Molecular mechanisms behind clinical benefits of intensive insulin therapy during critical illness: Glucose versus insulin

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High blood glucose levels have been associated with morbidity and poor outcome in critically ill patients, irrespective of underlying pathology. In a large, randomised, controlled study the use of insulin therapy to maintain normoglycaemia for at least a few days improved survival and reduced morbidity of patients who are in a surgical intensive care unit (ICU). Since the publication of this landmark study, several other investigators have provided support for, whereas others have questioned, the beneficial effects of intensive insulin therapy.

In this review, we discuss the investigated potential molecular mechanisms behind the clinical benefits of intensive insulin therapy. We first describe the molecular origin of hyperglycaemia and the impact of the therapy on insulin sensitivity. Next, the molecular basis of glucose toxicity in critical illness and the impact of intensive insulin therapy hereon are described, as well as other non-glucose-toxicity-related metabolic effects of intensive insulin therapy.

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Clinical benefits of intensive insulin therapy

Critical illness is hallmarked by numerous endocrine and metabolic disturbances, including the development of hyperglycaemia consequent to insulin resistance and increased hepatic glucose production, which is referred to as 'stress diabetes' or 'diabetes of injury'. High blood glucose levels...
have been associated with morbidity and poor outcome in critically ill patients, irrespective of underlying pathology. This association may merely reflect that the degree of hyperglycaemia is a marker of the severity of illness. Alternatively, it may indicate that hyperglycaemia itself contributes to the disease and that there is a causal relationship between hyperglycaemia and clinical complications.

For a long time, it was believed that a mild degree of hyperglycaemia would be beneficial for organs that largely rely on glucose for energy provision, but do not depend on insulin for glucose uptake. With the publication of the first, landmark Leuven randomised clinical study on intensive insulin therapy in adult surgical critically ill patients this concept was challenged. At the time of the study, standard care consisted of treating only excessive hyperglycaemia above the renal threshold (>220 mg dl⁻¹, known to induce osmotic diuresis and infectious complications) with infusion of exogenous insulin, which was discontinued when levels fell below 180 mg dl⁻¹. This approach was compared with strict glycaemic control to normal fasting blood glucose levels (80–110 mg dl⁻¹) with insulin infusion, labelled ‘intensive insulin therapy’. Intensive insulin therapy strikingly lowered mortality in-ICU and in-hospital, most pronounced for long-stay patients, and improved long-term outcome. Furthermore, it reduced the incidence of several common critical illness-associated clinical complications. These included the prevention of bloodstream infections, acute renal failure, critical illness polyneuropathy and hyperbilirubinaemia and reduced need for red blood cell transfusions and prolonged mechanical ventilation, all culminating in a reduced need for prolonged intensive care. The therapy also protected the central and peripheral nervous system from secondary insults and improved long-term rehabilitation of patients with isolated brain injury. Subsequently, two large randomised clinical studies were performed by the same investigators in a strictly medical population of critically ill adults and in critically ill children, again comparing standard care versus fasting glucose levels targeted to age-adjusted normal levels. These studies largely reproduced the previously observed clinical benefits of intensive insulin therapy. Since the publication of the first Leuven study, several other investigators have provided support for the beneficial effects of intensive insulin therapy, whereas in other studies no clinical benefits were seen. Due to these apparently conflicting results, the optimal level and modality of glucose control remain an area of heavy debate. In general, however, studies that were unable to detect clinical benefit already started with lower glucose levels in the standard care group than did the Leuven studies. This may suggest that prevention of excessive hyperglycaemia is what evokes the benefit, although the combination with inadequate glucose monitoring and poor achievement of the glycaemic targets may have played a role.

Hence, whether strict normoglycaemia should be maintained, as in the Leuven studies, or whether an intermediate glucose range should be targeted needs to be studied further using appropriate glucose monitoring tools. An indication for the answer to this question has been provided by multivariate analyses on the two adult randomised controlled trials performed in Leuven. Compared with the intermediate blood glucose levels of 110–150 mg dl⁻¹, mortality was higher with blood glucose >150 mg dl⁻¹ and lower with glucose <110 mg dl⁻¹. The largest benefit was gained by prevention of the excessive hyperglycaemia. However, the reduction of blood glucose levels below 110 mg dl⁻¹ seemed to be crucial for the prevention of events that cause morbidity such as bacteremia, anaemia and acute renal failure.

