Role of glucagon in protein catabolism

Steven E. Thiessen, Jan Gunst, and Greet Van den Bergh

Purpose of review
Glucagon is known as a key hormone in the control of glucose and amino acid metabolism. Critical illness is hallmarkied by a profound alteration in glucose and amino acid metabolism, accompanied by muscle wasting and hypoaminoacidemia. Here we review novel insights in glucagon (patho)physiology and discuss the recently discovered role of glucagon in controlling amino acid metabolism during critical illness.

Recent findings
The role of glucagon in glucose metabolism is much more complex than originally anticipated, and glucagon has shown to be a key player in amino acid metabolism. During critical illness, the contribution of glucagon in bringing about hyperglycemia appeared to be quite limited, whereas increased glucagon availability seems to contribute importantly to the typical hypoaminoacidemia via stimulating hepatic amino acid breakdown, without affecting muscle wasting. Providing amino acids further increases hepatic amino acid breakdown, mediated by a further increase in glucagon.

Summary
Glucagon plays a crucial role in amino acid metabolism during critical illness, with an apparent feedback loop between glucagon and circulating amino acids. Indeed, elevated glucagon may, to a large extent, be responsible for the hypoaminoacidemia in the critically ill and infusing amino acids increases glucagon-driven amino acid breakdown in the liver. These novel insights further question the rationale for amino acid administration during critical illness.

Keywords
amino acid metabolism, critical illness, glucagon, nutrition, protein catabolism

INTRODUCTION
The discovery of insulin in 1921 by Banting et al. [1] is seen as one of the most important discoveries in the history of medicine, providing a lifesaving treatment for a dreadful disease – diabetes mellitus. Of lesser prominence was the simultaneous identification of a ‘pancreatic pollutant’ which raised glycemia. This ‘pollutant’ was identified 2 years later by Kimball and Murlin as yet another hormone, which they named glucagon [2]. In the years after its discovery, glucagon lived in the shadow of its older brother insulin and did not receive a lot of attention from the scientific community. Only in the late 1950s, glucagon was sequenced and purified by Eli Lilly, and its physiological role as an important glucose-regulating hormone was only recognized in 1975 [3,4]. Also, among critical care physicians, glucagon remains an elusive hormone and drug, which is only considered as a treatment for severe hypoglycemia and as a last resort for beta-blocker intoxication [5,6]. However, recent findings reconsider the role of glucagon in human physiology, which is also of importance for the critical care physician [7,8,9]. In this review, we discuss these novel findings regarding the (patho)physiological role of glucagon in human glucose and amino acid metabolism, and focus on its role on amino acid metabolism during critical illness. Apart from its effect on glucose and amino acid homeostasis, glucagon also affects many other important metabolic pathways, such as lipolysis, fatty acid oxidation, ketogenesis, thermogenesis, and energy expenditure. This is, however, beyond the scope of this review and is nicely reviewed elsewhere [9].

GLUCAGON: AN OLD HORMONE WITH NEW TRICKS
Glucagon was first identified as an important regulator of glucose homeostasis [2]. Glucagon – a 29
Metabolic support

KEY POINTS

- High glucagon concentrations and low blood amino acid levels are observed in critically ill patients. Both are associated with worse outcome.

- The role of glucagon in glucose homeostasis is much more complex than originally anticipated, and glucagon is recently identified as a key player in amino acid metabolism.

- Recent findings suggest the existence of a feedback loop between glucagon and blood amino acid levels during critical illness. Elevated glucagon may induce low blood amino acid levels during critical illness by stimulating amino acid breakdown in the liver. Providing amino acids during critical illness may aggravate the hepatic amino acid breakdown by further increasing glucagon levels.

- This novel identified feedback mechanism between glucagon and amino acids during critical illness offers a pathophysiological explanation for several observations made in recent randomized controlled trials concerning amino acid supplementation during the acute phase of critical illness and questions the rationale of early amino acid administration during critical illness. These findings may have therapeutic implications for the critically ill patient, but further research is warranted.

Amino acids containing hormone mainly produced in the alpha-cells of the pancreas – is released in response to a decrease in glycemia. Upon release, glucagon binds to its receptor in the liver, hereby activating adenyl cyclase, resulting in the production of cyclic AMP, which in turn activates protein kinase A [10]. Activated protein kinase A up-regulates the transcription of glycogenolytic and gluconeogenic enzymes, such as glucose-6-phosphatase and phosphoenolpyruvate carboxykinase. Furthermore, glucagon also activates phospholipase C, as such increasing inositol 1,4,5-triphosphate (IP3), hereby increasing intracellular calcium concentrations [10]. Both these pathways stimulate hepatic glycogenolysis and gluconeogenesis, hereby enhancing the hepatic release of glucose, resulting in an increase in glycemia. As such, glucagon opposes the effect of insulin, the other important regulator of glucose homeostasis [8]. Based on these findings, a homeostatic model was proposed where glucagon and insulin regulate glycemia in a push–pull manner and where an imbalance in one of the two hormones would result in hypo or hyperglycemia [8].

