

MOLECULAR MECHANISMS BY WHICH METABOLIC CONTROL MAY IMPROVE OUTCOMES

Derek Le Roith, MD, PhD

ABSTRACT

Objective: To summarize available evidence providing potential explanations for metabolic and nonmetabolic abnormalities during conditions of acute stress and possible mechanisms whereby insulin therapy may affect these changes.

Results: Recent studies have demonstrated a remarkable effect of intensive insulin therapy and reductions in morbidity and mortality in patients in intensive-care units and other hospital settings. The mechanisms involved in these effects are under thorough investigation. Insulin therapy improves glucose and lipid homeostasis, both of which are deleterious to the tissues, especially during severe stress. In addition, insulin has direct effects on the levels of inflammatory cytokines and other proteins that may influence the overall outcome of patients undergoing various stressful conditions.

Conclusion: Analysis of published studies suggests that the beneficial effects of insulin therapy may be derived from both direct and indirect mechanisms. (*Endocr Pract.* 2004;10[Suppl 2]:57-62)

Abbreviations:

DCCT = Diabetes Control and Complications Trial; **FAs** = fatty acids; **GAPDH** = glyceraldehyde phosphate dehydrogenase; **ICAM-1** = intercellular adhesion molecule-1; **IL** = interleukin; **IRS** = insulin receptor substrate; **NF- κ B** = nuclear factor- κ B; **NO** = nitric oxide; **PAI-1** = plasminogen activator inhibitor-1; **PI3K** = phosphatidylinositol 3'-kinase; **ROS** = reactive oxygen species; **TNF- α** = tumor necrosis factor- α

INTRODUCTION

Recently, considerable interest has been expressed in the use of insulin as an important aspect of "in-hospital" therapy for patients without diabetes who have major acute stressful conditions, such as those in the intensive-care unit. These patients may be under stress as a result of myocardial infarction or acute inflammation, such as that seen in sepsis. This focus has drawn attention to the need for understanding the basic mechanisms involved in the setting of stress. Both metabolic (glycemic) and nonmetabolic processes have been implicated. Although these processes may be addressed separately, they also may be interrelated. This article will summarize some of the evidence that is thought to explain the metabolic and nonmetabolic abnormalities created under conditions of acute stress and the possible mechanisms whereby insulin therapy may affect these changes. The first part of this report will focus on the metabolic changes that can be corrected by insulin therapy. The second part will address the nonmetabolic changes that occur, which may also be affected by insulin therapy.

THE "VICIOUS CYCLE" OF INSULIN RESISTANCE

The biologic response to multiple acute and chronic stress-related conditions involves proinflammatory cytokines, such as tumor necrosis factor- α (TNF- α) and interleukin (IL)-6. One of the side effects (or "side benefits") of an increase in TNF- α levels is the induction of insulin resistance (Fig. 1). TNF- α has been shown to interfere with the actions of insulin in multiple insulin-sensitive tissues by inducing serine phosphorylation of the insulin receptor substrate (IRS) proteins. IRS proteins function as docking sites that interact with both the insulin receptor and various downstream signaling molecules. Phosphorylation of IRS proteins at specific serine residues can block the ability of the insulin receptor tyrosine kinase to phosphorylate IRS proteins on tyrosine residues, a process that is necessary for normal insulin action. Insulin resistance and increases in TNF- α levels enhance lipolysis. The resulting release of fatty acids (FAs) is associated with increased serine phosphorylation of IRS (1,2).

From the Diabetes Branch, National Institutes of Health, Bethesda, Maryland.

Presented at the American College of Endocrinology Inpatient Diabetes and Metabolic Control Conference, Washington, DC, December 14 and 15, 2003.

© 2004 AACE.

Although the exact mechanism by which serine phosphorylation of IRS occurs has not been fully elucidated, the I- κ β kinase has been identified as a possible mediator in this process. Thus, a vicious cycle may develop, whereby TNF- α induces lipolysis and, together with FAs released from adipocytes, causes insulin resistance in muscle, liver, and adipocytes, thereby leading to the further release of FAs (Fig. 1). One of the conclusions that may eventually be drawn from this hypothetical vicious cycle is that insulin therapy corrects the glycemic abnormality but concomitantly reverses FA-induced "lipotoxicity." Because adipocytes are exquisitely sensitive to insulin, they could respond to the antilipolytic effects of insulin even as muscle and liver maintain some degree of insulin non-responsiveness.

