studies suggest that hyperglycemia per se is toxic and that insulin has anti-inflammatory and cardioprotective actions. If this is true, it has wider clinical implications. Hyperglycemia is harmful: but why and how?

Persistent hyperglycemia increases the risk of myocardial infarction, peripheral vascular disease, stroke, renal damage, vision loss, cataract, etc. This is especially evident in patients with both type 1 and type 2 diabetes mellitus. Studies showed that when these patients are appropriately and adequately treated with diet restriction, exercise, oral hypoglycemic agents and/or insulin are protected from these complications. Both UKPDS (UK Prospective Diabetes Study done in type 2 diabetes mellitus) and DCCT (The Diabetes Control and Complications Trial of USA done in type 1 diabetes mellitus) studies clearly demonstrated that rigorous control of blood glucose to near normal levels decreases the progression of diabetic microvascular disease and improved the quality of life. This beneficial effect has been attributed to a reduction in the plasma glucose. If so, this suggests that hyperglycemia is harmful. But it does not explain why hyperglycemia should be harmful. A better understanding of the molecular mechanism(s) of harmful actions of hyperglycemia is expected to lead to development of newer methods of treatment not only to control hyperglycemia but also to prevent complications associated with raised blood glucose levels.

Studies performed in experimental animals and patients with diabetes mellitus revealed increased production of free radicals (especially superoxide anion, O₂⁻) and formation of excess of lipid peroxides especially when plasma glucose levels are high. Hyperglycemia is known to increase the production of O₂⁻ and other reactive oxygen species (ROS) inside cultured aortic endothelial cells. The importance of increased concentrations of ROS lies in the fact that they
have the ability to inactivate prostacyclin (PGI2) and nitric oxide (NO), which are secreted by endothelial cells and are potent vasodilators and platelet anti-aggregators. In this context, it is important to note that even in otherwise normal subjects glucose challenge (75 gm glucose in 300 ml water orally during fasting) produced a significant increase in leukocyte free radical generation above the basal level at the end of 2 hours. This increase in ROS was associated with a simultaneous increase in plasma lipid peroxides and fall in the plasma α-tocopherol. The increase in ROS was found to be due to activation of NADPH oxidase following glucose challenge. The fall in the concentrations of α-tocopherol can be attributed to its increased consumption as a result of enhanced release of ROS. These data suggest that glucose enhances ROS generation not only in diabetes but also in otherwise normal subjects. High plasma glucose concentrations have also been reported to increase leukocyte rolling, leukocyte adherence and leukocyte transmigration through mesenteric venules, events that suggest local low-grade inflammation. This increase in adherence of leukocytes to endothelial cells and other tissues is expected to lead to damage of exposed cells and tissues since leukocytes are the principal source of ROS. Any increase in the contact between leukocytes and endothelial cells and other tissues will lead to the exposure of latter to ROS released by leukocytes and so increase in cell and tissue damage. Increased rolling, adherence and transmigration of leukocytes in the presence of hyperglycemia is due to decreased release of endothelial NO (eNO) and increased expression of P-selectin on endothelial surfaces. On the contrary, local application of insulin completely attenuated these pro-inflammatory events, inhibited free radical generation, and NF-κB activation in mononuclear cells and reduced soluble intercellular adhesion molecule-1, monocytes chemoattractant protein-1 and plasminogen activator-1 production by enhancing NO synthesis.

This suggests that insulin suppresses ROS generation, enhances eNO formation and thus, has anti-inflammatory actions.

Hyperglycemia, cytokines, endothelial nitric oxide, and eicosanoids

If hyperglycemia has pro-inflammatory actions, is it possible that glucose modulates the production of pro-inflammatory cytokines? In a recent study, Esposito et al reported that in normal subjects when plasma glucose levels were acutely raised (~15 mmol/L) and endogenous insulin secretion was blocked, an increase in plasma interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and IL-18 levels occurred within 2 hours. It was reported that in subjects with impaired glucose tolerance (IGT) the fasting plasma IL-6 and TNF-α levels were higher compared to normal subjects. It is interesting to note that the increase in plasma pro-inflammatory cytokine levels in IGT occurred early and was sustained for a longer time compared to normal individuals. These increases in plasma pro-inflammatory cytokine concentrations could be completely abrogated by simultaneous infusion of glutathione, a known anti-oxidant, along with glucose pulses suggesting that hyperglycemia acutely increases pro-inflammatory cytokine concentrations by an oxidative mechanism and that this effect is more pronounced in subjects with IGT.

