

EFFECT OF INSULIN THERAPY ON NONGLYCEMIC VARIABLES DURING ACUTE ILLNESS

Irl B. Hirsch, MD

ABSTRACT

Objective: To review the possible mechanisms for the reported clinical finding of better outcomes for hospitalized and critically ill patients as the result of improved metabolic control.

Results: Insulin inhibits free fatty acids, proinflammatory cytokines, and inflammatory growth factors, all of which may be detrimental in critically ill patients. Furthermore, insulin enhances nitric oxide synthesis, which promotes vasodilation. The mechanisms of insulin regulation of these factors are complex, although insulin seems to have a direct effect on the transcriptional factor, nuclear factor- $\kappa\beta$ (NF- $\kappa\beta$). In turn, NF- $\kappa\beta$ modulates the proinflammatory cytokines, adhesion molecules, and chemokines. In a euglycemic or slightly hyperglycemic environment, NF- $\kappa\beta$ is suppressed by insulin; however, with more profound hyperglycemia, NF- $\kappa\beta$ is induced and the proinflammatory cytokines are thus increased.

Conclusion: Although considerable research must be completed to identify the apparent relationship between stringent metabolic control and improved outcomes in acutely ill patients, current evidence suggests that both the treatment (glucose-insulin-potassium infusion) and the resultant plasma glucose concentrations may be independent important components of the underlying mechanisms. (*Endocr Pract.* 2004;10[Suppl 2]:63-70)

Abbreviations:

AP-1 = activator protein-1; **CRP** = C-reactive protein; **Erg-1** = early growth response gene-1; **FFAs** = free fatty acids; **IL** = interleukin; **NF- $\kappa\beta$** = nuclear factor- $\kappa\beta$; **NO** = nitric oxide; **NOS** = nitric oxide synthase; **PAI-1** = plasminogen activator inhibitor-1; **ROS** = reactive oxygen species; **TNF- α** = tumor necrosis factor- α

INTRODUCTION

The discovery of insulin more than 80 years ago is still considered one of the greatest scientific achievements (1). Patients with type 1 diabetes—primarily children—were saved from certain death by injection of bovine or porcine soluble insulin. Over time, the insulin preparations were improved, the structure of insulin was identified, and the relationships between control of diabetes and occurrence of complications were clarified. Enhanced understanding of the physiologic role of insulin in regulating carbohydrate, protein, and fat metabolism has reinforced the importance of insulin therapy in managing both type 1 and type 2 diabetes; however, recent publication of several clinical trials suggests that insulin may have even broader application (2-6). Indeed, the use of insulin for acute myocardial infarction in a nondiabetic population was first introduced in the 1960s, and a meta-analysis of 9 studies (N = 1,932) revealed a reduction of absolute, relative, and proportional hospital mortalities of 4.9%, 23.3%, and 28%, respectively (7).

Evidence suggests that these outcomes may be attributed to more than lowered blood glucose concentrations. For example, the difference in blood glucose levels between the intensive insulin therapy and the control group in the landmark study by Van den Berghe et al (2) was only 50 mg/dL (2.8 mmol/L). Indeed, the more striking difference between the two study groups was that 99% of subjects in the intensive therapy group (N = 765) received an intravenous insulin infusion in comparison with only 39% in the control group (N = 783).

In another recently published trial from The Netherlands, patients with acute myocardial infarction were randomized to a glucose-insulin-potassium infusion as an adjunct to primary transluminal coronary angioplasty (N = 940) (8). Only 10.5% of the study subjects had diabetes mellitus, and the presence or absence of diabetes had no effect on 30-day mortality. For those patients without signs of heart failure (Killip class 1), 30-day mortality was 1.2% in the group that received glucose-insulin-potassium infusion compared with 4.2% in the control group (relative risk, 0.28; 95% confidence interval, 0.1 to 0.75). Of importance, no differences were noted in admission or in-hospital plasma glucose levels.

From the University of Washington School of Medicine, Seattle, Washington.

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If insulin has one or more functions distinct from the classic teachings of metabolism, especially the reduction of blood glucose concentration, what might those be? Several candidate hypotheses will be reviewed.

