Adipose-Lung Cell Crosstalk in the Obesity-ARDS Paradox

Ana Fernandez-Bustamante and John E Repine*
Department of Anesthesiology & Webb-Waring Center, University of Colorado SOM, USA

Abstract
Obesity is an increasingly frequent condition associated with increased adipose, systemic and pulmonary inflammation. There is an emerging and unexpected finding that obese individuals may not be at a greater risk for ARDS and, indeed, may even be partially protected against ARDS. This finding is known as the Obesity-ARDS Paradox. In this review we discuss the observations regarding this intriguing phenomenon and begin to elaborate on the theoretical rationale that obesity-triggered low-grade inflammatory processes may constitute pre-conditioning insults or trigger anti-inflammatory adaptive mechanisms that confer protection against ARDS.

The Obesity-ARDS Paradox
Obesity is an increasing global epidemic that increases cardiovascular abnormalities, diabetes, sleep apnea and mortality [1-3]. The Acute Respiratory Distress Syndrome (ARDS) is a highly fatal respiratory failure disorder [4,5]. Because both obesity and ARDS are characterized by increased inflammation and oxidative stress [6-12], obesity might reasonably be considered a risk factor for ARDS. However, the opposite seems to be the case. Although obese patients manifest a greater incidence of certain pro-inflammatory respiratory diseases, such as sleep apnea and asthma [13,14], they actually have a lower than expected incidence and/or severity of ARDS in a number of studies (Table 1) [15-21]. This unexplained finding is termed the “Obesity-ARDS Paradox.” Some of these studies are retrospective in nature or do not allow the investigation of the concept that has been termed “Metabolically Healthy Obesity” (MHO) [22,23] terminology that refers to obese individuals with no associated metabolic comorbidities (insulin resistance, atherosclerosis, liver dysfunction). Interestingly, this MHO concept has been linked to lower adipose-related inflammatory profiles and a lower mortality risk compared to individuals with metabolically unhealthy obesity [24]. How obesity may affect systemic and even pulmonary inflammatory responses is the key focus of this review. The differences in the metabolic and inflammatory backgrounds of patients in studies that do not fully support the Obesity ARDS Paradox phenomenon may explain their different conclusions. However, the finding of multiple studies showing a lower-than-expected mortality (including lower mortality [15,19,20] or similar mortality [17,18,21] than normal weight patients) in obese individuals after ARDS is provocative. Elucidating the causes of this observation should increase understanding of the causes of ARDS and, perhaps, lead to new and needed therapeutic approaches.

Pre-Conditioning as a Mechanism Contributing to the Obesity-ARDS Paradox
The underlying mechanism responsible for the Obesity-ARDS Paradox is unknown. Confronted with trying to elucidate a reason, we hypothesized earlier [25] that obesity induces a low-grade inflammation that generates a process that subsequently protects the lung against later insults. We termed this protective response the “pre-conditioning cloud” because the mechanism responsible for protection is nebulous at this time and likely to be multi-factorial in nature. However, the possibility of a pre-conditioning protective response is not new. The pre-conditioning or “tolerance” concept has been appreciated for many years. In its simplest presentation, pre-conditioning implies that a minor “first hit”---a relatively small inflammatory and/or oxidative insult that does not generate overwhelming damage---somehow creates a beneficial reaction that reduces the detrimental inflammation and/or oxidative stress a more aggressive and damaging “second hit”. Double (or multiple) hits are common in clinical settings; for example, when a patient sustains trauma (“first hit”) and then becomes infected (“second hit”). This double hit injury in which an initial insult (for example sepsis or trauma) primes and worsens the injury caused by a second insult (pneumonia or sepsis), rather than a first hit reducing the damage caused by a second hit, has also been observed in animal models and often used to create a greater lung injury [26,27].

