were also measured at the bedside for the study duration using a glucose analyzer (Beckman Instruments, Fullerton, CA).

Analytical Methods

Aliquots of blood for measurement of amino acid and glucose levels were placed in heparinized tubes. One-milliliter blood aliquots for measurement of glucagon and catecholamine levels were placed in tubes containing EDTA plus aprotinin and tubes containing glutathione, respectively. Blood aliquots for insulin, cortisol, and growth hormone (GH) were collected in tubes without additives for serum separation. All blood samples were placed on ice until the plasma or serum was prepared by centrifugation at 4°C (within 1.5 hours of sampling). All plasma and serum aliquots were frozen at −60°C until later analysis.

The d_2-glucose enrichment was measured by GCMS after preparation of the butyl-boronate derivative.[24] Samples were injected into a GCMS instrument (model 5970; Hewlett-Packard, Palo Alto, CA) operated using electron-impact ionization. The ion mass at *m/z* 297 and 299 was selectively monitored for unlabeled glucose and d_2-glucose, respectively. After addition of the appropriate internal standards ([$^2H_7$]leucine, [$^2H_2$]phenylalanine, and ketocaproate), amino and keto acids were isolated from 0.5-mL aliquots of plasma using cation-exchange columns as previously described.[25] Eluants from the columns were evaporated to dryness and derivatized to the *N*-heptafluorobutyrl, *n*-propyl (HFBP) amino acid ester derivatives for measurement of phenylalanine and leucine content.[23,26] Separate injections of HFBP-phenylalanine and -leucine were made into a GCMS instrument (model 5988A; Hewlett-Packard) operated in a negative chemical ionization mode. The [M-HF]$^-$ ions *m/z* 383, 385, and 387 were monitored for unlabeled, d_2- and d_5-phenylalanine, and the ions *m/z* 349, 352, and 356 were monitored for unlabeled, d_3- and d_7-leucine, respectively. To measure plasma glutamine enrichment, eluants from the columns were evaporated to dryness, and 25 μL *N*-methyl-*N*-(*t*-butyldimethylsilyl)-trifluoroacetamide (Pierce Chemical, Rockford, IL) and 25 μL acetonitrile (1:1) were added.[27] The vials were capped and allowed to set at room temperature overnight to form the *tris-t*-butyldimethylsilyl (TBDMS) derivative of glutamine. Injections of TBDMS-glutamine samples were made into a GCMS instrument (model 5970; Hewlett-Packard) operated using electron-impact ionization. The [M-57]$^+$ ions at *m/z* 431 and 432 were monitored for unlabeled glutamine and [2-^{15}N]glutamine,

respectively. TBDMS-glutamine was chromatographically resolved from TBDMS-glutamate.[28] The α-ketoisocaproic acid (KIC) enrichment and concentration were measured after eluants from the columns were derivatized to the trimethylsilyl-quinoxalinol derivatives.[23,26] The derivatives were injected into a GCMS instrument (model 5970; Hewlett Packard) operated using electron-impact ionization. The ions at *m/z* 259 and 262 were monitored for unlabeled and d_3-labeled KIC, respectively. The ketocaproate peak was resolved from the KIC peak and used for quantification of the KIC concentration. For all measurements, the background-corrected tracer enrichment in mole percent excess (mpe) was calculated as previously defined[25] for d_2-glucose, d_5-phenylalanine, d_3-leucine, d_3-KIC, and [2-^{15}N]glutamine.

Plasma glutamine concentrations were measured by enzymatic assay[29,30] as previously described.[31] A 0.7-mL aliquot of plasma was deproteinized using 0.7 mL ice-cold 1-mol/L perchloric acid and centrifuged, and the supernatant was neutralized with potassium hydroxide. The sample was then split into two aliquots. The aliquot for glutamate was incubated with glutamate dehydrogenase in a glycine-hydrazine buffer[29] to form ammonia, α-ketoglutarate, and NADH. The glutamate concentration was determined by the fluorometric measurement (excitation at 350 nm and emission at 450 nm) for appearance of NADH. The aliquot for glutamine was first treated with glutaminase[30] to convert all glutamine to glutamate, and then assayed as glutamate. The plasma glutamine concentration was taken as the difference between the measurement of glutamine and glutamate aliquot determinations.

Plasma hormone concentrations were measured by radioimmunoassay with commercial kits from Diagnostic Products (Los Angeles, CA) for insulin and glucagon, Incstar (Stillwater, MN) for cortisol, and Nichols Institute Diagnostics (San Juan Capistrano, CA) for GH. Catecholamine levels were measured by a single-isotope radioenzymatic method.[32]

Calculations

Glucose and amino acid kinetics were calculated using Steele's equations for the non–steady state.[33] The rate of appearance of the unlabeled substrates ([R_a] micromoles per kilogram per hour) was calculated using the equation,

$$R_a(t) = [i - V_d C(t)dE(t)/dt]/E(t), \quad \text{Eq 1}$$

where i is the infusion rate of the tracers as micromoles per kilogram per hour of tracer per se (ie, the product of the rate of tracer infusion times the enrichment of the tracer), V_d is the volume of distribution, C(t) is the plasma concentration of the tracee (micromolars) at time t, E(t) is the enrichment in plasma, and dE(t)/dt and dC(t)/dt are the rates of change with time in enrichment and concentration, respectively. The disappearance rate (R_d) is equal to R_a under steady-state conditions, but must be adjusted for the rate of change of an expanding or contracting pool of substrate,

$$R_d(t) = R_a(t) - V_d dC(t)/dt. \quad \text{Eq 2}$$

The value for the glucose V_d was assumed to be 0.16 L/kg. A glutamine V_d of 0.38 L/kg was used based on previous study concerning the tracer-miscible glutamine pool in healthy subjects.[34] For leucine and phenylalanine, the value for V_d was chosen to be body water (0.6 L/kg) because leucine and phenylalanine are distributed throughout this space. Inside cells, eg, in muscle,[35] the concentration is expected to be about 25% higher than in extracellular water. Therefore, the true V_d may be closer to 0.7 L/kg. The effect of increasing the V_d results in a higher R_d when the concentration is decreasing and magnifies the difference between R_a and R_d. For this reason, the more conservative value of 0.6 L/kg was chosen for the V_d to avoid the possibility of artifactually magnifying differences.

