Insulin: an endogenous cardioprotector

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This review discusses the myocardial protective property of the insulin/glucose-insulin-potassium regimen and the mechanisms involved in this beneficial action. Several recent studies suggest that insulin not only is useful to control hyperglycemia and maintain glucose homeostasis but also may have the unique property to protect the myocardium from reperfusion injury and ischemia and prevent apoptosis of myocardial cells. The insulin/glucose-insulin-potassium (GIK) regimen suppresses the production of tumor necrosis factor-α, interleukin-6, macrophage migration inhibitory factor and other pro-inflammatory cytokines, and free radicals; and enhances the synthesis of endothelial nitric oxide and anti-inflammatory cytokines interleukin-4 and interleukin-10. Thus, the insulin/GIK regimen brings about its cardioprotective action. This may also explain why the insulin/GIK regimen is useful in sepsis and septic shock, myocardial recovery in acute myocardial infarction, and critical illness. It is suggested that the infusion of adequate amounts of insulin to patients with acute myocardial infarction, congestive heart failure, cardiogenic shock, and critical illness preserves myocardial integrity and function and ensures rapid recovery. In view of the suppressive action of insulin on the synthesis of proinflammatory cytokines and free radicals, it is possible that the insulin/GIK regimen, when used in a timely and appropriate fashion, may also protect other tissues and organs and facilitate in the recovery of patients who are critically ill. Curr Opin Crit Care 2003, 9:375–383

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Abbreviations

AMI	acute myocardial infarction
CCF	congestive cardiac failure
DKA	diabetic ketoacidosis
eNO	endothelial nitric oxide
FFA	free fatty acids
HETE	hydroxyeicosatetraenoic acid
GIK	glucose-insulin-potassium
leukotriene B_4	LTB_4
MIF	migration inhibitory factor
NO	nitric oxide
PGI_2	prostacyclin
PKC	protein kinase C

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Introduction

Diabetic ketoacidosis (DKA) is managed with insulin, intravenous fluids, and potassium. Because the plasma glucose level invariably falls more rapidly than the plasma ketone level, insulin administration is continued, and glucose and potassium are infused simultaneously to prevent the occurrence of hypoglycemia and hypokalemia. This forms the glucose-insulin-potassium (GIK) regimen, which is also used to manage moderate degrees of hyperglycemia even in the absence of ketoacidosis [1••]. In most instances, especially when patients with moderate degrees of hyperglycemia—and at times those with DKA—are given insulin, the infusion of substantial amounts of glucose and potassium may not be necessary, although both glucose and potassium are given and are kept handy more as a precaution than out of necessity. This is because hypoglycemia and/or hypokalemia is unlikely to develop in most of these patients if they are monitored closely. In general, most patients with DKA recover when properly treated, although about 10% may still die because of late complications of DKA, such as myocardial infarction and infection. The other acute complications of DKA are vascular thrombosis and acute respiratory distress syndrome (ARDS). Patients with diabetes can have volume depletion, hyperosmolality, and increased viscosity of blood as a result of hyperglycemia and changes in clotting factors favoring vascular thrombosis. The cause of acute respiratory distress syndrome is not known, but it could be due to a hyperglycemia-induced or -associated increase in free radical generation and proinflammatory cytokines. Hyperglycemia also increases circulating free fatty acids (FFAs), which are toxic to the myocardium and induce arrhythmias. Hyperglycemia causes osmotic diuresis, and the resulting volume depletion may further compromise myocardial function.

Hyperglycemia is harmful

Why should hyperglycemia be treated? Several studies showed clearly that patients with persistent hyperglycemia are at increased risk of myocardial infarction, peripheral vascular disease, stroke, renal damage, vision loss, cataract, etc. On the other hand, those who have been treated with diet restriction; exercise, oral hypoglycemic agents and/or insulin are protected from the development of these diseases or at least able to postpone the development of these complications for substantial period. Both the UK Prospective Diabetes Study done in type 2 diabetes mellitus (UKPDS) and the Diabetes Control and Complications Trial of USA done in type 1 diabetes mellitus (DCCT) revealed that improved blood glucose control decreases the progression of diabetic mi-

crovascular disease and improves the quality of life [2,3]. In the UKPDS trial, at the end of 10 years of intensive treatment of type 2 diabetes with a sulfonyl-ureas (chlorpropamide, glibenclamide, or glipizide) or with insulin in such a manner that the fasting plasma glucose was less than 6 mmol/L (*ie*, <110 mg/dL) compared with a fasting plasma glucose below 15 mmol/L (*ie*, <270 mg/dL) in the conventional group (treated by diet alone, with drugs added only if fasting plasma glucose was >15 mmol/L), the risk in the intensive group was 12% lower for any diabetes-related endpoint; 10% lower for any diabetes-related death; and 6% lower for all-cause mortality. Most of the risk reduction was due to a 25% risk reduction in microvascular endpoints that included renal failure, death resulting from renal failure, retinal photocoagulation, or vitreous hemorrhage. In addition, intensive treatment of diabetes also reduced the incidence of stroke, myocardial infarction, and peripheral vascular disease. In the DCCT study of type 1 diabetes mellitus, done in the United States, intensive therapy with insulin reduced the risk of retinopathy by 76% and reduced the occurrence of microalbuminuria by 39%, that of albuminuria by 54%, and that of clinical neuropathy by 60%. In general, it was concluded that the beneficial actions of intensive blood glucose control in both type 1 and type 2 diabetes mellitus is due to a reduction in the plasma glucose. But it is not clear why hyperglycemia should be harmful. Recent studies suggest that hyperglycemia induces oxidative stress, enhances the production of proinflammatory molecules, and thus brings about its deleterious actions.

