Published in final edited form as:

Biochem Biophys Res Commun. 2012 May 11; 421(3): 442–448. doi:10.1016/j.bbrc.2012.03.146.

Injury-Induced Insulin Resistance in Adipose Tissue

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Abstract

Hyperglycemia and insulin resistance are common findings in critical illness. Patients in the surgical ICU are frequently treated for this ‘critical illness diabetes’ with intensive insulin therapy, resulting in a substantial reduction in morbidity and mortality. Adipose tissue is an important insulin target tissue, but it is not known whether adipose tissue is affected by critical illness diabetes. In the present study, a rodent model of critical illness diabetes was used to determine whether adipose tissue becomes acutely insulin resistant and how insulin signaling pathways are being affected. There was a reduction in insulin-induced phosphorylation of IR, IRS-1, Akt and GSK-3 β . Since insulin resistance occurs rapidly in adipose tissue, but before the insulin resistance in skeletal muscle, it may play a role in the initial development of critical illness diabetes.

Keywords

insulin resistance; adipose tissue; critical illness diabetes; injury; hemorrhage; insulin signaling

INTRODUCTION

Insulin resistance is a major underlying feature of Type 2 diabetes and many other chronic metabolic abnormalities, including obesity, dyslipidemia, non-alcoholic fatty liver, polycystic ovary syndrome, and hypertension [1–5]. An acute form of hyperglycemia and insulin resistance, referred to as “critical illness diabetes,” are common findings in patients following trauma, surgery, burn injury or infection [6–13]. The development of hyperglycemia in human critical illness diabetes has been considered an adaptive and beneficial response to stress. However, recent studies indicate that the development of hyperglycemia is associated with a poor outcomes following critical illness [9; 14–17], and

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intensive insulin therapy, used to reduce the hyperglycemia, reduces morbidity and mortality among at least a subset of this patient population [6; 9; 18; 19].

The binding of insulin to the extracellular subunit of its receptor activates the insulin receptor tyrosine kinase activity, stimulates autophosphorylation, and induces the phosphorylation of a number of target proteins. These include the insulin receptor substrate proteins (IRS) which serve as docking proteins for several downstream signaling molecules, facilitating the activation of multiple insulin responsive signal transduction cascades [20; 21]. The IRS/phosphatidylinositol 3-kinase (PI3K)/Akt pathway is the major signaling cascade that is activated following the insulin binding and involved in the regulation of metabolism by insulin [22–25].

White adipose tissue, liver, and skeletal muscle are the major targets of insulin action and play an important role in energy metabolism and insulin sensitivity. The development of insulin resistance in skeletal muscle following injuries has been studied [10; 26–29] and there are a few studies on the development of insulin resistance in liver [11; 12; 29; 30]. However, little is known about the development of insulin resistance in adipose tissue following injury. To address the question of whether adipose tissue becomes insulin resistant following injury and to determine the time course of any defects in insulin signaling, we assessed the phosphorylation of IR, IRS-1, Akt, and GSK-3 β following experimental injury. Here we provide evidence that insulin resistance develops in adipose tissue as early as 15 minutes following hemorrhage and is characterized by impaired insulin-stimulated signaling via the IR/IRS/PI3K/Akt pathway.

MATERIALS AND METHODS

Animal model of critical illness diabetes

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 and were approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham. An animal model of rat critical illness diabetes, trauma and hemorrhage, was used in this study as previously described [10–12; 27]. Male Sprague-Dawley rats (260–300g, Harlan, Indianapolis, IN) were fasted 18–20 hours before the experiment, but allowed water *ad libitum*. The rats were anesthetized with isoflurane inhalation (Mallinckrodt Veterinary, Mundelein, IL), a 5-cm ventral midline laparotomy was performed representing soft-tissue trauma and the abdomen was closed. 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. Trauma and hemorrhage (TH) rats were bled to a MAP of 35–40 mmHg within 10 minutes (= initial bleed). Once the MAP reached 40 mmHg, the timing of the hemorrhage period began and was maintained for the specified times. Trauma only (T) rats underwent the same surgical procedure (laparotomy and catheterization), but were not hemorrhaged.

