Effect of Hypoglycemia on Amino Acid and Protein Metabolism in Healthy Humans

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In response to hypoglycemia, healthy individuals rapidly antagonize insulin action on glucose and lipid metabolism, but the effects on protein metabolism are unclear. Because amino acids are an important substrate for gluconeogenesis and a fuel alternative to glucose for oxidation, we evaluated whether hypoglycemia antagonizes the hypoaminoacidemic and the antiproteolytic effects of insulin and changes the de novo synthesis of glutamine, a gluconeogenic amino acid. To this purpose, in 7 healthy subjects, we performed 2 studies, 3.5 h each, at similar insulin but different glucose concentrations (i.e., 4.9 ± 0.1 mmol/l [euglycemic clamp] or 2.9 ± 0.2 mmol/l [hypoglycemic clamp]). As expected, hypoglycemia antagonized the insulin suppression of glucose production achieved in euglycemia (from 21 ± 15 to 116 ± 12% of basal, $P < 0.001$), the stimulation of glucose uptake (from 207 ± 28 to 103 ± 7% of basal, $P < 0.01$) and the suppression of circulating free fatty acids (from 30 ± 5 to 80 ± 17% of basal, $P < 0.001$). In contrast, hypoglycemia increased the insulin suppression of circulating leucine (from 63 ± 1 to 46 ± 2% of basal, $P < 0.001$) and phenylalanine (from 79 ± 3 to 64 ± 3% of basal, $P < 0.001$) concentrations. Hypoglycemia did not change the insulin suppression of proteolysis (from 79 ± 2 to 82 ± 4% of basal, $P < 0.001$). However, hypoglycemia doubled the insulin suppression of the glutamine concentrations (from 84 ± 3 to 63 ± 3% of basal, $P < 0.01$) in the absence of significant changes in the glutamine rate of appearance, but it also caused an imbalance between glutamine uptake and release. This study demonstrates that successful counterregulation does not affect proteolysis. Moreover, it does not increase the availability of circulating amino acids by de novo synthesis. In contrast, despite the lower concentration of circulating amino acids, hypoglycemia increases the uptake of glutamine that can be used for gluconeogenesis and as a fuel alternative to glucose. *Diabetes* **49:1543–1551, 2000**

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FFA, free fatty acid; GCMS, gas chromatography–mass spectrometry; KIC, ketoisocaproic acid; R_a, appearance rate; R_d, disappearance rate; TBDMS, *Tris-t*-butyldimethylsilyl.

Insulin causes hypoglycemia by increasing the uptake of glucose from the peripheral tissues and by blocking the release of new glucose in the circulation (1,2). At the same time, insulin limits the breakdown of fat (3,4) and protein stores (5,6) that provide substrates for gluconeogenesis and fuel alternatives to glucose. When hypoglycemia develops, several counterregulatory hormones reestablish the proper rates of glucose production. All of these hormones may act simultaneously on glucose, fat, and protein metabolism. Glucagon has the potential to increase glucose production, proteolysis, glutamine uptake, and amino acid oxidation (2,7–16). Epinephrine can increase glycogenolysis and gluconeogenesis, lipolysis, and amino acid uptake (17–22). Cortisol can increase gluconeogenesis, proteolysis, glutamine uptake, and amino acid oxidation (23–25). Many studies have shown that the reversal of insulin-induced hypoglycemia not only involves changes in glucose kinetics but also is accompanied by a rebound increase in lipolysis (3,4). It is then reasonable to ask whether counterregulation to hypoglycemia also reverts the antiproteolytic effects of insulin in humans. Studies in the dog have suggested that, in response to hypoglycemia, a sharp increment in proteolysis provides amino acids to the tissues that may use them for oxidation and for gluconeogenesis (26). Based on these data and on the awareness that cortisol and glucagon may exert a proteolytic effect under certain conditions, it is somewhat common wisdom that the response to hypoglycemia involves antagonism of insulin action on protein metabolism (27). However, it must be stressed that experimental evidence to support this hypothesis is definitely lacking in humans. In addition, glucagon and epinephrine cause hypoaminoacidemia rather than increased availability in amino acids (9,13,16,18–22,28,29), and cortisol action is slow compared with the time-frame in which healthy individuals respond to hypoglycemia. Thus, there are reasons to believe that in humans the acute response to hypoglycemia does not restore the proteolytic rate to postabsorptive values. Nonetheless, given the importance of amino acids in supporting gluconeogenesis and in stimulating insulin and glucagon secretion, the effect of hypoglycemia on their availability needs to be clarified.

This study was designed to assess the effect of insulin-induced hypoglycemia on protein and amino acid metabolism in healthy subjects. To dissect the effects of insulin and hypoglycemia, we performed 2 studies at similar insulin but different glucose concentrations, in which we measured the kinetics of an essential amino acid, leucine, that reflects the rates of pro-

tein synthesis and degradation. In addition, we traced the kinetics of glutamine, a prominent gluconeogenic and anabolic amino acid, as representative of the effects of hypoglycemia on the release and the utilization of gluconeogenic amino acids. With this approach, we could determine that insulin-induced hypoglycemia does not increase the protein catabolism. In contrast, hypoglycemia stimulates the inward flux of gluconeogenic amino acids to compensate for their reduced release from proteolysis and to promote gluconeogenesis.

RESEARCH DESIGN AND METHODS

Materials. L-[1-^{13}C]leucine, L-[2-^{15}N]glutamine, and sodium [^{13}C]bicarbonate were purchased from MassTrace (Woburn, MA). Chemical and isotopic purity of the tracers was determined by gas chromatography–mass spectrometry (GCMS). Before each infusion study, sterile solutions of L-[1-^{13}C]leucine, L-[2-^{15}N]glutamine, and sodium [^{13}C]bicarbonate 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 filter before use. An aliquot of the sterile solution was initially verified to be pyrogen-free before administration to human subjects. Solutions were prepared no more than 24 h before use, and they were kept at 4°C before administration.

