Current recommended parenteral protein intakes do not support protein synthesis in critically ill septic, insulin-resistant adolescents with tight glucose control

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Objective: **To investigate the effects of insulin infusion and increased parenteral amino acid intakes on whole body protein balance, glucose kinetics, and lipolysis in critically ill, insulin-resistant, septic adolescents.**

Design: **A single-center, randomized, crossover study.**

Setting: **A medicosurgical intensive care unit in a tertiary university hospital.**

Patients: **Nine critically ill, septic adolescents (age 15.0 ± 1.2 yrs, body mass index 20 ± 4 kg m^{-2}) receiving total parenteral nutrition.**

Interventions: **Patients received total parenteral nutrition with standard (1.5 $g \cdot kg^{-1} \cdot day^{-1}$) and high (3.0 $g \cdot kg^{-1} \cdot day^{-1}$) amino acid intakes in a 2-day crossover setting, randomized to the order in which they received it. On both study days, we conducted a primed, constant, 7-hr stable isotope tracer infusion with [1-^{13}C]leucine, [6,6-$^{2}H_2$]glucose, and [1,1,2,3,3-$^{2}H_5$]glycerol, in combination with a hyperinsulinemic euglycemic clamp during the last 3 hrs.**

Measurements and Main Results: **Insulin decreased protein synthesis at standard amino acid and high amino acid intakes ($p < .01$), while protein breakdown decreased with insulin at standard amino acid intake ($p < .05$) but not with the high amino acid intake. High amino acid intake improved protein balance ($p < .05$), but insulin did not have an additive effect. There was significant insulin resistance with an M value of ~3 ($mg \cdot kg^{-1} \cdot min^{-1}$)/($mU \cdot mL^{-1}$) which was 30% of reported normal values. At high amino acid intake, endogenous glucose production was not suppressed by insulin and lipolysis rates increased.**

Conclusion: **The current recommended parenteral amino acid intakes are insufficient to maintain protein balance in insulin-resistant patients during tight glucose control. During sepsis, insulin decreases protein synthesis and breakdown, and while high amino acid intake improves protein balance, its beneficial effects may be offset by enhanced endogenous glucose production and lipolysis, raising concerns that insulin resistance may have been exacerbated and that gluconeogenesis may have been favored by high amino acid intakes. Dose-response studies on the effect of the level of amino acid intakes (protein) on energy metabolism are needed. (Crit Care Med 2011; 39:2518–2525)**

Key Words: children; clamp; insulin resistance; kinetics; protein; sepsis

During sepsis and inflammation, cytokine release through nuclear factor κB activation induces suppression of insulin receptor signaling via reduced tyrosine phosphorylation of insulin receptor substrate 1 and insulin receptor substrate 2, as well as decreased activation of phosphatidylinositol 3-kinase and protein kinase B, resulting in insulin resistance (1). Insulin has pleiotropic effects, and insulin resistance affects both protein and energy metabolism, in addition to multiple other processes (2).

The inflammatory response elicited by sepsis induces altered whole body protein turnover with increased hepatic synthesis of inflammatory/immune proteins and decreased synthesis of myofibrillar and sarcoplasmic muscle proteins (3), due to changes in translation initiation and reduction of translation efficiency (4, 5). Changes in protein turnover during sepsis are organ specific and are influenced by age (6, 7).

Sepsis also triggers a profound catabolic response characterized by increased muscle protein breakdown. Loss of lean body mass is caused by activation of the ubiquitin-proteasome proteolytic pathway in muscle initiated by activation of caspase 3 (8, 9). Furthermore, there appears to be a link between muscle wasting and insulin resistance (10, 11).

Most critically ill septic pediatric and adult patients present with insulin resistance and receive insulin therapy to maintain plasma glucose concentrations within an acceptable range (12). Nevertheless, protein and energy supplies are rather variable in pediatric critically ill patients and are constrained by fluid restriction, feeding intolerance, and lack of

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evidence of appropriate nutrient requirements under these conditions (13).

There is a close interrelationship between protein (nitrogen) and energy metabolism. Protein accretion will not occur without a sufficient energy supply, and a sufficient energy supply will not support anabolism in the absence of adequate protein (nitrogen) intake. We have shown that the parenteral protein requirements of critically ill children have been based on limited data (13) and that, at currently recommended enteral protein intakes, critically ill pediatric patients remain in significantly negative protein balance (14).

On the basis of the knowledge that amino acid availability is a key component in the drive for protein synthesis (15), we hypothesized that critically ill, insulin-resistant, septic adolescents would require higher parenteral amino acid intakes to maintain protein balance in the presence of insulin administration. For this purpose, we conducted a 2-day, prospective, randomized, crossover study to compare the effects of a high parenteral amino acid intake of 3 $g \cdot kg^{-1} \cdot day^{-1}$ vs. the standard recommended amino acid intake of 1.5 $g \cdot kg^{-1} \cdot day^{-1}$ (16) on the rates of protein turnover and glucose and lipid kinetics in critically ill, insulin-resistant, septic adolescents under basal conditions and while receiving a hyperinsulinemic euglycemic clamp (HEC), aiming at maintaining plasma glucose concentrations at rates equivalent to a tight glucose control regimen (90–110 mg dL^{-1}, 5.0–6.1 mM) (17). We conducted the HEC study during the isotope tracer infusion to assess for glucose disposal, which mostly occurs in skeletal muscle, and with the aid of stable isotope tracers of glucose, we could determine endogenous glucose production during the clamp. Hence, both peripheral and hepatic insulin resistances were assessed. Substrate metabolism and the response to inflammation and insulin differ within the pediatric population. There are known changes in insulin sensitivity during puberty (18). Hence, we focused on adolescents because this age group is metabolically different from infants and children, and it is important that metabolism in each pediatric age group is separately studied.