Notwithstanding the detrimental consequences of a double hit injury, many observations show that a prior insult, if occurring under the right conditions with the right timing, can provide protection against a second insult in animals [28-30] and humans [31,32]. Several observations in animals are relevant. With respect to lung injury, good examples exist in studies of the acute edematous lung injury (“ARDS”) that develops in rats exposed continuously to hyperoxia (100% oxygen). Pre-treating rats with endotoxin, TNFα and IL-1, or 85% oxygen increases the survival of rats in hyperoxia [28-30]. The underlying mechanism is not clear but a common feature of these initial insults is that they each produce an inflammatory and oxidative response of apparently manageable proportions. A second feature of this protective response appears to be that time is required between the first and second insult for optimal protection to be achieved. In addition, a “cross-tolerance” can occur whereby pre-treating with one insult (e.g. ozone) will diminish the injury seen by different insult (e.g. hyperoxia) [33]. The protective response is not simply related to increases in antioxidants, which do not increase following every insult.

Theoretical Pre-Conditioning Mechanisms Contributing to the Obesity ARDS Paradox
Several mechanisms have been proposed as responsible for pre-conditioning development, including an increase of TNFα and other cytokines [29,34,35], and of heme oxygenase-1 (HO-1) [36], among others [37-39]. Interestingly, obesity raises the adipose and plasma levels of TNFs and other cytokines [40] and of HO-1 [41,42]. For example, one could hypothesize that the adipose-triggered first hit inflammatory mediators (e.g. adipose-released TNFs or other cytokines) locally
the physiological negative control responses. The possibilities include inducing early p38-MAPK activation, degrading IκBα which leads to activation of NFκB, and inducing a late up-regulation of IκBβ that prevents a prolonged TNFα synthesis [34]. The TNFα preconditioning reaches the systemic circulation in ischemia-reperfusion models [34] and therefore could contribute to anti-inflammatory protection against reperfusion injury [34]. The TNFα preconditioning is intrinsically decreased rather than impacted by competing protective responses.

### Obesity Related Processes that Might Trigger the Pre-Conditioning Mechanisms

The unexpected finding that obesity creates an intrinsic resistance to ARDS is particularly intriguing because obesity actually worsens the outcome of other inflammatory related abnormalities such as hypertension, diabetes and sleep apnea [43-46]. One aspect that might underlie this difference is that ARDS is an acute disorder while hypertension, diabetes and sleep apnea are chronic conditions. However, the intensity and nature of the pre-conditioning insult, more than the timing, are likely critical in determining any increases in

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Description</th>
<th>Findings/Comments</th>
</tr>
</thead>
</table>
| **Obesity is associated with decreased incidence and/or severity of ARDS**

---

O'Brien, 2006 [20]

Retrospective multi-center study comparing BMI with in-hospital mortality in mechanically ventilated adult patients with ALI/ARDS (n=1,488 patients between 1995 and 2001).

- BMI was independently associated with in-hospital mortality. Survivors had greater average BMI and a higher proportion of the obese (BMI>30) patients were survivors.
- The risk of in-hospital mortality was significantly reduced in obese patients with BMI ranging from 30-39.9 compared to patients with normal or underweight BMIs, after adjusting for age, gender, race, SAPS II probability of survival, diagnosis or ICU-acquisition of renal or genitourinary diseases.

---

Morris, 2007 [19]

Prospective multi-center observational study analyzing the relationship between BMI at hospital admission and clinical outcomes in ALI patients (n=825 patients, 1999-2000).

- Observed mortality was highest in underweight patients. Mortality decreased as BMI increased.
- After adjusting for age, acute and chronic illness scores using the APACHE III score and ALI etiology (sepsis, trauma or other), no statistically significant difference was found in mortality between obese and normal-weight patients.
- ALI Patients with BMI>40 had longer adjusted hospital LOS, and ALI survivors had more prolonged adjusted ICU LOS and duration of mechanical ventilation than normal-weight patients.

---

Memtsoudis, 2012 [15]

Database study using the Nationwide Inpatient Sample (NIS) developed by the Agency for Healthcare Research and Quality (AHRQ) which the mortality in obese vs. non-obese patients with diagnoses of RI/ARDS after surgery (n=9,149,030 surgical admissions between 1998 and 2007).

- Obese patients after surgery had a decreased incidence of RI/ARDS, need for mechanical ventilation when diagnosed with RI/ARDS, in-hospital mortality when intubated for RI/ARDS compared to non-obese patients after surgery.
- Obesity was identified as an independent protective factor against in-hospital mortality after postoperative RI/ARDS (OR=0.31; CI=0.28-0.36) using multivariable regression analysis adjusted for age, gender, race, admission status, hospital characteristics, type of surgery, and comorbidity burden.