To define the leucine released from proteolysis, intracellular leucine enrichment was estimated also by the plasma d_3-KIC enrichment, which is derived from intracellular leucine (reciprocal pool approach).[36,37] We did not attempt to describe intracellular leucine kinetics using equations for the non–steady state, since this approach would imply many more assumptions than the simpler, monocompartmental approach already described. However, the changes of d_3-KIC enrichment within each study period were small, as reflected by the small coefficient of variation (7% to 14%), and thus we were able to calculate intracellular leucine release from proteolysis using the mean value for d_3-KIC enrichment within each study period and the standard steady-state equation,

$$R_a = I[E_i/E_p - 1], \quad \text{Eq 3}$$

where I is the leucine tracer infusion rate as micromoles per kilogram per hour, E_i is the enrichment of the d_3-leucine tracer, and E_p is d_3-KIC enrichment in plasma.

The glutamine R_a into the systemic circulation has two components: (1) glutamine from proteolysis and (2) glutamine from de novo synthesis. Glutamine release from proteolysis was estimated using leucine release from protein breakdown, corrected for the different concentrations of leucine and glutamine in body proteins (8 and 7 g/100 g protein, respectively). Then, the de novo component of the glutamine R_a was calculated by subtracting the glutamine released from protein breakdown from the total glutamine R_a.

Statistical Analysis

The data were analyzed using a balanced repeated-measures ANOVA (RMANOVA), followed by contrasts when significant differences were determined. When the data satisfied the conditions of normality and equal variance, parametric RMANOVA was performed; otherwise, RMANOVA on ranks was used. The mean values for data in the first and second half of the study period were analyzed separately. The statistical analysis tested whether the basal periods of the 3 study days were different, and whether, in study 0, significant differences between the basal and study periods reflected an effect of the pancreatic clamp per se. The effect of each glucagon dose was calculated as the difference between the study and basal period of each pancreatic clamp. These differences were then compared among the three studies. When RMANOVA defined a significant effect of glucagon, a dose-response relationship was calculated as follows: for each subject, the changes between basal and study periods were plotted against the increments in glucagon concentrations, and the slope of the regression line was calculated. The slopes of the individual regression lines were then averaged and tested for significant difference from zero by two-tailed *t* test.

RESULTS

Hormone Concentrations

Plasma hormone concentrations are shown in Table 1. The time course of glucagon, insulin, and epinephrine concentrations is shown in Fig 1. The values reflect the mean concentrations in the last hour of the basal period of the pancreatic clamp and during the 3.5-hour study period. There were no significant differences in the concentration of any of the hormones in the basal period for the three studies (0, 1, and 2). In all three studies, octreotide was infused to block endogenous pancreatic insulin and glucagon secretion while insulin and glucagon were infused intravenously. In each study, basal plasma glucagon and

Table 1. Hormone Concentrations

Hormone	Study 0		Study 1		Study 2	
	Basal	Study	Basal	Study	Basal	Study
Glucagon (pg/mL)	166 ± 8	164 ± 8	155 ± 7	233 ± 7	164 ± 5	328 ± 6
Insulin (μU/mL)	9.8 ± 0.5	8.5 ± 0.6	8.9 ± 0.6	9.1 ± 1.0	10.5 ± 0.6	11.0 ± 1.1
Cortisol (μg/dL)	8.5 ± 0.7	7.0 ± 0.6	9.1 ± 1.1	7.1 ± 1.0	8.5 ± 0.9	7.9 ± 0.9
Epinephrine (pg/mL)	18.8 ± 2.4	28.2 ± 4.0	20.7 ± 5.2	21.7 ± 2.2	13.6 ± 2.7	16.6 ± 2.5

NOTE. Values are the mean ± SE for the 7 subjects in the last hour of the basal period and in the study period.

insulin concentrations were appropriate for normal postabsorptive concentrations and did not change with time during the studies. The same was true of the other hormones measured. In studies 1 and 2, glucagon infusion increased the peripheral glucagon concentration by a factor of 1.5 and 2, respectively. Plasma GH was also measured in all studies. GH concentrations were less than the detection limits of the assay (0.1 ng/mL) in almost all of the samples drawn prior to the somatostatin clamp, as well as in the samples drawn during the somatostatin clamp.

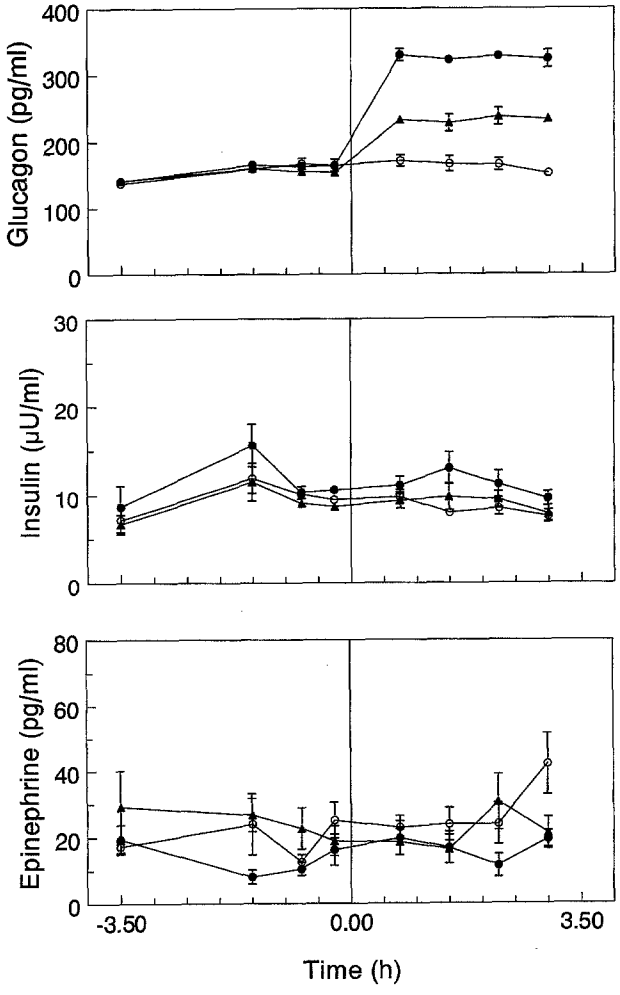

Fig 1. Time course of hormone concentrations: ○, study 0, ▲, study 1; ●, study 2. In studies 1 and 2, glucagon concentrations increased 50% and 100%, but insulin and epinephrine concentrations were unchanged with respect to study 0.

