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Stimulation of the endogenous incretin glucose-dependent insulinotropic peptide by enteral dextrose improves glucose homeostasis and inflammation in murine endotoxemia

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Abstract

Loss of glucose homeostasis during sepsis is associated with increased organ dysfunction and higher mortality. Novel therapeutic strategies to promote euglycemia in sepsis are needed. We have previously shown that early low-level intravenous (IV) dextrose suppresses pancreatic insulin secretion and induces insulin resistance in septic mice, resulting in profound hyperglycemia and worsened systemic inflammation. In this study, we hypothesized that administration of low-level dextrose via the enteral route would stimulate intestinal incretin hormone production, potentiate insulin secretion in a glucose-dependent manner, and thereby improve glycemic control in the acute phase of sepsis. We administered IV or enteral dextrose to 10-week-old male C57BL/6J mice exposed to bacterial endotoxin and measured incretin hormone release, glucose disposal, and proinflammatory cytokine production. Compared with IV administration, enteral dextrose increased circulating levels of the incretin hormone glucose-dependent insulinotropic peptide (GIP) associated with increased insulin release and insulin sensitivity, improved mean arterial pressure, and decreased proinflammatory cytokines in endotoxemic mice. Exogenous GIP rescued glucose metabolism, improved blood pressure, and increased insulin release in endotoxemic mice receiving IV dextrose, whereas pharmacologic inhibition of GIP signaling abrogated the beneficial effects of enteral dextrose. Thus, stimulation of endogenous GIP secretion by early enteral dextrose maintains glucose homeostasis and attenuates the systemic inflammatory response in endotoxemic mice and may provide a therapeutic target for improving glycemic control and clinical outcomes in patients with sepsis.

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Supplementary Data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.trsl.2017.11.001>.

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INTRODUCTION

Dysregulation of glucose control complicates approximately 12% of sepsis cases and increases both morbidity and mortality.^{1–3} Glucose homeostasis in critical illness is impacted by inflammation, insulin resistance, stress hormone release, calorie provision (intentional or unintentional), and insulin administration.^{4–7} Although initial clinical trials supported tight glycemic control using exogenous insulin to improve patient outcomes,^{8,9} subsequent trials highlighted a risk of hypoglycemia with this strategy.^{10–13} Thus, a treatment approach that maintains euglycemia in septic patients without increased hypoglycemic risk is needed. To date, such a strategy has presented a therapeutic challenge but is potentially achievable by activation of endogenous metabolic regulatory pathways.

Hyperglycemia in sepsis shares many characteristics with an accelerated form of type 2 diabetes mellitus: insulin resistance, pancreatic insufficiency, and impaired glucose disposal in the setting of systemic inflammation.^{4,14–16} Treatment of diabetes with exogenous insulin carries a risk of hypoglycemia,¹⁷ similar to insulin therapy in sepsis. New diabetic treatment strategies target incretin hormones, which stimulate endogenous insulin secretion in a glucose-dependent manner thereby minimizing the risk of hypoglycemia. The gut-derived incretin hormones glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide 1 (GLP-1) are released in response to enteral nutrients and signal to increase pancreatic insulin secretion.¹⁸ Additionally, from a therapeutic perspective, incretin hormones have pleiotropic effects with the potential to reduce inflammation and to increase insulin sensitivity (Si) of peripheral metabolic organs.^{19–23} We were interested in potential therapeutic benefits of manipulating enteral nutrients to stimulate endogenous incretin hormone production in sepsis, facilitating both calorie provision and appropriate insulin secretion to control hyperglycemia while preventing hypoglycemia. Thus, in this study, we tested the effects of acute low-level enteral dextrose provision on glucose disposal, incretin hormone release, and systemic inflammation in endotoxemic mice.

METHODS

Animal care

All experiments were performed in 10-week-old male C57BL/6J mice (Jackson Laboratory) and were carried out in strict accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Animal protocols were approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh. Mice were sacrificed via cervical neck dislocation in accordance with guidelines established by the American Veterinary Medical Association.

Vascular catheterization and gastric cannula placement

Detailed descriptions of catheterization techniques have been published previously.²⁴ Briefly, femoral arterial catheters (MRE-025; Braintree Scientific, Braintree, MA) were implanted in all mice for the purposes of hemodynamic monitoring, arterial blood sampling, and metabolic testing. Catheters were tunneled subcutaneously through the left upper back and connected to a dual channel swivel (Instech Laboratories Inc., Plymouth Meeting, PA) to

allow the mice to move freely throughout the experimental period. Catheter patency was maintained with a continuous infusion (7 $\mu\text{L/h}$) of sterile heparinized normal saline (20 U/mL; Sagent Pharmaceuticals Inc., Schaumburg, IL).

Mice then received femoral venous catheters or gastric cannulas depending on experimental group. Femoral intravenous (IV) catheters were implanted in a similar fashion to the arterial catheters. Gastric cannulas were fashioned from 60-cm Portex polythene tubing (Smiths-Medical, Dublin, OH) and were implanted via laparotomy: the abdominal cavity was opened with a 0.5–1.0-cm ventral midline incision on the right side followed by puncture of the greater curvature of the stomach with a 16-gauge sterile needle. Cannulas were inserted into the gastrostomy site, secured with 6.0 silk sutures, tunneled subcutaneously to the base of the neck, and connected to the swivel. All mice received a single dose of intramuscular penicillin G 12.5 mg (Pfizer, New York, NY) at the time of surgery.

Experimental protocol

In a first set of experiments, the effects of IV vs enteral dextrose infusions on metabolic outcomes were tested in an endotoxemia model. Mice were randomized to receive either a venous catheter or a gastric cannula. Mice were permitted 3 days to recover from surgery. On the day of metabolic testing, mice were randomized to receive either intra-arterial lipopolysaccharide (LPS, 1 mg/kg) or vehicle (100 μL of saline) followed immediately by dextrose or saline infusion at 100 $\mu\text{L/h}$. Food was removed from cages to control caloric intake. As a result, at the time of metabolic and inflammatory testing, mice receiving dextrose would have received approximately 10% of their daily caloric needs.