OVERVIEW

The endocrinologic features of acute and chronic illness are complex. The initial adaptive neuroendocrine response to critical illness consists of activated anterior pituitary function, whereas anabolic target organ hormones, with the exception of cortisol, are inactivated (9). In the chronic phase of critical illness, a uniformly reduced pulsatile secretion of these hormones has been observed in relationship to reduced levels of target hormones—again, with cortisol being the notable exception.

Advances in our understanding of this endocrine response to stress have raised provocative questions. For example, inflammatory cytokines have been investigated as possible mediators of the changes observed with growth hormone and insulin-like growth factor-I, the acute low triiodothyronine syndrome, and secretion of adrenocorticotrophic hormone (corticotropin) (9-11). Is it possible that these same cytokines could be involved in the pathogenesis of sepsis, cardiogenic shock, and thrombosis in critically ill patients? Additionally, inflammatory growth factors have been shown to be involved in various aspects of atherosclerosis. Free fatty acids (FFAs), at high levels in this hormonal milieu promoting lipolysis, have been implicated for years as having deleterious effects on arrhythmias and thrombosis. Finally, data on endothelial nitric oxide (NO) and endothelial function suggest its possible role in treating critically ill patients. Of interest, all four of these possibilities are regulated by insulin.

INFLAMMATORY CYTOKINES

Levels of circulating inflammatory markers are often high during severe illness and acute myocardial infarction (12). Epidemiologic studies have also found an increased risk of vascular disease with increased basal levels of cytokines, such as interleukin (IL)-6 and tumor necrosis factor- α (TNF- α) (13-15); cell adhesion molecules, such as intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 (16); and downstream acute-phase reactants, such as C-reactive protein (CRP), fibrinogen, and serum amyloid A (13,17,18).

Most of the cytokines are released from macrophages and monocytes, but they are also released from various other sites. Besides regulating the acute-phase proteins in acute illness, cytokines are responsible for controlling other neuroendocrine responses. For example, IL-6 is required for the final steps leading to fever (17). IL-6 also stimulates arginine vasopressin, an action that leads to hyponatremia in some inflammatory disorders. Proinflammatory cytokines stimulate corticotropin-releasing hormone, with eventual elevation of cortisol levels (17). In

addition, cytokines seem to be responsible for the anemia associated with chronic disease and the thrombocytosis of inflammation (19). The cachexia noted in patients with cancer apparently is attributable to IL-1, IL-6, TNF- α , and interferon- γ (20). TNF- α is implicated in inflammation, cell apoptosis and survival, cytotoxicity, production of IL-1 and IL-6, and induction of insulin resistance in numerous clinical settings.

Although an increase in cytokines can result in some adaptive functions, these cytokines can cause a fatal outcome, as seen with sepsis (21). Proinflammatory cytokines, such as TNF- α and IL-2, mediate the T-cell activation involved with fatty streak formation in atherosclerosis (22). Moreover, investigators have suggested that simultaneous administration of neutralizing antibodies to TNF- α and IL-1 β abolishes the cardioprotective action of exercise (23). TNF- α is responsible for the “cardiac cachexia” seen in conjunction with advanced congestive heart failure, which is similar to that in patients having chronic inflammation or a malignant lesion (24). Furthermore, a positive relationship seems to exist between circulating TNF- α levels and the clinical features of heart failure; after cardiac transplantation, TNF- α levels decrease (25). Interestingly, TNF- α has been found to decrease myocardial contractility in a dose-dependent manner (25).

TNF- α can cause endothelial dysfunction by enhancing the generation of free radicals. Indeed, TNF- α causes apoptosis of endothelial cells. By inducing damage to endothelial cells, this cytokine can also trigger procoagulant activity and fibrin deposition (26). TNF- α -induced synthesis of NO can have a beneficial or a detrimental role in heart failure. The NO may cause vasodilation, which is beneficial for counteracting the vasoconstriction induced by the sympathetic nervous system; nevertheless in some cases, the vasodilation may overcompensate and result in hypotension (27).

The cytokine IL-6 has also been shown to be involved in the pathogenesis of atherosclerosis. For example, IL-6 was injected in C57B1/6 and apolipoprotein E-deficient mice. Subsequently, IL-6, IL-1 β , TNF- α , fibrinogen, and lesion size increased in comparison with findings in saline-injected mice (28).

