Skeletal muscle insulin resistance after trauma: insulin signaling and glucose transport

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Strömmer, Lisa, Johan Perment, Urban Arnelo, Camilla Koehler, Bengt Isaksson, Jörgen Larsson, Inger Lundkvist, Marie Björnholm, Yuichi Kawano, Harriet Wallberg-Henriksson, and Juleen R. Zierath. Skeletal muscle insulin resistance after trauma: insulin signaling and glucose transport. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E351–E358, 1998.—Surgical trauma induces peripheral insulin resistance; however, the cellular mechanism has not been fully elucidated. We examined the effects of surgical trauma on insulin receptor signaling and glucose transport in skeletal muscle, a tissue that plays a predominant role in maintaining glucose homeostasis. Surgical trauma was induced by intestinal resection in the rat. Receptor phosphorylation was not altered with surgical trauma. Phosphotyrosine-associated phosphatidylinositol (PI) 3-kinase association was increased by 60 and 82% compared with fasted and fed controls, respectively (P < 0.05). Similar results were observed for insulin receptor substrate-1-associated PI 3-kinase activity. Insulin-stimulated protein kinase B (Akt kinase) phosphorylation was increased by 2.2-fold after surgical trauma (P < 0.05). The hyperphosphorylation of Akt is likely to reflect amplification of PI 3-kinase after insulin stimulation. Submaximal rates of insulin-stimulated 3-O-methylglucose transport were reduced in trauma vs. fasted rats by 51 and 38% for 100 and 200 µU/ml of insulin, respectively (P < 0.05). In conclusion, insulin resistance in skeletal muscle after surgical trauma is associated with reduced glucose transport but not with impaired insulin signaling to PI 3-kinase or its downstream target, Akt. The surgical trauma model presented in this report provides a useful tool to further elucidate the molecular mechanism(s) underlying the development of insulin resistance after surgical trauma.

insulin receptor; phosphatidylinositol 3-kinase; Akt/protein kinase B; surgical stress

TRAUMA-INDUCED METABOLIC STRESS from surgery or injury is associated with alterations in carbohydrate metabolism (13, 20), including increased glucose production (6) and decreased peripheral glucose utilization (5, 41). The most common form of posttraumatic insulin resistance is iatrogenic in nature, developing in the postoperative state. Several lines of evidence suggest that peripheral tissues are the major site of the trauma-induced insulin resistance (3, 31). Glucose clamp studies in postoperative patients revealed that impaired glucose disposal develops in peripheral tissues (3, 17, 41). Interestingly, the magnitude of surgically induced insulin resistance is related to the degree of surgical trauma, and this persists for 2–3 wk after uncomplicated abdominal surgery (32, 40–42). Despite the considerable progress in understanding the development of trauma-induced insulin resistance at the whole body level, the cellular mechanism remains unclear.

Peripheral insulin resistance after posttraumatic stress may occur in response to defects in early or intermediate components of the insulin signal transduction pathway and/or in response to defects at the level of glucose transport, a rate-limiting step in glucose utilization (10). One of the earliest postreceptor events leading to the metabolic effects of insulin includes the phosphorylation of the insulin receptor substrate-1 (IRS-1) on tyrosine residues (46). IRS-1 serves as a multiple docking site for proteins containing Src-homology 2 domains, such as phosphatidylinositol (PI) 3-kinase (46). Multiple lines of evidence suggest that PI 3-kinase plays a central role in mediating insulin signaling to glucose transport (8, 37). Recently, we have reported that reduced insulin-stimulated PI 3-kinase activity is coupled to reduced glucose transport in skeletal muscle from patients with non-insulin-dependent diabetes mellitus (2). Thus defects at the level of the insulin signal transduction pathway may contribute to impaired glucose transport (2, 14, 18, 19) and glucose transporter isoform GLUT-4 translocation (49), and this may result in reduced whole body glucose homeostasis. In adipocytes, insulin resistance after trauma appears to develop from a postreceptor lesion, since glucose uptake is decreased despite normal insulin binding to the insulin receptor (34). Whether the reduced glucose transport with surgical trauma is a primary defect due to altered GLUT-4 traffic from an intracellular pool to the plasma membrane or a secondary defect due to alterations in the insulin signal transduction cascade remains unknown.

