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Resveratrol ameliorates mitochondrial dysfunction but increases the risk of hypoglycemia following hemorrhagic shock

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Abstract

BACKGROUND—Hemorrhagic shock (HS) may contribute to organ failure, by profoundly altering mitochondrial function. Resveratrol (RSV), a naturally occurring polyphenol, has been shown to promote mitochondrial function and regulate glucose homeostasis in diabetes. We hypothesized that RSV during resuscitation would ameliorate HS-induced mitochondrial dysfunction and improve hyperglycemia following acute blood loss.

METHODS—With the use a decompensated HS model, male Long-Evans rats ($n = 6$ per group) were resuscitated with lactated Ringer's solution with or without RSV (30 mg/kg) and were killed before hemorrhage (sham), at severe shock, following resuscitation, and 18 hours after resuscitation. At each time point, the liver and kidney mitochondria were isolated to assess individual respiratory complexes (CI, CII, and CIV) and the production of reactive oxygen species (ROS). Blood samples were assayed for glucose, insulin, corticosterone, total glucagon-like

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AUTHORSHIP

H.W. contributed to the literature search, experimental procedures, data analysis, data interpretation, manuscript writing, and figures. Y.G. contributed to the experimental procedures and data analysis. A.L.W. contributed to the literature search and experimental procedures. L.B.B. contributed to the data analysis and data interpretation. J.A.B. contributed to the study design, data analysis, and data interpretation. P.M.R. contributed to the study design and data interpretation. C.A.S. contributed to the literature search, study design, data analysis, data interpretation, manuscript writing, and critical revision.

DISCLOSURE

The authors declare no conflicts of interest.

peptide (GLP-1), glucagon, and serum cytokine levels. The Homeostatic Model Assessment–Insulin Resistance index was used to quantify insulin resistance.

RESULTS—RSV supplementation following HS significantly improved mitochondrial function and decreased mitochondrial ROS production in both liver and kidney. RSV-treated animals had significantly lower blood glucose levels following resuscitation when compared with sham animals (116.0 ± 20.2 mg/dL vs. 227.7 ± 8.3 mg/dL, $p < 0.05$) or those resuscitated with lactated Ringer's solution (116.0 ± 20.2 mg/dL vs. 359.0 ± 79.5 mg/dL, $p < 0.05$). RSV supplementation was associated with significantly decreased plasma insulin levels (1.0 ± 0.4 ng/mL vs. 6.5 ± 3.7 ng/mL, $p < 0.05$), increased total GLP-1 levels (385.8 ± 56.6 ng/mL vs. 187.3 ± 11.1 ng/mL, $p < 0.05$), and a lower natural Log Homeostatic Model Assessment–Insulin Resistance index (1.30 ± 0.42 vs. 4.18 ± 0.68 , $p < 0.05$) but had minimal effect on plasma corticosterone, glucagon, or cytokine levels.

CONCLUSION—Resuscitation with RSV restores mitochondrial function and decreases insulin resistance but may be associated with increased hypoglycemia. The observed antiglycemic effects of RSV may be mediated by decreased mitochondrial ROS and increased GLP-1 secretion.

Keywords

Resveratrol; hemorrhagic shock; mitochondria; blood glucose; rats

Hemorrhagic shock remains a common cause of death in severely injured patients, with a significant number of deaths occurring as the result of multiple organ failure days to weeks after the initial injury.^{1,2} While the etiology of multiple organ failure following trauma is likely multifactorial, increasing data suggest that persistent mitochondrial dysfunction may play a significant role.^{3,4} Because mitochondria rely on oxygen to synthesize the majority of cellular adenosine triphosphate (ATP), impaired perfusion results in decreased energy stores and direct cellular damage.⁵ Moreover, with reperfusion, injured mitochondria cannot effectively transfer electrons, resulting in a deluge of reactive oxygen species (ROS) that further damage tissues and trigger apoptosis.⁶ Improving mitochondrial function, therefore, is an important step in mitigating hemorrhage-induced organ damage.

