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REVIEW

Hypothesis: normalisation of cytokine dysbalance explains the favourable effects of strict glucose regulation in the critically ill

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ABSTRACT

Recent trials investigating the effects of strict glucose regulation in critically ill patients have shown impressive reductions in morbidity and mortality. Although the literature focuses on the possible toxic effects of high blood glucose levels, the underlying mechanism for this improvement is unclear. We hypothesise that strict glucose regulation results in modulation of cytokine production, leading to a shift towards a more anti-inflammatory pattern. This shift in the cytokine balance accounts for the reduction in morbidity and mortality.

To support our hypothesis, effects of glucose and insulin on cytokine release and effects of glucose, insulin, and cytokines on host defence, cardiac function and coagulation will be reviewed.

INTRODUCTION

Diabetic patients are at risk for cardiovascular and various infectious diseases while infections will often run a complicated course.[1] The reason for this increased susceptibility to infections may be an impaired immune defence, microvascular and macrovascular comorbidity, the relative high number of medical interventions or – viewed from the microbial site – an altered adherence of micro-organisms to human cells under hyperglycaemic conditions.[2]

Reportedly, postoperative hyperglycaemia is an independent risk factor for the development of infectious complications.[4] A large recent study by Van den Berghe *et al.*[4] in critically ill patients showed that maintenance of blood glucose levels between 4.4 and 6.1 mmol/l results in a 42% reduction in mortality compared with conventional treatment aimed at blood glucose levels between 9.9 and 11.1 mmol/l. Patients with multiple-organ failure and a proven septic focus demonstrated the greatest improvement with intensive insulin therapy. In addition, in patients with acute myocardial infarction maintenance of blood glucose levels below 11.9 mmol/l has been shown to increase the success rate of thrombolysis,[5] preserves myocardial function,[6] and improves long-term outcome.[7,8]

How the strict control of blood glucose reduces morbidity and mortality is not known, but it is thought that the mechanism may be related either to a direct effect of normalisation of hyperglycaemia or to the concomitant higher insulin levels.

There is a striking similarity between the effects of high glucose levels on host defence, cardiac function and coagulation and the observation of elevated systemic levels of proinflammatory cytokines during critical illness. Cytokines, low-molecular-weight proteins produced by various immune-competent cells, have important modulating effects on the immune response. Cytokines have autocrine, paracrine and endocrine effects.[9] Whereas the local autocrine and paracrine effects may be beneficial in

containing the infection or tissue damage,[10-13] the systemic endocrine effects of the so-called proinflammatory cytokines, such as tumour necrosis factor-α (TNFα) and interleukin-1 (IL-1), are considered to be deleterious. During the normal response to infections or to noninfectious stressors, the tailored and balanced production of proinflammatory cytokines, aiming at stimulation of the innate immune response, and anti-inflammatory cytokines, curtailing the potential deleterious effects of inflammation, ensures a favourable outcome. However, when this balance is distorted towards an insufficient control of the infection or towards a dominant systemic proinflammatory status designated as 'lethal cytokinaemia',[14] the condition of the patient deteriorates and shock or death may develop. There are indications that glucose levels influence the type and quantity of cytokine release.

In the present article, we hypothesise that a better control of hyperglycaemia shifts the systemic cytokine profile towards a more anti-inflammatory balance resulting in a decreased morbidity and mortality of critically ill patients. This hypothesis is based on and derived from various *in vitro* and *in vivo* studies on the effect of glucose and insulin on immune functions, cardiac performance and coagulation.

THE HYPOTHESIS

Normalisation of hyperglycaemia in acute and critically ill patients will shift the cytokine profile from a systemic proinflammatory status to a more balanced anti-inflammatory condition. By this mechanism, normoglycaemia improves and restores host defence, haemodynamics and coagulation abnormalities and decreases morbidity and mortality.

EFFECTS OF GLUCOSE AND INSULIN ON HOST DEFENCE, ROLE OF CYTOKINES

While glucose is freely permeable across the cell membrane of leucocytes in diabetic patients, these cells display a decreased rate of glycolysis[15] and an increased glucose consumption after the addition of small amounts of insulin.[16] In diabetic patients, a variety of abnormalities in neutrophil function have been observed such as decreased chemotaxis, increased adherence to endothelium, decreased phagocytosis, decreased bacterial killing and overproduction of free radicals. These defects may result from short-term metabolic effects of hyperglycaemia and (relative) insulin deficiency or from long-term effects such as increased glycation.

