Hyperglucagonemia and the Immediate Fate of Dietary Leucine: A Kinetic Study in Humans

J. Sergio Marchini, Lisa M. Marks, Dominique Darmaun, Vernon R. Young, and Michel Krempf

The possible role of glucagon in determining the fate of dietary absorbed amino acids within the splanchnic bed was investigated in five healthy male volunteers. A kinetic study was performed involving a continuous 240-minute infusion of L-[5,5,5-$^2H_3$]leucine and D-[6,6-$^2H_2$]glucose by vein, while L-[1-^{13}C]leucine was infused by a feeding tube into the duodenum (intragut [IG]) along with a constant intravenous (IV) infusion of somatotropin release-inhibitory factor (SRIF) combined with insulin, growth hormone, and glucagon. In random order, glucagon was infused at a rate of 0.4 ng · kg^{-1} · min^{-1} in one experiment and 1.2 ng · kg^{-1} · min^{-1} in the other experiment, while insulin and growth hormone were kept at constant serum levels, respectively, 37 ± 13 pmol · L^{-1} and 5 ± 0.2 μg · L^{-1}. The diet was provided as an L-amino acid solution including 60 μmol · kg^{-1} · h^{-1} leucine without fat and carbohydrate. During the higher rate of glucagon infusion, there was an increase in plasma glucagon and glucose concentrations, glucose flux, and net dietary leucine release into the periphery from the splanchnic bed. Splanchnic removal and uptake of leucine were decreased with increased glucagon infusion. There were no statistical differences in the plasma leucine level and IV and IG leucine fluxes at the two glucagon levels, although leucine metabolic clearance increased (0.74 *v* 0.85 L · kg^{-1} · h^{-1}, $P = .08$) in the case of glucagon excess. Plasma glucose increased with glucagon excess and was negatively correlated ($P < .05$) with the plasma leucine level ($r = -.348$) and IV ($r = -.459$) or I.G. ($r = -.359$) leucine fluxes. The negative correlation between plasma glucagon and leucine levels was also significant ($r = -.684$). No significant correlation was found between dietary leucine splanchnic removal and glucose, glucagon, or leucine plasma concentrations. We conclude that glucagon in excess has only a small quantitative effect on the overall handling of dietary leucine, and hypothesize that more leucine is exported to the peripheral tissues under these hormonal conditions.

THE SPECIFIC ACTION OF GLUCAGON in determining the fate of dietary absorbed amino acids through anabolic and catabolic pathways is still unclear. Glucagon is a catabolic hormone under critical stress conditions.[1] The glucagonoma syndrome is characterized by a high level of glucagon and a low level of plasma leucine,[1-2] due to a high metabolic clearance of leucine, and an increase in urea synthesis.[2] However, these effects still appear to be generally within a normal range for healthy volunteers.[3]

The metabolic response to a glucagon infusion in healthy fasting subjects is characterized by an increase in plasma glucose, related to an increase in gluconeogenesis from amino acids,[4-6] and a decrease in plasma amino acid, including branched-chain amino acids.[5,7] This glucagon effect might be related to an increase in net amino acid transport from the extracellular to the intracellular space and an increased conversion of amino acids to glucose.[4] On the other hand, in vitro experiments have shown that high-dose glucagon increased the output of branched-chain amino acids from isolated liver,[8] presumably due to accelerated proteolysis.[6] Quantitative kinetic data in initially fasting humans are conflicting. Glucagon has been reported to diminish the amino acid–induced stimulation of protein synthesis[9] and to increase leucine flux and oxidation in insulin deficiency.[6] However, if insulin is kept constant, hyperglucagonemia has been reported to decrease leucine flux and increase its oxidation,[10-11] while other studies, apparently involving the same hormonal status, did not find any effect on the plasma concentration and turnover of branched-chain amino acids.[10-12]

For the fed state, the effects of glucagon on protein and amino acid metabolism are also contactory. In vitro data in fed rats showed that there was no increase in amino acid oxidation and no decrease in protein synthesis after glucagon in the heart or diaphragm.[13] In another fed rat study, the degree of inhibition of protein synthesis by glucagon infusion was not the same for different muscles.[14] In pigs, glucagon infusion stimulated whole-body proteolysis in both the fasting and fed state.[15]

These conflicting data for the fasted and fed states might be related to the fact that the effect of glucagon on protein metabolism depends, in part, on a specific response of amino acid metabolism within the splanchnic bed. Thus, a catabolic effect of glucagon may be due to an enhanced hepatic utilization of amino acids, leading to a decreasing plasma level of the amino acids and their mobilization from peripheral tissues.[7] However, the removal and metabolic uptake of dietary leucine within the splanchnic region decreases with added dietary carbohydrate, a condition associated with a decrease in plasma glucagon concentrations.[16] Hence, the discrepancies between in vivo and in vitro data and during the fasting and fed states are probably related to various confounding factors, including the status of splanchnic amino acid metabolism and prevailing insulin concentrations at the time of study.