Thus, insulin resistance is almost certainly common under conditions of acute stress. Such insulin resistance can increase blood glucose levels, and this hyperglycemia should be reversible by insulin therapy.

ACUTE MYOCARDIAL INFARCTION

In addition to their well-known long-term effects, high blood glucose levels have short-term effects on the vasculature. Under normal aerobic conditions, the myocardium utilizes FAs as fuel in the resting state (3). In

contrast, after a meal (when increased serum insulin levels inhibit fat cell lipolysis), glucose becomes the major source of myocardial fuel. Oliver and Opie (3) showed that FAs may impair myocardial function after acute myocardial infarction by increasing oxygen consumption (Fig. 2). This situation interferes with normal calcium channel function and increases the likelihood of reperfusion arrhythmias. The stress after the period of infarction is associated with a substantial increase in release of catecholamines, which, in turn, inhibits the release of insulin from pancreatic beta cells, increases lipolysis, and increases circulating FA levels.

During the postinfarction period, infusion of glucose, insulin, and potassium allows the ischemic myocardium to use glucose as its major fuel, by means of glycolysis. This process decreases reperfusion ventricular arrhythmias in humans and reduces infarct size in animals (4). This paradigm once again suggests that insulin therapy can have an important role in metabolic control under conditions of acute stress.

In the Diabetes and Insulin-Glucose Infusion in Acute Myocardial Infarction (DIGAMI) study, patients with diabetes who had an acute myocardial infarction underwent a randomized trial of insulin and glucose infusion, followed by intensive subcutaneous insulin treatment in the hospital (5). This treatment was then continued for several months

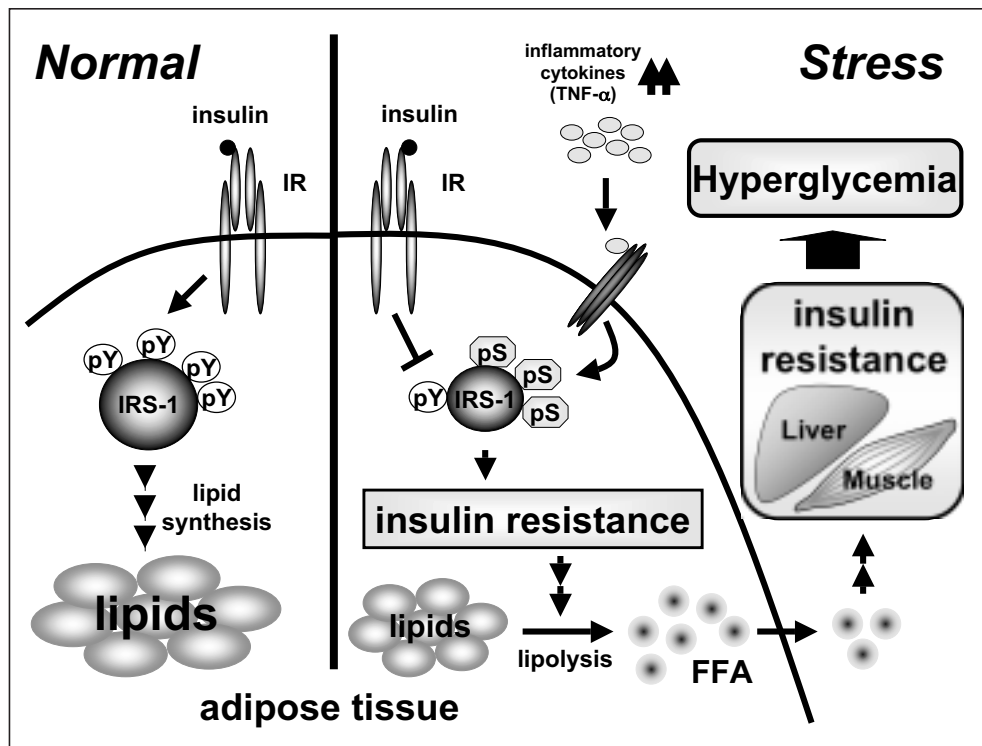


Fig. 1. Normally, through the insulin receptor (IR) signaling cascades, insulin leads to lipogenesis. In contrast, during stress, inflammatory cytokines can interfere with insulin signaling by increasing serine phosphorylation (pS) of insulin receptor substrate (IRS) molecules and preventing tyrosine phosphorylation (pY) of these proteins. This situation leads to lipolysis with release of free fatty acids (FFA) and a secondary effect on liver and muscle, worsening the insulin resistance in those tissues. TNF- α = tumor necrosis factor- α .

