Preoperative Feeding Does Not Reverse Postoperative Insulin Resistance in Skeletal Muscle in the Rat

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Metabolic studies on injured and postoperative patients have shown impaired glucose disposal in peripheral tissues after trauma. Using small-bowel resection as a model of surgical trauma, we investigated whether substrate availability could ameliorate the changes in muscle glucose uptake induced by trauma. We also studied the effect of preoperative feeding on postoperative insulin-stimulated insulin receptor substrate-1 (IRS-1)–associated phosphatidylinositol (PI) 3-kinase activity in both Wistar rats and genetically non–insulin-dependent diabetic Goto-Kakazaki rats (GK rats). Serum glucose, insulin, plasma epinephrine, lactate, and plasma nonesterified free fatty acids (NEFAs) were measured as indicators of the metabolic state and surgical stress. Insulin-stimulated glucose transport was significantly reduced in fed traumatized Wistar rats compared with fed nontraumatized rats ($P < .05$). Significant increases in in vivo insulin-stimulated IRS-1–associated PI 3-kinase activity were found in fed traumatized Wistar rats compared with fed nontraumatized Wistar rats and fasted traumatized Wistar rats, as well as fed traumatized GK rats compared with fed nontraumatized GK animals (all $P < .017$). Serum insulin concentrations were significantly reduced in fed traumatized Wistar and GK rats compared with the respective fed nontraumatized groups (both $P < .01$). Serum glucose levels were significantly elevated in fed traumatized GK rats compared with fed nontraumatized animals ($P < .01$). In the present study, preoperative feeding did not prevent a postoperative reduction in insulin-stimulated glucose transport in skeletal muscle. The finding that insulin-stimulated PI 3-kinase activity increased after trauma in both Wistar and GK rats indicates that postoperative insulin resistance is not caused by an impairment in the early steps of the insulin signaling pathway. The postoperative decreases in serum insulin despite high blood glucose suggest that trauma impairs the insulin response to hyperglycemia.

METABOLIC STRESS after surgery or injury is associated with alterations in carbohydrate metabolism. Glucose and insulin clamp studies in injured and postoperative patients have shown that impaired glucose disposal develops in peripheral tissues after trauma.[1-3] The severity of this insulin resistance is related to the magnitude of surgery.[4] However, the cellular mechanisms underlying the development of trauma-induced insulin resistance have not been determined. Some recent studies report that preoperative glucose supplementation reduces postoperative insulin resistance,[5,6] suggesting that postoperative glucose metabolism may be influenced by the availability of nutrients at the time of trauma.

Peripheral insulin resistance after trauma may occur in response to changes in early or intermediate components of the insulin signal transduction pathway and/or in response to alterations at the level of glucose transport. Some of the earliest postreceptor events leading to the metabolic effects of insulin are the phosphorylation of tyrosine residues on insulin receptor substrate-1 (IRS-1), the association of IRS-1 with phosphatidylinositol 3-kinase (PI 3-kinase), and the subsequent activation of PI 3-kinase.[7] Multiple lines of evidence suggest that PI 3-kinase plays a central role in the insulin signaling pathway that affects glucose transport.[8,9] Defects at this level of insulin signal tranduction have been shown to be associated with impaired glucose transport in animal models[10,11] and humans.[12,13] In patients with non–insulin-dependent diabetes mellitus and obese subjects, reduced insulin-stimulated PI 3-kinase activity is associated with impaired glucose transport and may result in reduced whole-body glucose utilization.[12,13] Conversely, increased PI 3-kinase activity has been shown to be associated with an increase in glucose transport.[14,15]

Improvements in anesthesia and surgical techniques have helped to reduce the stress response associated with surgery.[16-18] The relatively low stress response associated with modern surgery is demonstrated by the small to modest postoperative increases in counterregulatory hormones.[5] However, metabolic changes are still found after surgery, and current treatments for postoperative catabolic processes have limited benefits.[19] Because it is difficult to perform mechanistic experiments in human subjects, we recently developed an animal model involving small-bowel resection that is suitable for studying the cellular mechanisms responsible for postoperative insulin resistance.[20] This model results in modest postoperative increases in circulating counterregulatory hormones and reflects the magnitude of stress from modern surgery more accurately than previously described animal models.[21-23] When we used our model with fasted rats, we observed a postoperative decrease in insulin-stimulated glucose transport.[20] However, we found that the insulin-stimulated activity of IRS-1–associated PI 3-kinase and its downstream target protein Akt kinase (protein kinase B) in skeletal muscle increased after trauma,[20] rather than decreasing in parallel with the reduction in glucose transport.

