

Anti-inflammatory and Antiatherogenic Effects of Insulin

Paresh Dandona, BSc, MD, DPhil

*Distinguished Professor of Medicine and Pharmacology, School of Medicine and Biomedical Sciences
Division Head, Endocrinology and Metabolism, State University of New York at Buffalo, Buffalo, New York*

Ajay Chaudhuri, MD

*Assistant Professor of Medicine, Diabetes-Endocrinology Center of Western New York, Division of Endocrinology,
Kaleida Health/Millard Fillmore Hospital, Buffalo, New York*

Recent clinical and experimental studies have shown that chronic vascular inflammation is an important contributor to the development of atherosclerosis. Glucose is one of several stimuli that induce cellular and molecular mechanisms of inflammation. Elevated plasma glucose concentrations have been associated with increased risk of cardiovascular disease in patients with and without diabetes. Conversely, insulin produces a number of anti-inflammatory effects. Infusion of insulin reduces the production of reactive oxygen species (ROS) by mononuclear cells in obese but otherwise healthy individuals. Insulin suppresses the activity of nuclear factor (NF)- κ B (NF- κ B), a transcription factor for many inflammatory cytokines. It also reduces the production of tissue-damaging matrix metalloproteinases (MMPs) and of proteins like tissue factor that stimulate thrombosis. In patients receiving fibrinolytic therapy for acute myocardial infarction (AMI), infusion of insulin for 48 hours reduces several biochemical markers of inflammation and myocardial injury. Insulin may be a valuable adjunct to current therapies for AMI. Additional clinical research is needed to identify the optimal dose and timing for insulin administration.

GLUCOSE AND VASCULAR INFLAMMATION

Recent animal models and clinical studies have demonstrated that chronic vascular inflammation is an important contributor to the development of atherosclerotic plaque.¹ Markers of ongoing inflammation, such as C-reactive protein (CRP), are independent predictors of the risk of future cardiovascular disease among otherwise healthy adults.² A growing body of evidence also demonstrates that glucose is a pivotal proinflammatory and thrombogenic substance that may promote the development of coronary artery disease, and that insulin exerts rapid and powerful anti-inflammatory effects at normal physiologic concentrations. Recent clinical studies of patients with AMI have suggested that treatment with insulin may suppress inflammation and thrombogenesis and limit cardiac damage following ischemia.

At the molecular level, inflammation depends critically on the activity of the cytosolic protein NF- κ B.³ NF- κ B promotes the transcription of ~200 known genes, many of which trigger the production of inflammatory cytokines, ROS, chemokines that attract immune cells to sites of inflammation, and other inflammatory mediators. Under normal conditions, NF- κ B is sequestered within the cytosol by a family of NF- κ B inhibitors (the I κ B family). I κ B is phosphorylated by I κ B in response to endotoxin and other proinflammatory substances, liberating NF- κ B for transport to the nucleus.³

Thus, one definition of inflammation is an increase in NF- κ B in the nucleus and a decrease in I κ B in the cytosol.

The importance of glucose in the cellular and molecular mechanisms of inflammation was demonstrated in a study in which the formation of ROS by mononuclear cells (MNCs) and by polymorphonuclear leukocytes (PMNLs) was measured over 3 hours following the administration of a 75-g glucose challenge in 14 healthy subjects.⁴ In both MNCs and PMNLs, ROS generation increased to ~240% of baseline values within 2 hours of glucose administration, and began to gradually return to baseline after 3 hours (**Figure 1**). Glucose also increased the expression of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase—an enzyme which converts molecular oxygen to superoxide radical within 2 hours of exposure.

In subsequent studies, ROS generation was observed with administration of lipid and protein.⁵ Lipid intake induced ROS similar to that following glucose but to a lesser extent than with glucose while that following protein was much less. Glucose also increased the translocation of NF- κ B to the nucleus in MNCs and decreased the concentration of I κ B in the cytosol, demonstrating the inflammatory effects of glucose at the molecular level. Similar effects have now been shown following IV infusion of lipid and the elevation of free fatty acids to a level similar to that observed in the

