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J Clin Invest. 1987;79(4):1062-1069. <https://doi.org/10.1172/JCI112919>.

Research Article

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Differential Effects of Hyperinsulinemia and Hyperaminoacidemia on Leucine-Carbon Metabolism In Vivo

Evidence for Distinct Mechanisms in Regulation of Net Amino Acid Deposition

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Abstract

The effects of physiologic hyperinsulinemia and hyperaminoacidemia, alone or in combination, on leucine kinetics in vivo were studied in postabsorptive healthy subjects with primed-constant infusions of L-[4,5-³H]leucine and [1-¹⁴C]α-ketoisocaproate (KIC) under euglycemic conditions. Hyperinsulinemia (~ 100 μU/ml) decreased ($P < 0.05$ vs. baseline) steady state Leucine + KIC rates of appearance (Ra) from proteolysis, KIC (~ leucine-carbon) oxidation, and nonoxidized leucine-carbon flux (leucine → protein). Hyperaminoacidemia (plasma leucine, 210 μmol/liter), with either basal hormone replacement or combined to hyperinsulinemia, resulted in comparable increases in leucine + KIC Ra, KIC oxidation, and leucine → protein ($P < 0.05$ vs. baseline). However, endogenous leucine + KIC Ra was suppressed only with the combined infusion. Therefore, on the basis of leucine kinetic data, hyperinsulinemia and hyperaminoacidemia stimulated net protein anabolism in vivo by different mechanisms. Hyperinsulinemia decreased proteolysis but did not stimulate leucine → protein. Hyperaminoacidemia per se stimulated leucine → protein but did not suppress endogenous proteolysis. When combined, they had a cumulative effect on net leucine deposition into body protein.

Introduction

Both insulin and amino acids are widely considered as necessary for the stimulation of protein anabolism (1, 2) but their individual role in the regulation of protein metabolism in vivo has not yet been established. Net protein deposition in body tissues can be achieved by the suppression of endogenous protein degradation, by the stimulation of protein synthesis, or both. A number of in vitro studies have demonstrated that insulin inhibits protein degradation in the liver (3) and muscle (4), whereas it stimulates protein synthesis in muscle (4, 5) but doesn't seem to have any effect in the liver (3). In the perfused rat hindlimb, insulin also decreased the oxidation and the transamination of essential amino acids (5). On the other hand, amino acids have been shown to stimulate directly protein synthesis in muscle (6) and suppress proteolysis in perfused rat liver (7). Thus, both insulin

and amino acids can directly stimulate protein anabolism in vitro. The assessment of their role in vivo is complicated by the concomitant changes in their concentrations that accompany the experimental increments in insulin and amino acid levels. On the other hand, the simultaneous increase in insulin and amino acids occurs physiologically during meal absorption, i.e., in a situation where net protein anabolism is achieved.

In a number of recent studies (8–10) performed in vivo, it has been shown that hyperinsulinemia decreases proteolysis and the oxidation of essential amino acids. However, the effects of hyperaminoacidemia, alone or in combination with hyperinsulinemia, on whole-body protein turnover were not investigated. Therefore, in the present study, we examined the effects of euglycemic hyperinsulinemia, hyperaminoacidemia in the presence of basal insulin, and hyperinsulinemia and hyperaminoacidemia in combination on whole-body leucine kinetics in postabsorptive healthy men. The essential amino acid leucine was studied as representative of whole-body protein turnover (8–10), as it cannot be synthesized de novo and therefore can derive either from endogenous proteolysis or exogenous sources. Because a significant portion of leucine-carbon is released into the circulation as its transamination product, i.e., α-ketoisocaproate (KIC),¹ both in postabsorptive conditions and when exogenous leucine is infused (11), the kinetic of KIC was also investigated. Extrapolations to whole-body protein metabolism were made under the assumption that leucine kinetics reflect whole-body protein turnover (8–10).

Methods

Isotopes. L-[4,5-³H]Leucine (³H-leucine) (55 Ci/mmol) and L-[1-¹⁴C]-leucine (57 mCi/mmol) were purchased from Amersham International (Amersham, UK). [1-¹⁴C]α-ketoisocaproate (¹⁴C-KIC) was prepared from the L-[1-¹⁴C]-leucine and purified by high pressure liquid chromatography (HPLC) (12), with the exception that ethanol was substituted for acetonitrile in the running buffer. [¹⁴C]HCO₃ (50 mCi/mmol) was purchased from Amersham International. Isotopes were proven to be sterile and pyrogen-free before use.

Experimental design. Normal healthy volunteers of both sexes were studied in the postabsorptive state. The purposes and the potential risks of the study were explained in detail to each subject. Informed, written consent to the study was then signed by each volunteer. The study was part of a more comprehensive project approved by the Consiglio Nazionale delle Ricerche of Italy. The total amount of radioactive isotope administered was within the range approved by other institutions (11, 13). Whole-body radiation dosage was estimated to be < 60 mRad for each study (14). Subjects were studied only once. All subjects consumed

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Received for publication 13 February 1986 and in revised form 16 July 1986.