Because there is not a clear understanding of glucagon action on amino acid metabolism in the fed state, particularly branched-chain amino acids, this study was designed to help clarify the effect of glucagon on the fate of dietary amino acids within the

From the Centre de Recherche en Nutrition Humaine, Groupe Métabolisme, Université de Nantes, Nantes, France; Division of Clinical Nutrition, School of Medicine of Ribeirao Preto, Ribeirao Preto, Brazil; and Laboratory of Human Nutrition and Clinical Research Center, Massachusetts Institute of Technology, Cambridge, MA.

Submitted January 23, 1997; accepted August 8, 1997.

Supported by Nantes University, National Institutes of Health Grant No. DK15856 (V.R.Y.), and FAPESP-BR 95/9776-1 (J.S.M.).

Address reprint requests to Michel Krempf, MD, Clinique d'Endocrinologie, Maladies Métaboliques et Nutrition, Hôtel Dieu, 44093 Nantes cedex 1, France.

0026-0495/98/4705-0002$03.00/0

splanchnic bed. We performed a kinetic study involving continuous, simultaneous infusions of stable-isotope leucine tracers by vein and into the duodenum. This approach was used under precisely controlled hormonal conditions obtained with constant infusions of somatotropin release-inhibitory factor (SRIF), insulin, and growth hormone, while glucagon was given to achieve either basal or excess plasma glucagon concentrations.

SUBJECTS AND METHODS

Subjects

Five healthy male volunteers aged 21 to 26 years (mean body mass index, 20 ± 2 kg · m^{-2}) participated in the study. They were in good health as determined from physical examination, medical history, and routine biochemical screening of blood and urine samples. All had a fasting blood glucose less than 5.2 mmol · L^{-1}, and none had a family history of diabetes. For 3 days before entry into the study, subjects consumed an adequate, weight-maintenance diet supplying about 188 kJ · kg^{-1} · d^{-1} and 1.2 g mixed dietary protein · kg^{-1} · d^{-1}. The experimental protocol and potential risks involved were fully explained to the subjects before beginning the study. Written consent was obtained from each participant according to protocols approved by the Nantes University Hospital Ethics Committee.

Materials

L-[1-^{13}C]leucine (99 atom% ^{13}C), L-[5,5,5-$^{2}H_3$]leucine (99 atom% ^{2}H), and [6,6-$^{2}H_2$]glucose (99 atom% ^{2}H) were purchased from MassTrace (Woburn, MA). Isotopic and optical purity of amino acid tracers was verified by chemical ionization gas chromatography/mass spectrometry and nuclear magnetic resonance. Sterile solutions of the isotopes, tested for sterility and pyrogenicity before use, were prepared in normal saline and passed through a 0.22-μm Millipore filter (Millipore Products Division, Bedford, MA) into a sterile syringe. For each infusion, one sample of the infusate was analyzed to determine the exact isotope concentration. From previous study, the two leucine tracers used have been shown to yield equivalent estimates of amino acid flux in plasma when infused intravenously.[16]

SRIF was purchased from Duphar (Lyon, France). Insulin and glucagon were from Novo (Copenhagen, Denmark), and growth hormone was from Kabi Laboratories (Helsinki, Finland).

Experimental Protocol

Subjects were studied after a 12-hour overnight fast. At 7 AM on the study day, a feeding tube (Dobbhoff; Biosearch Medical, Somerset, NJ) was inserted into the duodenum. Its position was controlled by x-ray. The intravenous (IV) catheter for infusion of hormones was placed in an antecubital vein in one arm. A second catheter for blood sampling was inserted into a contralateral hand vein and the hand was warmed at 60°C. The procedure details, including the catheters, feeding tube, and infusions and priming doses of the tracers (L-[5,5,5-$^{2}H_3$]leucine, L-[1-^{13}C]leucine, and D-[6,6-$^{2}H_2$]glucose), have been described elsewhere.[16] The design of the tracer protocol is shown in Fig 1. In brief, for 240 minutes, L-[5,5,5-$^{2}H_3$]leucine and D-[6,6-$^{2}H_2$]glucose were infused by vein (IV) while L-[1-^{13}C]leucine was infused by feeding tube (intragut [IG]) after administration of priming doses of L-[5,5,5-$^{2}H_3$]leucine (4.7 μmol · kg^{-1}), L-[1-^{13}C]leucine (4.7 μmol · kg^{-1}), and D-[6,6-$^{2}H_2$]glucose (22 μmol · kg^{-1}) by their respective routes. The priming doses were administered alone and over a period of 1 to 2 minutes for both IV and IG tracers. The L-[5,5,5-$^{2}H_3$]leucine, L-[1-^{13}C]leucine, and D-[6,6-$^{2}H_2$]glucose tracers were infused by the venous and intraduodenal routes at a predetermined rate of 4.47 ± 0.08, 4.77 ± 0.08, and 100.2 ± 1.2 μmol · kg^{-1} · h^{-1}, respectively. From the start of the experiment, SRIF was infused IV at a rate of 250 μg · h^{-1}, growth hormone at 10 ng · kg^{-1} · min^{-1}, and insulin at 0.42 pmol · kg^{-1} · min^{-1}.

