Differential Regulation of Protein Dynamics in Splanchnic and Skeletal Muscle Beds by Insulin and Amino Acids in Healthy Human Subjects

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To determine the in vivo effect of amino acids (AAs) alone or in combination with insulin on splanchnic and muscle protein dynamics, we infused stable isotope tracers of AAs in 36 healthy subjects and sampled from femoral artery and vein and hepatic vein. The subjects were randomized into six groups and were studied at baseline and during infusions of saline (group 1), insulin (0.5 mU · kg^{-1} · min^{-1}) (group 2), insulin plus replacement of AAs (group 3) insulin plus high-dose AAs (group 4), or somatostatin and baseline replacement doses of insulin, glucagon and GH plus high dose of AAs (group 5) or saline (group 6). Insulin reduced muscle release of AAs mainly by inhibition of protein breakdown. Insulin also enhanced AA-induced muscle protein synthesis (PS) and reduced leucine transamination. The main effect of AAs on muscle was the enhancement of PS. Insulin had no effect on protein dynamics or leucine transamination in splanchnic bed. However, AAs reduced protein breakdown and increased synthesis in splanchnic bed in a dose-dependent manner. AAs also enhanced leucine transamination in both splanchnic and muscle beds. Thus insulin's anabolic effect was mostly on muscle, whereas AAs acted on muscle as well as on splanchnic bed. Insulin achieved anabolic effect in muscle by inhibition of protein breakdown, enhancing AA-induced PS, and reducing leucine transamination. AAs largely determined protein anabolism in splanchnic bed by stimulating PS and decreasing protein breakdown.
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Insulin exerts its anticatabolic effect on protein metabolism by its differential effects on protein synthesis (PS) and breakdown (PB) in many tissue beds (1–4). Human studies have demonstrated that muscle is in a catabolic state after an overnight fast and that it provides amino acids (AAs) to the systemic circulation (2). This AA supply is thought to be crucial for the synthesis of essential proteins, especially in liver (1). Insulin decreases the net efflux of AAs from the muscle bed mainly by inhibiting muscle PB (2). However, insulin has no effect on splanchnic PB and also decreases PS (2). It is unclear whether lack of any stimulatory effect on muscle and splanchnic PS is secondary to reduced intracellular AA concentrations. Our studies, and those of others, suggested that a reduction in circulating AAs (hypoaminoacidemia) or related changes in intracellular AA levels when insulin alone is infused may be the reason why insulin failed to stimulate muscle PS in skeletal muscle (5–8). Animal studies have reported that AAs, especially branched chain AAs, increased the sensitivity of muscle PS to insulin (9). In vitro experiments using large increases in insulin and AA concentrations have also stimulated muscle PS (10,11). Both insulin and AAs have been shown to have independent effects at the translational level of PS (12–15). AAs, especially branched chain AAs, have also been shown to have inhibitory effects on liver PB based on liver perfusion studies (16) and in vivo studies in rodents (12). The independent effects of AAs alone and in combination with insulin on PS and PB in splanchnic and muscle tissue beds in humans have not been determined. The current study was performed to determine the effect of AAs alone and in combination with insulin on muscle and splanchnic PB and synthesis. In addition, we have determined the role of AAs in the regulation of leucine transamination in splanchnic beds since this important biochemical process has been shown to be affected by insulin in nondiabetic (2) and type 1 diabetic patients (17). However, it remains to be determined whether this insulin effect is dependent on secondary changes in AA concentration.

In the current study, we simultaneously measured PS and PB and leucine transamination in splanchnic and leg (representing mostly skeletal muscle) to determine the role of AAs alone or in combination with insulin on regional protein metabolism. We tested the following hypotheses: *1*) AAs enhance PS in muscle and splanchnic bed and insulin further enhances this effect; *2*) AAs inhibit PB in both muscle and splanchnic bed and these effects are enhanced by insulin; and *3*) AAs accelerate leucine transamination in both splanchnic and muscle beds, whereas insulin inhibits transamination of leucine in these tissues.

RESEARCH DESIGN AND METHODS

Materials. L-[1-^{13}C,^{15}N]leucine (99 atom percent excess) and L-[ring-^{15}N]phenylalanine (99 atom percent excess) were purchased from Cambridge Isotope Laboratories (Andover, MA) and L-[ring-$^{2}H_4$]tyrosine (99 atom percent excess) and L-[^{15}N]tyrosine (99 atom percent excess) were purchased from Isotec (Maimisburge, OH). The chemical, isotopic, and optical purity of these

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AA, amino acid; GC/MS, gas chromatography/mass spectrometry; KIC, ketoisocaproate; PB, protein breakdown; PS, protein synthesis.

TABLE 1
Demographic data

Group	Age (years)	BMI (kg/m^2)	Fat mass (kg)
NS	26 ± 3	24.3 ± 1.1	18.4 ± 2.0
Insulin	27 ± 3	23.5 ± 1.4	17.5 ± 2.8
LoAA/Ins	25 ± 3	23.4 ± 1.0	15.9 ± 2.5
HiAA/Ins	26 ± 2	24.6 ± 2.1	17.2 ± 3.2
SRIH/AA	25 ± 3	24.8 ± 1.0	16.9 ± 1.2
SRIH/NS	28 ± 2	21.4 ± 1.0	16.3 ± 3.2

Data are means ± SE. Each group was composed of three men and three women. There were no significant differences among groups.

compounds were confirmed before use. Solutions were prepared under sterile precautions in the pharmacy and were shown to be bacteria and pyrogen free before use in human subjects.

Subjects. A total of 36 healthy subjects participated in the study (Table 1). The age, BMI, and fat mass were similar in each group, which had equal numbers of men and women. Each participant had a normal physical examination, normal hepatic and renal functions, and no biochemical abnormalities. Fasting blood glucose levels were normal in all participants (4–5.6 mmol/l). The study protocol was reviewed and approved by the Institutional Review Board of the Mayo Foundation. The purpose and potential risks of the study were explained to all subjects and informed written consent was obtained from each subject before participation.

Experimental design. All subjects received a standard weight-maintaining diet consisting of 20% protein, 50% carbohydrate, and 30% fat during the 3 days before the study from the General Clinical Research Center. They received dinner at 6 P.M. and a snack at 10 P.M. (5.5 kcal/kg) to avoid prolonged starvation. After 10 P.M., only water was allowed until the completion of the study. All subjects maintained their usual level of physical activity.

