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Intravenous Insulin Decreases Protein Breakdown in Infants on Extracorporeal Membrane Oxygenation

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Abstract

Background/Purpose—Infants requiring extracorporeal membrane oxygenation (ECMO) have the highest rates of protein catabolism ever reported. Recent investigations have found that such extreme protein breakdown is refractory to conventional nutritional management. In this pilot study, the authors sought to use the anabolic hormone insulin to reduce the profound protein degradation in this cohort.

Methods—Four parenterally fed infants on ECMO were enrolled in a prospective, randomized, crossover trial. Subjects were administered an insulin infusion using a 4-hour hyperinsulinemic euglycemic clamp followed by a control saline infusion on consecutive days in random order. Whole-body protein flux and breakdown were quantified using a primed continuous infusion of the stable isotope L-[1-¹³C]leucine. Statistical analyses were performed using paired *t* tests.

Results—Serum insulin levels were increased 15-fold during the insulin clamp compared with the saline control (407 ± 103 v 26 ± 12 μ U/mL; $P < .05$). During the insulin infusion, infants had decreased rates of total leucine flux (214 ± 25 v 298 ± 38 μ mol/kg/h; $P < .05$) and leucine flux derived from protein breakdown (156 ± 40 v 227 ± 54 μ mol/kg/h; $P < .05$) when compared with saline control. Overall, insulin administration produced a 32% reduction in protein breakdown ($P < .05$).

Conclusions—In this pilot study, the anabolic hormone insulin markedly reduced protein breakdown in critically ill infants on ECMO. Because elevated protein breakdown correlates with mortality and morbidity, the administration of intravenous insulin may ultimately have broad applicability to the metabolic management of critically ill infants.

INDEX WORDS

Protein; metabolism; insulin; hyperinsulinemic; euglycemic clamp; protein breakdown; nutrition; neonate; extracorporeal membrane oxygenation

Critically ill infants requiring extracorporeal membrane oxygenation (ECMO) have shown the highest rates of whole-body protein breakdown ever recorded.¹ These babies continue to manifest negative protein balance in the post-ECMO recovery phase.¹ At this rate of protein breakdown, a neonate loses 15% of his or her lean body mass during an ECMO course of 7 days. Because the loss of lean body mass has been correlated with overall mortality and

morbidity,^{2,3} it is possible that such extreme protein catabolism plays a significant role in the child's overall course of illness.

The limited options available to alter protein catabolism in this population have proven ineffective. The increased allotment of protein in parenteral nutrition regimens serves to increase the synthesis of new protein but has little effect on elevated rates of protein breakdown.^{4,5} Excessive carbohydrate provisions may worsen respiratory failure,⁶ and a recent study of ECMO neonates has correlated increased carbohydrate intake with further increases in protein breakdown.⁷

Therefore, investigation into an alternate means to alter protein metabolism in this cohort is clinically relevant. Our laboratory has focused recently on the anabolic hormone insulin, which has been used experimentally to reduce the protein loss associated with critical illness in the adult population.⁸⁻¹⁰ The hypothesis of the current study was that the intravenous administration of insulin would reduce the elevated rate of protein degradation commonly seen in infants on ECMO.

MATERIALS AND METHODS

Subjects

Infants were recruited from the 2 centers in New England that provide ECMO support: Children's Hospital Boston (CHB) and Mass-General Hospital for Children (MGH). The study design was approved by the Institutional Review Boards at both centers. Subjects were eligible for recruitment if they were cannulated on ECMO at less than 1 year of age and were considered hemodynamically stable by the treating attending physician. Exclusion criteria included significant intracranial hemorrhage or severe encephalopathy; a known fatal chromosomal anomaly; irreversible cardiopulmonary disease; renal insufficiency (serum creatinine concentration greater than 1.5 mg/dL); hepatic insufficiency (protime greater than 20 seconds before anticoagulation); and concomitant treatment with corticosteroids, insulin, growth hormone, or thyroid hormone. There were no maternal corticosteroids administered before delivery of any subject.

After meeting criteria for recruitment, parents were approached initially by a treating physician and, if permission was granted, by a study physician. Informed consent was then obtained, and the subject was enrolled in the study.

Study Design

The study protocol lasted 4 hours on each of 2 consecutive days. Upon enrollment, subjects were selected randomly as to the order in which they received insulin or control infusions. Randomization was performed by a computer-generated algorithm using an allocation ratio of 1:1 at each study center in a permuted blocks design. In this way, subjects underwent either the hyperinsulinemic euglycemic clamp on day 1 of the study followed by saline (control) infusion on day 2 or the reverse order. Each subject therefore served as his or her own control during the study period. Randomization was incorporated to minimize any potential difference in the critical illness between days of the study. The identical stable isotope infusion protocol was employed on both days of the study.

