

# Persistently Elevated Glucagon-Like Peptide-1 Levels among Critically Ill Surgical Patients after Sepsis and Development of Chronic Critical Illness and Dismal Long-Term Outcomes

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**BACKGROUND:** Glucagon-like peptide-1 (GLP-1) is a gut-derived incretin hormone that stimulates insulin secretion, cellular glucose uptake, and has immune-regulatory functions. Glucagon-like peptide-1 is markedly altered after trauma and sepsis, but the implications remain unclear.

**STUDY DESIGN:** We performed an analysis of a prospective, longitudinal cohort study of critically ill surgical patients with sepsis. Patient characteristics and clinical data were collected, as well as peripheral blood sampling for biomarker analysis, out to 28 days after sepsis onset. We prospectively adjudicated sepsis diagnosis, severity, clinical outcomes, and 6-month follow-up.

**RESULTS:** The cohort included 157 septic surgical patients with significant physiologic derangement (Maximum Sequential Organ Failure Assessment [SOFA] score 8, interquartile range [IQR] 4 to 11), a high rate of multiple organ failure (50.3%), and septic shock (24.2%). Despite high disease severity, both early death (<14 days;  $n = 4$ , 2.9%) and overall inpatient mortality were low ( $n = 12$ , 7.6%). However, post-discharge 6-month mortality was nearly 3-fold higher (19.7%). Both GLP-1 and interleukin [IL]-6 levels were significantly elevated for 21 days ( $p \leq 0.01$ ) in patients who developed chronic critical illness (CCI) compared with patients with a rapid recovery. Elevated GLP-1 at 24 hours was a significant independent predictor for the development of CCI after controlling for IL-6 and glucose levels ( $p = 0.027$ ), and at day 14 for death or severe functional disability at 6 months (WHO/Zubrod score 4-5,  $p = 0.014$ ).

**CONCLUSIONS:** Elevated GLP-1 within 24 hours of sepsis is a predictor of early death or persistent organ dysfunction. Among early survivors, persistently elevated GLP-1 levels at day 14 are strongly predictive of death or severe functional disability at 6 months. Persistently elevated GLP-1 levels may be a marker of a nonresolving catabolic state that is associated with muscle wasting and dismal outcomes after sepsis and chronic critical illness. (J Am Coll Surg 2019;229:58–68. © 2019 by the American College of Surgeons. Published by Elsevier Inc. All rights reserved.)

CME questions for this article available at <http://jacscme.facs.org>

**Disclosure Information:** Authors have nothing to disclose. Timothy J Eberlein, Editor-in-Chief, has nothing to disclose.

**Support:** This study was supported in part by National Institute of General Medical Sciences (NIGMS) grants: R01 GM-113945 (PE), T32 GM008721 (MC) and P50 GM-111152 (SB, PE, FM) awarded by the National Institute of General Medical Sciences (NIGMS). Support was also provided by National Institute on Aging (NIA) grants R03 AG056444 (SB), P30 AG028740 (SB).

ClinicalTrials.gov Identifier: NCT02276417

Presented at the Western Surgical Association 126th Scientific Session, San Jose del Cabo, Mexico, November 2018.

Received December 18, 2018; Revised March 27, 2019; Accepted April 3, 2019.

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### Abbreviations and Acronyms

CCI	= chronic critical illness
GLP-1	= glucagon-like peptide-1
IL-6	= interleukin-6
PICS	= persistent inflammation, immunosuppression, and catabolism syndrome
RAP	= rapid recovery
SOFA	= Maximum Sequential Organ Failure Assessment
UF	= University of Florida

Glucagon-like peptide-1 (GLP-1) is a gut-derived incretin hormone that plays an important role in glucose homeostasis, including stimulating insulin secretion, suppressing glucagon secretion, inhibiting gastric emptying, and decreasing appetite. Loss of glucose homeostasis as a result of the injury stress response and ongoing proinflammation is a frequent occurrence in critically ill surgical intensive care unit (ICU) patients.<sup>1-3</sup> Numerous studies have demonstrated the association between failure to control hyperglycemia and poor outcomes in the ICU patients, including increased incidence of deaths, nosocomial infections, wound complications, prolonged ICU stays, and the neuropathy of critical illness.<sup>4,5</sup> Additionally, glycemic control is variable despite aggressive implementation of insulin protocols, even in patients without previous evidence or history of diabetes.<sup>6-9</sup> The pathophysiologic mechanisms for this variability and the role of GLP-1 are poorly understood. Although the role of GLP-1 in glycemic control has been studied in trauma,<sup>10</sup> it has not been well studied in sepsis, and the epidemiology of sepsis has changed in recent years. With rapid delivery of evidence-based care, early hospital mortality has decreased substantially, but unfortunately, roughly one-third of the survivors progress into chronic critical illness (CCI) with dismal long-term outcomes.<sup>11-13</sup> Our ongoing studies indicate that this is often due to a failure to achieve immune homeostasis, leading to a persistent inflammation, immunosuppression, and catabolism syndrome (PICS).<sup>14-18</sup> In this study, we serially determined GLP-1 levels after sepsis, with the intent of determining how they were related to development of CCI-PICS and its dismal long-term outcome. We hypothesized that abnormal GLP-1 levels, as a biomarker of metabolic stress and loss of glycemic homeostasis, would be associated with poor outcomes, specifically, the development of CCI and death or disability at 6 months.

