Analysis of Thermal Injury-induced Insulin Resistance in Rodents

IMPLICATION OF POSTRECEPTOR MECHANISMS

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Burn injury is associated with insulin resistance. The molecular basis of this resistance was investigated by examining insulin receptor signaling in rats after thermal injury. The impaired insulin-stimulated transport of [3H]2-deoxyglucose into soleus muscle strips confirmed the insulin resistance following burns. In vivo insulin-stimulated phosphoinositide 3-kinase activity, pivotal in translocation of GLUT4, was decreased in burns when assessed by its insulin receptor substrate-1 (IRS-1)-associated activity. Insulin-induced tyrosine kinase activity of insulin receptor (IR) and tyrosine phosphorylation of IRS-1 were also attenuated. Immunoprecipitated IR, however, appeared to have normal insulin-responsive kinase activity. Finally, immunoprecipitated IRS-1 was tested for its effect on partially purified recombinant IR and was found to inhibit its kinase activity. This inhibitory effect of IRS-1 was abolished by prior treatment of IRS-1 with alkaline phosphatase, indicating that burn injury-related hyperphosphorylation of IRS-1 is similar to that observed in TNFα-induced inhibition of IR signaling. All of these changes were observed in the absence of quantitative changes in IR, IRS-1, and phosphoinositide 3-kinase. Alterations in postreceptor insulin signaling, therefore, may be responsible for the insulin resistance after thermal injury.

Insulin, the principle hormone maintaining glucose homeostasis, stimulates glucose influx into muscle, glycoegen synthesis in the liver, and fat deposition in adipocytes (1, 2). Insulin resistance is a major metabolic abnormality following burn injury (3–6). Insulin resistance of burn injury is indicated by elevated glucose production (gluconeogenesis) and blood glucose levels in association with normal or increased insulin levels. Muscle constitutes approximately 40% of the body mass, and insulin resistance results in muscle wasting due to the lack of anabolic effects. Indeed, insulin resistance as documented in skeletal muscle after burn injury results in enhanced gluconeogenesis and protein catabolism (3, 5, 7). Vigorous nutritional support to optimize the hypermetabolic state and promote anabolism has not been effective. Attempts have been made to correct this burn stress-induced pseudodiabetes, hyperglycemia, and muscle wasting with pharmacological doses of insulin, but this treatment resulted in fatty liver and increased wasted substrate cycling, enhanced CO₂ production, and attendant complications of hepatic and respiratory failure (8–10). The molecular mechanism of this abnormal insulin function has not been elucidated.

The binding of insulin to the α-subunit of the IRβ induces rapid autophosphorylation of its β-subunits, activating its tyrosine kinase activity (1, 2). This leads to tyrosine phosphorylation of cytosolic molecules, including IRS-1, whose phosphorylation is pivotal in the biological actions of insulin (11). The tyrosine phosphorylation of IRS-1 leads to the second intracellular step in insulin action, the association of phosphorylated IRS-1 with the enzyme PI 3-K, which propagates the distinct metabolic functions of insulin (12). The association occurs through phosphorylated YXXM or YXXX motifs on IRS-1 and the SH2 domains on the 85-kDa subunit of the PI 3-K, resulting in activation of the catalytic (110-kDa) subunit of PI 3-K (12). Treatment of cultured cells with wortmannin, a potent PI 3-K inhibitor, or overexpression of dominant negative p85, both of which inactivate endogenous PI 3-K activity, abolishes insulin-stimulated glucose uptake (13–15). These data, along with the time course and the dose response of production of PI 3,4-P2 and PI 3,4,5-P3 in response to insulin, are consistent with the hypothesis that PI 3-K is an important element of the signaling pathways of insulin action for glucose uptake (16). This notion is further substantiated by recent studies indicating that PI 3-K activity is critical in maintaining glucose homeostasis in muscle and adipose tissues (13, 17).

The molecular pathogenesis of insulin resistance in diabetes has been intensively studied. In non-insulin-dependent diabetes mellitus, IR phosphorylation is significantly attenuated and is paralleled by a reduction in IRS-1 phosphorylation in both liver and muscle (1). In insulin resistance of obese diabetic subjects, the IR and IRS-1 phosphorylation and PI 3-K activity are all decreased when tested in skeletal muscle strips (16–18). Ineffective functioning of IR, IRS-1, and PI 3-K can, therefore, alter the metabolic actions of insulin in vivo. Recently, it has been reported that increased expression of TNFα in adipose tissues of obese animals is, at least in part, responsible for this impaired insulin signaling (18, 19). Interestingly, the inhibitory effects of TNFα were due to serine rather than tyrosine phosphorylation of IRS-1; serine phosphorylation functioned as a dominant negative molecule for IR tyrosine kinase activity.