Study Design

Due to the considerable trauma incurred by anesthesia and opening of the abdominal cavity to perform the saline or insulin injections, it was impossible to have a completely untreated control group [10–12; 27]. Thus, the baseline group was selected in these experiments to be the trauma only animals (T0') that were subjected to anesthesia, laparotomy, and catheterization and then killed immediately. Additional trauma only animals (T groups) were subjected to these same procedures and then killed at 15 (T15'), 30 (T30') and 60 min (T60') after catheterization. Matched to these groups were the trauma and hemorrhage (TH)

groups that were subjected to the same procedures as the T groups, but also subjected to hemorrhage and then killed at 15, 30 or 60 min after the initial bleed.

At the specified time point, the abdominal cavity was opened again, the portal vein exposed, and insulin (5 U in 0.5 ml saline) or saline alone (0.5 ml) was injected into the portal vein. Epididymal fat pads were removed 1.5 min following the injection and quickly frozen in liquid nitrogen.

Immunoblot analysis

Epididymal adipose tissue from each animal (approximately 0.25 grams) was homogenized in 0.5 ml lysis buffer containing 50 mM Tris, pH 8.0, 150 mM NaCl, 50 mM NaF, 30 mM sodium pyrophosphate, 2 mM sodium orthovanadate, 0.1 µg/ml okadaic acid, 1 % Igepal, 0.5 % sodium deoxycholic acid, and 0.5X protease inhibitor cocktail (P2714; Sigma-Aldrich, St. Louis, MO). Tissue lysates were centrifuged twice at 12,000 x *g* for 10 minutes. Protein concentrations were determined using the Bio-Rad DC assay (Bio-Rad Laboratories, Hercules, CA). For Western blotting, 20 µg of protein per lane was resolved by SDS-PAGE and transferred to nitrocellulose membranes [10–12; 27]. The Western transfers were immunoblotted with polyclonal antiserum against phosphotyrosine (Y972) IR, phosphotyrosine (Y612) IRS-1 (Invitrogen's Biosource International, Carlsbad, CA), phosphoserine (S473) Akt, phosphoserine (S9) GSK-3β, and total-ERK (Cell Signaling Technology, Danvers, MA). Horseradish peroxidase-conjugated secondary antibody was added for detection of bound antibody by enhanced chemiluminescence (GE Healthcare, Piscataway, NJ). Each blot was stripped in Re-Blot Plus (Chemicon, Temecula, CA) before reprobing with a different antibody.

Statistical analysis

Enhanced chemiluminescent images of immunoblots were scanned and quantified using Zero D-Scan (Scanalytics, Fairfax, VA). All values are presented as mean density ± standard error. The values for the T and TH groups (in the absence and presence of insulin) are expressed as fold change compared to the T0 group in the absence of insulin (baseline). All data was analyzed by either Student's *t* test or one-way ANOVA, when appropriate, using the InStat statistical program by GraphPad Software (San Diego, CA).

RESULTS

Time course of alterations in insulin signaling in adipose tissue following initiation of Critical Illness Diabetes

The primary aim of this study was to determine whether adipose tissue becomes insulin resistant in a rat model of critical illness diabetes. Trauma and hemorrhage was performed and mean arterial pressure maintained at 35–40 mmHg for 15 min (TH15'), 30 min (TH30'), and 60 min (TH60'), respectively. Western blot data (Fig. 1A) indicated that there was an almost complete loss of insulin-induced phosphorylation of insulin receptor tyrosine 972 [P-IR (Y972)], as early as 15 min following hemorrhage which persisted up to 60 min following hemorrhage. Quantified data from multiple animals demonstrated that there was no consistent difference in the fold induction of insulin-stimulated P-IR (Y972) in epididymal adipose tissue (Fig. 1B–D) following trauma only (T0' vs T15', T30' and T60'), although following 30 min of trauma only (T30'; Fig. 1C), there was a transient increase in insulin-induced P-IR (Y972). Most importantly for this work, there was a consistent, large, and significant decrease in insulin-stimulated P-IR (Y972) in each of the trauma and hemorrhage (TH) groups (T0' vs TH15', TH30', and TH60'; Fig. 1B–D). Total ERK levels are shown as a control for loading and transfer. All blots were re-probed with the antibody for total ERK in all the experiments presented below, but are not shown for brevity.