**Mechanism of stress hyperglycaemia and blood glucose control**

**Insulin resistance and glucose uptake**

The stress imposed by any type of acute illness or injury results in insulin resistance, glucose intolerance and hyperglycaemia. Despite high blood glucose levels and abundantly released insulin, hepatic glucose production is up-regulated in the acute phase of critical illness. Hepatic insulin resistance is further characterised by elevated circulating levels of IGF-binding protein-1 (IGFBP-1). Elevated levels of cytokines, growth hormone, glucagon and cortisol might play a role in this increased gluconeogenesis. Several effects of these hormones oppose the normal action of insulin, resulting in an increased lipolysis and proteolysis and providing substrates for gluconeogenesis. Catecholamines, which are released in response to acute injury, also enhance hepatic glycogenolysis and inhibit
glycogenesis.27 The most severe cases of stress-induced hyperglycaemia1 and highest levels of circulating IGFBP-124–26 are observed in patients with the highest risk of death. Glucose uptake mechanisms are also affected during critical illness and contribute to the development of hyperglycaemia. As a result of the immobilisation of critically ill patients, exercise-stimulated glucose uptake in skeletal muscle disappears. Furthermore, because of impaired insulin-stimulated glucose uptake and impaired glycogen synthase activity, glucose uptake in heart, skeletal muscle and adipose tissue is compromised.28–31 In tissues not depending on insulin for glucose uptake, such as the nervous system, hepatocytes, gastrointestinal mucosa, pancreatic β-cells, renal tubular cells and endothelial and immune cells, hyperglycaemia may increase glucose uptake.1 The higher levels of insulin, impaired peripheral glucose uptake and elevated hepatic glucose production reflect the development of insulin resistance during critical illness.

The mechanism by which intensive insulin therapy lowers blood glucose levels in critically ill patients is not completely clear. Insulin actions in metabolism, cell growth and differentiation are mediated by intracellular signalling pathways. Insulin binding to its receptor can activate two signalling pathways: the ‘mitogenic’ pathway, which proceeds through Shc/Grb2 activation leading to activation of different mitogen-activated protein kinase (MAPK) isoforms, and the ‘metabolic’ pathway, which proceeds through the insulin receptor substrates (IRSs) and depends on the activation of PI3K.32 In critically ill patients, it was demonstrated that tight glycaemic control with intensive insulin therapy activated the metabolic insulin signalling pathway in muscle33 and increased the levels of mRNA encoding GLUT4 (transporter responsible for insulin-stimulated glucose uptake) and of hexokinase II (rate-limiting enzyme in intracellular insulin-stimulated glucose metabolism).34 By contrast, hepatic insulin signalling remained impaired under intensive insulin therapy.33 In addition, hepatic expression of phosphoenolpyruvate carboxykinase (rate-limiting enzyme in gluconeogenesis) and of glucokinase (rate-limiting enzyme for insulin-mediated glucose uptake and glycogen synthesis) was unaffected by insulin therapy.34,35 Moreover, circulating levels of insulin-like growth factor binding protein 1, which is normally under inhibitory control of insulin, was also refractory to the therapy.35 Intensive insulin therapy also increased the insulin-sensitising hormone adiponectin.33 These studies suggest that intensive insulin therapy does not affect hepatic insulin resistance and lowers blood glucose mainly through stimulation of skeletal muscle glucose uptake. Whether also other peripheral tissues are involved, remains to be investigated.

Blood glucose control or insulin?