Glucose Metabolism

Plasma glucose concentrations were similar in the basal period of the three studies (Table 2). In study 0, plasma glucose decreased progressively (Fig 2), as reflected by the means for each study period (Table 2). In two subjects, plasma glucose was prevented from decreasing to less than 70 mg/dL during the second half of the study by infusing dextrose. Table 3 shows the changes from basal that each of the three glucagon infusions produced in the first and second half of the study periods. In the first half of studies 1 and 2, glucagon dose-dependently stimulated the glucose concentration, which subsequently decreased. In the second half of studies 1 and 2, glucose concentrations were still higher than in study 0. The time course of d_2-glucose enrichment is also shown in Fig 2. In study 0, the glucose tracer enrichment increased with time. Increasing plasma glucagon in studies 1 and 2 decreased the glucose enrichment with respect to study 0.

The time courses of glucose R_a and R_d (calculated from glucose concentrations and tracer enrichments) are reported in Fig 3. In the two subjects who received intravenous glucose in the last part of study 0, the exogenous glucose infusion rate (0.13 and 1.41 mg/kg/min, respectively) was subtracted from the total R_a of glucose to yield the endogenous glucose R_a. The mean values for each period are reported in Table 2. The glucose R_a peaked at 3.82 ± 0.36 mg/kg/min in study 1 and 4.21 ± 0.33 in study 2 on the first time point at 0.25 hours from the beginning of the study period, and then it declined. In the second half of the studies, values for glucose R_a were not statistically different. However, in evaluating the second half of the studies in Fig 3, it is clear that glucose production diverged between studies, as reflected by the glucose R_a changing over time with a slope higher in study 2 than in study 0 (rate of change, +0.20 ± 0.21 *v* −0.39 ± 0.20 mg/kg/min, $P < .05$). After the initial peaks in glucose R_a and concentration, the glucose R_d increased and remained higher in the second half of studies 1 and 2.

Phenylalanine Metabolism

The time course of the phenylalanine concentration and enrichment is shown in Fig 4. Phenylalanine concentration showed a small but significant decrement with time in study 0 (Table 2), and was not significantly affected by glucagon (Table 3). Phenylalanine R_a and R_d decreased insignificantly during study 0 (Table 2) and were not significantly affected by glucagon (Table 3).

Table 2. Substrate Concentrations and Kinetics

Substrate	Study 0			Study 1			Study 2			Time Effect in Study 0 (*P*)	Differences Among Basal Periods (*P*)
	Basal	1st Half	2nd Half	Basal	1st Half	2nd Half	Basal	1st Half	2nd Half		
Glucose											
Concentration (mg/dL)	110 ± 3	100 ± 5*	91 ± 5*†	110 ± 2	122 ± 3	102 ± 4	111 ± 3	139 ± 6	108 ± 4	.001	NS
R_a (mg/kg/min)	2.11 ± 0.18	2.16 ± 0.13	1.64 ± 0.23	2.22 ± 0.20	2.54 ± 0.18	2.12 ± 0.21	2.13 ± 0.16	3.07 ± 0.25	2.14 ± 0.15	NS	NS
R_d (mg/kg/min)	2.29 ± 0.19	2.36 ± 0.16	1.99 ± 0.10	2.31 ± 0.21	2.47 ± 0.17	2.45 ± 0.25	2.19 ± 0.12	2.80 ± 0.23	2.63 ± 0.19	NS	NS
Phenylalanine											
Concentration (μmol/L)	58.4 ± 3.2	56.1 ± 3.1	55.5 ± 3.4	55.8 ± 2.4	52.2 ± 2.2	51.7 ± 2.7	56.1 ± 2.4	51.7 ± 2.6	50.4 ± 2.2	.047	NS
R_a (μmol/kg/h)	36.0 ± 3.0	34.3 ± 2.4	33.3 ± 2.1	33.8 ± 2.3	33.2 ± 2.2	32.2 ± 2.7	32.4 ± 2.5	32.2 ± 2.1	31.9 ± 2.3	NS	NS
R_d (μmol/kg/h)	36.1 ± 2.7	34.7 ± 2.6	33.8 ± 2.1	35.1 ± 2.2	34.1 ± 2.1	32.8 ± 2.5	33.8 ± 2.1	33.8 ± 2.2	31.4 ± 1.8	NS	NS
Leucine											
Concentration (μmol/L)	111 ± 8	106 ± 8*	105 ± 8*	104 ± 7	99 ± 7	97 ± 6	101 ± 4	92 ± 5	86 ± 5	.020	NS
R_a (μmol/kg/h)	73.7 ± 3.6	70.6 ± 4.4	71.9 ± 3.5	72.7 ± 4.1	72.0 ± 5.7	69.8 ± 6.6	72.9 ± 4.2	68.0 ± 2.7	68.4 ± 3.4	NS	NS
R_d (μmol/kg/h)	76.2 ± 3.1	72.0 ± 4.4	72.5 ± 3.6	74.5 ± 3.6	74.1 ± 5.5	69.7 ± 6.3	75.5 ± 5.0	72.2 ± 2.8	68.2 ± 3.3	NS	NS
Glutamine											
Concentration (μmol/L)	401 ± 43	396 ± 45	384 ± 45*	441 ± 27	420 ± 26	376 ± 27	438 ± 28	396 ± 34	325 ± 28	.047	NS
R_a (μmol/kg/h)	261 ± 21	246 ± 10	243 ± 13	273 ± 22	256 ± 14	246 ± 16	275 ± 31	272 ± 26	243 ± 20	NS	NS
De novo R_a (μmol/kg/h)	167 ± 19	157 ± 8	158 ± 12	184 ± 20	170 ± 12	163 ± 13	186 ± 30	187 ± 25	159 ± 19	NS	NS
R_d (μmol/kg/h)	269 ± 22	249 ± 10	243 ± 12	278 ± 21	264 ± 15	254 ± 16	280 ± 33	288 ± 25	254 ± 21	NS	NS

NOTE. Values are the mean ± SE for the 7 subjects in the last hour of the basal period and in the first and second half of the 3.5-hour study period. Differences between the periods in study 0 and between the basal periods of studies 0, 1, and 2 were tested by RMANOVA followed by contrasts when significant.

*$P < .05$ *v* basal.

†$P < .05$ *v* 1st half.