Western blot analysis was performed at the same time points to examine the phosphorylation of tyrosine 612 of IRS-1 (P-IRS-1 [Y612]), which is important for the activation of the PI3-kinase/Akt pathway [Fig. 2A; [31]]. In the trauma only groups there was little consistent change in insulin-induced P-IRS-1 (Y612; Figs. 2 B–D). Again, most important for the present work, for each of the trauma and hemorrhage time points, there was a complete loss of insulin-induced tyrosine phosphorylation of IRS-1 (Y612) following the combination of trauma and hemorrhage.

To further evaluate the IRS/PI3K/Akt signaling cascade in adipose tissue, insulin-induced phosphorylation of Akt on serine 473 (P-Akt [S473]; Fig. 3) and GSK-3 β on serine 9 (P-GSK-3 β [S9]; Fig. 4), a downstream substrate of Akt, were also examined. Following trauma alone there was no significant change in insulin-stimulated P-Akt (S473). However, there was a complete loss of insulin-stimulated P-Akt (S473; Fig. 3A) as early as 15 min (TH15'), as well as at 30 and 60 min following the combination of trauma and hemorrhage (Fig. 3 B–D). Likewise, as early as 15 min and at 60 min following the combination of trauma and hemorrhage following hemorrhage, there was a complete loss in insulin-induced P-GSK-3 β (Fig. 4 A–C). Thus, the data indicates a rapid onset of a severe epididymal adipose tissue defect of insulin induction of the IR/IRS/PI3K/Akt signaling pathway which occurred as early as 15 min following the initiation of an animal model of critical illness diabetes.

DISCUSSION

The overall endocrine response to injury is not well understood. There are only a limited number of studies on insulin signaling defects in skeletal muscle and liver following injury [10–12; 27–30; 32; 33]. Recently, we found that hepatic insulin resistance develops quickly in rats, within 15 min [11; 12; 27; 30], while skeletal muscle signaling defects develop more slowly (within 60 min) following trauma and hemorrhage [10; 27]. While decreased insulin sensitivity in adipose tissue is well-established in chronic diseases [34–39], little is known about the acute adipose tissue response to critical illness diabetes. One study reported alterations in insulin signaling in adipose tissue following burn injury in rats, but these measurements were performed 3 weeks after the injury [29]. In the present study, an insulin signaling defect, as measured by decreased insulin receptor autophosphorylation, developed in adipose tissue following a time course similar to liver, but prior to that in skeletal muscle. We also measured decreased activation/phosphorylation downstream of the insulin receptor, including insulin-stimulated phosphorylation of IRS-1, Akt and GSK-3 β , possibly due to the decreased activation of the insulin receptor.

The present work indicates the potential importance of adipose tissue in critical illness diabetes. Adipose tissue can produce and secrete both cytokines and adipokines, which have been implicated in insulin resistance associated with chronic diseases, such as Type 2 diabetes and obesity [40–45]. Several of these cytokines and adipokines can directly or indirectly interfere with insulin signaling [30; 46–53]. However, there is no information on protein or mRNA expression of adipose tissue cytokines and adipokines in critical illness diabetes, and since there are changes in adipose tissue insulin responsiveness, future experiments are planned to determine this. For instance, we previously demonstrated that circulating TNF- α , and hepatic levels of TNF- α mRNA and protein, increase by 90 min following the combination of trauma and hemorrhage [11; 30], but changes in adipose tissue TNF- α expression is unknown.

Numerous studies suggest that intensive insulin therapy, to reduce the hyperglycemia often observed in the critically ill, can be beneficial, reducing morbidity and mortality [9; 54–59]. However, there can also be an increase in hypoglycemic incidents, and it is not clear if all

patients will benefit from intensive insulin therapy [60–62]. Even though intensive insulin treatment to reduce hyperglycemia is now the norm in many ICU's, little has been learned about the causes of the acute insulin resistant state that leads to the hyperglycemia, which tissues are involved, and the cellular mechanisms which result in the acute insulin resistance of critical illness diabetes. Our additional studies suggest that there are likely different causative factors and cellular mechanisms for the acute development of insulin resistance in the three main insulin target tissues, liver, skeletal muscle and adipose tissue. The present study indicates that adipose tissue may play a role in the acute insulin resistant state in an animal model of critical illness diabetes.