Mitochondrial dysfunction also contributes to the phenomenon of postinjury hyperglycemia. In addition to directly damaging mitochondrial proteins and impairing function, ROS can activate various serine kinases that regulate insulin signaling.^{7,8} Moreover, postinjury insulin resistance and hyperglycemia can be minimized by blocking the production of ROS.⁹ Because hyperglycemia is an independent risk factor for adverse outcomes, including death in trauma patients,¹⁰ resuscitation strategies that either mitigate oxidative stress or improve glycemic control could prove beneficial.

Resveratrol (RSV), a naturally occurring polyphenol found in red wine, has been shown to promote mitochondrial function and regulate glucose in a variety of disease states.^{11,12} In models of obesity and diabetes, RSV supplementation has been shown to promote mitochondrial biogenesis, increase insulin sensitivity, improve glucose tolerance, and suppress inflammation and oxidative stress.^{11,13} When used in models of hemorrhagic shock, RSV has been shown to improve cardiac contractility, decrease hepatic dysfunction,

and reduce tissue inflammation.^{14–17} There is, however, limited information regarding the impact of RSV on mitochondrial function and glucose regulation following trauma and hemorrhagic shock. We hypothesized that RSV during resuscitation would ameliorate shock-induced mitochondrial dysfunction and improve hyperglycemia following acute blood loss.

MATERIALS AND METHODS

Experimental Protocol

All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania and in accordance with National Institutes of Health guidelines. Male Long-Evans rats (250–300 g) were housed using a 12-hour light-dark cycle with constant temperature and humidity. Animals were allowed to acclimate at least 2 days before surgery and given access to food and water ad libitum. Using a well-validated decompensated hemorrhagic shock model,¹⁸ animals were anesthetized (isoflurane, 2–4%) and femoral vascular catheters (PE50, Braintree Scientific, Inc., Braintree, MA) were placed. A 5-cm midline laparotomy was performed to simulate soft tissue trauma.¹⁸ Surgical sites were bathed in 1% lidocaine and closed in layers. Animals received 0.25% buprenorphine (0.05 mg/kg) and were allowed to emerge from anesthesia. Mean arterial pressure (MAP) and heart rate (HR) were continuously monitored and recorded throughout the experiment (Digi-Med Signal Analyzers, Louisville, KY). After full reversal of anesthesia and 30 minutes of hemodynamic stability, animals were passively bled via the femoral artery and maintained at a MAP of 40 mm Hg. When the blood pressure could no longer be maintained without fluid infusion, a MAP of 40 mm Hg was sustained by incrementally infusing 0.2-mL boluses of lactated Ringer's (LR) solution (Baxter Corporation, Toronto, Canada). Animals were considered to be in severe shock when 40% of the shed volume had been returned in the form of LR boluses. Animals were then resuscitated with four times the shed volume in LR solution with or without RSV (30 mg/kg, 50 mg/mL in 50% DMSO, Orchid Pharmaceuticals, Lalilab, Durham NC) over 60 minutes.^{14–16,19} Animals (n = 6 per group) were killed before hemorrhage (sham), at severe shock, following LR resuscitation or RSV + LR resuscitation (LR + RSV), 18 hours after LR resuscitation (18hr LR) or LR + RSV resuscitation (18hr LR + RSV). Before sacrifice, blood samples were taken and assayed for arterial blood gases, lactate, and hemoglobin (i-STAT, Abbot Point of Care Inc., Princeton, NJ).

Isolation of Mitochondria

At each time point, liver and kidney tissues (4–6 g) were excised immediately after euthanasia and immersed in ice-cold mitochondrial isolation buffer (MIB) (210-mM mannitol, 70-mM sucrose, 10-mM HEPES, 1-mM EDTA; final pH 7.2 with 0.5% fatty acid-free Bovine Serum Albumin). Tissue was minced and homogenized with additional MIB plus 0.5% BSA using Potter Elvehjem homogenizer and loose-fitting Teflon pestle. Mitochondrial isolation was performed using differential centrifugation at 4°C.²⁰ The mitochondrial pellet was resuspended in 15-mL MIB without BSA and centrifuged for an additional 10 minutes at 9,600 G. The final mitochondrial pellet was resuspended in MIB. Protein concentration was determined using the Biuret method as described previously.²⁰