The studies were conducted after an overnight fast in the General Clinical Research Center at Mayo Clinic. Catheter sheaths were placed in the right femoral artery and femoral vein between 7 and 8 A.M. Hepatic vein catheters were inserted under fluoroscopic guidance, and appropriate positioning was confirmed by nonionic contrast injection (18). A femoral artery catheter was inserted through the arterial sheath. A slow infusion of normal saline was used to maintain patency of the catheters. The femoral artery sheath was used for infusion of indocyanine green to measure blood flow in the leg and splanchnic regions using indicator dye dilution techniques (18,19). The femoral artery, femoral vein, and hepatic vein catheters were used to collect blood samples.

Priming doses of L-[1-^{13}C,^{15}N]leucine (1 mg/kg), L-[^{15}N]phenylalanine (0.75 mg/kg), L-[ring-$^2H_4$]tyrosine (0.6 mg/kg), and L-[^{15}N]tyrosine (0.3 mg/kg) were given intra-venously to achieve an early plateau. A continuous infusion of L-[1-^{13}C,^{15}N]leucine (1 mg · kg^{-1} · h^{-1}), L-[^{15}N]phenylalanine (0.75 mg · kg^{-1} · h^{-1}), and L-[ring-$^2H_4$] tyrosine (0.6 mg · kg^{-1} · h^{-1}) was started and maintained for 5 h. Blood samples from the femoral artery, femoral vein, and hepatic vein were collected at baseline, 120, 130, 140, 150, 330, 340, 350, and 360 min. Each participant was randomly assigned to one of six groups. Group 1 received saline only. Groups 2–4 received an intravenous infusion of insulin (0.5 mU · kg^{-1} · min^{-1}) through a peripheral vein between time 150 and 360 min. The blood glucose was clamped at the baseline blood glucose of each participant by infusion of 20% dextrose via a peripheral vein (20). Groups 3 and 4 also received an infusion of AAs (Trophamin 10%; B. Braun Medical, Bethlehem, PA). Group 3 received a dose of AAs aimed at maintaining AA levels at baseline (0.6 ml · kg^{-1} · min^{-1}) (Ins/LoAA) while group 4 received an AA infusion aimed at elevating AA levels two- to threefold above baseline (1.5 ml · kg^{-1} · min^{-1}) (Ins/HiAA). Groups 5 and 6 received an infusion of AAs (Trophamine 10%, 1 ml · kg^{-1} · min^{-1}) (SRIH/AA) or saline (SRIH/NS), while clamping hormones during somatostatin infusion (60 ng · kg^{-1} · min^{-1}), and baseline replacement doses of insulin (0.25 mU · kg^{-1} · min^{-1}), glucagon (0.65 ng · kg^{-1} · min^{-1}), and growth hormone (3 ng · kg^{-1} · min^{-1}). The insulin dose was chosen to be the lowest possible dose that would maintain normoglycemia during somatostatin infusion (data on file). The study was completed at 360 min, and the catheters were removed and homeostasis was obtained.

Analysis of samples. Blood glucose was measured by the glucose oxidase method using a glucose analyzer (Beckman Instruments, Fullerton, CA). Hormone assays were performed as previously reported (21). Plasma insulin and growth hormone were measured by a chemiluminescent sandwich assay (based on a kit from Sanofi Diagnostics, Chaska, MN), and glucagon and cortisol were measured by a radioimmunoassay technique (based on kits from Diagnostic Products, Los Angeles, CA). Indocyanine green concentration was measured using spectrophotometry, and whole-blood arterial AA concentrations were measured using reverse-phase high-performance liquid chromatography (22). Plasma concentrations and enrichment levels of [1-^{13}C,^{15}N]leucine, [1-^{13}C]leucine, [^{15}N]phenylalanine, [ring-$^2H_4$]tyrosine, and [^{15}N]tyrosine were determined using gas chromatography/mass spectrometry (GC/MS). Fifty microliters of an internal standard solution containing 30 μg/ml norleucine, 60 μg/ml [$^2H_5$]phenylalanine hydrochloride, and 20 μg/ml [$^{13}C_6$]tyrosine (Isotec, Miamisburg, OH) was added to six standards containing leucine, phenylalanine, and tyrosine (all from Sigma-Aldrich Chemicals) ranging from 0 to 300 mmol/l, as well as to 100-μl plasma samples. The AAs from the plasma samples were isolated by ion exchange chromatography and were dried. All standards and samples were derivatized with *N*-methyl-*N*(t-butyldimethylsilyl)-trifluoroacetamide + 1% t-butyl-dimethylchlorosilane in acetonitrile (both from Regis Technologies) at room temperature overnight. The amino derivatives were separated on a 30 mm × 0.25 mm × 0.25 μm DB5MS column (J&W Chromatography, Folsom, CA) at constant flow of helium (1.1 ml/min) with the following temperature programming: 120°C initially, then ramped to 210°C at 25°C/min, to 225°C at 10°C/min, to 255°C at 25°C/min, to 265°C at 10°C/min, to 300°C at 25°C/min, and finally to 325°C at 10°C/min and held for 2 min. Under electron ionization, fragment ions were monitored at m/z 200, 201, 300, 302, and 304 for leucine, m/z 302 for norleucine, at m/z 336 and 337 for phenylalanine, at m/z 341 for [$^2H_5$]phenylalanine, at m/z 466, 467, and 470 for tyrosine, and at m/z 472 for [$^{13}C_6$]tyrosine using a Hewlett Packard 5972A MSD (Hewlett Packard, Avondale, CA) to determine the isotopic enrichment as well as concentrations of leucine, phenylalanine, and tyrosine.

Plasma α-ketoisocaproate (KIC) enrichment was measured after derivatizing the extracted KIC as its quinoxalinol-trimethyl silyl derivative (23) under electron ionization conditions in a GC/MS monitoring m/z fragment ions at 233/232. KIC concentration was measured using α-ketovaleric acid as an internal standard.

Calculations. The isotope plateau was observed during the baseline period (120–150 min) and during the insulin infusion (330–360 min). The plateau status was determined based on the observation that when the isotopic enrichment values of leucine KIC, phenylalanine, and tyrosine in different sites were plotted against time, the slopes of each of these measurements were not significantly different from zero. Mean values of isotopic enrichment values at each plateau were used for all calculations of AA kinetics.

Regional leucine kinetics. For calculations of leucine carbon flux, leucine nitrogen flux, and leucine transamination (KIC reamination to leucine) across the leg and splanchnic bed, we used the equation as previously described (17,24).

Regional dynamics of phenylalanine across splanchnic and muscle bed. We used the equations originally described elsewhere for these measurements (17). Protein synthesis in leg is assumed to represent mostly skeletal muscle, although it is acknowledged that drainage from skin, fat, and other tissues may contribute to femoral venous drainage. Whole-body (total) muscle PS and PB were estimated assuming that leg contributes 25% of whole body.