MATERIALS AND METHODS

Patients

The study was approved by the Baylor College of Medicine Institutional Review Board (Houston, TX), and informed consent was obtained from the parents. Studies were conducted in the pediatric intensive care unit at Texas Children's Hospital (Houston, TX). Hyperglycemic (>120 mg dL^{-1}, >6.7 mM) adolescents (13–18 yrs of age) with a diagnosis of severe sepsis, septic shock, or systemic inflammatory response syndrome (19) and receiving total parenteral nutrition were considered eligible. Only adolescents aged 13–18 yrs were included in the study as we have previously observed that metabolic processes greatly vary with age (14). All patients had drawing and infusing intravascular catheters and had received complete parenteral feedings for at least 24 hrs before the study. All were assessed for severity of disease by the Pediatric Logistic Organ Dysfunction score (20), Pediatric Risk of Mortality III score (21), and sexual maturity was assessed by Tanner classification (22, 23). Four patients received hydrocortisone at stress doses of 2 mg/kg intravenously followed by 50 mg intravenously every 6 hrs for empirical treatment of adrenal insufficiency in eight patients and suspicion of adrenal suppression in one patient. Patients with metabolic diseases, diabetes mellitus, primary liver, or renal failure were excluded.

A total of 13 consecutive, hemodynamically stable, critically ill adolescents admitted to the pediatric intensive care unit were included.

Of the 13 adolescents, nine patients (age 15.0 ± 1.2 yrs, body mass index 20 ± 4 kg m^{-2}, Pediatric Risk of Mortality score 11 ± 4) were enrolled in the actual 2-day study and randomized to receive first either the standard (SAA) or high (HAA) amino acid intake, and the alternate level of parenteral amino acid intake was supplied over the next 24 hrs (Fig. 1). Four patients (age 16.5 ± 3.6 yrs, body mass index 25 ± 9 kg m^{-2}, Pediatric Risk of Mortality score 6 ± 3) with similar conditions participated in a 1-day pilot study to determine the contribution of ^{13}C from the dextrose infusion to the recovery of $^{13}CO_2$ from ^{13}C-labeled leucine oxidation (24). These pilot patients received an HEC *without* additional infusion of stable isotope tracers (see below).

The characteristics of the patients are described in Tables 1 and 2. One patient randomized to start first with the SAA intake group died the next day after the first study (SAA) was completed; therefore, the HAA intake arm of the study could not be conducted. In a second patient, a technical error occurred during the insulin infusion protocol on the study day with SAA intake. Therefore, complete 2-day study data were available on seven patients. Data available from the two incomplete studies were included according to the intention-to-treat principle. In three patients with severe insulin resistance, plasma glucose levels did not decrease within the first hour of the HEC study. Insulin infusion rates were increased until plasma glucose levels decreased to achieve normoglycemia. Due to this approach, the tracer infusion and clamp study in these three patients was extended to 4 hrs instead of 3 hrs to achieve steady-state normoglycemia for at least 1 hr. Interestingly, the same situation occurred on both study days for these three patients, who appeared to be extremely insulin resistant. Two of these patients were receiving glucocorticoids.

Experimental Design

The experimental design followed involved two 24-hr dietary study periods in a randomized, crossover fashion, where the subjects received for 24 hrs a specific parenteral level of amino acid intake (SAA or HAA) on study day 1, followed by the alternate parenteral level of amino acid intake on the next day (Fig. 1). Each patient received two dietary study days and two tracer-clamp studies.

Parenteral Dietary Intake

Patients were adapted to the level of study protein intake for at least 16 hrs before the tracer infusion study and continued to receive the randomized level of parenteral amino acid intake during the tracer infusion study period at the baseline and during the HEC period. The amino acid composition of the total parenteral nutrition provided to

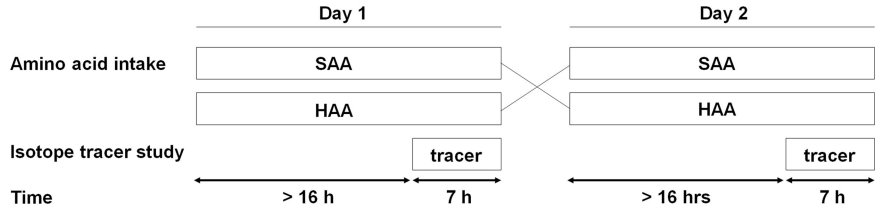

Figure 1. Schematic presentation of the study protocol in nine critically ill septic adolescents randomized to either standard or high parenteral amino acid intake in a crossover design: standard amino acid (*SAA*) intake, 1.5 $g \cdot kg^{-1} \cdot day^{-1}$; high amino acid (*HAA*) intake, 3 $g \cdot kg^{-1} \cdot day^{-1}$.

the patients is shown in Table 3. Energy intake provided as parenteral glucose and lipids was prescribed by the clinical team according to standard care. The total energy intake supplied was slightly higher in the high protein intake, because it is physically impossible to provide higher protein intake without modifying the energy provided by carbohydrates and fat. However, the difference in caloric intake for both study days was not significant (Table 1).