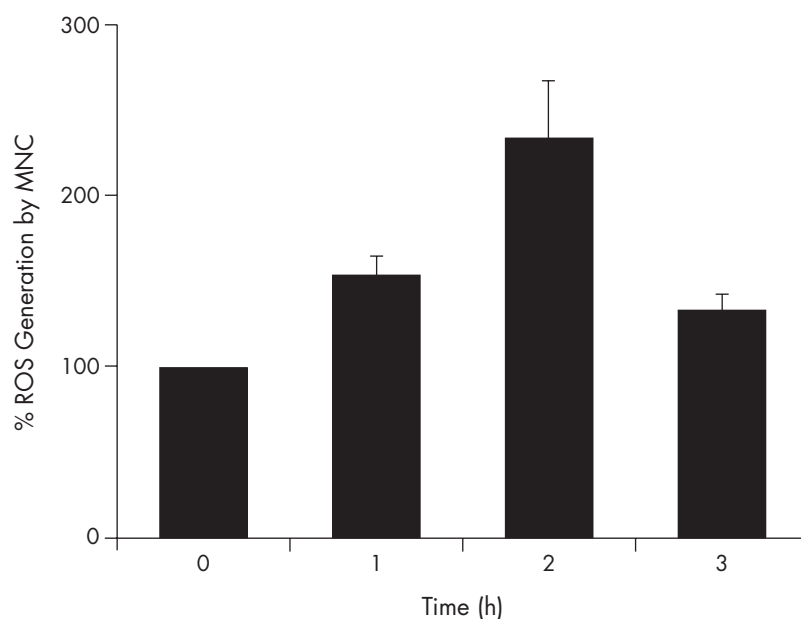


Figure 1. Increased reactive oxygen species (ROS) generation following glucose intake. MNC = mononuclear cell. Copyright 2000, The Endocrine Society. Reprinted from Mohanty P et al. Glucose challenge stimulates reactive oxygen species (ROS) generation by leucocytes. *J Clin Endocrinol Metab.* 2000;85(8):2970–2973.

obese. Glucose also induces an increase in activator-protein-1 and early growth response-1 (Egr-1), the 2 transcription factors which induce MMPs, tissue factor and plasminogen activation inhibitor protein-1 (PAI-1).⁶

In addition, clinical studies have shown that in patients with AMI, plasma glucose concentration at the time of hospitalization is related in a dose-response manner to mortality. This relationship has been noted for patients with and without diabetes. In a recent study in Germany, Meier et al⁷ examined survival over 48 months when patients with or without diabetes were grouped into tertiles on the basis of plasma glucose values at the time of hospitalization for AMI. For patients with diabetes, those in the highest plasma glucose tertile (plasma glucose values of 228–615 mg/dL) had a mortality rate of nearly 80% over the following 4 years. Mortality rates were approximately 40% to 50% in patients with diabetes in the lower glucose tertiles (glucose concentrations of 60–142 mg/dL and 143–227 mg/dL). Although the overall mortality rate was lower for patients without diabetes, nondiabetic patients who were in the highest plasma glucose tertile (plasma glucose values of 133–260 mg/dL) also had a probability of 40% mortality which was greater than that in patients in the 2 lower tertiles (50–105 mg/dL and 106–132 mg/dL). Similarly, in the Clinical Trial of Reperfusion and Metabolic Modulation in Acute Myocardial Infarction Treatment Evaluation—Estudios Cardiológicas Latin America Study Group (CREATE-ECLA)—a randomized clinical trial that compared the efficacy of glucose-insulin-potassium (GIK) infusion in more than 20,000 pa-

tients with AMI—baseline hyperglycemia was clearly associated with worse clinical outcomes.⁸ Mortality among patients in the lowest baseline plasma glucose tertile was 6.6%, compared with 8.5% and 14.0% for patients in the middle and highest tertiles, respectively.⁸ In addition to the proinflammatory effects of glucose, hyperglycemia is also associated with reduced coronary blood flow in AMI on angiography, reduced rates of recanalization following thrombolytic therapy and angioplasty, reduced rates of reversal of ST elevation following angioplasty or thrombolysis and diminished myocardial blush following angioplasty. (Myocardial blush is an index of capillary perfusion.) Treatment with insulin to restore euglycemia may reverse these effects of hyperglycemia and thus reduce myocardial damage.

