Hemorrhage induces the rapid development of hepatic insulin resistance

YUCHEN MA,1 PING WANG,1,2,3 JOACHIM F. KUEBLER,2
IRSHAD H. CHAUDRY,2,3 AND JOSEPH L. MESSINA1
1Department of Pathology, Division of Molecular and Cellular Pathology, 2Center for Surgical Research and Department of Surgery, and 3Department of Physiology and Biophysics, The University of Alabama at Birmingham, Birmingham, Alabama 35294

Submitted 7 June 2002; accepted in final form 10 September 2002

Ma, Yuchen, Ping Wang, Joachim F. Kuebler, Irshad H. Chaudry, and Joseph L. Messina. Hemorrhage induces the rapid development of hepatic insulin resistance. Am J Physiol Gastrointest Liver Physiol 284: G107–G115, 2003.—Hyperglycemia is an early metabolic response to trauma and hemorrhage. The role of hepatic insulin resistance to the development of this hyperglycemia is not well understood. The aim of this study was to determine whether the liver becomes insulin resistant and to identify the particular hepatic insulin signaling pathways that may be compromised following trauma and hemorrhage. Male adult rats were bled to a mean arterial pressure of 40 mmHg and maintained at that pressure for 90 min followed by resuscitation with Ringer lactate. Data showed that trauma and hemorrhage rapidly induced profound hyperinsulinemia in combination with significant hyperglycemia, suggesting the development of insulin resistance. After trauma and hemorrhage, hepatic insulin signaling via the insulin-induced phosphatidylinositol 3 (PI3)-kinase-Akt pathway was abolished, whereas ERK1/2 signaling was relatively normal. The regulation (inhibition) of a hepatic-, insulin-, and the PI3-kinase-dependent gene, IGF binding protein-1, was also lost. The present study provides convincing evidence of a rapid onset hepatic insulin resistance following a combination of trauma and hemorrhage.

VARIOUS PATHOPHYSIOLOGICAL conditions such as surgical trauma, hemorrhage, shock, sepsis, and burns often result in a hypermetabolic state in which energy expenditure is increased (5, 19, 38). As part of this physiological response, there is often increased protein degradation in muscle, at least partially due to development of muscle insulin resistance. Hyperglycemia ensues due to decreased peripheral glucose disposal, often coincident with hyperinsulinemia (27, 43, 51). The hyperglycemia, sometimes referred to as “stress diabetes,” could be readily explained by either insulin deficiency or insulin resistance. In less severe forms of stress, a transient decrease in circulating insulin is sometimes found (6, 21, 39). However, in most clinical and experimental situations, there is an increase in insulin in addition to the development of hyperglycemia (24, 44). This hyperglycemia in the face of elevated insulin is commonly referred to as insulin resistance.

Insulin resistance in peripheral tissues, primarily muscle and fat, results in decreased glucose uptake (reduced glucose clearance) by these tissues. Insulin is one of the primary suppressors of hepatic gluconeogenesis and hepatic glucose output, and the hyperinsulinemia should work to decrease hepatic gluconeogenesis. However, if the liver becomes resistant to insulin, the increased hepatic glucose output can contribute to the hyperglycemia. Recently, there is evidence that expression of at least one hepatic gluconeogenic gene (glucose-6-phosphatase) is altered following hemorrhage or sepsis (1, 31, 33), but the relationship between the development of hepatic insulin resistance and altered expression of this gene is unknown.

Although hyperglycemia is an early metabolic response to trauma and hemorrhage, the mechanisms responsible for this hyperglycemia remain unclear. By measurement of insulin-induced glucose uptake in isolated soleus muscle, Chaudry and co-workers (7) reported that following the combination of surgical trauma and hemorrhage, rat skeletal muscle insulin resistance was evident by as early as 2 h. However, it is unknown whether hepatic insulin resistance also occurs in this model.