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In the increase of the levels of TNFα is a well-known phenomenon following burn injury (21–23). Based on this background, it seemed reasonable to assess the mechanism of burn-induced insulin resistance by focusing on the in vivo activities of PI 3-K as well as on the functions of IR and IRS-1. We initiated this study based on the hypothesis that insulin resistance after severe thermal injury is induced by aberrant IR intracellular signaling. Here, we provide multiple lines of evidence indicating that postreceptor insulin signaling is impaired in skeletal muscle tissues after burn injury.

MATERIALS AND METHODS

The Burn Model and Timing of Experiments—The protocol for the studies was approved by the Institutional Animal Care Committee. The animal care facility is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. Male Sprague-Dawley rats (Taconic, Germantown, NY) weighing about 150–160 g were used. Six animals were used for each group (sham burn with or without insulin injection and burn with or without insulin injection). After clipping of the hair, a full thickness third degree burn injury was produced under anesthesia by immersing the back of trunk for 15 s and abdomen for 5 s in 80 °C water. A weight- and time-matched sham burn group (controls) was treated in the same manner as the trauma group, except that they were not immersed in the hot water. All biochemical experiments were performed in the gastrocnemius (fast) and/or soleus (slow) muscles excised from the animals at 3 days after burn or sham burn injury.

Soleus Muscle Glucose Transport—Glucose transport in soleus muscle was determined as described previously, with minor modifications (24). Rats were killed by cervical dislocation, and solei were surgically removed and tied via their tendons to stainless steel clips. Muscles were preincubated for 15 min in 10 ml of Krebs-Ringer bicarbonate buffer, pH 7.3, supplemented with 1% bovine serum albumin and 2 mM pyruvate. They were then incubated for 10 min in another flask containing 10 ml of Krebs-Ringer bicarbonate buffer, pH 7.3, supplemented with 1% bovine serum albumin containing [3H]-2-deoxyglucose (final 0.1 mM, 0.5 Ci/mmol, and [14C]glucose (final 0.1 mM) as a marker, with or without 100 mM insulin added to the mixture. All incubations were carried out at 37 °C in an atmosphere of 95% O2, 5% CO2. After incubation, the reaction was stopped by transferring the muscles to ice-cold phosphate-buffered saline. The 3H and 14C incorporated were counted by liquid scintillation spectrometry.

Insulin Receptor Signaling after Burns

Dephosphorylation of Immunoprecipitated IRS-1 and Its Effect on PI 3-K Activity—Aliquots of immunoprecipitated IRS-1 or p85 PI 3-K were incubated in the absence or presence of 100 μM NaCl and 1 mM EDTA. The pellets were resuspended in 50 μl of 10 mM Tris/HCl (pH 7.5) containing 100 mM NaCl and 1 mM EDTA.

Detection of Tyrosine Phosphorylation of IR and IRS-1 Molecules—Aliquots of resuspended precipitates of IR or IRS-1 prepared as described above were subjected to SDS-PAGE (6% T) in a miniature slab gel apparatus (Bio-Rad). After electrophoresis of protein from the gel to polyvinylidene difluoride membrane, the membrane was first exposed to blocking buffer (10 mM Tris/HCl (pH 7.5), 150 mM NaCl, 1% bovine serum albumin) for 20 min at 37 °C. The membrane was incubated for 20 min at 37 °C with 0.5 μCi of horseradish peroxidase-conjugated antihuman insulin receptor (25) were grown in 12 100-mm dishes to confluence. The supernatant, which was prebaked for 10 min. The plate was developed in CHCl3/methanol (1:1) and visualized, and bands were visualized as described above.