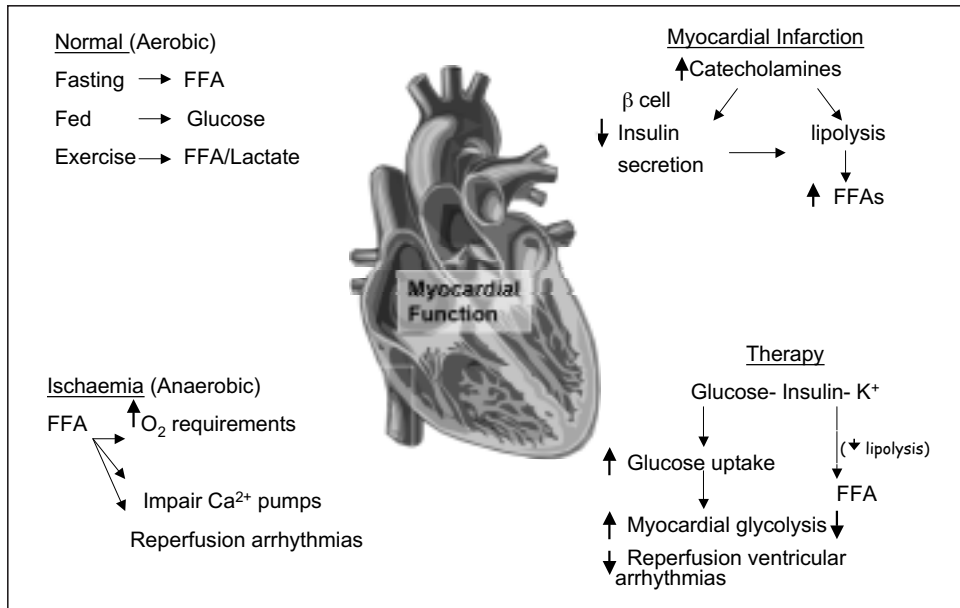


Fig. 2. Under normal aerobic conditions, the cardiac muscle utilizes free fatty acids (FFA) as fuel in the fasting or exercise state and glucose in the fed state. Acute myocardial infarction is often associated with catecholamine excess, leading to impaired insulin secretion and increased FFA release from adipocytes. When the myocardium is ischemic, FFA may cause cardiac impairment and arrhythmias. Thus, use of a glucose-insulin-potassium therapeutic regimen after acute myocardial infarction (ischemia) may prevent the arrhythmias and improve outcomes.

after their dismissal from the hospital. Short-term survival and, more interestingly, long-term survival were significantly improved by this regimen. The immediate short-term effect could be explained by the aforementioned mechanisms. The basis for improved long-term survival, however, remains unknown. One possible explanation is “metabolic memory”—that is, the intensive therapy given initially may have profound long-lasting effects, similar to that seen in the Epidemiology of Diabetes Interventions and Complications (EDIC) trial (6). This study was a follow-up of the Diabetes Control and Complications Trial (DCCT). Seven years after the DCCT, hemoglobin A1c levels in the previous intensive treatment and conventional treatment groups were similar (8.1% and 8.3%). The group that received previous intensive treatment, however, continued to exhibit a significant reduction (approximately 60%) in certain risk factors for vascular disease, such as the intima-media thickness of the carotid artery and coronary calcification (7).

In a recent example of the usefulness of insulin therapy in the acute intensive-care setting, Van den Berghe et al (8) demonstrated that insulin treatment was valuable for treating the hyperglycemic state. Even in patients without apparent diabetes, insulin treatment appreciably reduced mortality and morbidity. This result led to the proposal that insulin be used routinely (under strict supervision and with careful monitoring) for acutely ill patients in the inpatient setting. Nevertheless, the exact mechanism by which insulin improves outcomes in such patients has not been well defined and is the subject of intense investigation.

POSSIBLE NONGLYCEMIC EFFECTS OF INSULIN

Effects of Insulin on Blood Flow

Insulin increases blood flow in skeletal muscle beds, and this process is dependent on endothelial-derived nitric oxide (NO) (9). The increased NO production by insulin in these cells is mediated through the activated insulin receptor, by means of tyrosine phosphorylation of IRS proteins and the phosphatidylinositol 3'-kinase (PI3'K) pathway. The increased blood flow induced by insulin also contributes substantially to glucose disposal by delivering increased amounts of substrate to skeletal muscle.