It has been shown *in vitro* that D-glucose and insulin, alone or in combination, improve chemotaxis of neutrophils from healthy humans.[17,18] D-glucose alone is able to increase neutrophil locomotion in a dose-dependent fashion. However, as studied *in vivo*, N-formyl-methionyl-leucyl-phenylalanine (fMLP) and complement-induced chemotaxis is significantly lower in diabetic patients than in healthy controls, probably because of the increased expression of the adhesion promoting β2-integrins CD11b and CD11c.[19] Because in addition, glucose increases the expression *in vitro* of adhesion molecules on endothelium such as E-selectin, intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1),[20] hyperglycaemia will promote the binding of leucocytes to endothelium.[21] Recently, it was demonstrated that K_{ATP} channel blockade with glibenclamide inhibited fMLP and lipopolysaccharide-induced neutrophil activation and migration *in vitro* and *in vivo*,[22] suggesting that, independent of glucose and insulin, treatment of type 2 diabetic patients with glibenclamide could be detrimental to immune function. In type 2 diabetic patients circulating levels of cytokines and adhesion molecules are increased following a meal rich in carbohydrates.[23] This effect was inhibited by pretreatment with high doses of vitamin C and E, indicating that an oxidative mechanism mediates this effect.

Phagocytosis is impaired in diabetes.[24] This impairment is at least partly mediated by elevated levels of cytosolic calcium. In a prospective cohort study of patients with type 2 diabetes, higher fasting glucose levels were correlated with higher cytosolic calcium levels in neutrophils and with decreased phagocytic activity.[25] In type 2 diabetes mellitus this impaired phagocytic activity can be restored by glibenclamide therapy, either as a result of the glibenclamide-stimulated insulin production or decreased serum glucose levels. In diabetes, ingested bacteria seem to be protected from killing by intracellular bactericidal mechanisms.[26] Under hyperglycaemic conditions, proteins may be glycated resulting in the formation of so-called advanced glycation end products (AGEs). Recent studies have shown that these AGEs can bind to the corresponding receptor on neutrophils and impair intracellular bacterial killing.[18] Delamaire *et al.* reported that, although elevated in the unstimulated state, bactericidal activity of stimulated neutrophils was significantly depressed in diabetic patients.[19]

Neutrophils from diabetic patients show increased levels of basal free radical release as measured by chemoluminescence. This increased free radical production, in combination with increased adhesion, may induce local lesions in the vascular endothelium.[19]

Although all these defects in leucocyte function have been reported in diabetic patients, the exact mechanism and the effects of short-term glucose control are still not clear. We speculate that high glucose concentrations influence host defence by the modulation of cytokine synthesis.

This mechanism is sustained by a recent report by Morohoshi *et al.* that showed a glucose-dependent increase in basal TNFα and IL-6 production in human monocytes *in vitro*.[27] Similarly, studies by our group demonstrated a glucose-dependent increased production of TNFα by peripheral blood cells *in vitro* after stimulation with lipopolysaccharide, whereas glucose does not influence the production of the anti-inflammatory cytokine IL-10.[28] We also demonstrated that *in vivo* induced hypoglycaemia in hypoglycaemic human clamp models (achieved glucose levels 5.0, 3.5, and 2.5 mmol/l) resulted in a down-modulation of LPS-induced TNFα synthesis.[29] Together, these studies strongly suggest that glucose may stimulate the production of proinflammatory cytokines such as TNFα and IL-6, with no effect on the anti-inflammatory cytokine IL-10. Therefore, normalisation of hyperglycaemia may result in a shift of cytokine production towards a more balanced anti-inflammatory profile.

In addition to the effect of glucose on the production of pro- and anti-inflammatory cytokines, several effects of insulin on cytokine production have been shown.[30] Satomi *et al.* demonstrated that insulin blocks TNFα production by peritoneal macrophages.[31] Insulin has been shown to have a potential anti-inflammatory activity, since it inhibits the expression of the proinflammatory adhesion molecule ICAM-1 by endothelial cells[32] and increases the expression of the mRNA for macrophage migration inhibitory factor.[33] Rats treated with recombinant TNFα have a significant decrease in food intake, nitrogen balance and body weight compared with saline-treated control rats.[34] Concurrent insulin administration reversed all these changes. Five days of TNFα treatment caused severe interstitial pneumonitis, periportal hepatitis and increased wet organ weight of heart, lung, kidney and spleen. Concurrent insulin treatment led to near total reversal of these pathological changes. However, as systemic insulin administration results in lower glucose concentrations, the observed effects cannot be attributed to insulin per se.