Glucose increases monocytes adhesion to human aortic endothelial cells (HAECs) in vitro, and also triggered a 2-fold elevation in IL-8 secretion over control cells. Both glucose and IL-8 activated β2 integrin on the HAEC surface, which is necessary for the adhesion of monocytes to endothelial cells. Neutralizing antibody to IL-8 prevented glucose-mediated monocytes adhesion. Human IL-8 promoter possessed binding sites for NF-κB and AP-1, glucose stimulated IL-8 promoter activity, and inhibition of ROS production reduced glucose-mediated induction of IL-8 expression. It is interesting that IL-8 is a pro-inflammatory cytokine, activates polymorphonuclear leukocytes (PMNs) and augments chemotaxis, superoxide anion production, and enzyme release of PMN, and is also chemotactic to lymphocytes. But, it is not yet certain the exact sequence of events following the onset of hyperglycemia: is it the ROS generation, pro-inflammatory cytokine production or whether both these events occur simultaneously. A better understanding of the sequence of events will help to devise methods to block the initial event so that subsequent downstream events do not occur. Anti-oxidants and NO blunt the pro-inflammatory actions of hyperglycemia. This suggests that ensuring adequate cell or tissue anti-oxidant capacity or status plays an important role in prevention of the toxic actions of glucose. Several recent studies showed that external supplementation of anti-oxidant vitamin E is not of significant benefit in the prevention of cardiovascular disease. This indicates that methods should be designed to enhance the anti-oxidant capacity of cells/tissues endogenously rather than their supplementation from external sources. Moderate physical exercise enhances tissue/cell anti-oxidant capacity. Thus, exercise is anti-inflammatory in nature. This explains why regular exercise is such an effective method in the management of obesity, atherosclerosis, diabetes mellitus, cardiovascular disease and hypertension in which there is now evidence for the presence of low-grade systemic inflammation.

Further support to the role of hyperglycemia in inflammation is derived from the observation that dietary glycemic load enhanced plasma high-sensitivity C-reactive protein (hs-CRP) levels in healthy middle-aged women, independent of conventional risk factors for ischemic heart disease (IHD). Hyperglycemia per se increased serum amyloid A3 (SAA3), a marker of inflammation, in the adipose tissue. Both glucose and TNF-α stimulated NADPH oxidase activity, activated NF-κB, and increased intercellular adhesion molecule-1 (ICAM-1) expression in endothelial cells and enhanced free radical generation, which are pro-inflammatory events. There is a significant elevation in the activity of NADPH oxidase in patients with type 2 diabetes mellitus. In addition, patients with diabetes mellitus showed dysfunctional endothelial NO synthase activity, which forms an additional source of superoxide production. Dysfunctional NO synthase activity leads to decrease in NO generation and this in turn causes increase in superoxide anion levels since under physiological conditions NO quenches superoxide anion. Hence, in the absence of NO superoxide anion levels remain elevated. This decreased NO production in diabetes can be corrected by intracellular tetrahydrobiopterin (BH4), a cofactor that is necessary for NO production, whereas increased superoxide anion production was abrogated by PKC inhibition. This suggests that glucose and TNF-α enhance NADPH oxidase, PKC,
NF-κB, and ICAM-1 levels and thus initiate and sustain inflammation. Thus hyperglycemia induces increase in ROS, decreases in eNO production, augments IL-6, TNF-α, IL-8, and IL-18 generation and depletes intracellular anti-oxidant content that eventually causes endothelial damage and dysfunction. This ultimately leads to target organ damage seen in diabetes mellitus.