ANTI-INFLAMMATORY EFFECTS OF INSULIN

In vitro studies performed in cultured human aortic endothelial cells have shown that insulin induces the expression of the vasodilator and anti-inflammatory agent nitric oxide, and also inhibits NF- κ B production.^{9,10} These findings raised the question of whether insulin is anti-inflammatory and antiatherogenic in the human endothelium in vivo. The anti-inflammatory effects of insulin were examined in 10 obese but otherwise healthy adults.¹¹ The patients received infusions of insulin at a rate of 2.5 U/h for 4 hours, followed by an additional 2-hour monitoring period. To prevent an insulin-induced hypoglycemic state, insulin was infused with a 5% dextrose solution at a rate of 5 g/h, which is just sufficient

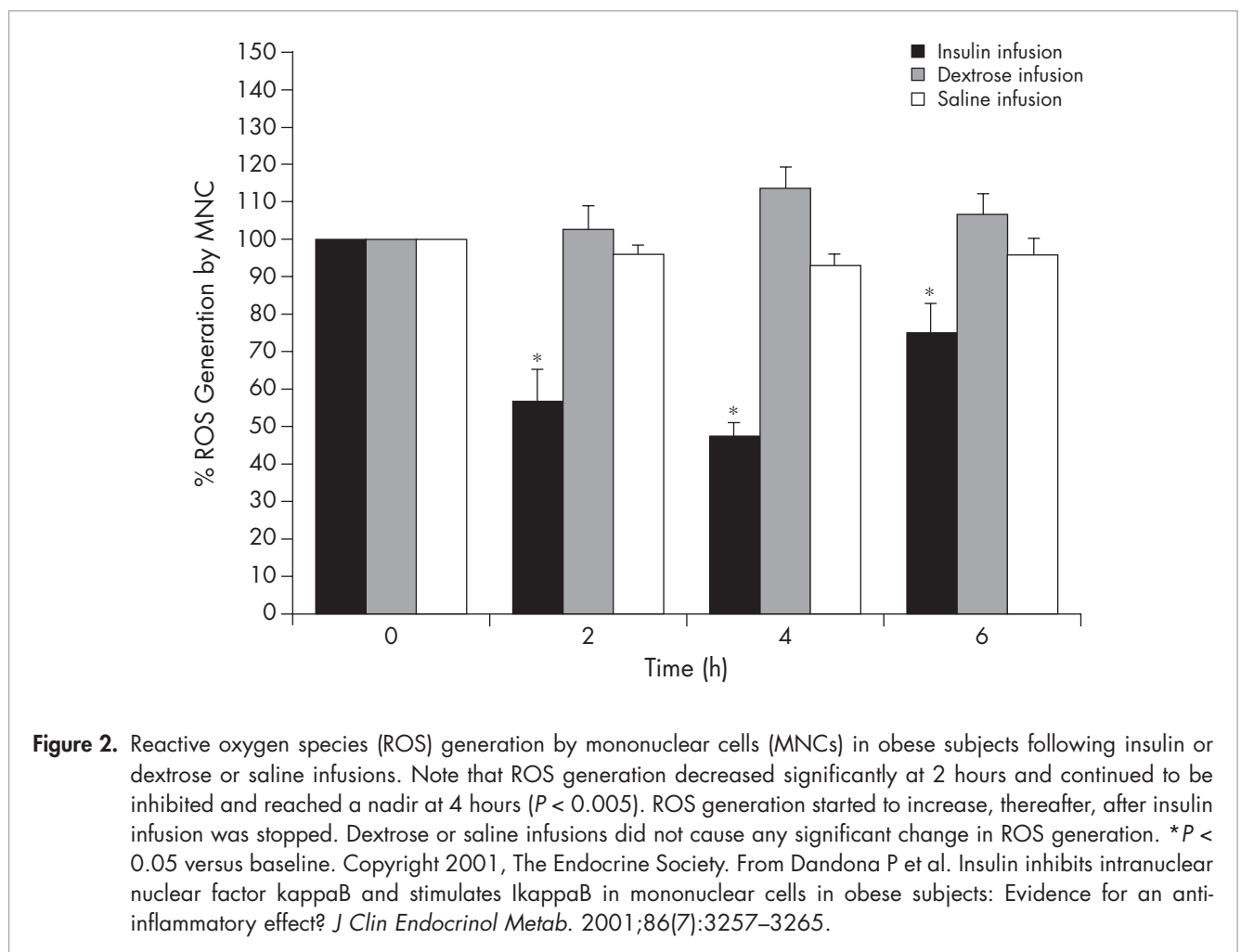
to prevent hypoglycemia. In addition, potassium chloride (10 mmol/h) was added to the infusate to prevent hypokalemia. After insulin infusion, the subjects returned 2 to 3 weeks later for control infusions of dextrose or saline solutions.

