Insulin as an Anti-Inflammatory and Antiatherogenic Modulator

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Data demonstrate the anti-inflammatory effects of insulin and proinflammatory effects of glucose. These data provide a mechanistic justification for the benefits of maintaining euglycemia with insulin infusions in hospitalized patients. Regimens that infuse fixed doses of insulin with high rates of glucose are usually associated with hyperglycemia, which may neutralize the beneficial effects of insulin. Therefore, we propose that such regimens should be avoided and instead replaced by insulin infusions that normalize and maintain blood glucose at a reasonably low level and ensure that plasma insulin is maintained at levels high enough to provide clinically relevant anti-inflammatory and cardioprotective effects. Trials to test this hypothesis are in progress. (J Am Coll Cardiol 2009;53:S14–20) © 2009 by the American College of Cardiology Foundation

Since its discovery in 1921, insulin has been considered a key metabolic hormone with profound effects on glucose and lipid metabolism as well as cardiovascular function, as reviewed elsewhere in this supplement. Additionally, over the past decade, data have emerged on the different effects of insulin and hyperglycemia on inflammatory processes. This article will summarize these data and discuss their implications for management of patients with acute myocardial infarction (AMI).

Proinflammatory Effect of Glucose

Our group has shown that acute challenge with 75-g glucose given orally causes an increase in superoxide radical generation by polymorphonuclear leukocytes and mononuclear cells and increased expression of p47phox, an essential component of the enzyme nicotinamide adenine dinucleotide phosphate hydrogen oxidase, which converts molecular O2 to superoxide radical (1). Downstream consequences include activation of redox-sensitive proinflammatory transcription factors such as nuclear factor-kappa B (NF-kB), activator protein-1, early growth response-1, and hypoxia-inducible factor-alpha, with concomitant increases in expression of matrix metalloproteinases and tissue factor by mononuclear cells (2,3). Likewise, Esposito et al. (4) demonstrated that the induction of steady-state hyperglycemia with intravenous glucose infusion with concomitant inhibition of endogenous insulin secretion with somatostatin led to an increase in plasma tumor necrosis factor-alpha and interleukin-6 concentrations. Finally, Monnier et al. (5) correlated acute glucose excursions with induction of oxidative stress in subjects with type 2 diabetes mellitus.

Hyperglycemia-related oxidative stress reduces the bioavailability of nitric oxide (NO) because superoxide radical combines with NO to form peroxynitrite. NO causes vasodilation and has inhibitory effects on platelet adhesion and aggregation (6,7). In addition, tissue factor is an activator of the extrinsic pathway of coagulation and is involved in the conversion of prothrombin to thrombin (3). Thrombin is a potent platelet aggregator and induces the conversion of fibrinogen to fibrin. Thrombin also triggers proinflammatory pathways, while platelets release CD40 ligand, a powerful inflammatory trigger (8). Thus, inflammation caused by elevated glucose concentrations induces a vasoconstrictive, prothrombotic state and thrombosis begets further inflammation. Moreover, elevated plasma levels of the inflammatory marker C-reactive protein (CRP) are associated with increased incidence of arrhythmias (9). These mechanisms may contribute to the adverse outcomes associated with hyperglycemia in patients with acute coronary syndromes (ACS) (10–13). Hyperglycemia is also associated with a reduction in spontaneous reperfusion, reduced rate of Thrombolysis In Myocardial Infarction (TIMI) flow grade 3, and a higher rate of no reflow after primary percutaneous coronary intervention in AMI (14,15). Moreover, chronic hyperglycemia and high hemo-
globin A1c levels are associated with a lower percutaneous coronary intervention success rate in people with diabetes (16).

**Reactive Oxygen Species Suppression and the Anti-Inflammatory Effects of Insulin**

Insulin induces expression of endothelial NO synthase through the activation of phosphatidylinositol kinase (PI3) and Akt kinase (protein kinase B), an insulin signaling mechanism similar to that which mediates the uptake of glucose through the glucose transporter (17). In turn, endothelial NO synthase generates NO. Human studies have demonstrated that insulin increases blood flow at the arterial, venous, and microcirculatory levels (18), effects that are NO-dependent. Insulin also inhibits platelet aggregation via a NO-dependent effect (7).

