

Nitric Oxide and Glycemic Control

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SUMMARY

Glycemic control is a critical process for the Intensive Care Unit (ICU) patient. Severe bacterial Infection (i.e. sepsis) or severe stress evokes a cytokine “storm” leading to pro-inflammatory imbalance and metabolic dysregulation. Following a seminal study in 2001 [1] intense and exogenous insulin therapy (glycemic control of 80-110 mg/dL) gained support for deterring end organ failure and mortality in the ICU patient population. However, conflicting evidence suggests a lack of benefit with this therapy and a potential risk for adverse effects associated with hypoglycemia [2]. Furthermore, the cell and molecular mechanisms which regulate insulin sensitivity in this setting remain an important area of interest. Recent studies demonstrate insulin resistance is determined, in part, by the overabundance of nitric oxide species generation following heightened innate immunity. This book chapter will provide new evidence for the regulation of insulin sensitivity by nitric oxide-dependent mechanisms during severe sepsis and key observations related to the topic, including: 1) Regulation of cardiovascular function by specific nitric oxide synthases, 2) Barriers of muscle glucose uptake and 3) Hepatic glucose output. Overall, insulin sensitivity during severe sepsis and hemodynamic shock is partially determined by the overabundance of nitric oxide species. Understanding the integrated physiological response to nitric oxide may, thus, help improve strategies against hypoglycemia and mortality in the ICU.

INTRODUCTION TO SEPSIS AND HYPOGLYCEMIA IN THE ICU

Epidemiology

Sepsis is the host response to microbial infection which can include hypoperfusion of tissues, physiological organ dysfunction, and hypotension. In 1995, the incidence of severe sepsis in the U.S was estimated at ~750,000 cases per year with a ~29% mortality rate [3]. Thus, ~215,000 deaths occur as a result of this condition each year. The average cost per case was \$19,330 in 2007 with an economic burden of \$23.4 billion annually to the U.S. health care system [4]. Overall, it ranks 10th as the leading cause of death in U.S [5]. Antibiotic resistance may also continue to increase the prevalence of sepsis in the future.

Molecular Physiology of Inflammation

Infection triggers the innate immune system to defend and adapt against noxious stimuli [6]. During physiological inflammation, increased blood flow and recruitment of leukocytes promote the restoration of homeostasis. Numerous advances have helped elucidate the cell and molecular pathways which respond during an acute inflammatory response.

Initially, resident macrophages are known to immediately respond via activation of the Toll-Like Receptor-4 (**TLR-4**). Due to its high affinity for TLR4, the bacterial cell wall component, Lipopolysaccharide (**LPS**), activates the resident macrophage (Figure 1). In 1979-80, this relationship was originally discovered in several mouse strains (3H/He], C57BL/10ScCr (**Cr**) and C57BL/10ScN) which possessed mutations in the TLR4 gene and were phenotyped as “LPS-resistant” or possessed low responsiveness [7-9]. Later, other cell surface co-receptors were shown to be required for the interaction of TLR4 and LPS, including CD14 and LPS Binding Protein (**LBP**) [10]. Subsequently, a pro-inflammatory signaling cascade initiates the intracellular phosphorylation of I-Kappa-Kappa (**IKK**), the ubiquitination and degradation of I-Kappa-Beta (**IKB**) and the release of NF-Kappa-Beta (p50/p65 subunits) for translocation into the nucleus (Figure 1). The downstream effect of NF-kappaB pathway is a potent transcription factor for numerous pro- and anti-inflammatory genes [11,12]. Following just a few hours of LPS stimulation, microarray expression profiling and transcription factor binding motif assays reveal TLR signaling in macrophages controls the expression of several hundred genes, including TNF-alpha, IL-6, IL-1beta, IL-10, iNOS, A20 and IKB [13]. This coordinated, but complex molecular program in macrophages is the foundation for an innate immune response during bacterial infection. (Figure 2) [14]. Cytokines further trigger hepatocytes and macrophages (Kupffer Cells) of the liver to synthesize Acute Phase Proteins (**APP**) as part of the early response to infection. APP proteins, such as C-Reactive Protein (**CRP**), haptoglobin, and Serum Amyloid A (**SAA**), promote a systemic milieu aimed for resolution and re-establishing homeostasis [15]. Following 24 hours, livers from Sprague Dawley rats receiving LPS (3mg/kg) were shown to differentially express 508 genes vs a control; whereby 248 were elevated and 260 were down regulated [16]. Thus, gene function in

liver shifts from metabolic to immunologic and demonstrates a dynamic role of this organ during systemic inflammation. In response to innate immune activation of liver, systemic inflammation due to LPS can generate elevated levels of pro-inflammatory cytokines in plasma, such as TNF-alpha, IL-6, IL-1beta. In C57/bl6 mice, this response is sensitive to the dosage of intravenous LPS administered (1 vs. 10mg/kg) [17]. Recently, Interleukin-3 (**IL-3**) has also been implicated in the progression of sepsis [18]. IL-3 deficiency in mice improved survival following a sepsis model of cecal ligation and puncture ($p<0.001$). In human sepsis patients, IL-3 plasma levels ≥ 89.4 pg/ml were also associated with decreased survival ($p=0.001$).