Although the regulation of these inflammatory cytokines is complex, one fact is clear: insulin will reduce the transcription of proinflammatory genes, adhesion molecules, chemokines, and the enzymes responsible for generating reactive oxygen species (ROS) by regulation of nuclear factor- κ B (NF- κ B) (29). For example, in humans without diabetes, insulin has been shown to suppress NF- κ B and induce an inhibitor of NF- κ B (I- κ B) in the cytosol (30) (Fig. 1). In that same study, low-dose insulin infusion resulted in a reduction of ROS, soluble intercellular adhesion molecule-1, monocyte chemoattractant protein-1, and plasminogen activator inhibitor-1 (PAI-1) (30). Similar results were reported in human aortic endothelial cells (31). Furthermore, CRP levels were reduced in the inten-

sive insulin therapy group in the landmark intensive-care unit study (32). Thus, insulin seems to have anti-inflammatory effects similar to those of glucocorticoids (33,34).

An immediate paradox arises when one notes that in cultured vascular smooth muscle cells, insulin, in the environment of glucose at a concentration of 450 mg/dL (25 mmol/L), actually activates NF- κ B (35) (Fig. 2). With this extreme hyperglycemia without hyperinsulinemia, NF- κ B activation was increased twofold, but after addition of 100 nmol/L of insulin and incubation for 24 hours, this activation was increased approximately sevenfold. Furthermore, this activation by insulin is dose-dependent (35). One interpretation of this paradox is that perhaps hyperinsulinemia in the context of euglycemia or mild hyperglycemia, as noted in the study reported by Van den Berghe et al (2), results in a down-regulation of NF- κ B with a subsequent reduction of proinflammatory cytokines, adhesion molecules, chemokines, and ROS. This possibility is further supported by the report that when insulin is suppressed by octreotide and plasma glucose is clamped at 270 mg/dL (15 mmol/L) for 5 hours, increases are noted in IL-6, TNF- α , and IL-18 (36) (Fig. 3).

In the Diabetes and Insulin-Glucose Infusion in Acute Myocardial Infarction (DIGAMI) study, after 24 hours of an intravenous insulin infusion, the blood glucose level decreased from 277 mg/dL (15.4 mmol/L) to 173 mg/dL (9.6 mmol/L); in contrast for the control group, the glucose level decreased from 283 mg/dL (15.7 mmol/L) to 211 mg/dL (11.7 mmol/L) (37). Although the patients receiving intravenous insulin infusion had hyperglycemia, the possibility that NF- κ B was still suppressed relative to the control group is compelling. This is supported by the data of Furnary et al (4), who reported that mortality more than doubled with subcutaneous administration of insulin and a mean blood glucose concentration of 213 mg/dL (11.8 mmol/L) in comparison with a 3-day insulin infusion and a mean blood glucose value of 177 mg/dL (9.8 mmol/L). No published in vitro studies have examined a threshold effect for blood glucose for NF- κ B changes relative to different insulin concentrations. Of further interest, patients treated with insulin have shown a reduction in hemoglobin A1c, from 11.8% to 8.6%, which also resulted in a decrease in CRP levels (38). That study, however, found no changes in IL-6 or TNF- α (38). Importantly, with insulin therapy, fasting plasma glucose averaged 178 mg/dL (9.9 mmol/L).