In the present study, we investigated whether substrate availability at the time of trauma can ameliorate the changes in muscle glucose uptake induced by trauma and whether trauma increases insulin-stimulated PI 3-kinase activity in fed Wistar rats as it does in fasted rats. We also measured IRS-1–associated PI 3-kinase activity in genetically non–insulin-dependent dia-

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betic Goto-Kakazaki rats (GK rats), which show moderately impaired insulin sensitivity.[24,25] The GK rat experiments provided an opportunity to determine whether trauma increases insulin-stimulated PI 3-kinase activity in animals known to have lower insulin-stimulated PI 3-kinase activity than Wistar rats.[26] Serum insulin and glucose levels were determined as an indication of the metabolic state of the rats. Epinephrine, lactate, and nonesterified free fatty acid (NEFA) levels were measured in plasma as a reflection of the degree of surgical stress.

MATERIALS AND METHODS

Animals

Male Wistar rats (3 months old, 320 to 350 g; B&K Universal, Stockholm, Sweden) and GK rats (3 months old, 320 to 350 g; Karolinska Institute, Stockholm, Sweden) were housed under controlled conditions with a 12-hour light-dark cycle (lights on 7 AM to 7 PM) with free access to standard laboratory chow and water. Food was withdrawn immediately before anesthesia in the fed groups and 16 hours before anesthesia in the fasted group. All procedures were performed with approval from the local ethics committee.

Study Design

There were 5 study groups: fed nontraumatized Wistar rats (n = 22), fed traumatized Wistar rats (n = 22), fasted traumatized Wistar rats (n = 7), fed nontraumatized GK rats (n = 13), and fed traumatized GK rats (n = 6). Serum glucose, serum insulin, and in vivo stimulation of IRS-1–associated PI 3-kinase activity in skeletal muscle were measured in all study groups. In vitro studies of glucose transport and IRS-1–associated PI 3-kinase activity in response to a range of insulin doses were performed with skeletal muscle tissue from fed nontraumatized and fed traumatized Wistar rats. Because of the differing protocols, not all studies were performed on all rats within a group.

Small-Bowel Resection

The rats in the trauma study groups underwent intestinal resection as previously described.[20] Briefly, the abdominal cavity was opened after the rats were anesthetized by an intraperitoneal injection of ketamine (70 mg/kg) and xylazine (10 mg/kg). A 5-cm small-bowel resection was then performed at a site 5 cm distal to the ligament of Treitz. The animals were returned to the individual cages with free access to water but not food.

Blood and Muscle Sampling

All rats were anesthetized by an intraperitoneal injection of ketamine (70 mg/kg) and xylazine (10 mg/kg). This was the only time that animals in the nontrauma study groups were anesthetized. Rats in the trauma groups, which were anesthetized before bowel resection, were re-anesthetized 2 hours after completion of the operation. A plastic catheter was inserted into the internal jugular vein of the anesthetized rats, and a blood sample was obtained for measurement of serum insulin and glucose levels.

In animals used for in vitro studies of glucose transport or IRS-1–associated PI 3-kinase activity, the right and left soleus muscles were removed and placed in the incubation buffer described later. The animals were killed by pentobarbital injection after blood samples were taken from the aorta for measurement of epinephrine, lactate, and NEFA levels.

In animals used for the IRS-1–associated PI 3-kinase activity experiments, 1 mL 0.9% NaCl was administered intravenously before a biopsy was taken from the right gastrocnemius muscle. Insulin (10 U/kg) was then injected intravenously through the jugular catheter. Four minutes later, a biopsy was obtained from the left gastrocnemius muscle. The rats were then killed by pentobarbital injection. Muscle specimens, which were frozen in liquid nitrogen upon excision, were ground to a fine powder with a mortar and pestle in liquid nitrogen and homogenized in ice-cold buffer as previously described.[20] Protein concentrations were determined and aliquots were stored at −70°C.