## METHODS

### Study design, site, and patients

This was a retrospective subgroup analysis of an ongoing prospective longitudinal, observational cohort study of

trauma and surgical ICU patients treated for sepsis at an academic, quaternary medical, and level-1 trauma center (UF Health, Gainesville, FL). The parent study started in January 2015 and overall study design and protocols for the University of Florida (UF) Sepsis and Critical Illness Research Center (SCIRC) program have been previously published.<sup>19</sup> Glucagon-like peptide-1 biomarker analysis was performed on a subgroup of 157 consecutively enrolled patients from the parent cohort between January 2015 and September 2016, which delineates the analytic cohort for this study. The GLP-1 analytic cohort was similar and representative of the parent program cohort across all domains (eTable 1). It should be noted that GLP-1 was not an a priori component or hypothesis of the parent sepsis cohort study. Instead, GLP-1 was added to the program's serial biomarker panel and analytic batches after the senior author (RSS) joined the UF faculty, based on his interest and previous work on investigating GLP-1 in trauma patients.<sup>11</sup> Although GLP-1 was added in ad hoc fashion after initiation of the parent cohort, from that point on, the patients were consecutively enrolled, sampled, batch analyzed (including GLP-1), and followed longitudinally in prospective fashion. The parent study received Institutional Review Board (IRB) approval upon program initiation in 2015, and IRB reapproval was obtained when GLP-1 was added as an ad hoc analyte. With an established IRB-approved precedent of initial enrollment with delayed consent for critically ill patients, we obtained signed informed consent from the patient or legal proxy within 96 hours of study enrollment. Patients included in this study were those ICU patients who screened positive for sepsis and were placed on our ICU sepsis protocol either on admission with, or subsequent development of sepsis. Sepsis screening was performed using the Modified Early Warning Signs-Sepsis Recognition System (MEWS-SRS).<sup>12</sup> All enrolled patients were managed using a standardized sepsis management protocol based on the Surviving Sepsis guidelines, supplemented by standardized evidenced-based ICU clinical care protocols, and executed with the assistance of electronic medical record (EMR)-based clinical care order sets to ensure high compliance and timely intervention.

### Patient enrollment, classification, and outcomes

Parent cohort inclusion criteria consisted of the following: age 18 years or older; presence in the surgical or trauma ICU; and clinical diagnosis of sepsis, severe sepsis, or septic shock, as defined by the 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference.<sup>20</sup> Sepsis diagnosis, severity, and source were clinically adjudicated in prospective fashion by Sepsis and Critical

Illness Research Center program investigators at weekly adjudication meetings. Exclusion criteria included refractory shock (death < 24 hours) or inability to achieve source control (eg unresectable dead bowel); pre-sepsis expected lifespan < 3 months; patient goals of care not consistent with aggressive management; severe congestive heart failure (NYHA Class IV); Child-Pugh Class C liver disease or pre-liver transplant; HIV with CD4+ count < 200 cells/mm<sup>3</sup>; chronic corticosteroids or immunosuppressive agent use, including organ transplant recipients; pregnancy; chemotherapy or radiotherapy within the past 30 days; severe traumatic brain injury (evidence of neurologic injury on CT scan and Glasgow Coma Scale < 8); or spinal cord injury resulting in permanent sensory and/or motor deficits. Baseline and inpatient clinical data collected prospectively during initial hospitalization included patient and infection characteristics, sepsis severity, electronic medical record-based clinical and laboratory data, complications, and inpatient disposition.

Based on established definitions, post-sepsis clinical trajectories were categorized as “early death,” “rapid recovery,” (RAP) or CCI. Early death was defined as those patients who died before day 14. Chronic critical illness was defined as an ICU length of stay (LOS) greater than or equal to 14 days, with evidence of persistent organ dysfunction, determined using components of the Sequential Organ Failure Assessment (SOFA) score (cardiovascular SOFA  $\geq$  1, or score in any other organ system  $\geq$  2). Rapid recovery patients were the remaining patients who had organ function recovery and discharge from the ICU within 14 days of sepsis onset. Inpatient outcomes included in-hospital mortality, hospital and ICU lengths of stay, ventilator days, presence and severity of organ dysfunction, development of CCI, and discharge disposition. All patients enrolled in the parent cohort, who survived to discharge, underwent scheduled follow-up visits, which were conducted at the UF Institute on Aging, the patient’s home, or via telephone, with a tiered priority structure, respectively. Six-month outcomes included performance status and mortality. Performance status was measured by the WHO/Zubrod scale, a 5-point scale that measures the performance status of a patient’s ambulatory nature: 0) asymptomatic (fully active), 1) symptomatic but completely ambulatory (restricted in physically strenuous activity), 2) symptomatic, <50% in bed during the day (ambulatory and capable of all self-care but unable to perform any work activities), 3) symptomatic, >50% in bed, but not bedbound (capable of only limited self-care), 4) bedbound (completely disabled, incapable of any self-care), and 5) death. Six-month mortality incidence underwent cross-check validation via the United States Social Security Death Index (SSDI).

### Biomarker analyses

All subjects enrolled in the parent cohort underwent peripheral blood sampling at 12 hours, 1, 4, 7, and 14 days after sepsis onset, and weekly thereafter while hospitalized. These samples were processed and stored for subsequent program a priori and ad hoc biomarker analysis. This included the biomarker of interest for this analysis GLP-1 (Luminex; MilliporeSigma) and interleukin-6 [IL-6] (ELISA; MilliporeSigma). We chose to use IL-6 as a covariate biomarker in this analysis because it is a well-established measure of the magnitude of the host innate pro-inflammatory response. We have also shown previously that IL-6 is a predictive biomarker for CCI after sepsis.<sup>16</sup>