Acknowledgments

This work is supported by grants from the National Institutes of Health (DK62071) and the Veterans Administration Merit Review to JLM. A University of Alabama Comprehensive Minority Faculty and Student Development Program Fellowship provided support for VLW. We would like to thank Drs. L. Li, J. Xu and L. H. Thompson for their insightful discussions and suggestions.

References

1. Berge RK, Tronstad KJ, Berge K, Rost TH, Wergedahl H, Gudbrandsen OA, Skorve J. The metabolic syndrome and the hepatic fatty acid drainage hypothesis. *Biochimie*. 2005; 87:15–20. [PubMed: 15733731]
2. Avramoglu RK, Basciano H, Adeli K. Lipid and lipoprotein dysregulation in insulin resistant states. *Clin Chim Acta*. 2006; 368:1–19. [PubMed: 16480697]
3. Mlinar B, Marc J, Janez A, Pfeifer M. Molecular mechanisms of insulin resistance and associated diseases. *Clin Chim Acta*. 2007; 375:20–35. [PubMed: 16956601]
4. Ueki K, Kondo T, Tseng YH, Kahn CR. Central role of suppressors of cytokine signaling proteins in hepatic steatosis, insulin resistance, and the metabolic syndrome in the mouse. *Proc Natl Acad Sci U S A*. 2004; 101:10422–10427. [PubMed: 15240880]
5. Arner P. The adipocyte in insulin resistance: key molecules and the impact of the thiazolidinediones. *Trends Endocrinol Metab*. 2003; 14:137–145. [PubMed: 12670740]
6. Li L, Messina JL. Acute insulin resistance following injury. *Trends Endocrinol Metab*. 2009; 20:429–435. [PubMed: 19800814]
7. Gore DC, Chinkes D, Heggers J, Herndon DN, Wolf SE, Desai M. Association of hyperglycemia with increased mortality after severe burn injury. *J Trauma*. 2001; 51:540–544. [PubMed: 11535907]
8. McCowen KC, Malhotra A, Bistrian BR. Stress-induced hyperglycemia. *Crit Care Clin*. 2001; 17:107–124. [PubMed: 11219223]
9. Van den Berghe G, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M, Vlasselaers D, Ferdinande P, Lauwers P, Bouillon R. Intensive insulin therapy in the critically ill patients. *N Engl J Med*. 2001; 345:1359–1367. [PubMed: 11794168]
10. Thompson LH, Kim HT, Ma Y, Kokorina N, Messina JL. Acute muscle-type specific insulin resistance following injury. *Mol Med*. 2008; 11–12:715–723.
11. Ma Y, Toth B, Keeton AB, Holland LT, Chaudry IH, Messina JL. Mechanisms of hemorrhage-induced hepatic insulin resistance: role of tumor necrosis factor- α . *Endocrinology*. 2004; 145:5168–5176. [PubMed: 15297437]
12. Ma Y, Wang P, Kuebler JF, Chaudry IH, Messina JL. Hemorrhage induces the rapid development of hepatic insulin resistance. *Am J Physiol Gastrointest Liver Physiol*. 2003; 284:G107–G115. [PubMed: 12388175]
13. Strommer L, Isaksson B, Wickbom M, Arnelo U, Ostenson C, Herrington M, Permert J. Effect of carbohydrate feeding on insulin action in skeletal muscle after surgical trauma in the rat. *Nutrition*. 2001; 17:332–336. [PubMed: 11369174]