In vitro studies showed that insulin suppressed intracellular adhesion molecule-1, monocyte chemoattractant protein-1 expression, and NF-kB binding in human aortic endothelial cells (19). In obese subjects, who typically are insulin resistant and have chronic inflammation, we further showed that low-dose insulin infusion (2 U/h) suppressed reactive oxygen species generation and p65\textsuperscript{phox} expression in mononuclear cells and suppressed NF-kB and early growth response-1 (20,21). Both the binding and expression of early growth response-1 and its protein were also suppressed, whereas inhibitor kappa B, the intracellular inhibitor of NF-kB, was increased. Plasma concentrations of intracellular adhesion molecule-1, monocyte chemoattractant protein-1, matrix metalloproteinase-2 and -9, tissue factor, and plasminogen activator inhibitor-1 also dropped significantly following the insulin infusion during which plasma glucose concentrations were maintained at a normoglycemic and constant level (20,21). We further showed in obese subjects that vascular endothelial growth factor, cytokine that may contribute to neovascularization of the retina in the pathogenesis of diabetic retinopathy and cause expansion of experimental MI extent in the rat (22) was also suppressed by insulin (23). Taken together, these findings support potent and comprehensive anti-inflammatory and antioxidant effects for insulin. Onset is rapid, within 2 h, with the magnitude of this observed effect similar to that of 100 mg of hydrocortisone given intravenously.

**Suppressive Effect of Insulin on Toll-Like Receptors (TLRs)**

TLRs are a class of pathogen-recognition receptors that bind to bacterial, fungal, and viral products and induce inflammation through the subsequent activation of proinflammatory transcription factors. TLR-4 is the specific receptor for the lipopolysaccharide moiety of endotoxin and, therefore, mediates inflammatory changes induced by endotoxins (24). TLR-4 has also been shown to mediate diet-induced obesity, insulin resistance, and vascular inflammation (25,26). Thus, it is plausible that TLR-4 suppression by insulin is implicated in endotoxin-mediated inflammation, diet-induced obesity, insulin resistance, vascular inflammation, and atherogenesis. TLR-2 binds to lipopeptides, glycolipids, and peptide glycerol, which might play a role in ischemia-reperfusion–induced myocardial injury (27,28). Our most recent studies in patients with type 2 diabetes have shown that insulin infusion (2 U/h) suppresses both TLR-2 and -4 and PU1, the key transcription factor involved in the biosynthesis of TLRs (29).

**Anti-Inflammatory and Cardioprotective Effects of Insulin in Experimental Models**

In a rat isolated heart preparation, the addition of insulin, even without glucose and potassium, at the time of reperfusion following the ligation of the anterior descending coronary artery reduced the size of the infarct by 45% (30). This was attributed to an antiapoptotic action of insulin, mediated via PI3 kinase, Akt, BAD, and NO synthase phosphorylation (31). In a canine model of low-flow ischemia, insulin improved contractile function and myocardial metabolic efficiency without alteration of adenosine triphosphate, phosphocreatine, and phosphate levels (32). In an in vivo canine model of AMI, glucose and potassium infusion induced hyperglycemia and was shown to increase infarct size, but the administration of insulin with glucose and potassium, or insulin alone, reduced infarct size and improved left ventricular function (33). In total, the benefits of insulin were attributed to its antiapoptotic effect, suggesting that insulin is the main beneficial component of glucose, insulin, and potassium (GIK) infusion.

Another study in an in vivo rat model with AMI showed that induced hyperglycemia during ischemia was associated with increased infarct size (34). Hyperglycemia also neutralized the benefits obtained by GIK infusion during reperfusion, because the antiapoptotic effect of insulin, mediated via a PI3 kinase-dependent pathway, was inhibited. These findings underscore the need to avoid hyperglycemia and maintain adequate insulin concentration during insulin infusion in ACS.

In other animal studies, insulin was shown to suppress endotoxin–induced proinflammatory transcription factors and genes regulated by them (35). These effects were shown during euglycemic conditions and, thus, are independent of any changes in glucose concentrations. A suppressive effect of insulin on proinflammatory factors has also been demonstrated in rats exposed to thermal injury (36).

In insulin treatment in a rat model of endotoxemia has been shown to decrease endotoxin-induced increase in poly(adenosine

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**Abbreviations and Acronyms**