Increased levels of FAs in vivo and the addition of FAs to cultured endothelial cells in vitro increase the serine phosphorylation state of IRS molecules. This scenario, in turn, blocks insulin activation of PI3'K and results in reduced activation of endothelial NO synthase and lower NO levels. This is yet another example of how FAs are implicated in blocking an important aspect of insulin action on vascular function, both in the acute setting and with respect to the chronic complications of diabetes. These effects of FAs on endothelial dysfunction also contribute to metabolic insulin resistance, by decreasing the delivery of substrate to skeletal muscle.

Insulin and the Vasculature

The effects of insulin on the vasculature may occur both directly, by affecting the production of proinflammatory cytokines, and indirectly, by lowering ambient blood

glucose levels. At the cellular level, nuclear factor- $\kappa\beta$ (NF- $\kappa\beta$) induces the expression of proinflammatory cytokines such as TNF- α , IL-6, and IL-1 β ; adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1; and chemokines such as monocyte chemoattractant protein-1 and C-reactive protein. Interestingly, in vascular-derived endothelial and smooth muscle cells, insulin inhibits the expression of these proinflammatory proteins (10).

Under normal conditions, insulin can induce the expression of NO synthase in endothelial cells. This outcome leads to the production of NO, which in turn results in vasodilation and anti-inflammatory effects. The induction of NO by insulin uses the PI3'K pathway (10), which is frequently inhibited in states of insulin resistance, such as obesity, type 2 diabetes, and acute stress. The mechanism by which this important insulin signaling pathway is inhibited involves high circulating levels of FAs, increased stores of tissue triglycerides, and long-chain FAs—so-called lipotoxicity. Apparently, long-chain FAs can also lead to serine phosphorylation of the IRS molecules. As discussed in the foregoing material, this process interferes with the normal tyrosine phosphorylation of IRS induced by the insulin receptor tyrosine kinase and explains the block in the downstream PI3'K signaling

pathway. The eventual results are inhibition of insulin-induced glucose uptake in muscle and fat cells and inhibition of production of NO in vascular-derived cells.

Vessel Wall Inflammatory Processes

Vessel wall injury leads to an “inflammatory” process, whereby proinflammatory cytokines induce the expression of adhesion molecules on endothelial cells and the secretion of chemokines, which attract and attach to monocytes. These activated monocytes generate superoxide (O_2^-) radicals. NF- $\kappa\beta$ is a redox-sensitive transcription factor that responds to reactive oxygen species (ROS) by enhancing the expression of proinflammatory cytokines.

ROS and cytokines induce phosphorylation of I- $\kappa\beta$, which releases NF- $\kappa\beta$ to the nucleus. In that site, it induces the gene expression of adhesion molecules, proinflammatory cytokines, and chemokines.

Hyperglycemia is also involved in the induction of proinflammatory molecules (Fig. 3). On one hand, glucotoxicity can inhibit insulin secretion and insulin action, whereas on the other hand, glucose has direct proinflammatory effects by reducing cytosolic I- $\kappa\beta$ levels, a situation that then allows the nuclear transfer of NF- $\kappa\beta$ in endothelial cells.