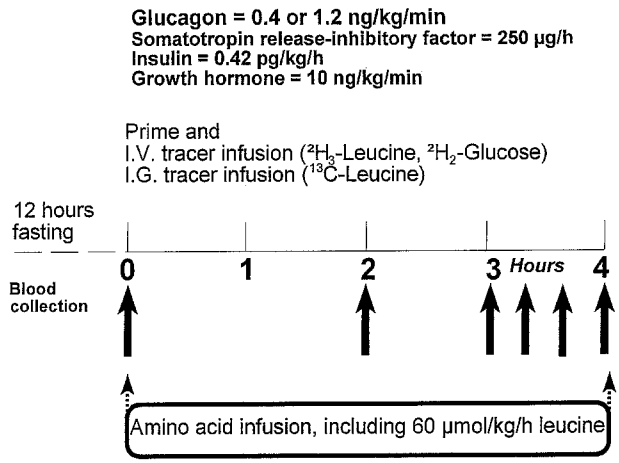

Fig 1. Experimental design.

In random order, glucagon was infused at a rate of 0.4 ng · kg^{-1} in one experiment and 1.2 ng · kg^{-1} · min^{-1} in the other experiment. All hormones were mixed in 20% albumin solution (Institut Pasteur, Lille, France). The diet on both days was delivered by feeding tube for 240 minutes and consisted of an amino acid solution (Vamine; Vitrum, Vitry sur Seine, France) supplying 60 μmol · kg^{-1} · h^{-1} leucine.

Blood samples were drawn for determination of isotopic enrichment in plasma leucine and glucose, substrate, and hormone concentrations before the start of each experiment and then at 120, 180, 210, 225, and 240 minutes.

Analytical Methods

Blood samples were collected in ice-cold heparinized tubes for subsequent determination of isotopic enrichment and substrate levels. Tubes containing EDTA and Trasylol (Sigma, St Louis, MO) were used for the collection of samples required for hormone assays. After centrifugation, the plasma was stored at −80°C until used for analysis. For measurements of isotopic enrichment, the heptafluorobutyryl-*n*-propyl ester and pentacetate derivatives were prepared for leucine and glucose, respectively. Isotopic enrichment was determined by selected ion–monitoring gas chromatography–mass spectrometry (model 5971A; Hewlett-Packard, Cupertino, CA). Electron ion impact ionization was used to monitor ions at *m/z* 240 and 242 for glucose (representing natural and $^{2}H_2$-labeled glucose, respectively) and 282, 283, and 285 for leucine (representing natural, 1-^{13}C-, and $^{2}H_3$-leucine, respectively).[16] Leucine, rather than α-ketoisocaproate (KIC), was measured in this study because of the focus on splanchnic uptake and disappearance of leucine.[17]

Blood glucose concentrations were measured using the glucose oxidase method (Analyzer II; Beckman Instruments, Fullerton, CA). Plasma leucine was determined by high-performance liquid chromatography using a reversed-phase column and postcolumn orthophthaldehyde derivatization (Beckman Instruments). Radioimmunoassay was used to measured plasma insulin and growth hormone (Oris, Gif/Yvette, France) and glucagon (Novo) levels.

Calculations

All calculations followed procedures previously described.[16] In brief, leucine and glucose fluxes were calculated using a steady-state isotope-dilution equation and a simplified pool model. Glucose hepatic production was calculated as previously reported.[18] Splanchnic uptake of dietary leucine was estimated from dose-corrected plasma enrichment ratios for the IG and IV tracers. The fraction, f, of a labeled tracer extracted during its first pass throught gut and liver tissue before

reaching the systemic circulation can be estimated from the flux obtained from the IV tracer (Q_{IV}) and the IG tracer (Q_{IG}) as follows: First-pass dietary amino acid uptake was calculated by multiplying the rate of leucine delivery into the duodenum by f.

Statistical Analysis

All data are presented as the mean ± SEM. Leucine and glucose fluxes and leucine splanchnic uptake were examined by ANOVA with the glucagon concentration as a fixed variable. Tukey's test was used for pair wise comparisons. Statistical calculations, including regression analysis, were performed using Instat Software (San Diego, CA).