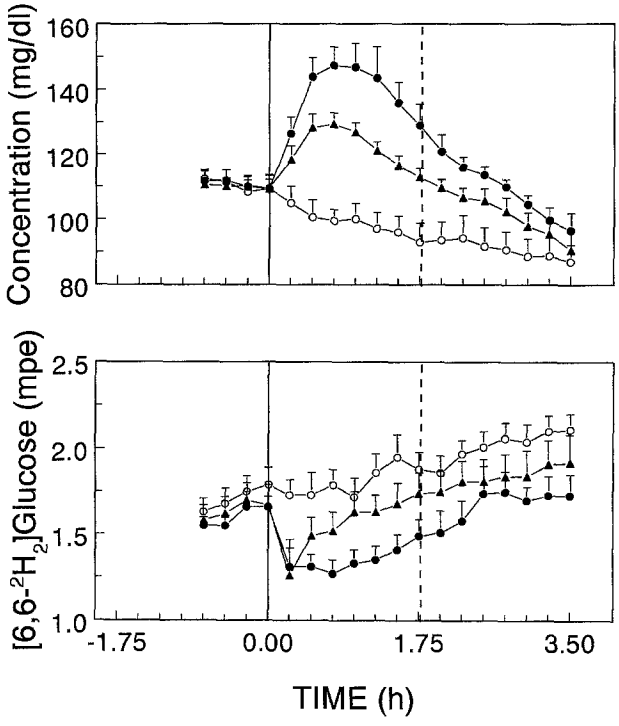

Fig 2. Time course of the glucose concentration and enrichment (mpe, mole percent excess): ○, study 0; ▲, study 1; ●, study 2. Glucagon transiently increased the glucose concentration and decreased the glucose tracer enrichment.

Leucine Metabolism

The time course of the leucine concentration and enrichment is shown in Fig 5. During study 0, the leucine concentration had a minor but significant decrement (−5%; Table 2). Glucagon increased the suppression of leucine concentration in a dose-dependent fashion (−7% and −15% in studies 1 and 2; Table 3). Leucine enrichment did not change with time in study 0, and was not affected by glucagon in studies 1 and 2. Glucagon did not significantly affect leucine R_a and R_d calculated with the leucine concentration and leucine tracer enrichment (primary pool approach; Table 3).

KIC concentration decreased slightly and comparably during all studies (Tables 4 and 5). The enrichment of d_3-KIC derived from the infused d_3-leucine tracer increased in study 0, but this increment was significantly smaller when glucagon was infused. d_3-KIC enrichments were used to calculate the intracellular leucine release from proteolysis (Tables 4 and 5). The leucine released from proteolysis decreased in study 0 (−11% from basal; Tables 4 and 5) and glucagon had a small but significant stimulatory effect on proteolysis (to −7% and −6% from basal).

Glutamine Metabolism

The time course of glutamine concentration and enrichment is shown in Fig 6. During study 0, the glutamine concentration had a small (−4%) but significant decrement in the second half of the study (Table 2). Both glucagon doses caused a progressive dose-dependent decrement in the glutamine concentration

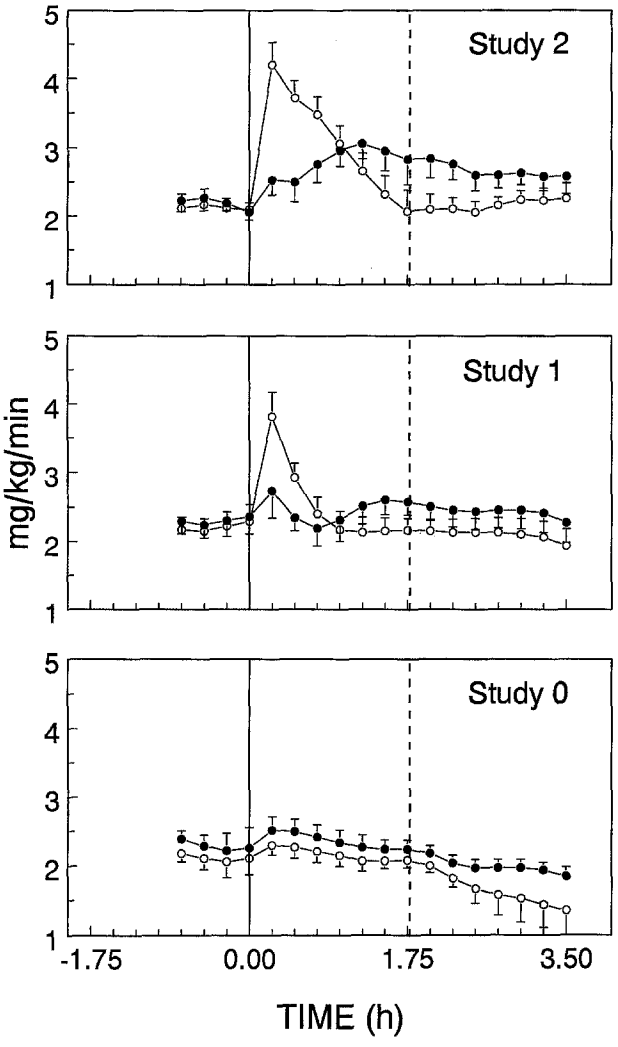

Fig 3. Time course of glucose kinetics in the 3 studies: ○, glucose R_a; ●, glucose R_d. Glucagon transiently increased the glucose production rate in the first half of studies 1 and 2. The peak in glucose R_a was followed by stimulation of the glucose R_d.

(Table 3) that did not reach a steady state by the end of the studies. Glutamine enrichment increased progressively during all studies. The time course of glutamine R_a and R_d is shown in Fig 7. Both glutamine R_a and R_d decreased slightly in study 0, and glucagon did not produce significant changes between studies. However, the balance between glutamine R_a and R_d changed from not significantly different from zero in study 0 (-1.9 ± 0.9 μmol/kg/h) to negative during studies 1 and 2 (-8.1 ± 1.1 and -13.6 ± 1.0 μmol/kg/h, RMANOVA $P = .001$; slope of the response $= -7.06 \pm 0.81$ μmol/kg/h per 100-pg/mL glucagon increase, $P = 0.001$). The metabolic clearance of glutamine did not change during study 0, but it increased dose-dependently with glucagon, up to 8% and 21% in the second half of studies 1 and 2 (RMANOVA $P = .001$).