Recent studies, however, challenge this relative simple homeostatic model [7**]. Excess insulin or insulin deficiency does indeed result in, respectively, hypo and hyperglycemia. However, the role of glucagon is much more complex. Hyperglucagonemia is seen in patients with a glucagon-stimulating tumor, called a glucagonoma [11]. Intriguingly, not all patients suffering from this rare disease have hyperglycemia, and diabetes mellitus is only observed in about 30% of the cases [7**]. Furthermore, infusion of glucagon in healthy individuals only resulted in a transient mild increase in glycemia [12]. So, it seems that glucagon excess does not necessarily result in hyperglycemia. On the basis of the proposed homeostatic model, one would expect that glucagon deficiency would result in a profound hypoglycemia. To challenge this hypothesis, several research groups have developed highly selective glucagon antibodies and created animal models in which the glucagon receptor was inactivated to evaluate the effect of glucagon deficiency on glycemia [13,14]. These interesting studies were able to show that glucagon deficiency did not result in hypoglycemia and that glucagon is not essential for the maintenance of normal fasting glucose levels. It actually seems that cortisol is much more important for the maintenance of normoglycemia by providing gluconeogenic substrates [15]. So, it seems that the glycemia-modulating role of glucagon is much more complex than originally thought.

In the landmark study that identified glucagonoma as a clinical identity, the most striking biochemical feature of excess glucagon was not hyperglycemia (which was not present in all patients suffering from a glucagonoma), but a profound hypoaminoacidemia, meaning low blood amino acid levels [11]. Furthermore, a study investigating the effect of a total pancreatectomy on metabolic parameters identified low glucagon and high amino acid blood levels in these patients [16]. These findings already hinted towards an important role for glucagon in amino acid homeostasis. To address this hypothesis, several research groups evaluated the effect of glucagon excess and glucagon deficiency on amino acid homeostasis.

Several studies clearly showed that exogenous glucagon administration resulted in a decrease in blood amino acid levels and an increase in ureagenesis, by stimulating hepatic amino acid catabolizing enzymes, and also hepatic enzymes involved in the urea cycle [17–19]. Glucagon deficiency, on the contrary, brought about by the administration of glucagon antibodies or genetic interference, resulted in increased plasma levels of amino acids, which resulted in the proliferation of alpha-cells, by an mTOR (mammalian target of rapamycin)-dependent pathway [18,20]. Interestingly, the acute administration of glucagon does not necessarily affect muscle wasting, because skeletal myofibers do not express the glucagon receptor [21].
The physiological role of glucagon on amino acid metabolism. Amino acids stimulate the secretion of glucagon from the alpha cells in the pancreas (black arrow). This increase in glucagon stimulates hepatic amino acid breakdown (black arrow) and ureagenesis (dotted arrow) by binding to its hepatic receptor and inducing enzymes involved in these processes. The activation results in a decrease in blood amino acid levels (white arrow), hereby inhibiting the glucagon release from the alpha cells [7**].

Therefore, glucagon influences the hepatic utilization of amino acids, but does not affect the provision of amino acids to the liver [7**]. However, prolonged glucagon excess might promote muscle wasting, because patients suffering from glucagonoma are hallmark by muscle wasting [11]. This might suggest that by enhancing the hepatic breakdown of amino acids, glucagon may diminish the precursor pool of amino acids available for protein synthesis, which could lead to muscle wasting [17], though this hypothesis remains to be tested.

These interesting findings seem to suggest that there exists a feedback loop between blood amino acid levels and glucagon, where glucagon activates hepatic amino acid breakdown and induces ureagenesis, and where amino acids stimulate glucagon release, at least in part, via inducing alpha cell hyperplasia (Fig. 1) [7**]. This means that when amino acids are administered to healthy individuals, this would lead to an increase of glucagon and hence hepatic amino acid breakdown, and increased ureagenesis, which was nicely shown in several studies [7**,17,18].

**GLUCAGON DURING CRITICAL ILLNESS: A KEY PLAYER IN AMINO ACID METABOLISM**

Critical illness is a life-threatening condition hallmarked by severe lean tissue wasting, hyperglycemia, and low circulating amino acid levels [22,23]. High levels of catecholamines and glucocorticoids have been documented during critical illness, and it is assumed that these hormones play a crucial role in the metabolic alterations seen during critical illness [24,25]. However, whether glucagon is elevated during critical illness and what the metabolic role is during critical illness are questions that have been poorly investigated, until recently [26*].