Hyperglycaemia has been identified as an independent risk factor for adverse outcome in numerous different clinical settings. Even a modest degree of hyperglycaemia occurring after admission to an intensive care unit (ICU) is associated with a substantial increase in mortality while in the hospital.36 Multivariate logistic regression analyses on the two adult Leuven studies indicated that blood glucose control, rather than the insulin dose administered, statistically explains most of the beneficial effects of insulin therapy on the outcomes of critical illness.8,23 However, a higher mean daily insulin dose for any given blood glucose level was associated with higher hospital mortality in the intention-to-treat population.8 This statistical association between a high insulin dose for any given blood glucose level and risk of death can point to the known association between severity of illness and degree of insulin resistance, or it may suggest that hyperinsulinaemia is deleterious. However, although patients under tight glycaemic control regimen received several-fold higher insulin doses to maintain normoglycaemia than patients under standard care or ‘conventional insulin therapy’, it was demonstrated that serum insulin levels were only transiently higher with intensive insulin therapy, despite the much lower blood glucose levels.33,37 Patients under conventional insulin therapy demonstrated elevated serum C-peptide levels, suggesting increased endogenous insulin release, which appeared to be counteracted by intensive insulin therapy.33,37

A recent animal study by Ellger et al., in which plasma insulin and blood glucose levels were manipulated independently over 7 days in burn-injured, parenterally fed rabbits, addressed the separate effects of glucose and insulin in detail.38 The study showed that reduction of glucose levels, independent from the circulating insulin levels was responsible for improved morbidity and mortality, although both factors contributed to improved leucocyte and myocardial functions.
Two possible pathways to organ failure in the critically ill are currently suggested. The first is a disturbed microcirculation due to endothelial dysfunction, leading to inadequate oxygen supply and cellular hypoxia. This compromises cellular energy metabolism and function and causes organ failure and death. The other pathway involves a disturbed mitochondrial oxygen utilisation labelled ‘cytopathic hypoxia’ as a cause of bioenergetic failure, rather than inadequate oxygen delivery. Such mitochondrial dysfunction has been related to lethal outcome in patients as well as in a resuscitated long-term rat model of sepsis. Intensive insulin therapy targeting strict normoglycaemia has been shown to protect the vascular endothelium and the mitochondrial compartment.

(i) Endothelial dysfunction and hypoxia

Reduced endothelial activation, as reflected by lower levels of adhesion molecules, related to prevention of organ failure and death with intensive insulin therapy in critically ill patients. This effect was likely mediated in part via inhibition of excessive nitric oxide release by inducible nitric oxide synthase (iNOS). Levels of the endogenous NOS inhibitor asymmetric dimethylarginine (ADMA) were also reduced, which was associated with a better outcome. Here, attenuation of the inhibition of the constitutively expressed endothelial nitric oxide synthase (eNOS), contributing to preservation of organ blood flow, may have played a role. In a rabbit model of critical illness, where blood glucose and insulin were independently manipulated to normal or high levels, it was demonstrated that it was the glycaemic control and not glycaemia-independent effects of insulin that mediated the observed endothelial protection. This included improved endothelium-mediated relaxation of aortic rings and maintenance of physiological ADMA levels in plasma and tissues by preservation of physiological activity of the ADMA metabolising enzyme dimethylarginine dimethylaminohydrolase (DDAH). However, no effect was seen on tissue perfusion and oxygen delivery.

(ii) Cytopathic hypoxia

Whereas liver biopsies taken from patients who received the conventional standard care glucose control regimen tolerating hyperglycaemia up to 215 mg dl\(^{-1}\) showed an abundant presence of severely abnormal mitochondria that were swollen, had an increased number of abnormal and irregular cristae and were up to 10-fold enlarged, patients who received intensive insulin therapy were virtually protected from such severe morphological damage to mitochondria. At the functional level, this correlated with higher activities of the mitochondrial respiratory chain enzyme complexes I and IV. No morphological or functional differences were observed in skeletal muscle. Like for the endothelial protection, glucose control appeared crucial to preserve mitochondrial integrity, as shown in critically ill rabbits. Severe morphological and/or functional damage to mitochondria was only observed in the presence of hyperglycaemia whereas maintenance of normoglycaemia, but not elevating insulin levels, attenuated or prevented such abnormalities. The role of glucose toxicity was further underscored by the strong correlations observed between tissue levels of glucose, strongly elevated in hyperglycaemic animals and mitochondrial enzyme activities. Importantly, mitochondrial enzyme activities correlated with biomarkers of hepatic, myocardial and renal damage, independent of oxygen delivery. Mitochondrial respiratory chain enzyme activities remained normal in skeletal muscle of these burn-injured critically ill rabbits, regardless of the glucose and insulin levels, which is in agreement with the absence of an effect of intensive insulin therapy in skeletal muscle of critically ill patients. The tissue-specific effects may be explained by the different mechanisms of glucose uptake, allowing cellular glucose overload in tissues with insulin-independent glucose transporters, whereas tissues that rely predominantly on insulin-dependent glucose uptake may be relatively well protected in view of the development of insulin resistance.