Hyperglycemia enhances free radical generation

Both in animal models of diabetes and in patients with diabetes mellitus, increased production of free radicals (especially the superoxide anion, $O_2^{\cdot-}$) and consequently the formation of excess of lipid peroxides were noted [4–6]. Hyperglycemia increases the production of reactive oxygen species inside cultured aortic endothelial cells [7]. Free radicals (especially the superoxide anion) has the ability to inactivate prostacyclin (PGI_2) and nitric oxide (NO), which are potent vasodilators and platelet antiaggregators [8–10]. In an elegant study, it was found that glucose challenge (given in the form of 75 g glucose in 300 mL water orally to fasting normal subjects) enhanced the generation of leukocyte free radicals almost 233 ± 34% above the basal level at 2 hours ($P < 0.001$) with a simultaneous increase in plasma lipid peroxides and a fall in the plasma α-tocopherol [11]. This increase in free radical generation after glucose challenge was found to be closely associated with an increase in the expression of $p47^{phox}$, the key component of NADPH oxidase, suggesting that the increase in free radical generation is due to the activation of NADPH oxidase. The simultaneous fall in the concentrations of α-tocopherol suggests that increase in free radical generation leads to consumption of this antioxidant. This is in agreement with the results of other studies reporting that patients with diabetes have low antioxidant capacity/activity [4,5,12]. These results suggest that glucose can directly activate NADPH oxidase to enhance free radical generation, which in turn may consume antioxidants, leading to pro-oxidative stress not only in patients with diabetes but also in normal individuals. Because free radicals have proinflammatory actions, this explains why chronic subclinical inflammation exists and persists in diabetes. This is supported by the observation that high glucose concentrations increased leukocyte rolling, leukocyte adherence, and leukocyte transmigration through mesenteric venules [13••]. These proinflammatory properties of leukocytes were seen to be associated with attenuation of endothelial NO release and increased expression of P-selectin on endothelial surfaces. By contrast, the local application of insulin completely attenuated these proinflammatory events. Insulin infusion also inhibited free radical generation and $p47^{phox}$ and NF-κB activation in mononuclear cells, and it reduced soluble intercellular adhesion molecule-1, monocytes chemoattractant protein-1 and plasminogen activator-1 production by enhancing NO synthesis [1••,14•,15,16•,17•].

Hyperglycemia, proinflammatory cytokines, and nitric oxide

Esposito *et al.* [18••] observed that in normal subjects, when plasma glucose levels were acutely raised and maintained at 15 mmol/L for 5 hours while endogenous insulin secretion was blocked with octreotide, plasma interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and IL-18 levels rose within 2 hours of the clamp but returned to basal values at 3 hours. By contrast, in subjects with impaired glucose tolerance, the fasting plasma IL-6 and TNF-α levels were higher than those of normal subjects, and the increase in plasma cytokine levels during the clamping lasted longer (4 hours *vs* 2 hours). In another study, the same normal subjects were given three consecutive pulses of intravenous glucose (0.33 g/kg) separated by 2-hour intervals, and the plasma cytokine levels were estimated at 3, 4, and 5 hours [18]. It was found that the plasma cytokine levels obtained at 3, 4, and 5 hours were higher than the corresponding values obtained during the clamp. It was also reported that the cytokine peaks in subjects with impaired glucose tolerance after the first glucose pulse were higher than those of the normal subjects. It is interesting that when both the normal subjects and those with impaired glucose tolerance received the same glucose pulses along with an infusion of glutathione, a known antioxidant, plasma cytokine levels did not show any significant change from baseline after the 3 glucose pulses, suggesting that hyperglycemia acutely increases proinflammatory cytokine concentrations by an oxidative mechanism, and that this effect is more pronounced in subjects with impaired glucose tolerance.

In another interesting study, Srinivasan *et al.* [19•] showed that glucose increases the adhesion of monocytes to human aortic endothelial cells *in vitro*. Human aortic endothelial cells cultured in the presence of high glucose (25 mmol/L for 7 days) had a twofold elevation in IL-8 secretion over control cells that were cultured in the presence of 5.5 mmol/L glucose. Both glucose and IL-8 activated β_1 integrin on the surface of the human aortic endothelial cells, suggesting that activation of $\alpha_5\beta_1$ integrin complexes on the endothelial surface that is necessary for the adhesion of monocytes to endothelial cells. The use of a neutralizing antibody to IL-8 prevented glucose-mediated monocyte adhesion. Analysis of the human IL-8 promoter revealed binding sites for NF-κB and AP-1, and further work revealed that glucose stimulated IL-8 promoter activity and that both the AP-1 element and the glucose response element were responsible for much of the glucose-mediated activation of IL-8 transcription. Inhibition of free radical production reduced the glucose-mediated induction of IL-8 expression. In this context, it is interesting that IL-8, a proinflammatory cytokine, is a potent activator of polymorphonuclear leukocyte functions such as chemotaxis, superoxide anion production, and enzyme release and is also chemotactic to lymphocytes. But it not yet certain which is the first event: whether increased production of proinflammatory cytokines occurs first, leading to enhanced free radical generation, or *vice versa*. It is possible that free radical generation and the production of proinflammatory cytokines occur simultaneously or are so closely linked that it may be virtually impossible to determine which occurs first. Because antioxidants and NO seem to negate the proinflammatory actions of hyperglycemia, it appears that cell or tissue antioxidant capacity or status has a major role in preventing the toxic actions of glucose.

Hyperglycemia and eicosanoids

In addition, hyperglycemia causes upregulation of cyclooxygenase-2, especially in endothelial cells, in such a manner that the production of thromboxane A_2 is increased, whereas that of PGI_2 is suppressed [20]. Glucose-induced activation of protein kinase C (PKC) resulted in the formation of peroxynitrite and tyrosine nitration of PGI_2 synthase enzyme, which resulted in decreased release of NO despite a twofold increase in endothelial nitric oxide (eNO) synthase and reduced PGI_2 formation. Antioxidants such as *N*-acetylcysteine and vitamin C not only prevented free radical formation but also restored NO release to a normal state by reducing the colocalization of nitrotyrosine and PGI_2 synthase. These results are interesting because hyperglycemia enhances the production of IL-8, which in turn stimulates 5-lipoxygenase, leading to the formation of leukotriene B_4 (LTB_4) and also activates 15-lipoxygenase to increase the production of 15-hydroxyeicosatetraenoic acid (15-HETE). LTB_4 is a proinflammatory molecule, whereas 15-HETE is antiinflammatory. 15-HETE specifically inhibits LTB_4 formation and LTB_4-induced chemotaxis of human neutrophils. Because IL-8 stimulates both LTB_4 and 15-HETE formations, the effect of IL-8 on inflammation depends on the relative stimulation of 5- and 15-lipoxygenases.

