Predictors of Circulating Insulin-Like Growth Factor-1 and Insulin-Like Growth Factor–Binding Protein-3 in Critical Illness*

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Objective : To characterize predictors of insulin-like growth factor-1 and insulin-like growth factor–binding protein-3 in acute critical illness with the hypothesis that acute factors associated with critical illness will more strongly predict circulating insulin-like growth factor-1 and insulin-like growth factor–binding protein-3 than chronic clinical or genetic factors.

Design: Observational study nested within a large prospective study using multivariable linear regression to model circulating insulin-like growth factor-1 and insulin-like growth factor–binding protein-3 with acute and chronic clinical variables, and genotype from five polymorphisms in insulin-like growth factor pathway genes.

Setting: ICUs from two large academic medical centers.

Patients: Five hundred forty-three Caucasian patients with risk factors for acute respiratory distress syndrome and available plasma from early in critical illness.

Interventions: None.

Measurements and Main Results: Total insulin-like growth factor-1 and insulin-like growth factor–binding protein-3 were measured in plasma using IMMULITE assays (Siemens, Malvern, PA). We examined age, gender, body mass index, cirrhosis, and diabetes, as well as Acute Physiology, Age, and Chronic Health Evaluation III score, acute hepatic dysfunction, pneumonia and aspiration, sepsis/septic shock, acute respiratory distress syndrome, and receipt of corticosteroids. Body mass index, cirrhosis, and acute respiratory distress syndrome were strongly associated with insulin-like growth factor-1 and insulin-like growth factor–binding protein-3 levels; Acute Physiology, Age, and Chronic Health Evaluation III was strongly associated with insulin-like growth factor-1 levels; and age was strongly associated with insulin-like growth factor–binding protein-3. Five polymorphisms (*IGF1*: rs1520220, rs35767, rs2946834; *IGFBP1*: rs4619; *IGFBP3*: rs2854746) were analyzed for associations with plasma levels. When genotypes were added to models, rs2854746 was significantly associated with plasma insulin-like growth factor–binding protein-3. Genotype explained an additional 2% of variability with an overall adjusted R-square of 0.18.

Conclusions: Despite the acute derangements of critical illness, both acute and chronic health factors significantly influence circulating levels of insulin-like growth factor-1 and insulin-like growth factor–binding protein-3 early in critical illness. rs2854746 is also significantly associated with insulin-like growth factor–binding protein-3 levels in this ICU cohort. Overall, phenotypic and genotypic factors explained only a modest amount of variability in

*See also p. 2695.

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insulin-like growth factor-1 and insulin-like growth factor–binding protein-3. Further research is needed to understand how to apply these findings to patient care. (*Crit Care Med* 2015; 43:2651–2659)

Key Words: acute respiratory distress syndrome; critical care; insulin-like growth factor-1; insulin-like growth factor–binding protein-3; molecular epidemiology; single-nucleotide polymorphisms

In acute critical illness, there are numerous derangements in endocrine pathways, many as direct markers of catabolism and stress. This has led to interest in various endocrine molecules as potential prognostic markers or even treatment options for acute critical illness (1, 2). Insulin-like growth factor (IGF)-1 and insulin-like growth factor–binding protein (IGFBP)-3 have been of particular interest because of their relationship with growth hormone (GH) and mediation of its anabolic actions (3–5). Both IGF-1 and IGFBP-3 are decreased in acute critical illness despite increasing GH due to peripheral GH resistance, decreased GH receptor expression, and down-regulation of hepatic synthesis and secretion of IGF-1 and IGFBP-3 (1, 2, 4, 6, 7). This cascade may be a key contributor to the catabolism of critical illness that could have major prognostic implications (2, 6, 8). In our previous work, we showed that lower levels of IGF-1 and IGFBP-3 were independently associated with acute respiratory distress syndrome (ARDS) in a cohort of at-risk patients, and lower levels were associated with mortality in ARDS cases (9).

IGF-1 is an anabolic peptide hormone with structural similarities to proinsulin (10). IGF-1 has also been linked to fibrogenesis, apoptosis, mitogenesis, and cell cycle regulation and differentiation (10, 11). In circulation, over 90% of IGF-1 is bound to IGFBP-3, the most abundant of the six known IGFBPs (5, 7). The anabolic actions of IGF-1 are controlled primarily by GH, and IGF-1 itself indirectly mediates anabolic actions of GH (3).

IGF-1 and IGFBP-3 levels vary considerably between individuals, and various population-based cohorts have studied factors associated with these differences (12–15). There has been consistent evidence that IGF-1 declines with age and that men have higher IGF-1 levels than women (12–14). IGFBP-3 tends also to decline with age and be lower in men, but evidence has been less striking than for IGF-1 (12, 13, 15). There has been less consistency in associations between circulating IGF-1 and IGFBP-3 and factors, such as body mass index (BMI), smoking, and dietary factors (12–15).

