Airway Smooth Muscle Function is Altered in Obesity to Enhance Airway Responsiveness to Carbachol

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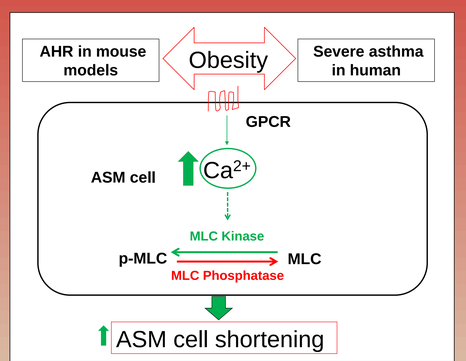
Abstract

Background: Obesity, an important public health issue, affects about one-third of the US population and represents a common asthma co-morbidity. Obese patients with asthma have poor response to treatment and their asthma symptoms reportedly improve upon successfully implementing weight loss measures. The mechanisms by which obesity causes asthma onset remains unknown. Findings from our laboratory suggest that the phenotype of airway smooth muscle (ASM) cells from obese donors is altered compared to that of non-obese donors. To elucidate the mechanisms that link obesity and asthma, we hypothesized that airway smooth muscle function is altered in obesity. **Methods:** C57/BL6 mice were started on either regular diet (RD, 15% dietary fat) or high-fat diet (HFD, 60% dietary fat) immediately after weaning. After 10 weeks on these diets, the mice were anesthetized and lung function was determined by methacholine dose-response experiment (airway resistance, R_l & dynamic compliance- C_{dyn}). Airway inflammation was determined by BAL fluid analysis. Human ASM (HASM) cells obtained from obese (BMI≥ 30 kg/m^2) or non-obese (BMI≤ 25 kg/m^2) lung donors were cultured *in vitro* and carbachol (10 uM)-induced cytosolic Ca^{2+} release was determined in using a fluorescent dye. In some experiments, HASM cells were exposed to carbachol (10 uM, 10 min) in the presence or absence of the metabolic hormone adiponectin (0.01-1 ug/ml, 24 h). Lysates were collected and myosin light chain (MLC) phosphorylation was determined by immune blotting. **Results:** Mice on HFD had significantly higher airway resistance (R_l for 50 mg/ml methacholine dose) (8.4±0.52 cmH_2O/ml/s, n=4) compared to the mice on RD (5.51±1.06 cmH_2O/ml/s, n=3). The dynamic compliance (C_{dyn} for 25 mg/ml methacholine) was significantly reduced in HFD-fed mice (0.005±0.0012 ml/cm H_2O) compared to that of RD-fed mice (0.010±0.0004 ml/cm H_2O). RD and HFD mice had comparable total BAL and differential cell counts. HASM cells from obese donors showed differentially higher carbachol-induced cytosolic Ca^{2+} release (area under the curve: 172500 ± 28880, n=10) compared to that of non-obese donors (94490 ± 17480, n=9). Further, carbachol induced differentially higher MLC phosphorylation in obese donor HASM cells (densitometry ratio: 4.521 ± 1.006, n=5) than in non-obese cells (1.693 ± 0.4279, n=5). The metabolic hormone adiponectin had little effect on carbachol-induced MLC phosphorylation in HASM cells from non-obese or obese donors (n=2). **Conclusions:** The *in vivo* results suggest that enhanced airway response to methacholine in HFD mice was independent of airway inflammation and is potentially mediated by airway structural cells. Differentially increased Ca^{2+} mobilization and MLC phosphorylation in HASM cells from obese donors support our hypothesis that ASM cell function is altered in obese individuals to enhance airway response to carbachol.

Obesity and Asthma

- The incidence of asthma is higher in obese subjects
- Obese patients have more severe disease and reduced response to therapy
- We demonstrate that airway smooth muscle (ASM) cells derived from obese subjects retain hyperresponsiveness as measured by agonist-induced Ca^{2+} release and phosphorylation of MLC.