Statistics. All values are given as mean ± SE. Statistical analysis was performed using a two-way ANOVA for repeated measurements with a level for statistical significance at $P < 0.05$. Post hoc testing was performed using Student's t test. The effects on changes in protein dynamics by different concentrations of insulin and AAs, in all subjects under study, were also analyzed using a stepwise multiple regression model.

RESULTS

Hormones, glucose, and AAs (Table 2). Insulin levels were increased above baseline (120–150 min) in groups 2 (Ins), 3 (LoAA/Ins), and 4 (HiAA/Ins) during the intervention phase of the study (330–360 min). No difference in insulin levels was found among the three insulin-infused groups (NS, Ins, LoAA/Ins, and HiAA/Ins) during the baseline period or during the intervention period among Ins, LoAA, and HiAA groups (Table 2). Plasma glucose, cortisol, and growth hormone were not different among groups at baseline or during intervention while higher levels of plasma glucagon were found in HiAA as compared with the other groups during the intervention. No significant differences in hormone levels were found between SRIH/AA and SRIH/saline groups during the baseline and intervention periods (Table 2).

Arterial plasma concentrations of total AAs, leucine, phenylalanine, and tyrosine are given in Table 2. Insulin infusion decreased concentrations of total AAs, leucine, phenylalanine, and tyrosine (Table 2). Plasma AA levels

TABLE 2
Plasma hormones and glucose during baseline and during intervention

Group	Insulin (pmol/l)	Cortisol (μg/dl)	Glucagon (pg/ml)	GH (ng/ml)	Glucose (mmol/l)	Arterial AAs (μmol/l)				
						Leucine	Phenylalaline	Tyrosine	Total AAs	KIC
NS										
Baseline	29 ± 4	14.3 ± 2.3	42 ± 5	2.2 ± 1.4	5.1 ± 0.1	131 ± 8	49 ± 3	46 ± 4	1,911 ± 162	34 ± 3
Intervention	22 ± 2	11.8 ± 2.5	41 ± 4	1.3 ± 0.4	4.9 ± 0.1	137 ± 7	49 ± 3	45 ± 4	1,886 ± 166	33 ± 3
Ins										
Baseline	32 ± 6	22.8 ± 4.9	49 ± 13	1.3 ± 1.0	5.0 ± 0.1	113 ± 6	48 ± 3	50 ± 9	1,611 ± 157	31 ± 2
Intervention	159 ± 8*	17.0 ± 3.2	48 ± 9	9.0 ± 4.3	4.6 ± 0.1	63 ± 5*	40 ± 2*	34 ± 4*	1,345 ± 152*†	15 ± 1*
LoAA/Ins										
Baseline	32 ± 2	12.4 ± 2.0	47 ± 6	3.1 ± 1.8	5.0 ± 0.1	112 ± 8	48 ± 2	55 ± 5	1,790 ± 149	28 ± 4
Intervention	171 ± 9*	13.2 ± 2.4	59 ± 10	7.4 ± 2.8	4.6 ± 0.1	168 ± 25	61 ± 5	48 ± 5	1,984 ± 170	24 ± 4
HiAA/Ins										
Baseline	33 ± 8	11.8 ± 2.3	51 ± 10	0.8 ± 0.1	5.0 ± 0.1	106 ± 8	44 ± 3	45 ± 5	1,796 ± 154	25 ± 2
Intervention	182 ± 10*	15.9 ± 0.8	97 ± 10*†	7.5 ± 3.2	4.8 ± 0.1	392 ± 19*	100 ± 6*	51 ± 7	2,937 ± 172*†	33 ± 3*
SRIH-AA										
Baseline	58 ± 6	14 ± 2	47 ± 9	0.7 ± 0.1	5.8 ± 0.5	112 ± 9	50 ± 2	46 ± 4	1,501 ± 119	26 ± 2
Intervention	59 ± 6	16 ± 2	58 ± 6	1.0 ± 0.2	5.0 ± 0.1	312 ± 14*	102 ± 3*	56 ± 3*	2,611 ± 299*†	35 ± 4*
SRIH/NS										
Baseline	59 ± 4	14 ± 4	44 ± 7	0.8 ± 0.1	5.2 ± 0.3	103 ± 4	50 ± 1	42 ± 3	1,320 ± 57	26 ± 3
Intervention	57 ± 4	14 ± 3	42 ± 7	0.9 ± 0.1	5.1 ± 0.3	90 ± 4	48 ± 1	42 ± 4	1,268 ± 62	20 ± 3

Data are means ± SE. *$P < 0.001$ vs. baseline; †$P < 0.01$ vs. all other groups. GH, growth hormone.

were not different among the groups during baseline period and did not change from baseline during the second phase of the saline infusion (NS). With insulin infusion alone (Ins), total AAs, leucine, phenylalanine, tyrosine, and KIC were lower than during baseline period. These levels were also lower compared with the saline group ($P < 0.05$). Plasma AAs were not significantly different from baseline or the saline group during LoAA/Ins, while plasma AAs were higher with HiAA/Ins than baseline and saline, except for glutamate, glutamine, glycine (not shown), and tyrosine. Similarly, plasma AAs were higher during SRIH/AA infusion than during SRIH/NS.

Plasma flow and plasma isotopic enrichment. Plasma flow at baseline (150–180 min) and during intervention (330–360 min) in leg and splanchnic beds are given in Table 3. Plasma enrichment values of isotopes at plateau are shown in Tables 4 and 5. These mean values were used for all calculations.

Protein dynamics

Regional protein dynamics: leucine, phenylalanine, and tyrosine net balance. The net balance of phenylalanine and leucine in leg decreased significantly with saline infusion while the change in tyrosine balance did not reach statistical significance (Fig. 1). Insulin infusion significantly altered net balances for all three AAs in comparison with saline. The LoAA/Ins and HiAA/Ins groups showed a positive balance for phenylalanine and leucine in all subjects and a positive balance for all three AAs during HiAA/Ins. In all cases, the changes were significantly different from that of NS. Similar results were found in SRIH/AA, while no change was noted with SRIH/saline. Splanchnic net balance of the above AAs did not change with saline infusion. A significant reduction in splanchnic phenylalanine net balance was found with insulin infusion alone, while no significant changes in net balance of tyrosine and leucine were found. In the LoAA/Ins and HiAA/Ins groups, dose-dependent increases in phenylalanine and leucine net balances in the splanchnic bed were observed, while no significant change was found for tyrosine net balance. Similarly, increased net balance in the splanchnic bed was also observed during SRIH/AA, while no change was found during SRIH/saline.