Prevalence of obesity diagnosis in the study population was 5.48%—a significantly lower percentage than the CDC national obesity estimate for adults, estimated to be 30% [15]. Possible explanations are surgical pre-selection bias and the use of billing-derived diagnosis codes, based on ICD-9-CM as used in Clinical Classification Software (CCS).

| **Obesity does not increase ARDS severity**

---

O'Brien, 2004 [21]

Retrospective multi-center study comparing BMI with in-hospital mortality in mechanically ventilated adult patients with ALI/ARDS (n=607).

- Ventilator-free days and mortality were similar to those in normal-weight patients. Underweight (BMI<18.5) and extremely obese patients (weight(kg)-to-height(cm)≥1.20) were excluded from the analysis.

---

Gong, 2010 [17]

Prospective multi-center study analyzing BMI as risk factor for ARDS development and severity in patients at risk for ARDS at ICU admission (n=1,795, 1999-2007).

- 30% of at-risk patients developed ARDS.
- Patients who developed ARDS had greater average BMI, and BMI was positively correlated with ARDS development. ARDS development in obese patients occurred later in the ICU stay than in normal-weight patients. Authors suggested that the observed ventilatory settings might have played a role in the delayed ARDS development in obese patients.
- Obesity was not associated with an increased ICU-mortality or with an increased 60-day mortality. Survivors had greater average BMI than non-survivors.

---

Anzueto, 2011 [18]

Secondary analysis of prospective observational multi-center study cohort of mechanically ventilated ICU patients designed to analyze the effect of BMI on outcomes of mechanical ventilation, including ARDS development (n=4,698, April 2004).

- Obese and severely obese patients (BMI>30) had an increased incidence of ARDS development, higher tidal volumes per predicted body weight and higher PEEP levels.
- Outcomes (duration of mechanical ventilation, weaning duration, ICU and hospital LOS and mortality) were not significantly different in obese compared to other BMI ranges. The authors observed a non-significant trend to lower mortality rates in patients with a BMI>30 compared to normal-weight patients.

| **Obesity is associated with lower inflammatory biomarkers during ARDS**

---

Stapleton, 2010 [16]

Retrospective analysis of the effect of BMI on plasma biomarkers and outcomes in ARDS patients evaluated in previous NHLBI ARDSNet trials (n=1,409).

- BMI was not associated with increased mortality.
- After adjusting for gender, APACHE III score, coexisting diabetes, and ALI risk factors, obese patients (BMI>30) had lower plasma IL-6, IL-8, and SP-D levels and higher plasma protein C and vWF levels, compared to normal-weight patients.

(AlI=Acute Lung Injury; ARDS=Acute Respiratory Distress Syndrome; ARDSNet= Acute Respiratory Distress Syndrome Network; BMI=Body Mass Index; ICU=Intensive Care Unit; LOS=Length of Stay; NHLBI=National Heart Lung and Blood Institute; RI=Respiratory Insufficiency; SAPS II=Simplified Acute Physiology Score II; SP-D=Surfactant Protein D; vWF=von Willebrand Factor)

Table 1: Studies related to the Obesity-ARDS Paradox concept.
protection against injury achieved through this mechanism. Obviously, no matter what level of protection might be generated by the increased inflammation, oxidative stress and/or other processes occurring in obesity, the presence of too many and/or potent simultaneous pro-inflammatory/oxidative stress "hits" might overwhelm any protective adaptation conferred by obesity.