Insulin signaling consists of a cascade of phosphorylation events carried out by protein and lipid kinases (41, 61). Autophosphorylation of the receptor further phosphorylates insulin receptor substrates (IRS), which serve as “docking” molecules, favoring the generation of intracellular signals (40, 60). There are at least two main branches that propagate insulin intracellular signaling: IRS/phosphatidylinositol 3-kinase (PI3-kinase)-Akt pathway and the Ras/mitogen-activated protein kinase (MAPK) pathway, which includes ERK1/2 (30, 41). Insulin resistance refers to a decreased capacity of target cells to respond to ordinary...
levels of circulating insulin due to impairment of one or more signaling pathways.

In the present study, a rat model of soft tissue trauma and hemorrhage has been used to delineate the cellular mechanisms of hepatic insulin resistance. Changes in insulin signaling pathways were characterized as well as changes in blood glucose and insulin levels following trauma and hemorrhagic shock. Defects in insulin signaling were selective and resulted in alterations in insulin-dependent, signaling pathway-specific, gene expression.

METHODS

Animal model of surgical trauma and hemorrhage. All procedures were carried out in accordance with the guidelines set forth in the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health. A model of trauma and hemorrhage in the rat, as previously described by Chaudry et al. (9) and Wang et al. (59) was used in this study with minor modifications. Male Sprague-Dawley rats (275–325 g, Charles River labs, Wilmington, MA) were fasted 18–20 h before the experiment but were allowed water ad libitum. The rats were anesthetized with ether inhalation. After rats were clipped and shaved, they were restrained in a supine position and were kept anesthetized by continued inhalation of 1.5% isoflurane (Mailleferd Veterinary, Mundelein, IL) and 98.5% air throughout the surgical procedure. While anesthetized, a 5-cm ventral midline laparotomy was performed representing soft tissue trauma. The abdomen was then closed in layers using 4/0 Ethilon sutures (Ethicon, Somerville, NJ), and the wounds were bathed with 1% lidocaine (Elkins-Sinn, Cherry Hill, NJ) throughout the surgical procedure to reduce postoperative pain. Polyethylene-50 catheters (Clay-Adams, Parsippany, NJ) were placed in the right and left femoral arteries and the right femoral vein for bleeding, monitoring of mean arterial pressure (MAP), and fluid resuscitation, respectively. Blood pressure was constantly monitored by attaching one of the catheters to a blood pressure analyzer (Micro-Med, Louisville, KY). The rats were then bled to a MAP of 40 mmHg within 10 min. Once MAP reached 40 mmHg, the timing of the hemorrhage period began and was maintained for 90 min. At the end of the hemorrhage period, the rats were resuscitated with four times the withdrawn blood volume using Ringer lactate infused by syringe pump (Harvard Apparatus, South Natick, MA) at a constant rate over 60 min. Sham-operated rats underwent the same surgical procedure (laparotomy and catheterization), but neither hemorrhage nor resuscitation was carried out.

Experimental design. Due to the considerable trauma incurred by anesthesia and opening of the abdominal cavity to perform the insulin injections (see next section), it was impossible to have a completely untreated control group. Thus the “baseline” animal was selected in these experiments to be the trauma-alone group (T 0’), which was subjected to anesthesia, laparotomy, and catheterization and then killed immediately. Additional trauma-alone groups were subjected to these same procedures and then killed at 90’ (T 90’), 210’ (T 210’) after catheterization. Matched to these groups were the trauma plus hemorrhage (TH) groups, which were subjected to the same procedures as the T groups, but were also subjected to hemorrhage and then killed at 90 min just at the end of the hemorrhage period (TH 90’) or 60 min following completion of the 60-min resuscitation period (TH 210’ = 90 min hemorrhage + 60 min resuscitation + 60 min recovery).

Blood and tissue harvesting procedures. At the 0’, 90’, or 210’ time points, the abdominal cavity was opened again, the portal vein was exposed, and 5 U insulin in saline or saline alone (0.5 ml) were injected into the portal vein. This large dose of insulin was chosen because it was found to be optimal for induction of insulin signaling pathways in liver as well as other peripheral tissues (17). Unless otherwise noted, 1 min following the injection, livers were removed and quickly frozen in liquid nitrogen.