Tracer Infusion Studies

On each study day, the patients received a 7-hr, primed, continuous, stable isotope tracer infusion (Fig. 2). The first 4 hrs was the basal period, and during the last 3 hrs of the tracer study, the patients received the HEC. Isotope tracers were purchased from Cambridge Isotope Laboratories (Andover, MA) and were tested for sterility and pyrogenicity. During each tracer study, the patients received an intravenous, primed, continuous, 7-hr tracer infusion of L-[1-^{13}C]leucine (6 $\mu mol \cdot kg^{-1}$, 6 $\mu mol \cdot kg^{-1} \cdot hr^{-1}$), D-[6,6-2H2]glucose (25 $\mu mol \cdot kg^{-1}$, 30 $\cdot \mu mol \cdot kg^{-1} \cdot hr^{-1}$), and 1,1,2,3,3-2H5]glycerol (30 $\mu mol \cdot kg^{-1}$, 3 $\mu mol \cdot kg^{-1} \cdot hr^{-1}$) for prime and constant infusion rates, respectively. The bicarbonate pool was primed with 2.1 $\mu mol \cdot kg^{-1}$ ^{13}C-labeled sodium bicarbonate as previously described (25). Four hours after initiation of the tracer infusions, an HEC study was conducted as described below (Fig. 2). At the end of the first study day, the parenteral amino acid intake was changed to the alternate level of amino acid intake, and again, the tracer infusion protocol was repeated (Fig. 1).

HEC

Four hours after the initiation of the tracer infusions, an HEC study was conducted as previously described (24, 26). In brief, a 3-hr infusion of insulin (Actrapid, Novo Nordisk, Princeton, NJ), dissolved in sterile isotonic sodium chloride, was started at 80 $mU \cdot m^2 \cdot min^{-1}$ to achieve both normoglycemia between 90 and 110 mg dL^{-1} (5.0–6.1 mM) and a plasma insulin concentration of >100 $\mu U \cdot mL^{-1}$. During the insulin infusion, the whole blood glucose concentration was monitored at the bedside every 5–10 mins. To maintain the plasma glucose concentration between 90 and 110 $mg \cdot dL^{-1}$ and the enrichment of D-[6,6-$^2H_2$]glucose at a steady state for the duration of the study, a 30% glucose solution (Baxter, Deerfield, IL) enriched at 3.5% with D-[6,6-$^2H_2$]glucose was infused as previously described ("hot Ginf") (26).

Table 1. Demographic and nutritional data in nine critically ill, septic adolescents[a]

Characteristic	Standard Amino Acid Intake	High Amino Acid Intake	*p*
Age (yrs)	15.0 ± 1.2		
Gender (male, female)	3, 6		
Body mass index ($kg \cdot m^{-2}$)	20 ± 4		
Tanner score	4.0 ± 0.9		
Resting energy expenditure according to Schofield (61) ($kcal \cdot kg^{-1} \cdot day^{-1}$)	29.3 ± 6.0		
Pediatric intensive care unit length of stay at the start of the study (days)	5.9 ± 3.6	7.0 ± 3.6	.53
Pediatric Logistic Organ Dysfunction score (24)	9 ± 11	6 ± 7	.61
Pediatric Risk of Mortality III score (25)	10 ± 4	8 ± 4	.65
C-reactive protein ($mg \cdot dL^{-1}$)	16.5 ± 9.4	15.1 ± 12.0	.61
Highest glucose from prior study ($mg \cdot dL^{-1}$)	182 ± 36	186 ± 81	.64
Catecholamines (n)	1	0	
Glucocorticoids (n)	4	3	
Protein intake ($g \cdot kg^{-1} \cdot day^{-1}$)	1.5 ± 0.2	2.8 ± 0.4	<.001
Caloric intake ($kcal \cdot kg^{-1} \cdot day^{-1}$)	32.7 ± 10.0	37.8 ± 9.9	.36
TPN leucine intake ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	45.5 ± 6.6	83.9 ± 15.6	<.001
TPN glucose intake (($mg \cdot kg^{-1} \cdot min^{-1}$)	4.4 ± 1.7	4.5 ± 1.5	.35
TPN glycerol intake ($\mu mol \cdot kg^{-1} \cdot hr^{-1}$)	26.8 ± 18.5	22.2 ± 16.3	.19
Protein calories ($kcal \cdot kg^{-1} \cdot day^{-1}$)	5.8 ± 0.8	11.2 ± 1.4	<.001
Carbohydrate calories ($kcal \cdot kg^{-1} \cdot day^{-1}$)	22.5 ± 9.5	23.8 ± 10.3	.35
Lipid calories ($kcal \cdot kg^{-1} \cdot day^{-1}$)	5.7 ± 3.9	4.7 ± 3.5	.19
Glucose before HEC ($mg \cdot dL^{-1}$)	168 ± 58	172 ± 62	.75
Glucose during HEC ($mg \cdot dL^{-1}$)	98 ± 6	101 ± 15	.78
Insulin plasma levels at the baseline ($\mu U \cdot mL^{-1}$)	32 (17–122)	51 (29–153)	.16
Insulin plasma levels during HEC ($\mu U \cdot mL^{-1}$)	144 (94–2385)	168 (93–2239)	1.0

TPN, total parenteral nutrition; HEC, hyperinsulinemic euglycemic clamp.
[a]All values are mean ± SD.