In the present study, we have adopted a small intestinal bowel resection model in fasted rats to delineate the underlying mechanism for the apparent peripheral insulin resistance that arises from surgical trauma. We have assessed early, intermediate, and final components of the insulin signal transduction pathway to glucose transport in skeletal muscle. Specifically, we have assessed insulin receptor binding and autophosphorylation, PI 3-kinase activity and association to phosphotyrosines, protein kinase B (Akt kinase) phosphorylation, and glucose transport to determine whether defects in insulin signaling contribute to impaired glucose homeostasis after postoperative stress.
METHODS

Animals. Male Wistar rats (320–350 g; B&K Universal, Stockholm, Sweden) were housed under controlled conditions with a 12:12-h light-dark cycle (lights-on 7 AM to 7 PM). Rats were provided standard laboratory chow and water ad libitum for 1 wk before experimentation. Rats were divided into three groups: fed (n = 25), fasted controls (n = 22), and trauma (n = 30). Food was withdrawn 16 h before experimentation from rats in the fasted and trauma groups and immediately before experimentation from rats in the fed group.

Operative procedure. All procedures were conducted with approval from the local ethical committee. Rats were anesthetized by an intraperitoneal injection of ketamine and xylazine (70 and 10 mg/kg, respectively). Blood samples were obtained for preoperative determination of glucose levels from the tail vein. An 8-cm incision was made along the abdomen. Five centimeters distal to the Treitz’s ligament, a 5-cm small bowel resection was performed. Thereafter, the bowel was sutured with absorbable sutures (6/0, Vicryl), and the abdominal cavity was closed using eight sutures (4/0, Vicryl). The animals were returned to individual cages and had free access to water. Two hours later, rats were reanesthetized, and a plastic catheter (Venflon, 0.8/25 mm) was inserted into the internal jugular vein. Blood samples were obtained for determination of glucose and insulin levels. Thereafter, a bolus injection of 1 ml of 0.9% NaCl was administered under 1 min (iv), and a muscle biopsy was obtained from the right gastrocnemius muscle. Insulin was injected intravenously (10 U/kg, in 1 ml of 0.9% NaCl and 0.01% BSA) as a bolus delivered under 1 min. Four minutes after the injection, a second biopsy was obtained from the left gastrocnemius muscle. The muscle specimens were immediately frozen on excision and stored in liquid nitrogen until analysis.

Blood chemistry. In a subgroup of rats, a catheter was inserted into the jugular vein immediately after the second anesthesia, and blood samples were collected for determination of blood glucose and plasma levels of insulin, nonesterified free fatty acids (NEFAs), epinephrine, cortisol, and lactate. Blood glucose and plasma lactate were determined enzymatically (YSI 2000 system; Kebo, Stockholm, Sweden). Plasma insulin was analyzed using a commercially available kit (Diagnostica, Falkenberg, Sweden). Plasma NEFAs were analyzed using a kit from Wako Chemicals (Neuss, Germany). Plasma epinephrine was determined by HPLC with electrochemical detection, with 3,4-dihydroxybenzylaminehydrochloride as an internal standard (16). The size of the chromatography column was 150 × 4.6 mm (Catecholamine column 5 SA; Machery-Nagel, Düren, Germany), and an EC 2000 electrochemical detector was utilized (Therm Separation Products, Riviera Beach, FL). Serum cortisol was measured in a solid-phase time-resolved fluoromunoassay (9), based on a competitive reaction between europium-labeled insulin and sample cortisol (Wallac Oy, Turku, Finland).