Some older studies have reported increased plasma glucagon concentrations during critical illnesses, such as trauma, burns, major surgery, sepsis, and hemorrhage [27]. These studies, however, used assays that could not discriminate between active glucagon and inactive glucagon, or other proglucagon derived hormones [28]. A recent study, performed by our group, confirmed these earlier findings using a novel assay that only quantified active glucagon [26*]. Interestingly, in this study, glucagon correlated positively with the severity of illness and was much higher in patients with sepsis than in those without.

The underlying mechanism of this increase in glucagon during critical illness remains unknown. Since critical illness is hallmarked by the development of hyperglycemia and hyperinsulinemia [24,25], both powerful suppressors of glucagon release from the alpha-cells [21], another mechanism is likely to be involved. Possible underlying pathways are the development of insulin resistance in the alpha-cells or the activation of the autonomic nervous system during critical illness. The alpha-cells contain alpha-1 and beta receptors, both stimulating glucagon release upon activation [27]. As such, critical illness could lead to the release of glucagon by a systemic catecholamine response or by a stimulation of the pancreatic islet sympathetic innervation, possibly mediated by an activation of the hypothalamic ventromedial nucleus. Further research is needed to investigate the role of this interesting pathway.

Also, the role of increased glucagon availability during critical illness has not been well studied until recently. It has been suggested that glucagon during critical illness could increase substrate (mainly glucose) availability, stimulate cardiac function (by a positive inotropic and chronotropic effect), decrease gut motility, and increase thermogenesis [25,27]. However, these assumptions are based on experimental studies performed in other disease states, such as trauma or on studies where somatostatin infusion was used to inhibit glucagon release, which also affects growth hormone and insulin availability. However, the recent development of highly selective glucagon antibodies allowed to study in more detail the role of glucagon during critical illness. Recently, our group has performed a

![Figure 1](image-url)
translational study to evaluate the metabolic role of glucagon during critical illness [26*]. In this study, we neutralized circulating glucagon, using highly selective glucagon antibodies, in a well validated mouse model of prolonged sepsis-induced critical illness, and evaluated its effect on mortality, glucose, and amino acid homeostasis, among others. In this model, critical illness resulted in an increased glucagon availability and low amino acid levels, resembling the human condition. Quite surprisingly, the complete abolition of glucagon during critical illness did not affect survival, only transiently affected glucose metabolism, and did not result in hypoglycemia at any time. These remarkable findings are in accordance with the novel insights in the (patho)physiology of glucagon, as discussed above. The most striking effect of glucagon neutralization during critical illness, however, was its effect on amino acid homeostasis. Neutralizing glucagon completely reversed the hypoaminoacidemia, even resulting in supra-physiological levels of amino acids. This effect on amino acidemia was due to the hepatic amino acid breakdown-stimulating effect of glucagon, and there was no effect on muscle breakdown. So, it seems that increased glucagon availability during critical illness is responsible for the low amino acid levels present in this condition, by stimulating hepatic amino acid breakdown, without affecting muscle wasting. Again, these at first sight surprising findings are in accordance with the novel insights in glucagon (patho)physiology, as mentioned above.

Next, we evaluated the effect of amino acid administration on glucagon availability and markers of hepatic amino acid breakdown in the mouse model, and also in a human randomized controlled trial (RCT). In both studies, amino acid administration resulted in an increase in plasma glucagon concentrations and increased markers of hepatic amino acid breakdown, without affecting muscle wasting. Interestingly, the administration of glucose together with insulin – two powerful suppressors of glucagon in healthy individuals – did not affect glucagon levels nor plasma amino acid levels in critically ill patients. So, recent studies identify enhanced glucagon availability as a key player in amino acid metabolism during critical illness, responsible for the hypoaminoacidemia, a hallmark of critical illness, without affecting muscle wasting.

**IMPLICATIONS OF THE AMINO ACID REGULATING ROLE OF GLUCAGON DURING CRITICAL ILLNESS**

These novel findings suggest that during critical illness, a feedback loop exists between glucagon and blood amino acid levels, as is shown in Fig. 2. The increased glucagon availability during critical illness results in an increased hepatic amino acid breakdown, resulting in an increased ureagenesis and low amino acid levels. Increasing amino acid levels during critical illness stimulates glucagon, resulting in even more hepatic amino acid breakdown and ureagenesis. At this moment, there is insufficient evidence to suggest that glucagon stimulates muscle wasting or that amino acid supplementation results in a decrease in muscle wasting during critical illness [26*,29].