Measurement of glucose and insulin levels. Just before insulin or saline injection, blood was withdrawn, and glucose levels were measured in 0.2 ml whole blood using a Radiometer ABL 700 Series (Radiometer American, Westlake, OH). For insulin levels, 0.5 ml blood were placed at room temperature, allowed to coagulate, and then centrifuged at 5,000 g for 15 min. The serum was stored at −80°C until insulin levels were measured by a rat insulin RIA kit (Linco Research, St. Charles, MO).

Western immunoblots. Liver tissue from each animal (~0.2 g) was homogenized in 1 ml lysis buffer containing 20 mM HEPES, pH 7.9, 1.5 mM MgCl₂, 20 mM KCl, 20 mM glycerol, 0.2 mM EDTA, 2 mM Na₃VO₄, 10 mM NaF, 1% Triton X-100, 0.2 mM PMSF, 10 mg/ml aprotinin, and 10 mg/ml leupeptin. Tissue lysates were centrifuged at 10,000 g for 10 min, and the supernatants were stored at −80°C until use. Tissue lysate protein concentrations were assayed (BioRad Laboratories, Hercules, CA), and lysates (15 µg/lane) were then resolved by SDS, 10% PAGE, and transferred to nitrocellulose paper. The Western transfers were immuno-blotted with anti-phospho-ERK1/2, anti-total ERK1/2, and anti-phospho-Akt (Cell Signaling Technology, Beverly, MA) followed by the addition of horseradish peroxidase-conjugated secondary antibody for detection of bound antibody by enhanced chemiluminescence (ECL; Amersham Pharmacia Biotech, Piscataway, NJ). Each blot was stripped 30 min at 50°C in stripping buffer (100 mM 2-mercaptoethanol, 2% SDS, 62.5 mM Tris-HCl, pH 6.7) and then reprobed with a different antibody.

Northern blot hybridization analysis. Total hepatic RNA was isolated by the UltraSpec RNA isolation system (Biotec Laboratories, Houston, TX). Total RNA (10 µg) from each animal was subjected to formaldehyde gel electrophoresis and transferred to nylon membrane (Ambion, Austin, TX) as described previously (36). IGF binding protein (IGFBP)-1 cDNA probe was radiolabeled with [32P]dCTP using Prime-It II random primer labeling kit (Stratagene, La Jolla, CA), and blots were incubated with this probe overnight, washed, and autoradiographed.

Densitometric and statistical analysis. ECL images of immunoblots and Northern signal intensity were scanned and quantified with a Scanalyses Zero D-Scan. All data were analyzed by one-way ANOVA using the InStat Statistical program (version 3) by GraphPad Software (San Diego, CA).

RESULTS

Trauma and hemorrhage induce hyperglycemia and hyperinsulinemia. Blood glucose levels following trauma alone increased slightly at both 90 and 210 min compared with T 0’ animals from 97 to 121 and 123 mg/dl, respectively. This increase in blood glucose was most likely due to a moderate degree of insulin resistance resulting from the continued anesthesia and/or developed over time following the trauma of the surgi-

AJP-Gastrointest Liver Physiol • VOL 284 • JANUARY 2003 • www.ajpgi.org
Peter C. Wago, G. Joseph Goff, and Harry J. Reid

TRAUMA, HEMORRHAGE AND INSULIN RESISTANCE

AJP-Gastrointest Liver Physiol • VOL 284 • JANUARY 2003 • www.ajpgi.org

Fig. 1. Increases in blood glucose and insulin concentrations following trauma alone or trauma and hemorrhage. Rats were subjected to trauma alone (T) or trauma and hemorrhage (TH). At 0' (following trauma but before any further treatment) or 90' (without or with hemorrhage) or 210' (without or with 60 min of recovery following 60 min of resuscitation), blood glucose (A) and serum insulin (B) concentrations were measured. Data are presented as the means ± SE of blood samples from 3 rats in each group. #P < 0.05 compared with the T 0' group; *P < 0.05 compared with the T groups of that same time point.