14. Bochicchio GV, Sung J, Joshi M, Bochicchio K, Johnson SB, Meyer W, Scalea TM. Persistent hyperglycemia is predictive of outcome in critically ill trauma patients. *J Trauma*. 2005; 58:921–924. [PubMed: 15920404]
15. Laird AM, Miller PR, Kilgo PD, Meredith JW, Chang MC. Relationship of early hyperglycemia to mortality in trauma patients. *J Trauma*. 2004; 56:1058–1062. [PubMed: 15179246]
16. Sung J, Bochicchio GV, Joshi M, Bochicchio K, Tracy K, Scalea TM. Admission hyperglycemia is predictive of outcome in critically ill trauma patients. *J Trauma*. 2005; 59:80–83. [PubMed: 16096543]
17. Yendamuri S, Fulda GJ, Tinkoff GH. Admission hyperglycemia as a prognostic indicator in trauma. *J Trauma*. 2003; 55:33–38. [PubMed: 12855878]
18. Lewis KS, Kane-Gill SL, Bobek MB, Dasta JF. Intensive insulin therapy for critically ill patients. *Ann Pharmacother*. 2004; 38:1243–1251. [PubMed: 15187219]
19. Pittas AG, Siegel RD, Lau J. Insulin therapy for critically ill hospitalized patients: a meta-analysis of randomized controlled trials. *Arch Intern Med*. 2004; 164:2005–2011. [PubMed: 15477435]
20. White MF. The IRS-signalling system: a network of docking proteins that mediate insulin action. *Molecular & Cellular Biochemistry*. 1998; 182:3–11. [PubMed: 9609109]
21. Flores-Riveros JR, Sibley E, Kastelic T, Lane MD. Substrate phosphorylation catalyzed by the insulin receptor tyrosine kinase. Kinetic correlation to autophosphorylation of specific sites in the beta subunit. *J Biol Chem*. 1989; 264:21557–21572. [PubMed: 2557333]
22. Shepherd PR, Nave BT, Rincon J, Haigh RJ, Foulstone E, Proud C, Zierath JR, Siddle K, Wallberg-Henriksson H. Involvement of phosphoinositide 3-kinase in insulin stimulation of MAP-kinase and phosphorylation of protein kinase-B in human skeletal muscle: implications for glucose metabolism. *Diabetologia*. 1997; 40:1172–1177. [PubMed: 9349598]
23. Alessi DR, James SR, Downes CP, Holmes AB, Gaffney PR, Reese CB, Cohen P. Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase. *Balpa Curr Biol*. 1997; 7:261–269.
24. Okada T, Kawano Y, Sakakibara T, Hazeki O, Ui M. Essential role of phosphatidylinositol 3-kinase in insulin-induced glucose transport and antilipolysis in rat adipocytes. Studies with a selective inhibitor wortmannin. *J Biol Chem*. 1994; 269:3568–3573. [PubMed: 8106400]
25. Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. *Nature*. 2001; 414:799–806. [PubMed: 11742412]
26. Strommer L, Permert J, Arnelo U, Koehler C, Isaksson B, Larsson J, Lundkvist I, Bjornholm M, Kawano Y, Wallberg H, Zierath JR. Skeletal muscle insulin resistance after trauma: insulin signaling and glucose transport. *Am J Physiol*. 1998; 275:E351–E358. [PubMed: 9688639]
27. Li L, Thompson LH, Zhao L, Messina JL. Tissue Specific Difference in the Molecular Mechanisms for the Development of Acute Insulin Resistance Following Injury. *Endocrinology*. 2009; 150:24–32. [PubMed: 18801909]
28. Ikezu T, Okamoto T, Yonezawa K, Tompkins RG, Martyn JA. Analysis of thermal injury-induced insulin resistance in rodents. Implication of postreceptor mechanisms. *J Biol Chem*. 1997; 272:25289–25295. [PubMed: 9312146]
29. Carter EA, Burks D, Fischman AJ, White M, Tompkins RG. Insulin resistance in thermally-injured rats is associated with post-receptor alterations in skeletal muscle, liver and adipose tissue. *Int J Mol Med*. 2004; 14:653–658. [PubMed: 15375597]
30. Xu J, Kim HT, Ma Y, Zhao L, Zhai L, Kokorina N, Wang P, Messina JL. Trauma and Hemorrhage-Induced Acute Hepatic Insulin Resistance: Dominant Role of Tumor Necrosis Factor (TNF)-alpha. *Endocrinology*. 2008; 149:2369–2382. [PubMed: 18187553]
31. Esposito DL, Li Y, Cama A, Quon MJ. Tyr(612) and Tyr(632) in human insulin receptor substrate-1 are important for full activation of insulin-stimulated phosphatidylinositol 3-kinase activity and translocation of GLUT4 in adipose cells. *Endocrinology*. 2001; 142:2833–2840. [PubMed: 11416002]
32. Altavilla D, Saitta A, Guarini S, Galeano M, Squadrito G, Cucinotta D, Santamaria LB, Mazzeo AT, Campo GM, Ferlito M, Minutoli L, Bazzani C, Bertolini A, Caputi AP, Squadrito F. Oxidative stress causes nuclear factor-kappaB activation in acute hypovolemic hemorrhagic shock. *Free Radic Biol Med*. 2001; 30:1055–1066. [PubMed: 11369495]