RESULTS

Substrate Concentrations

Table 1 lists blood glucose, plasma leucine, and serum hormone concentrations. Blood glucose increased significantly with feeding, and this increase was significantly greater in the case of glucagon excess. Leucine concentrations increased significantly from basal levels in both studies, with a greater change for the basal glucagon condition. Insulin concentrations remained unchanged and C-peptide was undetectable after SRIF infusion. There were no statistical differences between baseline, study I, and study II for insulin, as planned in the protocol. Growth hormone increased significantly compared with baseline in response to the amino acid intake, but levels remained in the normal range in both studies. Glucagon concentrations were markedly higher during the plateau in study II, again as anticipated by design of the experimental protocol.

Table 1. Plasma Glucose, Insulin, Glucagon, Growth Hormone, and Leucine Concentrations for the Basal State and During Feeding With the Amino Acid Diet With Basal Hormonal Conditions (study I) or Increased Plasma Glucagon (study II)

Parameter	Basal	Plateau	Change
Study I			
Glucose (mmol · L^{-1})	5.14 ± 0.41	7.07 ± 0.34*	1.92 ± 0.23
Insulin (pmol · L^{-1})	36.48 ± 15.60	32.94 ± 6.00	−3.54 ± 1.32
Glucagon (ng · L^{-1})	60.60 ± 14.70	60.20 ± 5.71	−0.40 ± 1.80
Growth hormone (μg · L^{-1})	1.40 ± 0.47	5.02 ± 0.19*	3.61 ± 0.41
Leucine (μmol · L^{-1})	129.60 ± 13.71	225.67 ± 9.25*	96.07 ± 7.71
Study II			
Glucose (mmol · L^{-1})	4.74 ± 0.08	8.19 ± 0.12‡	3.45 ± 0.12†
Insulin (pmol · L^{-1})	37.56 ± 10.80	34.98 ± 3.12	−2.58 ± 5.10
Glucagon (ng · L^{-1})	61.80 ± 14.09	96.04 ± 5.34*‡	34.24 ± 3.47†
Growth hormone (μg · L^{-1})	1.02 ± 0.09	4.67 ± 0.21*	3.64 ± 0.22
Leucine (μmol · L^{-1})	124.80 ± 7.46	209.33 ± 6.47*	84.53 ± 4.22

NOTE. Values are the mean ± SEM.
*$P < .05$, basal *v* plateau in the same study.
†$P < .05$, change between the 2 studies.
‡$P < .05$, plateau values, study I *v* study II.

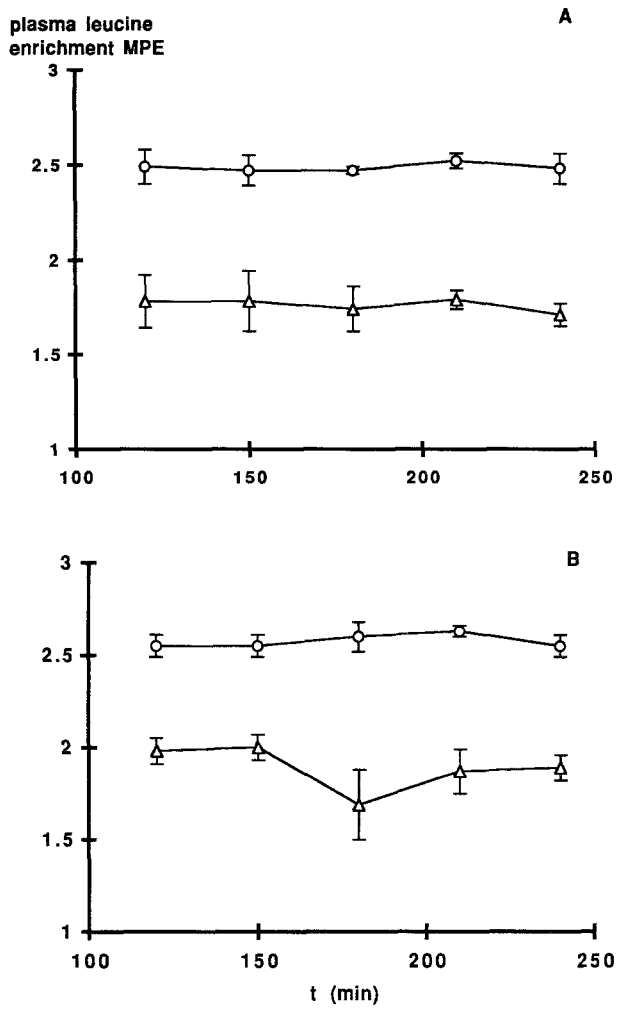

Fig 2. Plasma enrichment values measured in 5 men. L-[5,5,5-$^2H_3$]leucine was infused IV (○) and L-[1-^{13}C]leucine IG (△). (A) Low-rate glucagon infusion; (B) high-rate glucagon infusion.