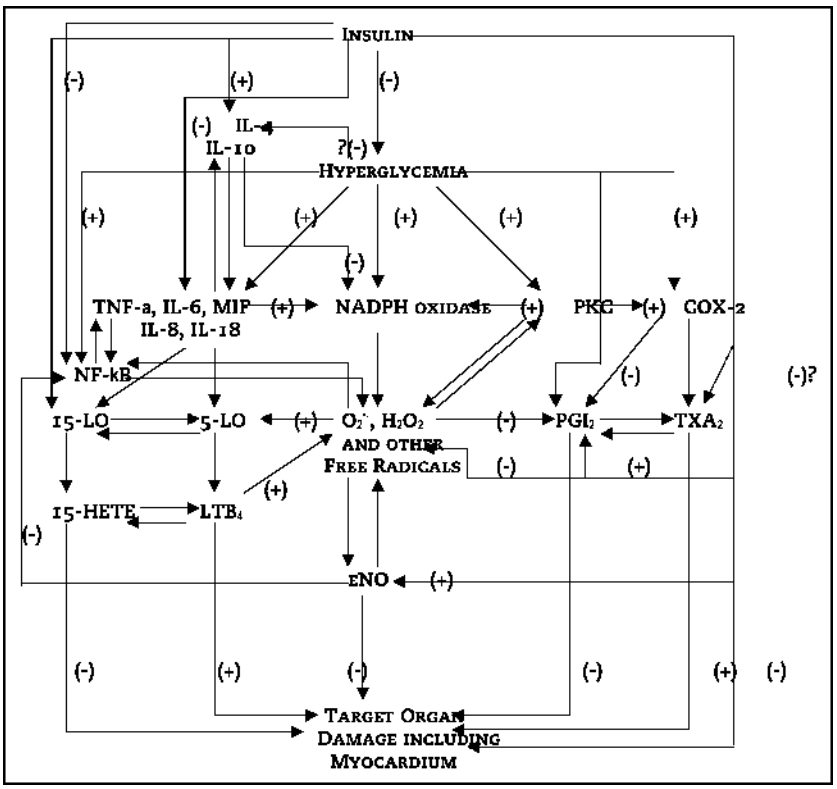
How glucose stimulates proinflammatory events

Although it is clear from the preceding discussion that hyperglycemia stimulates the production of proinflammatory cytokines and activates NADPH oxidase, NF-κB, and the eicosanoids pathway, it is not clear which is the first event that ultimately leads to glucose-induced inflammation. Indirect evidence suggests that possibly glucose initiates the inflammatory events first by stimulating the production of TNF-α. A positive and statistically significant association between dietary glycemic load and plasma high-sensitivity C-reactive protein was reported in healthy middle-aged women, independently of conventional risk factors for ischemic heart disease [21•]. Hyperglycemia *per se* but not hyperinsulinemia leads to a significant increase in serum amyloid A3 within a short time in adipose tissue [22]. Interestingly, older control animals, even those without diabetes, had a tendency for increased basal level expression of serum amyloid A3. Similarly to glucose, TNF-α enhanced free radical generation by augmenting polymorphonuclear leukocyte NADPH oxidase activity, activated NF-κB, and increased intercellular adhesion molecule-1 expression in endothelial cells [23]. By contrast, in patients with type 2 diabetes mellitus, both the activity of NADPH oxidase and the levels of NADPH oxidase protein subunits $p22^{phox}$, $p67^{phox}$, and $p47^{phox}$ were significantly elevated in both the veins and the arteries, enhanced PKC activity occurred, and venous and arterial endothelium showed dysfunctional NO synthase, leading to an additional source of superoxide production [24]. The decreased NO production by the diabetic vessels was corrected by intracellular tetrahydrobiopterin, a cofactor that is necessary for NO production, and the increased superoxide anion production was abrogated by PKC inhibition. Thus, both glucose and TNF-α have similar actions on NADPH oxidase, PKC, NF-κB, and intercellular adhesion molecule-1. This suggests that glucose initially enhances the production of IL-6, IL-8, IL-18, TNF-α, and C-reactive protein and thus brings about its proinflammatory actions. These events eventually cause endothelial dysfunction and lead to tissue and target organ damage in diabetes mellitus (Figure 1).

Stress hyperglycemia and acute myocardial infarction

Several studies revealed that patients with acute myocardial infarction (AMI) have raised blood glucose concentrations [17•,25–27]. In addition, a positive association between hyperglycemia and mortality from AMI has

Figure 1. Schematic showing how hyperglycemia causes tissue damage, whereas insulin prevents it



Plus sign indicates increase in synthesis, activation, and/or tissue damage. Minus sign indicates decrease in synthesis, activation, or tissue damage.

been reported [28]. This suggests that not just the presence of diabetes but even marginal increases in plasma glucose increase the risk of development of complications due to AMI, although the exact reason for this association is not clear. I suggest that this can be attributed in part to the proinflammatory actions of glucose [17•]. Intensive treatment with insulin to lower plasma glucose concentrations and maintain it under 110 mg/dL (<6.1 mmol/L) decreased overall mortality in patients with diabetes and AMI. In a prospective, randomized, controlled trial involving adults admitted to surgical intensive care units and receiving mechanical ventilation, intensive insulin treatment (wherein the maximum insulin dose was set at 50 IU/h) reduced mortality and morbidity and reduced the number of deaths resulting from multiple organ failure with sepsis [29••]. This suggests that hyperglycemia (defined here as plasma glucose >110 mg/dL) is harmful, whereas insulin therapy is beneficial not only in AMI but also in critical illness with or without diabetes mellitus. It is known that insufficient insulin (either qualitative or quantitative deficiency of insulin) associated with hyperglycemia causes a decrease in glycolytic substrate and an increase in FFAs, which reduce myocardial contractility and promote cardiac failure and arrhythmias [30], leading to poor outcome in such patients. Earlier, I proposed that insulin is an anti-inflammatory molecule and that when high rates of GIK are given (30% glucose, 50 IU of insulin, and 80 mmol/L potassium at the rate of >1.5 mL/kg./h, which is similar to the dosage of insulin used in [29••]) it suppresses circulating concentrations of FFAs and thus could be of benefit in critical illness, such as sepsis and septic shock [1••]. This seems to be true, as is evident from the work of Van den Berghe *et al.* [29••]. It was also reported that intensive insulin treatment suppressed the markers of inflammation, and that may have been responsible for its beneficial action.

The glucose-insulin-potassium regimen and acute myocardial infarction

The American College of Cardiology and the American Heart Association have recommended giving the GIK regimen to patients with AMI, especially those who are poor candidates for thrombolytic therapy and in whom the risk of bleeding is high [1••]. This is based on the results of studies showing that the GIK regimen is of benefit in AMI [1••]. GIK treatment (especially insulin) increases the uptake of glucose by the myocardium and thus may help to save the integrity and function of myocardial cells.