Mitochondrial Respiratory Capacity

Oxygen consumption was measured using high-resolution respirometry (Oxygraph-2k Oroboros Instruments, Innsbruck, Austria). A standard substrate/inhibitor titration protocol was used for the functional analysis of mitochondrial respiratory complexes after adding isolated mitochondria (0.15 mg) to a respiration medium (110-mM mannitol, 0.5-mM EGTA, 3-mM MgCl₂, 20-mM taurine, 10-mM KH₂PO₄, 60-mM K lactobionate, 0.3-mM DTT, and 0.1% BSA [fatty acid free], adjusted to pH 7.1, 37°C).⁴ Following stabilization (3–5 minutes), real-time oxygen flux data were collected continuously (DatLab software 4.3, Oroboros Instruments, Innsbruck, Austria). Complex I (CI)–dependent mitochondrial respiration was induced by adding glutamate (10 mM), malate (5 mM), and adenosine diphosphate (1 mM). To assess complex II (CII)–dependent respiration, rotenone (0.5 μM) was added to selectively inhibit CI followed by succinate (10 mM), a CII substrate. Antimycin A (5 μM) was then added to inhibit complex III (CIII) followed by the addition of TMPD (0.5 mM) and ascorbate (2 mM) as artificial electron donors for complex IV (CIV). To ensure that the respiratory capacity of CIV was not limited by cytochrome c depletion, respiration was measured after the addition of cytochrome c (10 μM).

Total Production of Mitochondrial-Derived ROS

Isolated mitochondria (10 μg) were suspended in 1 mL of buffer (250-mM sucrose, 20-mM 3-[N-morpholino] butane sulfonic acid, 10-mM Tris-base, 100-μM Pi [K], 0.5-mM Mg²⁺, pH 7.0, 30°C) containing CI substrates (malate/glutamate, 2.5/2.5 mM) and 2',7'-dichlorodihydro fluorescein diacetate (10 μM). Antimycin A was subsequently added to inhibit CIII and measure the inherent ROS production. Background fluorescence was subtracted from all readings. After incubation at 30°C for 1 hour, the fluorescent signal from dichlorofluorescein was detected (excitation, 488 nm; emission, 525 nm) and quantified using a Modulus Microplate Reader (Turner Biosystems, Sunnyvale, CA).

Measurement of Blood Glucose

Blood glucose was determined at each time point using a standard glucometer for rodents (AlphaTRAK, Abbott Laboratories, IL). Following resuscitation with LR or LR + RSV, glucose levels were measured every 15 minutes for 1.5 hours and then at 120, 240, and 360 minutes after the onset of resuscitation. Hypoglycemia was defined as blood glucose lower than 70 mg/dL.²¹

Measurements of Plasma Insulin, Total Glucagon-Like Peptide-1, Glucagon, and Corticosterone

Blood (1.0 mL) was collected before sacrifice, and plasma was stored at –80°C until analysis. Insulin, corticosterone, total glucagon-like peptide 1 (GLP-1), and glucagon were determined by the Radioimmunoassay and Biomarkers Core (Penn Diabetes Research Center, University of Pennsylvania) using commercially available enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay kits (Insulin and corticosterone ELISA, ALPCO Diagnostics, Windham, NH; total GLP-1 and glucagon radioimmunoassay, EMD Millipore, Billerica, MA). All samples were performed in duplicate.

Homeostatic Model Assessment–Insulin Resistance Index

Homeostatic Model Assessment (HOMA) is a mathematical model of the relationship between glucose and insulin that can be used to estimate insulin resistance and β -cell function. The natural Log (Ln) HOMA–Insulin Resistance (IR) index is used clinically to characterize abnormal glucose tolerance and assess insulin resistance using the equation, $\text{Ln}(\text{insulin level } (\mu\text{U/mL}) \times \text{glucose (mg/dL)})/405$.²²

Measurements of Plasma Tumor Necrosis Factor α and Interleukin 6

Tumor necrosis factor α (TNF- α) and interleukin 6 (IL-6) were measured from stored plasma samples using commercially available ELISA kits according to the manufacturer's guidelines. (Life Technologies, New York, NY). Each sample was run in triplicate with known standards.