Fig. 2 shows the changes in PS and PB in muscle and splanchnic beds between baseline intervention. PS in muscle decreased significantly during saline infusion, whereas no changes were observed with insulin infusion. PS increased from baseline during LoAA/Ins and HiAA/Ins. LoAA/Ins and HiAA/Ins also resulted in higher PS than during insulin. HiAA/Ins was associated with greater increase (average 75% from baseline) than the increase during LoAA/Ins (22%) ($P < 0.01$). SRIH/NS did not significantly change PS from baseline when SRIH/AA increased PS significantly from baseline and from that of SRIH/NS. The increment of SRIH/AA from baseline (45%) was lower than the increment (75%) during HiAA/Ins ($P < 0.05$). When the comparison is made with the same time points (i.e., 330–360 min) (HiAA/Ins vs. NS and SRIH/AA vs. SRIH/NS), the increments are significantly higher ($P < 0.01$) in HiAA/NS (150%) than in SRIH/AA (130%). PB in leg showed a clear trend to decrease during Ins ($P < 0.06$), but a significant decrease occurred only during LoAA/Ins, HiAA/Ins, and SRIH/AA. The change of skeletal muscle PB from the baseline during Ins was significantly different from that of NS ($P < 0.05$). In splanchnic bed, PS increased and PB decreased significantly only during the HiAA/Ins

TABLE 3
Mean plasma flow (ml/min)

	Femoral		Hepatic vein	
	Basal	Intervention	Basal	Intervention
NS	307 ± 33	352 ± 43	842 ± 75	1,069 ± 99
Insulin	283 ± 34	361 ± 30	978 ± 62	1,077 ± 18
LoAA/Ins	279 ± 38	317 ± 46	919 ± 83	1,156 ± 93
HiAA/Ins	336 ± 43	411 ± 43	1049 ± 74	1,198 ± 64
SRIH/AA	426 ± 103	398 ± 63	700 ± 52	982 ± 192
SRIH/NS	228 ± 30	224 ± 23	699 ± 69	814 ± 88

Data are means ± SE.

TABLE 4
Mean ± SE (of four time points) of isotopic enrichment of phenylalanine and tyrosine in the arterial vein (A), femoral vein (FV), and hepatic vein (HV) at the baseline (150–180 min) and during the last 30 min (330–360 min) of the infusion (intervention)

	[^{15}N]phenylalanine			[^{15}N]tyrosine			[$^{2}H_4$]tyrosine		
	A	FV	HV	A	FV	HV	A	FV	HV
NS									
Baseline	10.73 ± 0.57	8.52 ± 0.34	8.51 ± 0.31	1.54 ± 0.11	1.27 ± 0.11	1.58 ± 0.13	11.70 ± 0.48	9.15 ± 0.34	9.13 ± 0.40
Intervention	10.91 ± 0.48	8.95 ± 0.31	9.15 ± 0.33	1.44 ± 0.12	1.17 ± 0.13	1.58 ± 0.14	12.40 ± 0.48	9.90 ± 0.25	9.99 ± 0.37
Ins									
Baseline	10.87 ± 0.32	8.72 ± 0.14	8.61 ± 0.31	1.59 ± 0.10	1.28 ± 0.07	1.51 ± 0.09	12.30 ± 0.66	9.71 ± 0.53	9.70 ± 0.63
Intervention	12.52 ± 0.43	10.31 ± 0.21	9.88 ± 0.28	1.62 ± 0.07	1.24 ± 0.07	1.61 ± 0.07	15.08 ± 0.65	11.90 ± 0.57	12.10 ± 0.54
LoAA/Ins									
Baseline	10.88 ± 0.38	8.41 ± 0.47	8.72 ± 0.28	1.56 ± 0.06	1.26 ± 0.08	1.59 ± 0.05	11.31 ± 0.91	8.70 ± 0.80	9.16 ± 0.71
Intervention	9.02 ± 0.57	7.67 ± 0.48	7.92 ± 0.40	1.42 ± 0.10	1.17 ± 0.08	1.56 ± 0.07	11.90 ± 0.91	9.60 ± 0.79	9.86 ± 0.69
HiAA/Ins									
Baseline	10.46 ± 0.42	8.36 ± 0.23	8.45 ± 0.34	1.47 ± 0.11	1.15 ± 0.07	1.47 ± 0.13	12.00 ± 0.52	9.25 ± 0.37	9.35 ± 0.45
Intervention	6.50 ± 0.52	6.07 ± 0.47	6.09 ± 0.46	1.41 ± 0.10	1.26 ± 0.09	1.69 ± 0.14	12.53 ± 1.03	10.96 ± 0.84	9.72 ± 0.72
Som/AA									
Baseline	10.87 ± 0.41	8.82 ± 0.31	8.22 ± 0.27	1.79 ± 0.13	1.39 ± 0.08	1.81 ± 0.10	10.83 ± 0.88	9.34 ± 0.62	9.14 ± 0.75
Intervention	8.06 ± 0.29	7.39 ± 0.26	7.24 ± 0.26	1.69 ± 0.10	1.48 ± 0.06	2.10 ± 0.13	12.52 ± 0.92	10.94 ± 0.70	9.75 ± 0.67
Som/NS									
Baseline	11.43 ± 0.28	8.91 ± 0.21	8.86 ± 0.17	1.64 ± 0.15	1.35 ± 0.10	1.57 ± 0.21	12.92 ± 0.64	9.93 ± 0.38	9.87 ± 0.71
Intervention	12.13 ± 0.31	9.49 ± 0.24	9.85 ± 0.23	1.69 ± 0.15	1.23 ± 0.12	1.83 ± 0.21	13.70 ± 0.70	10.30 ± 0.35	10.90 ± 0.68

Data given as molar percent excess.