As expected, infusion of insulin solution, but not dextrose or saline, rapidly increased the plasma insulin concentration over the 4-hour infusion period, to a maximum of approximately double the baseline values. Due to the short half-life of insulin, insulin concentrations returned to baseline rapidly after discontinuation. The generation of ROS by MNC fell by ~40% within 2 hours of the start of insulin infusion and by ~50% after 4 hours, returning to ~25% below baseline value 2 hours after discontinuation (Figure 2).¹¹

Infusion of insulin, but not dextrose or saline, also suppressed several other markers of oxidation and inflammation, including p47^{phox} subunit of NADPH oxidase expression and NF- κ B binding in MNCs, demonstrating the anti-inflammatory effects of insulin infusions at the cellular level (Figure 3).¹¹ In contrast, the production of I κ B in MNCs was markedly increased following infusion of insulin (consistent with an anti-inflammatory effect), but not dextrose (5% dextrose, 100 mL/h) or saline solutions. Insulin infusion

decreased the mean plasma concentration of soluble intracellular adhesion molecule-1 and monocyte chemoattractant protein-1, which are important in the trafficking and adhesion of immune cells to sites of inflammation. Insulin infusion decreased transcription of the Egr-1 transcription factor, and 2 proteins that are regulated by Egr-1, tissue factor and PAI-1.¹² Tissue factor, an important component of the blood coagulation cascade, promotes the conversion of prothrombin to thrombin, which stimulates clot formation and platelet aggregation. PAI-1 is an endogenous inhibitor of fibrinolysis. These findings suggest that insulin may help to prevent the development of coronary thrombosis. In general, the broad range of anti-inflammatory effects with insulin infusion is similar to the anti-inflammatory effects of the glucocorticoids.¹¹

Insulin infusion also suppressed the production of vascular endothelial growth factor (VEGF),¹³ a cytokine that stimulates angiogenesis and that is thought to be an important contributor to the development of proliferative diabetic retinopathy.¹⁴ Some evidence has also suggested that VEGF may be important in heart disease. VEGF levels are elevated in patients with AMI¹⁵ and VEGF has been shown to