Genetic factors have also been studied as determinants of circulating IGF-1 and IGFBP-3 levels. Twin studies have reported estimated heritability of IGF-1 to be 38% and IGFBP-3 to be 60% (16). Numerous polymorphisms of *IGF1* and *IGFBP3* have been studied in association with IGF-1 and IGFBP-3 levels in several large cohorts (11, 17–20). Perhaps, the most consistent finding has been the association of rs2854746 (*IGFBP3*) with IGFBP-3 levels, although there have been many polymorphisms associated with alterations in circulating IGF-1 and IGFBP-3 (11, 17–20). Some of these associations have varied by gender or race/ethnicity. Thus, there is evidence that circulating IGF-1 and IGFBP-3 are complex traits with both significant genetic and nongenetic influences (21).

Genetic and other chronic factors, many unmodifiable, contribute significantly to IGF-1 and IGFBP-3 levels in patients without acute illness. However, acute derangements in the GH/IGF-1 axis during critical illness strongly affect circulating levels of IGF-1 and its binding proteins. Given the potential links to prognosis in acute and prolonged critical illness, we sought to further investigate the relative contributions of acute and chronic clinical factors, as well as genetic factors, to levels of IGF-1 and IGFBP-3 during acute medical critical illness. We hypothesized that acute factors associated with critical illness would more strongly predict circulating IGF-1 and IGFBP-3 than chronic clinical or genetic factors.

MATERIALS AND METHODS

Parent Study Population and Design

The current observational study is part of the ongoing prospective Molecular Epidemiology of ARDS Study of which study design has been described in depth previously (22). The Human Subjects Committees at the Massachusetts General Hospital (MGH), Beth Israel Deaconess Medical Center (BIDMC), and the Harvard School of Public Health approved this study. Recruitment of adult ICU admissions at MGH (Boston, MA) began in January 1999 and at BIDMC (Boston, MA) in January 2007. Enrollment for the current cohort continued through March 2009. Admissions were screened daily for clinical risk factors for ARDS: pneumonia, sepsis or septic shock, aspiration, massive transfusions, pulmonary contusion, or multiple fractures. Standard definitions of sepsis and septic shock were used (23). Eligible patients with at least one risk factor for ARDS were approached and enrolled after informed written consent was obtained from subjects or appropriate surrogates. Patients in the parent cohort were followed prospectively for development of ARDS, defined by meeting American-European Consensus Conference criteria (24). Thus, the cohort as a whole represents patients with significant critical illness defined by major risk factors for ARDS.

IGF-1 and IGFBP-3 Measurements

Details of blood drawing protocols and laboratory methods have been published elsewhere (9). Briefly, per the parent study protocol, blood was drawn within the first 2 days of ICU admission or within 2 days of onset of ARDS in patients with delayed onset of ARDS. In the parent cohort, onset of ARDS significantly delayed from ICU admission occurred in less than 10% of patients (9). Whole blood samples were centrifuged, and plasma was removed and stored at –80°C until analysis. The stability of IGF-1 and IGFBP-3 in frozen samples has been shown previously (25). Total IGF-1 and IGFBP-3 levels were measured using an automated IMMULITE assay on an

IMMULITE 1000 instrument (Siemens, Malvern, PA). Laboratory personnel were blinded to clinical information.

Genotyping

DNA was extracted using PureGene DNA Isolation Kits (Gentra Systems, Research Triangle, NC). Five single-nucleotide polymorphisms (SNPs) in the IGF pathway were previously chosen for analysis based on existing literature and information from the SNPseek Database (snp.wustl.edu/SNPseek/index.cgi; last accessed November 17, 2009), which included more than 90,000 putatively functional human SNPs. SNPseek integrated data from the International HapMap project to include minor allele frequency (MAF) distribution, population specificity, and genomic annotations. SNPs were chosen for being functional, presumed functional, or tagging and were also selected to be common with a MAF more than 0.05. The candidate SNPs ultimately included three polymorphisms on *IGF1* (rs35767, rs1520220, rs2946834) and one each on *IGFBP1* (rs4619) and *IGFBP3* (rs2854746) (**Table 1**). SNPs were not selected specifically for Caucasians, but all have MAF more than 10% in all racial populations available in HapMap.

In 2008, these five SNPs were genotyped using 5′ nuclease allelic discrimination assays (Taqman; Applied Biosystems, Foster City, CA) in over 1,400 patients from the parent cohort. With this platform, allele detection is achieved by exonuclease cleavage of taq polymerase using custom primers and probes. In 2010, the high-density (50 K) custom array designed by the Institute of Translational Medicine and Therapeutics, the Broad Institute, and the National Heart Lung and Blood Institute–supported Candidate gene Association Resource Consortium (ITMAT-Broad_CARe (IBC) array; Illumina, San Diego, CA) was used to genotype over 2,000 patients from the parent cohort. IBC samples were genotyped at the Center for Applied Genomics, Children's Hospital of Philadelphia (Philadelphia, PA), using the gene-centric high-density chip (26). With the IBC chip, the SNP allele is distinguished by enzymatic single-base extension to incorporate a labeled nucleotide.

For the current study, results of both Taqman and IBC genotyping platforms were combined to maximize patients with both biomarker and genotyping data available. Because of different technological designs that are associated with different allele-calling algorithms, a small number of discordant callings of genotypes among replicates are expected, typically less than 1%. In both methods, genotyping personnel were blinded to clinical information. For quality control, 10% of samples were randomly reanalyzed.

Statistical Analysis

All statistical analyses were performed using SAS Version 9.3 (SAS, Cary, NC).