Subjects. Seven healthy adults (3 males and 4 females, age 26 ± 2 years, weight 66 ± 5 kg, BMI 22 ± 1 kg/m^2) were studied at the San Raffaele Scientific Institute of Milan. A medical history, a physical examination, and biochemical laboratory screening tests were documented to verify that each subject was free of chemically evident metabolic, gastrointestinal, cardiovascular, neurological, and infectious disorders. The subjects were informed of the purpose, the benefits, and the risks of the study and gave their written consent in accordance with protocols approved by the institutional ethics committee.

Infusion protocol. Each subject participated in 2 consecutive infusion studies spaced 2–4 days apart. The sequence of the studies was randomized among subjects. Before admission, the subjects were placed on an adequate energy and protein (1.2 g · kg^{-1} · day^{-1}) diet and remained on this diet until the last study was completed. The diet was required to reduce intersubject variability induced by different subjects' dietary practices. The format of each of the infusion studies was similar and differed only in the amount of glucose that was infused. On the evening before each infusion study, the subjects consumed their evening meal by 8:30 P.M. and then drank only water until completion of the study on the next day at 2:00 P.M. At 7 A.M. on the infusion day, a catheter was placed in the subject's arm for infusion of the tracers of glucose and amino acids, of insulin and of dextrose when necessary. A second catheter was placed retrograde in a hand vein, and the subject's hand was placed in a warming box to obtain arterialized venous blood samples. The catheters were kept patent with a slow infusion of sterile saline. At the beginning of both studies, priming doses of [1-^{13}C]leucine (4.5 µmol/kg), [2-^{15}N]glutamine (4.5 µmol/kg), and D$_2$-glucose (12.0 µmol/kg) were administered intravenously and were immediately followed by the continuous infusion of the same tracers (4.5, 7.5, and 13.3 µmol · kg^{-1} · h^{-1}, respectively) for 6 h. After 2.5 h of equilibration, 1 of the following studies was performed. In study 1, a euglycemic-hyperinsulinemic clamp was performed for 3.5 h as previously described (30): insulin was infused at the rate of 0.5 mU · kg^{-1} · min^{-1} to achieve and maintain insulin concentrations of ~300 pmol/l, and 20% dextrose was infused at a variable rate to maintain the glucose concentration to 5 mmol/l. In study 2, a hypoglycemic-hyperinsulinemic clamp was performed as previously described (31): insulin was infused at the rate of 0.65 mU · kg^{-1} · min^{-1} for 3.5 h to achieve and maintain insulin concentrations similar to those produced in study 1. The insulin infusion rate was chosen to be 30% higher than in study 1 to compensate for a larger suppression of endogenous insulin secretion that was expected during hypoglycemia compared with euglycemia. Glucose concentrations were measured at the bedside every 5 min and were left free to fall in response to insulin. If and when glucose concentrations decreased to <2.4 µmol/l, enough 20% dextrose was infused to prevent any further reduction in glucose concentration. The glucose infusion was immediately discontinued if the glucose concentration exceeded 2.6 µmol/l.

In both studies, blood and breath samples were drawn just before the start and at 15-min intervals during the last 45 min of each of the 2.5-h tracer equilibration periods and through the 3.5-h study periods. Aliquots of blood were placed in tubes containing EDTA and stored on ice until the plasma was prepared by centrifugation at 4°C. A 0.5-ml aliquot was withdrawn, defined amounts of [$^2H_4$]alanine, [$^2H_7$]leucine, [$^2H_5$]phenylalanine, [$^2H_5$]glutamine, and ketocaproate were added as internal standards for quantitation of alanine, leucine, phenylalanine, and glutamine plasma concentrations, and the plasma was frozen at –60°C. Blood aliquots of 1 ml for the measurements of glucagon and the catecholamines were placed in tubes containing EDTA plus aprotinin and in tubes containing glutathione, respectively. Blood aliquots for insulin, cortisol, and growth hormone 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 h from drawing). All plasma and serum aliquots were frozen at –60°C until later analysis. Breath samples were placed into 20-ml evacuated tubes until measurement of $^{13}CO_2$ in the expired air by isotope ratio mass spectrometry. Each subject's CO_2 production rate was measured periodically for 15-min periods using an indirect calorimeter with a flow-through canopy system (model 2800 Z; Sensormedics, Palo Alto, CA). The CO_2 production rate was used to calculate leucine oxidation.

Analytical methods. The D$_2$-glucose enrichments were measured by GCMS after preparation of the butyl-boronate derivative (16). Injections of the samples were made into a GCMS instrument (model 5970; Hewlett-Packard, Palo Alto, CA) that was operated using electron impact ionization as previously described (16). Plasma amino and keto acid concentrations and enrichments were measured by electron impact GCMS. Before derivatization, amino and keto acids were isolated from plasma using cation-exchange columns as previously described (20). Amino acids eluted from the columns were evaporated to dryness and derivatized to form the *Tris-t*-butyldimethylsilyl (TBDMS) derivative (16). The [M-57]$^+$ ions at *m/z* 260 and 264 were monitored for unlabeled and [$^2H_4$]alanine, respectively. The [M-57]$^+$ ions at *m/z* 302, 303, and 309 were monitored for unlabeled, [1-^{13}C], and [$^2H_7$]leucine, respectively. The [M-57]$^+$ ions at *m/z* 336 and 338 were monitored for unlabeled and [$^2H_2$]phenylalanine, respectively. The [M-57]$^+$ ions at *m/z* 431, 432, and 436 were monitored for unlabeled, [2-^{15}N], and [$^2H_5$]glutamine, respectively. TBDMS-glutamine was chromatographically resolved from TBDMS-glutamate. The α-ketoisocaproic acid (KIC) enrichments and concentrations were measured after the eluant from the columns were derivatized to the trimethylsilyl-quinoxalinol derivative (16). Injections of the derivatives were made into a GCMS instrument (model 5970; Hewlett-Packard, Palo Alto, CA) that was operated using electron impact ionization. The ions at *m/z* 259 and 260 were monitored for unlabeled and [1-^{13}C]KIC, respectively. The ketocaproate peak was resolved from that of KIC and was used for the quantification of the KIC concentrations. For all measurements, the background-corrected tracer enrichments were calculated in mole percent excess (mpe) as previously defined.