33. Xin-Long C, Zhao-Fan X, Dao-Feng B, Jian-Guang T, Duo W. Insulin resistance following thermal injury: an animal study. *Burns*. 2007; 33:480–483. [PubMed: 17329027]
34. Reaven GM. Pathophysiology of insulin resistance in human disease. *Physiol Rev*. 1995; 75:473–486. [PubMed: 7624391]
35. Golay A, Ybarra J. Link between obesity and type 2 diabetes. *Best Pract Res Clin Endocrinol Metab*. 2005; 19:649–663. [PubMed: 16311223]
36. Hirosumi J, Tuncman G, Chang L, Gorgun CZ, Uysal KT, Maeda K, Karin M, Hotamisligil GS. A central role for JNK in obesity and insulin resistance. *Nature*. 2002; 420:333–336. [PubMed: 12447443]
37. Juhan-Vague I, Alessi MC, Mavri A, Morange PE. Plasminogen activator inhibitor-1, inflammation, obesity, insulin resistance and vascular risk. *J Thromb Haemost*. 2003; 1:1575–1579. [PubMed: 12871293]
38. Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *Am J Physiol Endocrinol Metab*. 2001; 280:E745–E751. [PubMed: 11287357]
39. Bosello O, Zamboni M. Visceral obesity and metabolic syndrome. *Obes Rev*. 2000; 1:47–56. [PubMed: 12119645]
40. Fantuzzi G. Adipose tissue, adipokines, and inflammation. *J Allergy Clin Immunol*. 2005; 115:911–919. [PubMed: 15867843]
41. Ailhaud G. Adipose tissue as a secretory organ: from adipogenesis to the metabolic syndrome. *C R Biol*. 2006; 329:570–577. [PubMed: 16860275]
42. Ahima RS, Flier JS. Adipose tissue as an endocrine organ. *Trends Endocrinol Metab*. 2000; 11:327–332. [PubMed: 10996528]
43. Garg A. Adipose tissue dysfunction in obesity and lipodystrophy. *Clin Cornerstone*. 2006; 8(Suppl 4):S7–S13. [PubMed: 17208666]
44. Rattarasarn C. Physiological and pathophysiological regulation of regional adipose tissue in the development of insulin resistance and type 2 diabetes. *Acta Physiol (Oxf)*. 2006; 186:87–101. [PubMed: 16497186]
45. Grundy SM. Obesity, metabolic syndrome, and cardiovascular disease. *J Clin Endocrinol Metab*. 2004; 89:2595–2600. [PubMed: 15181029]
46. Feinstein R, Kanety H, Papa MZ, Lunenfeld B, Karasik A. Tumor necrosis factor- α suppresses insulin-induced tyrosine phosphorylation of insulin receptor and its substrates. *J Biol Chem*. 1993; 268:26055–26058. [PubMed: 8253716]
47. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in tumor necrosis factor- α - and obesity-induced insulin resistance. *Science*. 1996; 271:665–668. [PubMed: 8571133]
48. Sell H, etze-Schroeder D, Kaiser U, Eckel J. Monocyte chemotactic protein-1 is a potential player in the negative cross-talk between adipose tissue and skeletal muscle. *Endocrinology*. 2006; 147:2458–2467. [PubMed: 16439461]
49. Rotter V, Nagaev I, Smith U. Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1 adipocytes and is, like IL-8 and TNF α , overexpressed in human fat cells from insulin-resistant subjects. *J Biol Chem*. 2003; 278:45777–45784. [PubMed: 12952969]
50. Senn JJ, Klover PJ, Nowak IA, Zimmers TA, Koniaris LG, Furlanetto RW, Mooney RA. Suppressor of cytokine signaling-3 (SOCS-3), a potential mediator of interleukin-6-dependent insulin resistance in hepatocytes. *J Biol Chem*. 2003; 278:13740–13746. [PubMed: 12560330]
51. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest*. 2003; 112:1796–1808. [PubMed: 14679176]
52. Harman-Boehm I, Bluher M, Redel H, Sion-Vardy N, Ovadia S, Avinoach E, Shai I, Kloting N, Stumvoll M, Bashan N, Rudich A. Macrophage infiltration into omental versus subcutaneous fat across different populations: effect of regional adiposity and the comorbidities of obesity. *J Clin Endocrinol Metab*. 2007; 92:2240–2247. [PubMed: 17374712]
53. Permana PA, Menge C, Reaven PD. Macrophage-secreted factors induce adipocyte inflammation and insulin resistance. *Biochem Biophys Res Commun*. 2006; 341:507–514. [PubMed: 16427608]