J. Clin. Invest.

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0021-9738/87/04/1062/08 \$1.00

Volume 79, April 1987, 1062–1069

1. Abbreviations used in this paper: BMI, body mass index; ¹⁴C-KIC, [1-¹⁴C]α-ketoisocaproate; HPLC, high pressure liquid chromatography; KIC, α-ketoisocaproate; ³H-leucine, L-[4,5-³H]leucine; Ra, rate of appearance; SA, specific activities.

a diet containing at least 50% carbohydrates for 3 d before the study. At 8 a.m., a forearm vein was cannulated with an 18-gauge polyethylene catheter and used for isotope, glucose, hormone, and amino acid infusions. A forearm vein of the opposite side was also cannulated and used for continuous blood glucose monitoring by the Biostator GC115, Miles Laboratories, Elkhart, IN (15). An additional wrist vein on the same side of the vein used for the Biostator was cannulated in a retrograde fashion and used for arterialized venous blood sampling (16). The subjects were studied according to one of the following protocols: in group A, euglycemic hyperinsulinemia, five subjects (four males, one female, age 35 ± 7 yr; body mass index (BMI), 22.3 ± 0.9) were infused with 0.9% NaCl from -150 to 0 min (basal period) and subsequently with insulin (17) at the rate of $0.05 \text{ U/m}^2 \cdot \text{min}$ from 0 to 180 min (infusion period). Blood glucose was clamped at euglycemia ($86 \pm 2 \text{ mg/dl}$; coefficient of variation, $\sim 11\%$) by the Biostator (15). In group B, hyperaminoacidemia with basal hormone replacement, four subjects (all males, age 22 ± 1 yr; BMI, 23.2 ± 0.8) were infused for 330 min with somatostatin (Stilamin, Sersono, Roma, Italy) at a constant rate of $250 \text{ } \mu\text{g/h}$, and replacement doses of insulin ($\sim 0.005 \text{ U/m}^2 \cdot \text{min}$) and glucagon (Novo, Bagsvaerd, Denmark) ($\sim 1 \text{ ng/kg} \cdot \text{min}$). In this group, 0.9% NaCl was infused from -150 to 0 min (basal period), thereafter an amino acid solution was administered intravenously from 0 to 180 min. The amino acid mixture contained 84.3 mmol/L L-alanine, 34.5 L-arginine, 6.1 L-phenylalanine, 120 glycine, 68.7 L-isoleucine, 15.5 L-histidine, 83.4 L-leucine, 41.6 L-lysine, 6.7 L-methionine, 69.6 L-proline, 47.6 L-serine, 37.8 L-threonine, 3.7 L-tryptophane, and 71.8 L-valine. This solution was infused at a constant rate of 0.867 ml/min (mean value), corresponding to $1.09 \pm 0.09 \text{ } \mu\text{mol/kg} \cdot \text{min}$ of leucine, by means of Harvard Pumps (Harvard Apparatus Co., South Natick, MA). The pH of the solution was ~ 4 . However, no changes in either blood pH or acid-base balance were observed when this solution was infused at the rates employed in the study. Plasma glucose concentrations remained at $\sim 90 \text{ mg/dl}$ (coefficient of variation, $\sim 15\%$) throughout the study (Table I). Finally, in group C, euglycemic hyperinsulinemia with hyperaminoacidemia, five subjects (four males, one female, age 35 ± 6 yr; BMI, 22.1 ± 0.7) were infused with 0.9% NaCl from -150 to 0 min (basal period). Thereafter, (between 0 and 180 min) insulin was infused at the same rate as in group A, and the amino acid solution at the rate of 1.41 ml/min , corresponding to $1.74 \pm 0.04 \text{ } \mu\text{mol/kg} \cdot \text{min}$ of leucine. On the basis of preliminary studies, this rate was chosen to achieve plasma leucine concentrations comparable with those of group B. Euglycemia ($88 \pm 1 \text{ mg/dl}$; coefficient of variation, $\sim 11\%$) was maintained by the Biostator. At 0 time, primed-continuous infusions of ^3H -leucine ($\sim 8,000 \text{ dpm/kg} \cdot \text{min}$) and of ^{14}C -KIC ($\sim 3,000 \text{ dpm/kg} \cdot \text{min}$) were initiated and continued throughout the studies. The priming doses were 30 times the continuous infusion rates. Bicarbonate pool was primed with $3 \text{ } \mu\text{Ci}$ of $[^{14}\text{C}]\text{HCO}_3^-$ (18). Plasma and breath samples were taken at frequent intervals in the basal period and during the in-

fusions. Steady state conditions in plasma leucine and KIC concentrations and specific activities (coefficient of variation, 2–6%) and in expired $^{14}\text{CO}_2$ (coefficient of variation, 5–10%) were attained at least in the final 30 min of both the basal and the infusion periods (Fig. 1).