Hypothesis

Obesity enhances excitation-contraction coupling in airway smooth muscle cells

High Fat Diet Increases Methacholine-induced Airway Narrowing in Mice

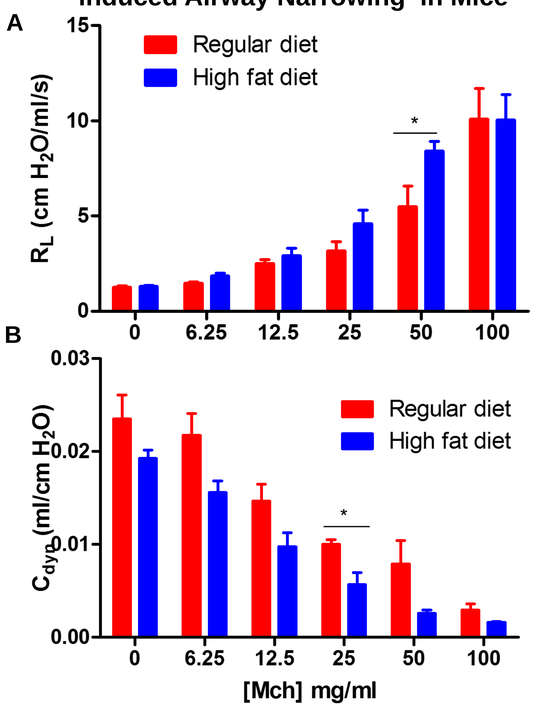


Figure 1. C57/BL6 mice fed with high-fat diet (HFD) or regular diet (RD) for 10 wks since weaning. HFD-fed mice showed A) elevated airway resistant (R_L, n=4 mice/group; *p=0.044) and B) reduced dynamic compliance (C_{dyn}, n=4 mice/group, *p=0.038) in response to methacholine (Mch), compared to that of RD-fed mice.

Carbachol-induced Myosin Light Chain Phosphorylation is Increased in HASM Cells from Obese Donors

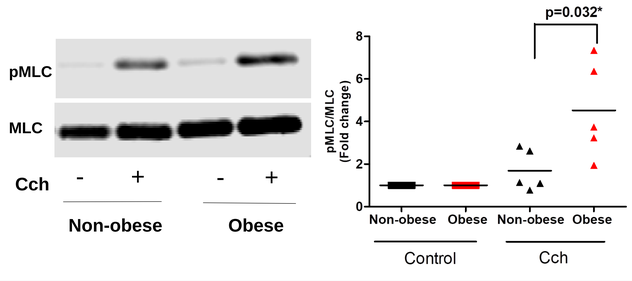


Figure 2. Left Panel- Representative blot: Carbachol (Cch) (10 μM/10minutes) induced enhanced phosphorylation of Myosin Light Chain (MLC) in HASM cells derived from obese donors. Right Panel-pMLC/total MLC densitometry ratio was significantly higher in HASM cells from obese donors (n=5 donors/ group; p=0.032).

Carbachol (Cch)- induced $[Ca^{2+}]_i$ is Significantly Elevated in HASM cells from Obese Donors

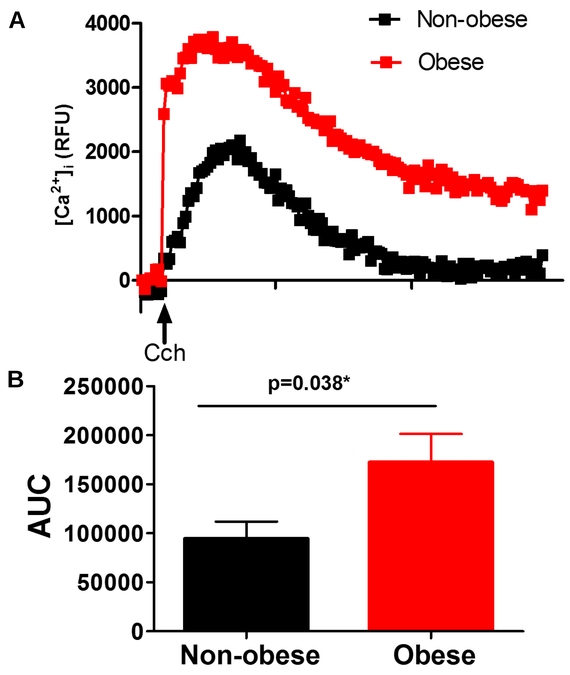


Figure 3. A) Representative Ca^{2+} trace: HASM cells derived from obese subjects showed enhanced $[Ca^{2+}]_i$ to Cch. B) Area under the curve (AUC) of Ca^{2+} trace: AUC of Cch-induced $[Ca^{2+}]_i$ was significantly higher in HASM cell from obese donors (n=10 donors) compared to that from non-obese (n=9 donors; (p=0.038).