Glucose Transport

Each soleus muscle was divided into 3 equal portions, and the 2 outer portions (20 mg each) were used to assess glucose transport using a modification of a previously described protocol.[27] Briefly, the muscle strips underwent a 15-minute recovery period in incubation buffer (Krebs-Henseleit buffer containing 5 mmol/L HEPES, 0.1% radioimmunoassay-grade bovine serum albumin (BSA), 2 mmol/L pyruvate, and 18 mmol/L mannitol). The strips were then placed for 30 minutes in incubation buffer to which 0, 100, 200, or 1,000 μU/mL insulin was added. Finally, the muscle strips were moved for 10 minutes to Krebs-Henseleit buffer containing 0.1% BSA, 8 mmol/L [^{3}H]3-*O*-methylglucose (2.5 μCi/mmol), 12 mmol/L [^{14}C]mannitol (26.3 μCi/mmol), and insulin (0, 100, 200, or 1,000 μU/mL). All incubations were performed at 35°C with continuous oxygenation (95% O_2/5% CO_2). Glucose transport activity was calculated as micromoles of [^{3}H]3-*O*-methylglucose accumulated per milliliter of intracellular water per hour.

Generation of Anti-IRS-1 Antibodies

cDNA corresponding to amino acids 424 to 521 of the mature IRS-1 protein[28] was amplified from rat skeletal muscle by polymerase chain reaction (PCR). The sequence of the upstream primer was 5′CGC GGG GGA TCC GAT GAG TAT GGC TCC/T AGT CC 3′, and the downstream primer was 5′CGC GGG GAA TTC GTG AGT TCT CTT T/CCG G/AAA CC 3′. The identity of the PCR product was confirmed using restriction enzyme digestion and nucleotide sequence analysis. The PCR product was ligated into the pGEX-2T vector (Pharmacia Biotech, Uppsala, Sweden) using T4 ligase (MBI Fermentas, Vilmus, Lithuania). The transformation of competent *Escherichia coli* strain BL21 was performed with recombinant pGEX-2T using the parental vector as a control. Glutathione S-transferase (GST)-IRS-1 fusion protein expression and purification was performed using the GST Gene Fusion System (Pharmacia Biotech) based on the method described by Smith and Johnson.[29] Polyclonal antibodies to IRS-1 were raised in rabbits according to standard protocols, using the purified GST-IRS-1 fusion protein as the immunogen. The antiserum was heat-inactivated at 56°C for 30 minutes and stored at −20°C.

IRS-1–Associated PI 3-Kinase Activity

Equal amounts of protein (2 mg) were immunoprecipitated overnight at 4°C with anti-IRS-1 antibody coupled to protein A–Sepharose. The immune complexes were washed and resuspended in 40 μL buffer (20 mmol/L HEPES, pH 7.5, and 180 mmol/L NaCl). PI 3-kinase activity was assessed directly on the protein A–Sepharose beads as previously described.[10] Bands corresponding to PI 3-phosphate were quantified using a phosphoimager (Fujix 2000; Fuji Photo Film, Fuji, Japan). Density data were adjusted using an internal standard and are expressed in arbitrary density units.

IRS-1–Associated PI 3-Kinase Activity In Vitro

Muscle strips were prepared and incubated as described for glucose transport except for the following modifications of the protocol: the doses of insulin were 0, 200, 1,000, and 20,000 μU/mL, no radioisotopes were used in the incubation, and the insulin incubation was only 4 minutes long.[30] After incubation, the muscle strips were placed in liquid nitrogen for storage until analysis. Density data were adjusted using an internal standard and are expressed in arbitrary density units.