TABLE 5
Mean ± SE (of four time points) of isotopic enrichment of leucine and KIC in the arterial vein (A), femoral vein (FV), and hepatic vein (HV) at the baseline (150–180 min) and during the last 30 min (330–360 min) of the infusion (intervention)

	[1-^{13}C, ^{15}N]leucine			Total [^{13}C]leucine			[^{13}C]KIC		
	A	FV	HV	A	FV	HV	A	FV	HV
NS									
Baseline	4.60 ± 0.28	2.69 ± 0.17	3.10 ± 0.19	8.69 ± 0.32	6.72 ± 0.20	6.85 ± 0.22	5.89 ± 0.20	5.51 ± 0.19	6.09 ± 0.20
Intervention	4.11 ± 0.24	2.49 ± 0.18	2.89 ± 0.14	8.71 ± 0.25	7.06 ± 0.22	7.18 ± 0.20	6.34 ± 0.19	5.85 ± 0.23	6.52 ± 0.19
Ins									
Baseline	4.35 ± 0.17	2.43 ± 0.09	2.81 ± 0.13	8.59 ± 0.22	6.68 ± 0.13	6.66 ± 0.18	6.19 ± 0.12	5.77 ± 0.11	6.32 ± 0.14
Intervention	6.77 ± 0.28	4.12 ± 0.15	3.94 ± 0.19	11.30 ± 0.41	8.71 ± 0.20	7.99 ± 0.23	7.28 ± 0.19	6.83 ± 0.14	7.41 ± 0.21
LoAA/Ins									
Baseline	5.01 ± 0.27	2.69 ± 0.27	3.29 ± 0.23	8.95 ± 0.50	6.62 ± 0.45	6.91 ± 0.39	6.06 ± 0.29	5.60 ± 0.33	6.34 ± 0.30
Intervention	3.80 ± 0.39	2.38 ± 0.24	2.76 ± 0.21	6.40 ± 0.61	5.27 ± 0.42	5.39 ± 0.39	4.91 ± 0.38	4.63 ± 0.34	5.06 ± 0.39
HiAA/Ins									
Baseline	4.90 ± 0.37	2.87 ± 0.18	3.28 ± 0.24	8.91 ± 0.42	6.84 ± 0.21	6.97 ± 0.28	6.01 ± 0.26	5.50 ± 0.22	6.14 ± 0.27
Intervention	2.02 ± 0.16	1.52 ± 0.12	1.70 ± 0.13	3.74 ± 0.31	3.51 ± 0.29	3.59 ± 0.29	3.30 ± 0.23	3.25 ± 0.22	3.33 ± 0.25
Som/AA									
Baseline	5.08 ± 0.45	3.11 ± 0.29	3.09 ± 0.18	9.20 ± 0.58	7.20 ± 0.41	6.67 ± 0.25	6.16 ± 0.25	5.85 ± 0.20	6.23 ± 0.23
Intervention	2.58 ± 0.13	1.89 ± 0.06	2.00 ± 0.08	5.14 ± 0.16	4.74 ± 0.12	4.66 ± 0.12	4.55 ± 0.34	4.42 ± 0.29	4.75 ± 0.36
Som/NS									
Baseline	5.66 ± 0.31	3.13 ± 0.18	3.46 ± 0.20	9.85 ± 0.39	7.22 ± 0.30	7.10 ± 0.33	6.30 ± 0.26	5.72 ± 0.24	6.50 ± 0.25
Intervention	6.19 ± 0.31	3.31 ± 0.24	3.92 ± 0.25	10.85 ± 0.39	7.81 ± 0.34	8.09 ± 0.37	7.03 ± 0.29	6.28 ± 0.26	7.34 ± 0.31

Data given as molar percent excess.