### Statistical analysis

Data are presented as frequency and percentage, or mean and standard deviation, or median and 25th/75th percentiles. Student’s *t*-test, ANOVA, and Kruskal–Wallis tests were used for comparison of continuous variables as appropriate. Chi-square test and Fisher’s exact test were used for comparison of categorical variables. Measured biomarkers were compared using nonparametric rank tests to determine significant differences between groups at each time point. Due to the similarity of clinical trajectory, the small number ( $n = 4$ ) of early death patients were combined with the CCI cohort for all biomarker analyses in this study. In order to determine if GLP-1 was associated with adverse outcomes independent of inflammation and glucose dysregulation, 2 multivariate logistic regression models were constructed to determine if GLP-1 was within 24 hours of the development of CCI, and at day 14 for death and severe disability (Zubrod score 4 to 5) at 6 months after sepsis onset after controlling for both IL-6 and peak glucose level on those days. Adjusted odds ratios (OR) with 95% confidence intervals (95% CI) and the area under the receiver operating characteristics curve values (AUC) and Hosmer–Lemeshow goodness-of-fit test were used. Due to suspicion of high collinearity of GLP-1 with the covariates, total SOFA, and Charlson comorbidity scores, we performed Spearman analysis to determine correlations between these continuous and ordinal variables. All significance tests were 2-sided, with  $p$  value  $\leq$  0.05 considered statistically significant. Statistical analyses were performed with SAS (v.9.4).

### RESULTS

During an 18-month period ending in September 2016, 157 consecutively enrolled study patients underwent serial GLP-1 biomarker analyses. Demographics, baseline

**Table 1.** Demographics, Baseline Characteristics, and Outcomes for All Study Patients

Parameter	Overall cohort (n = 157)
Age, y, median (25th, 75th)	62 (51, 70)
Male sex, n (%)	85 (54)
Race/ethnicity, n (%)	
Black or African American	13 (8)
Caucasian	139 (89)
Other	5 (3)
Charlson comorbidity index, median (25th, 75th)	4 (2, 6)
Interfacility hospital transfer, n (%)	68 (43)
Hospital admission diagnosis, n (%)	
Planned surgical procedure	28 (18)
Intra-abdominal sepsis	24 (16)
NSTI	23 (15)
Surgical site infection	16 (10)
Trauma	14 (9)
Other - noninfectious	12 (8)
Vascular disease - aorta/mesenteric	10 (7)
Other acute infection	7 (4.5)
Necrotizing pancreatitis	5 (3.2)
UTI	5 (3.2)
Pneumonia	4 (2.6)
Cholecystitis	3 (1.9)
Planned other surgery, not specified	2 (1.3)
Vascular disease, extremity	1 (0.6)
Primary sepsis diagnosis, n (%)	
Intra-abdominal sepsis	65 (42)
Pneumonia	28 (18)
NSTI	25 (16)
Surgical site infection	21 (14)
UTI	9 (6)
Catheter-related bloodstream infection	2 (1.3)
Bacteremia	1 (0.6)
Empyema	1 (0.6)
Other	2 (1.3)
Sepsis severity, n (%)	
Sepsis	57 (36)
Severe sepsis	62 (40)
Septic shock	38 (24)
APACHE II, median (25th, 75th)	17 (12, 23)
Maximum SOFA score, median (25th, 75th)	8 (4, 11)
Multiple organ failure,* n (%)	79 (50)
Ventilator days, median (25th, 75th)	2 (0, 6)
ICU LOS, median (25th, 75th)	8 (4, 17)
Hospital LOS, median (25th, 75th)	17 (8, 28)

(Continued)

**Table 1.** Continued

Parameter	Overall cohort (n = 157)
In-hospital mortality, n (%)	12 (7.6)
6-mo mortality, n (%)	31 (19.7)

LOS, length of stay; NSTI, necrotizing soft tissue infection; SOFA, sequential organ failure assessment; UTI, urinary tract infection.

\*Denver Multiple Organ Failure score  $\geq 3$

characteristics, in-patient outcomes, and long-term outcomes of these study patients are depicted in [Table 1](#). The overall study cohort consisted primarily of middle-aged and older adults (median age 62 years) with an equal biologic sex distribution, significant comorbidity burden, a high incidence and severity of physiologic derangement as measured by vasopressor requirements, APACHE II scores ([Table 1](#)). Roughly one-third carried a pre-existing diagnosis of diabetes mellitus requiring either oral or subcutaneous (ie insulin) hyperglycemic control medications. The majority of patients were admitted to the hospital with an acute, infection-related diagnosis and had intra-abdominal infection as the septic source ([Table 1](#)). The severity of organ dysfunction in this septic cohort was high, with a 50% incidence of multiple organ failure and a low in-hospital mortality of approximately 8% ([Table 1](#)).

The characterization of post-sepsis clinical trajectories is depicted in [Table 2](#). There were 4 (2.5%) early deaths, 98 (62.5%) RAP, and 55 (35%) CCI patients. There were no significant differences in hospital admission diagnosis or septic source between these groups. Chronic critical illness patients compared with RAP patients were older, had significantly higher incidence of septic shock, and a greater severity of organ dysfunction ([Table 2](#)). Although CCI patients carried a higher comorbidity burden than RAP patients, there was no difference in the rate of medication-dependent diabetes mellitus between CCI and RAP groups. The CCI patients had significantly worse clinical outcomes, including a greater number of ventilator days, longer ICU length of stay, and higher inpatient mortality ([Table 2](#)). Although approximately 85% of patients who developed CCI survived to discharge, 90% of these were discharged with a disposition to facilities associated with poor long-term outcomes (long-term acute care facility, skilled nursing facility, another hospital, or hospice). Additionally, prospective post-discharge follow-up revealed a much higher 6-month mortality for the CCI patients (40%) compared with RAP patients (5%, [Table 2](#)).