Demographic and clinical characteristics between groups were compared using chi-square tests for categorical variables and Student *t* tests and/or nonparametric tests for continuous variables. Correlations between plasma IGF-1 and IGFBP-3 and clinical variables were estimated using Spearman correlation.

IGF-1 and IGFBP-3 were log-transformed to approximate normality, and log-transformed values were used in all models. A two-phase approach was used for model building, first with phenotypic factors only and then with genotypic factors included. First, linear regression models were used to examine the association between the acute and chronic health factors of interest and circulating IGF-1 and IGFBP-3 levels. Chronic factors included age, gender, BMI, diabetes, cirrhosis, and smoking status (current, past, never). The acute factors included Acute Physiology, Age, and Chronic Health Evaluation (APACHE) III score as a marker of severity of illness; acute hepatic dysfunction; pneumonia including aspiration; presence of sepsis or septic shock; ARDS case status; and receipt of corticosteroids.

Acute hepatic dysfunction was defined as bilirubin at least 2.0 mg/dL within the first 24 hours of ICU admission. Because of the overlap between patients with cirrhosis and patients with elevated bilirubin at ICU admission, we considered models with separate variables for both cirrhosis and acute hepatic dysfunction (with cirrhotic patients excluded from the latter), as well as models with a single composite hepatic failure variable that included both cirrhosis and acute hepatic failure. ARDS case status was included based on our previous findings of an independent relationship between ARDS and circulating IGF-1 and IGFBP-3 (9). To avoid collinearity, score for age was removed from APACHE III in multivariable models also including age and score for Pa_{O_2}/F_{IO_2} was removed in models including ARDS status. Receipt of corticosteroids was defined as having received at least one dose of corticosteroid at any time during the first 3 days of critical illness. Multivariable models used a stepwise selection algorithm with conservative

TABLE 1. Single-Nucleotide Polymorphisms Genotyped

Gene	Chromosome	Single-Nucleotide Polymorphism	Gene Region	Minor Allele Frequency, %[a]
IGF-1	12	rs35767	Intergenic/upstream	16.2
IGF-1	12	rs1520220	Intron 3	19.4
IGF-1	12	rs2946834	Intergenic/downstream	35.2
IGFBP-1	7	rs4619	Exon	33.5
IGFBP-3	7	rs2854746	Exon-1	44.6

IGF = insulin-like growth factor, IGFBP = insulin-like growth factor–binding protein.
[a]Reported for the current cohort.

cutoffs of p up to 0.2 to enter and p up to 0.1 to stay. Models were refit with the surviving covariates.

In the second phase, linear regression models were used to examine the association between the SNPs of interest and circulating IGF-1 and IGFBP-3 levels. An additive genetic model was assumed for all SNPs. Univariate analyses examined relationships between IGF-1 and IGFBP-3 in association with genotype for each of the five SNPs of interest. Stepwise linear regression was then used to consider all relevant clinical covariates considered previously, along with genotypes of all five SNPs. Models were refit with the surviving covariates.

Final multivariable models thus considered the association of various factors to circulating IGF-1 and IGFBP-3 levels, with and without genetic contributions.

RESULTS

Patient Population

From January 1999 to March 2009, more than 44,111 consecutive ICU admissions were screened for possible inclusion (**Fig. 1**). In total, 2,397 patients were consented and enrolled in the parent study. Caucasians comprised more than 90% of the parent cohort, and given the lack of racial diversity of this cohort, the current nested study was limited to Caucasians to avoid population stratification.

There were 1,854 patients who did not have available plasma for IGF-1 and IGFBP-3 measurements. There were no significant differences between those with and without plasma in terms of gender, diabetes, cirrhosis, sepsis or septic shock, pneumonia or aspiration, need for red cell transfusion, or trauma. There were, however, significant between-group differences in several variables: proportion of ARDS cases (33.9% with plasma vs 25.6% without plasma; $p = 0.0002$), age (mean, 63.8 ± 17.0 with plasma vs 60.8 ± 17.6 without plasma; $p = 0.0007$), BMI (median, 26.1; interquartile range [IQR], 7.5 with plasma vs median, 26.6; IQR, 8.0 without plasma; $p = 0.039$); and APACHE III (mean, 73.3 ± 24.1 with plasma vs 65.5 ± 23.0 without plasma; $p < 0.0001$).

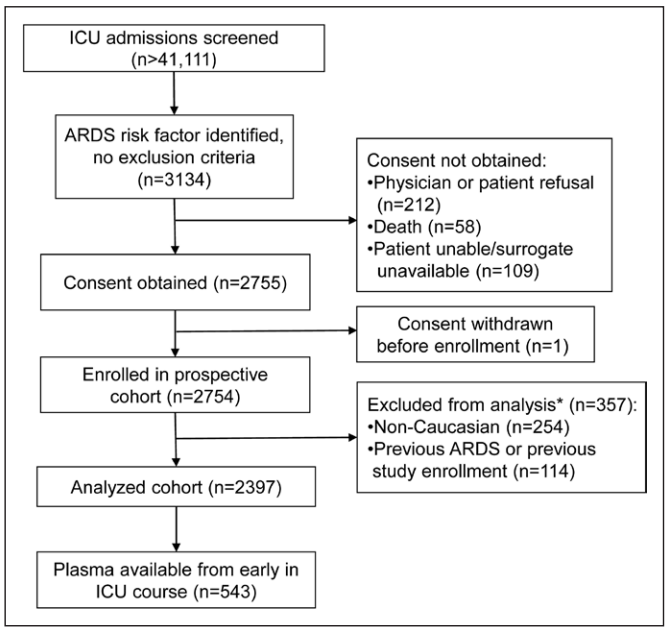

Figure 1. Study design and patient selection. *11 patients met both criteria for exclusion from current analysis. ARDS = acute respiratory distress syndrome.