Measurement of PI 3-K Activity—PI 3-K activity associated with immunoprecipitates of IRS-1 or p85 PI 3-K was measured in vitro as described previously by its effect on phosphoinositide 3-kinase (PI-3-kinase) phosphorylation at PI 3-adenylyltransferase activity at 15,000 rpm for 30 min. IR was immunoprecipitated with 1 ml of anti-IR mAb (Calbiochem) from aliquots of supernatant containing 10 μg of protein A-Sepharose (1-ml bed volume, Pharmacia). The Polytron homogenizer was operated at maximum speed for 30 s, and samples were left on ice for 30 min. Insoluble material was removed by centrifugation at 15,000 rpm for 30 min. IR was immunoprecipitated with 1 μg/ml of anti-IR mAb (Calbiochem) from aliquots of supernatant containing 10 μg of protein. IR-1 was immunoprecipitated from the same aliquots with 0.3 μg/ml of anti-IR-1 mAb, 1D6 (25). The P3-K molecules were immunoprecipitated from a sample of the same aliquot with a 1:300 dilution of anti-p85 PI 3-K. Preimmune mouse IgG (1 μg/ml) was used as a control. Following the addition of 50 μl of protein A-Sepharose 6MB (Pharmacia Biotech Inc.), immunoprecipitations were washed successively three times in phosphate-buffered saline containing 1% Nonidet P-40, three times in 10 mM Tris/HCl (pH 7.5) with 0.5 mM LiCl and 100 μM Na3VO4, and three times in 10 mM Tris/HCl (pH 7.5) incorporated with 100 mM NaCl and 1 mM EDTA. The pellets were resuspended in 50 μl of 10 mM Tris/HCl (pH 7.5) containing 100 mM NaCl and 1 mM EDTA.
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RESULTS

Insulin-stimulated Glucose Uptake Is Decreased in Skeletal Muscle after Burn Injury—In an attempt to demonstrate the presence of insulin resistance in our burn model, we initially examined insulin-stimulated in vitro glucose uptake in soleus muscle strips. Previous reports had indicated that insulin resistance was clearly observed at 2–7 days after burn injury (28–30). We chose 3 days after burn injury as the time for analysis of glucose uptake and insulin resistance.

Basal rates of glucose uptake in the muscle strip were significantly reduced (30%) in burned animals as compared with the sham burned animals at 3 days after burn (Fig. 1, p < 0.05). Consistent with other reports (30–32), glucose uptake was increased 5-fold in response to insulin in sham burned rats. Insulin-stimulated glucose uptake was, however, totally abolished in the muscle strips of burned animals compared with those of sham rats (Fig. 1, p < 0.001). These data directly demonstrated that insulin-stimulated glucose uptake was inhibited in burned animals at 3 days after burn and validated the use of 3 days after burn injury as an appropriate point for further analysis. The fact that insulin was ineffective even in vitro suggests that the inhibition may not be due to the presence of circulating extracellular inhibitory factors (e.g. glucosamine) that would inactivate the insulin signaling itself (33); such circulating factors might not be present under the in vitro conditions.

PI 3-K Activity Is Decreased in Muscle after Burns—We next investigated PI 3-K activity as an intracellular marker of insulin activation. As described in previous protocols (17), the skeletal muscles extracted from the hind limb were solubilized, and PI 3-K associated with IRS-1 was immunoprecipitated with anti-IRS-1 antibody. Insulin stimulation results in the binding of PI 3-K to IRS-1 (26); these experiments therefore specifically measure the insulin-stimulated activation of PI 3-K by IRS-1. Consistent with previous reports (17), sham rats showed a 5-fold increase of PI 3-K activity relative to basal value in response to insulin, giving a product that co-migrated with IRS-1-associ-
Insulin-mediated tyrosine phosphorylation of IRS-1 is decreased in burns. With the use of anti-IRS-1, it was confirmed that quantities of IRS-1 were the same between sham and burns (lower panel). Tyrosine phosphorylation (activation) of IRS-1 was assessed with the use of anti-phosphotyrosine antibody (upper panel). Basal (Insulin -) phosphorylation was absent in both sham burned (S1, S3) and burned (B1, B3) injured muscle samples. With insulin injected in vivo, phosphorylation of IRS-1 was observed (S2, S4). This action of insulin was not observed in burns (B2, B4).

Quantity Is Unchanged, but Tyrosine Phosphorylation of IRS-1 Is Impaired after Burns—The possibility that the attenuated PI 3-K activity was due to a quantitative change in IRS-1 was assessed in the following experiment. The immunoprecipitates of IRS-1 obtained with the anti-IRS-1 mAb were quantified by immunoblotting, using the anti-IRS-1 polyclonal antibody. The immunoreactivities of IRS-1 were the same between sham burn and burn animals (Fig. 4, lower panel). Thus, altered quantities of IRS-1 were not the cause of decreased PI 3-K activity. Next, the efficiency of tyrosine phosphorylation of IRS-1 was examined. The immunoprecipitates obtained with anti-IRS-1 antibodies were subjected to immunoblotting with an anti-phosphotyrosine (RC20H). The results (Fig. 4, upper panel) indicated that the tyrosine phosphorylation of IRS-1 was completely absent in burn-injured animals, in both the presence and absence of insulin. In contrast, tyrosine phosphorylation of IRS-1 in sham burned animals was enhanced by insulin.