Plasma hormone concentrations were measured by radioimmunoassay with commercial kits as previously described (32). The catecholamine concentrations were measured by a high-performance liquid chromatography method (33).

Calculations. The glucose and the glutamine kinetics were calculated using Steele's equations for the non–steady state (34), as we previously described (16). The appearance rate (R_a) of the unlabeled substrates (R_a [micromoles per kilogram per hour]) was calculated using the equation:

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

where i is the infusion rate of the tracers as micromoles per kilogram per hour of tracer per se (i.e., 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 (micromoles per liter) 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 of 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:

$$R_d(t) = R_a(t) - V_d\, dC(t)/dt \quad (2)$$

The value for the glucose V_d was assumed to be 0.16 l/kg. A glutamine V_d value of 0.38 l/kg was used, based on previous work concerning the tracer-miscible glutamine pool in healthy subjects (35).

We did not attempt to describe the intracellular leucine kinetics using equations for the non–steady state because this approach would imply many more assumptions than the simpler monocompartmental approach previously described. To define the leucine release from proteolysis, the intracellular leucine enrichments were estimated by the plasma [1-^{13}C]KIC enrichments, which are derived from intracellular leucine (reciprocal pool approach) (16) with the standard steady-state equation:

$$R_a = I\,[E_i/E_p - 1] \quad (3)$$

where I is the leucine tracer infusion rate as micromoles per kilogram per hour, E_i is the enrichment of the [1-^{13}C]leucine tracer, and E_p is the [1-^{13}C]KIC enrichment in plasma. The rate of excretion of the ^{13}C-leucine into exhaled $^{13}CO_2$ was calculated as follows:

$$F_{13C} = E_{13CO2} \cdot vCO_2 \quad (4)$$

where F_{13C} is the rate of oxidation of the ^{13}C-leucine to $^{13}CO_2$, E_{13CO2} is the CO_2 enrichment, and vCO_2 is the rate of CO_2 production. The oxidation of the dex-

trose infused with insulin in study 1 increased the breath $^{13}CO_2$ enrichment, because this sugar has a higher ^{13}C enrichment than the endogenous glucose (5). The contribution of the exogenous dextrose oxidation to the exhaled ^{13}C enrichment was determined in pilot experiments and was subtracted to the breath $^{13}CO_2$ enrichment in the present studies. The rate of leucine oxidation (Ox) is given by:

$$Ox = F_{13C}/E_{KIC}/0.81 \quad (5)$$

where 0.81 is the recovery factor of the label in exhaled CO_2 (36,37). Finally, the rate of leucine flux in protein synthesis was calculated as the difference between the leucine R_a and the rate of leucine oxidation (38).

The glutamine appearance rate into the systemic circulation has 2 components: *1*) glutamine from proteolysis and *2*) glutamine from de novo synthesis. The glutamine release from proteolysis was estimated using the 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 (16)

Statistical analysis. Comparisons between equilibration period and study period within each study and comparisons among studies were performed by means of 2-tailed paired Student's *t* test.

RESULTS

Hormone concentrations. The time courses of insulin and C-peptide are reported in Fig. 1. The insulin concentrations (basal and last 90min: 42 ± 9 and 298 ± 42 pmol/l in the euglycemic study, 33 ± 10 and 263 ± 21 pmol/l in the hypoglycemic study; NS) were similar among studies even though the insulin infusion rate was 30% greater during the hypoglycemic study. Insulin was infused at a higher rate because a greater suppression of endogenous insulin secretion was expected during hypoglycemia. In fact, in the last hour of the hypoglycemic study, the C-peptide concentration (an index of insulin secretion) was reduced more than that in the euglycemic study (–90 ± 1% vs. –28 ± 12% of basal, $P < 0.001$). The time-course of the counterregulatory hormones is reported in Fig. 2. Glucagon concentrations were reduced by –16 ± 5% in the last hour of euglycemia ($P < 0.05$ vs. basal) and were increased up to 52 ± 13% in the last hour of hypoglycemia ($P < 0.01$ vs. basal, $P < 0.01$ vs. euglycemia). Epinephrine concentrations did not change at euglycemia and increased 10-fold in the last hour of hypoglycemia ($P < 0.01$ vs. basal, $P < 0.01$ vs. euglycemia). Growth hormone concentrations did not change at euglycemia, and they peaked at 90 min of hypoglycemia ($P < 0.05$ vs. basal, $P < 0.05$ vs. euglycemia). Cortisol concentrations did not change at euglycemia and increased up to 179 ± 63% in the last hour of hypoglycemia ($P < 0.05$ vs. basal, $P < 0.01$ vs. euglycemia).

Glucose concentrations and kinetics. During the euglycemic study, the plasma glucose concentration was kept similar to the basal value (from 5.0 ± 0.3 to 4.9 ± 0.1 mmol/l) infusing 30.6 ± 3.2 μmol/kg/min of glucose (Fig. 3). During the hypoglycemic study, the plasma glucose concentration fell from 4.8 ± 0.3 to 2.9 ± 0.2 mmol/l within 30 min and remained constant thereafter. Of the 7 subjects, 2 (1 male and 1 female) required a small amount of exogenous glucose (0.6 and 3.8 μmol · kg^{-1} · min^{-1} in the last hour) to prevent plasma glucose from falling below 2.4 mmol/l, whereas the other 5 subjects could spontaneously prevent any decrement in plasma glucose concentration below this threshold. The rate of endogenous glucose production was almost completely suppressed in the last hour of euglycemia (from 11.2 ± 1.4 to 2.3 ± 2.9 μmol · kg^{-1} · min^{-1}, $P < 0.001$). In contrast, during the hypoglycemic study, the rate of endogenous glucose production reached a nadir within the first 30 min (from 11.4 ± 0.7 to 6.4 ± 1.5 μmol · kg^{-1} · min^{-1}, $P < 0.001$) and then it rebounded to values similar to the basal ones (13.4 ± 2.1 μmol · kg^{-1} · min^{-1} in the last hour, $P < 0.01$ vs. euglycemia). The rate of glucose uptake was stimulated almost 3-fold in the last hour of euglycemia ($P < 0.05$ vs. basal), whereas it remained similar to the basal rate in the last hour of hypoglycemia ($P < 0.05$ vs. euglycemia).