Of the several questions that can be addressed, perhaps the most intriguing is *how* glucose mediates the TNFα production. A possible explanation involves nuclear factor kappa-B (NFκB). The inducible transcription factor NFκB was discovered in 1986[35] and is present in the cytoplasm of many different cell types, such as leucocytes, endothelial and epithelial cells. Activation of this protein initiates its translocation into the nucleus where it binds to specific sequences in the promotor regions of target genes. This will result in an increased rate of transcription of the gene, more messenger RNA (mRNA) and more protein. NFκB plays a pivotal role in the regulation of the synthesis of various proinflammatory proteins, including cytokines, cell-adhesion molecules and inducible nitric oxide synthase. In sepsis, NFκB expression is upregulated and a persistently increased expression correlates with nonsurvival.[36] The production of NFκB is stimulated by lipopolysaccharide (LPS), TNFα, IL-1, and phorbol 12-myristate 13-acetate. The anti-inflammatory cytokine IL-10 inhibits NFκB formation. In type 2 diabetes it has been shown that IL-1β inhibits pancreatic β cell function and promotes Fas-triggered apoptosis in part by activating NFκB.[37] This suggests that an inflammatory process is involved in the pathogenesis of glucotoxicity in type 2 diabetes, which is mediated by NFκB.

In Gram-negative sepsis, LPS or endotoxin binds to several receptors of which CD14 is one of the most extensively studied. After binding to LPS, this receptor forms a complex with the toll-like receptor 4 (TLR4).[38] Activation of TLR4 results in the activation of the NFκB signalling pathway and subsequently to the production of cytokines such as TNFα, IL-1, IL-2, IL-6, IL-8, and IL-12, and interferon-β (IFNβ). TNFα, in a positive regulatory loop, can lead to further excessive activation of NFκB and to cytokine-mediated effects such as endothelial cell injury, disseminated intravascular coagulation, septic shock or death.

Hyperglycaemia rapidly induces activation of NFκB within hours of its onset.[39] NFκB activation induced by elevated concentrations of carbohydrates is associated with increased monocyte adhesion to endothelial cells and the generation of oxygen radicals.[40] Adherence of leucocytes to human umbilical vein endothelial cells was strongly augmented by elevation of the glucose concentration in the perfusate from 5 mmol/l to 30 mmol/l. In the same experiment it was shown that this effect was paralleled by an intense NFκB activation.[20] So, direct effects of glucose on NFκB activation may well be the initiator of the deleterious effects of increased systemic cytokine production. However, as NFκB is also involved in the stimulation of some anti-inflammatory cytokines, more subtle stimulation of intracellular pathways is likely to be involved. Oxidative stress-mediated cellular damage may be involved in hyperglycaemia-induced activation (reviewed in),[41] as was shown by treating diabetic patients with antioxidants that significantly suppressed NFκB activation. By inhibiting NFκB activation, transcription of genes under NFκB control, such as adhesion molecules and cytokines, is prevented.

Macrophage migration inhibitory factor (MIF) is a pro-inflammatory chemokine produced primarily by the anterior pituitary gland.[42] LPS-induced secretion of MIF from the pituitary gland and from circulating/tissue resident monocyte/macrophages is thought to play an important role in the pathogenesis of endotoxaemia and sepsis and might be an integral part of the host's systemic stress response. In mice that received endotoxin, there was a dramatic fall in the pituitary content of MIF, associated with a concomitant increase in plasma levels of MIF.[43,44] *In vivo* studies in rats have shown that MIF protein is released from the pituitary, adrenal gland, liver, spleen, lung, kidney and skin within six hours of LPS injection.[45] Upon release, MIF is directly proinflammatory by activating

or promoting cytokine expression (TNFα;[46,47] IL-1β, IL-2;[48] IL-6;[46,49] IL-8;[50] and interferon-γ[48,51]). From this data it is stated that 'MIF's position within the cytokine cascade is to act in concert with glucocorticoids to control the set point of the immune and inflammatory response'.[42] Similarly to TNFα, high MIF levels are closely linked with poor outcome in patients with systemic inflammatory response syndrome (SIRS).[52] Again, there is a link with glucose metabolism: the insulin-secreting β cells in the pancreas express MIF, and both MIF and insulin colocalise by immuno-cytochemistry within the secretory granules.[53] MIF is secreted in response to glucose stimulation in a time- and concentration-dependent fashion. In turn, exogenously administered MIF potentiates insulin release. MIF thus plays an important regulatory role in carbohydrate metabolism and its secretion is regulated by glucose. Through effects on MIF, strict glucose regulation could influence the pro-/anti-inflammatory cytokine balance.