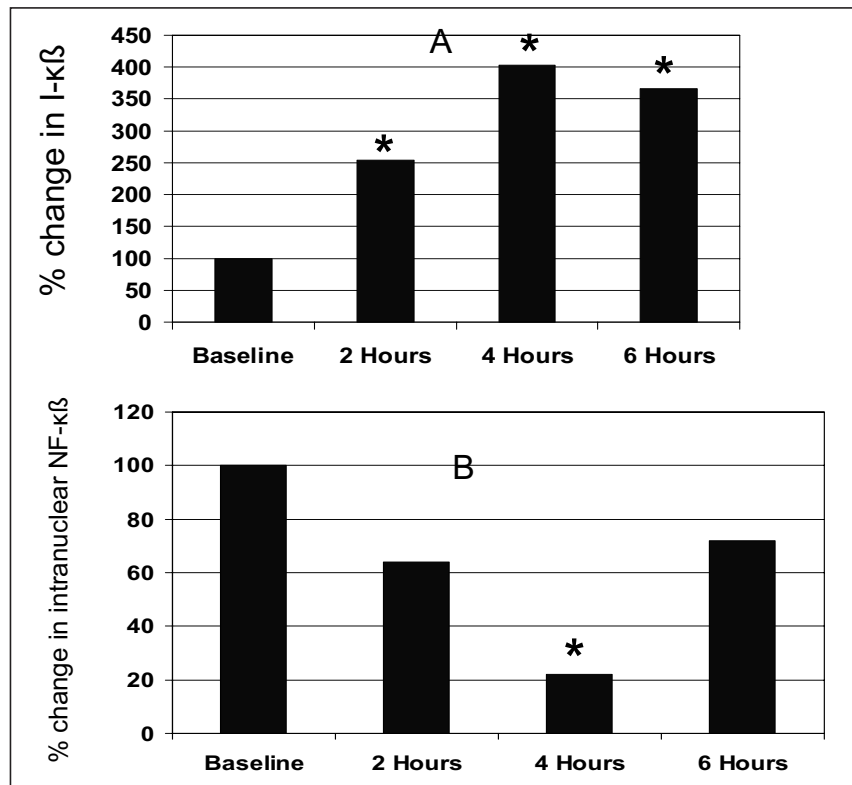


Fig. 1. Effect of a 4-hour insulin infusion on cytoplasmic inhibitor (*I-κB*) of nuclear factor- κ B (*NF-κB*) as well as on *NF-κB*. *A*, Results of mononuclear cell *I-κB* densitometric quantitative analysis. *B*, Relative *NF-κB* binding to double-stranded oligonucleotide containing *NF-κB* DNA binding site. All values were normalized to 100% for baseline levels. **P*<0.05 versus baseline. Reprinted with permission from Dandona et al (30).