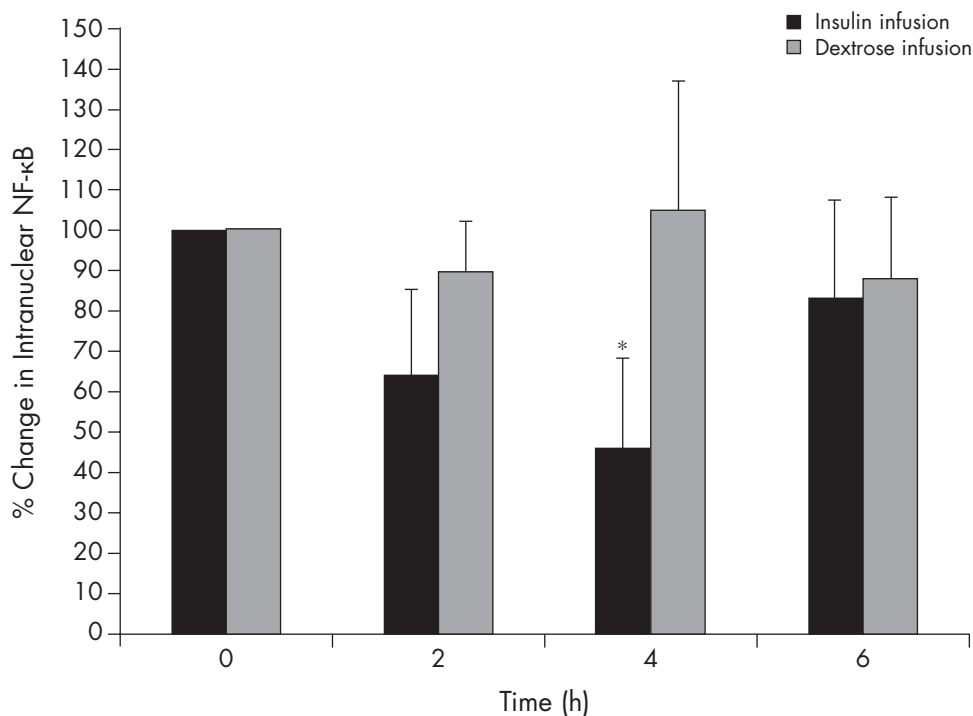


Figure 3. Relative nuclear factor (NF)- κ B binding to double-stranded oligonucleotide containing NF- κ B DNA binding site. All values were normalized to 100% for baseline levels and the following values were expressed as percent of basal. The results are presented as mean \pm SE. * $P < 0.05$ versus baseline. Copyright 2001, The Endocrine Society. From Dandona P et al. Insulin inhibits intracellular nuclear factor kappaB and stimulates IkappaB in mononuclear cells in obese subjects: Evidence for an anti-inflammatory effect? *J Clin Endocrinol Metab.* 2001;86(7):3257–3265.

increase infarct size in experimental myocardial infarction with a reduction in capillary perfusion.¹⁶ Neutralization of VEGF improves microcirculation in the infarcted myocardium and reduces the size of the infarct.

INSULIN IN ACUTE MYOCARDIAL INFARCTION

These studies, which demonstrated that insulin suppresses inflammation and other mechanisms of vascular disease, suggested the possibility that infusion of insulin may be beneficial as an acute treatment in patients with AMI. The effects of insulin infusion in this setting were evaluated in a recent study of patients with ST-elevation myocardial infarction.¹⁷ The patients were treated with fibrinolytic therapy with reteplase, IV heparin infusion, a statin, a β -blocker and either an angiotensin-converting enzyme inhibitor or an angiotensin-receptor blocker, and aspirin. They were then randomized to additional treatment with either an insulin regimen similar to that used in the studies described by us previously, or no additional treatment. Patients in the insulin infusion group received insulin at a dosage of 2.5 units per hour for 48 hours, with a 10% dextrose solution to maintain these patients in a euglycemic state. Treatment with the insulin infusion increased the plasma insulin concentration

within 2 hours, from a baseline value of 12 μ U/mL to more than 40 μ U/mL after infusion.

In the control group, CRP values began to increase after 6 hours, and continued to increase throughout the 48-hour infusion period. The CRP concentration also increased in the insulin infusion group, but the magnitude of the increase from baseline was ~40% less than that of the control group after 24 and 48 hours (**Figure 4**).¹⁷ At 48 hours, CRP concentration increased by a mean of 3.1 mg/mL in the insulin group and by 5.5 mg/mL in the control group ($P < 0.05$). This is a marked anti-inflammatory effect, and is among the most pronounced and rapid effects observed for any anti-inflammatory medication.¹⁷

A nearly identical pattern was observed for the systemic inflammatory marker amyloid A. Amyloid A increased in both groups during the 48 hours following hospitalization, but the magnitude of the increase was nearly 50% lower among the patients in the insulin group (24 mg/L) than among the control patients (46 mg/L; $P < 0.01$).¹⁷

Fibrinolytic medications induce the generation of PAI-1 by the endothelium, which is thought to at least partly counter the beneficial effects of fibrinolytic therapy in AMI.¹⁸ Infusion of insulin also significantly attenuated the increase in PAI-1 over 48 hours, compared with the control infusion.