Five hours after LPS or vehicle (time 0), 50 μL of blood was collected and processed for analysis of inflammatory cytokines circulating in the plasma, and the frequently sampled intravenous glucose tolerance test (FSIVGTT) was immediately conducted per our previously published protocol.²⁵ The minimal model was applied to the FSIVGTT glucose and insulin response curves to determine glucose disposal (area under the glucose curve [AUC_g]), acute insulin response to glucose, and Si.²⁶ Primary analysis compared endotoxemic mice receiving IV dextrose with those receiving enteral dextrose.

To examine the effect of continuous dextrose infusion on incretin hormone production, a separate group of mice exposed to LPS (1 mg/kg) were randomized to receive IV or enteral dextrose. After 5 hours, 100 μL of whole blood was collected in syringes rinsed with the protease inhibitor Diprotin A and transferred to Eppendorf tubes containing 10 μL of a preservative solution containing aprotinin (5000 KIU/mL), ethylenediaminetetraacetic acid (1.2 mg/mL), and the specific dipeptidyl-peptidase IV (DPP IV) inhibitor Ile-Pro-Ile (0.1 nmol) as previously described.²⁷ Blood was centrifuged at 10,000 rpm for 10 minutes, and plasma was frozen in liquid nitrogen for subsequent analysis of circulating GIP and GLP-1.

Based on results from the initial IV vs enteral dextrose experiments, a final set of experiments focused on GIP alone (not GLP-1). First, the therapeutic effect of exogenous GIP infusion was examined in a mouse model of impaired glucose tolerance. All mice in this experiment received femoral arterial and venous catheters followed in 3 days by intra-arterial LPS (1 mg/kg) and infusion of IV dextrose at 100 $\mu\text{L/h}$. Mice were randomized to

receive a concomitant continuous infusion of either (1) GIP [4 pmol/kg/min] or (2) vehicle [0.9% saline] at 100 μ L/h. Second, the effect of GIP blockade was tested in endotoxemic mice receiving enteral dextrose. Mice in this experiment received arterial catheters and gastric cannulas, and 3 days after surgery, all mice received LPS (1 mg/kg) followed by enteral infusion of dextrose at 100 μ L/h. Mice were randomized to receive either (1) intra-arterial GIP receptor antagonist 3Pro-GIP (25 nmol/kg) or (2) vehicle at the time of LPS administration. In all GIP experiments, blood was sampled and FSIVGTT was performed 5 hours after LPS as described previously. Group assignments are further detailed in Supplementary Table I.

Materials and biochemical assays

Escherichia coli O55:B5 lipopolysaccharide (Sigma-Aldrich, St. Louis, MO) was dissolved in normal saline (1 mg/mL). Pharmaceutical-grade dextrose solution (0.5 g/mL in sterile water) was purchased (Hospira, Lake Forest, IL) for enteral or IV infusion. Diprotin A was obtained from EMD Millipore (Billerica, MA). Aprotinin (5000 KIU/mL), ethylenediaminetetraacetic acid (1.2 mg/mL), and Ile-Pro-Ile (0.1 nmol) were obtained from Sigma-Aldrich. GIP (Catalog No. H-5645) and 3Pro GIP (Catalog No. 4061955) were obtained from Bachem (Torrance, CA).

Blood glucose was measured using a Prodigy Autocode Glucometer (~1 μ L of whole blood; Diagnostic Devices, Charlotte, NC). Plasma insulin was measured using the Crystal Chem Ultra-Sensitive Mouse Insulin Enzyme-Linked Immunosorbent Assay Kit (Crystal Chem Inc., Downers Grove, IL), and plasma cytokines were measured using BioRad 23-Plex Mouse Cytokine Multiplex Assay (Bio-Rad Laboratories, Hercules, CA). GIP was quantified by Rat/Mouse Total GIP enzyme-linked immunosorbent assay (EMD Millipore, Billerica, MA), and GLP-1 was measured by the MesoScale Discovery Active GLP-1 (version 2) kit (Rockville, MD). All assays were performed according to manufacturer protocols.

Statistical analyses

Statistical analyses were performed using GraphPad PRISM 6 for Windows (GraphPad Software Inc., La Jolla, CA) and Stata Version 13 (StataCorp LP, College Station, TX) in consultation with the statistical core at the University of Pittsburgh Clinical and Translational Science Institute. Data are presented as mean $\pm$ standard error of the mean, unless otherwise indicated. Primary analyses comparing mean metabolic and inflammatory end points between endotoxemic mice receiving IV or enteral dextrose were compared by unpaired two-sample *t* test or Mann-Whitney *U* test. Additional analyses comparing metabolic end points in IV and enteral dextrose infused groups to respective saline and vehicle controls were assessed via one-way analysis of variance or Kruskal-Wallis test as appropriate with prespecified post hoc analyses by Dunnett test. For incretin infusion and blocking experiments, differences in metabolic outcomes and cytokine levels were compared by *t* test or Mann-Whitney *U* test. Results were considered statistically significant for a *P*-value < 0.05, which was adjusted in post hoc analyses for multiple comparisons.

RESULTS

Enteral dextrose administration improves glucose disposal in endotoxemic mice

Control mice receiving vehicle and either IV or enteral saline demonstrated normal glucose disposal with blood glucose levels returning to baseline within 20 minutes after an IV dextrose bolus (1 g/kg) (Fig 1, A, open circles). Consistent with our previous studies,²⁸ neither IV dextrose infusion nor LPS alone induced profound glucose intolerance (Fig 1, B and C, respectively, open circles). In contrast, infusion of IV dextrose in the context of endotoxemia markedly impaired glucose tolerance compared with controls with higher peak glucose during metabolic testing and the inability to return to baseline (Fig 1, D, open circles). Endotoxemic mice receiving IV dextrose displayed the worst glucose tolerance of any group (AUCg $23.65 \pm 2.59 \text{ mg} \cdot 10^3/\text{dL} \cdot \text{min}$ vs $8.93 \pm 0.57 \text{ mg} \cdot 10^3/\text{dL} \cdot \text{min}$ in IV saline and vehicle group, $P < 0.01$, Table I).