Analytical methods. Plasma leucine and KIC concentrations and specific activities (SA) were determined as previously described (12). Plasma amino acid concentrations were determined as dansyl derivatives by HPLC (19). Plasma insulin (20), glucagon (21), and glucose (9) were also determined. Expired air was collected in plastic bags as previously described (11). All radioactive samples, i.e., those eluting from the HPLC (12) and ethanolamine solution containing the dissolved $[^{14}\text{C}]\text{bicarbonate}$ (11), were mixed into scintillation vials with a scintillation cocktail, allowed to equilibrate, and counted in a TRI Carb liquid scintillation spectrometer (Packard Instruments Co., Inc., Downers Grove, IL). Radioactivity was corrected for quench and ^{14}C spillover into the tritium channel with external standard methods (9).

Calculations and model assumptions. All the kinetic data were determined at steady state conditions of plasma substrate concentrations, SA, and expired $^{14}\text{CO}_2$ (Fig. 1), i.e., in the final 30 min of the basal and the infusion periods. Leucine and KIC rates of appearance (R_a) were calculated by dividing the isotope infusion rates ($\text{dpm/kg} \cdot \text{min}$) over the corresponding mean plasma specific activity (dpm/nmol) at steady state. The sum of leucine-plus-KIC R_a was also calculated as an index of total leucine-carbon entry (9, 22). Leucine-carbon oxidation was expressed as the oxidation of KIC, which was considered the direct precursor of leucine-carbon oxidation (9, 23). KIC oxidation was calculated by dividing the expired $^{14}\text{CO}_2$ ($\text{dpm/kg} \cdot \text{min}$) over plasma ^{14}C -KIC SA, and corrected for a $\sim 20\%$ fixation in body bicarbonate pools (24). This estimate of oxidation may be incorrect if intracellular ^{14}C -KIC SA does not equilibrate with that of plasma, where ^{14}C -KIC is infused. However, as KIC pool appears to be small and its turnover rate relatively high (11), plasma ^{14}C -KIC SA is likely to equilibrate fast with the intracellular one. In a previous study (25), we calculated leucine-carbon oxidation using both plasma ^{14}C -KIC and ^{14}C -leucine SA during the infusion of ^{14}C -KIC obtaining qualitatively but not quantitatively similar results. With respect to this issue, Schwenk et al. recently proposed the use of "reciprocal pools" of either leucine or KIC (22) as a more precise reflection of intracellular leucine or KIC SA, following the infusion of labeled KIC or leucine, respectively, to calculate both leucine-carbon appearance and oxidation (22). However, from in vitro studies (26) it has been suggested that oxidized leucine derived mainly from extracellular sources. Thus, plasma KIC (as well as ^{14}C -KIC), which can enter directly the oxidative pathway, could be reasonable indicators of the intracellular precursors of leucine-carbon oxidation during the infusion of ^{14}C -KIC. If, under the conditions of the present study, plasma ^{14}C -leucine SA had been used, it would have been diluted significantly by the entry of unlabeled leucine coming from proteolysis, leading therefore to a possible underestimation of the

Table I. Plasma KIC and Amino Acid Concentrations ($\mu\text{mol/l}$) in the Basal State and After Insulin (Ins), Amino Acid (AA), and Insulin Plus Amino Acid (Ins + AA) Infusions

	Group A		Group B		Group C	
	Basal	Ins	Basal	AA	Basal	Ins + AA
KIC	40 ± 3	$23 \pm 3^*$	34 ± 5	45 ± 1	34 ± 3	34 ± 3
Leucine	121 ± 13	$70 \pm 8^*$	133 ± 3	$208 \pm 6^*$	117 ± 5	$207 \pm 5^*$
Lysine	257 ± 48	$187 \pm 34^*$	211 ± 21	$340 \pm 56^*$	267 ± 13	$397 \pm 30^*$
Isoleucine	69 ± 5	$23 \pm 2^*$	56 ± 5	$138 \pm 9^*$	52 ± 4	$183 \pm 25^*$
Phenylalanine	99 ± 7	$57 \pm 7^*$	70 ± 8	65 ± 8	70 ± 3	$56 \pm 5^*$
Valine	235 ± 15	$128 \pm 10^*$	190 ± 24	$303 \pm 20^*$	221 ± 16	$459 \pm 43^*$
Alanine	257 ± 28	233 ± 19	287 ± 42	$349 \pm 55^*$	319 ± 37	$493 \pm 30^*$
Glycine	262 ± 18	$185 \pm 32^*$	214 ± 33	$364 \pm 48^*$	264 ± 36	$535 \pm 38^*$
Serine	84 ± 16	$46 \pm 4^*$	79 ± 14	95 ± 14	111 ± 4	$164 \pm 11^*$

* $P < 0.05$ or less from basal values (paired t test).