Until recently, adipose tissue-triggered inflammation was not considered to be especially influential systemically. However, it is increasingly clear that subtle changes in adipose tissue during obesity development constitute mechanisms that may have far reaching effects contributing to abnormalities in systemic and pulmonary circulations. For example, recent findings suggest that obesity suppresses adipose genes encoding proteins involved in transcription regulation, cell adhesion, and immune regulation. These genetic changes occur not only in adipocytes but also in macrophages [47]. Interestingly, this pattern of gene repression resembles the same responses that occur during endotoxemia in healthy humans, suggesting a pattern for an endogenous reactive protection reaction. In addition, there is a growing awareness of the responsiveness of the adipose tissue to ongoing systemic insults. During critical illness and, again for unknown reasons, adipose tissue macrophages shift from pro-inflammatory M1 to alternative or anti-inflammatory M2 phenotypes [48]. These examples suggest that a relatively fluid bidirectional cell-to-cell crosstalk exists between the adipose tissue and the systemic circulation. This interaction might be especially meaningful with respect to the circulation and function of the lung. The following proposed mechanisms have not been proven in the adipose-lung crosstalk. However, different findings suggest their potential role in either producing a direct immune-modulating effect in the lung and/or initiating an indirect response by triggering adaptive responses (as in the pre-conditioning models). Further research is needed to better understand the adipose-lung cell crosstalk pathways. This information may provide insight into the potential mechanisms contributing to the obesity generated pre-conditioning responses that may account for the Obesity-ARDS Paradox.

Pre-Conditioning Humoral Adipose-Lung Crosstalk Candidate Molecules

Adipokines

Hypertrophied adipocytes that characterize obesity secrete increased amounts of pro-inflammatory cytokines and chemokines, including leptin, TNFα, IL-6, Monocyte Chemoattractant Protein-1 (MCP-1) and osteopontin compared to adipocytes from non-obese subjects [49-51]. While TNFα, IL-6 and MCP-1 are ubiquitously produced, leptin is primarily secreted by white adipose tissue and leptin plasma levels are proportional to adiposity [52]. Interestingly, several findings suggest a role of leptin on lung inflammation. Leptin is increased in the plasma and bronchoalveolar lavage fluid of obese patients compared to healthy controls [52,53], but also in the BAL of non-obese ARDS patients compared to ventilated non-ARDS controls [54]. Several leptin receptor isoforms exist in different pulmonary cell types, including bronchial, alveolar epithelial and alveolar macrophages [55]. It is well known that leptin promotes the release of Th1-related cytokines [55], but recent findings suggest that leptin is also an immunomodulator in the lung with a role in efficient defense against infection [56,57] and exposure to smoke [58] in mice. Treating murine alveolar or peritoneal macrophages with leptin in vitro increases phospholipase A2 activity that in turn increases synthesis of leukotriene B4 (LTB4) and cysteinyl leukotrienes (LTC4, LTD4, LTE4, LTF4) [59]. Therefore, the adipose-originate leptin appears to have the capacity to convert distant alveolar macrophages towards pro-inflammatory phenotypes and is a direct adipose-lung crosstalk mediator. This obesity-triggered leptin-mediated inflammation modulates the pulmonary inflammatory background and may contribute to more efficient responses against further insults (being infection, smoke or others) as suggested earlier by other pre-conditioning methods. Leptin-related adaptations might stimulate protective responses that attempt diminishing inflammation and/or oxidative stress against further insults in the lung. These possible connections make leptin a reasonable candidate for involvement in the Obesity-ARDS Paradox, although the specific mechanisms remain to be elaborated.

Adiponectin, an anti-inflammatory adipocytokine, decreases during obesity development [60]. Adiponectin receptors also exist in the lung and their function is best linked to protection against allergen-related inflammatory responses [55,61]. Adiponectin-deficient mice show emphysematous-looking lungs and alveolar macrophages that produce increased TNFα and matrix metalloproteinase-12 (MMP-2) [62]. Interestingly, serum, more than pulmonary, adiponectin levels may modulate Lipopolysaccharide (LPS)-induced ARDS [63]. In a study by Konter et al. [63], adding adiponectin to pulmonary endothelial cells in vitro inhibited their LPS-induced IL-6 production, and ARDS was increased in adiponectin-deficient mice. The authors suggest that the obesity-associated serum adiponectin deficiency may explain the increased pulmonary vascular inflammatory responses and an increased risk to ARDS. However, the Obesity-ARDS Paradox is a clinical observation [15-21], and one could also hypothesize that the greater pulmonary vascular inflammation related to the decreased adiponectin during obesity leads to earlier or more efficient defensive responses against a later insult. Furthermore, recent findings are highlighting the differences in genetic inflammatory behavior between mice and humans [64] counsel caution interpreting any animal-based studies reflecting inflammation until confirmed in humans.