Five hundred forty-three patients had plasma measurements of IGF-1 and IGFBP-3 completed plus available genotype information for the SNPs of interest. Although the aim of the study was to analyze IGF-1 and IGFBP-3 at the earliest possible point in critical illness, 27 patients (< 5%) had analysis of IGF-1 and IGFBP-3 from blood samples collected after day 3 of ICU admission. These were all patients who developed ARDS delayed from ICU admission.

Baseline patient characteristics of the current cohort are shown in **Table 2**. Three hundred twenty-nine patients (60.6%) received at least one dose of corticosteroid within the first 3 days of ICU admission; of these, 328 had a diagnosis of sepsis, septic shock, or ARDS. Of the patients with septic shock, 250 (88.3%) received at least one dose of corticosteroids within the first 3 days of ICU admission. All 184 patients with ARDS received at least one dose of corticosteroid within the first 3 days of ICU admission.

Phenotypic Predictors of Circulating IGF-1 and IGFBP-3

In univariate analyses of log-transformed biomarkers, significant ($p < 0.05$) predictors of plasma IGF-1 were age (β, –0.0049; $p = 0.007$), BMI (β, 0.0089; $p = 0.031$), smoking status (β, –0.090; $p = 0.042$), diabetes (β, –0.19; $p = 0.008$), cirrhosis (β, –0.78; $p < 0.0001$), composite variable for acute and/or chronic hepatic dysfunction (β, –0.39; $p < 0.0001$), sepsis/septic shock (β, –0.28; $p = 0.001$), pneumonia/aspiration (β, –0.13; $p = 0.036$), ARDS (β, –0.28; $p < 0.0001$), APACHE III (β, –0.0096; $p < 0.0001$), and receipt of corticosteroids (β, –0.38; $p < 0.0001$).

Significant predictors of IGFBP-3 were similar: age (β, –0.0072; $p < 0.0001$), BMI (β, 0.013; $p = 0.0002$), female gender (β, 0.11; $p = 0.049$), cirrhosis (β, –0.78; $p < 0.0001$), acute hepatic dysfunction β, –0.062; $p = 0.46$), sepsis/septic shock (β, –0.28; $p = 0.0002$), pneumonia/aspiration (β, –0.14; $p = 0.009$), ARDS (β, –0.19; $p = 0.001$), APACHE III (β, –0.0058; $p < 0.0001$), and receipt of corticosteroids (β, –0.28; $p < 0.0001$).

The final models of phenotypic variables in association with IGF-1 and IGFBP-3 are shown in **Table 3**. BMI, cirrhosis, and ARDS were strongly associated with both IGF-1 and IGFBP-3; APACHE III was strongly associated with IGF-1; and age was strongly associated with IGFBP-3.

Acute hepatic dysfunction (exclusive of cirrhosis) was not significant in multivariable models. The composite variable for acute/chronic hepatic dysfunction was significant, but this appeared to be driven by cirrhosis with the parameter estimate for cirrhosis alone being much larger with a lower p value. The inclusion of cirrhosis versus composite hepatic dysfunction did not significantly affect parameter estimates

TABLE 2. Baseline Cohort Characteristics

Characteristic	*n* = 543
Age, mean ± SD	63.8 ± 17.0
Female gender, *n* (%)	210 (38.7)
Body mass index, median (IQR)	26.1 (7.5)
Smoking status, *n* (%)[a]	
Never smoker	155 (28.9)
Past smoker	171 (31.9)
Current smoker	110 (20.6)
Diabetes, *n* (%)	141 (26.1)
Cirrhosis, *n* (%)	25 (4.6)
Acute hepatic dysfunction, *n* (%)[b]	65 (12.0)
Acute Physiology, Age, and Chronic Health Evaluation III score, mean ± SD	73.3 ± 24.0
Sepsis syndrome, *n* (%)[c]	179 (33.0)
Pulmonary source, *n* (%)	109 (60.9)
Septic shock, *n* (%)[c]	283 (52.1)
Pulmonary source, *n* (%)	143 (50.5)
Pneumonia, *n* (%)	282 (51.9)
Aspiration, *n* (%)	42 (7.7)
Trauma, *n* (%)	39 (7.2)
Acute respiratory distress syndrome cases, *n* (%)	184 (33.9)
Total insulin-like growth factor-1 (ng/mL), median (IQR)	67.3 (59.9)
Insulin-like growth factor–binding protein-3 (ng/mL), median (IQR)	1,989.7 (1,626.1)

IQR = interquartile range.

[a]Data missing for 107 patients (19.6%).

[b]Defined by bilirubin ≥ 2.0 mg/dL within 24 hr of ICU admission and not including patients with chronic cirrhosis.