Enteral dextrose infusion was associated with preserved glucose disposal as evidenced by the lower peak glucose level and rapid return to baseline during the FSIVGTT (Fig 1, D, solid circles). Glucose tolerance was improved in endotoxemic mice receiving enteral dextrose (AUCg $9.80 \pm 0.45 \text{ mg} \cdot 10^3/\text{dL} \cdot \text{min}$, $P < 0.01$) compared with the IV dextrose group and was nearly identical to control mice receiving enteral saline that had not been exposed to LPS (AUCg $9.37 \pm 0.58 \text{ mg} \cdot 10^3/\text{dL} \cdot \text{min}$, $P = 0.91$).

Enteral dextrose administration improves insulin secretion and insulin sensitivity in endotoxemic mice

Insulin secretion (Fig 2, A) and insulin sensitivity (Table I) during FSIVGTT were comparable with previously published controls in mice receiving vehicle and IV saline.²⁸ Exposure to endotoxin in mice receiving IV saline decreased insulin sensitivity (77% reduction, $P < 0.01$) but increased insulin secretion (183% increase, $P = 0.01$); similar responses were noted in endotoxemic mice receiving enteral saline. Endotoxemic mice receiving IV dextrose demonstrated reduced insulin sensitivity (88% decrease, $P < 0.01$) but insulin secretion was unchanged ($P = 0.82$). In contrast, compared with an equivalent level of IV dextrose, enteral dextrose infusion in endotoxemic mice increased both insulin sensitivity (266% increase, $P < 0.01$) and insulin secretion (77% increase, $P = 0.02$).

Enteral dextrose improves hemodynamics and reduces systemic inflammation in response to endotoxemia

Endotoxin exposure increased circulating proinflammatory cytokines interleukin 1 beta (IL-1 β), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF- α) in mice receiving IV saline, enteral saline, and IV dextrose (Fig 3, A–C) compared with saline and vehicle controls. Compared with endotoxemic mice receiving IV dextrose, IL-1 β , IL-6, and TNF- α were reduced by 36% ($P = 0.02$), 65% ($P < 0.01$), and 39% ($P = 0.01$), respectively, in mice receiving enteral dextrose.

Similarly, mean arterial pressure decreased 5 hours after LPS administration in mice receiving IV dextrose ($88.82 \pm 3.42 \text{ mm Hg}$) compared with IV saline and vehicle controls

(101.20 ± 2.60 mm Hg, $P < 0.01$) but was preserved in the setting of enteral dextrose administration (99.57 ± 2.41 mm Hg, Fig 3, D).

Enteral dextrose increases circulating GIP but not GLP-1 in endotoxemic mice

Enteral dextrose infused over 5 hours increased circulating levels of the incretin hormone GIP in endotoxemic mice compared with IV dextrose (median 294 pg/mL [interquartile range 150, 518] vs 61 pg/mL [interquartile range 35, 63], $P = 0.04$, Fig 4), whereas circulating levels of GLP-1, which is released more distally in the intestine, did not differ significantly ($P = 0.87$).

Exogenous GIP infusion improves glucose tolerance, increases blood pressure, and decreases inflammation in endotoxemic mice receiving IV dextrose

Provision of IV dextrose after LPS administration impaired glucose tolerance (AUC_G 29.28 ± 5.13 mg·10³/dL·min, Fig 5, A); the addition of exogenous GIP infusion rescued glucose disposal (AUC_G 12.70 ± 1.86 mg·10³/dL·min, $P = 0.02$). Moreover, GIP infusion increased insulin secretion nearly threefold ($P = 0.004$, Table II) without a significant change in insulin sensitivity. Finally, concomitant with improved insulin secretion and glucose disposal, GIP infusion decreased circulating IL-6 by 67% ($P < 0.01$) and improved mean arterial blood pressure (79.43 ± 2.51 mm Hg vs 92.10 ± 0.97 mm Hg, $P < 0.01$) in endotoxemic mice receiving IV dextrose (Fig 5, E and F).

GIP signaling is necessary for the beneficial effects of enteral dextrose

Blockade of GIP signaling with 3ProGIP in endotoxemic mice receiving enteral dextrose impaired glucose tolerance (AUC_G 28.15 ± 2.58 mg·10³/dL·min [3ProGIP] vs 9.11 ± 0.53 mg·10³/dL·min [vehicle], $P < 0.01$, Fig 6, A) and reduced pancreatic insulin secretion in response to glucose during the FSIVGTT (acute insulin response to glucose: 85% decrease, $P < 0.01$) without significantly altering insulin sensitivity compared with enteral dextrose control mice (Table III).

Similarly, GIP blockade intensified the systemic proinflammatory response with increases in IL-1β (375%, $P < 0.01$), IL-6 (344%, $P < 0.01$), and TNF-α (67%, $P = 0.01$, Fig 6, C–E). Finally, blockade of GIP signaling decreased mean arterial blood pressure in endotoxemic mice receiving enteral dextrose 5 hours after LPS from 94.65 ± 3.45 to 79.73 ± 4.18 mmHg (Fig 6, F, $P = 0.02$).

DISCUSSION

The dysregulation of glucose homeostasis during sepsis represents a breakdown of the body's endogenous metabolic response and has important implications for patient outcomes. The development of hyperglycemia during an episode of sepsis is associated with increased morbidity and mortality.^{1,3} Furthermore, the development of hypoglycemia in critically ill patients (which may occur when exogenous insulin is used to control hyperglycemia) has also been associated with increased short- and long-term mortality.^{29–31} Maintaining euglycemia in septic patients remains challenging because of the complex balance between the effects of acute illness and inflammation, the disruption of endocrine pathways

responsible for glucose disposal, and the intentional and unintentional effects of therapeutic interventions employed during critical illness. To date, strategies to control glucose regulation in critically ill patients have focused on the use of exogenous insulin and have been limited by the unintended development of hypoglycemia.^{11,32} Our study suggests that glucose homeostasis in sepsis may be promoted by enteral dextrose-induced stimulation of endogenous incretin hormones which serve to increase pancreatic insulin secretion and attenuate the systemic inflammatory response.