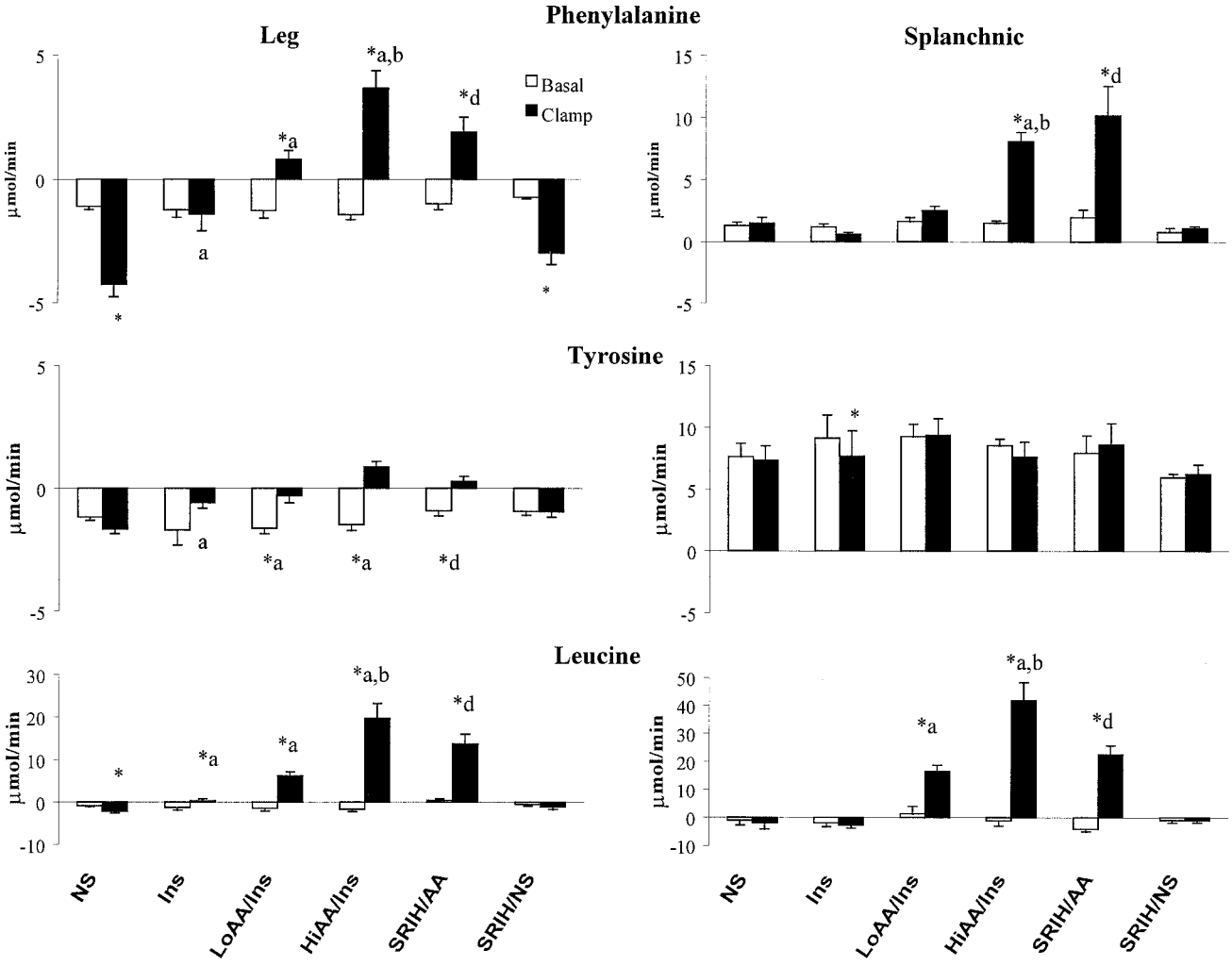

FIG. 1. Net AA balance for phenylalanine, tyrosine, and leucine across leg and splanchnic beds at basal (120–150 min) and clamp (330–360 min). The study conditions are saline infusion (NS), insulin alone (Ins), AA infusion to maintain plasma AAs during insulin infusion (LoAA/Ins), infusion at high physiological AA infusion with insulin (HiAA/Ins), and somatostatin with replacement of insulin, glucagon, and growth hormone with high physiological AAs (SRIH/AA) or saline (SRIH/S). *$P < 0.05$–01 from the baseline; a, the change from the baseline is different ($P = 0.05$–0.01) from that of NS; b, the changes are different from those of LoAA/Ins; d, the change from baseline in SRIH/AA is different from that of SRIH/NS ($P < 0.01$).

and SRIH/AA. The decrease during HiAA/Ins was greater than during Ins.

Leucine nitrogen flux and transamination. No change was found in leg leucine nitrogen flux with saline, although KIC transamination to leucine increased significantly (Fig. 3). A reduction in leucine nitrogen flux and KIC transamination to leucine was found with insulin infusion alone. With insulin, the change from baseline was significantly different from that of saline. A similar reduction was not seen during LoAA/Ins, whereas elevating AAs in HiAA/Ins increased leg leucine nitrogen flux and leucine reamination from KIC. Similarly, during SRIH/AA, an increase in leucine nitrogen flux and reamination from KIC were noted in leg, but no changes were observed with SRIH/saline. In the splanchnic bed, significant increases were found for leucine nitrogen flux and for leucine reamination with saline. Insulin infusion alone did not affect leucine nitrogen flux or leucine reamination, but the change in leucine reamination during insulin infusion was significantly different from that of saline. While LoAA/Ins did not affect leucine nitrogen flux and reamination from KIC, a marked increase was seen in the HiAA/Ins and SRIH/AA groups. No change was seen with SRIH/saline.

Multiple regression analysis. A stepwise multiple regression analysis was performed to determine the relative significance of changes in concentrations of insulin and AAs on protein dynamics (Table 6). The same results in the regression analysis were obtained regardless of whether plasma concentrations of total plasma AAs, essential AAs, phenylalanine, or leucine were used. The linearity of the regression was also tested with the addition of the square of each significant factor to the equation. However, this did not improve the regression model. Thus, there was a linear relationship throughout for the contributing variables. In Table 6, the results using changes in concentrations of leucine are shown. In summary, only changes in insulin concentrations were shown to be significant in determining leg PB while changes in both insulin and AA concentrations were shown to determine changes in leg PS and leg net balance of phenylalanine and leucine as well as leucine nitrogen flux and leucine reamination from KIC. Insulin and AAs have a significant interaction in

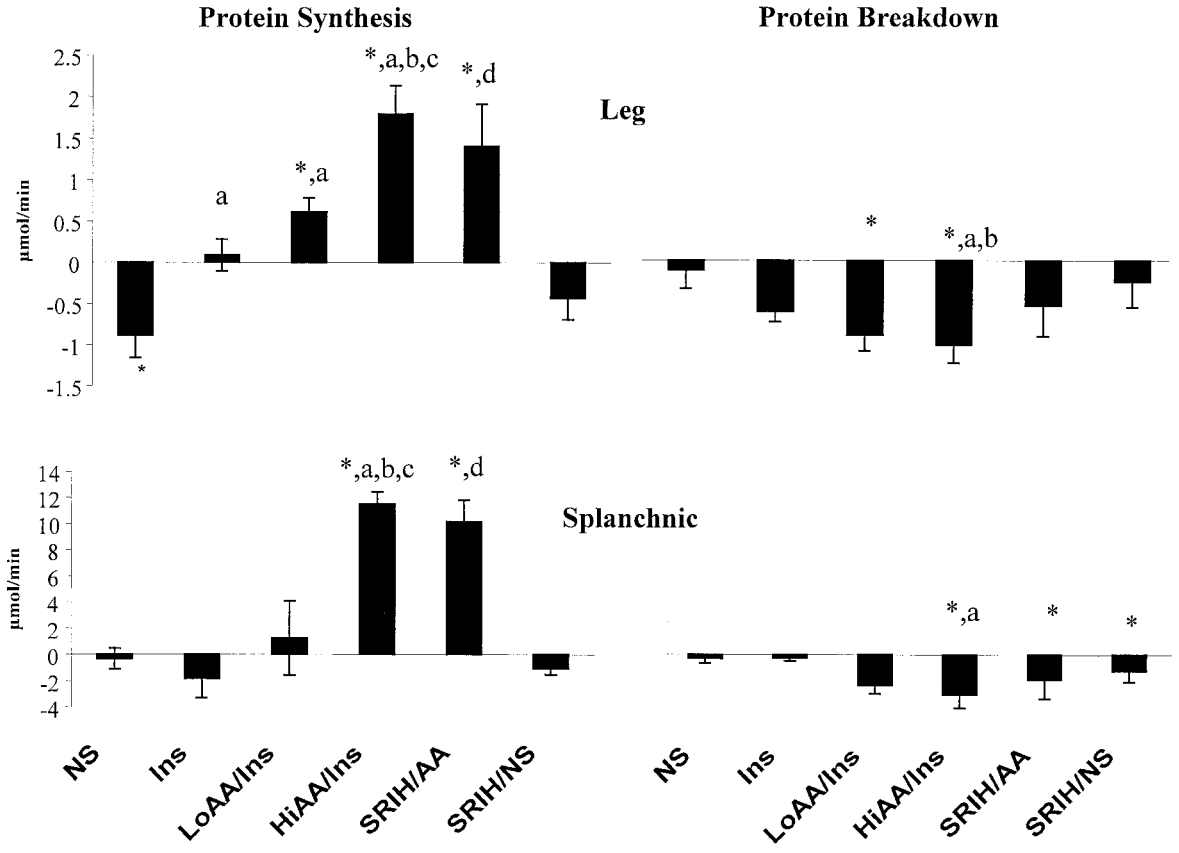

FIG. 2. Changes in PS and PB from baseline values. Both muscle and splanchnic regions are given. The calculation of muscle assumes that leg represents 25% of total muscle mass in the body. *difference from baseline, $P < 0.05–0.001$; a, different from NS ($P < 0.05–0.001$); b, different from Ins ($P < 0.05–0.001$); c, different from LoAA/Ins ($P < 0.001$); d, different from SRIH/NS.

determining leg PS. Insulin effects on leg net balance of phenylalanine were shown to be related to changes in AA concentrations (significant interaction factor, Ins* AA). In contrast, only changes in AA concentrations were shown to determine splanchnic PB and PS, leucine nitrogen flux, and net balance of phenylalanine. AAs have effects on all parameters of leucine kinetics in leg and splanchnic bed. Insulin has independent effects only on leucine nitrogen flux (leg), KIC to leucine flux (leg), net leucine balance in leg, and splanchinc regions. The insulin effect on KIC to leucine in splanchnic bed has interaction with AAs.

