

## Basic Science

### GM-CSF IMPROVES INNATE IMMUNE DYSFUNCTION AND SEPSIS SURVIVAL IN OBESE DIABETIC MICE

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**Background:** Sepsis is the leading cause of death in the intensive care unit, with an overall mortality rate of 20%. Obese, type 2 diabetic (T2D) individuals are physiologically frail making them especially vulnerable to sepsis mortality. Neutrophils (PMN) are essential for bacterial eradication and sepsis survival. The impact of obesity and T2D on PMN function and sepsis mortality is unknown. We hypothesize that obesity and T2D alters PMN cellular function, inhibits innate immune responses, and hinders bacterial clearance, which increases sepsis mortality.

**Methods:** C57BL/6 (lean) and Diet Induced Obese (DIO, diabetic) 30 week old mice underwent cecal ligation and puncture (CLP<sub>LD20</sub>) or sham procedure +/- recombinant mouse GM-CSF (100ng s.q. b.i.d.). Mice were followed for survival and weighed daily. At serial time points, n = 5 mice/group were euthanized. Peritoneal fluid was analyzed for bacterial counts. PMN and monocyte phagocytic ability and reactive oxygen species (ROS) generation were assessed by flow cytometry. Cytokine analysis was completed with Luminex<sup>TM</sup> technology. Genomic analysis of phagocytic pathways was completed with RT<sup>2</sup> Profiler Arrays.

**Results:** After sepsis from CLP, DIO mice had significantly more mortality ( $P < 0.05$ ) and lost more body weight ( $P < 0.0001$ ) compared to lean mice. Lean mice lost a maximum of 17% of their weight, but by day 7 had started regaining weight and were near baseline by day 18. DIO mice lost a maximum of 29% of their weight; however, this weight loss persisted through day 18. DIO mice failed to eliminate bacteria from the peritoneal cavity when compared to lean mice ( $P < 0.01$ ). DIO PMNs are immature and expressed less CD11b and CXCR3 compared to lean PMNs ( $P < 0.01$ ). DIO PMN and monocyte phagocytic and ROS ability were dramatically reduced out to 14 days after sepsis ( $P < 0.01$ ). Genomic analysis revealed significantly less *Mertk* and *Axl* transcripts in DIO PMNs, which regulate phagocytosis of necrotic and apoptotic cells and adaptive immune responses. DIO mice also produced far less plasma MIP1A and MCP-1 cytokine levels ( $P < 0.05$ ) and hence recruited significantly less M1, M2A, and M2B monocytes into the peritoneal cavity compared to lean mice ( $P < 0.05$ ). DIO mice splenic mass and myeloid cellularity were significantly less at 14 days compared to lean controls. GM-CSF administration starting 6 hours after sepsis initiation improved survival in DIO mice.

**Conclusions:** DIO mice have increased mortality compared to lean mice after sepsis. DIO mice demonstrate defects in PMN and monocyte phagocytosis and ROS generation, which enable bacterial persistence. In addition, DIO mice have inadequate emergency granulopoiesis, hindering downstream innate immune responses and overall survival. GM-CSF administration improves DIO sepsis survival and is a novel therapeutic strategy to improve overall survival in this vulnerable population.

## Clinical Abstracts

### THE ACUTE EFFECTS OF INSULIN ON WHOLE BODY FUEL METABOLISM AND SKELETAL MUSCLE BIOENERGETICS IN SEVERELY BURNED CHILDREN

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**Background:** Insulin resistance and altered skeletal muscle bioenergetics have been observed in patients with severe burns. Chronic insulin administration has been shown to improve insulin sensitivity and skeletal muscle mitochondrial function in burned children. However, the acute effects of insulin administration on fuel metabolism and muscle bioenergetics following burn trauma are unknown.

**Objective:** We set out to determine the acute effects of a hyperinsulinemic-euglycemic clamp on whole-body glucose and lipid metabolism, and skeletal muscle bioenergetics in burned children.

**Methods:** Following an overnight fast, nine severely burned children underwent a 4-h study during which stable isotopes of glucose, glycerol and palmitate were infused. During the last 2 h of the infusion, a hyperinsulinemic-euglycemic clamp was performed. Blood was collected throughout to quantify substrate metabolism, and muscle biopsies were taken immediately before and after the clamp to determine mitochondrial respiratory function.

**Results:** Patients were  $8 \pm 4$  years old with burns covering  $55 \pm 18\%$  of the body surface. In response to hyperinsulinemia, proinflammatory cytokines TNF-[alpha] ( $P < 0.01$ ) and IL-6 fell ( $P < 0.05$ ). During the hyperinsulinemic clamp, glucose was infused at a rate of  $7.6 \pm 4.3$  mg/kg/min to maintain euglycemia. Glucose uptake increased by  $64 \pm 35\%$  ( $P = 0.01$ ) and hepatic glucose production decreased by  $76 \pm 15\%$  ( $P < 0.001$ ) in response to hyperinsulinemia. Hyperinsulinemia decreased the rate of appearance of glycerol ( $9.4 \pm 4.3$  vs  $5.5 \pm 2.2$   $\mu\text{mol/kg/min}$ ,  $P = 0.001$ ) and FFA ( $20.4 \pm 8.0$  vs  $6.6 \pm 2.1$   $\mu\text{mol/kg/min}$ ,  $P < 0.001$ ) from adipose tissue into plasma, lowering plasma glycerol ( $P < 0.001$ ) and FFA concentrations ( $P < 0.01$ ). Hyperinsulinemia reduced mitochondrial ATP production ( $53.1 \pm 12.3$  vs  $40.6 \pm 10.3$  pmols/s/mg,  $P < 0.05$ ) as well as respiratory control in response to ADP ( $P < 0.05$ ), while it increased uncoupled respiration ( $P < 0.05$ ). Greater suppression of ATP production and the increase in uncoupled respiration during hyperinsulinemia correlated with a blunted suppression of hepatic glucose production ( $r = -0.72$  and  $-0.68$ ,  $P < 0.05$ ). Suppression of ATP production also correlated with decreased glucose clearance rate ( $r = -0.82$ ,  $P < 0.01$ ).

**Conclusion:** Insulin infusion decreased lipid turnover, blunted hepatic glucose output and stimulated peripheral glucose uptake, suggesting that insulin still exerts control over substrate metabolism in burned individuals. Interestingly, acute hyperinsulinemia uncoupled skeletal muscle mitochondria, indicating that alterations in systemic inflammation and/or increased muscle glucose availability may drive mitochondrial thermogenesis in muscle of burned individuals.

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