Cyclo-oxygenase-2 (COX-2) activity is up regulated by hyperglycemia in endothelial cells such that thromboxane A₂ (TXA₂) production is increased whereas that of PGI₂ is suppressed. Glucose-induced activation of protein kinase C (PKC) resulted in the formation of peroxynitrite and tyrosine nitration of PGI₂ synthase enzyme that leads to a decrease in the release of NO despite a 2-fold increase in eNO synthase and reduced PGI₂ formation respectively, events that can be prevented by anti-oxidants N-acetylcysteine and vitamin C. Both N-acetylcysteine and vitamin C prevented ROS generation and restored NO release to normalcy by reducing the co-localization of nitrotyrosine and PGI₂ synthase. Hyperglycemia-induced production of IL-8 stimulates 5-lipoxygenase leading to an increase in the formation of leukotriene B₄ (LTB₄) and also activation of 15-lipoxygenase to enhance the production of 15-hydroxyicosatetraenoic acid (15-HETE). LTB₄ is a pro-inflammatory molecule whereas 15-HETE is anti-inflammatory in nature. 15-HETE inhibits LTB₄ formation and LTB₄-induced chemotaxis of neutrophils. Since, IL-8 stimulates both LTB₄ and 15-HETE formation, the effect of IL-8 on inflammation depends on the relative stimulation of 5- and 15-lipoxygenases and other co-factors that have either suppressive or stimulatory actions on these enzymes. This suggests that the balance between 5- and 15-lipoxygenases in the tissues determine the degree of inflammation, initiation and/or persistence of inflammation and/or its resolution.

Myocardial suppressive action of hyperglycemia

A positive association exists between hyperglycemia and mortality from acute myocardial infarction (AMI) and even non-diabetic patients with AMI have raised blood glucose concentrations. This indicates that even marginal increases in plasma glucose increases the risk of development of complications due to AMI. These harmful actions of hyperglycemia on the myocardium can be attributed to the pro-inflammatory actions of glucose. The very presence of hyperglycemia (hyperglycemia is defined as plasma glucose > 110 mg%) is an indication that insufficient insulin (either qualitative or quantitative) is present in the circulation or more precisely there is peripheral insulin resistance, which is due to increased production of stress hormones such as corticosteroids, adrenaline and noradrenaline, and enhanced production of pro-inflammatory cytokines such as IL-6, IL-8, IL-18, TNF-α, and/or decreased production of anti-inflammatory molecules IL-4, IL-10, and transforming growth factor-β (TGF-β). Further, insufficient insulin causes a decrease in glycolytic substrate and an increase in FFAs (free fatty acids) that reduces myocardial contractility and promotes cardiac failure and arrhythmias leading to poor outcome in such patients. The source of cytokines could be from the myocardium itself and/or from leukocytes, lymphocytes, monocytes and macrophages. Ischemia stimulates the production of pro-inflammatory cytokines from the myocardium. Increased mesenteric venous pressure due myocardial dysfunction causes increased endotoxin absorption from the gut. Endotoxin is a potent stimulant of synthesis and release of pro-inflammatory cytokines from various cells/tissues especially macrophages. TNF-α and other pro-inflammatory cytokines depress myocardial function. This is further illustrated from the observation that in patients with congestive cardiac failure, CD14 concentrations (which are indicative of endotoxin-cell interaction) are elevated corresponding to the elevated levels of TNF-α and the degree of cardiac cachexia. Thus, ischemic myocardium produces TNF-α that in turn suppresses myocardial contractility, which results in increased mesenteric venous pressure and absorption of endotoxin from the gut. The absorbed endotoxin stimulates the production of TNF-α that induces further myocardial depression and exacerbates cardiac failure. Thus, a vicious cycle is initiated that may prove fatal.

Intensive treatment with insulin to lower plasma glucose concentrations and maintain it <110 mg% (< 6.1 mmol/L) has been reported to decrease overall mortality in patients admitted to surgical intensive care units and receiving mechanical ventilation. This suggests that plasma glucose > 110 mg% is harmful, whereas insulin therapy is beneficial in critically ill with or without diabetes mellitus. Earlier, I suggested that insulin is an anti-inflammatory molecule and that when GIK regimen is given (30% glucose, 50 IU of insulin, and 80 mmol/L potassium at the rate of >1.5 mL/kg per hour, which was similar to the dose of insulin used by Van der Berghe et al) it suppresses circulating concentrations of FFAs and production of pro-inflammatory cytokines TNF-α, IL-6 and ROS and enhances the production of IL-4, IL-10, eNO and PGI₂, and thus is of benefit in AMI, critically ill, and in sepsis and septic shock.