Hyperinsulinemic Euglycemic Clamp

The hyperinsulinemic euglycemic clamp was performed according to the published methods of DeFronzo et al.⁹ with modifications made for the added safety of the neonate. Ten minutes before the start of the stable isotope protocol, a 50% glucose infusion was initiated at a rate of

10 mg/kg/min. This glucose infusion was a supplement to the glucose in PN and served to maintain euglycemia during insulin administration.

The insulin solution was prepared by adding 0.75 mL of the patient's blood to the insulin mixture to prevent binding of insulin to the syringe and tubing.¹² The insulin pool then was primed with a bolus dose of U-100 regular insulin (Humulin R, Lilly, Indianapolis, IN) followed by a constant infusion of 120 mU/m²/min insulin (approximately equal to 0.6 U/kg/h and 10 mU/kg/min). Plasma glucose levels were monitored at the bedside every 5 to 10 minutes throughout the hyperinsulinemic euglycemic clamp using the glucose dehydrogenase reaction in duplicate (HemoCue B-Glucose Analyzers, HemoCue AB, Angelholm, Sweden). A normal blood glucose level of 100 mg/dL was targeted by varying the rate of glucose infusion. Serum potassium and arterial blood gas measurements were monitored every 30 to 60 minutes throughout the insulin infusion. After the 4-hour isotope protocol, the insulin infusion was discontinued immediately, and the supplemental glucose solution was weaned off over the subsequent several hours.

As determined by prior randomization, on the alternate day of the study, an intravenous infusion of normal saline was initiated at the start of the stable isotope protocol using the same infusion rate as calculated above for insulin administration.

A 2.0-mL blood sample was obtained at the end of the hyperinsulinemic euglycemic clamp and saline infusion to measure serum levels of insulin, lactic acid, and C-reactive protein (CRP).

Stable Isotope Protocol

An initial 0.5-mL blood sample was obtained from the neonate to measure the baseline enrichment of α -ketoisocaproic acid (α -KICA), the intracellular transamination product of leucine. A primed (12 μ mol/kg), 4-hour continuous intravenous infusion (9 μ mol/kg/hr) of L-[1-¹³C]leucine (¹³C isotopic enrichment 99%; Cambridge Isotope Laboratories, Andover, MA) was then administered into the ECMO circuit using a calibrated syringe pump (Perfusor S, B. Braun Medical Inc, Carrollton, TX) and infusion tubing with in-line 0.22-micron filter (Medex, Inc, Duluth GA). After 3 hours of the leucine infusion, 3 blood samples (0.5 mL each) were obtained at 20-minute intervals to measure isotopic enrichment of α -KICA at steady state. All blood samples were obtained from a previously placed arterial catheter and transferred into prechilled tubes. Blood samples were immediately centrifuged at 3,000 rpm for 10 minutes, and the plasma stored at -80°C until analyzed. Syringes containing isotope infusate were weighed before and after infusion to precisely quantify total volume delivered. All stable isotope infusates were prepared using aseptic technique and tested for pyrogenicity and sterility on a routine basis before administration. Two additional 0.5-mL blood samples were obtained immediately before and at the end of the stable isotope infusion to determine serum amino acid concentrations.

Sample Analysis

L-[1-¹³C] α -KICA was determined by conversion to the t-butyl-silylquinoxalinol derivative as previously described.¹³ Electron impact ionization was used on a Hewlett Packard 5989 gas chromatograph-mass spectrometer, with selected ion monitoring of m/z = 259 and 260 (MW-57 for unlabeled and labeled α -KICA, respectively). Enrichment was calculated as the mole fraction ¹³C excess for α -KICA after reaching plateau and corrected for natural levels of isotope present as previously described.¹⁴

Concentrations of the leucine infusate and serum amino acids were measured using ion exchange chromatography on a Hitachi 8800 high-pressure amino acid analyzer (Hitachi, Tokyo, Japan). Plasma levels of CRP were measured using the immuno-turbidimetric method (917 Chemical

Analyzer, Roche Diagnostics, Indianapolis, IN). Plasma lactate levels were measured using enzymatic calorimetry with lactate oxidase and 4-aminoantipyrine (Roche Diagnostics). Blood gas analyses were performed on the Bayer Rapidlab 860 analyzer (Bayer, Tarrytown, NY).