Table 2 also shows the de novo synthetic component of the glutamine R_a. In study 0, glutamine de novo synthesis accounted for the majority of glutamine flux and did not change significantly with respect to the basal period. Glucagon did not change the total glutamine R_a, and it caused only a minor increment in proteolysis. As a consequence, glucagon caused small changes in glutamine de novo synthesis that did not reach significance with RMANOVA (Table 3). However, plotting the changes in glutamine de novo synthesis against the increments in glucagon concentration for each study showed a trend for glucagon to decrease glutamine de novo synthesis (slope of the response $= -13.0 \pm 5.2$ μmol/kg/h per 100-pg/ml Glucagon increase, $P = .047$).

DISCUSSION

In agreement with previous studies,[38-40] we found that the effect of glucagon on glucose production peaked in the first minutes of hyperglucagonemia and then declined in a few hours. In animals and humans infused with high glucagon doses, a transient increase in glycogenolysis accounts for almost all of the response in the first minutes, whereas after a few hours gluconeogenesis predominates,[1,3] even though the liver glycogen stores are not depleted.[2] The dissipation of glucagon-induced glycogenolysis and the return of glucose production at typical postabsorptive rates do not imply that the effect of glucagon on glucose production has been terminated. Interestingly, both animals[1] and humans[41] infused with glucagon become dependent on the higher concentrations of glucagon to prevent glucose production from decreasing in the same time frame as in the present study.

Hyperglucagonemia stimulates hyperinsulinemia, which can antagonize glucagon's action. In this study, the effect of hyperinsulinemia was blocked by keeping insulin concentrations constant. In contrast, hyperglycemia probably contributed to the suppression of glucose production. However, in animals[42] and humans,[43] the effect of glucagon on glucose production

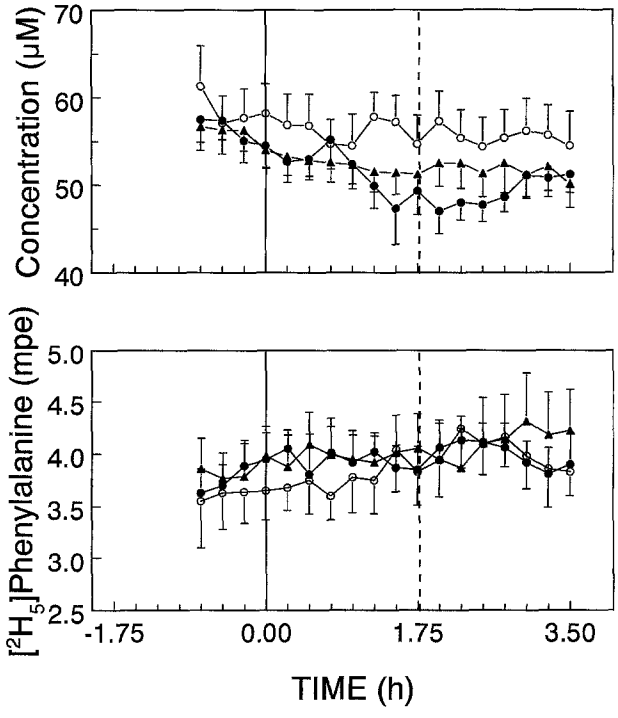

Fig 4. Time course of the phenylalanine concentration and enrichment (mpe, mole percent excess) in plasma: ○, study 0; ▲, study 1; ●, study 2.

Table 3. Changes From the Basal Period Induced by Glucagon

Parameter	1st Half of Study Period			Effect of Glucagon Dose (*P*)	Response to a 100-pg/mL Glucagon Increment	2nd Half of Study Period			Effect of Glucagon Dose (*P*)	Response to a 100-pg/mL Glucagon Increment
	Study 0	Study 1	Study 2			Study 0	Study 1	Study 2		
Glucose										
Concentration (mg/dL)	−10.3 ± 3.7	11.8 ± 1.8*	28.0 ± 3.5*	.001	23.3 ± 3.4	−19.5 ± 4.2	−8.3 ± 1.6*	−3.3 ± 2.5*	.003	9.9 ± 2.8
R_a (mg/kg/min)	0.05 ± 0.12	0.33 ± 0.13	0.95 ± 0.19*†	.001	0.55 ± 0.11	−0.48 ± 0.27	−0.10 ± 0.19	0.02 ± 0.15	NS	
R_d (mg/kg/min)	0.07 ± 0.07	0.16 ± 0.11	0.61 ± 0.19*†	.008	0.36 ± 0.13	−0.30 ± 0.18	0.14 ± 0.15	0.45 ± 0.12*	.025	0.48 ± 0.13
Phenylalanine										
Concentration (μmol/L)	−2.3 ± 1.0	−3.6 ± 0.7	−4.4 ± 1.4	NS		−2.9 ± 1.3	−4.1 ± 1.6	−5.7 ± 1.1	NS	
R_a (μmol/kg/h)	−1.7 ± 0.8	−0.6 ± 1.5	0.3 ± 2.7	NS		−2.7 ± 1.3	−1.6 ± 1.5	−0.6 ± 1.5	NS	
R_d (μmol/kg/h)	−1.5 ± 0.6	−1.1 ± 1.5	0.0 ± 0.6	NS		−2.3 ± 1.0	−2.4 ± 1.4	−2.5 ± 1.0	NS	
Leucine										
Concentration (μmol/L)	−5.1 ± 1.9	−5.14 ± 1.2	−9.1 ± 1.6	NS		−5.5 ± 2.2	−7.3 ± 3.1	−15.3 ± 2.0*†	.016	−7.1 ± 2.66
R_a (μmol/kg/h)	−3.1 ± 2.3	−0.8 ± 2.9	−4.9 ± 1.8	NS		−1.8 ± 2.1	−2.9 ± 4.5	−4.4 ± 1.2	NS	
R_d (μmol/kg/h)	−4.2 ± 2.5	−0.4 ± 3.0	−3.3 ± 2.7	NS		−3.7 ± 1.4	−4.8 ± 4.3	−7.3 ± 2.1	NS	
Glutamine										
Concentration (μmol/L)	−5.6 ± 4.5	−20.8 ± 13.7	−42.0 ± 9.5*	.048	−24.5 ± 7.2	−16.8 ± 6.7	−64.7 ± 11.7*	−113.1 ± 7.5*†	.001	−58.9 ± 5.56
R_a (μmol/kg/h)	−14.9 ± 13.4	−17.2 ± 9.9	−2.6 ± 8.3	NS		−18.5 ± 9.6	−26.7 ± 7.2	−32.0 ± 12.7	NS	
De novo R_a (μmol/kg/h)	−10.5 ± 12.5	−14.0 ± 10.2	0.7 ± 9.4	NS		−9.2 ± 8.7	−20.7 ± 7.7	−27.2 ± 12.8	NS	
R_d (μmol/kg/h)	−20.4 ± 14.0	−13.6 ± 8.9	8.9 ± 9.3	NS		−26.1 ± 10.6	−23.4 ± 7.4	−26.1 ± 14.0	NS	

NOTE. Mean ± SE changes from the basal period are compared between studies by RMANOVA followed by contrast. The response to the increment in glucagon concentrations was also tested by linear regression within each individual: the table reports the mean ± SE of the individual slopes.