Insulin suppresses TNF-α, MIF, and ROS, and enhances IL-4, IL-10, and eNO generation

TNF-α is secreted by a variety of cells/tissues including adipose tissue, macrophages and cardiac tissue. TNF-α plays a major role in insulin resistance, type 2 diabetes mellitus, hypertension, inflammation, and septic shock. TNF-α is released by myocardium in AMI. TNF-α directly decreases myocardial contractility in a dose-dependent manner, whereas following cardiac transplantation TNF-α levels decrease. Thus, there is a direct correlation between myocardial dysfunction and circulating TNF-α levels. Anti-TNF-α antibody reduces myocardial injury and dysfunction, whereas cardiac cachexia is due to an increase in the plasma levels of TNF-α. Furthermore, a direct correlation is known to exist between the circulating levels of TNF-α and the severity of congestive cardiac failure. TNF-α causes dysfunction and apoptosis of endothelial cells, decreases the production of eNO and enhances procoagulant activity and fibrin deposition. TNF-α stimulates endothelial cells and leukocytes to produce increased amounts of ROS that in turn inactivate eNO. Thus, TNF-α reduces the plasma levels of beneficial eNO. These actions of TNF-α ultimately are responsible for the various clinical features seen in AMI, and patients with sepsis and septic shock. But, paradoxically infusion of monoclonal anti-TNF-α antibody failed to improve prognosis and survival of patients with AMI and sepsis and septic shock. These negative results have been attributed to the fact that in these conditions there is an important role for several other mediators such as macrophage migration inhibitory factor (MIF), several...
other cytokines, TGF-β, ROS, eNO, adenosine, etc., which also should be either neutralized or enhanced to improve the patient's condition. If intensive insulin and/or GIK regimen is beneficial in patients who are critically ill, is it possible that insulin has a regulatory role in the production of pro- and anti-inflammatory cytokines, ROS, and NO?

**Exogenous administration of insulin blocked TNF-α by peritoneal exudate cells in a dose related manner from Propionibacterium acnes** primed mice compared to control. Reduced food intake, decreased body weight gain, severe interstitial pneumonitis, periportal inflammation in the liver and increases in the weights of the heart, lungs, kidney and spleen observed in TNF-α-treated experimental animals was completely prevented by concurrent administration of insulin. Insulin enhances eNO generation by activating Akt through the PI3-kinase (phosphatidylinositol 3'-kinase) pathway and suppresses superoxide anion generation. It was demonstrated that insulin therapy decreased serum IL-1β, IL-6, MIF and TNF-α concentrations after thermal injury and increased anti-inflammatory cytokines IL-4 and IL-10 as originally predicted by me. Insulin reduced pro-inflammatory signal transcription factors STAT-3 and C/EBP-β mRNA and increased anti-inflammatory signal transcription factor mRNAs expression of SOCS-3 and RANTES-7. These studies clearly showed that insulin is an anti-inflammatory molecule that explains the beneficial actions of GIK regimen in AMI and the critically ill, and in patients with sepsis and septic shock.

**Insulin and cardiac function**

Cardiovascular dysfunction is common in the critically ill. On the other hand, GIK regimen is known to improve myocardial function. Originally, it was thought that maintaining near-normal blood glucose is important to preserve myocardial function in the critically ill. But, studies showed that maintaining blood glucose at control levels (~140-200 mg %) by infusion of 50% glucose did not prevent myocardial dysfunction, whereas infusion of insulin at rates of 6 units/minute reversed all signs of myocardial failure and maintained normal performance. Despite wide ranges in glucose concentrations (5-120 mg %), this clearly suggested that it is insulin that improves cardiac performance but not glucose in the critically ill. In this context, it is noteworthy that during critical illness, infections, inflammation, sepsis and septic shock insulin resistance is common, which may render the cardioprotective actions of insulin ineffective.