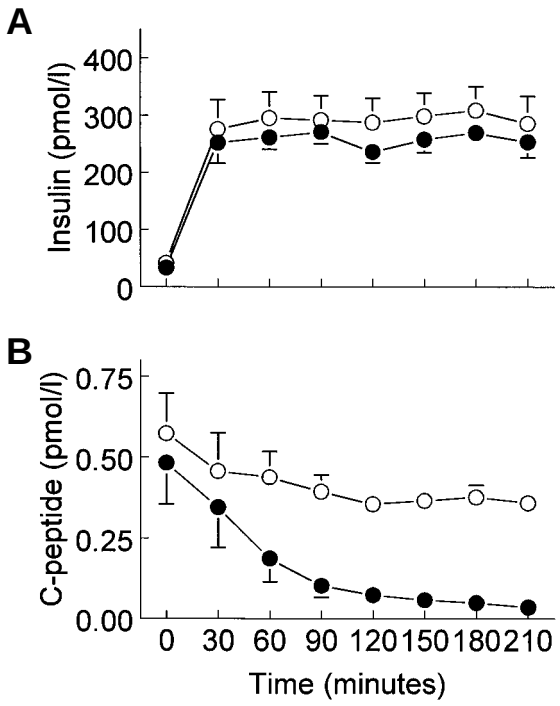

FIG. 1. The euglycemic (○) and the hypoglycemic (●) studies were conducted at similar insulin concentrations (*A*), despite a larger suppression of insulin secretion in hypoglycemia, which was indicated by an almost complete reduction in circulating C-peptide concentrations (*B*).

Amino acid concentrations. Figure 4 shows the time-course of leucine, α-KIC, and phenylalanine concentrations. The leucine concentration fell from 109 ± 10 to 69 ± 6 μmol/l in the last hour of euglycemia and from 133 ± 3 to 61 ± 2 μmol/l in the last hour of hypoglycemia ($P < 0.001$ vs. basal in both studies). Even though the absolute leucine concentrations were not different among studies, the percent suppression from basal was greater during hypoglycemia (–37 ± 1% during euglycemia and –54 ± 2% during hypoglycemia, $P < 0.05$). The KIC concentration was similarly suppressed among studies (from 45 ± 4 to 22 ± 2 μmol/l in the euglycemic study and from 43 ± 4 to 26 ± 4 μmol/l in the hypoglycemic study, $P > 0.001$ vs. basal in both studies). The phenylalanine concentration fell from 51 ± 2 to 41 ± 3 μmol/l during euglycemia and from 58 ± 3 to 37 ± 2 μmol/l during hypoglycemia ($P < 0.001$ vs. basal in both studies). Even though the absolute phenylalanine concentrations were not different among studies, the percent suppression from basal was greater during hypoglycemia (–21 ± 3% during euglycemia and –36 ± 3% during hypoglycemia, $P < 0.05$).

Figure 5 shows the time-course of the glutamine and the alanine concentrations. The glutamine concentration fell

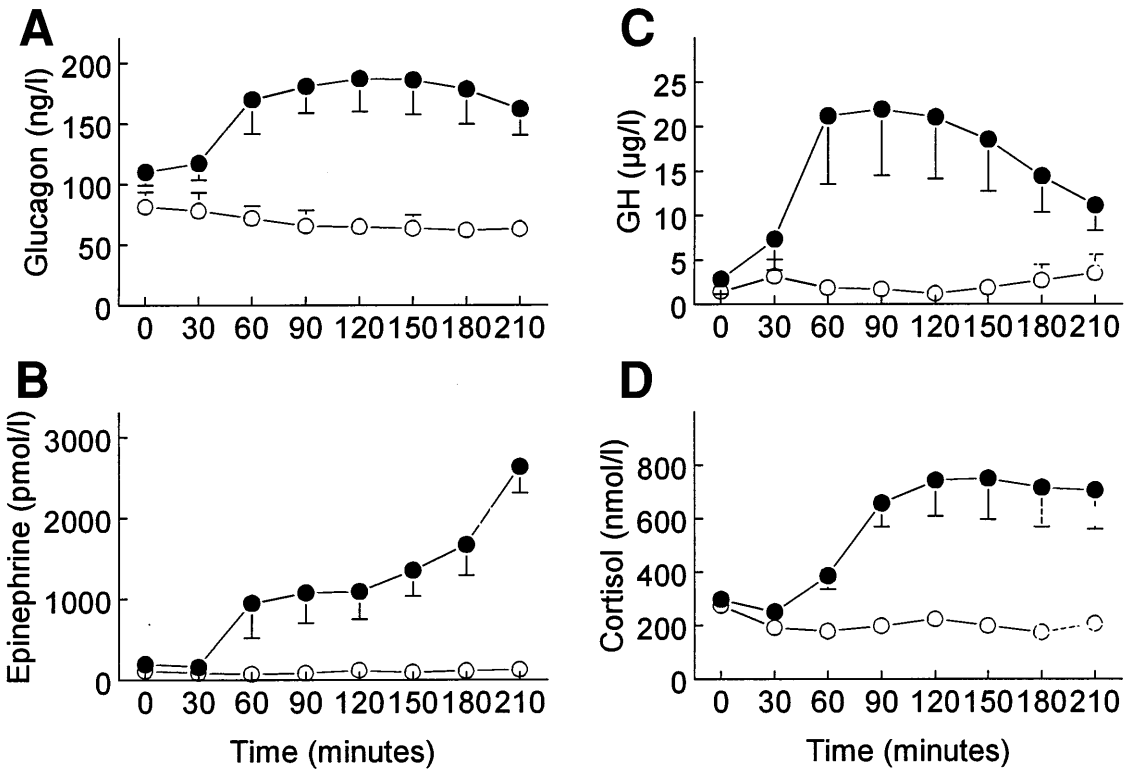

FIG. 2. Hypoglycemia (●) compared with euglycemia (○) stimulated marked increments in the concentrations of glucagon (*A*), epinephrine (*B*), growth hormones (*C*), and cortisol (*D*).

from 397 ± 49 to 366 ± 47 µmol/l ($P < 0.05$ vs. basal) during euglycemia and fell much more during hypoglycemia (from 376 ± 43 to 232 ± 19 µmol/l, $P < 0.01$ vs. basal, $P < 0.05$ vs. euglycemia). The percent suppression from basal was also greater during hypoglycemia (–37 ± 3%) than during euglycemia (–16 ± 3%, $P < 0.01$). In contrast, the alanine concentration was similarly suppressed among studies (from 315 ± 36 to 261 ± 25 µmol/l [–13 ± 10%], NS, in the euglycemic study and from 293 ± 28 to 228 ± 23 µmol/l in the hypoglycemic study [–22 ± 3%], $P > 0.01$ vs. basal).