DISCUSSION

The main objective of the current study was to investigate the effect of AAs alone or in combination with insulin on regional protein dynamics. The results from the study demonstrated that, like insulin (2), AAs have a differential effect on protein dynamics in muscle and splanchnic beds. AAs alone (with basal insulin levels) and in combination with high physiological insulin enhanced PS rates in both muscle and splanchnic beds. In muscle, multiple regression analysis showed that insulin had an inhibitory effect on PB, and AAs did not further increase this inhibitory effect. In contrast, insulin had no inhibitory effect on splanchnic PB, whereas AAs had a profound inhibitory effect on splanchnic PB. Insulin and AAs had an additive effect on muscle PS and net AA balance across leg, while no such additive effect was observed in the splanchnic bed.

The current study further confirmed that during the postabsorptive state, muscle released AAs to the systemic circulation due to an imbalance between PB and PS. As has been shown in a previous study (2), the splanchnic bed is in a positive balance for phenylalanine and tyrosine. A combination of insulin and AAs, following a mixed meal, resulted in a net positive AA balance in both the muscle and splanchnic beds. In muscle bed, a positive net AA balance was achieved mainly by the insulin effect on muscle PB and the AA effect on muscle PS. The net increase in AA balance in splanchnic bed resulted by an inhibition of PB and stimulation of PS by AAs.

The AAs were infused into individuals while at either a basal insulin level (SIRH+AA) or high physiological insulin levels (HiAA/Ins). It is, therefore, not possible to determine whether the effect of AAs is totally independent of insulin. However, studies performed in people with type 1 diabetes indicated that during insulin deficiency, high circulating AAs (mostly branched chain) are associated with increased AA uptake in the splanchnic bed and an increased PS rate (17). Together, these data indicate that AAs are predominant stimulants of splanchnic PS. This increased PS, however, does not seem to occur in liver tissue, because in a previous study, infusion of AAs along with insulin had no stimulatory effect on liver tissue PS (25). The splanchnic bed includes the gut, and it is possible that part of the increase in splanchnic PS is due to increased gut PS. A previous study in people with type 1 diabetes demonstrated that during insulin deprivation, gut mucosal PS is reduced (26). Rodents rendered diabetic by

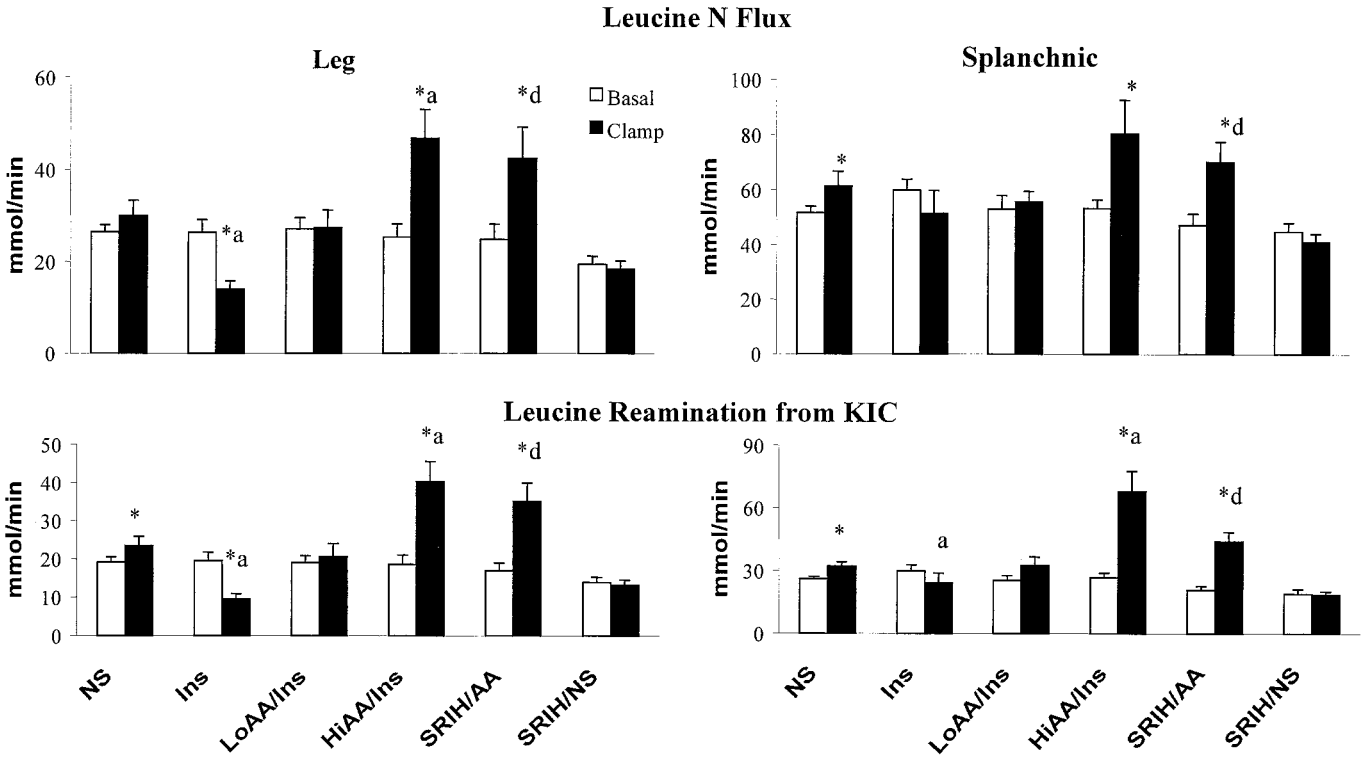

FIG. 3. Leucine nitrogen flux and leucine reamination from KIC are given in this figure. The abbreviations for different groups are the same as in Fig. 1. *the clamp period is different ($P < 0.05$–01) from the basal state; a, the changes from baseline in various groups are different from that of NS ($P < 0.05$–1); d, the change from baseline is different in SRIH/HiAA and SRIH/NS ($P < 0.01$).

streptozotozin are reported to have an increased gut protein content which was thought to be due to increased food intake, which would increase the AA load (27). Another possibility is that liver, and possibly gut, synthesizes proteins and exports them into the systemic circulation.