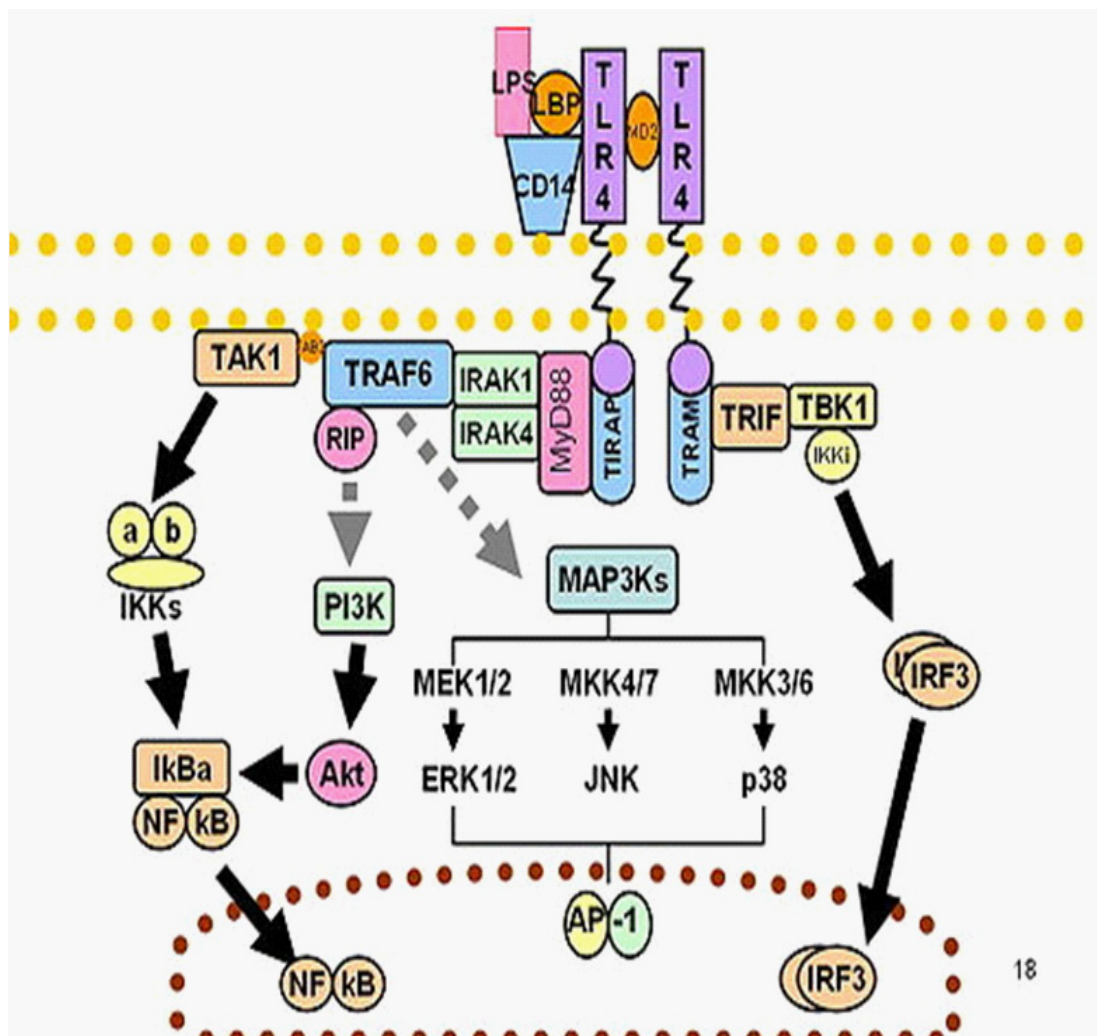


Figure 1: Activation of the Toll-like Receptor 4 (**TLR4**) by LPS and intracellular signaling. (https://upload.wikimedia.org/wikipedia/commons/8/85/Toll-like_receptor_pathways_revised.jpg)

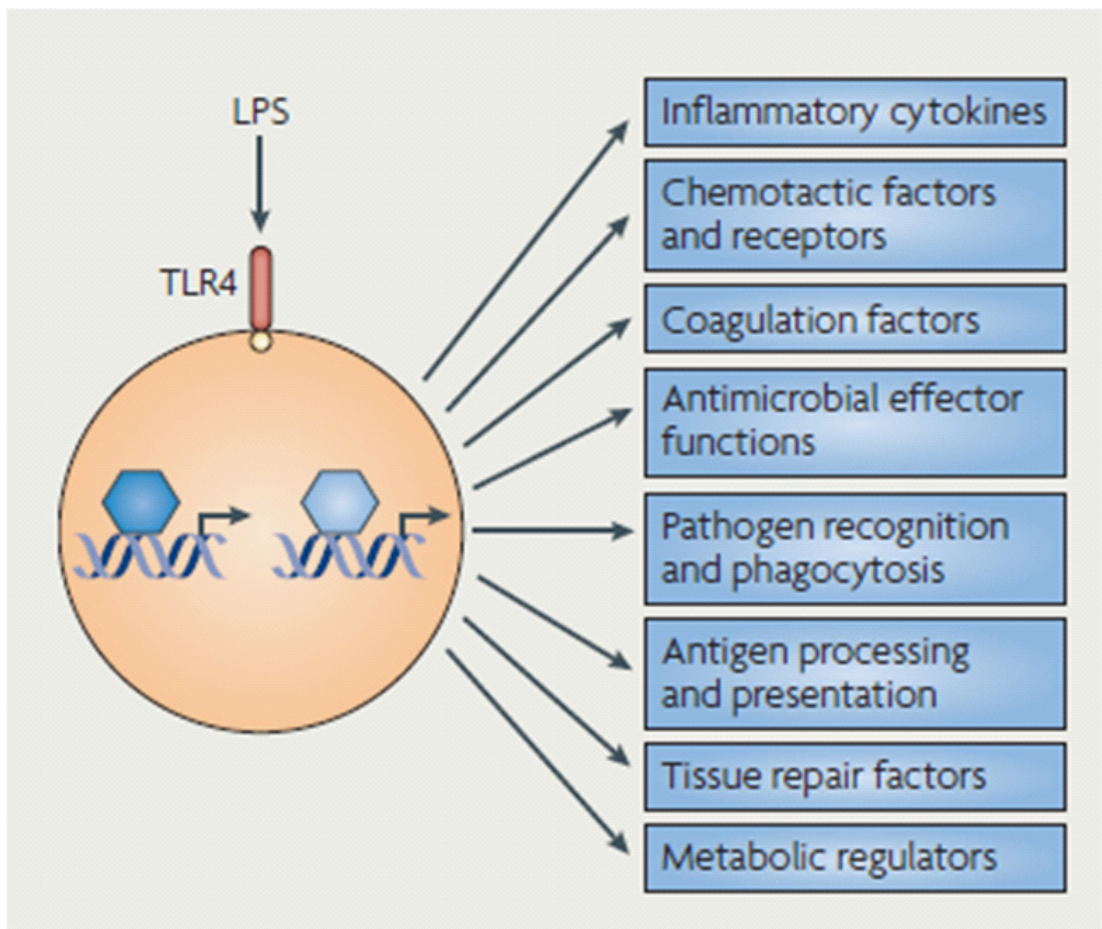


Figure 2: Cellular responses to LPS-induced activation of TLR-4. (Adapted and permission from Medzhitov R et al, Nature, 2009 and Macmillan Publishers Limited).

Resolution from a systemic inflammatory response is relatively less understood, but is facilitated by anti-inflammatory mediators. For example, increased IL-10 and IL-1ra (IL-1 receptor antagonist) expression serves to buffer the severity of an inflammatory reaction and lead to its resolution. Interestingly, IL-10 knockout mice develop severe colitis and IL-1ra reduces colitis in experimental models [19,20]. Intracellular signals can also have anti-inflammatory effects. In response to heightened NF- κ B transcriptional activity, I κ B and A20 gene expression are increased in macrophages and provides an inhibitory feedback loop [21]. Furthermore, up regulation of Suppressor of Cytokine Signaling-3 (**SOCS3**) can also provide similar inhibition of Interleukin-6 dependent signaling [22]. Taken together, an acute inflammatory response due to bacterial infection leads to several molecular pathways, including LPS-CD14-LPB / TLR4 / IKK / I κ B / NF- κ B, as well as secondary, anti-inflammatory mediators (IL-10, IL-1ra, I κ B, A20, SOCS3). However, an unbalanced pro-inflammatory response due to infection can manifest systemically and have pathophysiologic effects.