Insulin, tumor necrosis factor-α, macrophage migration inhibitory factor, free radicals, nitric oxide, and myocardium

TNF-α is secreted by adipose tissue, macrophages, and cardiac tissue, and it plays a major role in the pathogenesis of insulin resistance, type 2 diabetes mellitus, inflammation, and septic shock [8]. Early in the course of

AMI, TNF-α release occurs, which can directly decrease myocardial contractility in a dose-dependent manner [31]. Anti–TNF-α antibody reduces myocardial injury and dysfunction [32]. Cardiac cachexia is thought to be due to an increase in the circulating levels of TNF-α [33]. A direct correlation between the circulating levels of TNF-α and the clinical features of congestive cardiac failure has been reported. After cardiac transplantation, TNF-α levels decrease [31]. In addition, TNF-α causes dysfunction and apoptosis of endothelial cells, which in turn leads to decreased production of eNO and enhanced procoagulant activity and fibrin deposition [34]. TNF-α induces endothelial cells and leukocytes to produce large amounts of free radicals, which inactivate eNO. This causes vasoconstriction and ischemia.

Increased mesenteric venous pressure, which leads to intestinal edema and increased bowel permeability, is common in congestive cardiac failure (CCF). This causes an increase in endotoxin absorption from the gut, leading to an elevation in the circulating levels of endotoxin that activate macrophages and other cells to produce TNF-α [8,17•]. This is supported by the observation that in patients with CCF, 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 [8,17•,35]. These observations suggest that TNF-α plays a critical role in the pathogenesis of CCF and that methods designed to suppress its production and/or action could be of benefit not only in CCF but also in inflammation, sepsis, and septic shock [1••].

It is evident from the preceding discussion that TNF-α is harmful to the myocardium, whereas the GIK regimen is beneficial in AMI. Does this mean that the GIK regimen, and insulin in particular, can suppress TNF-α synthesis and action?

Insulin has anti-inflammatory actions and suppresses TNF-α and other cytokines

Satomi *et al.* [36] showed that the exogenous administration of insulin inhibited TNF-α production in a dose-related manner in animals that had been challenged with lipopolysaccharide. In an *in vitro* study, the addition of insulin to cultures of peritoneal exudate cells from mice primed with *Propionibacterium acnes* mice blocked TNF-α production, in comparison with control mice. Fraker *et al.* [37] reported that 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 could be completely prevented by the concurrent administration of insulin. The pneumonitis seen in these TNF-α–treated animals is similar to the adult respiratory distress syndrome that is common in patients with sepsis and septic shock—conditions in which the plasma concentrations of IL-1, TNF-α, and macrophage migration inhibitory factor (MIF) are elevated [38–40]. Furthermore, insulin enhances the production of eNO by activating *Akt* through the phosphatidylinositol 3′-kinase (PI3-kinase) pathway [41,42••] and suppresses superoxide anion generation [43]. *In vivo* insulin therapy significantly decreased the serum proinflammatory cytokines IL-1β, IL-6, MIF, and TNF-α concentrations after thermal injury and increased the anti-inflammatory cytokines IL-4 and IL-10 in a study by Jeschke *et al.* [44••], as predicted earlier by this author [1••]. In addition, insulin significantly reduced the proinflammatory signal transcription factors STAT-5 and C/EBP-β mRNA and increased the anti-inflammatory signal transcription factor mRNA expression of SOCS-3 and RANTES-7. Thus, insulin has hitherto unappreciated potent anti-inflammatory actions, which could be responsible for the beneficial actions of the GIK regimen in AMI and critical illness with sepsis and septic shock [1••,40,45•].

Cardiac dysfunction and insulin resistance in the critically ill, sepsis and septic shock

Cardiovascular dysfunction in the form of myocardial depression is present in septic shock [46]. Several studies showed that the GIK regimen improves myocardial function during sepsis and septic shock by enhancing cardiac output, stroke volume, arterial pressure, and oxygen consumption [47–49]. Cardiac dysfunction that occurred within 2 to 4 hours after endotoxin was not related to arterial-blood glucose concentrations [49]. Maintaining blood glucose at control or preshock levels by the infusion of 50% glucose did not prevent myocardial dysfunction, whereas intra-arterial infusions of insulin at rates of 6 units/min reversed all signs of myocardial failure and maintained normal performance despite wide ranges in glucose concentrations (5–120 mg/dL) [49,50]. This suggests that it is insulin that improves cardiac performance and that myocardial dysfunction is not precipitated or enhanced by the hypoglycemia of septic (endotoxin) shock. This indicates that the beneficial actions of insulin on cardiac performance are due to mechanisms other than myocardial glucose transport. In this context, it is important to note that insulin resistance is common during critical illness, infections, sepsis, and septic shock. Thus, the presence of insulin resistance for whatever reasons during a variety of clinical conditions may render the cardioprotective actions of insulin ineffective or less effective.

Insulin resistance in sepsis, liver cell failure, and multiorgan dysfunction syndrome, which occurs in septic shock, is associated with increased production of plasma TNF-α, IL-6, IL-1, IL-2, and MIF and possibly with decreased production of anti-inflammatory cytokines IL-4, IL-10, and TGF-β and enhanced activity of inducible nitric oxide synthase [1••]. The glucose utilization rate was increased significantly with exogenous insulin