This led me to suggest that continuous infusion of glucose and insulin might overcome insulin resistance and improve cardiac function and render critically ill patients to recover faster. This is somewhat similar to the use of insulin for DKA. “Low-dose” insulin schedules in which 8 to 10 units of insulin are infused intravenously each hour is the mainstay of treatment of DKA. Most patients with DKA respond to this regimen, though a minority of patients is “resistant” to this regimen. These non-responsive patients are given 25 to 50 units of insulin as an intravenous bolus, followed by an infusion of 15 to 25 units an hour until ketosis is reversed. This higher-dosage insulin schedule is expected to ensure saturation of the insulin receptors in the face of competing antibodies or other resistance factors. High amounts of insulin act via the insulin-like growth factor (IGF) receptor and reverses DKA. In a similar fashion, even in the critically ill continuous administration of the GIK could enhance tissue perfusion and glucose uptake, suppress lactate, FFA, glycerol production, and lipolysis and improve survival. GIK regimen also suppresses excess production of IL-1, IL-6, TNF-α and MIF, enhances the synthesis of eNO and anti-inflammatory cytokines IL-4 and IL-10. This facilitates improvement in myocardial function and recovery, which explains why GIK regimen is useful in AMI.

Studies showed that insulin but not glucose or potassium increases Akt and eNOS phosphorylation and enhanced NO production in ischemia/reperfusion myocardium. NO reduced myocardial apoptotic death and inhibited caspase-dependent Bcl-2 cleavage by nitrosating caspase-3, -6, -7, and -8 and blocked the release of mitochondrial cytochrome c. NO downregulated MKP-3 mRNA levels and thus, prevented the inactivation of ERK1/2, an anti-apoptotic member of the MAPK family which led to a reduction in apoptotic cell death of myocardial tissue. Several clinical studies showed that GIK regimen preserves myocardial function, though a minority of patients is “resistant” to this regimen. This may explain why insulin improves cardiac function, an effect that is independent of plasma glucose levels.

**HLA-DR expression, cytokines, ROS and the critically ill**

It is evident from the preceding discussion that insulin brings about its anti-inflammatory actions by decreasing the concentrations of intranuclear NF-κB and the generation of ROS, and increasing the levels of its inhibitor IκBα, and eNO. In addition, insulin administration produced a significant decrease in the concentrations of plasma soluble ICAM-1, monocyte chemoattractant protein-1, and plasmagen activator inhibitor-1 (PAI-1). Contrary to this, hyperglycemia not only has opposite actions on all these parameters compared to insulin but also increased the expression of P-selectin on endothelial surfaces which causes increased leukocyte rolling, leukocyte adherence, and leukocyte transmigration. These diametrically opposite actions of insulin and glucose explains their beneficial and harmful actions respectively especially in the critically ill.

In this context, it is interesting to note the relationship between human leukocyte antigen (HLA)-DR and CD11b expression, ROS, cytokines and development and recovery from postoperative or post-trauma-induced sepsis and other critical illnesses. In patients with an uneventful recovery from severe trauma or major surgery, the level of monocytes HLA-DR expression fell within hours of trauma or surgery, but returned to normal within a week. In those who developed infection but recovered, 3 weeks were required for HLA-DR expression to return to normal. Those who developed infection and sepsis and who died as a result, HLA-DR expression fell and never returned to normal. Similarly, after uncomplicated elective major abdominal surgery, expression of CD11b/CD18 (which is necessary for adhesion of neutrophils to endothelium) was unchanged throughout the postoperative period; in patients who developed post-operative sepsis, the expression of CD11b was significantly elevated within 24 hours of surgery. Even the production of hydrogen peroxide by neutrophils followed a pattern similar to that of CD11b expression in these two groups of patients. The production of hypochlorous acid, a marker of neutrophil activation, was reported to be decreased in patients who had uncomplicated abdominal surgery as compared to those who developed sepsis 7-10 days later, in whom its production was augmented to supranormal levels on postoperative day 1. It is noteworthy
that these changes in HLA-DR and CD11b expression, hydrogen peroxide and hypochlorous acid production were noted even when there was no evidence of infection. This suggests that changes in these parameters are an indication of the alterations in the immune system in response to surgery and trauma and are useful to predict the prognosis in these patients.