Severe Sepsis and Septic Shock

Systemic Inflammatory Response Syndrome (**SIRS**) is characterized as conditions involving a pro-inflammatory state which may have an infectious cause (bacterial sepsis) or non-infectious cause (trauma, burns, hemorrhage). Clinical symptoms of sepsis include a significant change in body temperature (> 101 F or 38.3°C ; or < 96.8 F or 36°C), heart rate > 90 bpm, respiratory rate > 20 cycles per min, and a high risk for infection [23]. Severe sepsis is characteristic of organ failure due to a reduction in tissue blood flow (i.e. brain or kidney). Additionally, an excessive vasodilatory response can promote poor venous return and decreased cardiac output. Septic shock is a concomitant impairment of cardiovascular performance via a reduction of mean arterial blood pressure (<65 mmHg) or systolic blood pressure (<90 mmHg) [24]. Furthermore, insulin resistance and metabolic perturbations may also develop during sepsis. Although the integrated mechanisms are not clearly understood, regulation of blood glucose (i.e glycemic control) through intense insulin therapy has previously been shown to reduce mortality in this setting.

Glycemic Control in the ICU

Intense insulin therapy

Insulin resistance and hyperglycemia in the ICU setting, or “diabetes of injury”, is an acute maladaptation. Since 2001, the effect of intense insulin therapy as a therapeutic intervention for improving glycemic control has been a central issue of debate. In a seminal study, Van De Berghe G et al demonstrated improved survivability of surgical ICU patients ($n=1548$) by 43% ($p<0.04$) with maintenance of blood glucose between 80-110 mg/dL following Intense Insulin Therapy (**IIT**) [1] (Figure 3). The most improved outcome due to IIT was associated with septic conditions and the deterrence of multi-organ failure. Based upon these initial observations, IIT and aggressive blood glucose management was widely adopted as standard critical care in the ICU [25].

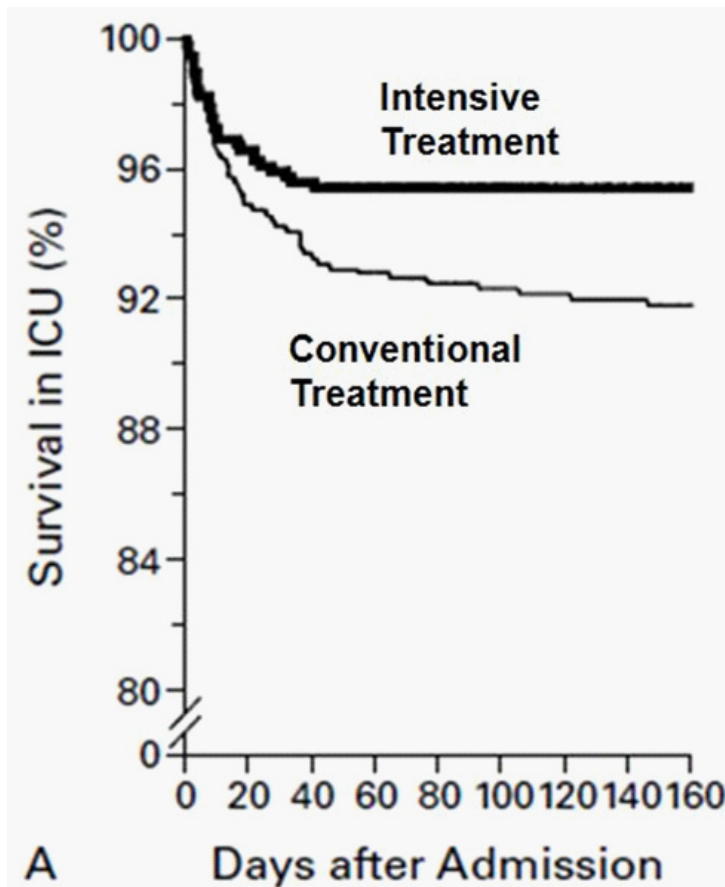


Figure 3: Intensive insulin treatment vs conventional and survival in critically ill patients. (Adapted and permission from Van Den Berghe G et al, NEJM, 2001 and Massachusetts Medical Society).

Risk of hypoglycemia

Subsequent studies have since reported conflicting data and spurred concern for the benefit of IIT [26]. For example, a large scale clinical trial including a group of IIT (80-110mg/dL or 4.5-6.0 mmol/L; n=3054) and conventional insulin therapy (<180mg/dL or <10mmol/L; n=3050) was evaluated in ICU adults [2]. Interestingly, the IIT group demonstrated a reduced probability of survival ($p<0.03$) and a significantly greater percentage of reported hypoglycemia (<40mg/dL or <2.2 mmol/L) ($p<0.001$) (Figure 4). However, predisposing risk factors for hypoglycemia in the ICU, such as diabetes, parenteral nutrition and ionotropic support, may confound these observations [27]. Taken together, these studies indicate glycemic control via IIT has the potential to improve survivability, but remains inconclusive due to an elevated risk of hypoglycemia. Studies addressing the molecular physiology of insulin resistance following acute inflammatory stress may help elucidate this paradox and improve critical care strategies.

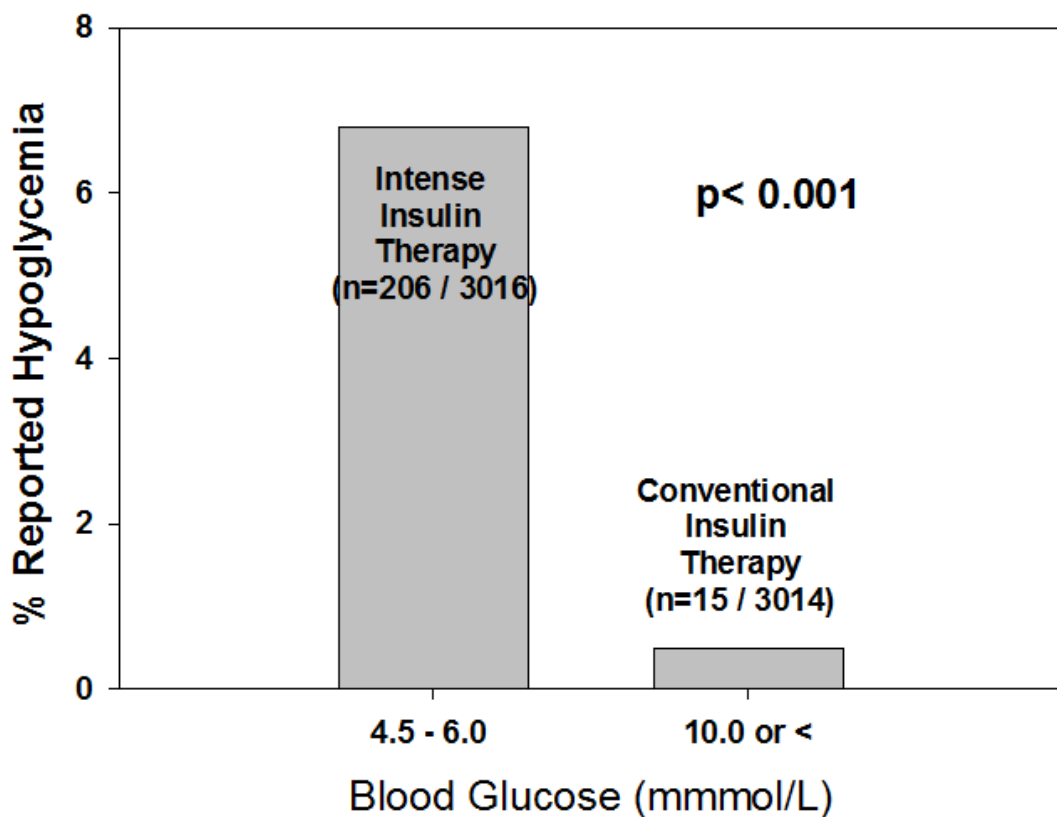


Figure 4: Risk of hypoglycemia following intense insulin therapy vs conventional and survival in critically ill patients (Adapted and permission from NICE-SUGAR Study Investigators, NEJM, 2009 and Massachusetts Medical Society).