(iii) Proximal pathways of glucose toxicity

Multiple mechanisms may contribute to glucose toxicity, as revealed by the diabetes literature. Several of them have been linked to a central event whereby the glycolytic enzyme glyceraldehyde-3-phosphate
Dehydrogenase (GAPDH) is inactivated, causing a block in glycolysis and a shift of glucose into proximal toxic pathways. These include an increased flux through the polyol and hexosamine pathways, activation of protein kinase C and increased formation of advanced glycation end products (AGES). GAPDH appeared to be not or hardly compromised by hyperglycaemia in critical illness, unlike in diabetes, as shown in burn-injured rabbits and critically ill patients. Furthermore, glycolysis was suggested to be increased rather than inhibited in critical illness, supported by animal data and increased pyruvate formation as a result of increased glucose turnover in critically ill patients. The described toxic pathways may however be fed by such overloading of the glycolytic pathway, as shown for the formation of methylglyoxal (the most important dicarbonyl precursor of AGES), being proportionate to the glycolytic flux. The dicarbonyl metabolism was disturbed in hyperglycaemic critically ill rabbits, with elevated dicarbonyl levels (glyoxal, methylglyoxal and 3-deoxyglucosone) and impaired detoxification as compared with normoglycaemic sick rabbits. Dicarbonyl levels strongly correlated inversely with mitochondrial enzyme activities, which may support involvement of this pathway in critical illness. Administration of dicarbonyls has been shown to reproduce the complications of diabetes. Furthermore, mitochondrial toxicity of dicarbonyls has been demonstrated and has included severe ultra-structural alterations, post-translational protein modification and inhibition of the citric acid cycle and respiratory chain.

(iv) Oxidative stress

Multiple organ failure in critical illness has been linked to increased oxidative stress that develops secondary to an increased production of reactive oxygen species and/or a decrease in its scavengers. Substantial evidence links diabetes and hyperglycaemia to the development of increased oxidative stress, and several mechanisms contribute to the elevated production of reactive oxygen species in the presence of high glucose concentrations. One of them is an increased mitochondrial superoxide production, which actually is central in the unifying hypothesis on hyperglycaemic damage pathways described above. Indeed, consequent DNA damage has been described to activate poly(ADP-ribose) polymerase (PARP) that in its turn inactivates GAPDH. Other sources of ROS production include transition metal-catalysed glucose oxidation, activation of NADPH oxidase and also AGES themselves are able to produce oxygen free radicals due to their strong reducing properties. Furthermore, antioxidant defence systems may be reduced. In critical illness, cytokines enhance the synthesis of nitric oxide, whereas hypoxia–reperfusion aggravates the production of superoxide, thus promoting the formation of peroxynitrite. This reactive nitrogen species may interfere with the normal function of several proteins, including the mitochondrial enzyme complex I of which the activity is suppressed by tyrosine nitration. Avoiding hyperglycaemia in critically ill rabbits prevented the rise of malondialdehyde, a marker of oxidative stress-induced lipid peroxidation, and the depletion of the oxidant scavenger reduced glutathione, which correlated with improved mitochondrial function.