Severe thermal injury caused a significant decrease in monocyte HLA-DR expression compared with healthy volunteers, and patients who developed sepsis showed much lower expression than non-septic patients. Plasma concentrations of IL-10 increased after thermal injury and were negatively correlated with HLA-DR expression on circulating B cells. HLA-DR expression was significantly reduced from days 6-14 after admission (day 1) in those who developed sepsis compared to that did not develop sepsis. In septic patients NK cell counts were significantly decreased from day 4 onwards. Analysis of surface expression of HLA-DR, CD11b, ICAM-1, CD66b, CD63, and CD64 on neutrophils and monocytes revealed increased expression of all markers except HLA-DR in patients with sepsis compared with age-matched healthy controls. Expression of CD11b and HLA-DR on neutrophils and ICAM-1 on monocytes was lower in patients who died compared to those who survived. In a prospective longitudinal clinical study, it was reported that in adult patients with blunt trauma serum IL-10 levels were elevated whereas monocyte HLA-DR expression was significantly lower compared to normal healthy controls. In patients who developed sepsis, serum IL-10 levels were greater on admission, and remained elevated during the study period compared with uncomplicated patients. These findings led to the conclusion that IL-10 has a negative regulatory control on HLA-DR expression and that excess release of IL-10 may have a role in the development of sepsis. Several other studies also reported the relative importance of HLA-DR expression in predicting the development of sepsis and its prognosis. For instance, Tschaikowsky et al noted a significant reduction in HLA-DR expression in both survivors and non-survivors at the onset of severe sepsis. However, percentages of HLA-DR+ lymphocytes were significantly increased during sepsis, especially in non-survivors. On the contrary, survivors of sepsis showed a continuous recovery of monocytic HLA-DR expression to \( > 70\% \) within 10 days, while non-survivors showed a second decrease in monocytic HLA-DR expression after day 7 or a permanent suppression. An inverse correlation was noted between peak of systemic inflammatory reaction, as documented by maximum serum concentrations of procalcitonin and C-reactive protein (CRP), and the expression of HLA-DR expression-peak of systemic inflammatory reaction coincided with the nadir of monocytic HLA-DR expression. Moreover, procalcitonin and CRP as well as scores on the Acute Physiology and Chronic Health Evaluation II (APACHE-II) and Sepsis Organ Failure Assessment (SOFA) were inversely correlated with the monocyte HLA-DR expression. These results suggest that severe the sepsis the higher the degree of decrease in monocytic HLA-DR expression. Satoh et al reported that in acute pancreatitis the low percentage of HLA-DR expressing cells in the monocytic population is a reliable indicator of the development of sepsis. But not all studies are supportive of these conclusions. Oczenski et al observed that in patients undergoing cardiac surgery the monitoring of pre- and immediate postoperative HLA-DR levels during the first 24 hours did not help to predict increased risk for postoperative sepsis or infectious complications. Similarly, Perry et al reported that low monocyte HLA-DR expression was not associated with a high mortality in patients with sepsis. They noted that high APACHE-II scores also did not correlate with low HLA-DR expression. It is interesting to note that Fumeaux and Pugin observed that the level of monocyte HLA-DR expression inversely correlated with the degree of severity of sepsis but found no significant decrease in the rate of transcription of HLA-DR between patients with sepsis and healthy controls. Further studies revealed that HLA-DR molecules are re-endocytosed and retained intracellularly in monocytes from patients with sepsis and that this phenomenon is partially mediated by IL-10. Contrary to this, rhGM-CSF (recombinant human granulocyte macrophage colony stimulating factor) upregulated HLA-DR expression on monocytes in patients with sepsis without any significant side-effects. These results suggested that HLA-DR expression in a given patient may depend on the circulating concentrations of IL-10 and GM-CSF. Thus, absence of significant relation between HLA-DR expression and the development of sepsis reported in some studies can be related to not so significant alterations in the plasma concentrations of IL-10 and GM-CSF in these patients. Furthermore, Hyninnen et al observed that in patients with sepsis, survivors and non-survivors differed significantly in HLA-DR expression at admission: survivor’s median 84% versus non-survivor’s median 62%. Similarly, statistically significant differences between survivors and non-survivors in admission plasma IL-10 levels and in admission SOFA and APACHE-II scores but not in IL-4 levels were noted. Monocytic HLA-DR expression and plasma IL-4 levels showed poor discriminative power in prediction of hospital mortality. The highest areas under receiver operative curves were those of APACHE-II and admission SOFA compared to HLA-DR expression and IL-10 and IL-4 levels in prediction of hospital mortality in critically ill patients with sepsis. On the other hand, Spittler et al observed that monocytes from patients with sepsis with high IL-6 plasma concentrations had higher HLA-DR, HLA-ABC, CD64, and CD71, and increased production of TNF-\( \alpha \) and IL-8, as well as increased monocyte phagocytic activity compared with septic patients with low plasma levels of IL-6. Follow up of these two groups of patients revealed that 4 of 8 patients with high levels of plasma IL-6 died while all 10 patients with low plasma IL-6 concentrations survived. It was observed that patients who died had constant high IL-6 concentrations during the first 3 days, whereas IL-6 levels in patients who survived decreased by 88% indicating that IL-6 levels may severe as a prognostic parameter in predicting the survival of patients with sepsis compared with HLA-DR expression.