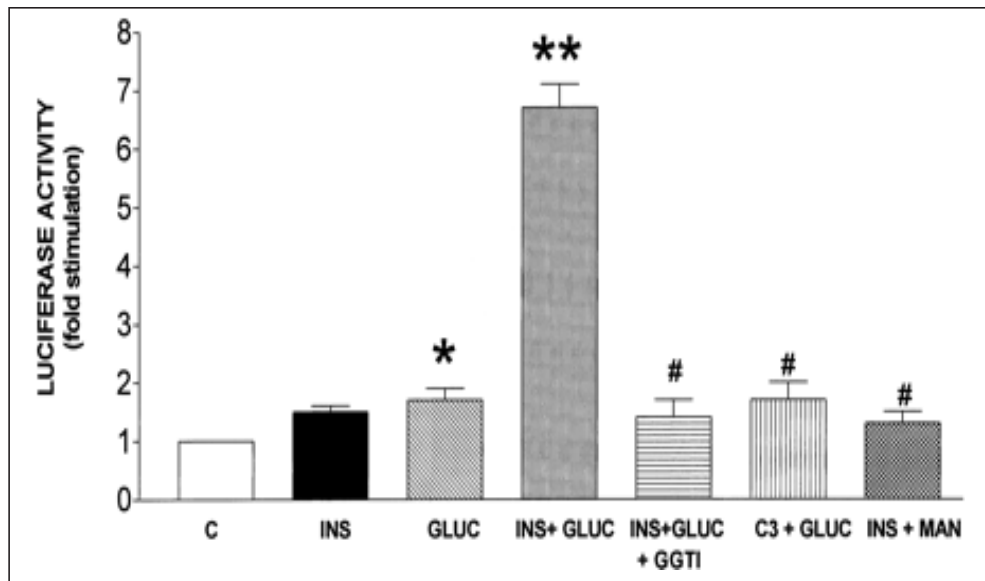


Fig. 2. Potentiating effect of insulin on the ability of hyperglycemia to activate nuclear factor- κ B (NF- κ B) response-element Luc construct in vascular smooth muscle cells. Preincubations of these cells with 100 nmol/L of insulin (*INS*) for 24 hours augmented NF- κ B-dependent transactivation by hyperglycemia from twofold (glucose alone [*GLUC*]) to approximately sevenfold (insulin + glucose [*INS+GLUC*]). A combination of insulin with mannitol (*INS+MAN*) in similar concentrations was without effect; thus, a nonspecific effect of hyperosmolarity was excluded. The priming effect of insulin on glucose was absent in the presence of GGTI-286 (*GGTI*), an indication that insulin potentiates NF- κ B-mediated transcription by means of the rho pathway. *C3+GLUC* = C-3 toxin + glucose (40 nmol/L). * $P < 0.05$ versus control (*C*); ** $P < 0.01$ versus glucose; # $P < 0.01$ versus insulin + glucose. Reprinted with permission from Golovchenko et al (35).

INFLAMMATORY GROWTH FACTORS

Two other proinflammatory growth factors are regulated by insulin. The first is early growth response gene-1 (Erg-1), a transcription factor that responds to various stimuli but seems to have an important role in the pathogenesis of atherosclerosis (39). Erg-1 regulates the expression of tissue factor and PAI-1 (Fig. 4). Tissue factor, in turn, will lead to the generation of thrombin, a platelet proaggregator that also converts fibrinogen to fibrin, a process that results in the eventual evolution of thrombus. Insulin has been shown to suppress Erg-1 and the expression of tissue factor and PAI-1 (40). These characteristics perhaps explain some of the beneficial effects of insulin during an acute myocardial infarction.

Another transcription factor, activator protein-1 (AP-1), regulates the expression of matrix metalloproteinases (Fig. 4). In turn, matrix metalloproteinases mediate the rupture of the atherosclerotic plaque, which triggers thrombosis (41). Insulin has also been shown to suppress AP-1 (42).

NITRIC OXIDE

Under physiologic conditions, continuous basal synthesis of NO from the vascular endothelium contributes to coronary flow. In addition, NO provides other effects that may protect against atherosclerosis. These potentially protective effects include inhibition of platelet adhesion and

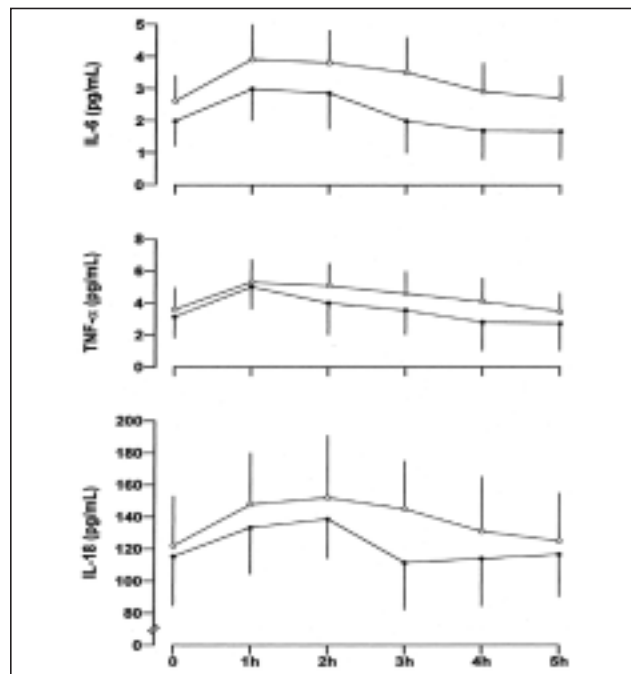


Fig. 3. Circulating cytokine levels during hyperglycemic clamps in 20 control subjects (*closed circles*) and in 12 subjects with impaired glucose tolerance (*open circles*). The increase in plasma cytokine levels during the clamping lasted longer in those with impaired glucose tolerance than in control subjects (4 hours versus 2 hours, respectively; $P < 0.01$), and circulating cytokines returned to basal levels at 5 hours. *IL* = interleukin; *TNF- α* = tumor necrosis factor- α . Reprinted with permission from Esposito et al (36).