Table 2. Characteristics of nine critically ill, septic adolescents included in the 2-day study

Patient	Diagnosis	Tanner Score	Body Mass Index ($kg\ m^{-2}$)	Gender	Mechanical Ventilation	Steroids	Age (yrs)	Pressors
1	Methicillin-resistant *Staphylococcus aureus* myositis	3	15.8	Male	Yes	Yes	17.0	Yes
2	Acute lymphatic leukemia, viral sepsis	5	24.2	Female	Yes	Yes	16.3	No
3	Peritonitis due to small bowel perforation	4	20.7	Female	Yes	No	13.6	No
4	Sepsis post lung and kidney transplantation	3	14.8	Male	Yes	Yes	15.9	No
5	Sepsis post spinal surgery	5	29.3	Female	Yes	No	14.4	No
6	Methicillin-susceptible *Staphylococcus aureus* pneumonia	4	21.4	Female	Yes	Yes	15.8	No
7	Peritonitis due to small bowel perforation	3	19.6	Male	Yes	No	13.9	No
8	Methicillin-resistant *Staphylococcus aureus* myositis	4	19.7	Female	Yes	No	14.4	No
9	Methicillin-susceptible *Staphylococcus aureus* pneumonia	5	18.5	Female	Yes	No	14.9	No

Measurements and Sample Analysis

Arterial blood samples were obtained at frequent intervals (Fig. 2), centrifuged (12 min, 3000 rpg, 4°C), and frozen at −80°C until analysis. We used plasma α-ketoisocaproate enrichment, which is intracellularly produced from the infused leucine tracer. Plasma isotope enrichment of [1-^{13}C]α-ketoisocaproate, D-[6,6-$^{2}H_2$]glucose, and [1,1,2,3,3-$^{2}H_5$]glycerol was determined as previously described (27–30). Carbon dioxide production was obtained with the respiratory profile monitor (CO_2SMO Plus, Novametrix Medical Systems, Wallingford, CT) during the last 40 mins of the baseline period and during the last 30 mins of the HEC period. Enrichment of $^{13}CO_2$ in whole blood was obtained as previously described (25, 31). Plasma samples for insulin were analyzed with standard human insulin specific radioimmunoassay techniques. Amino acids in plasma were determined as previously described (32) by anion exchange chromatography with ninhydrin detection on a Biochrom 30 amino acid analyzer (Biochrom, Cambridge, England).

Table 3. Amino acid composition of formulas administered to the adolescents[a]

Amino Acid	Aminosyn (10%) (n = 8)	Clinisol (15%) (n = 1)
Essential		
Histidine	300	894
Isoleucine	660	749
Leucine	1000	1040
Lysine	1050	1180
Methionine	172	749
Phenylalanine	298	1040
Threonine	400	749
Tryptophan	200	250
Valine	500	960
Nonessential		
Arginine	1018	1470
Alanine	993	2170
Asparagine	0	0
Aspartate	700	434
Cysteine	0	0
Glutamine	0	0
Glutamate	738	749
Glycine	500	1040
Proline	722	894
Serine	530	592
Tyrosine	270	39
Taurine	0	0

[a]All values are given in mg/100 mL. Composition provided by Aminosyn (Hospira, Lake Forest, IL) and Clinisol (Baxter, Deerfield, IL).

Calculations

Whole body kinetics of protein and lipids were calculated by conventional isotope dilution equations using a stochastic model during steady-state enrichment (33), and glucose kinetics were estimated using the Steele equation (34).

$^{13}CO_2$ Recovery During 30% Dextrose Infusion. To determine the contribution of ^{13}C originating from the 30% dextrose infused during the HEC period to $^{13}CO_2$ produced during leucine oxidation, we conducted an identical clamp study without the tracer infusion (24) in four patients with similar characteristics. Using linear regression, we established the $^{13}CO_2$ background enrichment in relation to the elevation of the glucose infusion rate during the HEC period. For every additional mg·kg^{-1}·min^{-1} of glucose infused during the HEC period in our pilot studies, $^{13}CO_2$ enrichment increased by 0.31 atoms percent excess × 10^3. Thus, for every additional mg·kg^{-1}·min^{-1} of glucose infused during the HEC period in the patients enrolled in the actual study, we subtracted 0.31 atoms percent excess × 10^3 enrichment of the $^{13}CO_2$ blood enrichment (atoms percent excess × 10^3), because this amount was contributed by the 30% dextrose infused, and the remaining enrichment originated from L-[1-^{13}C]leucine oxidation.

Glucose Metabolism. Estimates of whole body glucose kinetics were made at the isotopic steady state during the baseline and HEC periods. Mean values of plasma D-[6,6-$^{2}H_2$]glucose enrichment (mole fraction percent excess) and of the exogenous glucose infusion rate were used for data calculation. Under steady-state conditions, the total glucose rate of appearance is equal to the rate of disappearance (34). The rates of glucose disappearance reflect glucose utilization. Total endogenous glucose production rates (19), insulin sensitivity ([mg·kg^{-1}·min^{-1}]/[·μU·mL^{-1}]) (35), and insulin-stimulated glucose disposal (mg·kg^{-1}·min^{-1}) (36) were estimated as previously described (Supplemental Appendix [see Supplemental Digital Content 1, http://links.lww.com/CCM/A288]).