Blood Chemistry

Serum glucose and plasma lactate were determined enzymatically using glucose analyzer model 2700 (Yellow Springs Instrument, Yellow Springs, OH). Serum insulin was analyzed by radioimmunoassay (Diagnostica, Falkenberg, Sweden). Plasma NEFAs were analyzed using a kit from Wako Chemicals (Neuss, Germany). Plasma epinephrine was determined by high-performance liquid chromatography.[31]

Chemicals

Protein A was obtained from Calbiochem-Novabiochem (La Jolla, CA). Reagents for the protein assay (BCA* Protein Assay Reagent) were from Pierce (Rockford, IL). Radioisotopes were purchased from ICN Biomedical (Costa Mesa, CA), PI was from Avanti-Polar Lipids (Alabaster, AL), and aluminium-backed silica gel 60 thin-layer chromatographic plates were from Merck (Darmstadt, Germany). BSA and other standard chemicals and reagents were purchased from Sigma Chemical (St Louis, MO).

Statistical Analysis

Data are presented as the mean ± SE. The unpaired Student's *t* test was used to compare two means, with a *P* level less than .05 considered statistically significant. In vitro insulin stimulation data were analyzed by ANOVA with the Tukey-Kramer test for multiple comparisons. A *P* level less than .05 was considered statistically significant. In vivo PI 3-kinase activity data were analyzed by repeated-measures ANOVA with a between-group factor of 4 levels representing feeding, trauma, and rat strain. The between-subject repeated-measure factor of 2 levels represented basal and insulin-stimulated PI 3-kinase activity in each group. The Bonferroni correction for multiple comparisons was used as a post hoc test. A *P* level less than .017 was considered significant.

RESULTS

Glucose Transport in Isolated Soleus Muscle

Basal glucose transport (0 μU/mL insulin), as determined by the uptake of the nonmetabolized glucose analog [^{3}H]3-*O*-methylglucose, was reduced in fed traumatized Wistar rats compared with fed nontraumatized Wistar rats (1.30 ± 0.15 *v* 2.75 ± 0.13 μmol · mL^{-1} · h^{-1}, respectively, $P < .05$; Fig 1). The response to submaximal insulin stimulation (100 and 200 μU/mL) was also significantly decreased (2.46 ± 0.32 *v* 3.56 ± 0.34 and 2.88 ± 0.29 *v* 3.94 ± 0.35 μmol · mL^{-1} · h^{-1}, respectively, both $P < .05$). At the supraphysiological dose of insulin (1,000 μU/mL), the difference in glucose transport (4.26 ± 0.44 *v* 5.68 ± 0.57 μmol · mL^{-1} · h^{-1}) was not statistically significant.

IRS-1–Associated PI 3-Kinase Activity In Vivo

ANOVA showed a main effect of group ($F(3, 21) = 4.4$, $P < .05$). Insulin-stimulated PI 3-kinase activity was increased in skeletal muscle from fed traumatized Wistar rats compared with fed nontraumatized Wistar rats (PI 3-kinase × trauma interaction, $F(1, 21) = 54.2$, $P < .001$). Increased insulin-stimulated PI 3-kinase activity was also found in fed traumatized Wistar rats compared with fasted traumatized Wistar rats (PI 3-kinase × feeding interaction, $F(1, 21) = 12.7$, $P < .01$). Insulin stimulation of PI 3-kinase activity was significantly lower in fed nontraumatized GK rats versus fed nontraumatized Wistar rats (PI 3-kinase × strain interaction, $F(1, 21) = 31.9$, $P < .001$). When PI 3-kinase activity in another subgroup of fed nontraumatized GK rats was compared with the activity in fed traumatized GK rats, insulin-stimulated PI 3-kinase activity was significantly higher in traumatized GK rats ($P < .017$). Basal and insulin-stimulated values for IRS-1–associated PI 3-kinase activity are shown in Figs 2 and 3. The values for statistical significance reflect the results of repeated-measures ANOVA.