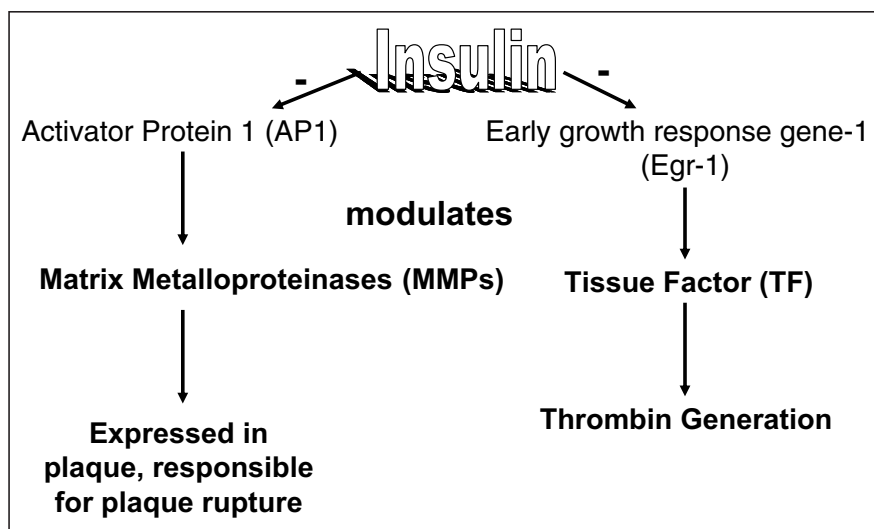


Fig. 4. The effect of insulin on inflammatory growth factors activator protein-1 and early growth response gene-1.

aggregation, reduction of cytokine-induced expression of tissue factor, inhibition of monocyte adhesion to the endothelium, and inhibition of intimal hyperplasia after endothelial injury.

NO, regulated by nitric oxide synthase (NOS), seems to be mediated by insulin (43,44) (Fig. 5). Insulin has been shown to increase NOS; the result is an acute vasodilatory action of NO on the vascular endothelium (45). Although endothelial dysfunction is known to be a hallmark of insulin resistance, insulin has been shown to improve forearm vasodilation (46). NO also has an antiaggregation

effect on platelets (47). Thus, it should not be surprising that this effect has been observed as an increase in blood flow of the lower (43) and upper (48) limbs, in addition to dilation of the carotid artery (49).

FREE FATTY ACIDS

Approximately 40 years ago, Randle et al (50) proposed that FFAs impair insulin-mediated glucose utilization. Since then, considerable information on the effects of FFAs has become available—particularly with respect to