infusion in control subjects but not in patients with sepsis, suggesting that sepsis impairs the action of insulin on endogenous glucose production and utilization, lipolysis and ketogenesis [51]. It was also observed that once transported into the cells and tissues, glucose is preferentially metabolized to lactate. Lind and Lithell [52] reported that basal serum lactate, glucose, and insulin were elevated in critically ill patients in the intensive care unit compared with healthy control subjects and were correlated to indices of severity of illness. Further, serum glucose, FFAs, glycerol, and triacylglycerols in serum, very low-density lipoprotein, and low-density lipoprotein were elevated, whereas high-density lipoprotein cholesterol was decreased in patients with sepsis in comparison with those without sepsis. Serum lactate and free glycerol (an indicator of lipolysis) were elevated in patients who did not survive in comparison with survivors [52]. These results suggest that glucose and lipid metabolism, which are influenced by insulin, are altered in sepsis and may be used as prognostic indicators. These metabolic abnormalities could be due to low insulin secretion, increased clearance, and peripheral insulin resistance, especially in the liver and skeletal muscle, although insulin production itself was not observed. In the early phase of sepsis, hyperglycemia occurs, and in the late stages hypoglycemia sets in [53,54]. These changes in the plasma glucose levels have been attributed to alterations in the concentrations of, and balance between, insulin and corticosterone during the various phases of sepsis and septic shock. This led to the suggestion that the continuous infusion of glucose and insulin might enhance tissue glucose uptake; suppress lactate, FFAs, glycerol production, and lipolysis; overcome corticosterone-dependent insulin resistance; and improve tissue perfusion and recovery [1••,52–54]. This is somewhat similar to the administration of insulin for DKA. Most patients with DKA are treated by low-dose insulin schedules in which 8 to 10 units of insulin are infused intravenously each hour. Most cases of DKA can be reversed adequately with this low-dose treatment, but some patients do not respond. Presumably, the insulin resistance that is characteristic of DKA is more pronounced in these patients than in responsive subjects. These "resistant" patients are given 25 to 50 units of insulin as an intravenous bolus, followed by an infusion of 15 to 25 units per hour until ketosis is reversed. This higher-dosage insulin schedule is believed to ensure saturation of the insulin receptors in the face of competing antibodies or other resistance factors. It is also possible that high amounts of insulin act via the insulin-like growth factor receptor and reverse DKA. In the same manner, even in the critically ill and in patients with sepsis and septic shock, continuous administration of the GIK cocktail could enhance tissue perfusion and glucose uptake; suppress lactate, FFAs, glycerol production, and lipolysis; and improve survival. It is also possible that the GIK regimen suppresses the production of the proinflammatory cytokines IL-1, IL-6, TNF-α and MIF; enhances the production of eNO and anti-inflammatory cytokines IL-4 and IL-10; and thus may facilitate improvement in myocardial function and recovery. This may also explain why the GIK regimen is useful in the management of AMI.

It is insulin, not glucose or potassium, that is critical to the heart

Although it is known that the GIK regimen improves myocardial function and protects the myocardium during endotoxic shock, AMI, and critical illness and in patients undergoing open heart surgery by improving cardiac output, stroke volume, mean arterial pressure, and oxygen consumption, the exact component that is responsible for this beneficial effect has been debated for some time. Cardiac dysfunction occurring within 2 to 4 hours after endotoxin was seen to be not related to arterial blood glucose concentrations [1••,40,47–49]. Maintaining blood glucose at control (preshock) levels by infusion of 50% glucose did not prevent myocardial dysfunction. By contrast, infusions of insulin reversed cardiac failure and maintained normal performance despite wide ranges in glucose concentrations (5–120 mg/dL), suggesting that it is the insulin that protects the myocardium and improves cardiac function, which can now be related to its ability to suppress TNF-α, IL-6, MIF, and superoxide anion production [1••].

Gao *et al* (42••) directly compared the cardioprotective effects of individual GIK components in an *in vivo* study and demonstrated that insulin, but not glucose or potassium, is the protective component. It was observed that when Sprague-Dawley rats were subjected to myocardial ischemia and reperfusion and treated with insulin, there were 2.6-fold and 4.3-fold increases in *Akt* and eNOS phosphorylation, respectively, with a significant increase in NO production in ischemia/reperfusion myocardium. This increase of NO production reduced myocardial apoptotic death. Although the exact mechanism by which this increase in NO generation induced by insulin exerts its antiapoptotic effects is not known, several possibilities have been suggested. NO can nitrosate caspase-3, -6, -7, and -8 and inhibit caspase-dependent *Bcl-2* cleavage and consequently the release of mitochondrial cytochrome *c* [55]. NO may downregulate MKP-3 mRNA levels, thereby preventing the inactivation of ERK1/2, an antiapoptotic member of the MAPK family and thus reducing apoptotic cell death of myocardial tissue [56]. This myocardial protective action of insulin has also been shown in several clinical studies [57–62].

Mauritz *et al.* [57] studied the hemodynamic and metabolic effects of the GIK regimen in 14 patients with peritonitis who were in hypodynamic septic shock (as evidenced by mean arterial pressure <50 mm Hg and cardiac index <3.5 1/min) despite a highly positive fluid

balance (greater than +2,000 mL during the last 12 hours) and use of catecholamines. The infusion of GIK (glucose 70% 1g/kg + 1.5 units/kg and potassium 10 mMol) led to significant increases in systolic (+53%) and mean (+61%) arterial pressures, cardiac index (+50%), right (+60%) and left (+109%) ventricular stroke work indices, and oxygen consumption index (+18%); heart rates remained unchanged, and serum glucose levels were elevated. Eventually, 2 patients were discharged from the hospital. Bronsveld *et al.* [58] did a similar study of septic shock in which a GIK regimen (glucose 50%, 1 g/kg body weight; insulin 1.5 units/kg; potassium 10 mMol) improved hemodynamic status, and eventually 4 of 15 patients were hospital survivors, suggesting that in hypodynamic septic shock refractory to volume loading and catecholamines, treatment with the GIK regimen may be useful.

Myocardial protective action of insulin in patients undergoing open-heart surgery

Several other studies suggested that the GIK regimen preserves systolic and diastolic function in ischemia and reperfusion [59] and protects the myocardium in patients undergoing open-heart surgery [60,61], although this is not without controversy [62••–65••]. It is not clear why the outcome was positive in only some studies whereas others failed to show a benefit from the GIK regimen against myocardial dysfunction in septic shock.

A close look at the studies [57–61,62••–65••] that used the GIK regimen to preserve myocardium revealed that those studies were not all comparable with one another. This is because the concentrations of glucose and insulin used in these studies were not uniform. Mauritz *et al.* [57] used glucose 70%, 1g/kg, and insulin 1.5 units/kg, whereas Bronsveld *et al.* [58] used glucose 50%, 1g/kg, and insulin 1.5 units/kg. Mauritz *et al.* [57] noted that plasma glucose increased in their patients after the GIK regimen, but they used a higher dose of insulin relative to the concentration of glucose infused than did Bronsveld *et al*(58). It is clear that studies in which higher concentrations of insulin were used showed better results than did studies that used lesser doses [59–61,62••–65••. For instance, studies in which 33% glucose with 120 units of insulin or 30% glucose with 300 units of insulin was used yielded positive results [60,61]. By contrast, results of studies that used a lower dose [63••,64••] were less favorable. This is because stress hyperglycemia, or even mild hyperglycemia with myocardial infarction, is associated with increased mortality, and intensive insulin treatment to maintain blood glucose levels between 80 and 110 mg/dL is highly beneficial and reduces morbidity and mortality among critically ill patients [66,29••]. Hence, it is likely that the negative results obtained with the GIK regimen were due to the low dose of insulin used, which invariably resulted in hyperglycemia (>110mg/dL) that is detrimental to the myocardium [1••,17•,63••–65••,66].