NITRIC OXIDE BIOLOGY

Discovery of Nitric Oxide (NO) and Nitric Oxide Synthases (NOS)

In 1980, the endothelium was determined to have an obligatory role in the vasodilatory response of acetylcholine through a newly discovered factor named, Endothelial-Derived Relaxing Factor (**EDRF**) [28]. Following a series of studies, including: 1) the release of EDRF from cultured endothelial cells following stimulation with bradykinin, 2) the inactivation of EDRF via superoxide ions, 3) the protection of EDRF by the antioxidant, Superoxide Dismutase (**SOD**) and 4) the biological resemblance of EDRF to nitric oxide during anti-aggregation in platelets, the newly described EDRF was determined to be Nitric Oxide (**NO**) [29]. Yet, the origin of NO from the endothelium was still undetermined.

A number of possibilities were considered to determine the source of nitric oxide generation. Intuitively, it was hypothesized that NO species in endothelial cells were generated from the amino acid, L-arginine, due to this observation previously made in active macrophages. Removal

of L-arginine from cell culture medium followed by its re-supplementation provided convincing evidence for the generation of nitric oxide through this pre-precursor. The biochemical pathways and enzymes, (NOS), required for NO generation from L-arginine were later established (30) (Figure 5A). Three genetic isoforms of Nitric Oxide Synthase (**NOS**), which converts L-arginine to NO and L-Citrulline, were found native to neurons (neuronal NOS; nNOS or NOS1), innate immune cells (inducible NOS; iNOS or NOS2) and endothelial cells (endothelial NOS; eNOS or NOS3) (Figure 5B). Major functions for each of these NOS isoforms include cellular communication, immunity, and vasodilation, respectively. In 1998, Robert F. Furchgott, Louis J. Ignarro and Ferid Murad were jointly awarded the Nobel Prize in Physiology or Medicine for their discovery of nitric oxide as a signaling molecule in the cardiovascular system. Several groundbreaking contributions on the cardiovascular impact of nitric oxide were also made by Dr. Salvador Moncada and was arguably a 4th scientist of the award [31].

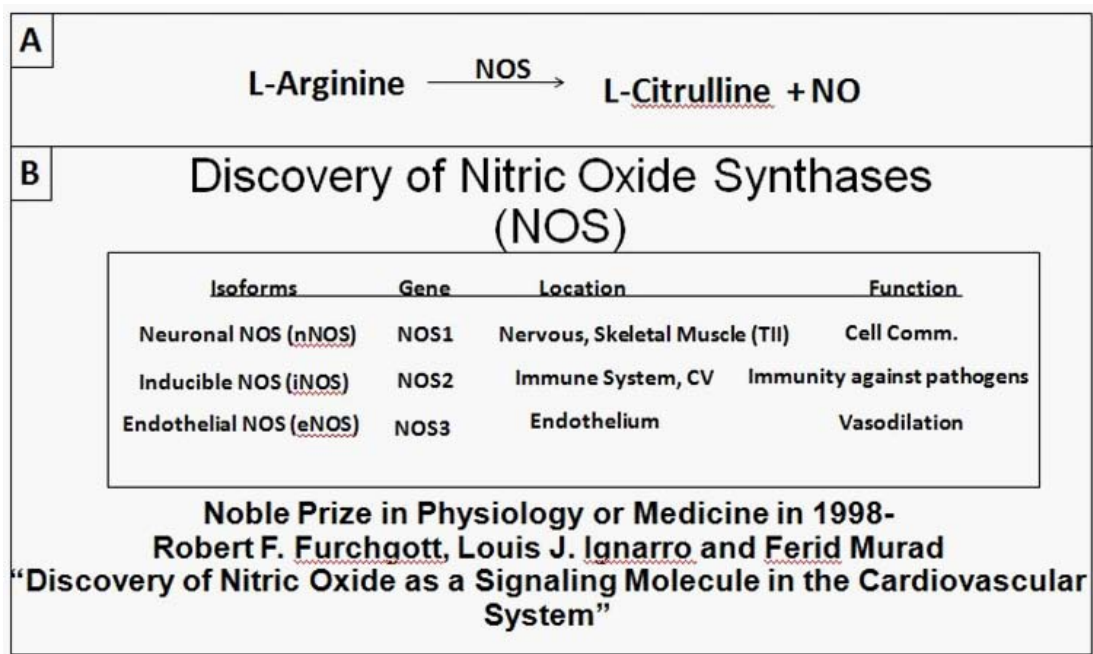


Figure 5: Discovery of Nitric Oxide, Nitric Oxide Synthases, and the 98’ Nobel Prize.

INTEGRATED PHYSIOLOGICAL RESPONSE TO NITRIC OXIDE DURING SEPSIS

Nitric Oxide and Cardiovascular Function

Non-specific inhibition

In vivo studies using pharmacologic methods have further characterized the ability of nitric oxide to regulate cardiovascular function. In Figure 6 panel A, the intravenous administration of endotoxin (12.5 mg/kg; E.Coli) in conscious mice effectively reduced Mean Arterial Blood

Pressure (**MABP**) from 94 ± 4 mmHg at $t=0$ hr to 76 ± 4 mmHg at $t=12$ hr. A concurrent increase in the concentration of plasma nitrite and nitrate within 2 hrs was observed and a peak concentration was measured at 12 hours post-administration of endotoxin [32]. Within the same study, intravenous infusion of the non-selective NO synthase inhibitor, NG-monomethyl-L-arginine (L-NMMA; 10 mg/kg/hr), protected mice against the development of hypotension and effectively blocked the generation of plasma NO species (nitrite, nitrate [μ M]) in response to endotoxin treatment (6mg/kg) (Figure 6-panel B,C). However, other studies using non-selective NOS inhibitors, such as L-NAME, have provided conflicting results with increased mortality and liver damage in a model of extremely high LPS dosage (70mg/kg) [33]. In humans, the intravenous infusion of L-NMMA hydrochloride (546C88) for 72 hours was successful in reducing septic shock in phase II clinical trials, but was later discontinued following increased risk of mortality [34,35]. Although non-specific inhibition failed to successfully translate in clinical trials, additional studies were performed to determine the selective inhibitory effects of NOS and discussed in the next section.