Insulin-mediated Association of IRS-1 with p85 PI 3-K Is Inhibited in Burns—To further confirm that the association of PI 3-K to IRS-1 is inhibited after burns, we assessed the direct interaction of IRS-1 with p85 PI 3-K molecules when stimulated by insulin in vivo. Following in vivo insulin stimulation, aliquots of muscle homogenates were immunoprecipitated with anti-IRS-1 mAb. The immunoprecipitates obtained in this manner were subjected to immunoblotting with anti-p85 antibody or anti-IRS-1 antibody. The total amount of IRS-1 was the same in the two groups (Fig. 5, lower panel), and thus the previous findings (Fig. 4, lower panel) that there were no quantitative changes in IRS-1 were confirmed. In the absence of insulin (Insulin -), p85 immunoreactivity associated with IRS-1 was absent in both sham and burn samples (Fig. 5, upper panel; S1, S3, B1, B3). The association of p85 with IRS-1 was promoted by the stimulation of insulin in vivo (Insulin +) in sham burn animals but was completely absent in burned animals. The results clearly indicate that despite the presence of normal amounts of IRS-1, the physical association of IRS-1 with p85 PI 3-K was deficient in burns.

IR Autophosphorylation Is Decreased in Burns—After insulin binds to the α-subunits of the IR, the next step in insulin signaling is the autophosphorylation of its β-subunit (IRβ) by its intrinsic tyrosine kinase activity. To verify if autophosphorylation of the IR occurs, insulin was administrated in vivo to burned and sham burned animals. The IR in the gastrocnemius muscle lysates of these animals was separated by immunoprecipitation with anti-IRβ antibody. These samples were then immunoblotted with an anti-phosphotyrosine antibody. As indicated in Fig. 6 (lower panel), the total quantities of IRβ were not different between sham burned and burned animals. In the absence of insulin (Insulin -), phosphorylation was absent in IR, both in sham (S1, S3) and burned (B1, B3) animals (Fig. 6, upper panel). Marked phosphorylation of IR (S2, S4) was observed following insulin in sham burned animals, but was completely absent in muscle samples from burned animals (B2, B4). These studies, thus, confirm that the autophosphorylation of IRβ is severely attenuated following burns, explaining some of the attenuated postreceptor signal transduction.

Tyrosine Phosphorylation of IR Is Intact after Burn Injury When Stimulated in Vitro—To further rule out the possibility that IR by itself is qualitatively or quantitatively changed after burn injury, IR from hind limb muscle of burned or sham burned animals was isolated by immunoprecipitation, and autophosphorylation was examined in the presence of 100 nM insulin in vitro. The samples were subjected to SDS-PAGE, and phosphorylated IR was detected as described previously. Basal tyrosine phosphorylation of IR was absent in all groups (Fig. 7, Insulin -). Insulin-stimulated tyrosine phosphorylation of IR was observed equally in the samples from burned and sham burned animals (Fig. 7, Insulin +). These results strongly suggest that action of insulin to activate IR is intact and that the defective insulin signaling probably occurs along the postreceptor intracellular signaling pathway.

Inhibitory Effect of IRS-1 on IR Kinase Activity after Burn Injury—Recently, it was reported that IRS-1 can inhibit IR kinase activity in the pathological state of obesity (20), an effect dependent on alkaline phosphatase-sensitive phosphorylation. We therefore examined whether the inhibitory form of IRS-1 was present in the muscle of burned animals. Tissues from insulin-resistant obese animals served as positive controls. For these experiments, IR was initially partially purified by wheat germ agglutinin column chromatography from CHO-IR cells.
stably overexpressing recombinant IR. Next, insulin-stimulated autophosphorylation of the recombinant IR was assessed in the presence of IRS-1 isolated from the hind limb of burned, obese, or sham burned animals. We found IR phosphorylation was markedly attenuated with the addition of IRS-1 from burned (90%) and obese (100%) animals, compared with that of sham burned rats (Fig. 8). These results indicate that IRS-1 from muscle tissue of rats with burns or obesity exhibits an inhibitory action on IR kinase activity.