Fig. 2. Time course of hepatic insulin signaling following insulin injection via portal vein in rat. In T 0' and TH 90' rats, either saline or 5 U of insulin was injected into the portal vein, and 1, 3, or 5 min later, the liver was removed and protein extracts were prepared, resolved by SDS-PAGE, and subjected to Western blotting as described in METHODS. A: Western blots were probed with antibodies specific for phospho-Akt (P-Akt) and total Akt (T-Akt). B: blots were reprobed with antibodies specific for phospho-ERK1/2 (P-ERK1/2) and total ERK1/2 (T-ERK1/2).

The ability of exogenous insulin treatment to induce phosphorylation of Akt and ERK1/2 following surgery combined with hemorrhage for 90 min (TH 90') resulted in a complete loss of insulin-induced P-Akt at 1, 3, and 5 min following insulin injection (Fig. 2A). In contrast, the maximal induction of insulin to P-ERK1/2 also occurred 1 min after insulin injection (Fig. 2B) and also returned to preinsulin treatment levels before injection. Because the 1-min insulin injection time point resulted in maximal P-Akt and P-ERK1/2 in trauma-only animals (T 0') and maximal P-ERK1/2 in TH animals, this time point was used in the ensuing studies.
With the use of antibodies that recognized both phosphorylated and unphosphorylated (total) forms of Akt and ERK, respectively, no significant changes in the total amounts of either Akt or ERK were found following insulin treatment or following trauma and hemorrhage. A consistent finding was that when insulin induced phosphorylation of Akt, it also caused a slight upward shift of the Akt protein. In agreement with the lack of Akt phosphorylation by insulin following trauma and hemorrhage is the absence of the upward shift of Akt following insulin treatment in the TH 90’ group.

Alterations in insulin-induced P-Akt signaling in the liver. The data presented in Fig. 2A indicated that there was a complete loss of the P-Akt response to exogenous insulin following TH at 90’ (Fig. 2A). Additional studies were performed and confirmed that there was little or no induction of P-Akt by insulin at both TH 90’ and TH 210’, the two time points following both trauma and hemorrhage, although there was no change in total Akt levels. However, most importantly, there was still an insulin induction of P-Akt at these same time points in the trauma-only groups (T 90’ and T 210’, Fig. 3A). When using the antibody that recognizes the total amount of Akt, it was clear that when the liver responded to insulin by an increase in P-Akt (at T 0’, T 90’, and T 210’), there was also an upward shift in the mobility of the Akt protein on Western blots. However, when the hepatic PI3-kinase-Akt pathway was unresponsive to insulin (TH 90’ and TH 210’), there was no upward mobility shift of the Akt protein (Fig. 3A).

Data from multiple animals in each group were quantified and presented as fold induction by insulin (+) compared with no insulin (−) injection at the same time points following trauma or both trauma and hemorrhage. In the T 0’ group, there was a 4.4-fold induction of P-Akt by 1 min of insulin injection. At the T 90’ and T 210’ time points, the effect of insulin in trauma-only groups decreased to 4.0- and 3.0-fold, respectively, but these slight changes were not significantly different from the T 0’ group. However, there was a much larger decrease of insulin’s ability to induce P-Akt in both the TH 90’ and TH 210’ groups, from 4.4- to 1.4- and 0.87-fold, respectively (Fig. 3B). This is indicative of a significant loss of insulin signaling via the IRS-PI3-kinase-Akt pathway within the 90-min hemorrhage period (TH 90’) that persists even 60 min following fluid resuscitation (TH 210’).

Alterations in insulin-induced P-ERK1/2 signaling in the liver. Unlike phosphorylation of P-Akt, insulin-induced phosphorylation of ERK1/2 was evident follow-