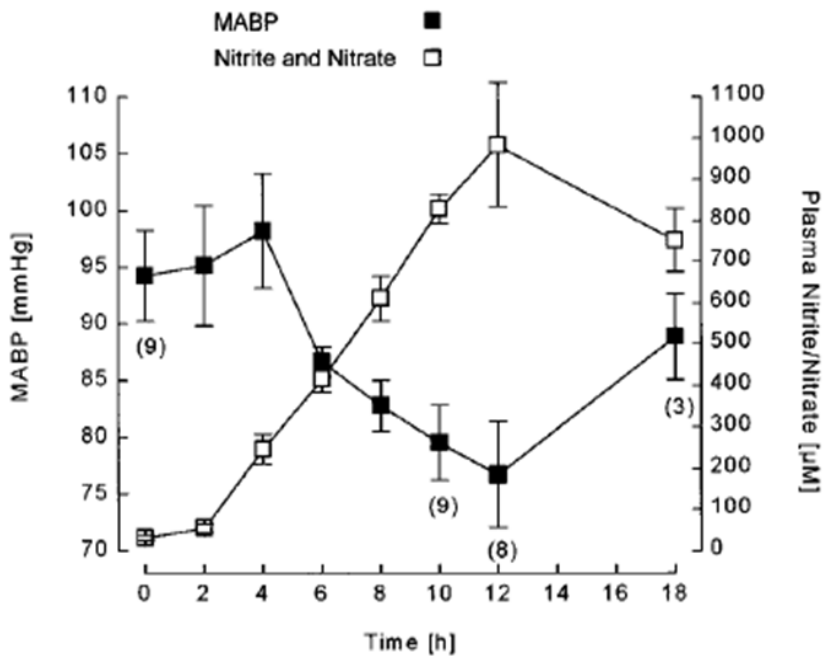


Figure 6A: Mean Arterial Blood Pressure (**MABP**) and concentrations of plasma NO_x following endotoxin (12.5 mg/kg) in mice. (Adapted and permission from Rees DD et al, British Journal of Pharmacology (1998) and Stockton Press).

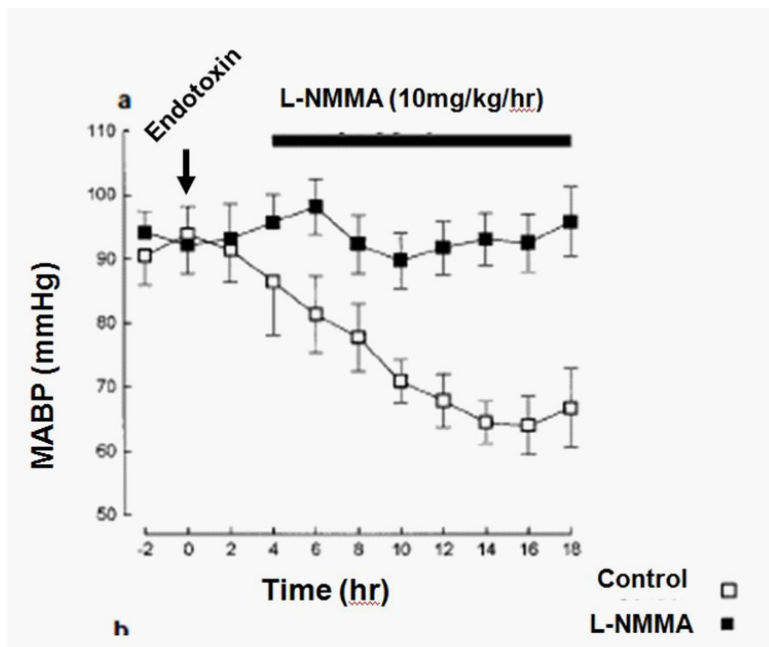


Figure 6B: Mean Arterial Blood Pressure (MABP) (a) and concentrations of plasma NO_x (b) following endotoxin (12.5 mg/kg) and L-NMMA (NOS inhibitor) in mice. (Adapted and permission from Rees DD et al, British Journal of Pharmacology (1998) and Stockton Press).

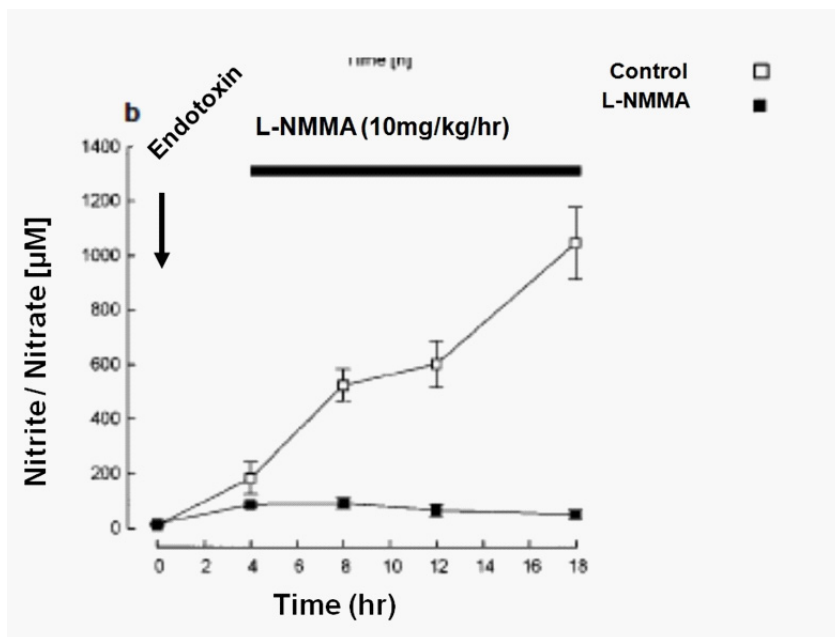


Figure 6C: Mean Arterial Blood Pressure (MABP) (a) and concentrations of plasma NO_x (b) following endotoxin (12.5 mg/kg) and L-NMMA (NOS inhibitor) in mice. (Adapted and permission from Rees DD et al, British Journal of Pharmacology (1998) and Stockton Press).

Inducible NOS

Mice with a genetic ablation for iNOS (iNOS KO) display an altered immune response towards LPS. Interestingly, iNOS KO mice were provided protected against LPS-induced mortality, but were less likely to survive following a cecal ligation puncture model of sepsis [36,37]. iNOS KO mice were protected against cardiovascular dysfunction and septic shock after 18hrs of endotoxin treatment (3mg/kg) (Figure 6-Panel D) [32]. Specific, competitive inhibitors of iNOS, such as Aminoguanidine (**AG**), have also proven beneficial in a rat model of cecal ligation puncture. AG at high dosages (17.5 and 175 mg/kg) blocked iNOS activity and the associated drop in MAP, but failed to inhibit eNOS activity in the thoracic aorta. Thus, selective inhibition of eNOS and nNOS also necessitate consideration during cardiovascular dysfunction in sepsis [38].