Amino acid kinetics. Table 1 shows the leucine and the glutamine kinetic values at baseline and in the last hour of the studies. The endogenous leucine flux, an index of proteolysis, was equally suppressed during both euglycemia and hypoglycemia. We could not define significant changes from basal leucine oxidation in either studies. The nonoxidative leucine disposal, an index of protein synthesis, was similarly suppressed during both euglycemia and hypoglycemia. The glutamine R_a and R_d decreased significantly only during hypoglycemia, even though there was no statistical difference with the euglycemic study in the last hour of the experiment. Figure 6 shows that the cumulative balance between glutamine R_a and R_d was not significantly different from 0 (–17.2 ± 9.7 µmol/kg) during euglycemia, but it changed to become significantly negative ($P < 0.0$ 1) during hypoglycemia (–64.7 ± 9.7 µmol/kg, $P < 0.05$ vs. euglycemia). The de novo glutamine flux did not change significantly from basal and it was not different among studies.

Plasma free fatty acid concentrations. In the last hour of euglycemia, the free fatty acid (FFA) concentration was suppressed to –70 ± 5% of the basal value ($P < 0.05$ vs. basal) (Fig. 7). In contrast, during hypoglycemia, the FFA concentration rebounded towards the basal value ($P < 0.05$ vs. euglycemia).

DISCUSSION

This study was designed to evaluate whether acute hypoglycemia affects protein and amino acid metabolism and increases the availability of amino acids. In this study, hypoglycemia was induced by insulin, and insulin per se has an effect on protein and amino acid metabolism. Thus, we dissected the effects of insulin and hypoglycemia by means of 2 experiments with similar insulin but different plasma glucose concentrations. As expected, we found that the hypoglycemic state was associated with the reversal of the effects of hyperinsulinemia on glucose kinetics and FFA concentrations. In contrast, we found that hypoglycemia has no effect on the insulin suppression of protein and leucine kinetics. In addition, hypoglycemia potentiates the insulin suppression of the glutamine concentration in the absence of changes in the glutamine flux, indicating that hypoglycemia accelerates the glutamine uptake without increasing its release from proteolysis or de novo synthesis.

We did not reproduce the results of a similar study in the dog, in which hypoglycemia markedly and rapidly increased whole-body protein breakdown, leucine concentration, and leucine oxidation (26). We calculated that we had the ability to detect a 6% difference in the insulin effect on proteolysis between euglycemia and hypogly-

TABLE 1
Leucine and glutamine kinetics

	Euglycemic study	Hypoglycemic study*
Endogenous leucine flux		
Basal	109 ± 3	110 ± 9
Study	86 ± 3	90 ± 10
% Change	–21 ± 2	–18 ± 4
P vs. basal	<0.001	<0.01
Leucine oxidation		
Basal	21 ± 1	19 ± 4
Study	22 ± 2	17 ± 2
% Change	7 ± 13	–6 ± 6
P vs. basal	NS	NS
Nonoxidative leucine flux		
Basal	89 ± 4	91 ± 9
Study	64 ± 3	73 ± 10
% Change	–28 ± 2	–20 ± 4
P vs. basal	<0.001	<0.01
Glutamine R_a		
Basal	326 ± 51	355 ± 58
Study	266 ± 24	287 ± 60
% Change	–15 ± 5	–21 ± 7
P vs. basal	NS	0.05
Glutamine R_d		
Basal	326 ± 51	355 ± 58
Study	267 ± 24	293 ± 58
% Change	–14 ± 6	–19 ± 7
P vs. basal	NS	<0.05
De novo glutamine flux		
Basal	238 ± 41	274 ± 57
Study	199 ± 24	240 ± 72
% Change	–11 ± 10	–18 ± 8
P vs. basal	NS	NS

Data are means ± SD and are expressed as μmol · kg^{-1} · h^{-1}. Hypoglycemia per se had no effect on the leucine and the glutamine fluxes in the last hour of the experiments. *The *P* values versus the euglycemic study were not significant.