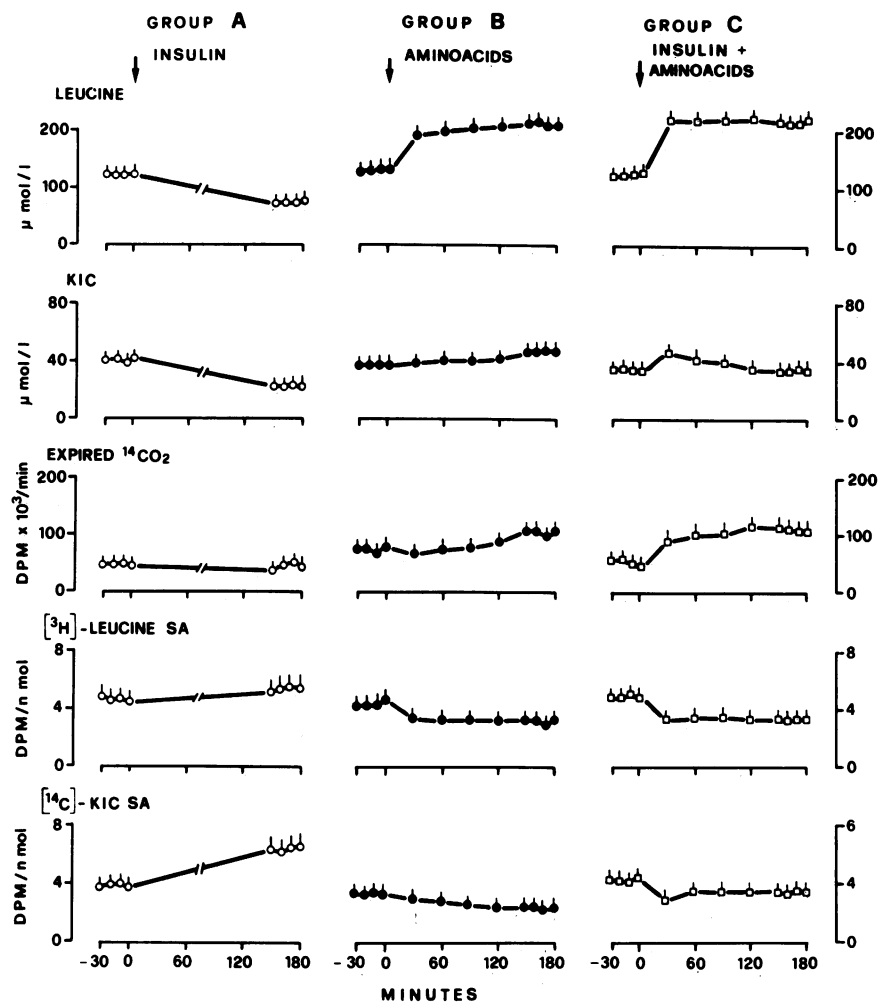


Figure 1. Plasma leucine and KIC concentrations, expired $^{14}\text{CO}_2$, $[^3\text{H}]$ leucine and $[^{14}\text{C}]$ KIC SA in the basal state and during the infusions of insulin (group A), amino acids (group B), and insulin plus amino acids (group C).

intracellular SA and overestimation of oxidation (26). In any case, it could be useful to compare our results with those in which ^{14}C -leucine had been infused and plasma ^{14}C -KIC SA had been used to calculate both turnover and oxidation rates. Nonoxidized leucine-carbon flux, an index of leucine incorporation into protein ($\text{Leu} \rightarrow \text{P}$), was calculated by subtracting KIC oxidation from $\text{Leu} + \text{KIC}$ Ra. Endogenous Leucine Ra and $\text{Leu} + \text{KIC}$ Ra, in groups B and C, were calculated by subtracting the exogenous leucine infusion rate ($\mu\text{mol/kg} \cdot \text{min}$) from either total Leucine Ra or $\text{Leu} + \text{KIC}$ Ra, respectively, at steady state.

The statistical analysis was performed using the two-tailed t test for paired data in the comparisons between values in the basal state (i.e., from -30 to 0 min) and following the infusions (i.e., from 150 to 180 min). The multiple comparisons between groups were performed using percent changes from basal values following the infusions applying the one-way analysis of variance and the Newmann-Keuls test (27). A $P \leq 0.05$ value was taken as statistically significant. All data have been expressed as mean \pm SEM.

Results

Plasma substrate and hormone concentrations, expired $^{14}\text{CO}_2$, $[^3\text{H}]$ -leucine and $[^{14}\text{C}]$ -KIC specific activities. Plasma glucose concentrations were stable throughout the study in all groups (Fig. 2). The glucose infusion rate to maintain euglycemia was about 30% lower in group C (hyperaminoacidemia combined with hyperinsulinemia) than in group A (hyperinsulinemia alone), and it has been reported elsewhere (28). No glucose infusion was

necessary to maintain euglycemia in group B. Plasma insulin concentration increased to similar levels in groups A and C (Fig. 2) (101 ± 14 vs. 88 ± 8 $\mu\text{U/ml}$, respectively), whereas it did not change in the group infused with amino acids and basal hormone replacement (group B). However, in the latter group, peripheral insulin concentration in the basal state (15 ± 1 $\mu\text{U/ml}$) was slightly but significantly greater ($P < 0.05$) than in the other two groups (9 $\mu\text{U/ml}$). Glucagon concentration (Fig. 2) did not significantly change from the basal state in groups B and C, whereas it decreased by $\sim 30\%$ ($P < 0.05$) in group A (from 119 ± 34 to 86 ± 25 pg/ml).