[c]Sepsis syndrome does not include patients with septic shock; the total number of patients in the cohort with sepsis and/or septic shock is 462 (85.1%) with 252 (54.6%) from a pulmonary source.

of other variables. Thus, cirrhosis alone was included in the final models.

Receipt of steroids appeared to act as a confounder for sepsis/septic shock and/or ARDS. Receipt of steroids was indeed highly correlated with the presence of septic shock and/or ARDS ($\rho = 0.83$; $p < 0.0001$).

IGF Pathway Genotyping

Of the 543 patients with plasma IGF-1 and IGFBP-3 measurements, 479 had genotyping using the IBC platform, and 445 had genotyping using the Taqman platform. There were 381 patients with genotypes from both platforms. Genotype discrepancies between platforms for the five SNPs of interest were found in less than 0.5% of patients, and these were excluded. Overall genotyping success rate was 99%.

IGF Pathway Genotypes and Circulating IGF-1 and IGFBP-3

There were no significant deviations from Hardy-Weinberg equilibrium for any polymorphisms tested. In univariate analyses, there was no significant association between log-transformed IGF-1 and any of the five candidate SNPs (rs35767, rs1520220, rs2946834, rs4619, rs2854746). For IGFBP-3, however, there was a significant association with rs2854746 genotype (β, 0.12; $p = 0.0025$).

In multivariable models that included both genotype and clinical variables, rs2854746 genotype remained a significant predictor of IGFBP-3 (**Table 4**). Because of the strong correlation between IGF-1 and IGFBP-3, we took a conservative approach and forced rs2854746 genotype into the final multivariable model of IGF-1, although it had been rejected in the selection algorithm (Table 4). Models were very stable with no changes in significant clinical variables, and with minimal change in parameter estimates for those variables when adding rs2854746 to the models. This suggests that genotype added additional information to the model of IGFBP-3 and is not a confounder. This is further supported by an increase in adjusted R-square with rs2854746 genotype explaining and additional 2% of variation in IGFBP-3 levels than clinical variables alone.

DISCUSSION

The somatotropic axis including GH, IGF-1, and IGFBP-3 has been of great interest in critical care because of its relationships to metabolism, catabolism, and nutritional status, factors that are important during critical illness. These factors may also have significant prognostic implications for recovery from critical illness. Thus, we sought to further elucidate derangements in the somatotropic axis during critical illness by better understanding contributors to IGF-1 and IGFBP-3 levels in acute critical illness.

In this ICU cohort, acute and chronic health factors and genetic factors were associated with circulating IGF-1 and IGFBP-3. Although there were numerous strong associations, the clinical and genetic variables explained only a modest amount of variability in IGF-1 and IGFBP-3, which is consistent with prior studies (13, 19). The most variability was explained for IGFBP-3, also consistent with prior studies (13, 19). However, perhaps most interesting is that age and gender, factors that have been strongly and consistently associated with IGF-1 (11–14, 16, 18, 19), were insignificant in the face of critical illness. This is consistent with a prior study of 88 patients with a range of illness severity showing no association between age or gender and IGF-1 measured early in critical illness (27). Gender was also not significantly associated with IGFBP-3 in our cohort. BMI has had very inconsistent and largely null associations with IGF-1 and IGFBP-3 outside of critical illness (12–14). Yet, there was a strong association between BMI and both IGF-1 and IGFBP-3 levels in our study. Given that IGF-1 and IGFBP-3 are hepatically synthesized, it is not surprising that there was a strong negative relationship between hepatic dysfunction and circulating levels. As above,

TABLE 3. Parameter Estimates for Multivariable Models of Plasma Insulin-Like Growth Factor-1 and Insulin-Like Growth Factor–Binding Protein-3

Variables	logIGF-1	*p*	logIGFBP-3	*p*
Age	NS		−0.0068	< 0.0001
Female	NS		NS	
Smoking	NS		NS	
Body mass index	0.010	0.010	0.010	0.003
Diabetes	−0.17	0.013	NS	
Cirrhosis	−0.61	< 0.0001	−0.76	< 0.0001
Sepsis/septic shock	NS		−0.19	0.009
Pneumonia/aspiration	NS		NS	
Acute Physiology, Age, and Chronic Health Evaluation III	−0.0064	< 0.0001	NS	
Acute respiratory distress syndrome	−0.22	0.0005	−0.19	0.0004
Adjusted R-square	0.145		0.160	

IGF = insulin-like growth factor, IGFBP = insulin-like growth factor–binding protein, NS = not significant (variables that did not survive the selection algorithm prior to model refit, and thus parameter estimates and *p* values were not generated in the final model).
Cells show parameter estimates (β) except as noted. IGF-1 is measured in ng/mL; IGFBP-3 is measured in µg/mL.

this effect appeared to be driven by cirrhosis and not acute hepatic dysfunction.

We also saw a strong relationship with severity of illness. For IGF-1, this manifested as a negative association with APACHE III; for IGFBP-3, there was a negative association with both pneumonia and sepsis. This was not seen in at least two prior studies in critically ill patients (2, 28). Consistent with our prior work in this cohort, ARDS was also significantly associated with lower IGF-1 and IGFBP-3 levels (9).