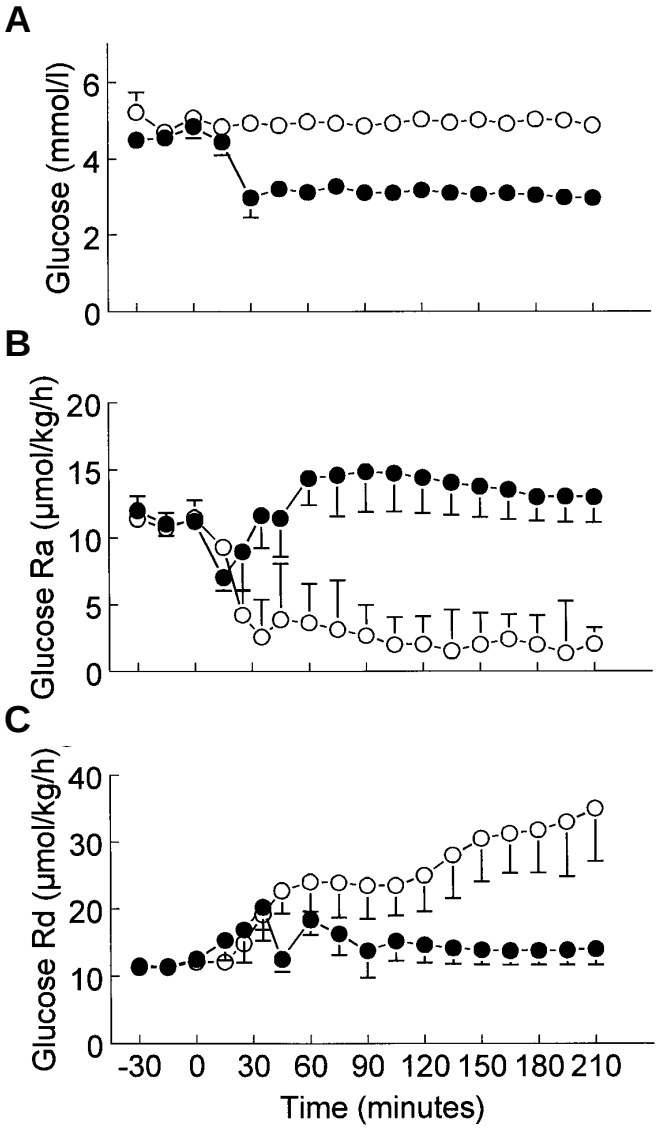

FIG. 3. ***A*: All subjects reached the hypoglycemic plateau in 30 min and maintained it for 3 h. The endogenous glucose R_a (*B*) was almost completely suppressed at euglycemia (○), but it rapidly rebounded to postabsorptive values within the first hour of hypoglycemia (●). The glucose R_d (*C*) was stimulated at euglycemia, but it returned to postabsorptive values within the first hour of hypoglycemia.**

cemia and a 30% difference in leucine oxidation. Thus, we could exclude the possibility that hypoglycemia has physiologically relevant effects on proteolysis, whereas we could not exclude small effects on leucine oxidation. However, such effects on leucine oxidation are not of the same magnitude as those observed in the dog. Possibly, the species difference is important in determining the response to hypoglycemia. However, we confirmed a report that, in humans, the concentration of the branched-chain amino acids progressively decline over several hours of hypoglycemia, but at a lesser degree than the gluconeogenic ones (27). In that study, paired control experiments with euglycemic hyperinsulinemia were not performed. Thus, it was impossible to ascertain whether the net effect of hypoglycemia is to increase the gluconeogenic amino acid consumption or, alternatively, to increase the branched-chain release with proteolysis. The present experiment demonstrates that hypoglycemia increases the uptake of the gluconeogenic amino acids and leaves unchanged the release of the branched chain. Our results are also in agreement with studies in humans that achieved hypoglycemia with a bolus of insulin (39) or with the discontinuation of the glucose infusion after a euglycemic clamp (40): in both of them, the leucine concentrations remained below the postabsorptive concentration in the first 2–3 h of hypoglycemia, despite a rapid discontinuation of hyperinsulinemia. In regard to the effect of hypoglycemia on the gluconeogenic amino acids, our results agree with a recent study from Meyer et al. (41) that showed that hypoglycemia potentiates the insulin suppression of the glutamine and the alanine concentrations. We did not find differences in the insulin suppression of glutamine fluxes among studies. We calculated that we had the power to detect 20% changes in the insulin effect on glutamine fluxes. Thus, we cannot exclude small effects of hypoglycemia on glutamine fluxes, but we can exclude changes in the order of magnitude of these induced by cortisol in the long term (42). We found, however, that the hypoglycemic

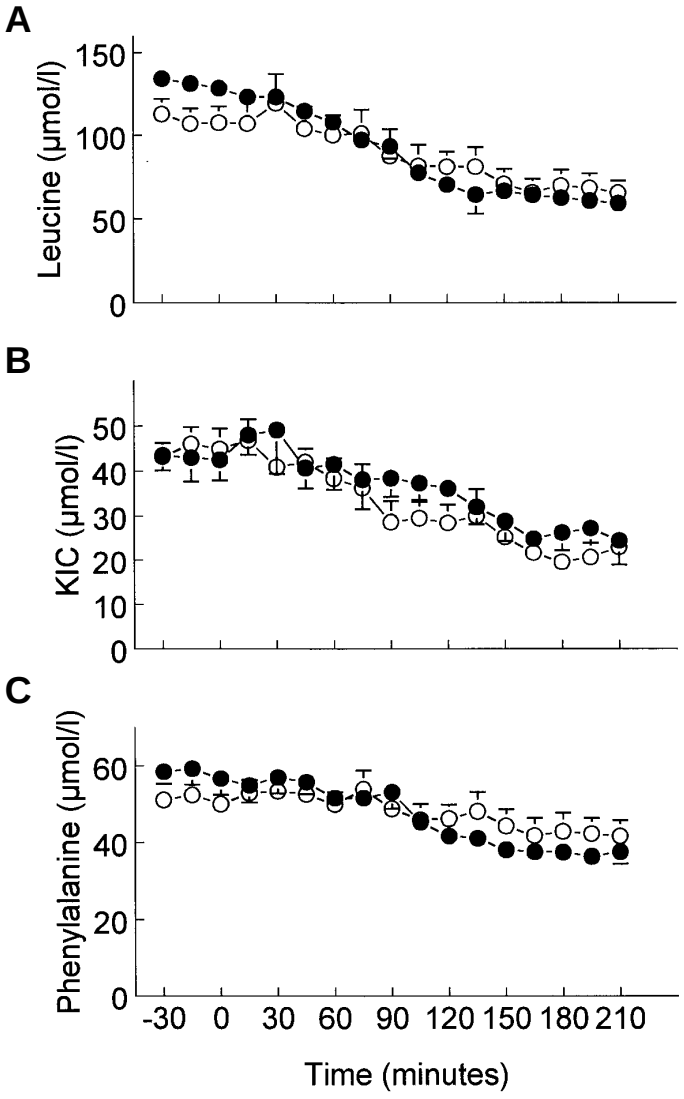

FIG. 4. The concentrations of leucine (*A*), KIC (*B*), and phenylalanine (*C*) were always similar in the 2 studies, even though the percent suppression from basal of leucine and phenylalanine was slightly but significantly increased during hypoglycemia (●) compared with euglycemia (○).