Heme oxygenase-1

Heme-oxygenase-1 (HO-1) is a heme-catalyzing enzyme that is elevated in the lungs of ARDS patients [65]. HO-1 induction has anti-inflammatory and antioxidant properties in a variety of inflammatory diseases including ARDS [66-68] but also obesity [69,70]. Increased monocyte/macrophage HO-1 is also associated with increased anti-inflammatory or M2 activation phenotypes (through arginase activity), greater phagocytic capacity, accelerated IL-10 production, and decreased macrophage inhibitory factor (MIF), TLR4, and iNOS activity via p38 MAPK [66,71-73]. For unknown reasons, HO-1 is increased in the adipose macrophages of obese humans compared to lean subjects [41]. Thus, obesity-initiated macrophage HO-1 increases could possibly represent a natural defensive attempt for controlling ongoing inflammation produced as a consequence of obesity. It is unclear if the obesity-induced increase in macrophage HO-1 expression concomitantly occurs in the alveolar macrophages, but this adaptation could constitute the vehicle for producing a protective pre-conditioning in the lung by attenuating inflammatory responses after later insults. The theoretical obesity-triggered HO-1 increase in the lung could contribute to the Obesity-ARDS Paradox by down-regulating neutrophil migration [74] and/or by down-regulating ICα-B and IFN-β [75,76]. The pro-inflammatory TNF-α, one of first cytokines in the NK-xβ cascade, is associated with alveolar neutrophil recruitment, increased insulin resistance, type 2 diabetes and obesity [77,78]. Therefore, increases in HO-1 may be an endogenous negative feedback process that can modulate the obesity-induced NF-xβ cascade and perhaps lead to attenuation of neutrophil recruitment, and ARDS development, following later insults.
**Peroxisome-Proliferator (PPAR-γ)**

The peroxisomal proliferator-activated receptor-γ (PPAR-γ) is a nuclear ligand-activated transcription factor of the nuclear receptor superfamily. PPAR-γ is endogenously activated by several unsaturated fatty acids [79], and is most prevalent in intestine and adipose tissue but also found in vascular endothelium and macrophages [80]. PPAR-γ has appreciable metabolic and pulmonary effects. PPAR-γ activation attenuates adipose inflammation and insulin resistance [81,82]. PPAR-γ stabilizes HO-1 mRNA [76], decreases IFN-β expression [76], and promotes M2 phenotype polarization in monocytes and macrophages [81]. In the lung, PPAR-γ activation decreases TNF-α, reduces oxidative stress and protects alveolar type II epithelial cells from LPS-induced apoptosis in vitro [83,84].

PPAR-γ is down-regulated by the obesity-mediated increases in leptin [85] a finding that may be responsible for the increased pro-inflammatory and fibroproliferative changes in lungs of leptin-resistant mice and human fibroblasts [8]. This phenomenon known as “leptin resistance” is a desensitization to circulating leptin that also occurs in obese patients [86,87]. Although the mechanism is unclear, both the leptin-resistant PPAR-γ increase, and the leptin-induced pro-inflammatory “first hit”, could theoretically participate in lung preconditioning and the Obesity-ARDS Paradox. The relationship of PPAR-γ to this phenomenon warrants further investigation.

**Cellular adipose-lung crosstalk**

Because of their capacity to quickly respond to broad stimuli, cells of the immune system travel through the circulation and communicate among themselves and other cell types. Consequently, inflammatory cells related to obesity are likely participants in any mechanism(s) involved in the Obesity ARDS Paradox. Nonetheless, despite our focus on inflammatory cells, other intermediary cells, such endothelial or epithelial cells, may likely contribute to the preconditioning process.

**Neutrophils:** Several findings suggest that obesity affects neutrophil count and function. First, neutrophil counts are increased in the blood of obese humans [88,89]; neutrophil counts also correlate with body mass index and waist circumference in obese female adolescents [88]. Despite their increased number, little is known about the functional activities of the neutrophils of obese patients. However, neutrophil recruitment is decreased in obese compared to lean mice in a murine LPS-induced ARDS model. In addition, neutrophils from unjured leptin-resistance and diet-induced obese mice show a decreased chemoattractant migration to chemokine KC, and a decrease in neutrophil CXCR2 expression was suggested as an involved mechanism [90]. In this study by Kordonowy et al. [90], the neutrophil surface expression of chemokine receptor CXCR2 (also known as IL-8 receptor β) was significantly reduced in obese compared to lean mice. Therefore, although not confirmed in humans, it is possible that an obesity-related neutrophil impairment contributes to the unexpected lower incidence of ARDS development in obese humans.