This inhibitory effect of IRS-1 obtained from muscle tissues of burned animals was abolished by enzymatic dephosphorylation of the IRS-1 with alkaline phosphatase prior to incubation (Fig. 8). These data confirm that the defective insulin action in burn injury is related to the alkaline phosphatase-sensitive phosphorylation of IRS-1, which is similar to that seen in obesity-induced insulin resistance.

**DISCUSSION**

The present study has indicated that glucose transport in soleus muscle is decreased following burn injury, confirming previous studies (28–30). We have used the in vitro system to analyze glucose metabolism. In vivo measurement of glucose metabolism is not a completely adequate system to assess insulin resistance, since glucose homeostasis in the intact body is regulated both by glucose production (mainly in liver) and by glucose uptake (mostly in muscle). Furthermore, insulin efficacy may also be affected by circulating inhibitory factors. Under the conditions we used, factors in the extracellular space that may inhibit insulin or the IR per se were eliminated. This permitted the conclusion that the observed insulin resistance was probably not due to a circulating factor but to a postreceptor mechanism. This notion is further supported by the evidence showing that the amount of IR in gastrocnemius muscles, as assessed by immunoblot with anti-IR antibody, was unchanged in burns (Fig. 6).

The in vitro ligand-dependent tyrosine phosphorylation of IR isolated by immunoprecipitation with anti-IR antibody was unchanged in burns (Fig. 7). This confirmed that there was no qualitative or qualitative change in the IR when it was isolated from the muscle tissue of burned rats and tested in vitro, suggesting the existence of some intracellular molecule inhibitory to IR kinase activity. The fact that insulin resistance was identified in limb muscles distant from the burned area correlates with other reports that burn injury causes systemic insulin resistance, including muscles that are distant from the burned area (30). The systemic inflammatory response, evidenced as an increase in acute phase reactant proteins, may cause these distant changes. Humoral factors, including epinephrine and/or cytokines, are likely candidates to play key roles in this process, since their concentrations are markedly increased in burns (5, 6). Our observation of insulin resistance at day 3 is consistent with other reports of insulin resistance and independent observations of elevations of TNFα during this period at 2–14 days after burn injury (3, 4, 5, 6, 21).

The second finding in our study was that IR failed to activate IRS-1 in response to insulin in vivo. We first employed the in vivo system and measured IRS-1-associated PI 3-K activity. PI 3-K was not activated by insulin injection in rats following thermal injury. As indicated previously, the activation of PI 3-K is pivotal for insulin action on glucose homeostasis (13, 15, 26). Our results suggest that the defect in signaling of insulin receptor in burns lies along the pathways from IR to PI 3-K. We found no changes in p85-associated PI 3-K activity, the amount of IRS-1, or the amount of IR. Although IR autophosphorylation was normal when tested in vitro, in vivo IR autophosphorylation and tyrosine phosphorylation of IRS-1 were markedly inhibited in burned animals.

There are some reports that TNFα can repress mRNA levels of GLUT4, the downstream target of PI 3-K, in 3T3-L1 adipocytes, as assessed by immunoblot with anti-IR antibody, was unchanged in burns (Fig. 6).
cytes (34, 35), but these studies were performed only in adipocytes in vitro. There is no report, however, of TNFα effect on GLUT4 in myocytes in vitro. In an in vivo study, Vary et al. (36) reported that the quantities of GLUT4 were not decreased in skeletal muscle after septic shock; both protein and mRNA levels of GLUT1, one of the isoforms of glucose transporters, were increased. In addition, in a rat model of endotoxic shock, both GLUT4 mRNA and GLUT1 mRNA were increased in skeletal muscle (37). Our results, taken together with these observations on critical illness models, thus support the conclusion that the system upstream of PI 3-K is a major derangement in the observed insulin resistance of burn stress.

What factor(s) inhibits insulin receptor kinase action inside the cells? The hyperglycemia and/or insulin resistance could be attributable to humoral factors, some of which are markedly increased in burns. The roles of adrenergic agonists are still controversial, since their antagonists did not inhibit hepatic production of glucose but rather increased it. Furthermore, the beneficial effects of β-blockers following burns were not observed relative to metabolic functions (38, 39). Contrary to expectations, adrenergic agonists in fact have beneficial effects on glucose and protein homeostasis in adipose tissue and muscle in stress states, when injected for longer periods (40, 41). Therefore, much remains to be understood concerning to the role of adrenergic agonists and antagonists in insulin resistance of burns.