Plasma amino acid and KIC concentrations are reported on Table I and Fig. 1. With euglycemic hyperinsulinemia and no amino acid infusion (group A), the concentrations of most amino acids and of KIC decreased significantly from basal by 30–70%, with the exception of alanine, which did not change. With amino acid infusion and basal hormone replacement (group B), the concentration of most amino acids increased by 60–150%, in part reflecting their content in the solution infused. On the contrary, the concentration of phenylalanine, infused at lower rates, decreased in three subjects and did not change in one, with an average decrease of $\sim 8\%$. Serine levels did not change, whereas KIC concentration increased by $\sim 30\%$ ($0.05 > P > 0.1$). In group C, plasma leucine concentration increased to an absolute value that was similar to that attained in group B. Also the percent increment from baseline in these two groups was not dif-

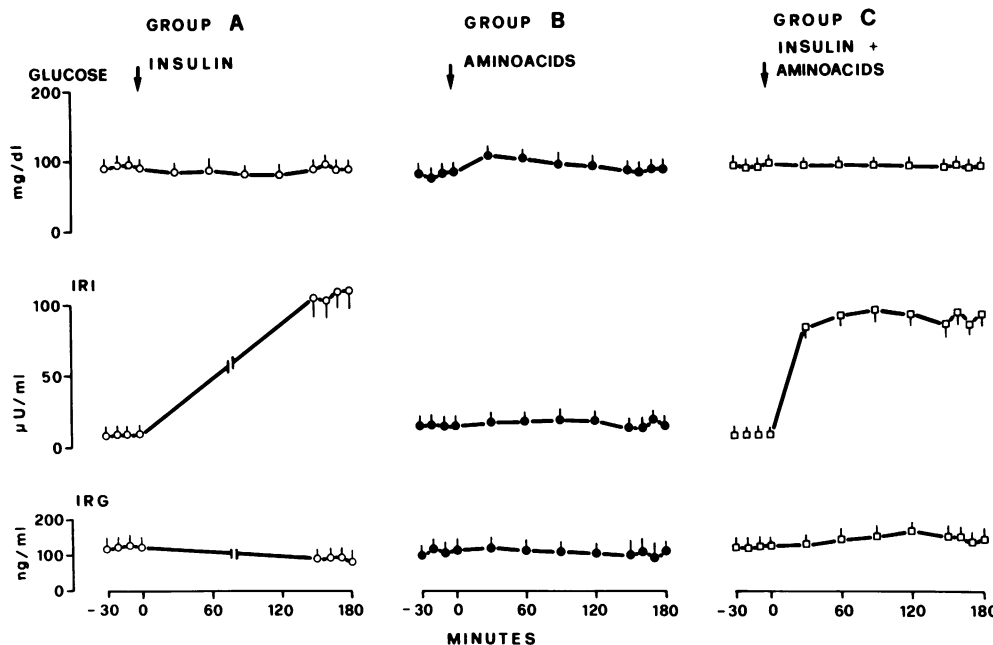


Figure 2. Plasma glucose, insulin (IRI) and glucagon (IRG) concentrations in the basal state and during the infusions of insulin (group A), amino acids (group B), and insulin plus amino acids (group C).

ferent. The concentrations of other amino acids increased by 50–250%, to an extent that was therefore somewhat greater than that achieved in group B. Plasma KIC concentration did not change from baseline values.

The data concerning plasma ^3H -leucine and ^{14}C -KIC SA, as well as the expired $^{14}\text{CO}_2$, are shown in Fig. 1. In group A (hyperinsulinemia), ^3H -leucine and ^{14}C -KIC SA increased, whereas expired $^{14}\text{CO}_2$ did not significantly change. In groups B and C ^3H -leucine and ^{14}C -KIC SA decreased, while expired $^{14}\text{CO}_2$ increased. Steady state conditions (see Methods) were achieved in the final 30 min of the basal period (i.e., from –30 to 0 min) and the infusion periods (150–180 min).

Leucine and KIC kinetics. Leucine Ra (Table II) decreased by $13 \pm 2\%$ with hyperinsulinemia (group A) ($P < 0.01$), whereas it increased ($P < 0.001$) by $37 \pm 7\%$ and $49 \pm 9\%$ following the amino acid infusions without (group B) or with hyperinsulinemia (group C), respectively. The percent increase in group B was not statistically different from that observed in group C. KIC Ra decreased by $36 \pm 8\%$ in group A ($P < 0.001$), and increased ($P < 0.001$) by $46 \pm 10\%$ and by $23 \pm 5\%$ in groups B and C, respectively (Table II). The percent increment in group B was two-fold greater than that of group C, and it did approach a statistically significant difference ($0.05 > P < 0.1$). Leu + KIC Ra (Table II) decreased by $23 \pm 4\%$ in group A ($P < 0.01$), and increased ($P < 0.001$) by $39 \pm 7\%$ and by $41 \pm 6\%$ in groups B and C, respectively. The percent increments in groups B and C were statistically similar (Fig. 3).