Insulin receptor binding and phosphorylation. Gastrocnemius muscle biopsies were ground to a fine powder with a mortar and a pestle in liquid nitrogen and immediately homogenized in ice-cold buffer as previously described (15). Homogenates were solubilized by gentle mixing for 30 min at 4°C. After centrifugation (35,000 g for 45 min at 4°C), the supernatant was removed, protein was determined (BCA protein assay kit, Pierce, Rockford, IL), and aliquots were stored at −70°C. Insulin receptor binding and phosphorylation were determined as described previously (15). A portion of the supernatant was immunoprecipitated overnight at 4°C with a monoclonal antibody directed against the β-subunit of the insulin receptor (IR 29B4, Santa Cruz Biotechnology, Santa Cruz, CA). The precipitate was further incubated for 30 min at 4°C with a secondary monoclonal antibody (anti-IgG1; DAKO, Copenhagen, Denmark), followed by incubation for 1 h at 4°C with 125 µl of protein A (Pansorbin cells). The immunoprecipitates were divided into four equal aliquots, washed, and resuspended in Tris buffer containing 1% Triton X-100 and 1 mM phenylmethylsulfonyl fluoride. A second aliquot of the supernatant (4 mg of protein) was used to immunoprecipitate tyrosine-phosphorylated proteins (overnight at 4°C) with the use of an anti-phosphotyrosine antibody (PY20; Signal Transduction Laboratories, Lexington, KY). The immunocomplex was incubated with secondary antibody (anti-IgG2b, DAKO) and protein G. The complex was washed three times and resuspended in buffer (50 mM Tris, 1% Triton X-100, 1 mM Pervanadate) and divided into four aliquots. In three aliquots of the insulin receptor immunoprecipitate, insulin receptors were assessed using 125I-labeled insulin (25,000 counts/min (cpm)) overnight. Non-specific binding was determined in one of the aliquots by addition of unlabeled insulin (60 µM). To detect insulin receptor tyrosine phosphorylation, the anti-phosphotyrosine immunoprecipitates were incubated with 125I-labeled insulin (25,000 cpm) overnight. The pellets were washed three times, and binding of labeled insulin in anti-insulin receptor (α-IR) and anti-phosphotyrosine (α-PY) immunoprecipitates was determined in a gamma counter. Bound-free quotients per milligram protein were calculated for the two different immunoprecipitated samples. Quotients of immunoprecipitates were used to calculate fraction of phosphorylated receptors, corrected for receptor binding under basal and insulin-stimulated conditions.

Tyrosine phosphorylation of IRS-1. Equal amounts of protein (2 mg) were immunoprecipitated overnight with IRS-1 antibody (gift from Dr. Morris White, Joslin Diabetes Center, Boston, MA) coupled to protein A-Sepharose. The immunoprecipitates were washed as described (14), resuspended in Laemmli sample buffer with 100 mM dithiothreitol, and heated (95°C) for 6 min. The proteins were separated by SDS-PAGE, transferred to nitrocellulose membranes, and blocked in Tris-buffered saline (TBS) (10 mM Tris, 0.1% Tween, pH 7.8) containing 3% BSA. The blotted membranes were immunoblotted with horseradish peroxidase-conjugated antiphosphotyrosine antibody (RC-20; Signal Transduction) and washed thereafter with TBS-T. IRS-1 was visualized by enhanced chemiluminescence (Amersham, Arlington Heights, IL) and quantified by densitometric scanning (Imagemaster, Pharmacia Biotech, Uppsala, Sweden).

Phosphotyrosine and IRS-1 association with the 85-kDa band. Plasma was separated by SDS-PAGE, transferred to nitrocellulose membranes, and blocked in Tris-buffered saline (TBS) (10 mM Tris, 0.1% Tween, pH 7.8) containing 3% BSA. The blotted membranes were immunoblotted with horseradish peroxidase-conjugated antiphosphotyrosine antibody (RC-20; Signal Transduction) and washed thereafter with TBS-T. IRS-1 was visualized by enhanced chemiluminescence (Amersham, Arlington Heights, IL) and quantified by densitometric scanning (Imaget (ImageMaster, Pharmacia Biotech, Uppsala, Sweden)).
RESULTS

Blood glucose and plasma insulin levels. Basal glucose was significantly lower in fasted control and traumatized rats compared with fed control rats (4.5 ± 0.3 and 4.3 ± 0.2 vs. 7.0 ± 0.4 mM, respectively; Table 1). After insertion of a catheter in the jugular vein, blood glucose was significantly increased in fed rats (7.0 ± 0.4 vs. 12.0 ± 0.5 mM), whereas no change was observed in fasted controls (4.5 ± 0.3 vs. 5.3 ± 0.4 mM). In traumatized rats, glucose levels were significantly elevated compared with fasted controls (4.3 ± 0.3 vs. 6.5 ± 0.4 mM, P < 0.05). Plasma insulin was significantly higher in fed rats compared with traumatized and fasted control rats (398 ± 94 vs. 56 ± 11 and 68 ± 7 pM, respectively; P < 0.01). The insulin values in fasted and traumatized rats were similar.