[Figure 1](#) depicts serial IL-6, GLP-1, and maximum measured daily glucose levels. Consistent with previously

**Table 2.** Post-Sepsis Clinical Trajectory Comparisons

Parameter	Early death (n = 4)	CCI (n = 55)	RAP (n = 98)	p Value*
Age, y, median (25th, 75th)	62 (51, 70)	65 (58, 72)	59 (48, 69)	0.010
Male sex, n (%)	2 (50)	36 (65.5)	47 (48)	0.045
Race, n (%)				0.54
Black or African American	1 (25)	4 (7.3)	8 (8.2)	
White	3 ((75)	50 (90.9)	86 (87.8)	
Other	0 (0)	1 (1.8)	4 (4.0)	
Charlson Comorbidity Index, median (25th, 75th)	4.5 (3.5, 6)	5 (3, 8)	3.5 (2, 6)	0.014
Comorbidity, diabetes, n (%)	1 (25)	21(38.2)	31 (31.6)	0.38
Interfacility hospital transfer, n (%)	3 (75)	29 (52.7)	36 (36.7)	0.032
Sepsis severity, n (%)				
Sepsis	1 (25)	11 (20)	45 (45.9)	0.001
Severe sepsis	0 (0)	25 (45.5)	37 (37.8)	
Septic shock	3 (75)	19 (34.5)	16 (16.3)	
Lactic acid (mmol/L), median (25th, 75th)	2.5 (1.6, 5.2)	2 (1.3, 3.6)	1.6 (1.2, 2.3)	0.033
APACHE II, median (25th, 75th)	27 (19, 38)	21 (15, 26)	14 (10, 21)	<0.001
Maximum SOFA score, median (25th, 75th)	17 (11, 22)	10 (8, 13)	6 (3, 8)	<0.001
Multiple organ failure <sup>†</sup> , n (%)	4 (100)	43 (78.2)	32 (32.7)	<0.001
Ventilator days, median (25th, 75th)	5.5 (5, 10)	7 (3, 15)	0 (0, 2)	<0.001
ICU LOS, median (25th, 75th)	5.5 (5, 9)	20 (15, 29)	5 (3, 9)	<0.001
Hospital LOS, median (25th, 75th)	6 (5.5, 9.5)	28 (20, 41)	12 (7, 20)	<0.001
Hospital mortality, n (%)	4 (100)	8 (14.5)	0 (0)	<0.001
Discharge disposition, n (%)				<0.001
Good	0 (0)	6 (11)	74 (76)	
Home	0 (0)	0 (0)	29 (30)	
Rehab	0 (0)	1 (1.8)	3 (3.1)	
Home health care service	0 (0)	5 (9.1)	42 (43)	
Poor disposition	4 (100)	49 (89)	24 (24)	
LTAC	0 (0)	24 (44)	1 (1)	
SNF	0 (0)	7 (13)	23 (24)	
Another inpatient hospital	0 (0)	8 (15)	0 (0)	
Hospice	0 (0)	5 (9.1)	0 (0)	
Death	4 (100)	5 (9.1)	0 (0)	
6-mo mortality, n (%)	4 (100)	22 (40)	5 (5.1)	<0.001

CCI, chronic critical illness; LOS, length of stay; LTAC, long-term acute care facility; RAP, rapid recovery; Rehab, inpatient rehabilitation facility; SNF, skilled nursing facility; SOFA, sequential organ failure assessment score.

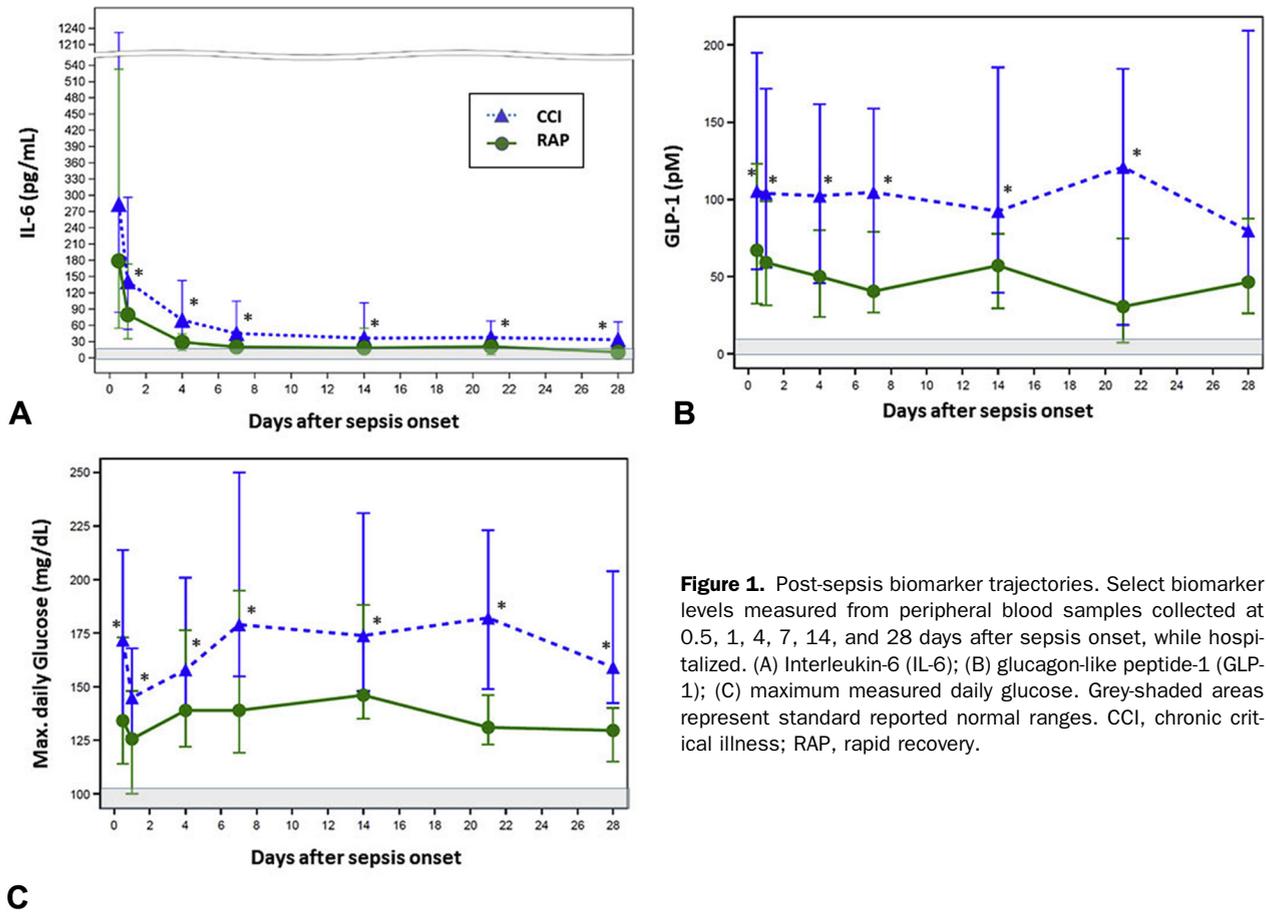
\*Comparison of chronic critical illness and rapid recovery groups.