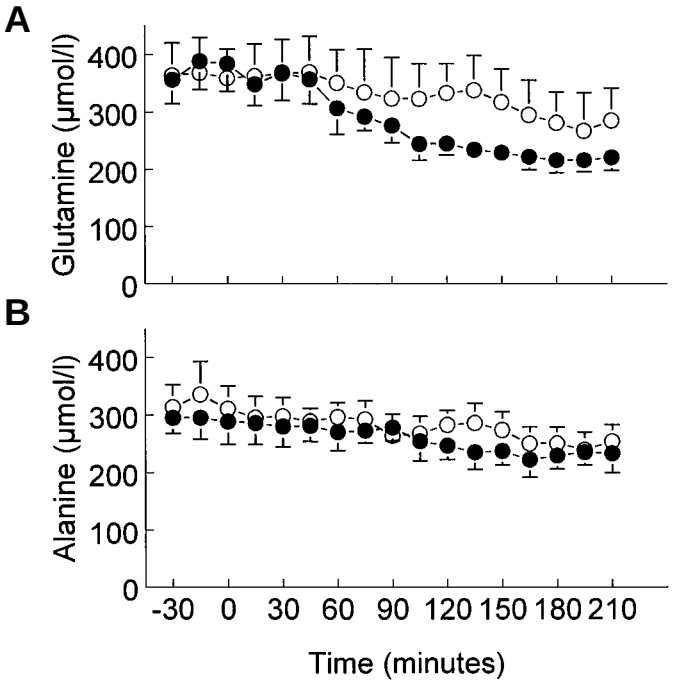

FIG. 5. The glutamine (*A*) versus the alanine (*B*) concentration was reduced during euglycemia (○). The suppression of the glutamine concentration was doubled during hypoglycemia (●).

state induced a significant imbalance between glutamine uptake and production, which was coupled to a decrement in glutamine concentrations. Even though Meyer et al. (41) did not present whole-body glutamine flux calculations, they reported an increased renal fractional extraction despite a reduction in arterial glutamine. This is consistent with our findings. Thus, both studies point to an increased glutamine uptake in the absence of an accelerated glutamine release during hypoglycemia.

Our findings may lead to the apparent paradox of hypoglycemia simultaneously antagonizing the insulin effects on glucose and FFA, leaving unchanged the effects on protein and leucine kinetics, and potentiating the insulin effects on glutamine metabolism. Nonetheless, the specific effects and the mode of action of the hormones involved in the response to hypoglycemia fit well with this view. We recently found that glucagon, the major counterregulatory hormone in healthy individuals, acutely increases the uptake of glutamine, but it does not increase its release with proteolysis or de novo synthesis, and it produces decrements in circulating glutamine and leucine (16). This finding is also supported by animal studies that found that both insulin and glucagon stimulate hepatic glutamine transport (43–46). In contrast, glucagon increases whole-body proteolysis only with insulin deficiency (13), but it has a very small effect at postabsorptive insulinemia (9,14,16). Thus, it is reasonable that we did not find increased proteolysis after insulin-induced hypoglycemia. Finally, glucagon has been shown to increase leucine oxidation only when leucine is in excess, not when it is at postabsorptive concentrations (14). This is compatible with our finding of unchanged leucine oxidation in hypoglycemia. Epinephrine decreases leucine and phenylalanine concentrations by increasing their uptake and leaving unchanged or decreasing proteolysis (18–22). Overall, the effect of epinephrine at doses greater than those achieved in this study is very small compared with that of hyperinsulinemia (<10% reduction in leucine and phenylalanine concentrations and flux [22]). In addition, epinephrine has minimal effects on the concentrations of gluconeogenic amino acids, including glutamine. Thus, epinephrine is not likely to play a role in the changes in amino acid metabolism seen in hypoglycemia, and the entire spectrum of metabolic changes on glucose and protein metabolism is comparable with that of glucagon alone. In the short term, growth hormones do not significantly affect whole-body protein metabolism (47). Cortisol is the hormone that has the potential to determine an important catabolic response to hypoglycemia, because it increases proteolysis, leucine oxidation, and glutamine flux from proteolysis and de novo synthesis (23–25). Cortisol also increases glucose production and substrate availability, lim-

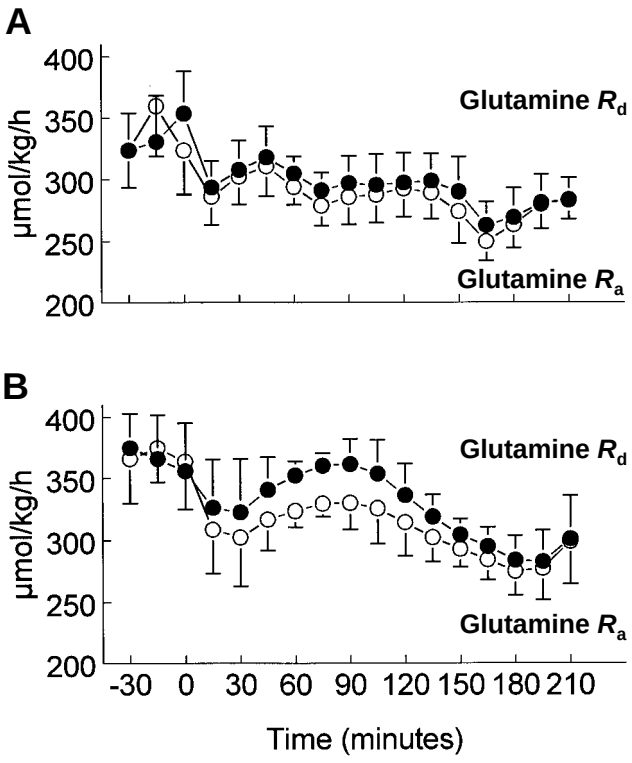

FIG. 6. The cumulative balance between the glutamine R_a (○) and R_d (●) changed from not different from 0 during euglycemia (*A*) to significantly negative during hypoglycemia (*B*).