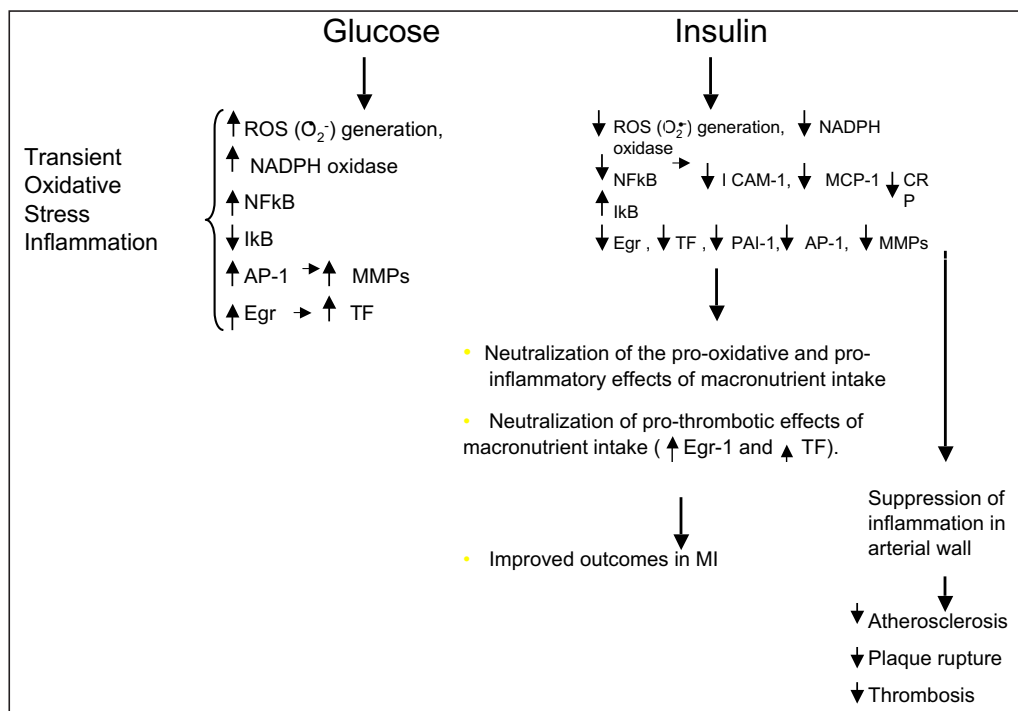


Fig. 3. Insulin may improve vascular wall structure and function by direct or indirect mechanisms. Excessive macronutrients, including glucose, have negative effects on vascular endothelial and smooth muscle cells by inducing the generation of reactive oxygen species (ROS) and many proinflammatory proteins, including early growth response (*Egr*) transcription factor and activator protein-1 (*AP-1*), that lead to increased production of matrix metalloproteinases (*MMPs*) and tissue factor (*TF*). Insulin indirectly potentially reverses these effects by reducing the circulating glucose and directly inhibits ROS, cell adhesion molecules such as intercellular adhesion molecule-1 (*ICAM-1*), and other proteins that are involved in the inflammatory process such as C-reactive protein (*CRP*) and plasminogen activator inhibitor-1 (*PAI-1*). *MCP-1* = monocyte chemoattractant protein-1; *MI* = myocardial infarction (ischemia); *NADPH* = reduced form of nicotinamide adenine dinucleotide phosphate; *NF- $\kappa\beta$* = nuclear factor- $\kappa\beta$.

The early growth response transcription factor also responds to oxidative stress, cytokines, and physical damage to blood vessels. It induces the expression of TNF- α , ICAM-1, plasminogen activator inhibitor-1 (PAI-1), matrix metalloproteinases, and other related molecules (11).

Insulin suppresses the activation of both NF- κ B and the early growth response transcription factor. It also suppresses the production of ROS, whereas glucose stimulates the production of ROS. Thus, in endothelial cells, insulin can stimulate I- κ B expression and inhibit NF- κ B binding activity; thereby the expression of PAI-1 and ICAM-1 is suppressed. This action also reduces the generation of ROS and inhibits the reduced form of nicotinamide adenine dinucleotide phosphate oxidase activity. The decrease in expression of the adhesion molecule ICAM-1 and the chemokine monocyte chemoattractant protein-1 is associated with increased induction of endothelial NO synthase and NO generation.

Insulin also has potential antithrombotic effects, in that it acts as a vasodilator, through generation of NO. In this way, insulin inhibits platelet function through the NO-cyclic guanosine monophosphate pathway.

Glucose enhances the production of ROS and facilitates their endothelial effects. Thus, reduction of the blood glucose levels by insulin therapy may partially explain some of the benefits to patients in the acute intensive-care setting.

Glucose and Oxidative Stress

Oxidative stress with increased ROS results in oxidized nucleic acids, protein, carbohydrates, and lipids (lipid peroxidation). O₂⁻ also inactivates NO. Glucose induces the reduced form of nicotinamide adenine dinucleotide phosphate oxidase and causes an increase in ROS, which in turn activate the NF- κ B, activator protein-1, and early growth response transcription factors. Thus, glucose and other macronutrients have proinflammatory effects.

One mechanism whereby hyperglycemia (and FAs) may increase transcription of PAI-1 is through the hexosamine pathway. Hyperglycemia increases glycolysis and generates mitochondrial superoxide anions. An excess of superoxide anions, in turn, inhibits glyceraldehyde phosphate dehydrogenase (GAPDH). This inhibition of GAPDH is mediated by poly(adenosine diphosphate-ribose) polymerase, which is activated after hyperglycemia-induced superoxide production and DNA strand breaks (11).