Substrate Kinetics

Values for the isotopic enrichment of plasma leucine were higher following IV tracer versus IG tracer (Fig 2 and Table 2). Glucose enrichment values were slightly but not significantly lower in study II compared with study I.

The calculated substrate fluxes are listed in Table 3. Glucose flux was higher compared with the level usually reported for the fasting state,[19-20] and was significantly increased in study II versus study I. Leucine IV flux tended to be higher in study II than in study I ($P < .08$), but no difference was observed

Table 2. Plasma Leucine and Glucose Enrichment (mol% excess) at Plateau

	Study I (physiological glucagon infusion)	Study II (high-dose glucagon infusion)
L-[1-^{13}C]leucine IG	1.8 ± 0.06	1.9 ± 0.05
L-[5,5,5-$^2H_3$]leucine IV	2.5 ± 0.05*	2.6 ± 0.04
D-[6,6-$^2H_3$]glucose	1.9 ± 0.12	1.7 ± 0.05

NOTE. Values are the mean ± SEM.
*$P < .05$, IV *v* IG enrichment for every experiment.

Table 3. Substrate Kinetics in Healthy Men With Maintenance (study I) or Increased (study II) Plasma Glucagon Concentrations

Parameter	Study I	Study II
Glucose flux (μmol · kg^{-1} · min^{-1})	15.6 ± 0.5	18.5 ± 0.4†
IV flux of leucine (μmol · kg^{-1} · h^{-1})	165.7 ± 5.4	176.6 ± 3.2*
IG flux of leucine (μmol · kg^{-1} · h^{-1})	264.3 ± 9.0	251.5 ± 8.4
Release from protein breakdown (from IG flux) (μmol · kg^{-1} · h^{-1})	204.3 ± 9.0	191.5 ± 8.4
First-pass splanchnic removal of dietary leucine (%)	35.3 ± 1.9	28.4 ± 2.1†
Net uptake of dietary leucine in splanchnic region (μmol · kg^{-1} · h^{-1})	21.6 ± 1.7	17.0 ± 1.5†
Net release of dietary leucine to periphery (μmol · kg^{-1} · h^{-1})	38.4 ± 1.7	43.0 ± 1.5†
Metabolic clearance of glucose (mL · kg^{-1} · min^{-1})	2.3 ± 0.1	2.3 ± 0.04
Metabolic clearance of leucine (L · kg^{-1} · h^{-1})	0.74 ± 0.03	0.85 ± 0.03†

NOTE. Values are the mean ± SEM.
*$P < .08$, †$P < .05$: study II *v* study I.

between the two studies for IG flux data. The percentage of removal of leucine within the splanchnic bed was lower ($P < .05$) when glucagon levels were increased (study II). Therefore, net dietary leucine uptake within the splanchnic bed was higher and net release was lower in study I compared with study II. The metabolic clearance of glucose was similar in both studies, but that of leucine was significantly higher ($P < .05$) with the higher glucagon infusion.

Correlations

As expected, a significant correlation was found between blood glucagon (x) and glucose (y) concentrations ($y = 6.24 + 0.019x$, $r = .418$, $P < .01$) and between glucose flux (y) and blood glucose (x) ($y = 2.45 + 0.30x$, $r = .596$, $P < .01$). The glucose (x) concentration also was correlated with the leucine (y) plasma concentration ($y = 280.34 - 8.18x$, $r = .348$, $P < .07$) and with IV ($y = 224.70 - 6.78x$, $r = -.459$, $P < .02$) or IG ($y = 339.88 - 10.79x$, $r = -.359$, $P < .02$) leucine fluxes (Fig 3). A significant correlation between glucagon and leucine plasma concentrations was also found ($y = 274.27 - 0.71x$, $r = -.684$, $P < .01$; Fig 3).

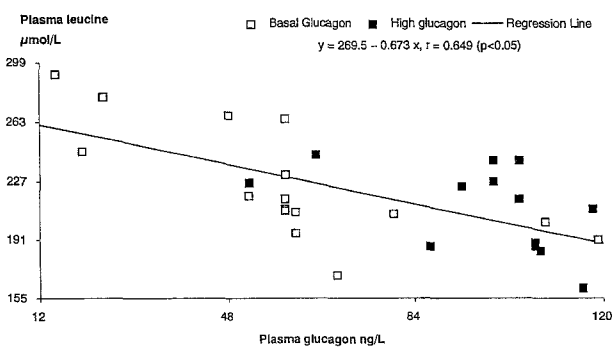

Fig 3. Regression between plasma glucagon and plasma leucine concentrations.