These results underscore the importance of various pro- and anti-inflammatory cytokines, HLA-DR expression, ROS, and CD11b levels in patients with sepsis. In view of the close interaction between cytokines, HLA-DR expression, and ROS, one need to exercise caution while interpreting results of these parameters in sepsis. It is likely that not all patients of sepsis are similar and comparable to each other. Hence, results obtained in different studies are not comparable. Further, it is possible that patients recruited and studied in different studies are probably not in the same phase of the septic process, which makes comparisons between these studies difficult. This indicates that there are some very clear
biologic variations in the response of different individuals to injury, surgery, infection, or sepsis that determines their ability or inability to recover from the onslaught of the initial event. The variations in neutrophil activation, HLA-DR expression, plasma concentrations of IL-4, IL-6, IL-8, IL-10, TNF-α, and GM-CSF and insulin resistance observed could account for some of this biologic variation. Because insulin and glucose influence neutrophil function, ROS generation, cytokine production and their action, and NO production and action, it is anticipated that in the presence of insulin resistance the production of these biological molecules will be variable that ultimately determines recovery from sepsis. It is predicted that intensive treatment with insulin infusion normalizes the production of various cytokines, ROS, NO, and HLA-DR expression and thus, facilitates recovery.

Conclusions

It is clear that insulin has myocardial protective action, which can be attributed in part, to its ability to control hyperglycemia. Insulin has anti-inflammatory actions17, 23, 93 and prevents apoptosis of myocardial tissue that facilitates myocardial recovery from ischemic insults. Many physicians are familiar with the use of insulin and so it is not difficult to extend insulin therapy to sepsis, septic shock and other critically ill patients to derive its benefits.93 It is possible that structural analogues of insulin can be developed that have selective myocardial protective action without hypoglycemic effect.

It is important to realize that apart from insulin there are several other endogenous molecules that possess myocardial protective actions. Adenosine has been demonstrated to have protective action against ischemia-induced myocardial damage. Adenosine is released during ischemia and is known to reduce infarct size.74 This myocardial protective action of adenosine is exerted by adenosine A1 and A3 receptors that selectively couple to phospholipases C and D, respectively. A1 receptor signals act via the low molecular weight GTPase RhoA to activate phospholipase D to bring about its myocardial protective action.14 Transgenic mice with cardiac-specific overexpression of adenosine A1 (both A1 and A3 receptors show myocardial protective action) showed metabolic and functional tolerance to myocardial ischemia. Microarray analysis revealed that myocardial protection exerted by A1 overexpression is associated with increased expressions of NADH dehydrogenase, GLUT4 glucose transporter, Na-K-ATPase, and BCL-x1, and decreased expression of caspase-8.95 This suggests that adenosine influences ROS generation and prevents apoptosis of myocardial cells.

High-density lipoproteins (HDLs) reduced ischemia-induced cardiac TNF-α expression and content, and improved functional recovery of the myocardium. HDLs enhanced ischemia-induced prostaglandin release that contributed to their myocardial protective action.96 This explains why low plasma HDLs are a risk factor for cardiovascular disease and is associated with excessive ischemia-reperfusion damage. In view of this negative relationship between low HDLs and increased expression of TNF-α, I suggest that subjects who have low plasma HDL concentrations are likely to have high plasma and tissue TNF-α levels. In this context, it is interesting to note that moderate physical exercise increases plasma HDL and reduces plasma and tissue TNF-α concentrations.97, 98 Similarly weight reduction either by diet control, exercise or both also resulted in reduction in plasma TNF-α levels.99 Based on these results, it is suggested that plasma TNF-α levels can be used as a marker to predict future development of cardiovascular disease, and adequacy of diet control and exercise in obese subjects.