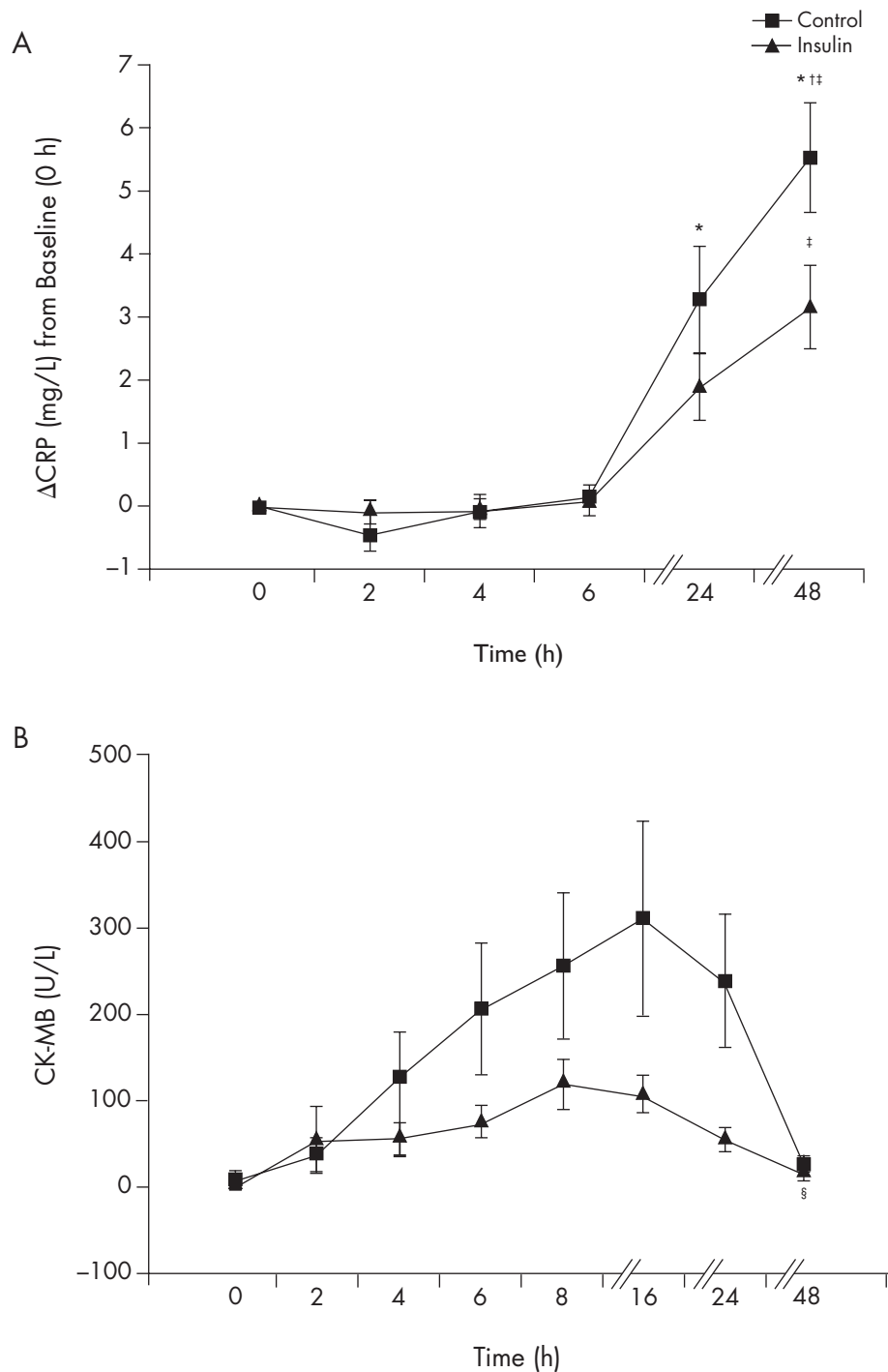


Figure 4. (A) Time course of the absolute difference in mean \pm SEM C-reactive protein (CRP) concentration from baseline (0 hour) over 48 hours. (B) Creative kinase-muscle and brain (CK-MB) in inferior wall myocardial infarction. Absolute values are presented as mean \pm SEM. * $P < 0.05$ between groups at 24 and 48 hours (2-way analysis of variance [ANOVA]); † $P < 0.05$ between groups at 48 hours (t test); ‡ $P < 0.01$ within group, 1-way repeated-measures ANOVA. § $P < 0.01$ between groups by 2-way ANOVA after logarithmic transformation. From Chaudhuri A et al. Anti-inflammatory and profibrinolytic effect of insulin in acute ST-segment-elevation myocardial infarction. *Circulation*. 2004;109:849–854; with permission.

This suggests that in addition to its anti-inflammatory effects, insulin may enhance the beneficial effects of fibrinolytic therapy in patients with AMI.

A decrease from baseline in the concentration of VEGF was noted for both the insulin and control infusion groups. This decrease may be related to the use of heparin, which is an inhibitor of VEGF.¹⁹ This study also examined the effects of insulin on the production of p47^{phox}, a marker for the generation of ROS by the enzyme NADPH oxidase. In the control patients, p47^{phox} release from MNCs increased steadily over 48 hours. This increase was prevented by infusion of the insulin solution.