Endogenous leucine Ra decreased by $19 \pm 3\%$ ($P < 0.01$) in group B, to an extent that was statistically similar to that observed in group A ($-13 \pm 2\%$) (Table II). In group C, endogenous leucine Ra decreased by $56 \pm 5\%$ ($P < 0.05$ or less vs. the other groups). However, when endogenous leucine-carbon Ra was estimated from Leu + KIC Ra, endogenous Leu + KIC Ra did not change from basal in group B ($0 \pm 5\%$, $P < 0.001$ vs. group A), whereas it decreased by $33 \pm 4\%$ in group C ($P < 0.001$ vs. group B), to an extent that was not statistically different from that observed in group A ($-23 \pm 4\%$) (Table II, Fig. 3).

KIC oxidation decreased by $46 \pm 7\%$ in group A ($P < 0.001$),

and increased ($P < 0.001$) by $142 \pm 30\%$ and by $203 \pm 68\%$ in groups B and C, respectively (Table II, Fig. 3). The percent increments in groups B and C were statistically similar (Fig. 3).

Non-oxidized leucine-carbon flux, a possible estimate of leucine incorporation into protein (Leu \rightarrow P) (Table II, Fig. 3), decreased by $19 \pm 4\%$ in group A ($P < 0.01$), and increased ($P < 0.001$) by $29 \pm 7\%$ and by $32 \pm 6\%$ with the amino acid infusion without (group B) or with hyperinsulinemia (group C), respectively. The percent increments in groups B and C were statistically similar.

Discussion

Our study suggests that both hyperinsulinemia and hyperaminoacidemia, within physiological ranges, stimulate net leucine deposition into body proteins, but to a different extent and with different mechanisms. Hyperinsulinemia had a slight net positive effect because it decreased leucine-carbon appearance from endogenous proteolysis to a larger extent than it restrained (surprisingly) the estimate of leucine incorporation into protein. Hyperaminoacidemia had a more marked effect because it stimulated leucine incorporation into protein without affecting endogenous proteolysis. Comparable degrees (with respect to the independent infusions) of hyperinsulinemia and hyperleucine-mia in combination resulted in a cumulative effect, due to the suppression of endogenous proteolysis and to the stimulation of leucine entering protein (Fig. 3).

In several published studies performed in vitro, it has been demonstrated that both insulin and amino acids exerted an anabolic effect on protein metabolism (5, 6). Recent studies in humans, utilizing tracer methodology, also showed that insulin inhibited protein degradation both in normal and diabetic subjects (9, 10, 30) but failed to stimulate the estimate of leucine entering protein (9, 10, 30). However, under the euglycemic, hyperinsulinemic conditions of those experiments, plasma amino acid concentrations were allowed to decrease. Therefore, the effects of changes in amino acid concentrations, with or without

Table II. Kinetics of Leucine and KIC in the Basal State (B) and Following Various Infusions (I) in Groups Studied

		Group A (Ins)	Group B (AA)	Group C (Ins + AA)
Leu Ra	B	1.82±0.10	1.99±0.13	1.64±0.09
	I	1.59±0.08*	2.70±0.08*	2.43±0.13*
	Δ	-0.23±0.04	+0.71±0.08	+0.79±0.13
KIC Ra	B	0.88±0.08	0.84±0.08	0.75±0.05
	I	0.55±0.05*	1.21±0.08*	0.92±0.06*
	Δ	-0.33±0.08	+0.37±0.08	+0.17±0.04
Leu + KIC Ra	B	2.70±0.16	2.83±0.15	2.39±0.13
	I	2.14±0.13*	3.91±0.09*	3.34±0.16*
	Δ	-0.56±0.11	+1.08±0.16	+0.96±0.14
Exogenous Leu infusion rate	—		1.09±0.09	1.74±0.04
Endogenous Leu Ra	I	1.59±0.08	1.61±0.16	0.69±0.10
	Δ	-0.23±0.04*	-0.38±0.07*	-0.92±0.10*
Endogenous Leu + KIC Ra	I	2.14±0.13	2.82±0.28	1.60±0.13
	Δ	-0.56±0.11*	-0.01±0.12	-0.78±0.12*
KIC oxidation	B	0.16±0.02	0.30±0.05	0.17±0.04
	I	0.08±0.01*	0.67±0.05*	0.45±0.06*
	Δ	-0.08±0.02	+0.38±0.02	+0.28±0.04
Leu → protein	B	2.55±0.17	2.53±0.16	2.21±0.16
	I	2.06±0.13*	3.24±0.14*	2.89±0.17*
	Δ	-0.49±0.12	+0.55±0.08	+0.68±0.11

The data are expressed as absolute values and as differences (Δ) from baseline. Ins, insulin; AA, amino acids.

All data are expressed as μmol/kg · min.