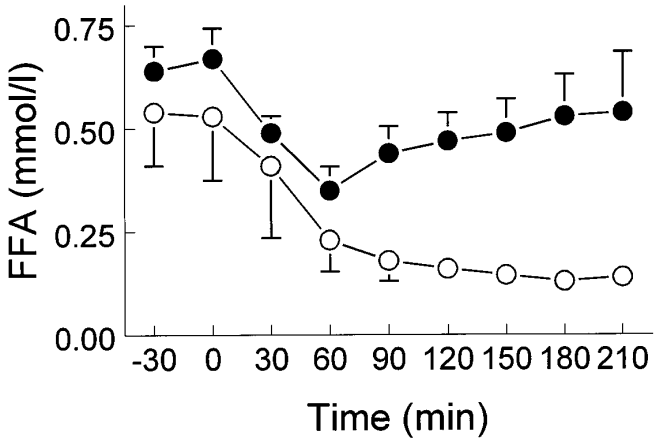

FIG. 7. Euglycemic hyperinsulinemia suppressed the concentration of circulating FFAs. During hypoglycemia (●) compared with euglycemia (○), the concentration of FFAs returned to postabsorptive values.

its peripheral glucose utilization and, thanks to these characteristics, plays a role in glucose counterregulation (48). Two factors, however, stand against an important contribution of cortisol to changes in amino acid and protein metabolism during acute hypoglycemia. First, the time course of cortisol action on glucose and protein metabolism is slow and requires hours to become evident (24). Accordingly, an effect of cortisol on glucose metabolism arises within a few hours in a model of slow developing and persistent hypoglycemia (48). Second, dose-response studies have shown that an increment in cortisol concentration similar to that in hypoglycemia should not produce important changes in protein and amino acid metabolism in 12 h (42). Thus, it is unlikely that if hypoglycemia had been expanded beyond 3.5 h we would have observed an important increment in amino acid concentrations, proteolysis, and glutamine flux because of cortisol action.

We found that amino acids are not mobilized to counteract acute hypoglycemia. This situation can be rationalized as a mechanism to survive starvation while sparing the protein mass. This finding also suggests that restoring amino acid availability is not advantageous to counteract hypoglycemia. Two considerations stand in favor of this conclusion. First, 2 different studies showed that the infusion of amino acids potentiates the response of glucagon, but it does not independently ameliorate the glucose response to hypoglycemia (40,49). Second, recent studies demonstrated that an elevation of cortisol (the only counterregulatory hormone shown to increase the availability of amino acids) has deleterious effects on subsequent glucose counterregulation (50,51).

The possibility that the depletion of circulating amino acids may act as a signal for successful glucose counterregulation should be evaluated. The common wisdom contrasting with this view is that amino acids reduce the insulin-stimulated glucose uptake by substrate competition (52–55) and by inhibiting the insulin stimulation of insulin receptor substrate 1 and phosphatidylinositol 3-kinase (56). Therefore, a large increment in amino acid availability could benefit the response to hypoglycemia by limiting the consumption of glucose in insulin-sensitive tissues. Nevertheless, it is also true that a simultaneous increment in leucine and glutamine availability could increase insulin secretion and could be detrimental to hypoglycemia. Recent studies found that, in the β-cell, leucine stimulates the rate of glutaminolysis, which in turn increases insulin secretion. The rate of β-cell glutaminolysis is inversely related to the ambient glucose concentration. Thus, this pathway is extremely active during hypoglycemia (57,58). This pathway may explain the leucine hypersensitivity of β-cells during hypoglycemia, which has a clinical relevance in subjects with insulinoma, subjects on sulfonylureas, or subjects with regulatory mutations of glutamate dehydrogenase (59,60). Leucine is not concentrated across the cell membrane. Thus, a depletion in circulating leucine may act as an intracellular signal to shut down the glutamate dehydrogenase pathway that, in the β-cell, leads to insulin secretion during hypoglycemia. As far as glutamine is concerned, the fact that we found a decrement in glutamine concentrations without changes in glutamine flux (neither in its release from proteolysis nor in its de novo synthesis compared with euglycemia) indicates that hyperinsulinemia plus hypoglycemia had the net effect to shift glutamine from plasma in the cells. Glutamine is antiproteolytic in the muscle and in the liver, and it is antiglycogenolytic in the liver. In the liver, an increased uptake of glutamine during hyperinsulinemia increases the proportion of glucose released from glutamine gluconeogenesis, during both euglycemia and hypoglycemia (41). Thus, an increased uptake of glutamine in hepatocytes could explain why the initial increment in glycogenolysis is rapidly reverted and followed by an increment in gluconeogenesis during hypoglycemia. Insulin does not stimulate glutamine uptake in the kidney (41), but

the kidney increases glutamine uptake and glutamine gluconeogenesis during hypoglycemia. This effect is not expected to pertain to glucagon (which does not stimulate glutamine uptake and gluconeogenesis in the kidney [61]), but it could be related to epinephrine (62).

Overall, our findings suggest that during insulin-induced hypoglycemia, an intracellular shift in circulating glutamine promotes gluconeogenesis and limits proteolysis, whereas leucine depletion blocks the effects of glutamine on insulin secretion. Because glucagon is the counterregulatory hormone responsible for most of this response, we expect that, in the absence of a glucagon response, hypoglycemia fails to increase glutamine uptake and gluconeogenesis and to suppress insulin secretion. In this case, an increment in proteolysis could represent a second-line defense triggered by cortisol, which is capable of limiting the effects of hyperinsulinemia on glucose uptake. We recently performed hypoglycemic clamp studies in islet recipients for type 1 diabetes (who completely lacked the response of glucagon secretion to hypoglycemia), confirming the existence of defective glucose production, defective glutamine uptake, increased proteolysis, and defective suppression of circulating C-peptide (63).

In conclusion, the present study demonstrates that during hyperinsulinemia and hypoglycemia the counterregulatory response rapidly reverts the suppressive effects of insulin on glucose production and lipolysis, but it leaves unchanged the antiproteolytic effects of insulin. Even though hyperinsulinemia limits the release of gluconeogenic amino acids as glutamine, hypoglycemia causes a rapid shift of glutamine from plasma into the cells, presumably into the liver, via glucagon action, and into the kidney via epinephrine action.

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