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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ACS</td>
<td>acute coronary syndromes</td>
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<td>AMI</td>
<td>acute myocardial infarction</td>
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<td>CABG</td>
<td>coronary artery bypass graft</td>
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<td>CRP</td>
<td>C-reactive protein</td>
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<tr>
<td>GIK</td>
<td>glucose, insulin, potassium</td>
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<td>ICU</td>
<td>intensive care unit</td>
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<tr>
<td>NF-kB</td>
<td>nuclear factor-kappa B</td>
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<tr>
<td>NO</td>
<td>nitric oxide</td>
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<tr>
<td>SAA</td>
<td>serum amyloid A</td>
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<td>TLR</td>
<td>toll-like receptor</td>
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amounts of glucose to maintain normoglycemia (39). There were twice as high as those at baseline, necessitating small amounts of glucose to maintain normoglycemia (39). There was an impressive 40% reduction in plasma CRP and serum amyloid A (SAA) concentrations at 24 h, which was maintained after 48 h of insulin infusion (39). Insulin prevented an increase in plasminogen activator inhibitor-1 concentrations induced by the thrombolytic agent reteplase and prevented the marked increase in P47phox expression in mononuclear cells observed in patients who were given reteplase alone. In addition, insulin infusion reduced promatrix metalloproteinase-1 concentrations (39,40) and prevented a heparin-induced increase in plasma free fatty acid concentrations, which probably is secondary to heparin-induced lipolysis in AMI. It did not lower basal elevated concentrations of free fatty acids in these patients (40).

The profound suppressive effects of insulin on CRP concentrations in patients with AMI shown by us have been confirmed by other groups (41). Insulin has also been shown to induce a 40% reduction in CRP and SAA concentrations in patients undergoing coronary artery bypass grafts (CABGs), in whom the increase in CRP and SAA is >30-fold than observed in AMI (42). In CABG patients, the cessation of insulin infusion, with resultant increases in glucose concentrations, also led to an increase in CRP and SAA within hours of insulin discontinuation. Furthermore, CRP-suppressive effects were observed only when insulin was infused continuously, and not when injected as an intravenous bolus to maintain normoglycemia (43). This is most likely a function of the maintenance of a steady and higher concentration of insulin when it is given as a continuous infusion. Because elevated CRP concentrations appear to be related to the size of the infarct in patients with AMI, this effect may be considered cardioprotective. In our study, insulin infusion reduced creatine kinase-myocardial band in subjects with inferior MI, representing the largest single subgroup (39). C-reactive protein may have an important and independent role in the pathogenesis of infarct size because CRP injection in experimental MI increased infarct size, but treatment with small molecules, specifically synthesized to bind CRP and block its action, reduced the infarct size (44). Clearly, a close relationship exists among glycemia, insulin, CRP, and SAA concentrations.

In patients with type 2 diabetes, insulin infusion to control glucose within 12 h of admission to the coronary care unit for ACS reversed the platelet hyperaggregatory effects of hyperglycemia, an effect not seen with subcutaneous insulin (45). In the ICU setting, insulin infusion lowered circulating levels of intracellular adhesion molecule-1 and E-selectin and suppressed inducible NO synthase expression (46). In this study, increased plasma NO metabolite concentrations were predictive of mortality in ICU patients. In another study, mitochondrial changes in the inner membrane and cristae induced by oxidative stress in ICU patients were also inhibited by insulin infusion, which helped to maintain euglycemia (47). Thus, further evidence supports the protective effects of insulin on mitochondrial function and structure in patients with hyperglycemia and ACS.

Antiatherosclerotic Effects of Insulin

Several epidemiological studies associated fasting hyperinsulinemia with an increase in cardiovascular morbidity and mortality (48,49). However, no animal model or cellular experiments have convincingly demonstrated an increase in either atherosclerosis or key atherogenic factor(s) in vitro induced by pathophysiologically relevant insulin concentrations. The consistent stimulatory effects of insulin on smooth muscle cell proliferation with concomitant increase in p47mitogen-activated protein kinase have been shown only at concentrations of 10 to 100 nmol/l (equivalent to 1,400 to 14,000 μU/ml) (50). These concentrations are far greater than in the grossly obese or in very insulin-resistant populations (fasting insulin of 25 to 50 μU/ml). Moreover, patients with insulin-secreting tumors with persistent hyperinsulinemia do not develop atherosclerosis (51).

In contrast, intensive insulin therapy, as demonstrated in the DCCT (Diabetes Control and Complications Trial) study, revealed a trend toward lower cardiovascular events. This was confirmed several years later in the EDIC (Epidemiology of Diabetes Interventions and Complications) study, an extension of DCCT (52). The EDIC study also demonstrated a significantly lower progression of carotid intima-media thickness, an index of atherosclerosis (53).

More recently, studies in apolipoprotein E knockout mice showed that the usual development of atherosclerosis is reduced by insulin administration (54). Furthermore, in insulin receptor substrate-2 knockout mice, interference with insulin signal transduction lead to atherogenesis (55). Taken together, these data support the antiatherogenic effects of insulin.