*$P < .05$ *v* study 0.

†$P < .05$ *v* study 1.

dissipates also when glucose concentrations are kept constant. Recently, experiments in the perfused rat liver showed that hyperglycemia cannot inactivate glucagon stimulation of glycogen phosphorylase and of glucose output.[44] Thus, factors different from hyperglycemia and hyperinsulinemia contribute to the dissipation of glucagon-induced hyperglycemia.

Hyperglucagonemia decreases glutamine concentrations.[4] We estimated that a 100-pg/mL increment in circulating glucagon decreases the concentration of glutamine by 60 μmol/L in approximately 3 hours. In a study similar to the present one, glucagon was able to suppress the concentrations of other amino acids involved in glucose production, such as alanine, glycine, serine, and proline,[5] suggesting that glucagon can decrease the bulk of the circulating gluconeogenic amino acids.

Our study provides an explanation as to why glucagon decreases the concentration of glutamine. Glucagon caused a significant imbalance in glutamine kinetics such that glutamine uptake was greater than glutamine release. Despite the fact that glutamine concentrations were reduced in studies 1 and 2, glutamine was still removed at the same rate as in study 0. In other words, glucagon increased the metabolic clearance of glutamine. With the present protocol, we could not assess which site was responsible for the increased uptake of glutamine during hyperglucagonemia. However, in humans, it is a common view that splanchnic tissues, including the liver and gut, are the principal site of glutamine disposal.[45,46] In the dog, glucagon decreases the glutamine concentration by increasing glutamine uptake by the liver and gut.[16] In isolated rat hepatocytes, glucagon rapidly stimulates the transport of glutamine.[47,48] In the rat, glucagon also promotes the disposal of intracellular glutamine by activating the rat hepatic glutaminase.[49] In contrast, glucagon does not stimulate glutamine transport in the rat muscle.[50] Thus, considerable evidence in the literature supports the idea that glucagon stimulates uptake of glutamine by the liver and gut.

We specifically traced the metabolism of glutamine because we expected that an increased splanchnic uptake of glutamine

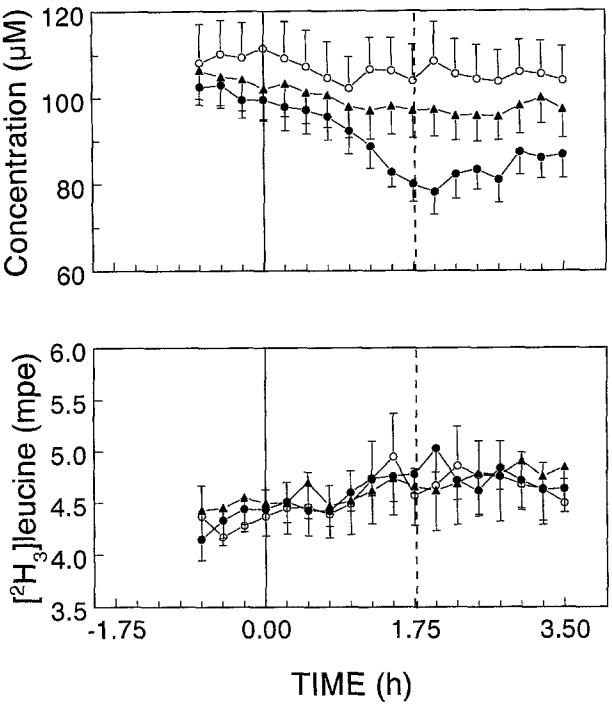

Fig 5. Time course of the leucine concentration and enrichment (mpe, mole percent excess) in plasma; ○, study 0; ▲, study 1; ●, study 2. Glucagon dose-dependently decreased leucine concentrations.

Table 4. KIC Concentration and Enrichment and Leucine Flux Calculated With the Reciprocal Pool Model

Parameter	Study 0			Study 1			Study 2			Time Effect in Study 0 (*P*)	Differences Among Basal Periods (*P*)
	Basal	1st Half	2nd Half	Basal	1st Half	2nd Half	Basal	1st Half	2nd Half		
Concentration (μmol/L)	36.5 ± 3.7	33.4 ± 3.6*	29.4 ± 3.0*†	35.6 ± 3.5	31.9 ± 3.5	27.4 ± 2.0	33.8 ± 1.5	30.2 ± 1.6	27.5 ± 1.2	.001	NS
Enrichment (mpe)	2.82 ± 0.17	3.11 ± 0.10*	3.28 ± 0.12*†	3.10 ± 0.13	3.23 ± 0.15	3.35 ± 0.18	3.06 ± 0.08	3.16 ± 0.07	3.26 ± 0.08	.001	NS
Intracellular leucine flux (μmol/kg/h)	109 ± 4	102 ± 3*	97 ± 3*†	102 ± 3	98 ± 3	95 ± 4	101 ± 2	98 ± 2	95 ± 2	.001	.018

NOTE. Differences between the periods in study 0 and between the basal periods in studies 0, 1, and 2 were tested by RMANOVA followed by contrasts when significant. The basal leucine flux in study 0 was slightly but significantly higher than in both studies 1 and 2.

*$P < .05$ *v* basal.

†$P < .05$ *v* 1st half.

has an important effect on glucose metabolism. Both alanine and glutamine are important gluconeogenic precursors in humans, but glutamine is more important than alanine for adding new carbon to the glucose pool, whereas alanine cycles extensively with glucose.[18,19] However, glutamine can promote cellular swelling, which in turn can activate glycogen synthesis in the liver[51] and consequently divert the newly synthesized glucose to glycogen.[52] We speculate that glucagon fuels gluconeogenesis and promotes hepatic glutamine uptake, but glutamine in the liver counteracts the glycogenolytic action of glucagon.[53] This putative antagonism between glucagon and glutamine on glycogenolysis can help to explain why the glycogenolytic response to glucagon is short-lived and gluconeogenesis rapidly becomes more important in sustaining glucose production.