Insulin stimulates the activity of the enzymes Δ6 and Δ5 desaturases. These enzymes are essential in the formation of long-chain polyunsaturated fatty acids gamma-linolenic acid (GLA) and dihomo-gamma-linolenic acid (DGLA) from dietary linoleic acid (LA), and of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from alpha-linolenic acid (ALA).100, 101 DGLA and EPA are precursors of prostaglandin E1 (PGE1) and prostaglandin I3 (PGI3), respectively. PGE1 and PGI3 are potent platelet anti-aggregators and vasodilators. Furthermore, GLA, EPA, DHA, and PGE, suppress the synthesis of TNF-α and IL-2 by human T cells.102, 103 This could be yet another potential mechanism by which insulin functions as an endogenous anti-inflammatory molecule. Furthermore, diets that are rich in EPA and DHA, and continuous tube feeding or intravenous infusion of these fatty acids improved survival of experimental animals challenged with endotoxin.104, 105 Previously my colleagues and I showed that patients with sepsis have low concentrations of GLA, DGLA, arachidonic acid, ALA, and EPA in their plasma.106 A deficiency of these fatty acids may lead to enhanced production of TNF-α and other pro-inflammatory cytokines due to the absence of their negative feed back control.

What are the clinical implications of the knowledge gained so far? It is evident that sepsis is a complex process. Based on the present knowledge, it is possible to predict, prevent and prognosticate the outcome in a given subject with sepsis. Serial measurements of plasma pro- and anti-inflammatory cytokines, ROS, eNO, HLA-DR expression and CD11b/CD18 will be useful to predict prognosis in sepsis. For instance, in patients with an uneventful recovery from severe trauma, surgery or infection, the level of monocyte HLA-DR expression falls; plasma concentrations of ROS, TNF-α, IL-6, IL-8, IL-1, and IL-2 and ICAM-1 increase; that of IL-4, and IL-10, GLA, DGLA, AA, EPA, and DHA decrease; insulin resistance sets in, and the expression of CD11b/CD18 remain unchanged within hours of trauma, surgery or infection, but return to normal within a week. In those who developed serious infection or become critically ill but recovered, 3 weeks are required for these abnormalities to return to normal. Finally, in those who develop sepsis and septic shock and who died as a result, these molecular abnormalities never return to normal. If this prediction is true, it may be worthwhile to measure some of these parameters in critically ill so that they may aid in implementing appropriate measures to improve their survival. This also implies that administration of adequate amounts of L-arginine, the precursor of NO, ω-3 fatty acids, HDL, adenosine, and intensive insulin infusion to correct some of these molecular abnormalities in the critically ill facilitates their recovery.

Recently, it was shown that drotrecogin alfa (activated), or recombinant human activated protein C (Xigris, Eli Lilly) is an effective adjuvant therapy for severe sepsis. Activated protein C reduced mortality in the Recombinant Human Activated Protein C Worldwide Evaluation in Severe Sepsis (PROWESS) trial.107-112 Use of activated protein C in severe
sepsis enhanced the risk of serious hemorrhage during the infusio

Human protein C is a plasma serine protease that plays a significant role in maintaining normal homeostasis. The thrombin-activated form of protein C acts as a feedback inhibitor of the coagulation cascade and shows antithrombogenic activity. The recombinant human protein C targets the effects of microvascular coagulation. It was shown that recombinant activated human protein C suppressed antithrombotic activity. The recombinant human protein C acts as a significant role in maintaining normal homeostasis.

In addition, it also modulated several genes in the endothelial apoptotic pathway, including Bcl-2 resulting in inhibition of apoptosis of endothelial cells, and enhanced the expression of endothelial nitric oxide (eNO).

Thus, activated protein C not only inhibits microvascular coagulation but also modulates TNF-α (and possibly MIF)-induced endothelial dysfunction, shows anti-inflammatory action and prevents endothelial cell death and thus, brings about its beneficial actions. Hence, it will be interesting to study whether combining administration of activated protein C with adenosine, L-arginine, ω-3 fatty acids, HDL, and intensive insulin infusion is beneficial to those who are critically ill. Such studies may prove interesting.

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