Insulin Infusion in AMI: Clinical Trials

The DIGAMI (Diabetes Mellitus Insulin-Glucose Infusion in Acute Myocardial Infarction) 1 trial randomized 620
patients with type 2 diabetes and AMI to conventional coronary unit management or insulin-glucose infusion for ≥24 h followed by multidose subcutaneous insulin for ≥3 months (56). At discharge, blood glucose was significantly lower in the insulin-infusion group than in the conventional therapy group (148 mg/dl vs. 162 mg/dl, p < 0.01). At 1 year, hemoglobin A1c decreased significantly more in the insulin-infusion group (0.9% vs. 0.35%, p < 0.05). The 1-year mortality rate was 18.6% and 26.1% in the insulin-infusion and conventional therapy groups, respectively (p = 0.027). More recently, however, 2 major trials of insulin-glucose infusion in patients with ACS failed to demonstrate a mortality benefit (57,58). The DIGAMI 2 trial randomized 1,253 AMI patients with concomitant type 2 diabetes to insulin-glucose infusion followed by insulin-based long-term glucose control, insulin-glucose infusion plus standard glucose control, or routine metabolic management (57). All 3 groups had similar glycemic control throughout the study and, in particular, patients in the group receiving the combination of insulin–glucose infusion plus insulin-based long-term therapy did not reach their target goal (fasting glucose 90 to 126 mg/dl). At 24 h, fasting glucose concentrations in the 2 insulin-glucose infusion groups were lower than in the third group but still relatively high (164 mg/dl vs. 180 mg/dl). Because glucose is proinflammatory and proapoptotic, as previously discussed, it is possible that hyperglycemia masked the benefits of insulin therapy. The CREATE-ECLA (Clinical Trial of Reviparin and Metabolic Modulation in Acute Myocardial Infarction Treatment and Evaluation–Estudios Clinicos Latino America) study randomized 20,201 patients with ST-segment elevation MI to high-dose GIK infusion plus usual care or usual care alone (58). The GIK regimen, using 25 to 30 g of glucose and 6 U/h of insulin resulted in hyperglycemia (mean glucose level: 187 mg/dl). When baseline glucose levels in the control group were divided into tertiles, those in the lowest tertile of glucose (<126 mg/dl) had a mortality rate of 6.6%, the middle tertile (126 to 144 mg/dl) of 8.5%, and the highest tertile (>144 mg/dl) of 14%. In a reanalysis of the CREATE-ECLA data regarding hyperglycemia-related mortality, we calculated that the GIK group in CREATE-ECLA should have demonstrated an excess of mortality by 22% due to hyperglycemia induced by the GIK infusion (10% real mortality vs. 12.2% mortality as expected from the induced hyperglycemia) in GIK-treated patients (59). However, there was no increase in mortality in the GIK group when compared with the control group not infused with GIK. This led us to hypothesize that insulin in the GIK infusion protected patients from the adverse effects of hyperglycemia. This observation underlines the importance of maintaining euglycemia when considering insulin infusion treatment. Indeed, in a reanalysis of the CREATE-ECLA and the OASIS (Organization for the Assessment of Strategies for Ischemic Syndromes)–6 trial data, it was found that an increase in the incidence of mortality and heart failure was shown in patients infused with GIK in the first 3 days after MI, a phase during which hyperglycemia occurred (60). On the other hand, mortality and heart failure were significantly lower in 3- and 30-day periods during which hyperglycemia induced by GIK had resolved, and presumably, the cardioprotective effect of insulin was manifest. In these 2 studies, 40% of the subjects became hyperglycemic (post-randomization blood glucose >144 mg/dl) for 24 h after admission, and of this group, 62% of the subjects had been randomized to the GIK arm. The increase in mortality and heart failure in the first 3 days observed in the GIK group was attributed by the investigators to hyperglycemia, fluid overload, and hyperkalemia induced by GIK infusion (60). A post-randomization glucose of >144 mg/dl showed increased mortality at 3 days by 2.5-fold compared with those with post-randomization glucose of <126 mg/dl.

Another possible reason for the lack of benefit of GIK in CREATE-ECLA may have been the time of initiation of GIK after reperfusion therapy in the majority of patients. A recent study of GIK infusion at the time of reperfusion with thrombolytic agents (mean of 3 h following chest pain) demonstrated an impressive 88% reduction in major cardiovascular events at 1 year (61).