Innate immunity, inflammation and coagulation

High glucose levels compromise all major components of innate immunity, including polymorphonuclear neutrophil function and intracellular bactericidal and opsonic activity. Hyperglycaemia can lead to glycosylation and thus inactivation of immunoglobulins, hence contributing to the risk of infection. This may play a role in the increased risk of infections observed for patients who are exposed to such high glucose levels. Tight glycaemic control with intensive insulin therapy has been shown to prevent nosocomial infections and lethal sepsis in critically ill patients. Independent of its effect on prevention of infection, intensive insulin therapy also lowered excessive inflammation, as illustrated by the lower levels of C-reactive protein (CRP) and mannose-binding lectin. In critically ill children, tight glycaemic control with intensive insulin therapy attenuated the inflammatory response as evidenced by the time course of CRP. Secondary infections were reduced, most importantly, pulmonary and bloodstream infections. Similar observations were seen in an animal model of prolonged critical illness induced by a third-degree burn injury, where glucose control with insulin beneficially affected innate immunity by preservation of phagocytosis and the oxidative burst function of monocytes and attenuated the CRP response. When glucose and insulin were manipulated independently in this rabbit model of critical illness, a partial rescue by insulin and not tight glycaemic control could be demonstrated on leucocyte dysfunction. These data indicate that both prevention of
hyperglycaemia and direct insulin effects appear to contribute to the anti-inflammatory effects of intensive insulin therapy.

In view of the link between inflammation, endothelial dysfunction and coagulation abnormalities, and as both insulin therapy and glucose control may affect coagulation and fibrinolysis, one could expect that intensive insulin therapy may prevent severe coagulation abnormalities, thereby contributing to organ protection and improved survival. However, in a post hoc analysis on a subset of the second Leuven trial on medical ICU patients, no effect of insulin therapy was found on the tested fibrinolytic and coagulation parameters.37 By contrast, in a prospective randomised clinical trial in patients with sepsis, a small but significant improvement of fibrinolysis was observed in patients receiving tight glycaemic control.86

Anabolic actions of insulin

Muscle protein synthesis is stimulated, whereas protein breakdown is inhibited by insulin, illustrating its anabolic properties. Hence, insulin administration may counteract the hypercatabolic state and feeding-resistant wasting syndrome of prolonged critical illness.89 From clinical observation, such an anabolic effect was not obvious, but intensive insulin therapy increased the total protein content in the skeletal muscle of critically ill patients.44 Improvement of insulin sensitivity may have played a role.33 Whereas insulin therapy in diabetes mellitus was able to restore the levels of the anabolic insulin-like growth factor-1 (IGF-1), altered regulation at the level of the growth hormone axis appeared not to be involved in the beneficial effects of insulin during critical illness, as IGF-1 levels surprisingly were further suppressed.35 Beneficial effects of insulin were also observed in animal models. For instance, it prevented weight loss in a rabbit model of prolonged critical illness90 and suppressed muscle proteolysis in burn-injured rats, with involvement of the ubiquitin–proteasome pathway and lysosomal proteases.91 However, when considering potential anabolic effects of intensive insulin therapy, a contribution of glycaemic control may not be excluded as it has been shown that hyperglycaemia induces catabolic responses.92,93 Maintaining normoglycaemia may therefore be required to obtain anabolic effects with insulin administration.

Myocardial protection by insulin

Infusion of insulin together with glucose and potassium, labelled GIK therapy, emerged several decades ago as a metabolic cocktail to reduce early mortality and morbidity of patients with myocardial infarction.94 It has also been shown to protect and improve myocardial function during open heart surgery, endotoxic shock and other critical conditions.95 Initially promising results were, however, followed by the disappointing results of two recent large randomised clinical studies in patients with myocardial infarction where GIK failed to improve outcome.97,98 Direct anti-apoptotic properties of insulin, independent of glucose uptake and involving insulin signalling, may play a role in myocardial protection.99 However, avoiding hyperglycaemia is likely crucial for insulin to exert any beneficial effect on the myocardium.95 Hyperinsulinaemia was able to increase myocardial contractility in critically ill rabbits, but only when blood glucose levels were maintained normal.38 Furthermore, simultaneous toleration of hyperglycaemia compromised contractility and mitochondrial function.38,48 These data, suggesting that insulin administration in the absence of adequate blood glucose control may be deleterious, may offer an explanation for the controversy surrounding GIK and the disappointment of the recent clinical studies. Indeed, neither of the studies performed or succeeded in tight blood glucose control.97,98 Similarly, GIK infusion after acute stroke failed to reduce mortality in the absence of glycaemic control.100 By contrast, intensive insulin therapy targeting and reaching age-adjusted normal glucose levels provided myocardial protection in critically ill children.7