54. Egi M, Bellomo R, Stachowski E, French CJ, Hart GK, Hegarty C, Bailey M. Blood glucose concentration and outcome of critical illness: the impact of diabetes. *Crit Care Med*. 2008; 36:2249–2255. [PubMed: 18664780]
55. Ellger B, Debaveye Y, Vanhorebeek I, Langouche L, Giulietti A, Van EE, Herijgers P, Mathieu C, Van den Berghe G. Survival benefits of intensive insulin therapy in critical illness: impact of maintaining normoglycemia versus glycemia-independent actions of insulin. *Diabetes*. 2006; 55:1096–1105. [PubMed: 16567534]
56. Hafidh SA, Reuter MD, Chassels LJ, Aradhyula S, Bhutto SS, Alpert MA. Effect of intravenous insulin therapy on clinical outcomes in critically ill patients. *Am J Med Sci*. 2007; 333:354–361. [PubMed: 17570988]
57. Scalea TM, Bochicchio GV, Bochicchio KM, Johnson SB, Joshi M, Pyle A. Tight glycemic control in critically injured trauma patients. *Ann Surg*. 2007; 246:605–610. [PubMed: 17893497]
58. Sleiman I, Morandi A, Sabatini T, Ranhoff A, Ricci A, Rozzini R, Trabucchi M. Hyperglycemia as a predictor of in-hospital mortality in elderly patients without diabetes mellitus admitted to a sub-intensive care unit. *J Am Geriatr Soc*. 2008; 56:1106–1110. [PubMed: 18482306]
59. Tamaki M, Shimizu T, Kanazawa A, Tamura Y, Hanzawa A, Ebato C, Itou C, Yasunari E, Sanke H, Abe H, Kawai J, Okayama K, Matsumoto K, Komiya K, Kawaguchi M, Inagaki N, Watanabe T, Kanazawa Y, Hirose T, Kawamori R, Watada H. Efficacy and safety of modified Yale insulin infusion protocol in Japanese diabetic patients after open-heart surgery. *Diabetes Res Clin Pract*. 2008; 81:296–302. [PubMed: 18556085]
60. Finfer S, Chittock DR, Su SY, Blair D, Foster D, Dhingra V, Bellomo R, Cook D, Dodek P, Henderson WR, Hebert PC, Heritier S, Heyland DK, McArthur C, McDonald E, Mitchell I, Myburgh JA, Norton R, Potter J, Robinson BG, Ronco JJ. Intensive versus conventional glucose control in critically ill patients. *N Engl J Med*. 2009; 360:1283–1297. [PubMed: 19318384]
61. Merz TM, Finfer S. Pro/con debate: Is intensive insulin therapy targeting tight blood glucose control of benefit in critically ill patients? *Crit Care*. 2008; 12:212. [PubMed: 18466639]
62. Vriesendorp TM, DeVries JH, Hoekstra JB. Hypoglycemia and strict glycemic control in critically ill patients. *Curr Opin Crit Care*. 2008; 14:397–402. [PubMed: 18614902]