Another major effect of AAs in the current study was on splanchnic PB. Liver perfusion studies have shown that AAs play a key role in the regulation of liver PB (28,29). AAs, especially branched chain AAs, are reported to be the key inhibitors of liver PB in these perfusion studies (16). Consistent with these previous liver perfusion studies, the results from the current study demonstrated that, in humans, AAs are key inhibitors of splanchnic PB. The study did not specifically demonstrate whether these changes occur in liver or other abdominal organs. It is also possible that these proteins include structural proteins and circulating proteins that are usually catabolized in liver. Evidence based on in vitro studies indicates that long-lived proteins, which constitute 90% of total cellular proteins in liver, are degraded in lysosomes through

TABLE 6
Regression analysis of factors affecting regional protein dynamics (phenylalanine model) and leucine kinetics

	ΔInsulin		ΔLeucine		ΔInsulin * ΔLeucine		Regression (r^2)
	β ± SEM	*P* value	β ± SEM	*P* value	β ± SEM	*P*	
Phenylalanine model							
PB leg	−0.023 ± 0.007	0.004		NS		NS	0.20
PB splanchnic		NS	−0.007 ± 0.003	0.028		NS	0.11
PS leg	0.03 ± 0.01	0.008	0.007 ± −0.001	0.0001		10.08	0.62
PS splanchnic		NS	0.05 ± 0.005	0.0001		NS	0.76
Net bal leg	0.068 ± 0.011	0.0001	0.011 ± 0.002	0.0001	−0.0002 ± 0.00005	0.012	0.77
Net bal splanchnic		NS	0.073 ± 0.005	0.001		NS	0.85
Leucine model							
Leu N flux leg	−0.236 ± 0.094	0.017	0.017 ± 0.107	0.0001		NS	0.77
Leu N flux splanchnic		NS	0.119 ± 0.019	0.0001		NS	0.51
KIC to Leu leg	−0.212 ± 0.073	0.007	0.102 ± 0.008	0.0001		NS	0.83
KIC to Leu splanchnic		0.61	0.102 ± 0.021	0.0001		0.022	0.79
Net bal leg	0.228 ± 0.041	0.0001	0.067 ± 0.004	0.0001		NS	0.90
Net bal splanchnic	0.346 ± 0.087	0.0004	0.139 ± 0.009	0.0001		NS	0.89

The results represent the significant effects of changes (Δ) in insulin, leucine, and interaction of leucine and insulin (ΔInsulin * ΔLeucine) on changes in PB, PS, and net balance (Net bal) in leg and splanchnic beds. Similar data are provided for leucine (Leu), nitrogen (N) flux, KIC, and leucine transamination and net balance.

macro- and microautophagic processes, and these proteins are regulated by AAs (30). In contrast, short-lived proteins ($t_{1/2}$ = 30 min) are unlikely to be controlled in this degradation pathway by AAs (31). It is, therefore, likely that the inhibitory effect of AAs on liver proteins is not a global effect, but is a selective effect.

The stimulatory effect of AAs on splanchnic and muscle PS is clearly demonstrated in the current study. It has been reported that this stimulatory effect occurs at the posttranscriptional level (12). It has been shown that AAs increase the phosphorylation of phosphorylated heat and acid stable protein-1 (PHAS-1) and P70 kinase in skeletal muscle independently of insulin (13).

Another important action of AAs was on leucine transamination. AAs substantially increased leucine transamination in both splanchnic and muscle beds. As has been shown in our previous study (2), insulin decreased leucine transamination in both splanchnic and liver tissues. When people with type 1 diabetes were studied, a substantial increase in leucine transamination was observed during insulin deprivation (17), and insulin infusion decreased leucine transamination in a dose-dependent manner in nondiabetic people (2). Because circulating AA levels decreased in those studies, it was not clear whether the changes in leucine transamination are secondary to an insulin-induced fall in circulating AAs. The current study clearly demonstrated that insulin has an independent effect (Table 6) on leucine transamination in the muscle bed but had no significant effect on the splanchnic bed. In contrast, AAs have independent effects on leucine transamination in both the splanchnic and muscle beds. These results are consistent with the observation in a previous study that isotopic enrichment of circulating KIC is lower than in muscle tissue fluid when a mixed meal was administered (32). An increased AA flux in the portal vein (32) following a mixed meal might stimulate leucine transamination in liver. Insulin has an additive effect on KIC conversion to leucine in the splanchnic bed, thus facilitating increased leucine formation in splanchnic bed. These studies confirm that when leucine/KIC ratio decreases, leucine transamination decreases and vice versa. This will ensure the maintenance of leucine concentration in the circulation. This process may be important in maintaining the supply of this essential AA in the splanchnic bed (more specifically in liver) to facilitate the increased PS, since PB in muscle and, thus, net AA output from muscle bed are decreased by insulin and AAs.

The net balance of three separate AAs is given in Fig. 1. Phenylalanine and tyrosine balance in the muscle bed reflects the differences between PS and PB, since these two AAs are either incorporated into protein or released from protein during protein degradation (breakdown). In contrast, tyrosine balance in the splanchnic bed represents the balance of four processes—PB and phenylalanine conversion to tyrosine on one side and PS and tyrosine catabolism on the other side. Leucine balance in both the muscle and splanchnic bed represents the difference between PB and leucine transamination (KIC to leucine) minus PS and leucine transamination (leucine to KIC). It is, therefore, not surprising that the net balances of these different AAs did not show a similar pattern. However, all three AA balances showed similar directional changes in the muscle bed consistent with the directional changes in PS and PB.

In summary, the current study demonstrated that AAs are key regulators of PS and PB in leg muscle beds. In the muscle bed, AAs have a profound effect on PS independently and have an additive effect with insulin. In the case where splanchnic bed AAs are the major regulators of PS and PB, insulin plays no significant role. These results indicate that AA supply is crucial for both splanchnic and muscle beds for synthesizing proteins. Amino acids also are key regulators of the transamination of leucine, a key process involved in nitrogen transfer between organs and among AAs. Overall, it appears that AAs and insulin play complementary roles. While insulin is the key regulator of muscle PB, AAs are crucial to maintain muscle PS. Amino acids are the key regulators of both splanchnic PS and PB.

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