Calculations for Leucine Kinetics

The principles for the determination of whole-body protein kinetics using amino acid tracer techniques are well established.¹⁵ Because leucine is an essential amino acid in humans, leucine enters the plasma-free amino acid pool by only 2 sources: from the breakdown of endogenous protein stores (B) and from PN (I) as all subjects were fed exclusively with PN. Leucine exits the plasma amino acid pool by incorporation into body protein (S) or through leucine oxidation (O). At steady state, the total leucine flux (Q), or the total appearance of leucine into the free plasma pool, is equal to $I + B = S + O$. Q can be calculated from the isotopic enrichment of α -KICA, the intracellular transamination product of leucine. α -KICA is measured in the plasma, and, in this way, total leucine flux is calculated as:

$$Q(\mu\text{mol/kg/h}) = \text{inf} \times [(E_I/E_p) - 1]$$

Where *inf* is the infusion rate of 1-[¹³C]leucine ($\mu\text{mol/kg/h}$), E_I is the enrichment of the 1-[¹³C]leucine infusate (mole percent excess), and E_p is the average isotopic enrichment of α -KICA (mole percent excess) as measured in plasma at steady state.

The leucine content in the PN solution (2.2% amino acid formulation: TrophAmine, B. Braun Medical, Irvine, CA) is standardized, and dietary intake of leucine from PN is known to be 111 $\mu\text{mol/kg/h}$ for every 2.5 g/kg/d of Trophamine. In this way, endogenous leucine flux (or the leucine appearance in the plasma as derived from protein breakdown) was calculated by subtracting the known rate of exogenous leucine administered in PN from total leucine flux (Q) as follows: $B = Q - I$ ($\mu\text{mol/kg/h}$). Whole-body protein kinetics then were calculated by multiplying the values for leucine kinetics by the conversion factor of 590 μmol leucine per 1 g body protein.¹⁶

Statistical Analyses

Protein kinetic and clinical data are expressed as mean $\pm$ SEM followed by the range in parentheses or brackets where appropriate. Comparisons of protein metabolism and laboratory values between insulin and control days of the study were performed using paired *t* tests. Statistical significance was set at $P < .05$.

RESULTS

Four infants (3 boys, 1 girl) on ECMO were studied including 3 neonates beginning on day of life 5 ± 1 and 1 infant at 6 months of age. The mean gestational age of subjects was 37 ± 1 weeks with a mean weight at time of study of 3.43 ± 0.73 (range 2.50 to 5.60) kg. The following primary diagnoses were present in 1 patient each: persistent pulmonary hypertension of the newborn, congenital diaphragmatic hernia, fetal hydrops, and complex congenital cardiac disease. In the 2 subjects undergoing operative repair of their primary diagnoses, the study was initiated on postoperative day 6.

At the time of the study, 2 infants were on venoarterial ECMO, and 2 were on venovenous ECMO. Flow rates ranged from 290 to 1,500 mL/min, usually at 0.1 L/kg/min, according to institutional ECMO protocol. The average time on the ECMO circuit at the start of the experimental protocol was 4 ± 1 days.

Care was taken to maintain the same PN regimen on both days of the study protocol, and there were no statistical differences in nutritional composition. On the treatment day of the study, the PN consisted of 62 ± 9 kcal/kg/d, 1.3 ± 0.3 g/kg/d protein, and 1.7 ± 0.3 g/kg/d lipid (20% lipid formulation: Intralipid, Baxter Healthcare/Fresenius Kabi Clayton LP, Clayton, NC). On the control day of the study, the PN consisted of 68 ± 15 kcal/kg/d, 1.6 ± 0.6 g/kg/d protein, and 1.6 ± 0.6 g/kg/d lipid. The glucose infusion rates before initiation of the hyperinsulinemic euglycemic clamp were similar between experimental and control study days (8.57 ± 1.44 [4.32–10.43] v 9.54 ± 1.74 [5.08–13.52] mg/kg/min). The mean glucose infusion rate at steady state was 5.88 ± 0.44 (5.21–7.15) mg/kg/min, and, therefore, the mean total glucose infusion rate including supplemental and PN glucose at steady state was 15.41 ± 1.40 mg/kg/min.