Carbachol-induced $[Ca^{2+}]_i$ in HASM Cells Showed Positive Correlation with Body Mass Index (BMI) of the Donor

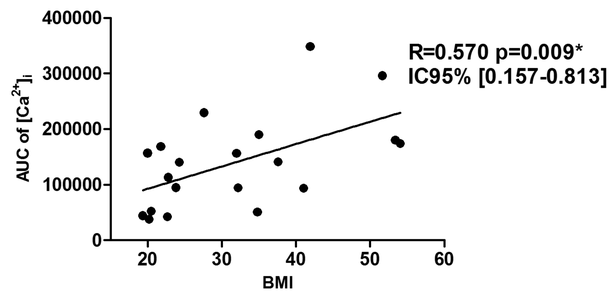


Figure 4. There was positive correlation between the donor BMI and Cch-induced $[Ca^{2+}]_i$ in HASM cells (n=20 donors; R=0.570 [IC 95%: 0.157-0.813]; P=0.009).

Gender Modulates Carbachol-induced $[Ca^{2+}]_i$ in HASM Cells from Obese Donors

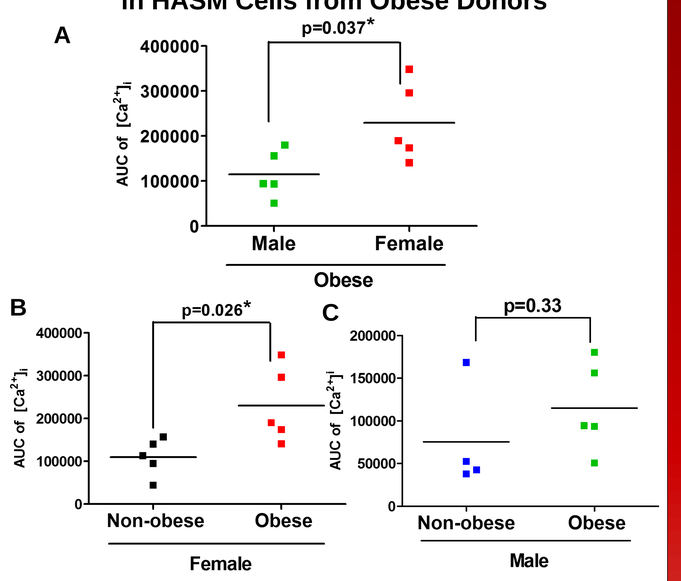


Figure 5. A) HASM cells from obese female donors showed significantly higher Cch-induced $[Ca^{2+}]_i$ than that from obese male donors (n=5 donors/group; p=0.037). B) HASM cells from Obese female donors showed significantly elevated Cch-induced $[Ca^{2+}]_i$ compared to the cells from non-obese female donors (n=5/group; p=0.026). C) HASM cells from non-obese and obese male donors showed comparable cch-induced $[Ca^{2+}]_i$ (n=4-5 donors; p=0.33)

Summary

- Obese mice showed increased lung resistance and decreased dynamic compliance compared to non-obese mice.
- HASM cells from obese donors were hyper responsive to carbachol, as measured by increased MLC phosphorylation and carbachol-induced $[Ca^{2+}]_i$, compared to those from non–obese donors.
- Gender modulates carbachol-induced $[Ca^{2+}]_i$ in HASM cells from obese donors

Significance

Airway smooth muscle cells from obese human donors have hyperresponsive phenotype which is maintained *in vitro*. Obesity-related systemic alterations (i.e: inflammatory, endocrine changes) may modulate ASM cell function to elicit this hyperresponsive phenotype. Identifying the underlying molecular mechanisms of this hyperresponsive phenotype will provide new therapeutic targets to improve clinical outcome in asthma patients with obesity.

Acknowledgments

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