<sup>†</sup>Denver Multiple Organ Failure score  $\geq 3$

published data on the known dysfunctional inflammatory response to sepsis, IL-6 levels were significantly elevated among all study patients, as compared with healthy age, sex, and ethnicity matched controls out to 28 days after sepsis onset (data not shown). Additionally, IL-6 levels were significantly elevated in CCI patients as compared with the RAP group at all measured time points between 24 hours and 28 days after sepsis onset (Fig. 1A). Levels of GLP-1 were significantly elevated among CCI patients at all measured time points from sepsis onset out to 21 days, as compared with RAP (Fig. 1B). Maximum daily blood glucose levels were significantly elevated in CCI patients

as compared with the RAP group across all measured time points (Fig. 1C).

Table 3 depicts the results of the multivariate models for association of GLP-1 with CCI and 6-month outcomes. Across all time points, GLP-1 and maximal daily blood glucose levels were modestly, but significantly, correlated (Spearman correlation = 0.27,  $p < 0.0001$ ). However, GLP-1 level at 24 hours after sepsis onset was independently associated with CCI after controlling for IL-6 and blood glucose levels. Similarly, after controlling for IL-6 and blood glucose levels, persistently elevated GLP-1 level at day 14 was associated with death or severe



**Figure 1.** Post-sepsis biomarker trajectories. Select biomarker levels measured from peripheral blood samples collected at 0.5, 1, 4, 7, 14, and 28 days after sepsis onset, while hospitalized. (A) Interleukin-6 (IL-6); (B) glucagon-like peptide-1 (GLP-1); (C) maximum measured daily glucose. Grey-shaded areas represent standard reported normal ranges. CCI, chronic critical illness; RAP, rapid recovery.

disability (ie Zubrod score 4 or 5) at 6 months (Table 3). Inclusion of total insulin as a covariate did not affect these models. Of note, Charlson comorbidity index score and total SOFA score at 24 hours and 14 days are strong composite (ie multidomain) predictors of both CCI and 6-month mortality and eliminate all serum biomarkers

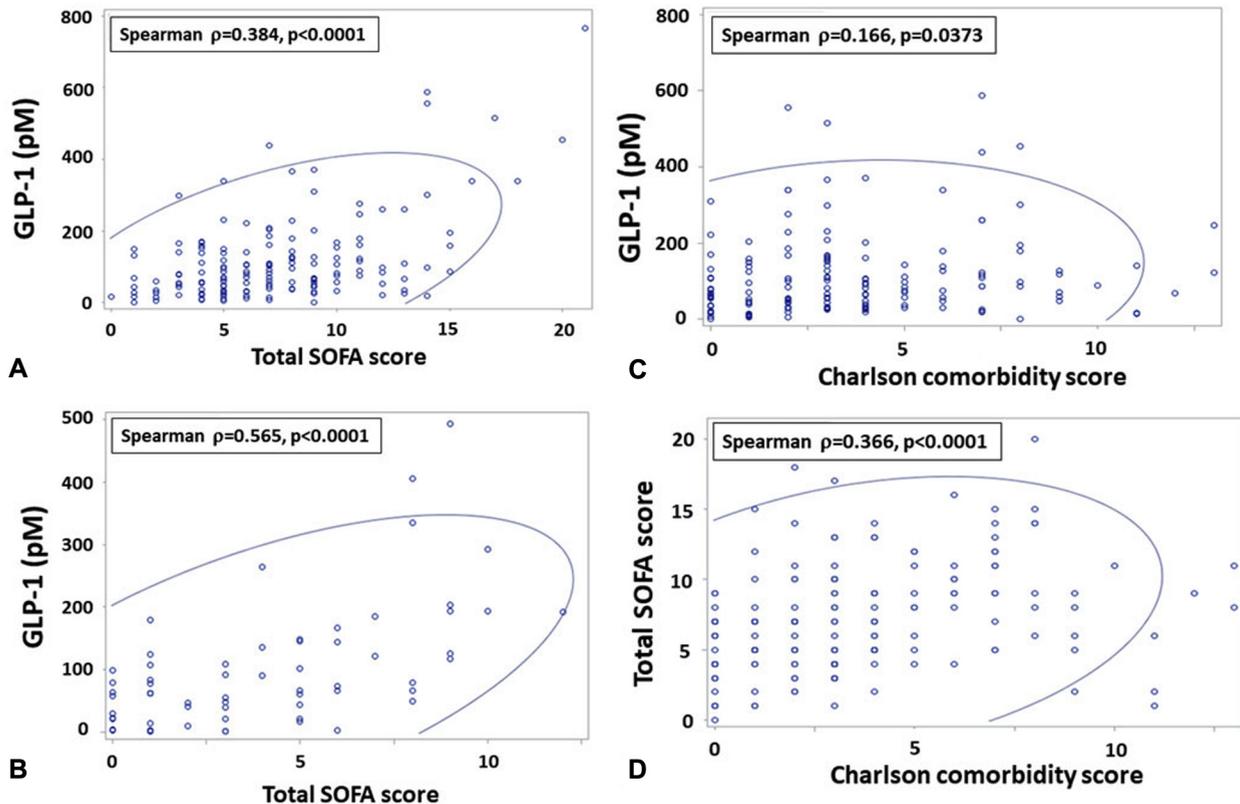
(IL-6, glucose, GLP-1) when included in the stepwise selection models. We therefore tested correlations of GLP-1 with baseline Charlson comorbidity index and total SOFA score at 24 hours and 14 days. Levels of GLP-1 were strongly correlated with total SOFA score at 24 hours (Spearman rho = 0.384, p < 0.0001; Fig. 2A)