This study showed that the glucagon-induced increase in glutamine uptake was not met with an increased release. Glutamine appears in the systemic circulation primarily from skeletal muscle via three mechanisms: (1) glutamine release from muscle proteolysis, (2) muscle glutamine de novo synthesis from other amino acids, and (3) release from the large free intracellular muscle glutamine pool. The source for glutamine de novo synthesis is most likely amino acids from proteolysis, tying the response of mechanisms 1 and 2 together. We found that glucagon induced a small increment in proteolysis. This effect was not adequate to release enough glutamine directly to counteract the decreasing glutamine concentrations, because the total glutamine R_a did not change with glucagon, suggesting that the effect of glucagon, if any, was to decrease the amount glutamine released from the other two sources. We speculate that this inability of glucagon to provide more fuel for gluconeogenesis in the face of an increased uptake is an important mechanism that limits the hyperglycemic (diabetogenic) action of glucagon in healthy humans. This mechanism can explain why, after a few hours of hyperglucagonemia, the maintenance of glucose production only at a postabsorptive rate is dependent on glucagon's driving the gluconeogenic amino acids into gluconeogenesis.

The small but significant increment in proteolysis at the end of the studies is also interesting in light of the possible catabolic effects of glucagon. Using the KIC enrichments and the reciprocal pool model, we had enough statistical power to detect small changes in proteolysis. Phenylalanine R_a is another index of proteolysis, but the measurements for phenylalanine enrichment were noisier than for KIC. Using phenylalanine kinetics, the statistical power was not adequate to evaluate the small differences that were assessed with KIC. With phenylalanine, we found a trend in proteolysis (with glucagon reversing the −8% suppression of proteolysis in study 0 to −1.8% suppression in study 2) similar to that found using KIC, but it was not significant. In addition, we possibly underestimated the changes in proteolysis, because phenylalanine plasma enrichment may not reflect intracellular enrichment, unlike KIC. Increased proteolysis was previously found only when hyperglucagonemia was coupled with insulin deficiency.[11] It should be reiterated that the detection of such small effects can depend on the

Table 5. Changes From the Basal Period Induced by Glucagon

Parameter	1st Half			Effect of Glucagon Dose (*P*)	Response to a 100-pg/mL Glucagon Increment	2nd Half			Effect of Glucagon Dose (*P*)	Response to a 100-pg/mL Glucagon Increment
	Study 0	Study 1	Study 2			Study 0	Study 1	Study 2		
Concentration (μmol/L)	−3.1 ± 1.0	−3.7 ± 0.8	−3.6 ± 0.8	NS	—	−7.1 ± 1.5	−8.2 ± 1.7	−6.4 ± 1.3	NS	—
Enrichment (mpe)	0.29 ± 0.10	0.12 ± 0.03	0.10 ± 0.02	NS	—	0.46 ± 0.10	0.25 ± 0.08*	0.20 ± 0.03*	.014	−0.15 ± 0.06
Intracellular leucine flux (μmol/kg/h)	−7.1 ± 1.9	−3.9 ± 0.9	−3.1 ± 2.2	NS	—	−12.4 ± 1.5	−7.2 ± 2.3*	−6.5 ± 0.8*	.011	3.42 ± 0.87

NOTE. Changes from the basal period are compared between studies by RMANOVA followed by contrasts. The response to increments in the glucagon concentration was also tested by linear regression: the table reports the mean ± SE of the individual slopes.

*$P < .05$ *v* study 0.

†$P < .05$ *v* study 1.

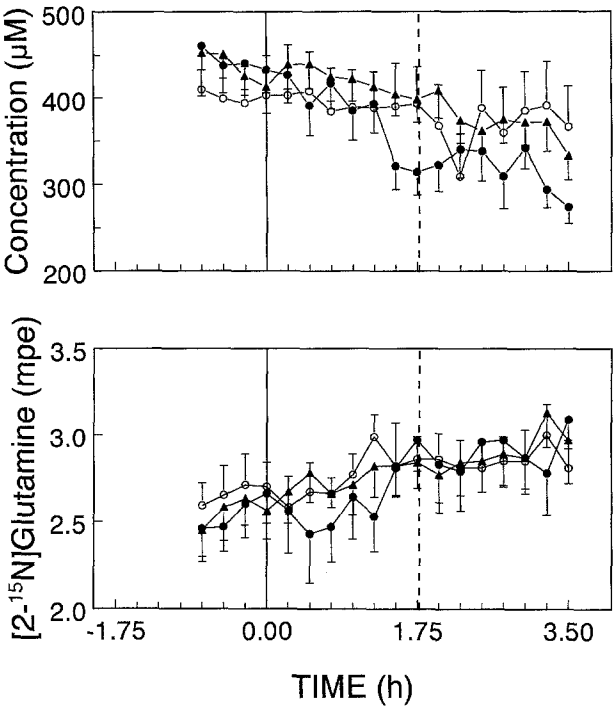

Fig 6. Time course of the glutamine concentration and enrichment (mpe, mole percent excess) in plasma: ○, study 0; ▲, study 1; ●, study 2. Glucagon decreased the glutamine concentration without changing glutamine enrichment.

experimental protocol and on the statistical power. For example, a previous study conducted with fewer subjects provided similar numbers but did not reach statistical significance.[5] In contrast, recent studies reported that during a pancreatic clamp, proteolysis decreased after administration of glucagon.[9,10] Our results show that this decrement in proteolysis was due to an effect of the pancreatic clamp itself, and that the net effect of glucagon is to increase, not decrease, proteolysis. Overall, the proteolytic effect of glucagon observed in the time frame of this study is small, and substantially confirms that glucagon does not contribute much to protein catabolism in the short-term. Nonetheless, the proteolytic effect of glucagon increased with time and did not subside at the conclusion of the study, suggesting that it is long-lasting and could further increase over time. The absence of significant changes in leucine enrichment and (primary pool) kinetics coupled with the small decrements in the leucine concentration suggest that the amino acids mobilized by the small increase in proteolysis were immediately disposed in the cells and did not mix with the circulating leucine and phenylalanine tracers. Other studies that used carbon-labeled amino acid tracers suggested that glucagon promotes the disposal of amino acids via oxidation and not protein synthesis.[8,10,11,13]