Inhibition of GAPDH results in the shunting of fructose-6-phosphate through the hexosamine pathway and increases uridine diphosphate-*N*-acetylglucosamine. The end result is *O*-*N*-acetylglucosamine acylation of Sp1, a transcription factor that is activated and enhances PAI-1 gene expression as well as the expression of certain growth factors in aortic endothelial cells. Reversal of this effect may be one mechanism whereby insulin reduces PAI-1 levels, by reversing the hyperglycemia (12).

“OPPOSING VIEWPOINT”

One possible explanation for the increased expression of adhesion molecules on vascular endothelial cells in patients with insulin resistance was provided by Montagnani et al (13). Insulin resistance is associated with inhibition of the PI3'K pathway, which interferes with the ability of insulin to stimulate metabolic events in muscle, fat, and liver. This block in PI3'K is also seen in endothelial cells and may be associated with increased signaling through the mitogen-activated protein kinase pathway as a result of the hyperinsulinemia. Activation of this pathway in these cells is associated with enhanced expression of vascular cell adhesion molecule-1 and E-selectin. Thus, this model proposes that hyperinsulinemia, attributable to insulin resistance, is the cause of increased expression of adhesion molecules on vascular endothelial cells. This process may be relevant to both the acute complications exhibited by inpatients and the chronic complications of diabetes.

How to reconcile these findings with those described in the foregoing material remains a mystery. Further studies should help to explain these observations.

CONCLUSION

The use of insulin therapy in patients in an acute stressful setting in the hospital and the ensuing improvement in clinical outcomes raise interesting questions about the potential glycemic and nonglycemic events that occur under these conditions. Both glucose and FAs can be implicated as major players in the effects on vascular endothelial cells that lead to potential complications for these patients. In addition, increasing evidence suggests that insulin may have direct effects— independent of its metabolic functions—that influence endothelial cell function, thrombosis, and blood flow. How the effects of insulin improve outcomes in this setting is as yet unknown, as are the basic mechanisms underlying the actions of insulin at the cellular level. Overall, these are exciting times for collective assessment of information from the clinical and basic science settings.

REFERENCES

1. Liu YF, Paz K, Herschkovitz A, et al. Insulin stimulates PKCzeta-mediated phosphorylation of insulin receptor substrate-1 (IRS-1): a self-attenuated mechanism to negatively regulate the function of IRS proteins. *J Biol Chem.* 2001;276:14459-14465.
2. Hotamisligil GS, Murray DL, Choy LN, Spiegelman BM. Tumor necrosis factor alpha inhibits signaling from the insulin receptor. *Proc Natl Acad Sci U S A.* 1994;91:4854-4858.
3. Oliver MF, Opie LH. Effects of glucose and fatty acids on myocardial ischaemia and arrhythmias. *Lancet.* 1994;343:155-158.
4. Jonassen AK, Sack MN, Mjøs OD, Yellon DM. 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.

5. **Malmberg K (Diabetes Mellitus, Insulin Glucose Infusion in Acute Myocardial Infarction [DIGAMI] Study Group).** Prospective randomised study of intensive insulin treatment on long term survival after acute myocardial infarction in patients with diabetes mellitus. *BMJ.* 1997;314:1512-1515.
6. **Writing Team for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group.** Effect of intensive therapy on the microvascular complications of type 1 diabetes mellitus. *JAMA.* 2002;287:2563-2569.
7. **Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group.** Intensive diabetes therapy and carotid intima-media thickness in type 1 diabetes mellitus. *N Engl J Med.* 2003;348:2294-2303.
8. **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.
9. **Aljada A.** Endothelium, inflammation and diabetes. *Metab Syndrome Related Disord.* 2003;1:3-22.
10. **Montagnani M, Quon MJ.** Insulin action in vascular endothelium: potential mechanisms linking insulin resistance with hypertension. *Diabetes Obes Metab.* 2000;2:285-292.
11. **Du X, Matsumura T, Edelstein D, et al.** Inhibition of GAPDH activity by poly(ADP-ribose) polymerase activates three major pathways of hyperglycemic damage in endothelial cells. *J Clin Invest.* 2003;112:1049-1057.
12. **Du XL, Edelstein D, Rossetti L.** Hyperglycemia-induced mitochondrial superoxide overproduction activates the hexosamine pathway and induces plasminogen activator inhibitor-1 expression by increasing Sp1 glycosylation. *Proc Natl Acad Sci U S A.* 2000;97:12222-12226.
13. **Montagnani M, Golovchenko I, Kim I, et al.** Inhibition of phosphatidylinositol 3-kinase enhances mitogenic actions of insulin in endothelial cells. *J Biol Chem.* 2002;277:1794-1799.