Previous work in our laboratory supports the hypothesis that an inability to maintain intact endogenous endocrine function worsens outcomes in mouse models of sepsis. First, we demonstrated that the inability to raise endogenous insulin production in response to hyperglycemia predicts mortality in endotoxemic mice.³³ Subsequently, we demonstrated that the development of stress hyperglycemia in mouse models is induced by two “hits,” combining an inflammatory insult with an exogenous caloric source, such as parenteral dextrose, which, when combined, disrupt both pancreatic insulin secretion and peripheral insulin sensitivity.²⁸ In addition, we demonstrated that the effect of exogenous dextrose on glucose metabolism does not follow a traditional dose-dependent relationship. Rather, previous data suggest that septic mice will either maintain normal glucose disposal or undergo a rapid transition to a state of insulin resistance and relative pancreatic insufficiency resembling an acute and accelerated form of type 2 diabetes mellitus.³⁴ The current study expands our understanding of metabolic function and dysfunction in sepsis by demonstrating that the metabolic and inflammatory responses to exogenous dextrose vary based on route of delivery. Our results demonstrate that administration of dextrose by the enteral route prevents the dysfunction induced by parenteral dextrose in endotoxemic mice. The data further suggest that glucose homeostasis in sepsis is critically dependent on secretion of the endogenous incretin hormone GIP.

Incretin hormones are secreted by intestinal neuroendocrine cells in response to enteral nutrients and stimulate pancreatic insulin secretion in a glucose-dependent manner.³⁵ GIP is released by K cells, which reside predominantly in the proximal small intestine, and GLP-1 is released by L cells in the distal ileum and colon.¹⁸ Incretins differ in their actions on the pancreatic alpha cell as endogenous GLP-1 inhibits glucagon release, whereas GIP does not; thus, GIP may be beneficial compared with GLP-1 with respect to hypoglycemia.^{35,36} Furthermore, incretins have also been shown to modulate systemic inflammation both in vitro and in vivo.^{19–21,37,38} New classes of antidiabetic medications targeting incretin hormones have emerged recently including the long acting GLP-1 receptor analogs (eg, liraglutide and exenatide) and inhibitors of DPP IV (eg, sitagliptin and linagliptin), the enzyme responsible for incretin degradation.³⁹ GLP-1-based therapies have been developed initially as diabetic individuals demonstrate decreased endogenous GIP signaling⁴⁰; however, exogenously infused GIP may still remain active in the setting of type 2 diabetes.⁴¹ Our research highlights a novel role for GIP in the setting of sepsis. Initial clinical trials of exogenous incretin hormones in critically ill populations have not demonstrated improvements in glycemic control but have been complicated by the use of enteral nutrients stimulating endogenous incretin production in the placebo group. Importantly, the trials confirm that the risk of hypoglycemia is low in critically ill patients with incretin-targeted therapies,^{42,43} since incretin hormones promote insulin release in a glucose-dependent

manner. As such, incretin hormones represent attractive potential therapeutic targets in critical illness.^{44–47}

In our model, continuous enteral dextrose infusion in endotoxemic mice increased GIP secretion, improved glucose disposal, increased insulin secretion, improved mean arterial pressure, and attenuated the systemic inflammatory response over 5 hours compared with continuous IV dextrose. Interestingly, we did not observe an increase in the secretion of GLP-1, possibly because the low level of dextrose infused was unable to stimulate the K cells located in the distal small intestine. Exogenous GIP infusion demonstrated therapeutic benefits in endotoxemic mice receiving IV dextrose (who would otherwise have no endogenous GIP release) with improvements in glucose disposal, insulin secretion, and the proinflammatory response. Importantly, the suppression of insulin release, worsened glucose tolerance, and increased proinflammatory cytokines in mice receiving enteral dextrose but with GIP receptor blockade highlight the critical role glucose-dependent insulinotropic peptide plays in the regulation of glucose metabolism in endotoxemia. Our results suggest that exploitation of the endogenous incretin signaling axis by manipulation of enteral nutrients, perhaps as simple as enteral dextrose, may provide a viable therapeutic strategy for patients with sepsis.

Several considerations should be noted in the interpretation of our study. First, the relationship between incretin hormones and systemic inflammation may be more complex than suggested in our manuscript. Recent studies by Kahles et al. demonstrate that proinflammatory cytokines including IL-6 and IL-1 β induce incretin hormone release, and that 3ProGIP, in the absence of enteral dextrose as utilized in our study, decreases proinflammatory cytokines in response to LPS.^{48,49} Notably, in the Kahles et al. study, 3ProGIP does not decrease insulin secretion, suggesting other incretin pathways, including GLP-1, remain active. In our study, enteral dextrose increases endogenous GIP (but not GLP-1), increasing insulin secretion and decreasing proinflammatory cytokines—findings that are abrogated by 3ProGIP. The specific induction of GIP alone by enteral dextrose in our model may explain differences in cytokine and insulin secretion compared with Kahles et al. Furthermore, the intraperitoneal LPS model utilized by Kahles et al. may influence incretin release through a local peritoneal inflammation that would not be present with our model of intra-arterial LPS.