Protein Metabolism. Whole body plasma leucine flux, an index of protein metabolism, and leucine oxidation were calculated as previously described (30). We have shown that 69.18 is the correction factor for $^{13}CO_2$ under recovery obtained from parenterally fed critically ill children (31). Whole body protein turnover was calculated from the model described by Waterlow (37).

Lipid Metabolism. As part of their parenteral nutrition, the patients were provided with lipids intravenously which contained 2.25 mg·mL^{-1} glycerol. Lipolysis was calculated by subtracting the glycerol intake (tracer infusion + glycerol intake through parenteral nutrition) from the glycerol flux (rate of appearance) (28).

Statistical Analysis

From our previous data on protein turnover at random different levels of protein intake in critically ill children (38), we estimated that eight patients with complete data would detect a difference of 20% (80% power, type I error of 5%) in protein balance. The Shapiro-Wilk normality test was used to determine data normality. Comparison between the two different amino acid intakes at the baseline and during the HEC period was made using the repeated measurements analysis of variance, after which Student's paired *t* test was used for normally distributed data. For nonparametric data, the Wilcoxon matched pairs test was used. Data are presented as the mean ± SD. Statistical significance was considered at $p < .05$. Repeated measures analysis of variance was used to analyze the effect of insulin on the parameters of glucose, lipid, and protein metabolism over time and between normal and HAA intake. Data were analyzed with Graphpad Prism 5.0.3 (Graphpad Software, LA Jolla, CA).

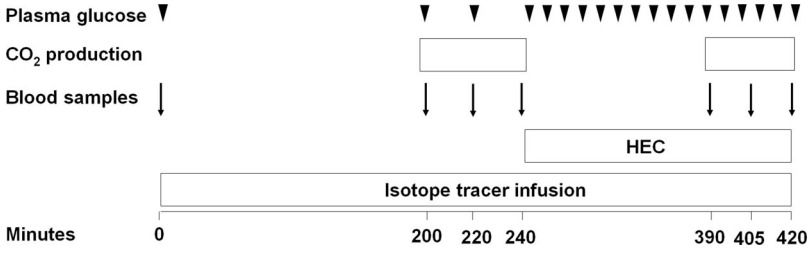

Figure 2. Schematic presentation of the tracer infusion studies during both study days in nine critically ill septic adolescents receiving either standard or high parenteral amino acid intake. *Black triangles* indicate time points of plasma glucose measurements, *arrows* indicate time points for plasma collection for isotopic enrichment measurements, and *open rectangles* represent the time period in which carbon dioxide production measurements take place. *CO_2*, carbon dioxide; *HEC*, hyperinsulinemic euglycemic clamp.

RESULTS

Patients

Demographic and nutritional data of the nine critically ill, septic adolescents are presented in Table 1. There were no differences in the total energy intake provided on both study days (Table 1).

Insulin Sensitivity and Glucose Kinetics

The maximum plasma glucose concentrations did not differ between both study days before the onset of the tracer studies (Table 1). As expected, the plasma glucose concentration differed between the baseline and HEC periods for SAA and HAA intakes ($p < .001$; Table 1). Plasma insulin concentrations for the baseline period did not differ between the dietary groups (Table 1). However, there was considerable variability in the plasma insulin concentrations during the HEC period in both the SAA and HAA groups because of three highly insulin resistant patients.

Endogenous glucose production decreased significantly with insulin administration during the HEC period for both the SAA and HAA intakes ($p < .05$), but it was not fully suppressed during the HAA intake (Fig. 3*A*).

Insulin-stimulated glucose disposal, an index of peripheral insulin sensitivity (35), was not different between the dietary groups ($p > .05$). However, these values were about 30% to 35% of the reported values of 14.2 $mg \cdot kg^{-1} \cdot min^{-1}$ in healthy adolescents (39), confirming that our patients presented significant peripheral insulin resistance (Fig. 3*B*).

There was no difference in the glucose infusion rate required during the HEC period to maintain normoglycemia at the steady state (6.2 ± 1.9 $mg \cdot kg^{-1} \cdot min^{-1}$ vs. 5.6 ± 2.2 $mg \cdot kg^{-1}$ min^{-1}, respectively, for SAA and HAA; $p > .05$). Insulin sensitivity did not differ between study days (M value 3.1 ± 1.7 vs. 3.0 ± 1.7 $[mg \cdot kg^{-1} \cdot min^{-1}]/[mU \cdot mL^{-1}]$, $p > .05$, respectively, for SAA and HAA) (Fig. 3*C*).