Insulin-treated patients also exhibited lower levels of MMPs and free fatty acids than controls. At the time of hospital admission, free fatty acid concentration was ~1 mmol/L in both groups, which is about 3 times the normal value. Heparin infusion, which stimulates lipolysis, triggered an additional rise in free fatty acid concentration, reaching a peak of ~1.6 mmol/L after 2 hours. This increase in free fatty acid concentration was prevented by insulin infusion.

Several measures also suggested that insulin infusion provided significant cardioprotection in AMI in patients with inferior wall infarcts, the largest single group in this study. Measurements of inferior wall creatine kinase (CK) and CK muscle and brain (CK-MB) fraction revealed increased values for both markers within 2 to 4 hours after presentation, reaching a peak at about 16 hours and declining toward baseline after 48 hours (Figure 4). The increases in CK and CK-MB were significantly lower among the patients who received the insulin infusion. A similar effect was observed for myoglobin, another protein released from the injured myocardium after AMI.

A very similar pattern of results on CRP and serum amyloid-A (SAA) concentrations was noted in a recent study in which patients who underwent coronary artery bypass grafting (CABG) received perioperative insulin and glucose for at least 25 hours after surgery.²⁰ The CABG procedure produces an extensive systemic inflammatory response as a result of invasive surgery, extensive manipulation of the chest cavity, and the use of extracorporeal circulation. Although CABG produces a 30 times greater increase in plasma CRP concentration than is associated with AMI, these investigators found that insulin infusion using a protocol very similar to the infusion protocol used in patients with AMI significantly reduced serum concentrations of CRP and SAA. Both inflammatory markers were ~40% lower in the insulin-treated group than among controls, a finding that closely parallels the effects of insulin infusion among patients with AMI. The suppressive effect of insulin has also been confirmed in patients with AMI in a study from Australia.²¹

CONFLICTING DATA FROM VARIOUS CLINICAL STUDIES UTILIZING INSULIN GLUCOSE REGIMENS

Although there are studies showing improvements in clinical outcomes following the infusion of insulin with glucose and potassium, there are several studies showing no beneficial effects of such a regimen. However, there are no studies showing an adverse effect of such a regimen. Such contradictory findings need to be explained. Some of the factors which may have contributed to these observations are: (1) induction of hyperglycemia following the use of the old GIK regimen which infuse as much as 25 to 30 g/h of glucose in contrast with 5 g/h glucose or less in our work; and (2) the interval between the onset of ischemic pain and the initiation of insulin infusion. Our study was based on insulin infusion which was started 90 minutes after the onset of pain. In the CREATE-ECLA study, the infusion was started a mean 24 hours after the onset of pain. In the Hyperglycemia: Intensive Insulin Infusion In Infarction study the infusion was started 13 hours after the onset of pain. This is important since irreversible myocardial damage occurs within 6 hours after the onset of pain. Therefore, initiation of therapy after that time is not likely to be very effective in protecting the myocardium.

It is important to point out that despite the induction of hyperglycemia by the GIK regimen of an order similar to that in the highest tertile in the CREATE-ECLA trial, mortality was not enhanced by the regimen in this study. Thus, there may be a "hidden" protective effect of insulin.

Clearly, future studies based on insulin regimens which restore euglycemia, accompanied by glucose infusions at rates just sufficient to prevent hypoglycemia, given at early and appropriate times, need to be tested to define insulin's beneficial effects in AMI.

SUMMARY AND CONCLUSIONS

Elevated plasma glucose triggers inflammation by activating cellular signaling pathways that are similar to those used by endotoxin or other proinflammatory substances. In patients with AMI, the risk of long-term mortality is approximately doubled for patients in the highest plasma glucose concentration tertile at the time of hospitalization. Insulin infusion following an AMI reduces markers of inflammation, thrombosis, and myocardial injury; similar results have been observed in patients undergoing CABG surgery. Thus, the reduction of glucose concentration by insulin infusions may constitute an effective cardioprotective strategy during AMI.

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Address correspondence to: Paresh Dandona, BSc, MD, DPhil, Division Head, Endocrinology and Metabolism, State University of New York at Buffalo, Buffalo, NY 14209. E-mail: pdandona@kaleidahealth.org