Although endogenous GLP-1 is not increased in our model, given the similarities to GIP, targeting GLP-1 either through endogenous stimulation with enteral nutrients or exogenous GLP-1 agonists (which are already in use for the treatment of diabetes mellitus) may have similar beneficial effects.^{50,51} Indeed, a recent study demonstrated that pretreatment with either exogenous GLP-1 agonists or with inhibitors of DPP IV (which should increase levels of both GIP and GLP-1) improved survival after LPS administration in mice.⁵² Moreover, stimulation of both GIP and GLP-1 may have additive beneficial inflammatory and metabolic effects, but has not as yet been studied in critical illness.⁵³

Of note, the current studies initiated IV and enteral infusions immediately after the proinflammatory insult, allowing us to determine the effects of early dextrose infusion in the midst of the acute inflammatory response—a process that is underway in patients with sepsis

when they present to the intensive care unit. Additionally, studies were performed in lean nondiabetic mice, and endogenous incretin responses to enteral nutrients may be blunted in diabetic individuals. Thus, septic diabetics may require exogenous infusions of GIP or GLP-1 to improve glycemic control in a similar manner. Further studies in animal models and human studies are needed to continue addressing the role of the incretin hormone pathway in sepsis.

In conclusion, the benefits of early enteral support in critically ill patients are believed to include maintenance of intestinal integrity and reduction in the risk of bacterial translocation during acute illness.^{54–56} Our data suggest that an additional potentially important effect of early enteral nutrition (at trophic levels) may include the activation of incretin hormones, particularly GIP, which promotes euglycemia and modulates the systemic inflammatory response. Further research, including well-designed clinical trials, will be important for establishing a role for targeting the incretin hormone axis in critical illness.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Conflicts of Interest: The authors have no financial conflicts or personal relationships with organizations that could potentially influence the presented research.

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Abbreviations

AUCg	area under the glucose curve
DPP IV	dipeptidyl-peptidase IV
FSIVGTT	frequently sampled intravenous glucose tolerance test
GIP	glucose-dependent insulintropic peptide
GLP-1	glucagon-like peptide-1
IV	intravenous
LPS	lipopolysaccharide (also referred to as endotoxin)
Si	insulin sensitivity

References

1. Krinsley JS. Association between hyperglycemia and increased hospital mortality in a heterogeneous population of critically ill patients. *Mayo Clin Proc.* 2003; 78:1471–8. [PubMed: 14661676]

2. Kyle UG, Coss Bu JA, Kennedy CE, Jefferson LS. Organ dysfunction is associated with hyperglycemia in critically ill children. *Intensive Care Med.* 2010; 36:312–20. [PubMed: 19882139]
3. Leonidou L, Michalaki M, Leonardou A, et al. Stress-induced hyperglycemia in patients with severe sepsis: a compromising factor for survival. *Am J Med Sci.* 2008; 336:467–71. [PubMed: 19092319]
4. Marik PE, Raghavan M. Stress-hyperglycemia, insulin and immunomodulation in sepsis. *Intensive Care Med.* 2004; 30:748–56. [PubMed: 14991101]
5. Dungan KM, Braithwaite SS, Preiser JC. Stress hyperglycaemia. *Lancet.* 2009; 373:1798–807. [PubMed: 19465235]
6. Mechanick JI. Metabolic mechanisms of stress hyperglycemia. *JPEN J Parenter Enteral Nutr.* 2006; 30:157–63. [PubMed: 16517960]
7. Mehta NN, McGillicuddy FC, Anderson PD, et al. Experimental endotoxemia induces adipose inflammation and insulin resistance in humans. *Diabetes.* 2010; 59:172–81. [PubMed: 19794059]
8. Krinsley JS. Effect of an intensive glucose management protocol on the mortality of critically ill adult patients. *Mayo Clin Proc.* 2004; 79:992–1000. [PubMed: 15301325]
9. van den Berghe G, Wouters P, Weekers F, et al. Intensive insulin therapy in critically ill patients. *N Engl J Med.* 2001; 345:1359–67. [PubMed: 11794168]
10. Arabi YM, Dabbagh OC, Tamim HM, et al. Intensive versus conventional insulin therapy: a randomized controlled trial in medical and surgical critically ill patients. *Crit Care Med.* 2008; 36:3190–7. [PubMed: 18936702]
11. Griesdale DE, de Souza RJ, van Dam RM, et al. Intensive insulin therapy and mortality among critically ill patients: a meta-analysis including NICE-SUGAR study data. *CMAJ.* 2009; 180:821–7. [PubMed: 19318387]
12. Finfer S, Chittock DR, et al. Investigators N-SS. Intensive versus conventional glucose control in critically ill patients. *N Engl J Med.* 2009; 360:1283–97. [PubMed: 19318384]
13. Ling Y, Li X, Gao X. Intensive versus conventional glucose control in critically ill patients: a meta-analysis of randomized controlled trials. *Eur J Intern Med.* 2012; 23:564–74. [PubMed: 22863436]
14. Michie HR. Metabolism of sepsis and multiple organ failure. *World J Surg.* 1996; 20:460–4. [PubMed: 8662135]
15. Tribl B, Sibbald WJ, Vogelsang H, Spitzauer S, Gangl A, Madl C. Exocrine pancreatic dysfunction in sepsis. *Eur J Clin Invest.* 2003; 33:239–43. [PubMed: 12641542]
16. Xiu F, Stanojic M, Diao L, Jeschke MG. Stress hyperglycemia, insulin treatment, and innate immune cells. *Int J Endocrinol.* 2014; 2014:486403. [PubMed: 24899891]
17. Zammitt NN, Frier BM. Hypoglycemia in type 2 diabetes: pathophysiology, frequency, and effects of different treatment modalities. *Diabetes Care.* 2005; 28:2948–61. [PubMed: 16306561]
18. Drucker DJ. The biology of incretin hormones. *Cell Metab.* 2006; 3:153–65. [PubMed: 16517403]
19. Iwai T, Ito S, Tanimitsu K, Udagawa S, Oka J. Glucagon-like peptide-1 inhibits LPS-induced IL-1 β production in cultured rat astrocytes. *Neurosci Res.* 2006; 55:352–60. [PubMed: 16720054]
20. Yanay O, Bailey AL, Kernan K, Zimmerman JJ, Osborne WR. Effects of exendin-4, a glucagon like peptide-1 receptor agonist, on neutrophil count and inflammatory cytokines in a rat model of endotoxemia. *J Inflamm Res.* 2015; 8:129–35. [PubMed: 26244029]
21. Lee YS, Jun HS. Anti-inflammatory effects of GLP-1-based therapies beyond glucose control. *Mediators Inflamm.* 2016; 2016:3094642. [PubMed: 27110066]
22. Nielsen LL, Young AA, Parkes DG. Pharmacology of exenatide (synthetic exendin-4): a potential therapeutic for improved glycemic control of type 2 diabetes. *Regul Pept.* 2004; 117:77–88. [PubMed: 14700743]
23. Zander M, Madsbad S, Madsen JL, Holst JJ. Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. *Lancet.* 2002; 359:824–30. [PubMed: 11897280]
24. Alonso LC, Watanabe Y, Stefanovski D, et al. Simultaneous measurement of insulin sensitivity, insulin secretion, and the disposition index in conscious unhandled mice. *Obesity (Silver Spring).* 2012; 20:1403–12. [PubMed: 22331130]