Although our cohort is made up exclusively of patients with risk factors for ARDS, it is generally representative of the medically critically ill patient population with high severity of illness and 88% of patients meeting criteria for sepsis and/or septic shock, pneumonia, or aspiration. Although our cohort does

TABLE 4. Parameter Estimates for Multivariable Models of Plasma Insulin-Like Growth Factor-1 and Insulin-Like Growth Factor–Binding Protein-3, Including Genotype at rs2854746 (*IGFBP3*)

Variables	logIGF-1	*p*	logIGFBP-3	*p*
Age	NS		−0.0067	< 0.0001
Female	NS		NS	
Smoking	NS		NS	
Body mass index	0.011	0.008	0.011	0.002
Diabetes	−0.18	0.012	NS	
Cirrhosis	−0.61	< 0.001	−0.77	< 0.0001
Sepsis/septic shock	NS		−0.18	0.014
Pneumonia/aspiration	NS		NS	
Acute Physiology, Age, and Chronic Health Evaluation III	−0.0063	< 0.0001	NS	
Acute respiratory distress syndrome	−0.23	0.0003	−0.21	0.0001
rs2854746[a]	0.075	0.081	0.13	0.0006
Adjusted R-square	0.148		0.179	

IGF = insulin-like growth factor, IGFBP = insulin-like growth factor–binding protein, NS = not significant (variables that did not survive the selection algorithm prior to model refit, and thus parameter estimates and *p* values were not generated in the final model).
[a]Genotype forced into model for logIGF-1. Cells show parameter estimates (β) except as noted. IGF-1 is measured in ng/mL; IGFBP-3 is measured in µg/mL.

include 39 patients with major trauma as a risk factor for ARDS, seven of those patients also had pneumonia, aspiration, sepsis, or septic shock, or a combination of these. Thus, only 5.9% of our cohort had trauma without other medical critical illness.

With the large number patients with sepsis and ARDS, we found that the majority of our patients received corticosteroids within the first 3 days of ICU admission, consistent with standard of care for septic shock and ARDS during most of the enrollment period represented. There is evidence that glucocorticoid treatment can down-regulate expression of IGF-1 and its receptor (IGF-1R). However, it has been shown that glucocorticoid treatment does not affect circulating free or total IGF-1 nor circulating IGFBP-3 (29). Thus, we do not believe our results are significantly altered by corticosteroid administration, and any small effect that might be present would be well-distributed across the cohort.

Most studies measuring IGF-1 in critically ill patients have not measured IGFBP-3 (1, 27, 28, 30). The few existing ICU studies measuring both IGF-1 and IGFBP-3 are all small (2, 3, 8). In these studies, IGFBP-3 has shown less consistent decreases than IGF-1, perhaps because IGFBP-3 has a long half-life, and although critically ill patients are acutely catabolic, the effects of protein catabolism may not be reflected in lower IGFBP-3 levels right away (6). We did see low levels of both IGF-1 and IGFBP-3 in this study, however, which may speak to the high level of illness severity in our patient cohort.

In our study, the minor allele of rs2854746 (Ala32Gly) in *IGFBP3* was associated with increasing circulating IGFBP-3. rs2854746 is a nonsynonymous missense polymorphism that is a putative exon splicing silencer and enhancer. rs2854746 is in the same haplotype block as rs2854744, the most extensively studied *IGFBP3* SNP, and these two SNPs are in strong linkage disequilibrium. Phenotypically, rs2854746 has been associated with increases in circulating IGFBP-3 in several studies (11, 17, 18, 20). Cheng et al (18) hypothesized that higher levels of IGFBP-3 due to genetic variation may decrease IGF-1 bioavailability ultimately affecting its bioactivity in circulation and tissues. This may be particularly relevant in critical illness when there is global depression of IGF-1 and IGFBP-3. We did not see any significant relationships, either in univariate or multivariate analyses, between IGF-1 or IGFBP-3 and any other polymorphisms tested. In studies of noncritically ill patients, rs35767 has been associated with higher IGF-1 in at least one study (17), with no significant association in other studies (11, 20). Results for rs1520220 and rs2946834 have been similarly mixed with higher IGF-1 seen in some studies (11, 17), particularly among women (19), but no relationship seen elsewhere (20). rs4619 has been less well-studied, but it has been of interest because it is a nonsynonymous missense polymorphism in a coding region. No consistent relationship has been found between rs4619 and circulating IGF-1 or IGFBP-3 (17, 20).

Although caution must be taken in interpreting the significance of limited SNP data in a single study, our findings are bolstered by the associations between rs2854746 and increased circulating IGFBP-3 observed in noncritically ill cohorts (11, 17, 18, 20). But just as with IGF-1 levels, it is likely that there are many genetic (and nongenetic) determinants of IGFBP-3 that remain undefined (31). It is clear that circulating IGF-1 and IGFBP-3 are complex traits, and it may be that the small effects of individual polymorphisms are overshadowed by the influences of acute critical illness.

This study has several strengths including that, to our knowledge, this is the largest study examining both IGF-1 and IGFBP-3 in critically ill patients and the first study to examine genetic polymorphisms in the IGF pathway in critically ill patients. This is also the first study to combine both phenotypic and genotypic data to model the IGF pathway in an ICU cohort. The cohort is a prospectively enrolled, thoroughly characterized cohort with minimal misclassification of clinical variables. Biomarker measurements were done using state-of-the-art methodology by personnel blinded to clinical information. Assay variability was minimal, and although assay errors cannot be excluded, such errors would result in random misclassification, biasing toward the null. Genotyping was high quality with minimal failures or discrepancies between the two platforms used.