Continuous intravenous infusion of insulin is superior to subcutaneous administration to control hyperglycemia, especially in patients with diabetes mellitus during the preoperative and postoperative periods [67]. During both the infusion period and the entire observation period (day of surgery and postoperative days 1 and 2), the GIK regimen resulted in lower blood glucose levels within the intended range of 90 to 180 mg/dL (5–10 mmol/L) in comparison with conventional subcutaneous insulin administration. Improved diabetic control results in fewer wound infections and better wound healing. I have suggested that the beneficial effects of GIK regimen may extend beyond the control of hyperglycemia alone [1••,17•,40,45•,68]. GIK infusion may salvage myocardium, improve cardiac function, and decrease mortality by an absolute 10%, provided that hyperglycemia is prevented [69,70]. This beneficial effect is independent of glucose [54,71•,72,73]. This assumption is supported by the results of a large trial conducted by Van den Berghe *et al.* [29••], who reported that intensive insulin therapy to avoid hyperglycemia (blood glucose maintained ≤110 mg/dL) in predominantly nondiabetic patients admitted to surgical intensive care units and receiving mechanical ventilation led to a decrease in morbidity and mortality in comparison with less intensively treated patients (blood glucose maintained between 180 and 200 mg/dL). The results of this study suggested that maintaining blood glucose concentrations at or below 110 mg/dl is critical to deriving the benefits of insulin treatment. This is supported by the observation that cardiac dysfunction induced by endotoxin administration was not related to arterial blood glucose concentrations, and that infusions of insulin but not glucose reversed cardiac failure and maintained normal performance [49,50]. This suggests that myocardial function is not influenced by hypoglycemia of endotoxin shock.

Conclusion

It is evident from the preceding discussion that insulin has a myocardial protective action. This explains the beneficial action of the GIK regimen in AMI. Hyperglycemia can initiate and perpetuate inflammation and thus bring about its harmful actions on the myocardium. The cardioprotective action of insulin is due, at least in part, to its ability to control hyperglycemia. In addition, insulin has anti-inflammatory actions. Insulin suppresses NF-κB expression, free radical generation (especially superoxide anion), and MIF production. It enhances eNO generation and thus inhibits inflammatory process [74•,75•]. It protects the myocardium by preventing apoptosis of myocardial tissue. This could be the reason why intensive insulin therapy and the GIK regimen are beneficial in critical illness, AMI, sepsis, and septic shock (Figure 1). Many physicians are familiar with the

use of insulin. Hence, it is not difficult to extend insulin therapy in these conditions. It remains to be seen whether structural analogues of insulin can be developed that show myocardial protective action without hypoglycemic action.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • Of special interest
- •• Of outstanding interest

1 Das UN: Is insulin an anti-inflammatory molecule? Nutrition 2001, 17:409–413.

2 Prospective Diabetes Study UK (UKPDS) Group: Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 1998, 352:837–853.

3 The Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993, 329:977–986.

4 Mohan IK, Das UN: Oxidant stress, anti-oxidants and nitric oxide in non-insulin dependent diabetes mellitus. Med Sci Res 1997, 25:55–57.

5 Das UN, Kumar KV, Mohan IK: Lipid peroxides and essential fatty acids in patients with diabetes mellitus and diabetic nephropathy. J Nutr Med 1994, 4:149–155.

6 Nishikawa T, Edelstein D, Du XL, et al.: Normalizing mitochondrial superoxide production blocks three pathways of hyperglycemic damage. Nature 2000, 404:787–790.

7 Giardino I, Edelstein D, Brownlee M: BCL-2 expression or antioxidants prevent hyperglycemia-induced formation of intracellular advanced glycation end products in bovine endothelial cells. J Clin Invest 1996,97:1422–1428.

8 Das UN: Free radicals, cytokines and nitric oxide in cardiac failure and myocardial infarction. Mol Cell Biochem 2000,215:145–152.

9 Laight DW, Kaw AV, Carrier MJ, et al.: Interaction between superoxide anion and nitric oxide in the regulation of vascular endothelial cells. Br J Pharmacol 1998, 124:238–244.

10 Das UN: Nutritional factors in the pathobiology of human essential hypertension. Nutrition 2001, 17:337–346.

11 Mohanty P, Hamouda W, Garg R, et al.: Glucose challenge stimulates reactive oxygen species (ROS) generation by leukocytes. J Clin Endocrinol Metab 2000, 85:2970–2974.

12 Ceriello A, Bortolotti N, Motz E, et al.: Meal-generated oxidative stress in type 2 diabetic patients. Diabetes Care 1998, 21:1529–1533.

13 Booth G, Stalker TJ, Lefer AM, et al.: Elevated ambient glucose induces acute inflammatory events in the microvasculature: effects of insulin. Am J Physiol 2001, 280:E848–E856.

14 Dandona P, Aljada A, Mohanty P, et al.: Insulin inhibits intranuclear nuclear factor kappa B and stimulates IkappaB in mononuclear cells in obese subjects: evidence for an inflammatory effect? J Clin Endocrinol Metab 2001, 86:3257–3265.

15 Aljada A, Saadesh R, Assian E, et al.: Insulin inhibits the expression of intercellular adhesion molecule-1 by human aortic endothelial cells through stimulation of nitric oxide. J Clin Endocrinol Metab 2000, 85:2572–2575.

16 Aljada A, Ghanim H, Saadesh R, et al.: Insulin inhibits NFκB and MCP-1 expression human aortic endothelial cells. J Clin Endocrinol Metab 2001, 86:450–453.

17 • Das UN: Is insulin an endogenous cardioprotector? Crit Care 2002, 6:389–393.
In this review the authors presented arguments that insulin is an endogenous cardioprotective agent because it has antiinflammatory actions and hence is of benefit in AMI, sepsis, and septic shock.

18 •• Esposito K, Nappo F, Marfella R, et al.: Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress. Circulation 2002, 106:2067–2072.
The authors presented evidence that hyperglycemia enhances the concentrations of proinflammatory cytokines and produces oxidative stress.

19 • Srinivasan S, Yeh M, Danziger EC, et al.: Glucose regulates monocytes adhesion through endothelial production of interleukins-8. Circulation Res 2003, 92:371–377.
In this elegant study, the authors showed that glucose stimulates the production of proinflammatory IL-8 by endothelial cells, which in turn is responsible for the adhesion of monocytes to the endothelial cells. This results in damage to the endothelial cells and inn endothelial dysfunction.