Table 1. Plasma and serum concentrations of hormones and metabolites

<table>
<thead>
<tr>
<th></th>
<th>Fed (n = 10)</th>
<th>Fasted (n = 8)</th>
<th>Trauma (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose, mM</td>
<td>7.0 ± 0.4</td>
<td>4.5 ± 0.3</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td>Posttrauma</td>
<td>12.0 ± 0.5*</td>
<td>5.3 ± 0.4</td>
<td>6.5 ± 0.4*</td>
</tr>
<tr>
<td>Plasma insulin, pM</td>
<td>398 ± 94</td>
<td>56 ± 11</td>
<td>68 ± 7</td>
</tr>
<tr>
<td>Serum cortisol, mM</td>
<td>310 ± 29</td>
<td>271 ± 43</td>
<td>331 ± 64</td>
</tr>
<tr>
<td>Plasma NEFA, mM</td>
<td>1.9 ± 0.3†</td>
<td>0.7 ± 0.2</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Plasma lactate, mM</td>
<td>1.1 ± 0.2</td>
<td>0.9 ± 0.1</td>
<td>0.8 ± 0.1</td>
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</tbody>
</table>

Values are means ± SE. In control rats, trauma is defined as anesthesia and insertion of a venous catheter. NEFA, nonesterified fatty acid. *P < 0.05, pre- vs. posttrauma. †P < 0.01, fed vs. fasted control and trauma.

Thiourea transport. Media were prepared from oxygenated (95% O2-5% CO2) Krebs-Henseleit buffer (KHB) containing 5 mM HEPES and 0.1% BSA (RIA grade). Immediately after the second anesthesia, isolated soleus muscles were removed from a subgroup of rats. The muscle was divided into three equal portions, and the two outer portions (20 mg each) were used to assess glucose transport (45). The muscle specimens were initially incubated (30°C) for 15 min in KHB containing 2 mM pyruvate and 18 mM mannitol. Thereafter, muscles were incubated in the presence of insulin (0, 100, 200, and 2 mM) for 10 min in KHB containing 20 mM mannitol. Glucose transport was assayed using 3-O-[methyl-3H]glucose (2.5 µCi/mmol) and 12 mM [14C]mannitol (26.3 µU/mmol). The muscles were processed as described (45). Glucose transport activity is expressed as micromoles of glucose analog accumulated per milliliter of intracellular water per hour.

Chemicals. Human insulin (Adtrapid) was purchased from Novo Nordisk (Bagsvaerd, Denmark). Protein G-Sepharose-4 fast flow was from Pharmacia (Uppsala, Sweden), and protein A (Pansorbin) was from Calbiochem-Novabiochem (La Jolla, CA). All reagents for SDS-PAGE were from Novex (San Diego, CA), and reagents for the protein assay were from Pierce. [125I]-insulin was from Amersham (Buckinghamshire, UK). All other radiotopes were purchased from ICN Biochemical (Costa Mesa, CA). Phosphatidylinositol was from Avanti-Polar Lipids (Alabaster, AL). Aluminum-backed silica gel-60 thin-layer chromatographic plates were from Merck (Darmstadt, Germany). Other standard chemicals and reagents, including BSA, were purchased from Sigma (St. Louis, MO).

Statistical analysis. Data are presented as means ± SE. Statistical analysis was performed using the Student’s unpaired t-test for pre- and postglucose values and for Akt phosphorylation. Comparisons between the groups were performed with ANOVA two-way multivariate analysis followed by the Bonferroni correction (PI 3-kinase) or Dunnet one-tailed post hoc test (glucose transport).
tion (Table 2). Anti-insulin receptor immunoblots of insulin receptor precipitates failed to reveal any reduction in insulin receptor content before and after insulin stimulation (data not shown). Thus the decrease in insulin receptor binding in fed control rats was not due to an actual reduction in the number of receptors in the insulin-stimulated state. The ratio of the bound-free quotients of $^{125}$I-insulin bound in immunoprecipitates in insulin-stimulated state. The ratio of the bound-free ratio to an actual reduction in the number of receptors in the insulin receptor binding in fed control rats was not due stimulation (data not shown). Thus the decrease in insulin receptor precipitates failed to reveal any reduction in insulin receptor content before and after insulin stimulation (Table 2).

