Obesity-induced Endoplasmic Reticulum Stress Causes Lung Endothelial Dysfunction and Promotes Acute Lung Injury

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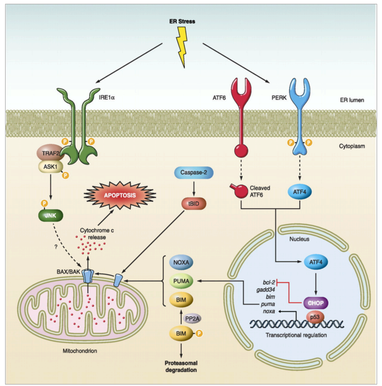
Introduction

- Obesity is a significant risk factor for the acute respiratory distress syndrome (ARDS): the mechanisms underlying this association remain poorly understood.[1,2]
- Diet-induced obesity (DIO) leads to profound changes in the vascular endothelium of the mouse lung including enhanced expression of leukocyte adhesion proteins and decreased expression of endothelial junctional proteins.[3]
- These cellular changes were associated with increased susceptibility to acute lung injury in obese mice (models ARDS in humans), suggesting obesity renders the lung more susceptible to ARDS.
- Chronically elevated levels of fatty acids contribute to organ dysfunction in obesity.[4-7] Cellular functions particularly vulnerable to "lipotoxic" effects of fatty acids (FAs) include the synthesis, folding and secretion of proteins by the endoplasmic reticulum (ER).



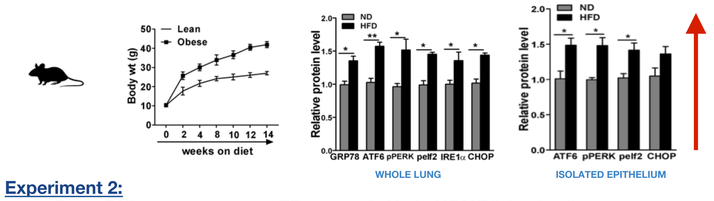
Study Design

- Mice studies: Male (3-weeks-old) AKR/J mice fed either a…
  - normal chow diet (13.5% calories from fat, 58% carb, 28.5% protein) or a
  - high fat/western-style diet (TD.08811, 45% calories from fat, Harlan Laboratories, USA) for a total of 14 weeks.
- Murine Model of Acute Lung Injury (ALI): One-time dose of LPS (100 mcg) or isotonic saline was instilled orotracheally.
- Western blotting: Protein bands were visualized using the Odyssey infrared imaging system (Li-Cor Biosciences, Lincoln, NE).
- Lung endothelial cell isolation: Lung tissue was dissociated into a single-cell suspension using a mouse lung dissociation kit.
- Cell culture: Human lung microvascular endothelial cells (HPMEC) and medium were purchased from ScienCell Research Laboratory, (Carlsbad, CA). Cells were utilized for no more than 5 passages.
- Preparation of fatty acid-BSA complex: The fatty acid-BSA complex used in experiments was prepared according standard protocol. Cells were exposed to the free fatty acid palmitate (PA) or oleic acid (OA) complexed to bovine serum albumin (BSA) in a ratio of 6:1 (free fatty acid:BSA) in full culture medium.
- In vitro endothelial permeability assay: In vitro endothelial permeability was performed as described previously (20).
- Statistical analysis: Statistics were performed using GraphPad Prism 5.0 software. Two-group comparisons were analyzed by unpaired Student's t-test while multiple-group comparisons were performed using one-way analysis of variance followed by Tukey post hoc analysis. Statistical significance was achieved when $p < 0.05$ at 95% confidence interval.