The hyperinsulinemic euglycemic clamp increased serum insulin levels 15-fold at steady state compared with saline infusion (407 ± 103 [185–662] v 26 ± 12 [9–59] μ U/mL; $P < .05$; Fig 1). There were no episodes of hypoglycemia in any subject during the study period. The insulin infusion did not change acid-base balance or serum levels of lactic acid compared with control. Euglycemia was maintained for each subject throughout the insulin and saline infusions.

The 4-hour experimental protocol achieved physiologic steady state for [$1\text{-}^{13}\text{C}$] α -KICA between 180 and 240 minutes (Fig 2). The coefficient of variance of α -KICA enrichment at plateau was 8.4%. Total leucine turnover (leucine R_a) was significantly reduced with the hyperinsulinemic euglycemic clamp compared with saline infusion (214 ± 25 [166–274] v 298 ± 38 [230–382] μ mol/kg/h; $P < .05$, Fig 3A). Exogenous leucine from PN was then subtracted from total leucine turnover for each day of the study, and the endogenous leucine turnover (ie, the plasma leucine derived from endogenous protein breakdown) was reduced by 32% with the hyperinsulinemic euglycemic clamp (156 ± 40 [83–250] v 227 ± 54 [116–342] μ mol/kg/h; $P < .05$, Fig 3B). When converted to whole-body protein units, the reduction in protein breakdown was 3.1 ± 0.7 g/kg/d. All 4 subjects showed a substantial reduction in endogenous leucine turnover with the intravenous insulin infusion.

The plasma-free amino acid concentration was decreased significantly after insulin infusion (159 ± 19 [108–197] v 233 ± 26 [162–286] μ mol/dL; $P < .05$). In particular, the plasma concentration of the essential amino acid leucine was significantly decreased with the intravenous insulin infusion (9 ± 1 [7–10] v 16 ± 2 [10–18] μ mol/dL; $P < .05$). There were no differences in CRP (5.05 ± 1.94 [1.77–17.00] v 4.43 ± 1.86 [1.21–16.90] mg/dL; P value, not significant), a marker of critical illness severity, between insulin and control respectively.

DISCUSSION

It is well established that the systemic response to critical illness includes a dramatic mobilization of host amino acids, thereby accelerating the rate of whole-body protein breakdown. In the critically ill neonate, studies have reported a significant negative protein balance.^{17,18} Neonates on ECMO have been found to have the highest rate of protein catabolism ever reported with a net negative protein balance of -2.3 g/kg/d.¹ The increase in protein catabolism is in contrast to the positive protein balance that the healthy neonate requires (1.5 g/kg/d). This problem is further compounded by limited neonatal body protein stores.¹⁹ Thus, in addition to the ongoing disease process, the critically ill neonate has distinct metabolic and nutritional challenges.

Attempts at affecting protein balance in critically ill infants using conventional nutritional management have made only modest gains. Exogenous protein and caloric provisions through parenteral nutrition help to increase the synthesis of new protein but have little effect on the body's increased protein degradation.^{4,5} Excessive caloric allotments may increase CO_2 production rates and thereby exacerbate the ventilatory burden of the child.⁶ One study of

ECMO neonates has shown a possible paradoxical increase in protein breakdown with the administration of increased carbohydrate regimens.⁷ Thus, research into alternative means to promote positive protein balance in this population is of metabolic interest and clinical relevance.

The current pilot study used the intravenous administration of insulin to pharmacologically modulate the rate of protein breakdown in neonates dependent on ECMO. In the data presented here, the hyperinsulinemic euglycemic clamp was associated with significant reductions in overall amino acid turnover and endogenous leucine flux, a marker of protein breakdown. The rates of protein catabolism were measured by a previously validated stable isotopic tracer technique using L-[1-¹³C]leucine.^{1,13,19} Supporting the isotopic findings, insulin significantly reduced the serum concentrations of total amino acids and the essential amino acid leucine, presumably caused by the decreased degradation of protein into its constituent amino acids. Importantly, the mitigating effects of intravenous insulin on protein catabolism took place with no associated hypoglycemic complications or changes in lactate or acid-base status. The substantial reduction in protein degradation was observed after just 4 hours of insulin infusion; using these measurements, whole-body protein breakdown was reduced by 32% and equivalent to 3.1 g/kg/d.

The anabolic effects of insulin have been described previously, and numerous studies have used insulin infusions to promote protein accretion in adult volunteers and burn patients.^{8–10,20,21} In the pediatric population, one previous study measured the effects of insulin on protein metabolism in a cohort of 4 extremely low-birth-weight neonates.²² The investigators showed a 20% reduction in protein breakdown, although this was associated with a corresponding decrease in protein synthetic rate. The current study therefore represents the first reported use of an intravenous insulin infusion to reduce protein catabolism in a critically ill infant population.