Fig. 3. Decrease in insulin-induced phosphatidylinositol 3-kinase (PI3-kinase)-Akt but no change in MEK-ERK1/2 signaling in rat liver following trauma alone or trauma and hemorrhage. At the same time points and treatment regimens described in Fig. 1, either saline (−) or 5 U insulin (+) were injected into the portal vein. A: after 1 min, the liver was removed and P-Akt, T-Akt, P-ERK1/2, and T-ERK1/2 protein were measured using specific antibodies as described in Fig. 2 by reprobing the same Western blots with the 4 different antibodies. B: P-Akt was measured, and the degree of induction of P-Akt was quantified by scanning densitometry of the autoradiographs. The data are presented as means ± SE fold induction of P-Akt by insulin compared with no insulin treatment at that time point (3 rats/group). *P < 0.05 compared with the T 0’ group; †P < 0.05 compared with the T groups of that same time point. C and D: P-ERK1/2 was measured, and the degree of induction of P-ERK1/2 was quantified by scanning densitometry of the autoradiographs. The data are presented as means ± SE fold induction of P-ERK1/2 with 3 rats/group. C: presents the changes in basal level of P-ERK1/2 with no exogenous insulin added. D: The open bars present the induction of P-ERK1/2 in the trauma alone and the trauma and hemorrhage groups following insulin injection via the portal vein. Also shown is the basal level of P-ERK1/2 in the T 0’ with no insulin treatment, which was arbitrarily set to 1. The fold induction at the other time points is presented as fold increase compared with this T 0’ basal [without insulin (~Ins)] level. *P < 0.05 compared with the T 0’ time point without exogenous insulin (~Ins); †P < 0.05 compared with the T groups of that same time point. The filled bars present the fold effects of insulin injection in the TH groups following subtraction of the elevated basal levels of P-ERK1/2 obtained in C to indicate the fold induction by exogenous insulin on top of the already elevated of P-ERK1/2.
ing trauma and hemorrhage. When the same blots used to measure changes in P-Akt were reprobed with an antibody specific for P-ERK1/2, the results were markedly different. In the trauma-only groups, there was little observable change in basal P-ERK1/2 (without insulin) at either of the time points (T 90' or T 210'). However, after the combination of trauma and hemorrhage, there was a significant increase of basal (without insulin) P-ERK at both 90 and 210 min, with no change in total ERK1/2 (Fig. 3A). When the results from multiple experiments were averaged, there were 10-fold increases in basal P-ERK2 following trauma and hemorrhage compared with the T 0' group as well as the time-matched trauma-only groups (Fig. 3C).

Induction of P-ERK1/2 by insulin injection was then examined in the animals subjected to trauma and hemorrhage. In Fig. 3A, it is evident that there was still a significant induction of P-ERK1/2 following 1 min of insulin injection, although basal levels were elevated (Fig. 3C). When multiple experiments were quantified, injections of insulin resulted in a 17- to 20-fold increase in P-ERK2. This effect varied little in animals immediately following surgery (T 0' + insulin) or in the other trauma-only groups (T 90' and T 210'; Fig. 3D). However, after trauma and hemorrhage, the final increase was 39- and 31-fold in the TH 90' and TH 210' groups, respectively, compared with T 0' rats not treated with insulin. This level is significantly increased compared with the time-matched trauma-only animals. These data indicate the continued responsiveness to insulin following hemorrhage. If the elevated baseline in the trauma plus hemorrhage animals without exogenous insulin (9.5- to 10.5-fold) were first subtracted, exogenous insulin resulted in the increase of P-ERK2 of 22- to 23-fold (Fig. 3D, right). This level of fold induction is similar to the effects of exogenous insulin in the trauma-only animals and demonstrates that when administered via the portal vein to rats following the combination of trauma and hemorrhage, exogenous insulin was still capable of inducing a large increase in P-ERK1/2 on top of the already elevated P-ERK1/2 levels.

Altemations in IGFBP-1 gene expression in rat liver following hemorrhage. Insulin normally inhibits the expression of IGFBP-1 mRNA (46, 47) and requires the PI3-kinase pathway for this effect. Because there was a defect in the PI3-kinase signaling pathway following TH, it was asked whether this loss of PI3-kinase signalning resulted in a loss of insulin inhibition of the IGFBP-1 gene. Northern analysis indicated that there was an increase of 1.9- and 6.7-fold of IGFBP-1 mRNA in the T groups at the 90' and 210', respectively, compared with the T 0' group (Fig. 4). This was not surprising because there was development of a mild insulin resistance in response to the stress of surgery. However, there was a much greater induction of IGFBP-1 gene expression, rising to 21- and 28-fold by 90' and 210' in the TH groups, respectively (Fig. 4).