25. Alonso LC, Yokoe T, Zhang P, et al. Glucose infusion in mice: a new model to induce beta-cell replication. *Diabetes*. 2007; 56:1792–801. [PubMed: 17400928]
26. Bergman RN, Prager R, Volund A, Olefsky JM. Equivalence of the insulin sensitivity index in man derived by the minimal model method and the euglycemic glucose clamp. *J Clin Invest*. 1987; 79:790–800. [PubMed: 3546379]
27. Hansotia T, Baggio LL, Delmeire D, et al. Double incretin receptor knockout (DIRKO) mice reveal an essential role for the enteroinsular axis in transducing the glucoregulatory actions of DPP-IV inhibitors. *Diabetes*. 2004; 53:1326–35. [PubMed: 15111503]
28. Watanabe Y, Singamsetty S, Zou B, et al. Exogenous glucose administration impairs glucose tolerance and pancreatic insulin secretion during acute sepsis in non-diabetic mice. *PLoS ONE*. 2013; 8:e67716. [PubMed: 23826335]
29. Ali NA, O'Brien JM Jr, Dungan K, et al. Glucose variability and mortality in patients with sepsis. *Crit Care Med*. 2008; 36:2316–21. [PubMed: 18596625]
30. Park S, Kim DG, Suh GY, et al. Mild hypoglycemia is independently associated with increased risk of mortality in patients with sepsis: a 3-year retrospective observational study. *Crit Care*. 2012; 16:R189. [PubMed: 23062226]
31. van Vught LA, Wiewel MA, Klein Klouwenberg PM, et al. Admission hyperglycemia in critically ill sepsis patients: association with outcome and host response. *Crit Care Med*. 2016; 44:1338–46. [PubMed: 26958752]
32. Arabi YM, Tamim HM, Rishu AH. Hypoglycemia with intensive insulin therapy in critically ill patients: predisposing factors and association with mortality. *Crit Care Med*. 2009; 37:2536–44. [PubMed: 19623047]
33. Woodske ME, Yokoe T, Zou B, et al. Hyperinsulinemia predicts survival in a hyperglycemic mouse model of critical illness. *Crit Care Med*. 2009; 37:2596–603. [PubMed: 19623043]
34. Singamsetty S, Shah FA, Guo L, et al. Early initiation of low-level parenteral dextrose induces an accelerated diabetic phenotype in septic C57BL/6J mice. *Appl Physiol Nutr Metab*. 2016; 41:12–9. [PubMed: 26624964]
35. Nauck MA, Heimesaat MM, Behle K, et al. Effects of glucagon-like peptide 1 on counterregulatory hormone responses, cognitive functions, and insulin secretion during hyperinsulinemic, stepped hypoglycemic clamp experiments in healthy volunteers. *J Clin Endocrinol Metab*. 2002; 87:1239–46. [PubMed: 11889194]
36. Pederson RA, Brown JC. Interaction of gastric inhibitory polypeptide, glucose, and arginine on insulin and glucagon secretion from the perfused rat pancreas. *Endocrinology*. 1978; 103:610–5. [PubMed: 744105]
37. Dai Y, Dai D, Wang X, Ding Z, Mehta JL. DPP-4 inhibitors repress NLRP3 inflammasome and interleukin-1 β via GLP-1 receptor in macrophages through protein kinase C pathway. *Cardiovasc Drugs Ther*. 2014; 28:425–32. [PubMed: 25022544]
38. Hirano T, Mori Y. Anti-atherogenic and anti-inflammatory properties of glucagon-like peptide-1, glucose-dependent insulinotropic polypeptide, and dipeptidyl peptidase-4 inhibitors in experimental animals. *J Diabetes Investig*. 2016; 7(Suppl 1):80–6.
39. Drucker DJ, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet*. 2006; 368:1696–705. [PubMed: 17098089]
40. Vilsboll T, Krarup T, Madsbad S, Holst JJ. Defective amplification of the late phase insulin response to glucose by GIP in obese type II diabetic patients. *Diabetologia*. 2002; 45:1111–9. [PubMed: 12189441]
41. Wice BM, Reeds DN, Tran HD, et al. Xenin-25 amplifies GIP-mediated insulin secretion in humans with normal and impaired glucose tolerance but not type 2 diabetes. *Diabetes*. 2012; 61:1793–800. [PubMed: 22522617]
42. Kar P, Cousins CE, Annink CE, et al. Effects of glucose-dependent insulinotropic polypeptide on gastric emptying, glycaemia and insulinaemia during critical illness: a prospective, double blind, randomised, crossover study. *Crit Care*. 2015; 19:20. [PubMed: 25613747]