Based on the results of this study, additional clinical investigation is warranted into the effects of intravenous insulin on the protein metabolism of critically ill infants. Although insulin appears to reduce protein breakdown in neonates on ECMO, the pattern must be confirmed in a larger population of critically ill babies. In addition, because protein metabolism is dependent on protein synthesis as well as breakdown, studies designed to measure insulin's effects on protein synthesis are ongoing. Interestingly, several clinical studies in adults have shown an increase in protein synthesis when insulin is administered with parenteral amino acids.^{9,10,23} If this finding is confirmed in parenterally fed infants, the protein-sparing effects associated with insulin would be even more substantial.

The data presented here are consistent with those reported in previous studies^{22,24} and suggest that the experimental use of insulin infusions may be performed safely and effectively in neonates. Given the absence of nutritional methods to reduce protein breakdown, insulin may ultimately have broad applicability to the metabolic support of critically ill infants.

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Discussion

W. Chwals (Cleveland OH): This work is potentially important because it was conducted during the catabolic phase of the injury response. The study results provide a putative protein-sparing advantage during acute metabolic stress, but, if you also consider the insulin administration data that have been generated in adults and older children recovering from burns (Herndon and Wolfe), there's a putative advantage in increasing protein synthesis as well as decreasing breakdown, thus, promoting earlier growth recovery if a lower insulin dose is used. The amount of insulin you gave was 10 $\mu\text{U/kg/min}$. In the burn studies in the adults and older children, when 7.8 $\mu\text{U/kg/min}$ were given for 7 days, there was a substantial glucose administration requirement to maintain appropriate glucose levels and avoid hypoglycemia. When insulin infusion was decreased to 2.6 $\mu\text{U/kg/min}$ for 2 days in a similar population, muscle protein synthesis was increased without any associated proteolysis and without the requirement of excessive exogenous glucose administration. In this regard, and from a practical standpoint, how would you propose to employ insulin clinically in this patient population to optimize protein metabolic advantages while avoiding caloric overload resulting from large quantities of exogenous glucose required to maintain euglycemia? Also, why did you choose not to measure leucine oxidation, which would establish the irreversible loss of leucine carbon and would, thus, be a better indicator of catabolism? Have you considered a second clamp later in the clinical course (during the recovery stage) to help establish the degree of insulin resistance and whether that equates to acute metabolic stress?

P.J. Javid (response): Thank you for those questions. Our main principle in this study was to demonstrate that insulin at a relatively high dose can reduce rates of protein breakdown in this population. Once this principle has been established in a cohort of critically ill pediatric patients, further studies may be performed to construct an insulin dose response curve. In this way, the optimal concentration of insulin to reduce protein catabolism but limit the risk of hypoglycemia and the amount of exogenous glucose required can then be elucidated. The second question, pertaining to leucine oxidation, is a very good question, because if you can measure leucine oxidation, you can also measure protein synthesis, which we have not included in this pilot data. Leucine oxidation is difficult to measure in this population because you need to give a small amount of isotopic bicarbonate and then measure the isotopic enrichment of carbon dioxide in that subject's expired breath. It is difficult to perform this experiment in subjects on ECMO because it requires the removal of sweep gas from the ECMO membrane. Subsequently, these babies on ECMO develop an alkalemia. We are employing a new stable isotope technique using the conversion of phenylalanine to tyrosine to measure the rate of protein synthesis with the insulin infusion. And the final question about performing an additional insulin clamp in the study introduces an interesting concept because this would allow us to measure changes in both insulin resistance and protein metabolism throughout the course of acute illness. It is an experiment that we have not yet performed, but it would clearly provide valuable data in this study.

Fig 1.
The hyperinsulinemic euglycemic clamp increased the serum concentration of insulin by 15-fold when compared with the control saline infusion. (* $P < .05$).

Fig 2.
As measured by mass spectrometry, a steady state was achieved using 1- ^{13}C α -KICA during the last hour of the stable isotope infusion protocol.

Fig 3.

The hyperinsulinemic euglycemic clamp reduced the total leucine flux and the endogenous leucine flux (a marker of the leucine derived from protein breakdown) by an average of 32%, equivalent to 3.1 g/kg/d of protein (**P* < .05). In the figures, “Insulin” and “Saline” correspond to the intravenous insulin infusion and the control saline infusion, respectively.