The proposed homeostatic mechanism could potentially explain several unresolved issues during critical illness, help guide clinical practice at the bedside, and lead to possible novel therapeutic interventions in critically ill patients. It could explain at least a part of the apparent resistance against anabolism during critical illness seen in several RCTs concerning nutrition. Indeed, our group has shown via a large RCT, with the acronym EPaNIC, that early parenteral nutrition is unable to attenuate muscle catabolism. The study also showed that the extra amount of nitrogen administered via the parenteral route was for more than 60% wasted via the urine throughout the first 2 weeks of critical illness [29,30]. Furthermore, as shown by four other large RCTs, both glutamine supplements and higher doses of amino acids also resulted in a large increase in plasma urea without bringing about the proposed beneficial effects [31–34]. The homeostatic mechanism that we propose would suggest that the administration of amino acids, glutamine, or other results in an increase in systemic glucagon availability, which triggers a further hepatic amino acid breakdown, precluding any potential beneficial effect of these amino acids, while enhancing ureagenesis – a potentially toxic degradation product. Apart from that, amino acids are powerful suppressors of autophagy – a damage-removal process that was found to be crucial in mediating recovery from critical illness-induced organ failure and muscle weakness [35,36]. Recent evidence has confirmed autophagy suppression by providing amino acids early to critically ill patients and animals [29,37]. Moreover, detailed secondary analyses from two large RCTs have implicated the extra amino acids, and not the supplementary glucose or lipids, as culprit macronutrient statistically explaining the clinical harm evoked by early parenteral nutrition [33,38]. Altogether, this raises serious concerns about the practice of administering high doses of amino acids during the acute phase of critical illness. On the basis of pathophysiological reasoning and on recent scientific data, we would advocate against the administration of high doses of amino acids,
during the acute phase of critical illness, especially via the parenteral route [39*]. Whether determination of plasma glucagon concentrations could help guide nutritional therapy, by determining the time point at which it becomes well tolerated to administer amino acids or increase the dose hereof, remains to be investigated.

Because amino acids are vital building blocks and signaling molecules for many important functions during critical illness, a prolonged hypoaminoacidemia during critical illness might, in theory, be detrimental [23,39*]. On the basis of our proposed homeostatic mechanism, instead of administering exogenous amino acids, the impact of diminishing glucagon availability might be considered in prolonged critical illness. Interestingly, drugs are available that can attenuate glucagon action, such as glucagon-like peptide-1 [21]. Whether pharmacological glucagon suppression during critical illness could improve outcome, by

FIGURE 2. The pathophysiological role of glucagon on amino acid metabolism during critical illness. (a) During critical illness, glucagon is elevated, possibly due to increased sympathetic tone, hereby increasing hepatic amino acid breakdown and ureagenesis. This results in low amino acid levels, despite the increased release of amino acids from the muscle, which is mediated by increased catecholamines and cortisol, among others. (b) Providing amino acids during critical illness increases circulating levels of amino acids and hereby further stimulates glucagon and hepatic amino acid breakdown and ureagenesis. Notice that there are no available data to suggest that amino acid administration during critical illness results in a decrease in muscle wasting.
reversing hypoaminoacidemia, is an intriguing hypothesis that requires further research.

CONCLUSION

Glucagon is an intriguing, but often forgotten, hormone. Novel insights in the (patho)physiology of glucagon point to a crucial role for this hormone in amino acid metabolism, by stimulating hepatic amino acid breakdown. Hypoaminoacidemia – a hallmark of critical illness – has been interpreted as a result of an increased utilization of amino acids by organs and tissues with an inability of muscle wasting to keep up with the increased amino acid demand. Therefore, practice guidelines have advocated to administer high amounts of amino acids to meet this increased demand. A recent animal study, however, clearly indicates that these low amino acid levels during critical illness are the result of increased glucagon availability that stimulates hepatic amino acid breakdown. Interestingly, in critically ill patients, this increase in glucagon cannot be suppressed by administering glucose and insulin, and is even intensified by administering amino acids early during critical illness, which does not affect muscle wasting. On the basis of these results, a homeostatic link between glucagon and blood amino acid levels appears to be present during critical illness, where glucagon and amino acids affect each other in opposite directions. Furthermore, it seems that enhanced glucagon availability, possibly, in part, mediated by increased sympathetic tone and insulin resistance, is responsible for the low amino acid levels observed uniformly during critical illness. These novel findings question the rationale of providing (high doses of) amino acids, in particular, early during the course of critical illness and may open new perspectives for treatment.

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Conflicts of interest

The authors have no conflicts of interest.

A clear and comprehensive review summarizing the current evidence concerning amino acid supplementation during critical illness.