The elevation in IGFBP-1 mRNA seems to correlate well with the elevation in plasma insulin concentra-

![Fig. 4. Induction of IGF binding protein-1 (IGFBP-1) mRNA in rat liver following trauma alone or trauma and hemorrhage. At the same time points and treatment regimens described in Fig. 1, livers were removed, total RNA was isolated and was subjected to Northern analysis using an IGFBP-1 probe. A: top is a representative Northern blot. Bottom is a reprobing of the same blot with cyclophilin indicating equal RNA loading of the lanes (10 µg/lane). B: autoradiographs were quantified by scanning densitometry, and the data are presented as means ± SE of 3 rats in each group. In each experiment, the relative densitometric signal from T 0' was arbitrarily set to 1 and the changes at other time points are presented as fold increase compared with T 0'. †P < 0.05 compared with T 0'; *P < 0.05 compared with the T groups of that same time point.](http://ajpgi.physiology.org/)](http://ajpgi.physiology.org/)

**DISCUSSION**

Hyperglycemia occurs following sepsis (11, 33), surgery (35, 37, 45), and hemorrhage (33). This injury/infection-induced hyperglycemia, often referred to as “stress diabetes,” could be readily explained by either insulin deficiency or insulin resistance. There is an increasing body of literature concerning insulin resistance in muscle and adipose tissue, with much less...
known about hepatic insulin resistance. In some models of sepsis, hepatic insulin resistance has been observed, whereas in other reports, no hepatic insulin resistance was found even with demonstrable peripheral insulin resistance (23, 29). Although the sequelae of trauma and hemorrhage remain a major cause of mortality in surgical intensive care units, it is unknown whether hepatic insulin resistance occurs, and if so, what are the cellular mechanisms responsible for this resistance. In this study, a well-established rat model of trauma and hemorrhage (3, 8, 9, 58) was used to examine changes of blood glucose and insulin levels and to determine whether hepatic insulin resistance develops. The cellular mechanisms of hepatic insulin resistance was also investigated by examining insulin signal-transduction pathways and insulin regulation of hepatic gene expression.

In the present study, three time points were selected: at the end of the soft-tissue injury (T 0’); at the end of a hemorrhage period, if performed (TH 90’ or T 90’), which allows examination of the effects of hemorrhage without the effects of fluid resuscitation; or 60 min after completion of the 60-min resuscitation period (TH 210’ if hemorrhage was performed; T 210’ if no hemorrhage). Previous work suggests that cytokines, such as TNF-α, which may be one of the causes of insulin resistance, are significantly increased by the end of the hemorrhage period and for 4–6 h after resuscitation (2). Additionally, ischemia-reperfusion injury of organs, such as the heart, can occur 1–2 h after reperfusion (57).

The data presented imply that a combination of anesthesia and surgical trauma alone induced mild hyperglycemia, whereas anesthesia/surgery in combination with hemorrhage induced more severe hyperglycemia, both at the end of the hemorrhage period (90’) and 60 min after fluid resuscitation (210’). It is unlikely that the hyperglycemia was from ingested food. Animals were fasted for 18–20 h, the basic pre-operative and surgical procedures lasted at least 0.25 h, and the hemorrhage period lasted 90 min. Thus 19.75–21.75 h had elapsed since the rats were last permitted access to food. Although elevation of epi-nephrine and glucagon can stimulate glycogen breakdown, glycogenolysis is also an unlikely source for the extreme hyperglycemia of the hemorrhaged animals. Previous studies suggest that hepatic glycogen content is reduced over 90% following a 20- to 24-h fast (28). In support of this contention, data from Maitra et al. (33) suggest that adrenergic blockers and inhibition of glucagon had no effect on hemorrhage-induced hyperglycemia in rats fasted for 20 h. However, breakdown of liver or muscle glycogen may have contributed to the increase in blood glucose. Hyperglycemia, when combined with the severe hyperinsulinemia obtained following trauma and hemorrhage, is strongly suggestive of a rapid development of insulin.