DISCUSSION

In this study in healthy young men, we have explored the effect of increasing plasma glucagon levels to within the high physiological range on the metabolic fate of dietary leucine, a nutritionally indispensable ketogenic amino acid, while growth hormone and insulin concentrations were maintained constant. The approach we used involved simultaneous infusion of two leucine tracers via IV and intraduodenal routes. In summary, our results suggest that during the amino acid infusion (fed state), the high-rate infusion of glucagon increased plasma glucose concentration and flux. The metabolic clearance of leucine and the net appearance of dietary leucine into the periphery from the splanchnic bed were also increased with elevated glucagon levels. Conversely, first-pass splanchnic removal of dietary leucine was decreased. Even though the plasma level of leucine during the high-rate glucagon infusion was similar to the level during physiological glucagon infusion, when we pooled all data points together, we observed a negative correlation between plasma leucine and glucagon levels (Fig 3).

The experimental approach used in this study does not reproduce the physiological fed state, because somatostatin was used and hormones other than glucagon were kept at constant levels. However, this scheme provides an approach for isolating the possible role of a hormone on dietary leucine uptake and metabolism. The rate of glucagon infusion used in this study achieved a 50% increase in plasma glucagon levels compared with baseline, which is comparable to that reported for previous studies.[5-12] It is also probably true that study I was performed during hypoglucagonemia in the liver, although physiological concentrations were obtained by IV infusion, because a substantial amount of glucagon is catabolized in the liver. However, the change in glucagon concentrations within the liver between the two experiments could explain the observed differences. At these elevated glucagon concentrations, glucose flux significantly increased, as expected.[21-22] The increased plasma glucose concentration during the basal glucagon experiment (study I) was not entirely expected. It could be explained by a lower insulin concentration in the portal blood compared with baseline, because insulin was infused in a peripheral vein. It could also be explained by the ingestion of gluconeogenic amino acids and the increment in the growth hormone level versus baseline.[23-24] This increase in growth hormone might explain the higher flux of leucine observed with IV tracer.[25-26] It must be pointed out that the level of plasma growth hormone was the same in both experiments, and the only hormonal difference observed in this protocol was the higher level of glucagon, as planned. However, although it was increased, the plasma growth hormone concentration remained within the physiological range.

As previously reported, the plasma leucine concentration was lower when the glucagon concentration was increased significantly over baseline.[12] Although plasma leucine was not significantly lower in study II, there was a significant negative correlation between plasma leucine and serum glucagon concentrations when data from both studies were pooled together (Fig 3). The present data on the uptake and release of dietary leucine within the splanchnic region may offer a clue as to the

underlying metabolic basis for the glucagon-induced reduction in plasma leucine. Indeed, we anticipated an increased leucine uptake within the splanchnic bed in the case of glucagon excess. However, we observed the opposite effect, namely a 12% increase in dietary leucine escape from the splanchnic bed during the high-rate glucagon infusion. Nevertheless, the metabolic clearance rate for leucine was calculated to be higher with increased peripheral glucagon levels. This implies a possible increase in the disposal of leucine in peripheral tissues, including muscle and perhaps adipose tissue, where leucine oxidation also occurs. An increase in the transamination of leucine could, at least in part, explain this result. A good correlation has been reported between KIC production from leucine transamination or KIC oxidation and plasma glucagon in sheep maintained on a high- or low-energy diet for 2 weeks.[27] Similar findings were reported in men fed a protein-rich meal[28] or in patients with septic disease,[29] although conflicting results were reported in perfused rat heart.[30] Glucagon has also been reported to stimulate amino acid oxidation in the muscle of rats.[13] Hence, the decreased plasma leucine concentration may be due, at least in part, to a greater rate of plasma leucine disposal via oxidation in the skeletal muscles. Charlton et al[31] have reported recently that glucagon increases leucine oxidation, but the organ site at which this occurred was not determined.

Additionally, with respect to the fate of dietary leucine, the decrease in uptake by the splanchnic bed when plasma glucagon was high occurred in association with increased blood glucose and diminished plasma leucine concentrations. This altered splanchnic uptake agrees with our previous study,[16] and may be related to the plasma leucine concentration. Although it is difficult to dissect the specific roles of plasma glucose and leucine, when we plotted the leucine plasma level against leucine splanchnic uptake (Fig 4) from data reported for the fed state in this and previous fed-state studies using the same tracer approach,[16-17,32-33] we observed a significant positive correlation ($r = .887$) between the rate of dietary leucine splanchnic uptake and the prevailing plasma leucine concentration. Thus, dietary leucine escape from the splanchnic bed appears to increase when the plasma leucine concentration is reduced. This could reflect a mechanism designed to prevent an even greater decline in the circulating concentration and the consequence of a reduced availability of leucine to peripheral tissues and organs. It might be noted that the first-pass disappearance of leucine within the splanchnic region found in the present study is higher than the values generally reported in the literature, which range from about 7% to 25% of leucine absorbed.[34-36] The reason for this difference may be due to the fact that in the present study