### Table 2. Insulin receptor autophosphorylation and binding in phosphotyrosine and insulin receptor immunoprecipitates per mg protein

<table>
<thead>
<tr>
<th>Phosphorylation, per mg protein</th>
<th>Fed (n = 10)</th>
<th>Fasted (n = 8)</th>
<th>Trauma (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>2.4 ± 0.3</td>
<td>2.7 ± 0.2</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>Insulin stimulated</td>
<td>33.2 ± 3</td>
<td>62.1 ± 4*</td>
<td>56.6 ± 3*</td>
</tr>
<tr>
<td>Phosphorylation per receptor, bound-free ratio $10^{-3}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>213 ± 8</td>
<td>238 ± 13</td>
<td>214 ± 8</td>
</tr>
<tr>
<td>Insulin stimulated</td>
<td>171 ± 9†</td>
<td>240 ± 12</td>
<td>215 ± 8</td>
</tr>
<tr>
<td>Phosphorylation per receptor, bound-free ratio $10^{-3}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>11 ± 1.2</td>
<td>12 ± 1.5</td>
<td>8.8 ± 0.9</td>
</tr>
<tr>
<td>Insulin stimulated</td>
<td>193 ± 14</td>
<td>267 ± 28*</td>
<td>264 ± 16*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05, trauma and fasted control vs. fed control; †P < 0.05 basal vs. insulin stimulation.

results were observed in IRS-1 immunoprecipitates; surgical trauma led to a 60% increase in insulin-stimulated PI 3-kinase association to IRS-1 (226 ± 26 vs. 362 ± 36% for fed control vs. traumatized rats, respectively; P < 0.05).

Phosphotyrosine- and IRS-1-associated PI 3-kinase activity. Recent studies provide evidence for several PI 3-kinase adapter subunit variants, which exhibit different insulin-induced PI 3-kinase elevating responses (1, 21, 22, 38). Thus the increase in p85 bound to phosphotyrosine-phosphorylated proteins or to IRS-1 noted in muscle from surgically traumatized rats may not translate to increased activity of PI 3-kinase. Consequently, we
assessed phosphotyrosine- or IRS-1-associated PI 3-kinase activity in muscle from control or traumatized rats. Basal PI 3-kinase activity was similar among all groups. Insulin-stimulated IRS-1-associated PI 3-kinase activity (Fig. 3) was significantly greater in traumatized vs. fed control rats (417 ± 41 vs. 716 ± 50%, respectively; P < 0.05). PI 3-kinase activity was also greater in traumatized rats compared with fasted control rats (417 ± 41 vs. 558 ± 44%); however, with Bonferroni correction, the latter difference did not reach statistical significance. A similar trend was observed in anti-phosphotyrosine immunoprecipitates; insulin-stimulated phosphotyrosine-associated PI 3-kinase activity tended to be increased in skeletal muscle after surgical trauma (244 ± 46 vs. 335 ± 63% for fed control vs. traumatized rats, respectively; not significant).

Akt phosphorylation. The serine/threonine kinase Akt (protein kinase B/Rac), a downstream target of PI 3-kinase, has been implicated to play a role in growth factor signaling to glucose transport and glycogen synthesis (4, 7, 12, 25–27, 39, 44). Thus we assessed basal and insulin-stimulated Akt phosphorylation in skeletal muscle from control or traumatized rats (Fig. 4). Insulin induced a marked phosphorylation of Akt kinase in skeletal muscle from both control and traumatized rats. Multiples of insulin stimulation could not be calculated because of the complete lack of Akt phosphorylation under basal conditions (Fig. 4). Surgical trauma resulted in a 2.2-fold increase (P < 0.05) in insulin-stimulated Akt phosphorylation (100 ± 9 vs. 221 ± 51% for control vs. traumatized rats, respectively).

Glucose transport in isolated soleus muscle. Glucose transport was studied in fasted controls (n = 7) and traumatized (n = 7) rats (Fig. 5). Basal (no insulin) glucose transport was similar between the groups (0.77 ± 0.12 vs. 0.73 ± 0.13 µmol·ml⁻¹·h⁻¹ for traumatized vs. fasted control rats, respectively). Furthermore, the response of glucose transport to a supraphysiological concentration of insulin was similar between the groups (2.87 ± 0.32 vs. 3.70 ± 0.41 µmol·ml⁻¹·h⁻¹ for traumatized vs. fasted control rats, respectively). The insulin dose response for 3-O-methylglucose trans-
port was significantly different between fasted control and traumatized rats (2-way ANOVA, \( P < 0.005 \)). Post hoc analysis revealed that glucose transport activity after submaximal insulin stimulation (100 and 200 \( \mu U/ml \)) was significantly lower (\( P < 0.05 \)) in traumatized vs. fasted rats.