Outcomes

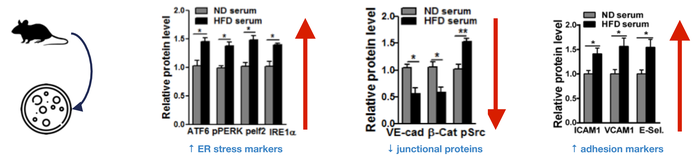
Experiment 1:

- **Does DIO promote ER stress in whole lung & isolated endothelium?**

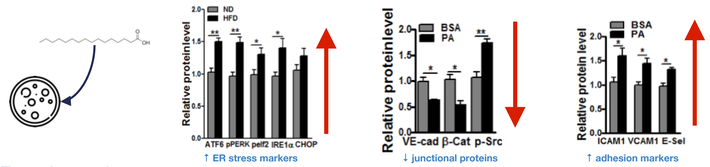


Experiment 2:

- **Does DIO serum promote ER stress & Δ's in HPMEC *in vitro*?**

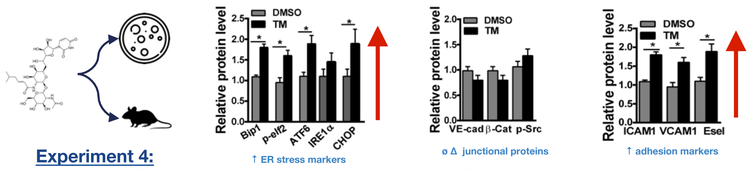


- **Do the FFA components (saturated) of DIO serum promote ER stress & Δ's in HPMEC *in vitro*?**



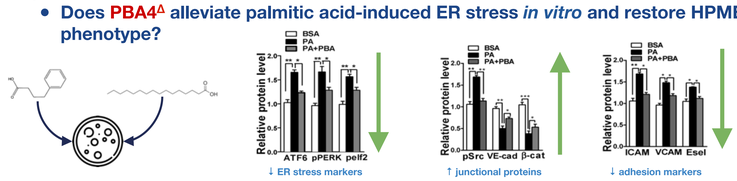
Experiment 3:

- **Does tunicamycin* cause Δ's in HPMEC (as FFAs *in vitro* & *in vivo*)?**

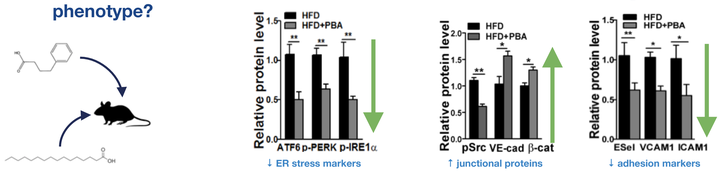


Experiment 4:

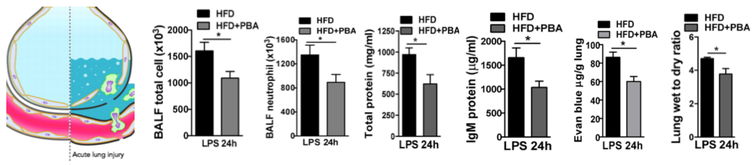
- **Does PBA4Δ alleviate palmitic acid-induced ER stress *in vitro* and restore HPMEC phenotype?**



- **Does PBA4Δ alleviate DIO-induced ER stress *in vivo* and restore lung endothelium phenotype?**



- **Does restoration of lung endothelium phenotype by PBA4Δ protect against ALI?**



Discussion

- DIO enhances expression of major sensors for misfolded proteins within the ER (PERK, IREα & ATF6), in whole lung and in lung endothelial cells in mice.
- Lung endothelial cells exposed to serum from DIO mice, or to saturated fatty acids (mimicking obese serum), enhanced expression ER stress markers and induction of other biological responses that typify the lung endothelium of DIO mice.
- Similar changes were observed in lung endothelial cells and in whole lung tissue after exposure to tunicamycin, a compound that causes ER stress by blocking N-linked glycosylation; indicating that ER stress causes endothelial dysfunction in the lung.
- Treatment with 4-PBA, a chemical protein chaperone that reduces ER stress, restored vascular endothelial cell expression of adhesion molecules and protected against LPS-induced acute lung injury in DIO mice.

Conclusion

- Lung endothelial dysfunction in DIO mice coincides with increased endoplasmic reticulum (ER) stress.
- Fatty acids in obese serum induce ER stress in the pulmonary endothelium leading to pulmonary endothelial cell dysfunction.
- Reducing protein load in the endoplasmic reticulum of pulmonary endothelial cells might protect against ARDS in obese individuals.

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