20 Fogh K, Larsen CG, Iversen L, et al.: Interleukin-8 stimulates the formation of 15-hydroxyeicosatetraenoic acid by human neutrophils in vitro. Agents Actions 1992, 35:227–231.

21 • Liu S, Manson JE, Buring JE, et al.: Relation between a diet with a high glycemic load and plasma concentrations of high-sensitivity C-reactive protein in middle-aged women. Am J Clin Nutr 2002, 75:492–498.
In this clinical study, authors demonstrated that a high glycemic load enhances the plasma concentrations of C-reactive protein, an indicator of inflammation. Although the concentrations of proinflammatory and antiinflammatory cytokines were not evaluated in this study, it can be concluded on the basis of this and other studies that continued consumption of a high-energy diet induces proinflammatory events that may trigger the development of metabolic syndrome X.

22 Lin Y, Rajala MW, Berger JP, et al.: Hyperglycemia-induced production of acute phase reactants in adipose tissue. J Biol Chem 2001, 276:42077–42083.

23 Fan J, Frey RS, Rahman A, et al.: Role of neutrophil NADPH oxidase in the mechanism of tumor necrosis factor-α-induced NF-κB activation and intercellular adhesion molecule-1 expression in endothelial cells. J Biol Chem 2002, 277:3404–3411.

24 Guzik TJ, Mussa S, Gastaldi D, et al.: Mechanism of increased vascular superoxide production in human diabetes mellitus: role of NAD(P)H oxidase and endothelial nitric oxide synthase. Circulation 2002, 105:1656–1662.

25 Cruikshank N: Coronary thrombosis and myocardial infarction, with glycosuria. BMJ 1931, 1:618–619.

26 Sewdarsen M, Jialal I, Vythilingum S, et al.: Stress hyperglycemia is a predictor of abnormal glucose tolerance in Indian patients with acute myocardial infarction. Diabetes Res 1987, 6:47–49.

27 Oswald GA, Smith CCT, Betteridge DJ, et al.: Determinants and importance of stress hyperglycemia in non-diabetic patients with myocardial infarction. BMJ 1986, 293:917–922.

28 Yudkin JS, Oswald GA: Hyperglycemia, diabetes and myocardial infarction. Diabetes Med 1987, 4:13–18.

29 Van den Berghe G, Wouters P, Weekers F, et al.: Intensive insulin therapy in critically ill patients. N Engl J Med 2001, 345:1359–1367.

30 Oliver MF, Opie LH: Effects of glucose and fatty acids on myocardial ischaemia and arrhythmias. Lancet 1994, 343:155–158.

31 Cain BS, Harken AH, Meldrum DR: Therapeutic strategies to reduce TNF-alpha mediated cardiac contractile depression following ischemia and reperfusion. J Mol Cell Cardiol 1999, 31:931–947.

32 Li D, Zhao L, Liu M, et al.: Kinetics of tumor necrosis factor alpha in plasma and the cardioprotective effect of a monoclonal antibody to tumor necrosis factor alpha in acute myocardial infarction. Am Heart J 1999, 137:1145–1152.

33 Levine B, Kalman J, Mayer L, et al.: Elevated circulating levels of tumor necrosis factor in congestive heart failure. N Engl J Med 1990, 323:236–241.

34 Meldrum DR, Donnahoo KK: Role of TNF in mediating renal insufficiency following cardiac surgery: evidence of a post bypass cardiorenal syndrome. J Surg Res 1999, 85:185–199.

35 Ferrari R: Tumor necrosis factor in CHF: a double facet cytokine. Cardiovasc Res 1998, 37:554–559.

36 Satomi N, Sakurai A, Haranaka K: Relationship of hypoglycemia to tumor necrosis factor production and antitumor activity: role of glucose, insulin, and macrophages. J Natl Cancer Inst 1985, 74:1255–1260.

37 Fraker DL, Merino MJ, Norton JA: Reversal of the toxic effects of cachectin by concurrent insulin administration. Am J Physiol 1989, 256(6 Pt 1):E725–E731.

38 Riedemann NC, Guo R-F, Ward PA: Novel strategies for the treatment of sepsis. Nature Med 2003, 9:517–524.

39 Martin TR: MIF mediation of sepsis. Nature Med 2000, 6:140–141.

40 Das UN: Cytokines, NF-κB, activated protein C, oxidized phospholipids, inducible cyclo-oxygenase eicosanoids, adenosine and insulin in sepsis and septic shock. Critical Care Shock 2003, 6:40–49.

41 Kuboki K, Jiang ZY, Takahara N, et al.: Regulation of endothelial constitutive nitric oxide synthase gene expression in endothelial cells and in vivo: a specific vascular action of insulin. Circulation 2000, 101:676–681.

42 Gao F, Gao E, Yue T-L, et al.: Nitric oxide mediates the antiapoptotic effect of •• insulin in myocardial ischemia-reperfusion: The roles of PI3-Kinase, *Akt*, and endothelial nitric oxide synthase phosphorylation. Circulation 2002, 105:1497–1502.
In this elegant *in vivo* study, it was found that the cardioprotective actions of insulin are due to its ability to enhance endothelial NO generation and antiapoptotic action on myocardial tissue.

43 Boichot E, Sannomiya P, Escofier N, et al.: Endotoxin-induced acute lung injury in rats: role of insulin. Pulm Pharmacol Ther 1999, 12:285–290.

44 Jeschke MG, Einspanier R, Klein D, et al.: Insulin attenuates the systemic •• inflammatory response to thermal trauma. Mol Med 2002, 8:443–450.
This study showed that insulin suppresses the production of TNF-α, IL-6, and MIF and at the same time enhances that of anti-inflammatory cytokines IL-4 and IL-10.

45 Das UN: Insulin and the critically ill. Crit Care 2002, 6:262–263.
In this review the authors summarized the anti-inflammatory actions of insulin and suggested that it could be useful in critically ill, AMI, sepsis, and septic shock.

46. Court O, Kumar A, Parrillo JE, Kumar A. Clinical review: Myocardial depression in sepsis and septic shock. Critical Care 2002, 6 (DOI 10.1186/cc1822).

47 Bronsveld W, van Lambalgen AA, van Velzen D, et al.: Myocardial metabolic and morphometric changes during canine endotoxin shock before and after glucose-insulin-potassium. Cardiovasc Res 1985, 19:455–464.