**Table 3.** Multivariate Models for Association of Glucagon-Like Peptide-1 with Chronic Critical Illness and 6-Month Outcomes

Model/covariate	Odds ratio (95% CI)	p Value	AUC
24-hour level predicting chronic critical illness			
GLP-1	1.004 (1.000–1.007)	0.027	0.689
IL-6	1.000 (1.000–1.000)	0.076	
Glucose	1.005 (1.000–1.010)	0.035	
Day-14 level predicting 6-month death/severe disability*			
GLP-1	1.011 (1.002–1.020)	0.014	0.717
IL-6	0.995 (0.986–1.004)	0.27	
Glucose	1.003 (0.994–1.013)	0.50	

Odds ratio represents the odds increase for an increase in a 1-unit increase in biomarker level: GLP-1 (nM), IL-6 (pg/mL), glucose (mg/dL). AUC, multivariate model area under the curve; GLP-1, glucagon-like peptide-1; IL-6, interleukin-6.

\*Zubrod score of 4 (severe disability) or 5 (death)



**Figure 2.** Correlation analysis of glucagon-like peptide-1 (GLP-1), organ dysfunction, and comorbidity burden. (A) Correlation plot of GLP-1 level and total Sequential Organ Failure Assessment (SOFA) score at 24 hours. (B) Correlation plot of GLP-1 level and total SOFA score at 14 days. (C) Correlation plot of GLP-1 level at 24 hours and Charlson comorbidity score. (D) Correlation plot of total SOFA score and Charlson comorbidity score. Blue oval represents 95% prediction ellipse.

and 14 days (Spearman  $\rho = 0.59$ ,  $p < 0.0001$ ; Fig. 2B). Levels of GLP-1 were modestly correlated with baseline Charlson comorbidity score (Spearman  $\rho = 0.37$ ,  $p < 0.0001$ ; Fig. 2C). Total SOFA score at 24 hours and 14 days was also significantly correlated with baseline comorbidity (Fig. 2D).

## DISCUSSION

Gut hormones, such as GLP-1, facilitate metabolism of glucose through stimulation of insulin secretion and various other mechanisms. It has been well established that trauma and sepsis result in loss of glycemic homeostasis and that the resulting hyperglycemia adversely affects immune function, wound healing, and metabolism, resulting in poor outcomes.<sup>1-9,21-24</sup> The mechanisms responsible for this glucose dysregulation are complex. However, based on the normal physiologic role of GLP-1, it should play an important role regarding metabolic regulation in septic ICU patients.<sup>25-31</sup> Indeed, Deane and colleagues<sup>32-34</sup> demonstrated that the exogenous

administration of GLP-1 analogues in critically ill populations has a modulating effect on glucose homeostasis. Our evaluation of septic surgical patients confirms these observations. Both GLP-1 levels and glucose levels were consistently and persistently elevated in patients who develop CCI. Levels of GLP-1 and blood glucose showed modest, but significant, correlation in this group. The most straightforward explanation for this finding is that elevated GLP-1 levels were in response to high blood glucose. This is the well documented function of the incretin system, as GLP-1 receptors present on pancreatic islet cells increase insulin secretion. However, extrapancreatic functions of GLP-1 appear to be involved in the response to sepsis.

Additionally, our multivariate models clearly demonstrated that an elevated GLP-1 level at 24 hours was independent of both IL-6 and maximum glucose levels, and is associated with development of CCI. Similarly, elevated GLP-1 levels at 14 days after the onset of sepsis independently is associated with death or severe disability in the subsequent 6 months. This suggests that GLP-1 response

after sepsis is independent of both systemic inflammation and glucose regulation. To our surprise, this analysis showed that elevated GLP-1 level is a better predictor of outcomes than IL-6. Although CCI compared with RAP patients were older, more likely to require vasopressor support, and had more medical comorbidities, they did not have a greater incidence of pre-sepsis diabetes requiring pharmacologic control. Again, this is suggestive of factors other than simple hyperglycemia causing compensatory elevation of GLP-1 levels.