* $P < 0.05$ or less from basal values (paired t test).

hyperinsulinemia, on estimates of whole body proteolysis and protein synthesis were not examined. In the present study, plasma leucine concentration, as well as Leu + KIC Ra, were raised to the same extent in the presence of either basal insulin (group B) or physiologic hyperinsulinemia (group C) by means of appropriate exogenous infusions. The suppression by hyperinsulinemia of endogenous leucine-carbon appearance, estimated by using Leu + KIC Ra, was slightly but not significantly augmented when plasma amino acid concentrations were raised above basal levels (group C vs. group A). Therefore, hyperaminoacidemia per se, alone (group B) or in combination to insulin (group C), doesn't appear to inhibit endogenous proteolysis. It follows that no enhancement of the insulin-induced suppression of endogenous proteolysis would have been observed also if exogenous amino acids had been infused at rates sufficient to clamp their concentrations at basal levels during the hyperinsulinemic, euglycemic clamp or, alternatively, if they had been infused in group C at a rate similar to that of group B. The increases above baseline in the estimates of leucine entering protein were similar in groups B and C, in which comparable plasma leucine concentrations were achieved. Therefore, insulin per se, as well as

the exogenous leucine delivery rate, did not seem to affect the increase in protein synthesis, which appeared to depend mainly on circulating plasma leucine concentrations. As the increments in Leu → P in groups B and C were similar, despite the greater exogenous amino acid infusion rate in group C, it might appear at first that in the latter group hyperinsulinemia actually decreased the rate of leucine entering protein. However, it has to be remembered that Leu → P decreased with hyperinsulinemia alone (Table II, Fig. 3), so the relative increment when exogenous amino acids were infused in combination to insulin (group C) was actually greater than that observed. Similarly, KIC (approximate leucine-carbon) oxidation increased to similar levels in groups B and C, despite a greater exogenous leucine infusion in group C. Again, as KIC oxidation decreased with hyperinsulinemia alone, the relative increase in KIC oxidation in group C could have been actually greater than that observed. The possible greater increments in both Leu → P and KIC oxidation in group C with respect to group B could be related to the higher leucine infusion rate in group C, even if a stimulatory effect of hyperinsulinemia per se under conditions of substrate availability cannot be strictly ruled out. With regard to this, insulin has been found to stimulate leucine oxidation under fasted but not fed conditions (5).

In a recent study (30), we found a significant negative correlation during sequential euglycemic hyperinsulinemic clamps between glucose disposal rates and both KIC oxidation and Leu + KIC Ra in normal man. Thus, the enhanced glucose utilization in groups A and C could have contributed to the decrease in oxidation observed in group A and could have possibly restrained oxidation in group C. The increase in glucose utilization could also have been, at least in part, responsible for the suppression of endogenous Leu + KIC Ra in groups A and C. On the other hand, the reduced glucose disposal rates observed in group C as compared with group A could have modulated the effects of hyperinsulinemia on both KIC oxidation and endogenous Leu + KIC Ra.

As mentioned above, the choice of the model of leucine-carbon metabolism is critical in the interpretation of the results. In fact, if leucine entry had been measured only by leucine Ra, a significant inhibition on endogenous proteolysis by hyperaminoacidemia in the presence of either basal insulin (group B) or hyperinsulinemia (group C), would have been observed (Table II). Also an enhancement by hyperinsulinemia on leucine entering protein (calculated by subtracting KIC oxidation from leucine Ra; data not shown) could have been observed. However, in our opinion, KIC kinetic must also be taken into account, as KIC Ra increased significantly when exogenous leucine was infused in group B and, to a lesser extent, group C, as well as in a previous study (11). KIC concentrations also tended to increase in group B, even if the change did not achieve statistical significance. Therefore, leucine-carbon appearance could be better expressed by the sum of Leu + KIC Ra, as discussed in recent studies (22, 30).

In a previous study (11) we did not observe significant changes in Leu + KIC Ra, KIC oxidation, and Leu → P between 3 and 6 h of saline infusion in normal volunteers. Therefore, isotope recycling and time-effects do not apparently affect leucine metabolism in experiments of such duration.

All measurements were performed in steady state conditions. Therefore, an equilibrium after the induced perturbations with respect to time was achieved, even if discrepancies between