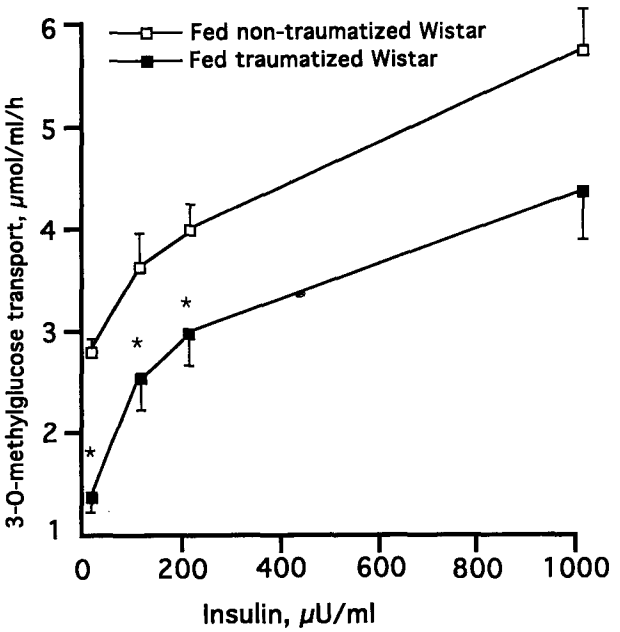

Fig 1. 3-*O*-Methylglucose transport in soleus muscle from fed nontraumatized Wistar rats (n = 8) and fed traumatized Wistar rats (n = 8). Results are the mean ± SE. *$P < .05$ *v* fed nontraumatized.

IRS-1–Associated PI 3-Kinase Activity In Vitro

There were no statistically significant differences in IRS-1–associated PI 3-kinase activity between traumatized and nontraumatized fed Wistar rats under basal conditions or after insulin stimulation at 200 or 20,000 μU/mL. At 1,000 μU/mL insulin, the 2-fold increase in activity found in fed traumatized rats was

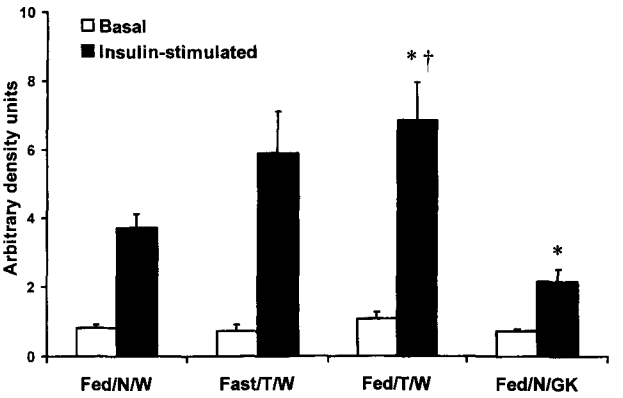

Fig 2. Basal and insulin-stimulated IRS-1–associated PI 3-kinase activity in fed nontraumatized Wistar rats (fed/N/W, n = 7), fasted traumatized Wistar rats (fast/T/W, n = 7), fed traumatized Wistar rats (fed/T/W, n = 7), and fed nontraumatized GK rats (fed/N/GK, n = 5). Results are the mean ± SE. *$P < .017$ *v* fed/N/W; †$P < .017$ *v* fast/T/W.

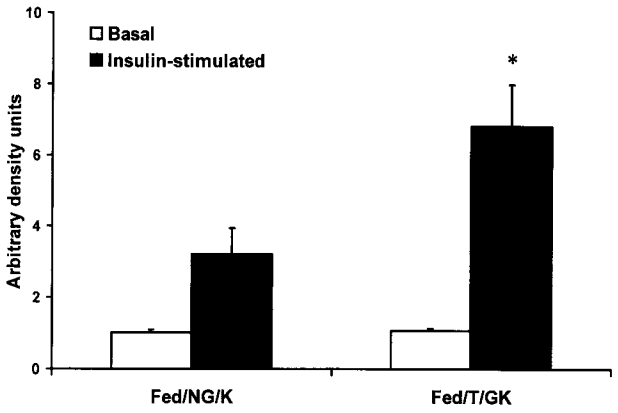

Fig 3. Basal and insulin-stimulated IRS-1–associated PI 3-kinase activity in fed nontraumatized GK rats (fed/N/GK, n = 8) and fed traumatized GK rats (fed/T/GK, n = 6). Results are the mean ± SE. *$P < .017$ *v* GK nontraumatized.

significantly higher than the 1.5-fold increase in fed nontraumatized rats ($P < .05$) (Fig 4).