Effect of insulin on the lipid profile

Lipid metabolism in critically ill patients is strongly deranged with an elevated triglyceride level (due to an increase in very-low-density lipoprotein (VLDL)) and very low levels of high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol.101,102 These disturbances were almost
completely (hypertriglyceridaemia) or partially reversed (HDL and LDL cholesterol) by insulin therapy. Insulin treatment also decreased serum triglycerides and free fatty acids in burned children. The important role of lipoproteins in transportation of lipid components (i.e., cholesterol, triglycerides, phospholipids and lipid-soluble vitamins) and scavenging of pro-inflammatory bacterial endotoxin may have contributed to the improved outcome. Furthermore, multivariate logistic regression analysis revealed that improvement of the dyslipidaemia with insulin therapy explained a significant part of the reduced mortality and organ failure in critically ill patients.

Cholestatic liver dysfunction and biliary sludge are common problems in critically ill patients. In animal studies, it was shown that hyperglycaemia induces cholestasis. In humans, gallbladder dysmotility is also associated with hyperglycaemia as well as with insulin resistance. Intensive insulin therapy significantly reduced both the cholestatic liver dysfunction and biliary sludge, but not ischaemic hepatitis in prolonged critically ill patients.

The mechanism behind the improved lipid profile with intensive insulin therapy remains unresolved although the ameliorated liver and gallbladder function is likely involved. One could also expect an increased peripheral lipid uptake or a decreased lipid release.

Function of the adrenal axis

An adequate cortisol response to critical illness is vital, as both excessive and insufficient activation of the hypothalamic–pituitary–adrenal axis is associated with increased mortality. Insulin theoretically could have an ambiguous effect on the cortisol response to critical illness. A stimulatory effect of insulin on free cortisol levels could be inferred by its ability to suppress the levels of cortisol-binding globulin. However, attenuation of the cortisol response may also be expected in view of the anti-inflammatory effects of insulin, which may reduce the levels of cytokines that directly drive cortisol secretion and metabolism. Circulating total and free cortisol levels were lowered by intensive insulin therapy, without altering cortisol-binding activity. This effect statistically related to the improved outcome of the patients who received intensive insulin therapy. The mRNA expression of the glucocorticoid receptor splice variants in postmortem liver and skeletal muscle biopsies of critically ill patients was not affected by insulin therapy.

Summary

Hyperglycemia in critical illness has been associated with increased mortality. Simply maintaining normoglycemia with insulin therapy improves survival and reduces morbidity in ICU patients, as shown by several randomised controlled studies. Multivariate logistic regression analyses on the two Leuven studies indicated that blood glucose control, rather than the insulin dose administered, statistically explains most of the beneficial effects of insulin therapy on the outcome of critical illness. Amelioration of insulin sensitivity thereby improving glucose uptake in insulin-sensitive tissues appears to play a major role in the mechanism by which blood glucose is lowered.

Several molecular pathways are beneficially affected by intensive insulin therapy. Prevention of hyperglycaemia-induced bioenergetic failure likely plays an important role in organ protection by this therapy. In addition, other metabolic effects of insulin therapy contributed to the clinical benefits, although most often with the requirement of simultaneously avoiding hyperglycaemia. Insulin therapy appeared to improve innate immunity and lowered excessive inflammation, prevented weight loss and ameliorated muscle mass, protected the myocardium and had an impact on the cortisol response.

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Conflicts of interest

The authors report to have no conflict of interest.

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