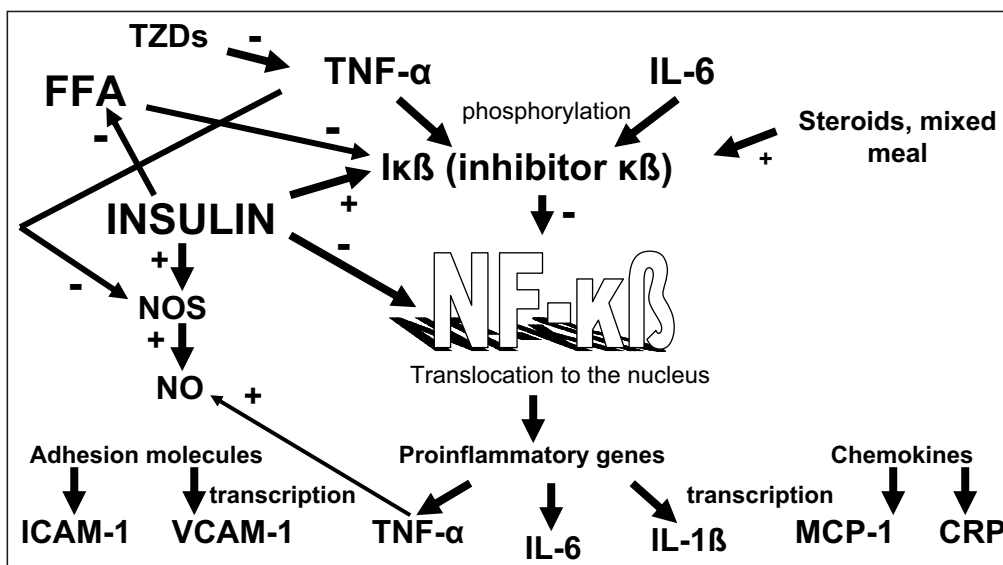


Fig. 5. The relationship of insulin to free fatty acids (FFA), nitric oxide (NO), adhesion molecules, chemokines, and proinflammatory cytokines. Insulin inhibits FFAs, stimulates nitric oxide synthase (NOS), and inhibits nuclear factor-κβ (NF-κβ), which in turn modulates adhesion molecules, proinflammatory cytokines, and chemokines. CRP = C-reactive protein; I-κβ = inhibitor of NF-κβ; ICAM-1 = intercellular adhesion molecule-1; IL = interleukin; MCP-1 = monocyte chemoattractant protein-1; TNF-α = tumor necrosis factor-α; TZDs = thiazolidinediones; VCAM-1 = vascular cell adhesion molecule-1. See text for further details.

production of glucose from the liver (51). In acutely ill patients, relative insulin deficiency, increased catecholamines, and TNF- α all stimulate lipolysis and raise plasma FFA levels. Evidence associating increased FFA concentrations and fatal arrhythmias was first recognized in 1966 (52). Thirty-five years later, this finding was confirmed in the Paris Prospective Study (53). Further support of this finding comes from a report that the infusion of FFAs can induce ventricular fibrillation (54).

More recently, increased levels of FFAs have been associated with cardiac sympathetic overactivity and an increase in oxidative stress in patients with type 2 diabetes (55,56). High concentrations of FFAs were also shown to cause endothelial dysfunction in a dose-dependent relationship (57). Furthermore, FFAs have been shown to increase PAI-1 transcription and secretion by endothelial cells in vivo (58).

FFAs are inhibited by insulin (Fig. 5), and, not surprisingly, this normal inhibition is resistant in obese patients with normotension or hypertension (59). Changes in insulin-induced FFA suppression in different degrees of illness have not been studied.

POTENTIAL NONGLYCEMIC ROLE OF INSULIN IN ACUTE ILLNESS

Several hypotheses have been formulated to explain the association between glucose-insulin-potassium infusion and improved outcomes in acutely ill hospitalized patients. Insulin is likely involved in many different cell systems, perhaps some of which have not yet been identified.

Insulin both inhibits FFAs and stimulates NOS, actions that result in an increase in NO (Fig. 5). As discussed earlier, these factors yield various positive effects. In the environment of euglycemia or mild hyperglycemia, insulin will enhance a cytoplasmic inhibitor of NF- $\kappa\beta$ (I- $\kappa\beta$); the result will be a reduction of NF- $\kappa\beta$. In a hyperglycemic environment, however, the available evidence suggests that insulin will enhance NF- $\kappa\beta$.

NF- $\kappa\beta$ modulates the transcription of the adhesion molecules, the proinflammatory cytokines, and the chemokines. There is a regulation on NF- $\kappa\beta$ through feedback of some of these cytokines (for example, TNF- α and IL-6). The "cytokine storm" noted in severely ill patients also results in an increase in acute-phase proteins. Furthermore, TNF- α enhances the synthesis of NO. This effect could be considered adaptive to promoting vasodilation in an acutely ill patient, but it may be maladaptive and result in shock in others.

Insulin has also been reported to suppress AP-1 and Erg-1, transcription factors involved with plaque rupture and thrombosis, respectively (Fig. 4). Finally, insulin therapy has been shown to improve endothelial function, perhaps through the suppression of FFAs.

CONCLUSION

After many years of controversy, strong available evidence indicates that strict metabolic control improves outcomes in acutely ill patients. The studies are not usually able to distinguish the role of the treatment (the glucose-insulin-potassium infusion) from the role of the resultant plasma glucose levels. Nevertheless, compelling data now suggest that *both* the treatment and the plasma glucose concentrations may be independently influential components of the mechanisms of the improved outcomes. Clearly, additional studies must be undertaken to investigate how metabolic control, with particular reference to insulin therapy, may influence outcomes in hospitalized patients with diabetes.

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