Changes of serum insulin levels can vary in different injury models and with different time courses. In less severe forms of injury or very early following hemorrhage, serum insulin concentrations were sometimes found to decrease or not increase (13, 33, 45). However, in the later stages of the hemorrhage period or following resuscitation, there is a uniform responsiveness of pancreatic beta cells to glucose and plasma insulin concentrations rise in an attempt to control the hyperglycemia.

Many researchers (10, 15, 45) have demonstrated muscle insulin resistance in different animal models of injury. They include impairment of insulin stimulation of IRS-1, PI3-kinase, and Akt in damaged human skeletal muscle (15). The liver is a main source of gluconeogenesis, and insulin is one of the primary suppressors of hepatic gluconeogenesis and hepatic glucose output. However, little is known about hepatic insulin resistance following injury, and if it becomes resistant to insulin, the liver may contribute to the hyperglycemia. There is evidence that soon after hemorrhage and resuscitation, hepatic expression of the gluconeogenic gene glucose-6-phosphatase increases, suggesting insulin resistance in the liver (33); but this might be due to rising glucocorticoids, another regulator of glucose-6-phosphatase expression (32). Therefore, a direct examination of hepatic insulin signaling and any changes following hemorrhage was necessary to characterize hepatic insulin resistance.
Development of insulin resistance can be explained by changes in the number of insulin receptors, impaired insulin binding, faulty docking protein association with the activated insulin receptor, or a number of postreceptor defects, including disruption of one or more of the intracellular signaling pathways used by insulin. The two most widely studied and understood insulin signal-transduction pathways are the IRS1/2-PI3-kinase-Akt pathway and the Ras-MEK-ERK1/2 pathway. The present study indicated that insulin-induced PI3-kinase-Akt signaling was abolished by the end of the hemorrhage period and was still absent even following fluid resuscitation. At the same time points, exogenously added insulin continued to be capable of stimulating phosphorylation of ERK1/2 roughly 20-fold. This induction of P-ERK1/2 by insulin was approximately the same as the effects of insulin injection in animals before hemorrhage (T 0') and in animals subjected to surgery alone (T 90' and T 210'). This was true even though there was an elevation of basal ERK activation/phosphorylation following hemorrhage. Thus, even in the presence of highly elevated endogenous insulin, the liver was still responsive to exogenous insulin. Although not directly measured, this suggests that there were sufficient insulin receptors in the liver for a full response to insulin and that the insulin receptors were capable of activating at least some of the insulin-induced signaling pathways. Thus hemorrhage-induced hepatic insulin resistance differentially affects the PI3-kinase-Akt and MEK-ERK1/2 signaling pathways. This also suggests that effects of insulin mediated via the MEK-ERK pathway may still be functional following trauma and hemorrhage even when insulin responses mediated via PI3-kinase-Akt have been compromised.

The data presented demonstrate that insulin-induced PI3-kinase signaling was abolished following hemorrhage, although further study is needed to explore the mechanism of this impairment. Following hemorrhage, sepsis, or ischemic injury to the liver, serum levels of many proinflammatory cytokines increase dramatically (2, 48, 49). It is known that proinflammatory cytokines, such as TNF-α, can result in insulin resistance of fat and muscle associated with Type 2 diabetes or obesity (14, 55, 62). However, several studies failed to confirm these findings (18, 52), and thus the effect of TNF-α on insulin resistance may be tissue specific or model specific. Further studies are needed to explore whether increased cytokines may contribute to the defects of insulin-induced PI3-kinase signaling following trauma and hemorrhage.