**Clinical Trials With Insulin Therapy in ICU and CABG Patients**

The first evidence on the outcomes of glycemic control with low-dose insulin infusions came from a study by Van den Berghe et al. in 2001 (62). In a prospective trial of 1,548 patients in a surgical ICU with a stay of >5 days, one-half of patients were infused with insulin, rendering them relatively euglycemic (fasting blood glucose: <110 mg/dl). Total mortality was reduced by 48%, the incidence of bacteremia by 46%, renal failure requiring dialysis by 41%, ICU neuroopathy by 44%, and the need for red blood cell transfusions by 50% when compared with control patients. Conversely, classical anti-inflammatory drugs such as the glucocorticoids have been shown to increase mortality in ICU patients (63) and, despite its anabolic effect, human growth hormone therapy has not shown improved outcomes (64); both therapies also increase plasma glucose concentrations.

Van den Berghe et al. (62) also showed that outcomes improved with low-dose insulin infusions and the reduction in CRP was more dependent on reduced plasma glucose levels than on the dose of insulin administered. Van den Berghe et al. (62) focused on restoration and maintenance of euglycemia; they did not examine the effects of maintaining high insulin concentrations, which would exert an independent anti-inflammatory effect and benefit of insulin beyond that achieved through blood glucose normalization. Indeed, in a subanalysis, they showed that plasma insulin concentrations were similar in the intensively treated and controlled subjects (65).

In a prospective study designed to reduce and maintain glucose <110 mg/dl in a medical ICU population requiring
admission for >3 days, Van den Berghe et al. (66) also showed that insulin therapy decreased mortality by 18% and reduced the duration of mechanical ventilation and incidence of renal injury compared with control patients (66). Other benefits included reduced length of stay in the ICU and the hospital. This is consistent with Kinsley’s (67) observations in the medical ICU of a community teaching hospital.

The role of lowering glucose concentrations in patients with diabetes undergoing CABG has been shown in a large observational study using historical controls (68). Improved glycemia (reduction of glucose from 213 to 177 mg/dl) was associated with a reduction in mortality from 5.3% to 2.5% (68). In a second, smaller randomized controlled trial, maintaining good glycemic control with insulin led to reductions in mortality, cardiac failure, and arrhythmias (69). A third, randomized trial in patients without diabetes undergoing CABG showed better myocardial function, decreased incidence of low cardiac output state, and a reduction in myocardial injury following treatment with GIK (70).

In a recent trial of intensive insulin therapy to improve glucose control in subjects with sepsis, controlling glucose to <110 mg/dl was not associated with an improvement in mortality (71). Hypoglycemia, which occurred more frequently in the intensively treated group, was an independent risk factor for death in this study. Hypoglycemia on admission or during hospitalization has also been identified as being associated with increased mortality in retrospective analyses of studies in ACS (72). It is possible that choosing a higher blood glucose target, between 110 and 130 mg/dl, and monitoring blood glucose more frequently reduces the incidence and duration of hypoglycemia while providing the benefit of lowering glucose and insulin administration in ACS.

**Conclusions**

Rapidly accumulating data demonstrate that the proinflammatory and pro-oxidant actions of glucose, in addition to the reactive oxygen species-suppressive and anti-inflammatory actions of insulin, likely play an important role in the pathogenesis and treatment of complications in patients with ACS (Fig. 1). Thus, maintenance of euglycemia with the help of insulin infusion may have a role in management of these patients. Treatment of hyperglycemia with insulin results in dual benefits—it lowers the proinflammatory effects of glucose and induces the anti-inflammatory effects of insulin. As observed in several studies of patients with AMI, regimens that infuse fixed doses of insulin with high rates of glucose infusion tend to be associated with hyperglycemia. The induction of hyperglycemia may potentially neutralize the benefits of insulin and thus these regimens should be avoided. This area clearly needs further definitive investigation. We are currently conducting a study to test whether low-dose insulin infusion that restores normoglycemia in

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**Figure 1 Rationale for the Use of Insulin in ACS**

The anti-inflammatory, antiapoptotic, cardioprotective, and neuroprotective effects of insulin have been demonstrated both in humans and in animal models. The vasodilatory, reactive oxygen species (ROS)-suppressive, antplatelet, antithrombotic, and profibrinolytic effects have been demonstrated in humans. ACS = acute coronary syndromes; cAMP = cyclic adenosine monophosphate; CRP = C-reactive protein; eNOS = endothelial nitric oxide synthase; FFA = free fatty acid; ICAM = intracellular adhesion molecule; IKB = intracellular kappa B; MCP = monocyte chemoattractant protein; NFkB = nuclear factor-kappa B; NO = nitric oxide; PAI = plasminogen activator inhibitor; TF = tissue factor.
patients with AMI will provide cardioprotection and improve clinical outcomes. We look forward to sharing these results with the cardiology community.

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REFERENCES


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