Western Immunoblot Analysis

Total protein lysates were prepared from frozen liver and kidney samples using RIPA buffer containing protease and phosphatase inhibitors. After determining protein concentrations by Bicinchoninic Acid Assay (Pierce Biotechnology, Rockford, IL), 40 μg of protein were loaded on a 4% to 12% polyacrylamide gel and separated by electrophoresis. Proteins were transferred onto a nitrocellulose membrane (Bio-Rad, Richmond, CA) and blocked with Tris (0.01 M), NaCl (0.15 M), 0.05% Tween-20, and 5% dry milk for 1 hour at room temperature. Individual membranes were incubated with the anti-phospho-Y612-IRS-1 (1:1,000, Invitrogen Biosource International, Carlsbad, CA) and actin (1:1000, Sigma-Aldrich, St. Louis, MO). After washing, membranes were incubated with peroxidase-linked donkey anti-rabbit IgG secondary antibodies or peroxidase-linked sheep anti-mouse IgG (1:10,000, Amersham, Buckinghamshire, United Kingdom) for 1 hour at room temperature. Signals were developed by enhanced chemiluminescence (PerkinElmer Life Sciences, Boston, MA). Bands were quantified by densitometry using ImageJ software (National Institutes of Health, Bethesda, MD).

Statistical Analysis

Analyses were performed using SPSS version 16.0 (SPSS Inc., Armonk, NY) and GraphPad Prism 4 (Graphpad Software Inc, San Diego, CA). Data were analyzed using one-way analysis of variance with a post hoc least significant difference test. Results are presented as mean $\pm$ SEM unless otherwise stated. A $p < 0.05$ was considered statistically significant.

RESULTS

Physiologic and Laboratory Parameters

Decompensated hemorrhagic shock resulted in severe lactic acidosis. Resuscitation with LR + RSV resulted in significantly less lactate production and improved HCO_3^- levels (Supplemental Digital Content 1, <http://links.lww.com/TA/A475>) but did not significantly impact the MAP (Fig. 1). There were no significant differences between treatment groups 18 hours after resuscitation.

RSV-Restored Mitochondrial Function and Decreased Mitochondrial ROS Production

Hemorrhagic shock significantly impaired the function of all liver and kidney mitochondrial complexes (CI, CII, and CIV) (Fig. 2A and B). Resuscitation with RSV, however, robustly restored the respiratory capacity of all complexes in liver and significantly improved CII- and CIV-dependent respiratory function 18 hours after resuscitation (Fig. 2A). In the kidney, treatment with RSV during resuscitation significantly improved CII- and CIV-dependent respiratory capacity and notably improved the function of all complexes 18 hours after resuscitation (Fig. 2B).

RSV supplementation also significantly decreased liver and kidney mitochondrial ROS production following resuscitation (Fig. 2C and D). Although treatment with RSV continued to mitigate ROS production in the liver 18 hours after resuscitation, the generation ROS in the kidney normalized by 18 hours and levels did not differ between resuscitation strategies (Fig. 2D).

RSV Significantly Lowered Blood Glucose, Decreased Insulin Levels, and Improved Insulin Resistance Following Resuscitation

As previously described,⁹ hemorrhagic shock resulted in severe hyperglycemia, hyperinsulinemia, and increased insulin resistance. Compared with resuscitation with LR alone, RSV significantly lowered blood glucose levels following resuscitation (116.0 ± 20.2 mg/dL vs. 359.0 ± 79.5 mg/dL, $p < 0.05$, Fig. 3A). Blood glucose continued to be significantly lower in the RSV-treated animals at 45, 60, and 90 minutes following resuscitation but returned to levels observed in LR-treated animals within 360 minutes of resuscitation (Fig. 3B). Blood glucose levels were also significantly lower in the RSV-treated animals when compared with the sham animals (116.0 ± 20.2 mg/dL vs. 227.7 ± 8.3 mg/dL, $p < 0.05$, Fig. 3A). The incidence of hypoglycemia in the LR + RSV group was 33.3% (2 of 6 animals), whereas no animal in any other group developed hypoglycemia.

RSV was associated with significantly lower plasma insulin levels (1.0 ± 0.4 ng/mL vs. 6.5 ± 3.7 ng/mL, $p < 0.05$, Fig. 3C) and a decreased Ln HOMA-IR index following resuscitation (1.30 ± 0.42 vs. 4.18 ± 0.68 , $p < 0.05$, Fig. 3D). All parameters, however, were similar between the treatment groups 18 hours after resuscitation (Fig. 3A, C, and D).