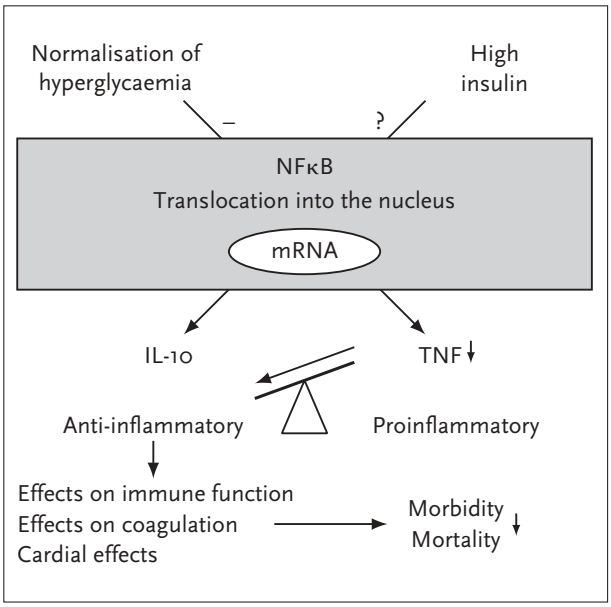

Figure 1

Representation of the hypothesis. Correction of high glucose concentration results in less NFκB activation and down-modulation of TNFα production, resulting in less spill-over to the systemic circulation. The production of the anti-inflammatory cytokine IL-10 is not influenced by glucose. This leads to a new pro- and anti-inflammatory cytokine balance with favourable effects on host defence, myocardial function, and coagulation. In the presence of little experimental evidence, the possible role of insulin remains questionable.

EFFECTS OF GLUCOSE AND INSULIN ON CARDIAC FUNCTION, ROLE OF CYTOKINES

Inflammation plays a pathogenic role in the development of acute coronary syndromes.[54,55] Increased levels of TNFα, IL-1β, IL-6, IL-8 and MIF have been observed after acute myocardial infarction[56,57] and postinfarction IL-6 levels correlate significantly with the extent of myocardial tissue damage.[58] TNFα production forms part of an important intrinsic myocardial stress response system to injury. Within the myocardium TNFα is not only confined to the infarct and peri-infarct zone, but is also expressed in cardiac myocytes of the contralateral myocardium.[59] As can be concluded from a study in mice, TNFα has, apart from its pathogenic role in the acute inflammatory response during the acute ischaemia, also a protective role in limiting the ischaemia-induced apoptosis.[60] Several studies have suggested that treatment of acute myocardial infarction with infusions of glucose, insulin and potassium is beneficial.[61-63] It remains unclear whether normalisation of hyperglycaemia or the higher insulin levels account for these beneficial effects. Insulin has been shown to attenuate infarct size in the isolated rat heart, independent of the presence of glucose,[64] and it was also demonstrated that infarct size was linearly related to blood glucose concentration during acute hyperglycaemia independent of insulin concentration in dogs.[65] The DIGAMI study was initiated to test the hypothesis that rapid improvement of metabolic control in diabetic patients with acute myocardial infarction improves short- and long-term outcome.[7,8] Patients were randomised to insulin-glucose infusion followed by multidose subcutaneous insulin therapy or conventional therapy. In the intensive therapy group mortality rate was reduced by 30% after one year. This effect is most likely the result of better local metabolic control in the myocardium or, in accordance to the presently presented hypothesis, to an altered cytokine-mediated inflammatory response.

EFFECTS OF GLUCOSE AND INSULIN ON COAGULATION, ROLE OF CYTOKINES

In critically ill patients, derangement of the coagulation system comprises enhanced activation of coagulation, depression of inhibitory mechanisms of coagulation, and inhibition of fibrinolysis. An antifibrinolytic endothelium phenotype is associated with multiorgan failure and mortality.[66] Derangement of the coagulation system appears to be mediated by various cytokines and may contribute to the pathogenesis of multiple organ failure. In human umbilical vein endothelial cells, TNFα alters the coagulant activity by stimulation of the production and surface expression of tissue factor and by inhibition

of anticoagulant mechanism, particularly the activation of the protein C system.[67,68] In healthy human volunteers, infusion of TNFα activates the coagulation system identically to what is seen in septic patients or in volunteers after endotoxin infusion,[69,70] and this activation of the coagulation seems to be, at least initially, dependent on activation of the extrinsic pathway of blood coagulation.[71] In septic patients, the fibrinolytic system becomes initially activated and subsequently inhibited.[72] Blockade of TNFα with monoclonal antibodies inhibits the fibrinolytic response upon the administration of endotoxin.[73-75] The mechanisms underlying the effects of TNFα on the fibrinolytic system are not completely clear. It is suggested that the rapid effects are not due to enhanced gene expression observed *in vitro*,[76,77] but to release of stored plasminogen activators probably from the vascular endothelium and endotoxin-activated platelets.