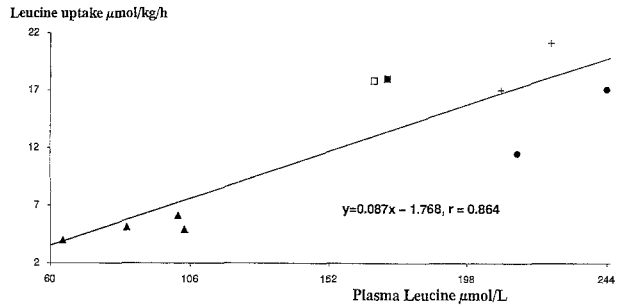

Fig 4. Relationship between the plasma level and splanchnic uptake of leucine (data from this study and others[16-17,32-33]). (+) Present study; (▲) Cortiella et al[32]; (●) Krempf et al[16]; (□) Hoerr et al[17]; (■) Boirie et al.[33]

we fed the amino acid mixture without additional carbohydrate or lipid, whereas published, fed-state values were derived from studies involving mixed meals.

Finally, the correlations found between blood glucose and leucine fluxes despite a lack of change in the insulin concentration must be emphasized and need further study, as these results are in conflict with previous data showing no interaction between the glucose plasma level and leucine kinetics.[37]

In conclusion, this study suggests that a significant increase in the plasma glucagon concentration, with constant levels of insulin and growth hormone, has a small effect on overall whole-body leucine metabolism, and specific and opposite effects on different tissues may occur. Unfortunately, we were not able to assess the effect of increased glucagon levels on whole-body leucine oxidation in this study, but it is reportedly increased in response to high glucagon.[31] Changing blood glucose concentrations related to glucagon excess might play a role in the fate of dietary leucine metabolism, even when basal insulin and growth hormone concentrations are sustained. Our findings point to a greater response of leucine metabolism to glucagon within the peripheral rather than the splanchnic territory, where amino acids or glucose concentrations seem to play a key role. This hypothesis could be tested using a combined arteriovenous substrate and isotope balance study across the limbs and the splanchnic bed.

ACKNOWLEDGMENT

We thank Nadine Denoual for secretarial assistance, and the Biochemistry Departments of Angers and Montpellier Hospitals for leucine and glucagon measurements. We also thank Kabi Laboratories for providing growth hormone.

REFERENCES

1. Lefebvre PJ: Glucagon and its family revisited. Diabetes Care 18:715-730, 1995

2. Almdal TP, Heindorff H, Bardram L, et al: Increased amino acid clearance and urea synthesis in a patient with glucagonoma. Gut 31:946-948, 1990

3. Klein S, Jahoor R, Baba H, et al: In vivo assessment of the metabolic alterations in glucagonoma syndrome. Metabolism 41:1171-1175, 1992

4. Boden G, Tappy L, Jadali F, et al: Role of glucagon in disposal of an amino acid load. Am J Physiol 259:E225-E232, 1990

5. Marliss EB, Aoki TT, Unger RH, et al: Glucagon levels and metabolic effects in fasting man. J Clin Invest 49:2256-2270, 1970

6. Nair KS, Halliday D, Matthews DE, et al: Hyperglucagonemia during insulin deficiency accelerates protein catabolism. Am J Physiol 253:E208-E213, 1987

7. Landau RL, Lugibihl K: Effect of glucagon on concentration of several amino acids in plasma. Metabolism 18:265-276, 1969

8. Mallette LE, Exton JH, Park CR: Effects of glucagon on amino acid transport and utilization in the perfused rat liver. J Biol Chem 244:5724-5728, 1969

9. Charlton MR, Nair KS: The effect of amino acid infusions on protein metabolism. The role of glucagon. Diabetologia 38:A23, 1995 (suppl, abstr)

10. Hartl WH, Miyoshi H, Jahoor F, et al: Bradykinin attenuates glucagon-induced leucine oxidation in humans. Am J Physiol 259:E239-E245, 1990

11. Tessari P, Inchiostro S, Barazzoni R, et al: Hyperglucagonemia stimulates phenylalanine oxidation in humans. Diabetes 45:463-470, 1996

12. Couet C, Fugakawa NK, Matthews DE, et al: Plasma amino acid kinetics during acute states of glucagon deficiency and excess in healthy adults. Am J Physiol 258:E78-E85, 1990