There are several limitations to this study. This is an observational study with measurements of IGF-1 and IGFBP-3 measured at one time point only, although the earliest point in critical illness was chosen because the majority of ICU patients demonstrate acute and often severe derangements at this early point, whereas derangements at later time points would be expected to vary substantially based on the trajectory of illness toward recovery, death, or chronic critical illness. We have not examined variables that may be more reflective of muscle weakness as a consequence of protein catabolism, and IGF-1 and IGFBP-3 levels at later time points may be relevant to such variables, particularly in patients with prolonged critical illness and those who develop significant muscle weakness.

Although our patient cohort is very well-characterized, we do not have information about significant nondiabetic endocrine comorbidities, such as thyroid or adrenal disease, both of which could affect the IGF pathway. We also do not have detailed information on hormone supplements, such as oral contraceptives or postmenopausal hormone replacement therapy.

We did not measure GH levels, and thus we cannot comment on the true relationship between GH and the biomarkers measured. Despite this, GH has been well-characterized as consistently elevated in acute critical illness, absent any primary disorders of GH. We do not have detailed data on insulin administration around the time of blood sampling; although when IGF-1 is measured in outpatient clinical settings, patients are not generally instructed to avoid insulin. In uncontrolled type 1 diabetes, IGF-1 levels are known to be low and subsequently rise with adequate insulin treatment, but the story in type 2 diabetes is much less clear with no consistent changes in IGF-1 levels compared with nondiabetic controls (31). It seems the more relevant factor may be nutritional status, specifically malnutrition or recent starvation. We did not have any dietary information from the time around ICU admission, and

we know that nutritional status is an important regulator of circulating IGF-1 in particular with low levels seen in acute starvation and chronic malnutrition (31). We would expect relative starvation with poor nutritional intake as part of the immediate prodrome of acute critical illness extending into the early phase of ICU admission (corresponding with timing of blood draws). However, it is an important limitation that we were unable to include nutritional variables in our analyses.

Although we have data on smoking status, the categorical smoking variable is imprecise, and there are missing data for almost 20% of the cohort.

We only tested associations with a limited number of candidate SNPs, and there may be additional information genotypes or haplotypes not tested here.

Finally, we acknowledge the possibility of selection bias given some significant differences between patients with available plasma and genotyping data and those without this information. This stems primarily from difficulty in rapidly enrolling and obtaining blood samples after ICU admission, a challenge commonly encountered in critical care research. We did include those variables that differed between groups (age, BMI, APACHE III, and ARDS case status) in our multivariable models. It is still possible that there are additional unmeasured variables that differ between these groups. Such differences, however, would be more likely to affect generalizability than internal validity.

CONCLUSION

Both acute and chronic health factors significantly influence circulating levels of IGF-1 and IGFBP-3 early in critical illness. rs2854746 is also significantly associated with IGFBP-3 levels in this ICU cohort, even after adjustment for other relevant covariates. Overall, these combined phenotypic and genotypic factors explain only a modest amount of variability in IGF-1 and IGFBP-3. Further research is needed to understand how these findings may be directly applied to patient care.