Recently, it has been reported that TNFα can play a central role in insulin resistance of obesity (42). In fa/ fa rats, neutralization of TNFα alleviated insulin resistance (19). Interestingly, the source of this factor was attributable to the local production (adipocyte), where insulin resistance was observed. In addition, using cultured cells, these investigators and others showed that insulin resistance could be induced by TNFα treatment (43). It is of interest to note that TNFα-induced insulin resistance in adipocytes was observed only after at least a 3-day exposure of this cytokine. This may suggest that prolonged activation of certain cytokines may be necessary for the process. The question therefore arises whether TNFα plays a role in the induction of insulin resistance of burns. There are several lines of evidence suggesting that this may be the case. First, it was reported that burn-induced insulin resistance was not observed at day 1 after injury (7) but was observed at day 5 after burns and that the resistance persisted for weeks. This observation may suggest that induction of certain cytokine-related genes may be necessary to induce insulin resistance. Second, in burns, the concentration of TNFα in the blood is usually elevated for prolonged periods (22, 44). Third, in preliminary data, we found elevated mRNA expression of TNFα in muscles of burned animals by ribonuclease protection assay but not in shams (data not shown). This indirect evidence supports the view that locally produced TNFα may play a role in inducing insulin resistance of burns. TNFα can convert IRS-1 to a hyper-serine-phosphorylated form and render it an inhibitory molecule to IR kinase. The hyper-serine-phosphorylated form can be easily reversed by dephosphorylation with alkaline-phosphatase treatment (20).

We investigated the possibility that the IRS-1 in muscles following burns would have similar characteristics to that seen in obese-induced insulin resistance. IRS-1 in burns not only possessed an inhibitory effect on insulin receptor kinase but also lost its inhibitory function when treated with alkaline phosphatase presumably by virtue of its dephosphorylation. Since this characteristic is shared with that of IRS-1 seen in fa/ fa rats and TNFα-treated cells, the results strongly support the view that TNFα might be involved in insulin resistance in burns too. Currently, we are investigating whether insulin resistance in burns can be corrected by injection of TNFα-neutralizing agents in rats. If TNFα plays as great a role in the insulin resistance of burns as in obesity, it could turn out that TNFα would be the major factor in the insulin resistance, even in other forms of stress states. In this regard, our burn model, together with the obesity model, may serve as a useful system to analyze the molecular mechanism of insulin resistance in stress/injury-induced insulin resistance. The burn injury model could be also useful to characterize the molecular details of alkaline phosphatase-sensitive phosphorylation of IRS-1. We expect the muscle extracts from burned animals to possess this activity, allowing more thorough investigation of this newly identified chemical modification of IRS-1.

Our findings in the present study have some clinical implications. Insulin stimulates IR kinase activity, which is turned off and negatively regulated by the IRS-1 in muscles after burn. It is therefore not surprising that insulin signaling is desensitized at certain periods after burns. Thus, two major metabolic functions of insulin, regulation of glucose homeostasis and protein anabolism, are both expected to be attenuated because of the presence of an inhibitory form of IRS-1 on insulin receptor kinase action. This speculation is well supported by the report of Clark et al. (30) that changes in glucose utilization and the rate of proteolysis in muscle are linked in their studies, where they used a rat model of thermal injury.

In sepsis, it is still controversial whether insulin function of regulating protein catabolism is inhibited, although the desensitization of its function in terms of glucose regulation is well established. Jahoor et al. (45) reported that insulin propagates signals to regulate protein catabolism in septic states equally well as in normal states. In contrast, Vary and co-workers (46) reported that insulin function in regulating protein catabolism is also desensitized. Since the major role of TNFα has been well characterized in sepsis in terms of dysregulation of homeostasis, it is expected that an inhibitory form of IRS-1 may be present also in tissues of animals with sepsis. Our approach, as presented here, would serve as a good system for analyzing the molecular defect in this pathologic state.

Recent studies have suggested that IRS-1-like docking proteins such as IRS-2, Gab1, and pp60 may play an important role in insulin signaling (47–49), since these molecules are tyrosine-phosphorylated and bind PI 3-kinase in response to insulin. In addition, a recent report has questioned the role of IRS-1 phosphorylation in mediating glucose transport (50). Thus, it would be of interest, in future studies, to analyze insulin-stimulated tyrosine phosphorylation of IRS-2, Gab1, and pp60 in skeletal muscle samples from thermally injured animals to determine the function of these molecules on insulin receptor kinase and downstream signaling molecules.

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