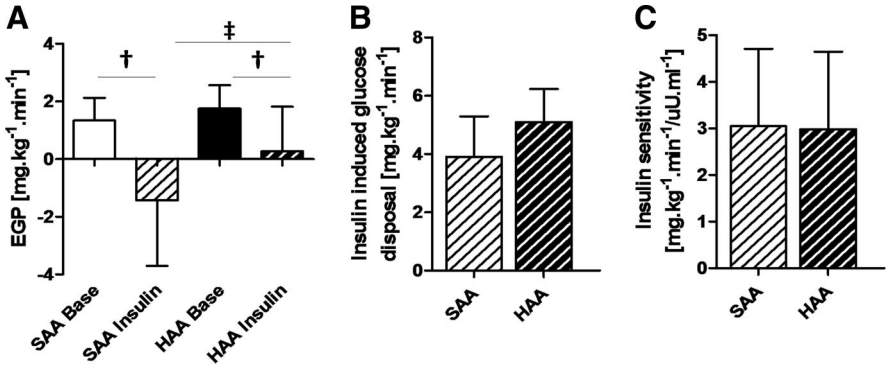

Figure 3. Glucose kinetics in nine critically ill septic adolescents with standard and high parenteral amino acid intake at baseline and during hyperinsulinemic euglycemic clamp: (*A*) endogenous glucose production (*EGP*) ($mg \cdot kg^{-1} \cdot min^{-1}$), (B) insulin-induced glucose disposal ($mg \cdot kg^{-1} \cdot min^{-1}$), (*C*) insulin sensitivity depicted as the M value ($[mg \cdot kg^{-1} \cdot min^{-1}]/[mU \cdot mL^{-1}]$). Values are mean ± SD. $^{\dagger}p < .05$, baseline vs. hyperinsulinemic euglycemic clamp. $^{\ddagger}p < .05$, standard amino acid (*SAA*) intake vs. high amino acid (*HAA*) intake.

Table 4. Plasma amino acid concentrations[a]

	Standard Amino Acid Intake		High Amino Acid Intake	
	Baseline	Hyperinsulinemic Euglycemic Clamp	Baseline	Hyperinsulinemic Euglycemic Clamp
Leucine	134 ± 50	122 ± 42	197 ± 82[c]	185 ± 91[c]
Isoleucine	77 ± 28	73 ± 26	120 ± 48[c]	112 ± 51[c]
Valine	193 ± 74	174 ± 64	268 ± 102[c]	256 ± 114[c]
Alanine	296 ± 115	295 ± 116	363 ± 142[c]	364 ± 180[c]
Arginine	110 ± 57	133 ± 85	149 ± 61[c]	172 ± 87
Ornithine	91 ± 38	87 ± 33	127 ± 53	120 ± 47
Citrulline	13 ± 10	12 ± 10	16 ± 17	16 ± 18
Asparagine and aspartate	48 ± 14	45 ± 13	54 ± 19	52 ± 16
Glutamine and glutamate	456 ± 131	455 ± 148	517 ± 103	504 ± 146
Glycine	203 ± 65	208 ± 70	236 ± 72	243 ± 96
Methionine	25 ± 28	22 ± 28	28 ± 26	24 ± 19
Cystine	6 ± 5	8 ± 6	8 ± 6	9 ± 8
Phenylalanine	104 ± 44	95 ± 38	109 ± 37	111 ± 41
Tyrosine	64 ± 33	55 ± 32[b]	57 ± 27[c]	59 ± 36
Lysine	222 ± 98	231 ± 97	293 ± 82[c]	305 ± 116[c]
Histidine	63 ± 21	66 ± 20	78 ± 19	76 ± 23
Threonine	154 ± 80	155 ± 76	162 ± 74	172 ± 71
Serine	99 ± 31	102 ± 29	128 ± 36[c]	131 ± 49[c]
Proline	236 ± 85	239 ± 62	376 ± 124[c]	361 ± 131[c]
Taurine	39 ± 36	30 ± 28	27 ± 20	30 ± 20

[a]Plasma amino acids depicted as μmol L^{-1}. Values are mean ± SD; [b]$p < .05$, baseline vs. hyperinsulinemic euglycemic clamp; [c]$p < .05$, standard amino acid intake vs. high amino acid intake.

Protein Turnover

Plasma Amino Acid Concentrations. As expected, the HAA intake resulted in higher ($p < .05$) plasma amino acid concentrations for many but not all amino acids. Insulin administration only decreased the tyrosine levels at SAA ($p < .05$), while none of the measured plasma amino acid concentrations decreased under hyperinsulinemia at HAA intake (Table 4).

Leucine Rate of Appearance and Oxidation. As shown in Figure 4*A*, the plasma leucine rate of appearance increased when the amino acid intake was higher, as would be expected, both at the baseline and during the HEC period ($p < .01$, SAA vs. HAA), and during the HEC period, the rate of appearance of leucine decreased at both SAA intake and HAA intake ($p < .05$) (Fig. 4).

The oxidation rates of leucine were not affected by insulin infusion but did increase with the higher amino acid intake at the baseline ($p < .001$) and during the HEC period ($p < .001$) (Fig. 4).

Protein Metabolism. As shown in Figure 5*A*, whole body protein synthesis rates significantly decreased when insulin was administered at SAA intake ($p < .01$), and this effect persisted even in the presence of HAA intake ($p < .01$). The protein synthesis rates were improved by the HAA intake during the baseline and HEC periods ($p < .05$). In contrast, as shown in Figure 5*B*, whole body protein breakdown was not affected by the amino acid intake but significantly decreased ($p < .05$) with insulin administration during the SAA intake and the HAA intake. Protein balance improved ($p < .05$) when the HAA intake was given, but insulin administered during the HEC period at the HAA intake did not further improve the protein balance. Likewise, insulin administration during SAA intake failed to significantly improve the protein balance (Fig. 5*C*).