RSV Increased Plasma GLP-1 Levels Following Resuscitation

Hemorrhagic shock resulted in significantly elevated plasma GLP-1, glucagon, and corticosterone levels (Fig. 4A–C). Compared with LR alone, RSV supplementation during resuscitation was associated with significantly higher GLP-1 levels (385.8 ± 56.6 ng/mL vs. 187.3 ± 11.1 ng/mL, $p < 0.05$, Fig. 4A) but did not influence glucagon or corticosterone values (Fig. 4B and C). At 18 hours, hormone levels were similar regardless of resuscitation strategy (Fig. 4A–C).

RSV Did Not Influence Plasma TNF- α and IL-6 Levels

The inflammatory cytokines TNF- α and IL-6 were significantly elevated following hemorrhagic shock and resuscitation. Resuscitation with RSV, however, did not significantly reduce the level of either cytokine (Fig. 5A and B).

RSV Supplementation Preserved the Active Form of IRS-1 in Both Liver and Kidney Tissues

Phosphorylated tyrosine 612 IRS-1 (pY612 IRS-1) is the active form of IRS-1 that activates several insulin signaling pathways. Following resuscitation with LR, the expression level of pY612 IRS-1 in liver and kidney tissues was significantly reduced. Resuscitation with RSV, however, preserved the expression of phosphorylated IRS-1 ($p < 0.05$, Fig. 6A and B). The expression level of pY612 IRS-1 did not differ between the treatment strategies 18 hours after resuscitation (Fig. 6A and B).

DISCUSSION

RSV, a naturally occurring polyphenol with known antioxidant and antiglycemic properties, has been shown to be protective in various models of human disease.^{14,15,23} Given that hemorrhagic shock is frequently complicated by mitochondrial dysfunction, exuberant ROS production, and hyperglycemia, we hypothesized that adding RSV to standard fluid resuscitation would prove beneficial.^{4,9} In our model of decompensated hemorrhagic shock, RSV supplementation significantly ameliorated liver and renal mitochondrial dysfunction and decreased blood glucose levels. RSV also seemed to improve insulin sensitivity following severe blood loss and was associated with decreased mitochondrial ROS production, enhanced GLP-1 secretion, and preserved IRS-1 phosphorylation—all factors that play a critical role in insulin signaling. RSV, however, may increase the risk of hypoglycemia following acute blood loss. Nonetheless, RSV seems to hold promise as a therapeutic adjunct during resuscitation.

Currently, resuscitation with standard crystalloid solutions inadequately targets ischemia-reperfusion injury and does not prevent the oxidative stress that follows hemorrhagic shock.²⁴ In this study, the use of LR failed to restore mitochondrial respiratory capacity and was associated with markedly elevated mitochondrial ROS production. Because impaired mitochondria can further exacerbate cellular damage and organ failure by decreasing aerobic ATP production and increasing ROS production,³ resuscitative agents that preserve mitochondrial function could prove highly beneficial. While a number of studies have demonstrated the antioxidant benefits of RSV following hemorrhagic shock,^{15–17} this study demonstrates that supplementing RSV during resuscitation actually preserves mitochondrial function. RSV administration in our study effectively restored CI-, CII-, and CIV-dependent respiratory capacity in liver and kidney tissues for at least 18 hours after hemorrhagic shock. In addition to potentially preserving oxidative phosphorylation and ATP production, the improved function in CI, CII, and CIV in RSV-treated animals likely contributed to the observed decrease in ROS following resuscitation. While CI is the major site of superoxide generation, both CII and CIV are important in modulating ROS production.^{25,26} As such, improved mitochondrial function should lead to a decrease in the generation of mitochondria-derived ROS. Our study demonstrates that RSV seems to protect mitochondrial function following hemorrhagic shock and may be a more useful strategy than standard crystalloid solutions alone.

Although a number of mechanisms have been implicated, the rapid generation of ROS also seems to play a key role in the development of postinjury hyperglycemia and acute insulin

resistance.^{9,27} ROS from damaged mitochondria is widely considered to be the primary initiating event in the development of diabetes and its subsequent complications,²⁸ and blocking the increase in ROS after injury seems to mitigate postinjury hyperglycemia.⁹ ROS may also promote insulin resistance by serving as a second messenger in the insulin signaling pathway.^{8,29} In the current study, RSV supplementation not only decreased mitochondrial ROS production but also preserved the expression of tyrosine phosphorylated IRS-1 following hemorrhagic shock. Because only phosphorylated IRS-1 is capable of activating downstream insulin signaling pathways,³⁰ it is likely that the observed decrease in insulin resistance following resuscitation with RSV was, in part, secondary to the preservation of insulin signaling.