**DISCUSSION**

Here we show that surgical trauma in combination with fasting leads to increased insulin receptor phosphorylation and PI 3-kinase activity and reduced insulin-stimulated glucose transport. Furthermore, surgical trauma results in a moderate increase in the plasma concentrations of glucose and epinephrine, with no change in cortisol levels. The reduced submaximal insulin-stimulated glucose transport in skeletal muscle from the traumatized rats suggests that the small bowel resection employed in the present study was sufficient to induce altered glucose homeostasis, a hallmark characteristic of surgical stress. Our finding of reduced insulin-stimulated glucose transport in skeletal muscle is consistent with previous reports in which marked peripheral insulin resistance has been observed after different forms of catabolic stress, including burns, sepsis, accidental trauma, and surgery in humans (3, 17, 23, 30, 41, 47, 48).

Fasting increases insulin signaling in skeletal muscle at the level of the insulin receptor, IRS-1 (37) and PI 3-kinase (19). The increase in insulin receptor phosphorylation after surgical stress in combination with fasting was accompanied with an increase in PI 3-kinase activity and an increased association of p85 to phosphotyrosines. Despite these changes, insulin-stimulated glucose transport was reduced. Such a dissociation between intermediate and final components of insulin signaling has been observed previously. With streptozotocin-induced diabetes, tyrosine phosphorylation of IRS-1 and IRS-1-associated PI 3-kinase activity is markedly increased in skeletal muscle (11) despite severe insulin resistance (45). Thus trauma-induced insulin resistance in skeletal muscle is neither at the level of the insulin receptor nor at PI 3-kinase but may lie downstream from PI 3-kinase. Recently, the serine/threonine kinase Akt (protein kinase B/Rac) has been suggested to play a role in the signaling pathway to glucose transport (27, 39, 44). We have recently provided evidence that reduced insulin-stimulated Akt activity is associated with reduced glucose transport in muscle from diabetic Goto-Kakizaki rats (28). Interestingly, restoration of glycemia completely normalized insulin action on Akt kinase (28). Consequently, we hypothesized that reduced Akt kinase activity may lead to decreased glucose transport in muscle after surgical trauma. Our finding that surgical stress potentiates the effect of insulin on PI 3-kinase and Akt kinase in skeletal muscle does not support the hypothesis that early or intermediate signaling defects contribute to reduced glucose transport after surgical trauma. Alternatively, the exocytotic machinery for GLUT-4 translocation may be altered by surgical stress such that GLUT-4 vesicles fail to fuse or dock to the plasma membrane, and thus glucose entry into the cell may be impaired. Furthermore, hyperphosphorylation of early components of the insulin signal transduction pathway may lead to negative feedback that inhibits downstream signaling to glucose transport (11).

In the present study, insertion of a venous catheter in fed (i.e., a minor trauma) but not in fasted rats resulted in a marked increase in blood glucose. When fasted rats were subjected to a more severe trauma (small bowel resection), blood glucose was significantly elevated. Thus the degree of posttraumatic hyperglycemia appears to be dependent on both the magnitude of trauma and on substrate availability. This finding is consistent with previous findings observed in an animal model of trauma and starvation (31).

Increased levels of NEFAs may reduce insulin action in skeletal muscle (33). However, in the present study, NEFA levels were lower in traumatized rats compared with fasted controls. Our finding is consistent with previous observations in hyperglycemic postoperative patients (24) and with reports of unchanged NEFA levels in septic patients (47). Furthermore, we found no significant differences in plasma lactate concentrations. Severe trauma may alter the circulatory levels of insulin and insulin-antagonistic hormones such as epinephrine and cortisol, which can affect glucose metabolism (29). However, plasma insulin concentrations were similar between the fasted and traumatized rats. Thus the defect in glucose transport was not due to reduced insulin levels. After intestinal resection, plasma concentrations of epinephrine were moderately increased, whereas cortisol levels were not altered compared with time-matched controls. The endocrine response found in the present study is similar to that observed in postoperative patients, in which epinephrine and cortisol levels are unaltered or moderately increased (17, 32, 41). Thus increased levels of these hormones may be of minor importance for the development of insulin resistance associated with sepsis or moderate surgical stress in humans (32, 47).

In conclusion, insulin resistance in skeletal muscle after surgical trauma is associated with reduced glucose transport but not with impaired insulin signaling to PI 3-kinase. The surgical trauma model presented in this report will be a useful tool to further elucidate the molecular mechanisms underlying the development of insulin resistance after surgical trauma.

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**REFERENCES**