Plasma Epinephrine, NEFA, and Lactate

Plasma epinephrine concentrations were all less than 0.3 nmol/L in fed nontraumatized Wistar rats (n = 4), whereas the values in fed traumatized Wistar rats were 1.2, 1.3, and 2.3 nmol/L in 3 rats and less than 0.3 nmol/L in 2 rats. Plasma NEFA was significantly lower in fed traumatized Wistar rats compared with fed nontraumatized Wistar rats (0.3 ± 0.01 *v* 1.9 ± 0.3 mmol/L, respectively, $P < .01$), but plasma lactate concentrations were not significantly different between the groups (1.1 ± 0.2 *v* 1.0 ± 0.1 mmol/L, respectively).

Serum Insulin and Glucose

In response to trauma, serum insulin levels decreased by almost 75% in fed Wistar rats ($P < .01$) and by 53% in fed GK rats ($P < .01$). The difference in serum glucose levels between traumatized and nontraumatized Wistar rats was not significant,

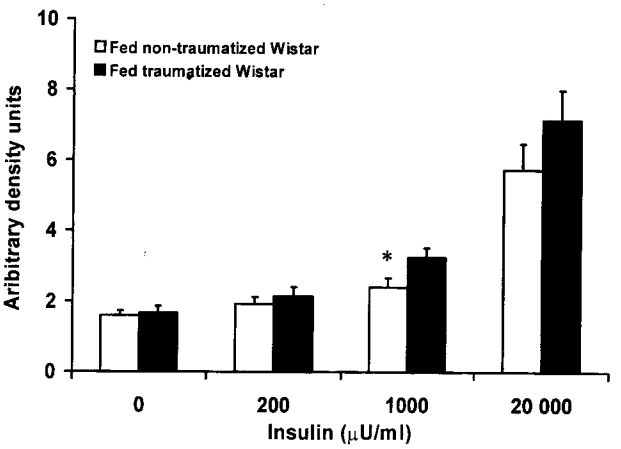

Fig 4. In vitro basal and insulin-stimulated IRS-1–associated PI 3-kinase activity in fed nontraumatized Wistar rats (n = 7) and fed traumatized Wistar rats (n = 7). Results are the mean ± SE. *$P < .05$ *v* fed nontraumatized.

but serum glucose in GK rats significantly increased after trauma ($P < .01$) (Table 1).

DISCUSSION

In the present study, trauma-induced insulin resistance was not reversed by preoperative feeding. The decrease in insulin-stimulated glucose transport in soleus muscle in fed traumatized Wistar rats compared with fed nontraumatized animals was similar to that found previously in fasted traumatized Wistar rats compared with fasted nontraumatized rats.[20] Paradoxically, this decrease in glucose uptake was associated with a postoperative increase in IRS-1–associated PI 3-kinase activity in both in vivo and in vitro experiments.

The observed reduction in glucose transport is consistent with our findings in the fasted rat using this trauma model,[20] as well as data from in vitro studies of isolated adipocytes from postoperative patients[32] and in vivo studies of posttraumatic insulin resistance in humans.[2-4] The reduced insulin-stimulated glucose transport found in fed traumatized rats at submaximal doses of insulin suggests decreased insulin sensitivity in skeletal muscle. Severe trauma may influence glucose metabolism by altering the circulatory levels of insulin-antagonistic hormones such as epinephrine.[33] Epinephrine has been found to inhibit insulin-stimulated glucose transport in rat skeletal muscle[34] and in man,[35] although at much higher concentrations (>10 nmol/L) than those found in traumatized rats in the present study. However, it is possible that trauma increased the sensitivity of muscle tissue to epinephrine and/or to tissue factors such as cytokines which would be present in the muscle under both in vivo and in vitro conditions.

Long-term fasting in humans[36,37] and rats[38] is associated with alterations in glucose utilization. Even short-term fasting induces peripheral insulin resistance in man,[39,40] and an overnight fast may aggravate the development of postoperative insulin resistance after elective surgery.[41] Recent studies have suggested that preoperative glucose delivery may reduce the development of postoperative insulin resistance.[5,6] Studies using a hyperinsulinemic-euglycemic clamp showed significantly smaller decreases in postoperative whole-body glucose disposal in patients given an oral glucose drink before surgery compared with those undergoing a routine overnight fast.[6] However, in the present study, preoperative feeding did not affect the reduction in glucose transport found after trauma. The conflicting data from these studies may reflect differences in study design (eg, the type and amount of nutrients used). The data obtained from in vivo studies may represent net changes in