Secretion of GLP-1 has been demonstrated to rapidly increase in response to cytokines, particularly IL-6. Ellinggaard and colleagues<sup>35</sup> showed that administration of IL-6 stimulates GLP-1 secretion from intestinal L cells and pancreatic alpha cells. This mechanism increased insulin secretion and improved glycemic control. This group concluded that IL-6 mediates crosstalk between insulin sensitive tissues, intestinal L cells, and pancreatic islet cells to adapt to changes in insulin demand. LeBrun and associates<sup>36</sup> noted that GLP-1 suppresses inflammation and promotes gut mucosal integrity. Furthermore, this group demonstrated that GLP-1 levels increased rapidly after the administration of lipopolysaccharide in mice. This phenomenon was detected before measurable changes in cytokine levels or lipopolysaccharide. A similar response was noted after gut ischemia.<sup>34</sup> Leberherz and coworkers<sup>37</sup> previously demonstrated the predictive importance of GLP-1 levels in critically ill patients. This group measured GLP-1 levels in critically ill patients admitted to an ICU, patients with chronic kidney disease on hemodialysis, and a control group without acute inflammation or kidney disease. Critically ill patients had a 6-fold increase in GLP-1 levels compared with the control group. Those requiring hemodialysis exhibited a 4-fold higher GLP-1 level compared with controls. This group concluded that both chronic and acute inflammatory states, including sepsis, increase circulating GLP-1 levels. Furthermore, this group demonstrated *in vivo* that serum from critically ill patients had a strong potential for inducing GLP-1 secretion. This group concluded that elevated GLP-1 levels independently predicted mortality in critically ill and end-stage renal disease patients.

Given these findings, it is easy to hypothesize that elevated GLP-1 levels are merely another compensatory biomarker of dysregulated glucose control after an acute, severe pro-inflammatory stressor. However, another possibility may be that GLP-1 elevation is representative of (or even contributory to) persistently deranged metabolism and a catabolic state after sepsis. Given the findings here of high GLP-1 correlation with both early and persistent organ dysfunction severity and comorbidity burden, it is possible that elevated GLP-1 levels after sepsis may

represent noninsulin-dependent metabolic dysregulation driven by organ dysfunction and exacerbated by pre-existing comorbidities.

Based on substantial clinical and basic research data available in 2012, we proposed that failure to achieve immune homeostasis after a septic insult results in a pathophysiologic syndrome of persistent inflammation, immunosuppression, and catabolism (PICS).<sup>14</sup> We believe PICS is the driving mechanism for the development of CCI and its dismal long-term outcomes after surgical sepsis. Our subsequent and ongoing work strongly supports this hypothesis.<sup>15-18</sup> Of note, our studies show that nonrecovery from acute kidney injury (AKI) is the strongest predictor of dismal outcomes in CCI patients, and we believe this perpetuates inflammation and immunosuppression through the sustained local and systemic release of damage-associated molecular patterns (DAMPs), cytokines, and the expansion of myeloid-derived suppressor cells (MDSCs) and through metabolic reprogramming (ie aerobic glycolysis). Normal cells metabolize glucose through glycolysis, and under normoxic conditions, the pyruvate that is generated is further oxidized in mitochondria to produce adenosine triphosphate (ATP). When oxygen is limited, mitochondrial oxidative metabolism is restricted and pyruvate is only converted to lactate, with minimal ATP production. In tumor cells, this latter process is dominant even when oxygen is plentiful and is known as “the Warburg effect” or aerobic glycolysis. As active innate immune effector cells rely on glucose primarily for cellular fuel, a switch to aerobic glycolysis is thought to occur as a compensatory mechanism. After sepsis, persistently elevated levels of GLP-1 may represent an underlying shift in metabolic programming toward aerobic glycolysis, and serve as a metabolic marker of the development of PICS pathophysiology and prognosticate a high risk of poor long-term CCI outcomes.

## CONCLUSIONS

Elevated circulating GLP-1 levels within 24 hours of sepsis are strongly associated with early death or the development of CCI independent of the known modulating effects of IL-6 and glucose dysregulation. Similarly among early survivors, persistently elevated GLP-1 levels at day 14 was also independently associated with death or severe functional disability at 6 months. Of note, elevated GLP-1 levels appear to be more strongly associated with a poor outcome than IL-6. Given significant correlation with organ dysfunction severity and pre-existing comorbidities, persistently elevated GLP-1 levels may be a marker of ongoing stress metabolism and a nonresolving catabolic

state, associated with muscle wasting and dismal CCI outcomes after sepsis. Future work should focus on elaborating these underlying mechanisms.

### Author Contributions

Study conception and design: Brakenridge, Moore, Smith

Acquisition of data: Brakenridge, Wu

Analysis and interpretation of data: Brakenridge, Moore, Mercier, Cox, Wu, Moldawer, Mohr, Efron, Smith

Drafting of manuscript: Moore, Smith

Critical revision: Brakenridge, Moore, Mercier, Cox, Wu, Moldawer, Mohr, Efron, Smith

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## Discussion



**DR JOHN FILDES** (Las Vegas, NV): The authors are to be commended on a carefully designed and well-executed study of glucagon-like peptide 1 (GLP-1) and its association with clinical outcomes in sepsis. They have shown that GLP-1 levels are markedly altered after sepsis, but the implication is unclear. Persistently elevated GLP-1 levels are strongly predictive of death and severe functional disability at 6 months. The methods are well designed, statistical analysis is rigorous, and the results are both clearly stated and strongly supported by the data. It is clear that GLP-1 is correlated with both inflammation, as is interleukin (IL)-6, and metabolic disturbances such as glucose and insulin metabolism, and it functions as a strong biomarker, but its role in the mechanistic understanding of this syndrome is not complete.