Because there was a defect in the PI3-kinase signaling pathway, we examined whether this loss of signaling resulted in a loss of insulin action. The hepatic action of insulin needed to be one solely dependent on the PI3-kinase pathway. One of the hepatic actions of insulin, working via activation of the Akt kinase, is to Ser/Thr phosphorylate and inactivate forkhead transcription factor(s). Of particular importance for this discussion, forkhead rhabdomyosarcoma transcription factor (FKHR) is a major regulator of IGFBP-1 mRNA (20, 42, 50, 53, 54). Nonphosphorylated FKHR in the nucleus activates IGFBP-1 transcription. When FKHR is phosphorylated in response to insulin stimulation of the PI3-kinase pathway, it is excluded from the nucleus, the stimulatory action of FKHR is inhibited, and IGFBP-1 transcription and mRNA decrease. However, if the normal induction of the PI3-kinase pathway by insulin is compromised (when insulin levels are low or when the liver cannot respond well to insulin due to insulin resistance), the FKHR transcription factor remains in the nonphosphorylated state, and transcription of the IGFBP-1 gene is induced. Because there was a defect in the PI3-kinase signaling pathway following trauma and hemorrhage, it was asked whether this loss of PI3-kinase signaling resulted in a loss of insulin inhibition of the IGFBP-1 gene. Because there was a large increase of circulating insulin concentrations following hemorrhage, the measured increase in IGFBP-1 mRNA was not due to a deficit of insulin but must be due to hepatic insulin resistance. The strong correlation between insulin levels, or the glucose/insulin ratio, two measures of insulin resistance, and IGFBP-1 mRNA, a proposed measure of hepatic insulin resistance, suggests that IGFBP-1 mRNA may be a sensitive indicator of insulin resistance and, in particular, a defect in the induction of PI3-kinase signaling by insulin shortly after trauma and hemorrhage. An elevation in plasma IGFBP-1 is commonly observed in a variety of catabolic conditions (16, 25, 26). Its physiological effects following trauma and hemorrhage are unknown. However, recent work (4, 12) does suggest that IGFBP-1 can modify the effects of IGF-I as well as having biological actions that are IGF-I independent. Further work is needed in the future to determine whether IGFBP-1 has a protective role following trauma and hemorrhage.

One hypothesis of the physiological importance of insulin resistance following injury suggests that the resulting hyperglycemia enhances the glucose concentration gradient, increasing insulin-insensitive facilitative glucose transport into injured tissues and organs involved in the immunological response to stress (5, 34, 37). A second premise is that after hemorrhage, plasma IGFBP-1 is commonly observed in a variety of catabolic conditions (16, 25, 26). Acute increased hepatic gluconeogenesis could result from glycogenolysis or increases in blood lactate and glycerol immediately following trauma and hemorrhage. However, if the insulin resistance is prolonged, the protective effect of hyperglycemia is costly in terms of tissue protein loss, because muscle proteolysis acts as a source of amino acids for hepatic conversion to glucose resulting in muscle wasting (22).

These studies present data of early time points following trauma alone or trauma and hemorrhage. Thus we do not know whether hepatic insulin resistance also occurs at later time points following hemorrhage and, if so, whether the mechanism(s) resulting in insulin resistance changes with time. We also do not know whether insulin resistance in other tissues, such as muscle and fat, are due to similar or dissimilar alter-
ations in insulin signaling. This early hepatic insulin resistance may occur concurrently with insulin resistance in other tissues, such as muscle and fat. This multitissue insulin resistance is the likely cause of hyperglycemia. Future studies will be needed to focus on these questions.

Although there are obvious differences between the rat model used in the present studies and the human patient, there are numerous parallels in the development of insulin resistance. In a recent publication (56), intensive insulin therapy was used to overcome insulin resistance and reduce blood glucose levels, which resulted in decreased morbidity and mortality among critically ill patients. Although these studies will need to be repeated and extended to other patient groups, this work suggests that a useful treatment protocol for future trauma patients may be intensive insulin therapy or treatment with pharmacological agents that can increase tissue sensitivity to endogenous insulin, resulting in greater long-term survival.

We thank Z. F. Ba for assistance with the surgical techniques, the Univ. of Alabama Birmingham Clinical Nutrition Research Center and Dr. B. Gower for the insulin measurements, and Dr. M. Rechler for providing the IGFBP-1 cDNA probe. This work was supported by grants from the American Diabetes Association (to J. L. Messina) and National Institutes of Health Grants DK-40466 (to J. L. Messina), GM-53008 (to P. Wang), and GM-R37–39519 (to I. H. Chaudry).

REFERENCES