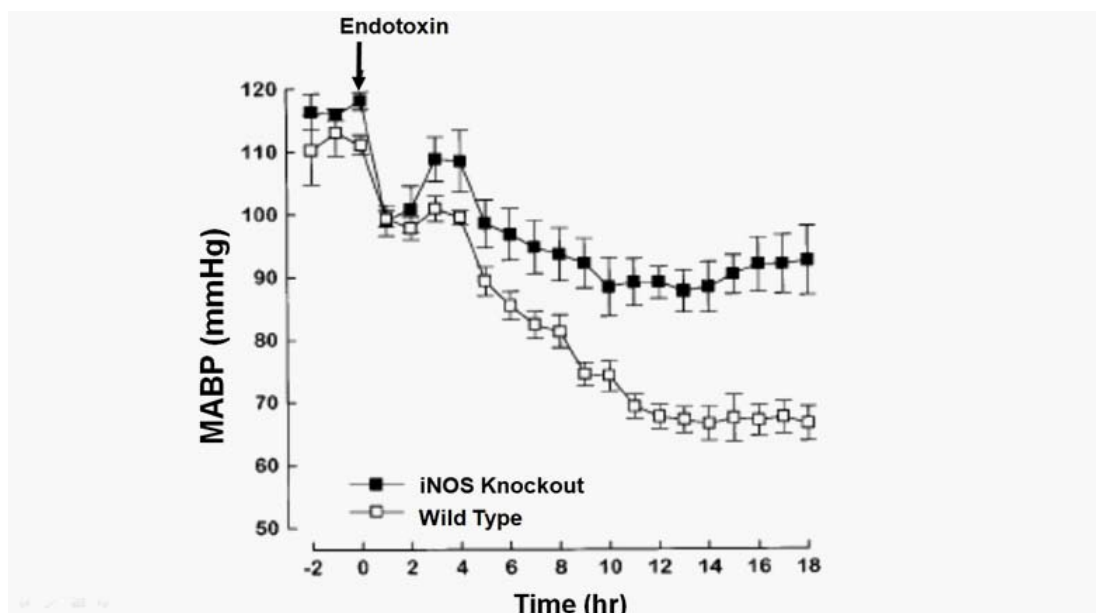


Figure 6D: Mean Arterial Blood Pressure (**MABP**) and concentrations of plasma NO_x following endotoxin (12.5 mg/kg) in iNOS knockout mice. (Adapted and permission from Rees DD et al, British Journal of Pharmacology (1998) and Stockton Press).

Endothelial NOS

eNOS knockout mice (eNOS KO) were utilized to determine the effect of this isoform on cardiovascular dysfunction during sepsis [39]. eNOS KO mice were protected against hypotension following the intravenous injection of LPS (12.5mg/kg) for 18 hours. The putative mechanism underlying this response included the reduction of both NO_x species in plasma and iNOS protein expression in liver, lung, heart, and aorta. Additional *in vitro* experiments using HUVEC cells demonstrated eNOS phosphorylation in response to LPS occurs through AKT signaling and likely precedes iNOS protein expression. Thus, eNOS may be required as an initial step prior to the heightened protein expression of iNOS during sepsis.

Neuronal NOS

nNOS deletion in mice (nNOS KO) were less likely to survive against sepsis following cecal ligation puncture. This response was coupled with impaired bacterial clearance and the up regulation of mRNAs for iNOS, TNF-alpha, and IL-6 in spleen vs a wild type control [39]. Although few studies have evaluated whether nNOS influences cardiovascular function during sepsis, pre-treatment with the nNOS inhibitor, 7-nitroindazole, improved the vasoconstriction response to norepinephrine and phenylephrine in isolated aortas following cecal ligation puncture [40].

Nitric Oxide and Glucose Metabolism

Barriers of muscle glucose uptake

Insulin resistance is the impairment of cells and tissues to effectively respond to the normal actions of insulin. For example, skeletal muscle mediates ~70% of insulin-stimulated glucose disposal and insulin resistance of this tissue due to a pro-inflammatory imbalance can promote hyperglycemia [41]. Thus, Muscle Glucose Uptake (**MGU**) represents a therapeutic target of whole body insulin sensitivity in the ICU. Previous studies have demonstrated skeletal muscle glucose uptake *in vivo* is a complex product determined by several control systems. Although there has been considerable debate identifying the rate limiting step in Muscle Glucose Uptake (**MGU**), three regulatory processes are known to be involved [42] (Figure 7). #1) Tissue blood flow and microcirculation contributes to the diffusion of glucose from the blood to the interstitial space. #2) Skeletal Muscle Insulin Signaling and GLUT4 in the sarcolemma allow glucose entry into the muscle. #3) Phosphorylation of intracellular glucose by Hexokinase-II generates glucose-6-phosphate and is an irreversible step. Each of these regulatory sites (substrate delivery, membrane transport and intracellular phosphorylation) is implicated in skeletal muscle glucose uptake. Although insulin resistance is not completely understood *in vivo*, functional defects in one or more of these 3 regulatory steps is likely to promote a decrease in glucose uptake in muscle. By understanding the mechanisms of insulin sensitivity and control of muscle glucose uptake during sepsis, new aims for improving glycemic control in this setting can be tested.

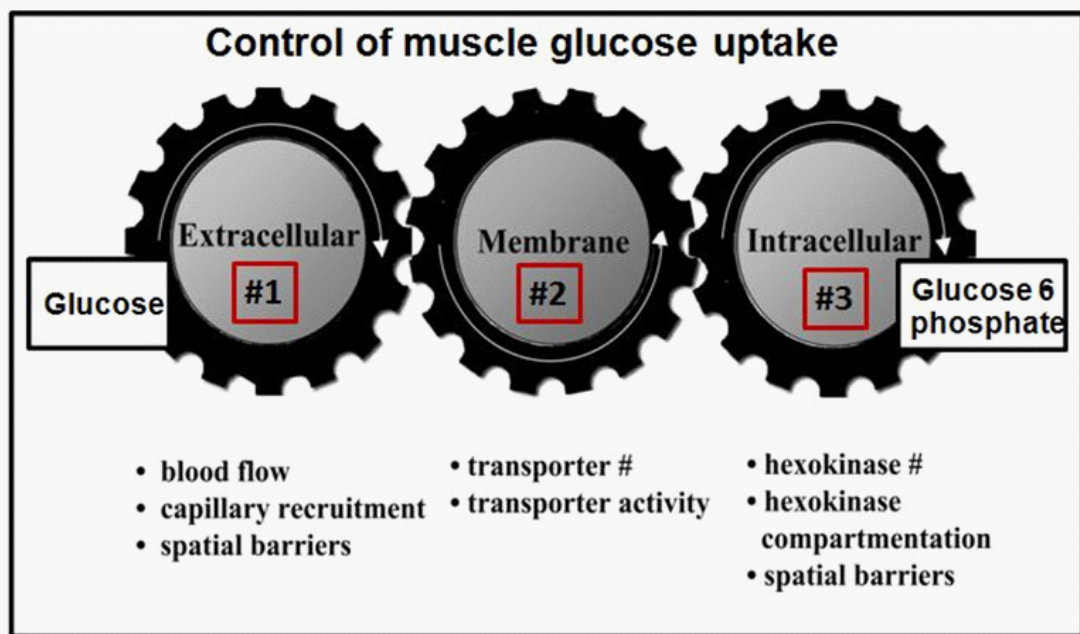


Figure 7: Control of Muscle Glucose Uptake (Adapted and permission from Wasserman, D. H. et al, Clin Exp Pharm Physiol Vol 32, p.319–323, April 2005; and John Wiley and Sons Inc).