HIGHLIGHTS

- a rodent model of critical illness diabetes was used
- adipose tissue became acutely insulin resistant in this model
- within minutes, there was a reduction in insulin-induced phosphorylation of IR, IRS-1, Akt and GSK-3 β .
- insulin resistance occurred rapidly in adipose tissue, prior to the insulin resistance in skeletal muscle

Figure 1. Decreased insulin-stimulated tyrosine phosphorylation of the insulin receptor (IR) in rat epididymal adipose tissue following trauma and hemorrhage

Rats were subjected to trauma only (T), or trauma and hemorrhage (TH). At 0', 15', 30' or 60', either saline (-) or 5U insulin (+) was injected via the hepatic portal vein and the epididymal adipose tissue was removed after 1.5 min. Tissue lysates were subjected to Western blotting with antibody specific for phospho-IR tyrosine 972 (P-IR Y972). (A) Representative Western blots are presented. Breaks in the images indicate where samples were run on different portions of the same gel. (B-D) Autoradiographs were quantified by scanning densitometry. The phosphorylated protein levels in T0' without insulin treatment were arbitrarily set to 1 and the data are presented as the means $\pm$ SEM, fold induction of P-IR (Y972) by insulin of three rats ($n = 3$) in each group. #, $p < 0.001$; *, $p < 0.01$; †, $p < 0.05$. For this and all figures, T, trauma only; TH, trauma and hemorrhage; Ins, insulin. Total

ERK levels are shown as a control for loading and transfer. All blots were re-probed with the antibody for total ERK in all the experiments presented below, but are not shown for brevity.

Figure 2. Decreased insulin-stimulated tyrosine phosphorylation of IRS-1 in rat epididymal adipose tissue following trauma and hemorrhage

Rats were subjected to trauma only (T) or trauma and hemorrhage (TH) as described in Figure 1 except tissue lysates were subjected to Western blotting with antibody specific for phospho-IRS-1 tyrosine 612 (P-IRS-1 Y612). (A) Representative Western blots are presented. Breaks in the images indicate where samples were run on different portions of the same gel. (B–D) Autoradiographs were quantified by scanning densitometry. The phosphorylated protein levels in T0' without insulin treatment were arbitrarily set to 1 and the data are presented as the means $\pm$ SEM, fold induction of P-IRS-1 (Y612) by insulin of three rats ($n = 3$) in each group. §, $p < 0.0001$; #, $p < 0.001$; *, $p < 0.01$; ‡, $p < 0.05$; NS, not significant.

Figure 3. Decreased insulin-stimulated serine phosphorylation of Akt in rat epididymal adipose tissue following trauma and hemorrhage

Rats were subjected to trauma only (T) or trauma and hemorrhage (TH) as described in Figure 1 except tissue lysates were subjected to Western blotting with antibody specific for phospho-Akt serine 473 (P-Akt S473). (A) Representative Western blots are presented. Breaks in the images indicate where samples were run on different portions of the same gel. (B–D) Autoradiographs were quantified by scanning densitometry. The phosphorylated protein levels in T0' without insulin treatment were arbitrarily set to 1 and the data are presented as the means $\pm$ SEM, fold induction of P-Akt (S473) by insulin of three rats ($n = 3$) in each group. §, $p < 0.0001$; *, $p < 0.01$.

Figure 4. Decreased insulin-stimulated serine phosphorylation of GSK-3 β in rat epididymal adipose tissue following trauma and hemorrhage

Rats were subjected to trauma only (T) or trauma and hemorrhage (TH) as described in Figure 1 except tissue lysates were subjected to Western blotting with antibody specific for phospho-GSK-3 β serine 9 (P-GSK-3 β [S9]). (A) Representative Western blots are presented. Breaks in the images indicate where samples were run on different portions of the same gel. Autoradiographs were quantified by scanning densitometry. The phosphorylated protein levels in T0' without insulin treatment were arbitrarily set to 1 and the data are presented as the means $\pm$ SEM, fold induction of P-GSK-3 β (S9) by insulin of three rats (n = 3) in each group. §, $p < 0.0001$; #, $p < 0.001$; ‡, $p < 0.05$.