48 Tuynman HA, Thijs LG, Straub JP, et al.: Effects of glucose-insulin-potassium (GIK) on the position of the oxyhemoglobin dissociation curve, 2.3-diphosphoglycerate, and oxygen consumption in canine endotoxin shock. J Surg Res 1983, 34:246–253.

49 Hinshaw LB, Archer LT, Benjamin B, et al.: Effects of glucose or insulin on myocardial performance in endotoxin shock. Proc Soc Exp Biol Med 1976, 152:529–534.

50 Archer LT, Beller BK, Drake JK, et al.: Reversal of myocardial dysfunction in endotoxin shock with insulin. Can J Physiol Pharmacol 1978, 56:132–138.

51 Chambrier C, Laville M, Rhzioual Berrada K, et al.: Insulin sensitivity of glucose and fat metabolism in severe sepsis. Clin Sci 2000, 99:321–328.

52 Lind L, Lithell H: Impaired glucose and lipid metabolism seen in intensive care patients is related to severity of illness and survival. Clin Intensive Care 1994, 5:100–105.

53 Maitra SR, Wojnar MM, Lang CH: Alterations in tissue glucose uptake during the hyperglycemic and hypoglycemic phases of sepsis. Shock 2000, 13:379–385.

54 Maitra SR, Wang S, Baithwaite CE, et al.: Alterations in glucose-6- phosphatase gene expression in sepsis. J Trauma 2000, 49:38–42.

55 Kim YM, Bombeck CA, Billiar TR: Nitric oxide as a bifunctional regulator of apoptosis. Circ Res 1999, 84:253–256.

56 Rossig L, Haendeler J, Hermann C, et al.: Nitric oxide down-regulates MKP-3 mRNA levels: involvement in endothelial cell protection from apoptosis. J Biol Chem 2000, 275:25502–25507.

57 Mauritz W, Schindler I, Zadrobilek E, et al.: Glucose-potassium-insulin in hypodynamic septic shock. Anaesthetist 1986, 35:623–627.

58 Bronsveld W, van den Bos GC, Thijs LG: Use of glucose-insulin-potassium (GIK) in human septic shock. Crit Care Med 1985, 13:566–570.

59 Zhu P, Lu L, Xu Y, et al.: Glucose-insulin-potassium preserves systolic and diastolic function in ischemia and reperfusion in pigs. Am J Physiol Heart Circ Physiol 2000, 278:H595–H603.

60. Rudez I, Sutlic Z, Husedzinovic I, Biocina B, Ivancan V. The importance of glucose-insulin-potassium with cardiopulmonary bypass prior to cardioplegic arrest in open-heart surgery. Lijed Vjesn 1995, 117(Suppl2):105–106.

61 Girard C, Quentin P, Bouvier H, et al.: Glucose and insulin supply before cardiopulmonary bypass in cardiac surgery: a double-blind study. Ann Thorac Surg 1992, 54:259–263.

62 Lell WA, Nielsen VG, McGiffin DC, et al.: Glucose-insulin-potassium infusion •• for myocardial protection during off-pump coronary artery surgery. Ann Thorac Surg 2002, 73:1246–1251.
This study and [63–65] showed both positive [62,63] and negative [64,65] results on the cardioprotective action of insulin (essentially used as GIK cocktail). These studies emphasize the importance of using the correct dose of insulin to bring about its cardioprotective action. A close look at these studies suggests that an appropriate ratio between insulin and glucose needs to be maintained to protect the myocardium.

63 Bruemmer-Smith S, Avidan MS, Harris B, et al.: Glucose, insulin and potassium for heart protection during cardiac surgery. Br J Anaesth 2002, 88:489–495.

64 Rao V, Christakis GT, Weisel RD, et al., for the ICT Investigators.: The Insulin Cardioplegia Trial: myocardial protection for urgent coronary artery bypass grafting. J Thorac Cardiovasc Surg 2002, 123:928–935.

65 Diaz-Arya G, Nettle D, Castro P, et al.: Oxidative stress after reperfusion with primary coronary angioplasty: lack of effect of glucose-insulin-potassium infusion. Crit Care Med 2002, 30:417–421.

66 Capes SE, Hunt D, Malmberg K, et al.: Stress hyperglycemia and increased risk of death after myocardial infarction in patients with and without diabetes: a systematic overview. Lancet 2000, 355:773–778.

67 Christiansen CL, Schurizek BA, Malling B, et al.: Insulin treatment of the insulin-dependent diabetic patient undergoing minor surgery: continuous intravenous infusion compared with subcutaneous administration. Anaesthesia 1988, 43:533–537.

68 Das UN: Possible beneficial action(s) of glucose-insulin-potassium regimen in acute myocardial infarction and inflammatory conditions: a hypothesis. Diabetologia 2000, 43:1081–1082.

69 Diaz R, Paolasso EA, Piegas LS, et al.: Metabolic modulation of acute myocardial infarction. The ECLA (Estudios Cardiologicos Latinoamerica) Collaborative Group. Circulation 1998, 98:2227–2234.

70 Van Campen CMC, Klein LJ, Visser FC: Glucose-insulin-potassium imaging: the past and the future? Heart Metabol 2001, 12:14–18.

71 Jonassen AK, Sack MN, Mjos OD, et al.: Myocardial protection by insulin at reperfusion requires early administration and is mediated via Akt and p70s6 kinase cell survival signaling. Circ Res 2001, 89:1191–1198.

72 Rao V, Meranta F, Weisel RD, et al.: Insulin stimulates pyruvate dehydrogenase and protects human ventricular cardiomyocytes from stimulated ischemia. J Thorac Cardiovasc Surg 1998, 116:485–494.

73 Girard C, Quentin P, Bouvier H, et al.: Glucose and insulin supply before cardiopulmonary bypass in cardiac surgery: a double-blind study. Ann Thorac Surg 1992, 54:259–263.

74 Dandona P, Aljada A, Mohanty P: The anti-inflammatory and potential anti- • atherogenic effect of insulin: a new paradigm. Diabetologia 2002, 45:924–930.
This article and [75] review the mechanisms involved in the anti-inflammatory actions of insulin and propose that insulin also prevents atherosclerosis [74], whereas in [75] it was suggested that insulin may have not only cardioprotective but also neuroprotective actions. Thus, insulin may have a role in the pathobiology of Alzheimer disease and other dementias.

75 Das UN: Insulin and inflammation: further evidence and discussion. Nutrition 2002, 18:526–527.