The effect of glucagon on protein catabolism in human muscle is controversial. There is an apparent discrepancy between the study by Pozefsky et al,[54] which infused glucagon directly in the arterial supply of the muscle and did not find an effect on amino acid exchange, and the study by Pacy et al[8] that infused glucagon systemically and found that glucagon worsened protein balance across the forearm. These studies can be

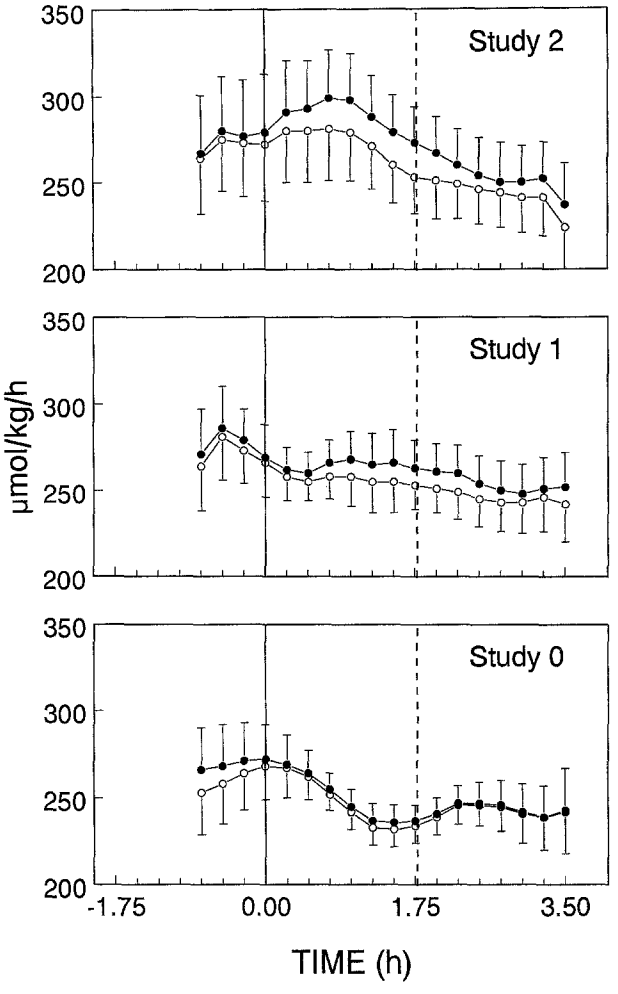

Fig 7. Time course of glutamine kinetics in the 3 studies: ○, glutamine R_a; ●, glutamine R_d. Glucagon caused a dose-dependent imbalance between the glutamine R_d (uptake) and glutamine R_a.

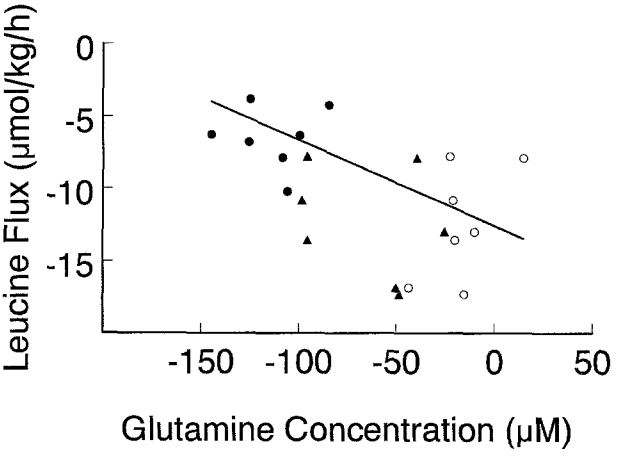

Fig 8. Changes from basal proteolysis (intracellular leucine flux) and glutamine concentration in the second half of the study periods: ○, study 0; ▲, study 1; ●, study 2. Changes in proteolysis (endogenous leucine flux) and in glutamine concentrations were inversely related, as shown by the regression line calculated from the mean of the individual slopes (−5.92 ± 1.58 μmol/kg/h per 100-μmol/L decrement in glutamine, $P < .01$).

reconciled if glucagon does not have a direct effect on protein metabolism in muscle but increases muscular proteolysis via an indirect mechanism, ie, a signal originating outside the muscle.

In this study, we found that proteolysis was highest when the concentration of glutamine was lowest. Figure 8 shows that the changes in proteolysis were significantly and inversely related to changes in the concentration of glutamine. We speculate that the low glutamine concentrations stimulated proteolysis. Animal data show that glucagon increases glutamine transport in the liver but not in the muscle,[50] and thus, we expect that when glucagon decreases glutamine concentrations it also limits glutamine uptake in the muscle. The muscle normally releases more glutamine than it removes, and a reduction in glutamine uptake can deplete free glutamine in the muscle. In agreement with this idea, a low content of free glutamine has been found in the muscle of subjects with glucagonoma[55] and other catabolic illness.[56-58] However, administration of glutamine reduced protein catabolism in the muscle after surgery[59,60] and improved the nitrogen balance after bone marrow transplantation,[61] suggesting that glutamine depletion is responsible for protein catabolism. In the muscle of the rat, intracellular glutamine is a strong antiproteolytic agent,[20] and its depletion leads to protein catabolism.[21,22] Thus, one explanation for glucagon-induced proteolysis is that glucagon decreases the availability of glutamine, and glutamine depletion acts as a signal to increase proteolysis in the muscle. Glutamine depletion is also a feature of acidosis induced by insulin deficiency. This may explain why insulin deficiency increases the catabolic action of glucagon, and suggests that insulin deficiency may also increase glucagon's effects on glucose metabolism.

In conclusion, increasing the glucagon concentration at the upper physiologic levels in humans causes transient hyperglycemia but persistently suppresses glutamine concentrations and slowly increases protein breakdown. Overall, these results show that glucagon promotes the uptake of gluconeogenic substrates but does not proportionally increase their release, supporting the idea that glucagon-induced hyperglycemia is short-lived because of the failure of glucagon to provide more fuel for gluconeogenesis. Nonetheless, the small but persistent increase in proteolysis coupled with the depletion of circulating amino acids prove that even modest hyperglucagonemia can contribute to protein catabolism.

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