RSV supplementation may also improve glycemic control by stimulating the secretion of GLP-1.³¹ GLP-1 is a gut-derived hormone that enhances insulin sensitivity by inducing glucose-dependent insulin secretion while suppressing glucagon release.³² Although we observed an increase in GLP-1 following resuscitation with RSV, we did not see a concomitant increase in insulin levels. GLP-1, however, has been shown to have independent insulin-like effects on glucose metabolism in rat liver, muscle, and fat.³³ Even in depancreatized animals, GLP-1 can still potentiate glucose use in peripheral tissues and has been shown to increase the expression and phosphorylation of IRS-1.^{34,35} As such, the increased secretion of GLP-1 observed in our study may have contributed to lower glucose values and the increase in phosphorylated IRS-1 observed in RSV-treated animals.

Consistent with previous studies,³⁶ hemorrhagic shock in our model resulted in significantly elevated TNF- α and IL-6 levels, which may have directly contributed to the development of hyperglycemia and insulin resistance.^{36,37} Although RSV has been shown to mitigate inflammation and reduced blood TNF- α and IL-6 levels in diabetic rats treated with enteral RSV for 30 days,³⁸ cytokine levels did not decrease in our study. Increased dosing regimens and longer observation periods may be needed to determine if RSV improves the cytokines profile in hemorrhagic shock.

Although the antiglycemic effects of RSV are well described, significant hypoglycemia does not seem to be a commonly reported complication.³⁹ In our model, however, intravenous administration of RSV led to two episodes of hypoglycemia. The clinical significance of this observation is unclear but certainly warrants heightened awareness and further investigation.

Our observations are subject to several limitations. First, our study did not use a hyperinsulinemic-euglycemic clamp to assess insulin sensitivity. While this is considered to be a criterion standard method of assessing insulin sensitivity, the frequent blood samples required would have affected the hemorrhage model, and the continuous insulin and glucose infusions may have potentially affected mitochondrial function. Instead, we used the Ln HOMA-IR index, which has been shown to correlate well with the hyperinsulinemic-euglycemic clamp method and is frequently used as a surrogate of insulin sensitivity.⁴⁰ Second, we did not treat hypoglycemic animals. Glucose administration may provide additional benefit during resuscitation with RSV but requires further study. Finally, RSV was only given at one time point. Longer-term studies and possibly increased dosing

regimens are needed to determine if the improvements seen in mitochondrial function translate into improved mortality or organ function.

In this study, we explored the effects of RSV on mitochondrial injury and glucose dysregulation following hemorrhagic shock. RSV supplementation during resuscitation restored liver and kidney mitochondrial function, decreased oxidative stress, and improved hyperglycemia. Adding RSV to standard fluid resuscitation seems to be beneficial and warrants further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

RSV does not influence mean blood pressure. Blood pressure was maintained at a fixed MAP of 40 mm Hg during shock. Compared with LR alone, LR + RSV resuscitation did not significantly improve the mean blood pressure. *LR*, resuscitation with LR solution; *LR + RSV*, resuscitation with LR solution plus RSV (30 mg/kg); *SS*, severe shock; *R*, after resuscitation. Values are mean $\pm$ SEM. $n = 6$.

Figure 2.