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REFERENCES

1. Takala J, Ruokonen E, Webster NR, et al: Increased mortality associated with growth hormone treatment in critically ill adults. *N Engl J Med* 1999; 341:785–792
2. Schuetz P, Müller B, Nusbaumer C, et al: Circulating levels of GH predict mortality and complement prognostic scores in critically ill medical patients. *Eur J Endocrinol* 2009; 160:157–163
3. Timmins AC, Cotterill AM, Hughes SC, et al: Critical illness is associated with low circulating concentrations of insulin-like growth factors-I and -II, alterations in insulin-like growth factor binding proteins, and induction of an insulin-like growth factor binding protein 3 protease. *Crit Care Med* 1996; 24:1460–1466
4. Van den Berghe G: Growth hormone secretagogues in critical illness. *Horm Res* 1999; 51(Suppl 3):21–28
5. Elijah IE, Branski LK, Finnerty CC, et al: The GH/IGF-1 system in critical illness. *Best Pract Res Clin Endocrinol Metab* 2011; 25:759–767
6. Lang CH, Frost RA: Role of growth hormone, insulin-like growth factor-I, and insulin-like growth factor binding proteins in the catabolic response to injury and infection. *Curr Opin Clin Nutr Metab Care* 2002; 5:271–279
7. Mesotten D, Van den Berghe G: Changes within the GH/IGF-I/IGFBP axis in critical illness. *Crit Care Clin* 2006; 22:17–28, v
8. Weber-Carstens S, Deja M, Koch S, et al: Risk factors in critical illness myopathy during the early course of critical illness: A prospective observational study. *Crit Care* 2010; 14:R119
9. Ahasic AM, Zhai R, Su L, et al: IGF1 and IGFBP3 in acute respiratory distress syndrome. *Eur J Endocrinol* 2012; 166:121–129
10. Clemmons DR: Involvement of insulin-like growth factor-I in the control of glucose homeostasis. *Curr Opin Pharmacol* 2006; 6:620–625
11. D'Aloisio AA, Schroeder JC, North KE, et al: IGF-I and IGFBP-3 polymorphisms in relation to circulating levels among African American and Caucasian women. *Cancer Epidemiol Biomarkers Prev* 2009; 18:954–966
12. Morimoto LM, Newcomb PA, White E, et al: Variation in plasma insulin-like growth factor-1 and insulin-like growth factor binding protein-3: Personal and lifestyle factors (United States). *Cancer Causes Control* 2005; 16:917–927
13. DeLellis K, Rinaldi S, Kaaks RJ, et al: Dietary and lifestyle correlates of plasma insulin-like growth factor-I (IGF-I) and IGF binding protein-3 (IGFBP-3): The multiethnic cohort. *Cancer Epidemiol Biomarkers Prev* 2004; 13:1444–1451
14. Kaklamani VG, Linos A, Kaklamani E, et al: Age, sex, and smoking are predictors of circulating insulin-like growth factor 1 and insulin-like growth factor-binding protein 3. *J Clin Oncol* 1999; 17:813–817
15. Kaklamani VG, Linos A, Kaklamani E, et al: Dietary fat and carbohydrates are independently associated with circulating insulin-like growth factor 1 and insulin-like growth factor-binding protein 3 concentrations in healthy adults. *J Clin Oncol* 1999; 17: 3291–3298
16. Harrela M, Koistinen H, Kaprio J, et al: Genetic and environmental components of interindividual variation in circulating levels of IGF-I, IGF-II, IGFBP-1, and IGFBP-3. *J Clin Invest* 1996; 98: 2612–2615
17. Patel AV, Cheng I, Canzian F, et al; Breast and Prostate Cancer Cohort Consortium: IGF-1, IGFBP-1, and IGFBP-3 polymorphisms predict circulating IGF levels but not breast cancer risk: Findings from the Breast and Prostate Cancer Cohort Consortium (BPC3). *PLoS One* 2008; 3:e2578
18. Cheng I, DeLellis Henderson K, Haiman CA, et al: Genetic determinants of circulating insulin-like growth factor (IGF)-I, IGF binding protein (BP)-1, and IGFBP-3 levels in a multiethnic population. *J Clin Endocrinol Metab* 2007; 92:3660–3666
19. Al-Zahrani A, Sandhu MS, Luben RN, et al: IGF1 and IGFBP3 tagging polymorphisms are associated with circulating levels of IGF1, IGFBP3 and risk of breast cancer. *Hum Mol Genet* 2006; 15:1–10
20. Su X, Colditz GA, Willett WC, et al: Genetic variation and circulating levels of IGF-I and IGFBP-3 in relation to risk of proliferative benign breast disease. *Int J Cancer* 2010; 126:180–190
21. Jernström H, Deal C, Wilkin F, et al: Genetic and nongenetic factors associated with variation of plasma levels of insulin-like growth factor-I and insulin-like growth factor-binding protein-3 in healthy premenopausal women. *Cancer Epidemiol Biomarkers Prev* 2001; 10:377–384
22. Gong MN, Thompson BT, Williams P, et al: Clinical predictors of and mortality in acute respiratory distress syndrome: Potential role of red cell transfusion. *Crit Care Med* 2005; 33:1191–1198
23. Bone RC, Sibbald WJ, Sprung CL: The ACCP-SCCM consensus conference on sepsis and organ failure. *Chest* 1992; 101: 1481–1483

24. Bernard GR, Artigas A, Brigham KL, et al: The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination. *Am J Respir Crit Care Med* 1994; 149:818–824
25. Ito Y, Nakachi K, Imai K, et al; JACC Study Group: Stability of frozen serum levels of insulin-like growth factor-I, insulin-like growth factor-II, insulin-like growth factor binding protein-3, transforming growth factor beta, soluble Fas, and superoxide dismutase activity for the JACC study. *J Epidemiol* 2005; 15(Suppl 1):S67–S73
26. Guo Y, Lanktree MB, Taylor KC, et al; IBC 50K SNP array BMI Consortium: Gene-centric meta-analyses of 108 912 individuals confirm known body mass index loci and reveal three novel signals. *Hum Mol Genet* 2013; 22:184–201
27. Kyle UG, Jolliet P, Genton L, et al: Clinical evaluation of hormonal stress state in medical ICU patients: A prospective blinded observational study. *Intensive Care Med* 2005; 31:1669–1675
28. Hajsadeghi S, Khamseh ME, Gholami S, et al: IGF-I concentration and changes in critically ill patients. *J Res Med Sci* 2011; 16:170–178
29. Frystyk J, Schou AJ, Heuck C, et al: Prednisolone reduces the ability of serum to activate the IGF1 receptor in vitro without affecting circulating total or free IGF1. *Eur J Endocrinol* 2013; 168:1–8
30. Kythreotis P, Kokkini A, Avgeropoulou S, et al: Plasma leptin and insulin-like growth factor I levels during acute exacerbations of chronic obstructive pulmonary disease. *BMC Pulm Med* 2009; 9:11
31. Clemmons DR: Clinical utility of measurements of insulin-like growth factor 1. *Nat Clin Pract Endocrinol Metab* 2006; 2:436–446