Tissue blood flow and microcirculation: Under physiologic conditions, NO promotes insulin-stimulated vasodilation and increased total blood flow to skeletal muscle [43]. This response is mediated through increased cGMP activity of vascular smooth muscle and helps regulate the 1st “gear shift” of MGU. However, during sepsis an overabundance of NO generation is implicated in the development of both macrovascular (hypotension) and microvascular (capillary) dysfunction. Interestingly, skeletal muscle microvascular dysfunction can occur in the presence of normotensive sepsis following fluid resuscitation in a cecal ligation model of sepsis [44]. Using intravital microscopy of the extensor digitorum longus muscle, stopped-flow capillary density increased and perfused capillary density decreased in response to sepsis. Thus, microvascular dysfunction during sepsis is not necessarily dependent upon hypotension. Sepsis-induced microvascular dysfunction has also been shown to limit Oxygen (O_2) diffusion and shunting via the heterogeneous stoppage of skeletal muscle capillaries [45]. Translational studies have used a spectral imaging technique to semi-quantitatively measure sublingual microvascular dysfunction in patients with sepsis. Capillary perfusion of septic patients was significantly reduced using this technique (Figure 8) [46].

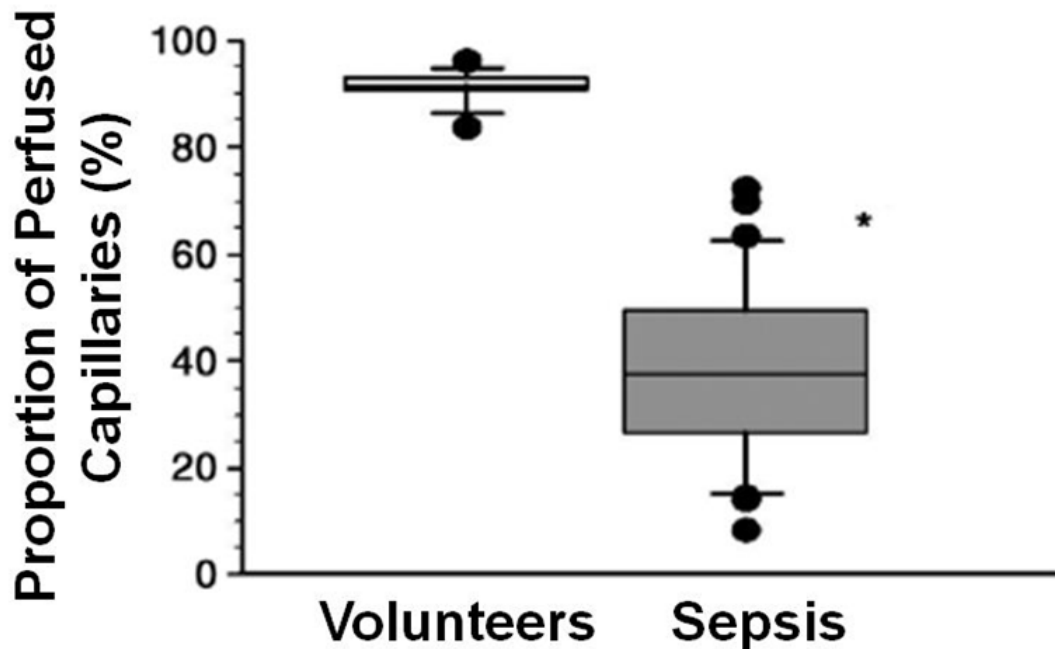


Figure 8: Sublingual capillary perfusion (%) in patients with severe sepsis (Adapted and permission from De, Backer D et al, Am.J.Respir.Crit Care Med., 2002).

Due to its potent vasodilatory effects, the overproduction of NO during sepsis may have a significant role in insulin resistance and skeletal muscle microvascular dysfunction. In order to determine whether NO can directly impair MGU without inflammation, MGU was determined in the presence of hypotension alone using a NO donor (sodium nitroprusside) [47]. Indexes of insulin sensitivity using a hyperinsulinemic-euglycemic clamp, such as glucose infusion rates and muscle 2-deoxyglucose uptake, were decreased in the NO donor group without a change in total skeletal muscle blood flow. It seems plausible that skeletal muscle microvascular dysfunction may contribute to this impairment. Plasma insulin levels were decreased with NO donor treatment suggesting enhanced insulin clearance. Markers of skeletal muscle insulin signaling, such as AKT-phosphorylation, were not changed with NO donor treatment. Interestingly, discontinuation of the NO donor infusion acutely restored MAP within 10 minutes and reversed whole body insulin resistance as determined by the glucose infusion rate (Figure 9A and Figure 9B). Taken together, NO-induced hypotension can induce whole body insulin resistance and impaired MGU without changes in total muscle blood flow or modifications to muscle insulin signaling. NO treatment increased insulin clearance and can acutely impaired whole body insulin action through macrovascular dysfunction. Future studies are required to determine the role of NO on skeletal muscle microvascular dysfunction and insulin-stimulated MGU.

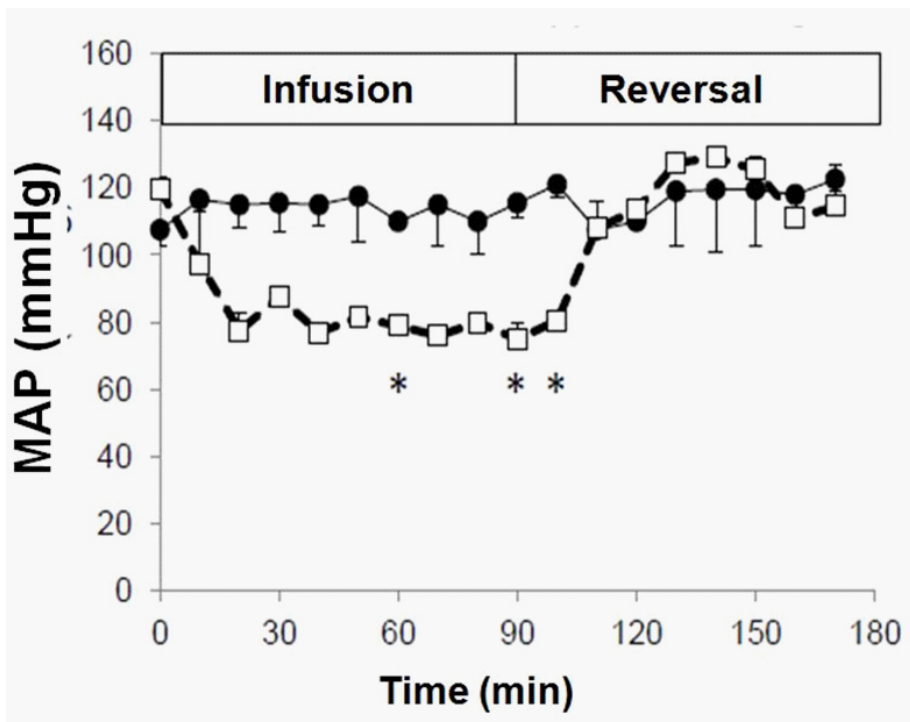


Figure 9A: Acute reversal of MAP following discontinuation of NO donor (SNP). (Adapted and permission from House LM et al, Cardiovas Diabet, 2015).