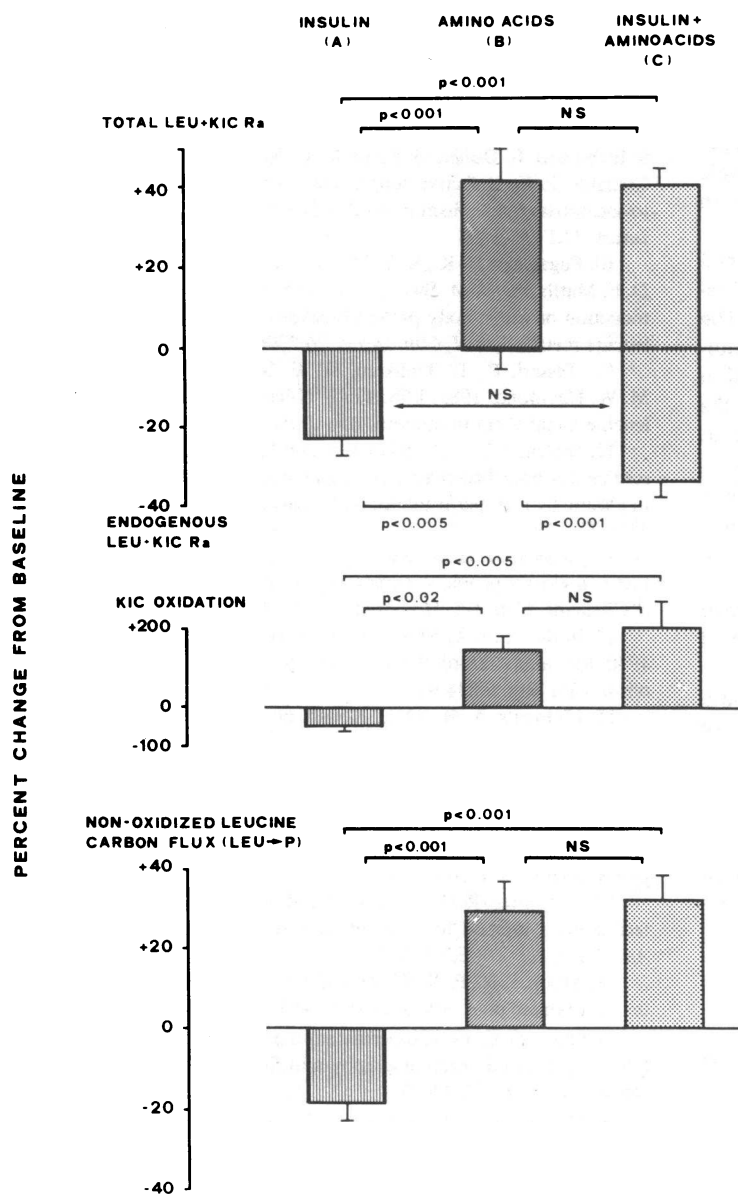


Figure 3. Percent changes from basal values of total leucine plus KIC (Leu + KIC) R_a and endogenous Leu + KIC R_a (top), KIC oxidation (middle), and Leu → P (bottom) after the infusion of insulin (group A), amino acids (group B), and insulin plus amino acids (group C).

plasma and intracellular SA could have persisted, because the tracers were infused into plasma. These possible discrepancies could have been diminished in the experiments where exogenous amino acids were infused (thus possibly affecting the estimates of the relative changes from baseline) because of a better equilibration between intra- and extracellular specific activities, as a consequence of a flooding effect by the increased plasma leucine concentrations. However, from in vitro data, it appears that intra- and extracellular specific activities equilibrate at much greater amino acid concentrations than those achieved in our study (26, 31). Thus, possible slight variations in the disequilibrium of specific activities between the intracellular and extracellular milieu should not have significantly affected our results. Also the sites of infusion and sampling deserve some comments. It would have been theoretically preferable to infuse into a central artery and sample from a central vein (32) to ensure a balanced tracer as well as substrate delivery to all organs and/or tissues, and to

obtain samples of well-mixed venous blood, but this is not feasible in humans. Therefore, as we infused into a peripheral vein, the lungs and heart were the first organs to be reached by the labeled and unlabeled substrates. The contributions of such organs to whole-body leucine turnover is unknown, at least to our knowledge, and a different experimental design, i.e., using the so called A-VC model according to Katz (32), theoretically might provide different results.

The effects of hyperaminoacidemia were studied under conditions in which insulin concentrations were maintained at basal levels. Therefore, no conclusions can be drawn on the possible effects of hyperaminoacidemia per se when also plasma insulin had been maximally suppressed. It is theoretically possible that hyperaminoacidemia per se stimulates leucine incorporation into protein even in the absence of circulating insulin. In conditions of insulin-deficiency, like type I diabetes, whole-body protein synthesis doesn't seem to be impaired, as recent studies suggest

(33, 34), possibly because of increased circulating amino acid levels.

Glucagon concentration was stable in all groups except group A, where it decreased by 30%. As glucagon has been demonstrated to increase leucine oxidation in isolated muscle preparations (35), this change in glucagon levels could be responsible at least in part for the decrease in leucine-carbon oxidation in this group.

The amino acid mixture used in the present studies contained relatively high amounts of branched-chain amino acids, and reduced or absent aromatic amino acids and methionine. The choice of such a solution, instead of a complete one, was motivated by the proposed role of branched-chain amino acids in the stimulation of protein anabolism (36, 37) and to test the efficacy of a mixture partially deprived of some essential amino acids on whole-body protein turnover. Such solutions are currently used in the parenteral nutrition of patients with liver failure (37, 38). Our results show that such solutions, even if incomplete, may stimulate net leucine deposition into body tissues. It would be of interest to compare their effects with those of complete amino acid mixtures to improve our knowledge of interactions among different amino acids in the regulation of protein metabolism in vivo.

In conclusion, our study underscores the importance of both hyperinsulinemia and hyperaminoacidemia in the regulation of whole-body amino acid and possibly protein metabolism in vivo. The substrates and hormone appear to exert different and complementary effects. Moreover, the kinetic of any compound into which an infused amino acid is converted, like KIC in the case of leucine, might have to be accounted for in the interpretation of the results. The modulation by glucose utilization on amino acid metabolism in vivo also requires further investigation.

Acknowledgments

This work was supported in part by National Research Council (CNR) of Italy grants 84-02551-56 and 85-00793-56.

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