The level of plasminogen activator inhibitor type I (PAI), an inhibitor of fibrinolysis, is higher in diabetic patients as compared with nondiabetics, and associated with the occurrence of myocardial infarction.[78] Systemic insulin treatment decreases the plasma activity of PAI in type 2 diabetic patients.[79] Human administration of recombinant IL-6, another proinflammatory cytokine, resulted in an increased thrombin generation[80] and antibodies directed against IL-6 are able to completely abolish the endotoxin-induced activation of coagulation in chimpanzees.[75] Also, diabetes has been associated with platelet hyperactivity to agonists *in vitro*, and alterations in a number of mechanisms involved in platelet activation occur in diabetic platelets. These alterations include increased presence of glycoprotein receptors for agonists and adhesive proteins on the platelet surface, increased fibrinogen binding, decreased membrane fluidity, enhanced arachidonate pathway activation with increased thromboxane A2 formation, and increased phosphoinositide turnover leading to increased inositol triphosphate production, calcium mobilisation, and protein phosphorylation.[81] Tight glycaemic control with insulin therapy reduces the excretion levels of a thromboxane metabolite by approximately 50%.[82] Again, the mechanism responsible for this effect is unclear.[83] In accordance with our hypothesis, the effect of systemic insulin treatment could by explained by better glucose control, subsequent inhibition of proinflammatory cytokines, resulting in a reduced cytokine-induced procoagulatory state.

Recently, it was shown that suppletion of the anticoagulatory protein recombinant activated protein C (aPC) reduces the mortality in critically ill patients.[84] Apart from the insulin study by Van de Berghe *et al*.[4] this is the only immune-modulatory strategy so far that has proven to be efficacious. The effects of aPC, however, appear to involve more than its antithrombotic activity. Similar to one of the effects of strict glucose control, aPC influences translocation of NFκB and cytokine production in an anti-inflammatory way.[85] Normalisation of hyperglycaemia may therefore be beneficial either directly or by a restoration of the cytokine balance, through a positive effect on the immune function, through the improvement of haemodynamics or through a decrease of the undesirable procoagulatory state.

Table 1
Summary of the effects of hyperglycaemia (compared with normoglycaemia)

EFFECTS ON HOST DEFENCE	
NfκB induction	↑
Production of proinflammatory cytokines, MIF	↑
Production of anti-inflammatory cytokines	=
Expression of adhesion molecules	↑
Binding of leucocytes to endothelium	↑
Phagocytic activation	↓
Intracellular bacterial killing	↓
EFFECTS ON CARDIAC FUNCTION	
Infarct size	↑
Postinfarction mortality	↑
EFFECTS ON COAGULATION	
PA-1 concentration	↑
Thromboxane concentration	↑
Platelet activity	↑

CONCLUSION AND FUTURE DIRECTIONS

Strict control of plasma glucose in acute and critically ill diabetic and nondiabetic patients has been shown to improve outcome in several clinical settings. The mechanism of action of these beneficial effects is not known, although in critically ill patients, the control of glucose levels rather than the absolute levels of insulin appear to account for the positive effects of strict regulation.[86] From the literature and our own studies it can be concluded that hyperglycaemia augments the production of proinflammatory cytokines. There is a striking similarity between the effects of glucose on host defence, cardiac performance and coagulation and the proinflammatory cytokine status associated with critical illness. Although in acute or critically ill patients various mutually dependent overlapping and redundant pathophysiological mechanisms are known to influence the clinical course in a complex fashion, we claim that the modulation of cytokines explains the beneficial effect of a more strict glycaemic control. Cognisant the

beneficial local autocrine and paracrine action and the potentially deleterious systemic endocrine effect of pro-inflammatory cytokines, we suggest that future studies to test this hypothesis encompass local and systemic cytokine production. Further understanding of the mechanism by which intensive therapy with insulin reduces morbidity and mortality in acute and critically ill patients will probably take place at the crossroads of metabolic control, immune response, cardiac performance and coagulation.

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