Table 1. Serum Glucose and Insulin Values in Fed Wistar and GK Rats

	Fed Wistar		Fed GK	
Parameter	Nontrauma (n = 7)	Trauma (n = 7)	Nontrauma (n = 12)	Trauma (n = 7)
Serum glucose (mmol/L)	13.9 ± 0.6	14.2 ± 0.6	22.9 ± 1.0	30.8 ± 1.4†
Serum insulin (μU/mL)	66 ± 17	17 ± 2*	49 ± 5	23 ± 2†

NOTE. Values are the mean ± SE.
*$P < .01$, fed trauma Wistar *v* nontrauma Wistar.
†$P < .01$, fed trauma GK *v* fed nontrauma GK.

a number of parameters, including hepatic glycogenolysis and glucagon release,[40] while the in vitro method used in the current study provides specific data about the changes in glucose uptake in skeletal muscle alone.

Although insulin-stimulated glucose uptake was decreased after trauma, a paradoxical increase in insulin-stimulated PI 3-kinase activity was found in skeletal muscle in fed traumatized Wistar rats in both the in vivo and in vitro experiments. An increase in insulin-stimulated PI 3-kinase activity was also found after trauma in the moderately insulin-resistant GK rat, although the magnitude of the increase was smaller than that in the Wistar rat. The demonstration of a posttraumatic increase in PI 3-kinase activity even in animals known to have a lesser PI 3-kinase response to insulin than other rats supports the idea that enhanced PI 3-kinase activity is one of the effects of trauma on metabolic pathways. However, the decrease in glucose transport indicates that trauma has other effects as well. The processes that are affected may be located downstream from PI 3-kinase, may be on other signaling pathways that affect glucose transport,[42] or may involve the machinery for GLUT4 translocation. An altered regulation of glycogen synthase might also contribute to the change in glucose transport.

Insulin concentrations were reduced postoperatively in both the Wistar and GK rats. This is consistent with previous observations in rats subjected to thermal injury stress[43] and with the impaired insulin response to intravenous glucose in injured rats and in human burn patients.[22,44] It has been suggested that the small reductions in serum insulin found previously in injured patients were due to insulin degradation.[45] However, the reductions in serum insulin concentrations after trauma were very marked in the present study (75% and 53% in fed Wistar and fed GK rats, respectively), and it is unlikely that changes of this magnitude could be caused by increased degradation and clearance of insulin. Blood glucose was elevated compared with the levels in unfed rats,[20] with the highest concentrations found in the fed traumatized GK rats. The low insulin levels found postoperatively in the present study in the presence of high blood glucose concentrations are likely an indication of an impaired insulin response to hyperglycemia.

Counterregulatory hormones have classically been considered responsible for the reduction in glucose-stimulated insulin release that is found after injury.[46] However, counterregulatory hormones are only moderately increased in postoperative patients when modern anesthetics and surgical techniques are used.[5] In addition, no major postsurgery changes in plasma epinephrine were found in the present study or in our previous experiments with rats that were fasted before trauma.[20] This suggests that trauma alters other circulating factors and/or mechanism(s) that influence insulin secretion. One possible mechanism could be the activation of sympathetic intrapancreatic nerve fibers. Pancreatic islets are richly supplied with sympathetic nerve fibers which may inhibit insulin secretion via the release of neuropeptides such as NPY and galanin.[47] The effects of trauma also may be mediated by changes in the concentration of glucocorticoids. Elevated corticosterone levels have been observed in animal models of trauma,[23] and physiological concentrations of corticosterone have been shown to inhibit glucose-stimulated insulin secretion in perfused rat pancreas and isolated pancreatic islets.[48,49]

In the present study, preoperative feeding did not prevent a postoperative reduction in insulin-stimulated glucose transport in skeletal muscle. In addition, insulin-stimulated PI 3-kinase activity increased after trauma, suggesting that postoperative insulin resistance is not caused by an impairment of an early step in insulin signaling. Further mechanistic studies may provide information on the complex effects of trauma on carbohydrate metabolism and may lead to effective methods to prevent or minimize the insulin resistance observed after surgery.

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