43. Lee MY, Fraser JD, Chapman MJ, et al. The effect of exogenous glucose-dependent insulinotropic polypeptide in combination with glucagon-like peptide-1 on glycemia in the critically ill. *Diabetes Care*. 2013; 36:3333–6. [PubMed: 23835687]
44. Deane AM, Chapman MJ, Fraser RJ, Burgstad CM, Besanko LK, Horowitz M. The effect of exogenous glucagon-like peptide-1 on the glycaemic response to small intestinal nutrient in the critically ill: a randomised double-blind placebo-controlled cross over study. *Crit Care*. 2009; 13:R67. [PubMed: 19439067]
45. Gitt AK, Bramlage P, Schneider S, Tschope D. A real world comparison of sulfonylurea and insulin vs. incretin-based treatments in patients not controlled on prior metformin monotherapy. *Cardiovasc Diabetol*. 2015; 14:13. [PubMed: 25645672]
46. Heine RJ, Van Gaal LF, Johns D, et al. Exenatide versus insulin glargine in patients with suboptimally controlled type 2 diabetes: a randomized trial. *Ann Intern Med*. 2005; 143:559–69. [PubMed: 16230722]
47. Nauck MA, Duran S, Kim D, et al. A comparison of twice-daily exenatide and biphasic insulin aspart in patients with type 2 diabetes who were suboptimally controlled with sulfonylurea and metformin: a non-inferiority study. *Diabetologia*. 2007; 50:259–67. [PubMed: 17160407]
48. Kahles F, Meyer C, Diebold S, et al. Glucose-dependent insulinotropic peptide secretion is induced by inflammatory stimuli in an interleukin-1-dependent manner in mice. *Diabetes Obes Metab*. 2016; 18:1147–51. [PubMed: 27350651]
49. Kahles F, Meyer C, Mollmann J, et al. GLP-1 secretion is increased by inflammatory stimuli in an IL-6-dependent manner, leading to hyperinsulinemia and blood glucose lowering. *Diabetes*. 2014; 63:3221–9. [PubMed: 24947356]
50. Anandhakrishnan A, Korbonits M. Glucagon-like peptide 1 in the pathophysiology and pharmacotherapy of clinical obesity. *World J Diabetes*. 2016; 7:572–98. [PubMed: 28031776]
51. Holst JJ, Deacon CF, Vilsboll T, Krarup T, Madsbad S. Glucagon-like peptide-1, glucose homeostasis and diabetes. *Trends Mol Med*. 2008; 14:161–8. [PubMed: 18353723]
52. Steven S, Hausding M, Kroller-Schon S, et al. Gliptin and GLP-1 analog treatment improves survival and vascular inflammation/dysfunction in animals with lipopolysaccharide-induced endotoxemia. *Basic Res Cardiol*. 2015; 110:6. [PubMed: 25600227]
53. Finan B, Muller TD, Clemmensen C, Perez-Tilve D, DiMarchi RD, Tschop MH. Reappraisal of GIP pharmacology for metabolic diseases. *Trends Mol Med*. 2016; 22:359–76. [PubMed: 27038883]
54. Ammori BJ. Importance of the early increase in intestinal permeability in critically ill patients. *The Eur J Surg*. 2002; 168:660–1. author reply 2. [PubMed: 12699109]
55. Jabbar A, Chang WK, Dryden GW, McClave SA. Gut immunology and the differential response to feeding and starvation. *Nutr Clin Pract*. 2003; 18:461–82. [PubMed: 16215082]
56. Kudsk KA. Current aspects of mucosal immunology and its influence by nutrition. *Am J Surg*. 2002; 183:390–8. [PubMed: 11975926]

AT A GLANCE COMMENTARY

Shah FA, et al.

Background

Dysregulation of glucose control during sepsis is associated with increased morbidity and mortality. Therapies targeting tight glycemic control in septic patients have been limited in part by the development of hypoglycemia. Novel treatment strategies that promote glucose homeostasis in sepsis are needed.

Translational Significance

In this study, we demonstrate that early enteral dextrose improves glucose disposal, increases insulin secretion, and attenuates systemic inflammatory responses in endotoxemic mice by increasing the incretin hormone glucose-dependent insulinotropic peptide. Our results suggest that targeting the endogenous incretin hormone axis may provide a novel therapeutic strategy for improving outcomes in patients with sepsis.

Fig 1.

Glucose disposal in endotoxemic mice is worsened with intravenous (IV) dextrose infusion but is preserved with enteral dextrose. Glucose-time curves from the frequently sampled intravenous glucose tolerance test (FSIVGTT) in control mice receiving vehicle and saline infusion by either IV or enteral route demonstrate the expected glucose response during metabolic testing in that blood glucose rises acutely in response to a dextrose bolus (1g/kg) and returns to baseline within 20 minutes (**A**). The infusion of dextrose instead of saline in the absence of endotoxin results in minor differences in glucose disposal varying by the route of administration but blood glucose levels return to baseline within a similar time frame (**B**). Mice receiving endotoxin (LPS 1 mg/kg) and saline start with lower blood glucose at baseline, but glucose disposal is similar to control mice (**C**). Infusion of IV dextrose in endotoxemic mice results in severely impaired glucose disposal characterized by an inability to return to baseline blood glucose levels in FSIVGTT (**D**, open circles). In contrast, infusion of enteral dextrose preserves glucose tolerance in endotoxemic mice (**D**, closed circles). LPS, lipopolysaccharide.

Fig 2.

Insulin secretion during metabolic testing is increased in endotoxemic mice receiving enteral dextrose compared with intravenous dextrose infusion—control mice receiving vehicle and saline infusion demonstrate an appropriate increase in insulin secretion after dextrose challenge in FSIVGTT. Mice receiving intravenous (IV) saline demonstrate a slightly higher peak of insulin secretion compared with mice receiving enteral saline (**A**). Insulin secretion does not differ significantly between mice receiving IV dextrose and enteral dextrose (**B**), but does increase in saline-infused mice challenged with endotoxin (**C**). Insulin secretion is inappropriately low in endotoxemic mice receiving IV dextrose infusion given the degree of hyperglycemia (**D**, open circles), but is notably increased in endotoxemic mice receiving enteral dextrose. LPS, lipopolysaccharide.

Fig 3.