**Monocytes/macrophages:** During the development of obesity, there is an increase in migration and infiltration activities of blood circulating monocytes, which are attracted by adipose tissue products: MCP-1, CXC1L14, osteopentin, Angn2, etc [91]. Macrophage infiltration in adipose tissue occurs before the development of insulin resistance in animal models [82,92]. Monocytes/macrophages are very heterogeneous in their activation profiles and function, primarily reflecting their local metabolic and immune microenvironment [83,93,94]. Macrophage activation phenotypes can modulate the inflammatory cascade by releasing different substances with a more pro-inflammatory profile (i.e. TNFa, IL-1β, IL-6) in M1 phenotypes, vs. a M2 profile (i.e. IL-4, IL-10) with anti-inflammatory, immunomodulatory or pro-healing properties. Obesity induces a switch in adipose tissue macrophages from M2 to M1 phenotypes [8]. Whether from adipocytes or adipose macrophages, TNFa and other pro-inflammatory mediators (as discussed earlier) keep re-emerging as possible mediators of an obesity-related pre-conditioning response. Nevertheless, it is not known if or how macrophage changes in the adipose tissue affect the remote lung, but macrophage-derived TNFa locally induces lipolysis in adipocytes via their TNFa receptor, releasing saturated fatty acids that in turn further enhance inflammatory changes via the TLR4 receptor [95]. Because new macrophage phenotypes are still being described [94], their functions and secretory profiles could also have roles in the process underlying the Obesity-ARDS Paradox.

**Lymphocytes:** Lymphocyte subpopulations have recently attracted attention for their potential role in obesity. Lymphocytes could possibly be involved in the crosstalk between the adipose tissue and the lung during obesity development. Blood T lymphocytes increase with obesity [88,96], although the mechanisms responsible for these increases are not well understood. In adipose tissue, CD8+ effector T lymphocytes play a critical role in initiating and maintaining adipose-related inflammation. Nishimura et al. [97] recently showed that CD8+ T cells increase in the adipose tissue in diet-induced murine obesity and in human obesity. In this key study Nishimura et al. [97] also showed that CD8+ T cell infiltration precedes macrophage infiltration and enhances M1 macrophage phenotypes and adipose inflammation. Macrophage chemoattractant protein (MCP-1) is a key messenger in this CD8+ T-mediated macrophage recruitment [97]. Paradoxically, a recent flow cytometry study reported normal levels of CD8+ T lymphocytes in the peripheral blood, but increased CD4+ T cells, in morbidly obese patients compared to lean patients [98]. The increased CD4+ cells had a more Treg- and Th2-phenotype, which suggests a shift towards more anti-inflammatory profiles [98]. Whether this anti-inflammatory shift exists in all degrees of obesity needs to be confirmed, but it strongly suggests a complex still under-explored crosstalk of different T cell subpopulations infiltrating the adipose tissue at different time points during obesity. The long-distant effect of lymphocyte subpopulations in the systemic circulation and the lung is still unknown and there is insufficient evidence regarding the involvement of T-lymphocytes in ARDS.

**Conclusions**

The surprising observation and well-named Obesity-ARDS Paradox potentially offers important insights into the causes, treatment and prevention of ARDS. This provocative observation is further underscored by another unanticipated observation showing that patients with diabetes also have a decreased incidence of ARDS [99,100]. Both of these clinical findings were likely unexpected at the time, although it had been shown previously that higher glucose can impair neutrophil bactericidal function and this may have impaired their ability to cause lung damage as well [101]. Whether the mechanism(s) are triggered by adiposity itself or adipose-related hyperglycemia, the reality is that the human body appears to have the capacity to develop its own endogenous ways of protecting itself against a highly lethal insult like ARDS. These protective responses are impressive as well as under-explored, and likely hold meaningful clues for providing an effective treatment or prevention strategy for ARDS.

**References**