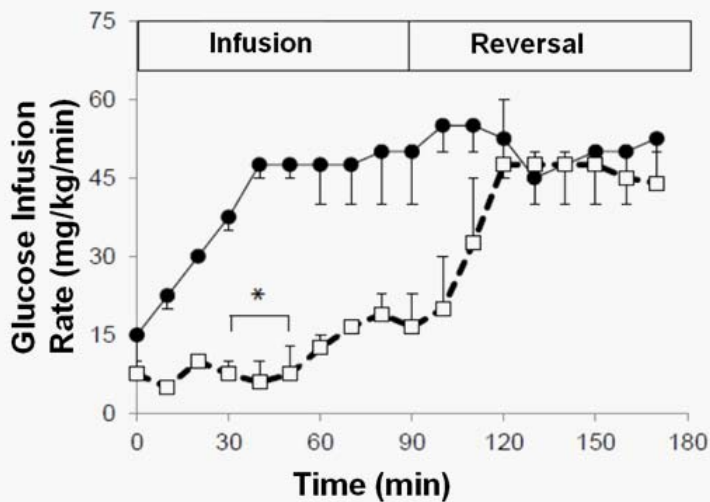


Figure 9B: Acute reversal of insulin resistance following discontinuation of NO donor (SNP). (Adapted and permission from House LM et al, Cardiovas Diabet, 2015).

Skeletal muscle insulin signaling and GLUT4: Muscle tissue has evolved a highly specialized transporter system for glucose entry. Insulin-mediated glucose uptake via sugar transporters was first discovered by a series of papers in 1980-81 [48-50] and has since become the subject of intense study [51]. Transport of glucose can rapidly increase by 10-40 folds within minutes of exposure to insulin. The ability of insulin to promote glucose entry into the muscle cell is dependent upon the number and activity of glucose transport proteins, or GLUT4, at the cell surface. The Insulin Receptor / IRS / PI3 kinase / AKT signaling pathway play a substantial role in activating the movement of GLUT4 vesicles to the plasma membrane [52]. In the absence of high blood insulin levels, glucose transport across the membrane produces the highest resistance to muscle glucose uptake among the 3 systems in Figure 2. However, insulin increases permeability of glucose entry into the muscle cell (step #2) (i.e. decreases resistance) and shifts the barrier of glucose uptake towards delivery (step #1) and intracellular phosphorylation (step #3) (i.e increases resistance). Previous studies have demonstrated that muscle glucose uptake becomes more dependent upon delivery and phosphorylation of glucose during high flux states. Thus, muscle glucose uptake *in vivo* is not limited to a single process or event, but rather distributed during either basal or high flux conditions [53,54].

The effect of sepsis and NO-dependent impairments on insulin receptor signaling has been assessed as a potential mechanism of insulin resistance. Following 5 hours of an intravenous bolus of LPS (10mg/kg), Wild Type (**WT**) mice demonstrated whole body insulin resistance and reductions of MGU via hyper insulinemic-euglycemic clamps. However, phosphorylation of the muscle insulin signaling pathways, such as IRS-1 (Tyr895, Ser307), AKT (Ser 473), and GSK-3 β (Ser9), were unaffected by LPS treatment [17]. This study provided evidence against skeletal muscle insulin signaling ("gear shift" #2) as a limiting step for MGU during sepsis.

Other post-translational modifications, such as protein S-nitrosylation, appear to regulate intracellular signaling events [55]. Due to its overproduction and biochemical reactivity, the role of NO-dependent protein nitrosylation in skeletal muscle insulin signaling was evaluated in WT and iNOS KO mice following 8 hours of endotoxin treatment (10mg/kg) [56]. Using western blot analysis and a biotin switch method, skeletal muscle AKT phosphorylation in the LPS group was significantly decreased with a concomitant increase in S-nitrosylation of AKT. This impairment was reversed in the iNOS KO group treated with LPS. Although MGU data was not collected, these observations support skeletal muscle insulin signaling events ("gear shift #2) as a limiting step in glycemic control during sepsis.

Phosphorylation of intracellular glucose by Hexokinase-II: Following insulin stimulation or exercise, the capacity to phosphorylate glucose has a crucial role in regulating muscle glucose uptake. After entering the muscle, glucose becomes phosphorylated primarily by Hexokinase-II (**HK-II**) and is committed to being metabolized as Glucose 6-Phosphate (**G6P**) in glycolysis. Skeletal muscle over expression of HK-II in mice enhances MGU following a hyper insulinemic-euglycemic clamp, but few studies have determined whether HK-II is a rate limiting step of MGU during sepsis.

Hepatic glucose output

In the post absorptive (fed) state, insulin is capable of inhibiting hepatic glucose production. The molecular pathway leading to down regulation of hepatic gluconeogenesis includes the direct blockade of FOXO1 transcriptional activity [57]. However, during insulin resistance the liver has an impaired ability to blunt this response and contributes to hyperglycemia. In order to determine whether NO facilitates increased hepatic glucose production, adult Sprague Dawley male rats were co-treated with LPS (20mg/kg) and a selective inhibitor of iNOS, Aminoguanidine (AG) (500mg/kg). LPS treatment significantly increased hepatic glucose production and this response was abolished by co-treatment with AG. Also, the activity of Glycogen Phosphorylase and PEPCK were both decreased with AG treatment suggesting iNOS-dependent stimulation of pathways leading to increased glucose output [58]. Although the effects of IIT on the hepatic glucose production in critically ill patients are still unclear, treatment with IIT in non-surviving patients of the ICU failed to reduce PEPCK mRNA within the liver [59]. Taken together, these studies suggest that iNOS activity can influence hepatic glucose output, but future studies are required to elucidate its role in humans.

CONCLUSIONS

Glycemic control through intensive insulin therapy can improve survival in sepsis, but may also increase the risk of hypoglycemia in these patients. Understanding the mechanisms of acute insulin resistance during sepsis (or “diabetes of injury”) may help improve this intervention. Nitric oxide is abundantly generated during an innate immune response and, if severe enough, can facilitate insulin resistance. Studies involving the inhibition of nitric oxide synthases for the treatment of hemodynamic shock and insulin resistance have shown promising results. Yet, initial clinical trials using nitric oxide synthase inhibitors have not been successful. Future studies will require new and integrative approaches to determine the basis for this important disconnect [60].

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