13. Buse MG, Biggers JF, Drier C, et al: The effect of epinephrine, glucagon, and the nutritional state on the oxidation of branched chain amino acids and pyruvate by isolated hearts and diaphragms of the rat. J Biol Chem 248:697-706, 1973

14. Preedy VR, Garlick PJ: The effect of glucagon administration on protein synthesis in skeletal muscles, heart and liver in vivo. Biochem J 228:575-581, 1995

15. Ostaszewski P, Nissen S: Effect of hyperglucagonemia on whole-body leucine metabolism in immature pigs before and during a meal. Am J Physiol 254:E372-E377, 1988

16. Krempf M, Hoerr RA, Pelletier VA, et al: An isotopic study of the effect of dietary carbohydrate on the metabolic fate of dietary leucine and phenylalanine. Am J Clin Nutr 57:161-169, 1993

17. Hoerr RA, Matthews DE, Bier DM, et al: Leucine kinetics form [$^{2}H_{3}$]- and [^{13}C]-leucine infused simultaneously by gut and vein. Am J Physiol 260:E111-E117, 1991

18. Wolfe RR: Radioactive and Stable Isotope Tracers in Biomedicine. Principles and Practice of Kinetic Analysis. New York, NY, Wiley-Liss, 1992, pp 283-315

19. Bier DM, Arnold KJ, Sherman WR, et al: In vivo measurement of glucose and alanine metabolism with stable isotopic tracers. Diabetes 26:1005-1011, 1977

20. Wolfe RR, Herndon DN, Jahoor F, et al: Effect of severe burn injury on substrate cycling by glucose and fatty acids. N Engl J Med 317:403-408, 1987

21. Jahoor F, Herndon DN, Wolfe RR: Role of insulin and glucagon in the response of glucose and alanine kinetics in burn-injured patients. J Clin Invest 78:807-814, 1986

22. Liljenquist JE, Mueller GL, Cherrington AD, et al: Evidence for an important role of glucagon in the regulation of hepatic glucose production in normal man. J Clin Invest 59:369-374, 1977

23. Douglas RG, Humberstone DA, Haystead A, et al: Metabolic effects of recombinant human growth hormone: Isotopic studies in the postabsorptive state and during total parenteral nutrition. Br J Surg 77:785-790, 1990

24. Moller N, Jorgensen JO, Abildgard N, et al: Effects of growth hormone on glucose metabolism. Horm Res 36:32-35, 1991 (suppl 1)

25. Yarasheski KE, Campbell JA, Smith K, et al: Effect of growth hormone and resistance exercise on muscle growth in young men. Am J Physiol 262:E261-E267, 1992

26. Yarasheski KE, Zachwieja JJ, Campbell JA, et al: Effect of growth hormone and resistance exercise on muscle growth and strength in older men. Am J Physiol 268:E268-E276, 1995

27. Nielsen S, Ostaszewski P: Effects of supplemental dietary energy on leucine metabolism in sheep. Br J Nutr 54:705-712, 1985

28. Schauder P, Schroder K, Langenbeck U: Serum branched-chain amino and keto acid response to a protein-rich meal in man. Ann Nutr Metab 28:350-356, 1984

29. Snelling CF, Woolf LI, Groves AC, et al: Amino acid metabolism in patients with severe burns. Surgery 91:474-481, 1982

30. Hildebrandt EF, Buxton DB, Olson MS: Acute regulation of the branched-chain 2-oxo acid dehydrogenase complex by adrenaline and glucagon in the perfused rat heart. Biochem J 250:835-841, 1988

31. Charlton MR, Adey DB, Nair KS: Evidence for a catabolic role of glucagon during an amino acid load. J Clin Invest 98:90-99, 1996

32. Cortiella J, Matthews DE, Hoerr RA, et al: Leucine kinetics at graded intakes in young men: Quantitative fate of dietary leucine. Am J Clin Nutr 48:998-1009, 1988

33. Boirie Y, Cachon P, Beaufrère B: Splanchnic and whole body leucine kinetics in young and elderly men. Am J Clin Nutr 65:489-495, 1997

34. Biolo G, Tessari P, Inchiostro S, et al: Leucine and phenylalanine kinetics during mixed meal ingestion: A multiple tracer approach. Am J Physiol 262:E455-E463, 1992

35. Castillo L, Chapman TE, Yu YM, et al: Dietary arginine uptake by the splanchnic region in adult humans. Am J Physiol 265:E532-E539, 1993

36. Horber FH, Haymond MW: Human growth hormone prevents the protein catabolic side effects of prednisone in humans. J Clin Invest 86:265-272, 1990

37. Heiling VJ, Campbell PJ, Gottesman IS, et al: Differential effects of hyperglycemia and hyperinsulinemia on leucine rate of appearance in normal humans. J Clin Endocrinol Metab 7:203-206, 1993