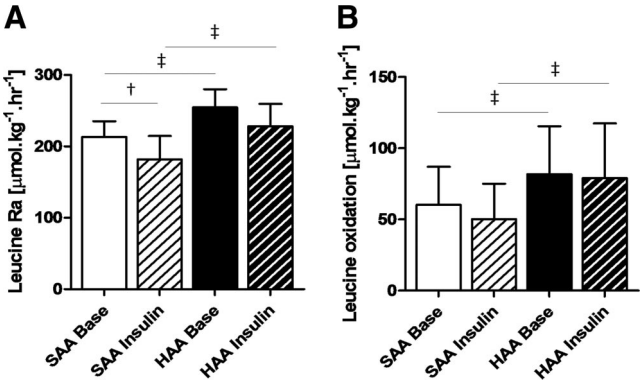

Figure 4. Leucine kinetics in nine critically ill septic adolescents with standard and high parenteral amino acid intake at baseline and during hyperinsulinemic euglycemic clamp: (*A*) leucine rate of appearance (*Ra*), (*B*) leucine oxidation. Values are given in $\mu mol \cdot kg^{-1} \cdot hr^{-1}$ (mean ± SD). $^{\dagger}p < .05$, baseline vs. hyperinsulinemic euglycemic clamp. $^{\ddagger}p < .05$, standard amino acid (*SAA*) intake vs. high amino acid (*HAA*) intake.

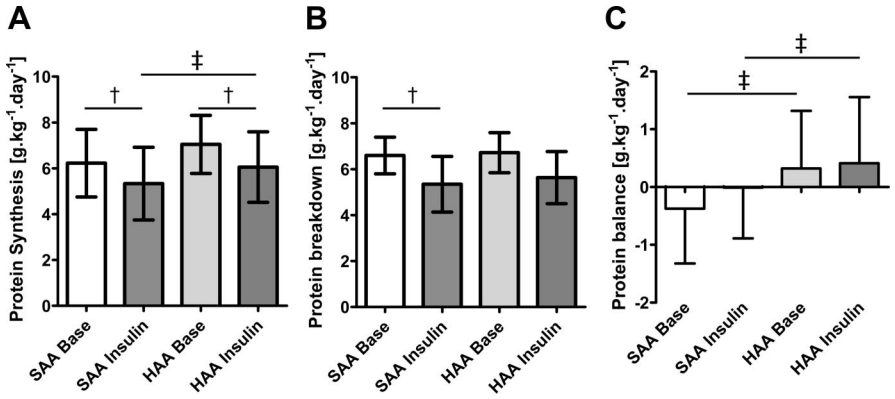

Figure 5. Protein metabolism in nine critically ill septic adolescents with standard and high parenteral amino acid intake at baseline and during hyperinsulinemic euglycemic clamp (*A*) protein synthesis, (*B*) protein breakdown, (*C*) protein balance. Values are given in $g \cdot kg^{-1} \cdot day^{-1}$ (mean ± SD). $^{\dagger}p < .05$, baseline vs. hyperinsulinemic euglycemic clamp. $^{\ddagger}p < .05$, standard amino acid (*SAA*) intake vs. high amino acid (*HAA*) intake.

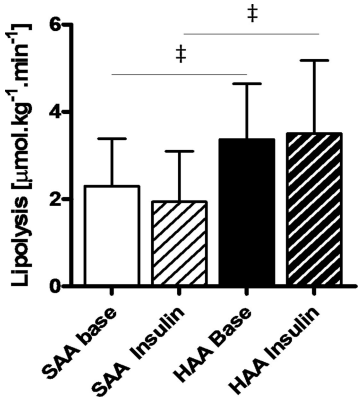

Figure 6. Rate of lipolysis in nine critically ill septic adolescents with standard and high parenteral amino acid intake at baseline and during hyperinsulinemic euglycemic clamp. Values are given in $\mu mol \cdot kg^{-1} \cdot min^{-1}$ (mean ± SD). $^{\ddagger}p < .05$, standard amino acid (*SAA*) intake vs. high amino acid (*HAA*) intake.

Lipolysis

The rates of lipolysis were not affected by insulin during SAA or HAA intake. Interestingly, the HAA intake increased lipolysis ($p < .05$) (Fig. 6).

DISCUSSION

Protein Turnover. The negative whole body protein balance observed at SAA intake in the present study is consistent with previous data by us and others (40, 41). Our data showed that parenteral amino acid intakes of 1.5 $g \cdot kg^{-1} \cdot day^{-1}$ per the recommended guidelines (16) are insufficient to support protein turnover and balance in septic, insulin-resistant adolescents at baseline conditions and while receiving insulin.

In critically ill adults, increasing the protein intake from about 1 to 1.5 $g \cdot kg^{-1} \cdot day^{-1}$ improved but did not normalize the whole body protein balance, while a further increase to 2 $g \cdot kg^{-1} \cdot day^{-1}$ did not lead to a further improvement (42). In contrast, our study demonstrated that, in these septic adolescents, increasing the parenteral amino acid intake from 1.5 to 3.0 $g \cdot kg^{-1}$ day^{-1} in the presence of adequate caloric intake showed a strong trend toward stimulation of protein synthesis and significantly improved the whole body protein balance, even in the absence of insulin administration.

It is not surprising that whole body protein synthesis was increased with higher amino acid intakes, since amino acids, especially branched-chain amino acids such as L-leucine and cationic amino acids such as L-arginine, act as nutrient signals themselves, modulating cellular processes that lead to protein synthesis via augmented messenger RNA translation initiation (15).