Enteral dextrose infusion attenuates the systemic inflammatory response following endotoxin and improves mean arterial blood pressure—endotoxin administration increases circulating levels of the proinflammatory cytokines interleukin 1 beta (IL-1 β), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF- α) in mice infused with either saline or intravenous (IV) dextrose (A–C). Infusion of enteral dextrose attenuates the proinflammatory response in endotoxemic mice with reductions in systemic cytokines of 40%–60%. Blood pressure measured 5 hours after lipopolysaccharide and just before FSIVGTT is increased in endotoxemic mice receiving enteral dextrose compared with those receiving IV dextrose (D). [* and ** denote difference in IV saline and vehicle group with $P < 0.05$ and < 0.01 , respectively. † and †† denote difference in enteral saline and vehicle group $P < 0.05$ and < 0.01 , respectively].

Fig 4. Circulating glucose-dependent insulintropic peptide (GIP) is increased in endotoxemic mice receiving enteral dextrose compared with those receiving intravenous dextrose. Circulating levels of the incretin glucagon-like peptide 1 (GLP-1) are not significantly different between the two groups.

Fig 5.

Infusion of exogenous glucose-dependent insulinotropic peptide (GIP) rescues impaired glucose tolerance and reduces interleukin 6 (IL-6) in endotoxemic mice receiving IV dextrose—GIP infusion improves glucose disposal (**A**) associated with increases in insulin secretion during metabolic testing (**B**), decreases in circulating levels of the proinflammatory cytokine IL-6 (**E**), and improvements in mean arterial pressure measured just before metabolic testing (**F**). Levels of interleukin 1 beta (IL- β) and tumor necrosis factor alpha (TNF- α) are not significantly changed with GIP infusion (**C** and **D**).

Fig 6.

Disruption of GIP signaling with 3ProGIP blocks the beneficial effects of enteral dextrose in endotoxemic mice—glucose disposal is markedly worsened by 3ProGIP (A) associated with impairments in insulin release during metabolic testing (B). Furthermore, GIP blockade in endotoxemic mice results in increases in interleukin 1 beta (IL- β), tumor necrosis factor alpha (TNF- α), and interleukin 6 (IL-6), and decreases in mean arterial pressure at the time of metabolic testing (C-F).

Table I

Minimal model results for intravenous vs enteral infusion experiments in endotoxemic mice^{//,†,‡}

Variable	IV saline and vehicle	Enteral saline and vehicle	IV dextrose and vehicle	Enteral dextrose and vehicle	IV saline and LPS	Enteral saline and LPS	IV dextrose and LPS	Enteral dextrose and LPS	Absolute difference*	P-value [‡]
Number (n)	6	8	8	8	7	7	9	8	—	—
Area under the glucose curve (mg·10 ³ /dL·min)	8.93 ± 0.57	9.37 ± 0.58	12.8 ± 0.61 [§]	9.99 ± 0.55	9.30 ± 0.81	9.30 ± 0.71	23.65 ± 2.59	9.80 ± 0.45 [¶]	-13.90	<0.01
Acute insulin response to glucose (mU·10 ² /mL)	8.60 ± 2.61	3.09 ± 0.60	3.46 ± 0.89	4.60 ± 0.98	18.10 ± 2.50 [§]	11.54 ± 1.21 [¶]	6.63 ± 1.72	11.74 ± 0.88 [¶]	+5.11	0.02
Insulin sensitivity (mU/mL·min)	14.54 ± 5.27	10.94 ± 1.96	4.48 ± 1.54	8.95 ± 1.72	3.41 ± 0.86 [§]	3.42 ± 0.52 [¶]	1.74 ± 0.49 [§]	6.36 ± 1.26 [¶]	+4.62	<0.01

Boldface values were considered statistically significant ($P < 0.05$).

* Absolute difference represents difference between the value of the minimal model variable in the combined enteral dextrose and endotoxin (lipopolysaccharide [LPS]) group minus the value in the combined intravenous (IV) dextrose and LPS group.

[†] P-value for absolute difference as tested by unpaired two sample *t* test.[‡] Denotes a difference compared to IV saline and vehicle control group with $P < 0.05$.[§] Denotes a difference compared to IV saline and vehicle control group with $P < 0.01$.[¶] Denotes a difference compared to enteral saline and vehicle group with $P < 0.05$.[¶] Denotes a difference compared to enteral saline and vehicle group with $P < 0.01$.

Table II

Minimal model results for incretin infusion experiments in endotoxemic mice receiving IV dextrose

Variable	Vehicle	GIP 4 (pmol/kg/min)	P-value
Number (n)	5	5	
Area under the glucose curve (mg·10 ³ /dL·min)	29.28 ± 5.13	12.70 ± 1.86	0.04
Acute insulin response to glucose (mU·10 ² /mL) *	2.90 (1.13, 4.88)	11.81 (9.03, 14.67)	0.02
Insulin sensitivity (mU/mL·min) *	0.45 (0.01, 4.53)	1.54 (0.92, 3.08)	0.40

Abbreviations: GIP 4, glucose-dependent insulinotropic peptide 4; IV, intravenous.

All mice in this experiment were challenged with LPS and IV dextrose.

Boldface values were considered statistically significant ($P < 0.05$).

* Data represented as median (interquartile range) instead of mean ± standard error.

Table III

Minimal model results for incretin blocking experiments in endotoxemic mice receiving enteral dextrose

	Vehicle	3ProGIP (25 nmol/kg)	<i>P</i> -value
Number (n)	6	6	
Area under the glucose curve (mg/dL·min·10 ³)	9.11 ± 0.53	28.15 ± 2.58	<0.01
Acute insulin response to glucose (mU/mL·10 ²)	10.50 ± 1.32	1.58 ± 0.76	<0.01
Insulin sensitivity (mU/mL·min)	6.20 ± 2.22	2.21 ± 1.09	0.40

All mice in this experiment were challenged with lipopolysaccharide and enteral dextrose.

Boldface values were considered statistically significant ($P < 0.05$).