RSV supplementation during resuscitation restores mitochondrial function and decreases the production of ROS in the liver and kidney. The respiratory capacity of individual complexes in isolated intact mitochondria was assessed by high-resolution respirometry. Following hemorrhagic shock, mitochondrial CI, CII, and CIV in the liver and kidney demonstrated decreased activity. **A**, In the liver, RSV supplementation during resuscitation robustly restored the respiratory function of all complexes and significantly improved CII- and CIV-dependent respiratory capacity over baseline at 18 hours after resuscitation. **B**, RSV supplementation significantly restored kidney CII- and CIV-dependent respiration following resuscitation and improved the respiratory capacity of all mitochondrial complexes 18 hours later. Mitochondrial ROS production was detected by measuring the fluorescent signal from dichlorofluorescein. RSV supplementation significantly reduced liver (**C**) and kidney (**D**) mitochondrial ROS production following resuscitation. *LR*, resuscitation with LR solution; *LR + RSV*, resuscitation with LR solution plus RSV (30 mg/kg); *18hr LR*, 18 hours after resuscitation with LR solution; *18hr LR + RSV*, 18 hours after resuscitation with LR solution plus RSV (30 mg/kg). Values are mean $\pm$ SEM. $n = 6$. * $p < 0.05$ versus sham; † $p < 0.05$, LR versus LR + RSV; ‡ $p < 0.05$, 18hr LR versus 18hr LR + RSV.

Figure 3.

RSV treatment significantly lowers blood glucose and reduces insulin resistance.

Decompensated hemorrhagic shock resulted in severe hyperglycemia, hyperinsulinemia, and a significantly elevated Ln HOMA-IR index. A, Animals treated with LR + RSV had

significantly lower blood glucose levels than those in the sham group. B, RSV was associated with significantly lower blood glucose values 45, 60, and 90 minutes after the

onset of resuscitation. C, RSV supplementation resulted in significantly decreased plasma insulin levels, as determined by ELISA. D, RSV supplementation was associated with a

significantly lower Ln HOMA-IR index ($\text{Ln} [\text{insulin} \times \text{glucose}/405]$). LR, resuscitation with LR solution; LR + RSV, resuscitation with LR solution plus RSV (30 mg/kg); 18hr LR, 18

hours after resuscitation with LR solution; 18hr LR + RSV, 18 hours after resuscitation with LR solution plus RSV (30 mg/kg). Values are mean $\pm$ SEM. n = 6. * p < 0.05 versus sham; † p < 0.05, LR versus LR + RSV.

Figure 4.

RSV treatment significantly increases GLP-1 following resuscitation but has no effect on glucagon or corticosterone. A, Compared with LR alone, LR + RSV significantly increased GLP-1 levels following hemorrhagic shock but did not influence glucagon or corticosterone values (B and C). GLP-1 and glucagon were measured by radioimmunoassay; corticosterone was measured by ELISA. LR, resuscitation with LR solution; LR + RSV, resuscitation with LR solution plus RSV (30 mg/kg); 18hr LR, 18 hours after resuscitation with LR solution; 18hr LR + RSV, 18 hours after resuscitation with LR solution plus RSV (30 mg/kg). Values are mean $\pm$ SEM. n = 6. * p < 0.05 versus sham; † p < 0.05, LR versus LR + RSV.

Figure 5.

RSV treatment does not change serum TNF- α and IL-6 levels. RSV treatment did not significant decrease the levels of TNF- α or IL-6 when compared with LR resuscitation alone (A and B). TNF- α and IL-6 were measured by ELISA. *LR*, resuscitation with LR solution; *LR + RSV*, resuscitation with LR solution plus RSV (30 mg/kg); *18hr LR*, 18 hours after resuscitation with LR solution; *18hr LR + RSV*, 18 hours after resuscitation with LR solution plus RSV (30 mg/kg). Values are mean $\pm$ SEM. n = 6. * p < 0.05 versus sham.

Figure 6.

RSV supplementation restores activation of IRS-1 in both the liver and the kidney. The phosphorylated tyrosine 612 of IRS-1 (pY612 IRS-1) is the active form of IRS-1 that participates in insulin signaling. Western blotting for this modification indicates that hemorrhagic shock and resuscitation with only LR are associated with significantly decreased phosphorylation of IRS-1 in liver and kidney tissues. Resuscitation with LR + RSV robustly preserved the phosphorylation of IRS-1 in both the liver (A) and the kidney (B). *LR*, resuscitation with LR solution; *LR + RSV*, resuscitation with LR solution plus RSV (30 mg/kg); *18hr LR*, 18 hours after resuscitation with LR solution; *18hr LR + RSV*, 18 hours after resuscitation with LR solution plus RSV (30 mg/kg). Values are mean $\pm$ SEM. $n = 4-5$. * $p < 0.05$ versus sham; † $p < 0.05$, LR versus LR + RSV.