Under physiologic conditions, the major regulator of muscle protein synthesis is increased amino acid availability (particularly leucine), rather than insulin (43). Hence, dietary amino acid intake and intracellular transport play a key role in this process. However, under conditions of sepsis and cytokine release, muscle protein synthesis is inhibited by decreased mammalian target of rapamycin kinase activity in muscle (5). In regard to the effects of insulin on protein synthesis during sepsis, our data showed that, during sepsis and perhaps exacerbated by the use of glucocorticoids, insulin decreased whole body protein synthesis, even when HAA intake was provided. These data are in agreement with those of septic animal models (5), insulin-dependent diabetics (44), and critically ill newborns (45). Lang and co-workers (5) reported that insulin failed to stimulate protein synthesis in an animal model of sepsis via a defect in insulin signaling to a step in translation initiation mediating the assembly of the active eukaryotic initiation factor 4F complex, which is a key protein complex in translation initiation. Therefore, sepsis induces insulin resistance to protein synthesis.

We observed that at SAA intakes insulin administration decreased protein breakdown. Insulin decreased protein breakdown in critically ill newborns (45, 46) and in adults with diabetes mellitus (44). Using leucine kinetics in healthy humans, it has been shown that hyperinsulinemia decreased proteolysis but did not stimulate protein synthesis. By con-

trast, elevation of plasma levels of amino acids, by infusion of an amino acid mixture, stimulated the protein synthesis but did not suppress endogenous proteolysis (47). Thus, amino acids and insulin appear to exert different and complementary effects in stimulating protein anabolism. In healthy humans, insulin is "permissive" for protein synthesis and suppressive for protein breakdown (48).

In septic adolescents, insulin suppresses protein synthesis and breakdown, while HAA intake enhances protein synthesis but abolishes the insulin effect on protein breakdown.

Furthermore, there is evidence that insulin resistance is associated with muscle breakdown. During inflammation, insulin resistance decreases phosphoinositide 3-kinase activity, and this subsequently reduces the level of phosphorylated protein kinase B (11). A low protein kinase B level relieves the inhibition of the expression of specific E3 ubiquitin-conjugating enzymes atrogin-1/muscle atrophy F-box and muscle ring finger 1 in muscle. Expression of these E3 enzymes is found in conditions causing loss of lean body mass (11). The ubiquitin proteasome pathway together with autophagy is the main route that cells use for degrading intracellular proteins.

Endogenous glucocorticoids and impaired insulin signaling are required for stimulation of muscle breakdown in inflammation (49). These conditions were found in our patients, and currently, a large proportion of critically ill patients are managed with glucocorticoids, which exacerbates insulin resistance and protein breakdown.

Insulin did not have an additive effect to the HAA intake in terms of net protein balance in the septic adolescents. This was mainly due to the lack of an effect of insulin in suppressing protein breakdown at HAA intake. This observation may have been due to our limited sample size and lack of power to detect an effect. However, high protein intakes are known to induce insulin resistance (50).

Glucose Metabolism and Insulin Sensitivity. All septic patients showed considerable insulin resistance with an about 65% decrease in insulin sensitivity when compared to values of ~11 ($mg \cdot kg^{-1} \cdot min^{-1}$)/($microunit \cdot mL^{-1}$) reported in fasting healthy adolescents (51, 52), and the degree of insulin resistance is comparable to that observed in critically ill adults (4.6 [$mg \cdot kg^{-1} \cdot min^{-1}$]/[$mU \cdot mL^{-1}$]) (53). Despite significant insulin resistance, when the SAA intake was supplied in the presence of insulin, endogenous glucose production was suppressed, which is in agreement with previous studies in critically ill adults (54–57).

Interestingly, when HAA intake was provided to our patients, insulin failed to suppress the rates of endogenous glucose production and lipolysis rates were increased. This observation, in addition to the lack of insulin effect to suppress protein breakdown at HAA intakes, raised concern that HAA intake may exacerbate insulin resistance and that a greater supply of gluconeogenic amino acids could preferentially be used for glucose production (58). Amino acid infusion in healthy subjects to achieve hyperaminoacidemia decreases insulin sensitivity (59). In contrast, we observed no effect of increased amino acid intake on insulin plasma levels or insulin sensitivity, but our sample size was limited.

Hence, although HAA intake increased protein synthesis, this effect may be offset by a negative impact on glucose and lipid homeostasis.

Lipolysis. Insulin did not decrease lipolysis, and this is consistent with data in critically ill adults (56, 60). Furthermore, lipolysis increased at HAA intakes, again raising concern for increased insulin resistance when the HAA intake was provided.

Finally, we acknowledged that our sample size was limited, and we cannot rule out that we might have missed relevant findings due to the limited sample size.

CONCLUSION

We confirmed that critically ill, septic adolescents are markedly insulin resistant and conclude that recommended parenteral amino acid intakes in these patients are insufficient to maintain the protein balance. Increasing amino acid intakes to 3 $g \cdot kg^{-1} \cdot day^{-1}$ improved protein synthesis and balance, but it may have favored gluconeogenesis and stimulated lipolysis, raising concern that this level of amino acid intake may have enhanced insulin resistance. Insulin infusion and tight glucose control at SAA intakes decreased both protein synthesis and breakdown and did not affect protein balance. Dose-response studies on protein administration and their effects on energy metabolism and insulin resistance are needed.

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