First, in the dysfunctional endocrine milieu associated with sepsis, how big a player is GLP-1 when compared with insulin or IL-6? The manuscript would imply that it is on equal footing in terms of the number of organs and the intensity of response that it provokes from receptor activation. Second, is this one of many stress response biomarkers elaborated in this complex response? Or is this a proximal signal for the overall pathologic response—one that could attenuate or amplify the stress response of sepsis? Third, as an investigator in the use of anti-TNF alpha antibody in the early 1990s, I came away from those trials, those failed trials, believing that the forces of human evolution allowed the expression of metabolic disturbance and inflammation ultimately because they confer some sort of beneficial survival effect on humans. Could it be that your rapid recovery group demonstrates the intended course of the disease? Could it be that your rapid recovery group has characteristics that might provide more

information about where this process goes awry? Most of your attention has been paid to those who failed to respond.

**DR STEPHEN SMITH** (Gainesville, FL): Frankly, we are very much at the beginning of this process. We are just scratching the surface in determining the role of GLP-1 and the response to illness, sepsis in this case, and injury. There are 3 working diagnoses, and, quite honestly, in our group, we do not completely agree on the hypothesis regarding how GLP-1 plays out in sepsis. The first hypothesis is, as I think you stated in one of your questions, GLP-1 is just one of those many biomarkers that occurs after serious illness, and it probably plays no role as a mechanistic component to the response to sepsis. Based on our information and the pre-existing literature, we think that is the least likely hypothesis. The second hypothesis is that GLP-1 is really an important factor in the metabolic reprogramming, which my colleagues in the sepsis center have done a lot of work on, and it is a mediator for continued aerobic glycolysis. This hypothesis is actually supported by our findings that GLP-1 levels at 14 days remain elevated, and lactate similarly remains elevated at 14 days. The third hypothesis is that elevated levels of GLP-1 are actually beneficial and are a part of the recovery process through their anti-inflammatory effects and other effects that are not incredibly clear at this point.

Certainly, GLP-1 levels are elevated in a variety of conditions that cause severe stress. Quite honestly, I favor this final hypothesis because the preponderance of the pre-existing literature documents that in animal trials, and some limited human trials, patients generally do better with exogenous GLP-1 administration. I look forward to hopefully establishing this third hypothesis as the correct one, but to answer your question, at this point, we simply do not know. There are not enough data, either from our paper or from the existing literature in critical illness, to answer this question.

Adam Deane's group in Adelaide has administered GLP-1 in the ICU setting and has found a beneficial effect for critically ill patients in a fairly small series. And there is a ton of animal literature for either GLP-1 or GLP-1 analog that are now commercially available, have been administered, and it has been universally protected not only for sepsis, but for ischemic reperfusion injury and a variety of other conditions. I think this again supports that last hypothesis.

**DR LEIGH NEUMAYER** (Tucson, AZ): Did the etiology of the sepsis come into play at all in this series?

**DR STEPHEN SMITH** (Gainesville, FL): The etiology of the sepsis was broad spectrum, typically seen in a surgical or trauma ICU. It was not limited, for example, to intra-abdominal sepsis vs pneumonia that develops subsequently.

**DR JAMES MADURA** (Scottsdale, AZ): I am familiar with GLP-1 through the mechanism of diabetes resolution after bariatric surgery, where rapid presentation of food into the distal small intestine is thought to be the causative mechanism of elevated GLP-1, subsequent elevated level of insulin, increased beta cell activity, and resolution of diabetes within a very short time period, within days after a gastric bypass, for example. I am wondering if, in your study, you have sicker patients and it has also been replicated by tube feeds.

**eTable 1.** Demographics of Glucagon-Like Peptide-1 Analytical and Parent Program Cohorts

Parameter	GLP-1 cohort (n = 157)	Parent cohort (n = 345)
Age, y, median (25th, 75th)	62 (51, 70)	62 (50, 71)
Male sex, n (%)	85 (54)	184 (53.3)
Race/ethnicity, n (%)		
Black or African American	13 (8)	32 (9.3)
Caucasian	139 (89)	307 (89)
Other	5 (3)	6 (1.7)
Charlson comorbidity index, median (25th, 75th)	4 (2, 6)	3 (1, 5)
Interfacility hospital transfer, n (%)	68 (43)	141 (40.9)
Primary sepsis diagnosis, n (%)		
Intra-abdominal sepsis	65 (42)	138 (40)
Pneumonia	28 (18)	55 (16)
NSTI	25 (16)	50 (14)
Surgical site infection	21 (14)	36 (10)
UTI	9 (6)	36 (10)
Catheter-related bloodstream infection	2 (1.3)	3 (1)
Bacteremia	1 (0.6)	5 (1)
Empyema	1 (0.6)	6 (2)
Other	2 (1.3)	16 (5)
Sepsis severity, n (%)		
Sepsis	57 (36)	103 (29.9)
Severe sepsis	62 (40)	147 (42.6)
Septic shock	38 (24)	93 (27)
APACHE II, median (25th, 75th)	17 (12, 23)	17 (11, 23)
Maximum SOFA score, median (25th, 75th)	8 (4, 11)	7 (5, 10)
Multiple organ failure*, n (%)	79 (50)	145 (42)
Ventilator days, median (25th, 75th)	2 (0, 6)	2 (0, 6)
ICU LOS, median (25th, 75th)	8 (4, 17)	7 (3, 17)
Hospital LOS, median (25th, 75th)	17 (8, 28)	15 (8, 26)
In-hospital mortality, n (%)	12 (7.6)	29 (8.4)
6-mo mortality, n (%)	31 (19.7)	61 (17.7)

GLP-1, glucagon-like peptide-1; LOS, length of stay; NSTI, necrotizing soft tissue infection; SOFA, sequential organ failure assessment; UTI, urinary tract infection.

\*Denver Multiple Organ Failure score  $\geq 3$