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High fat feeding protects mice from ventilator-induced lung injury, via neutrophil-independent mechanisms

Michael R Wilson, PhD, Joanne E Petrie, FRCA, Michael W Shaw, FRCA, Cong Hu, MRes, Charlotte M Oakley, BVSc, Samantha J Woods, BSc, Brijesh V Patel, FRCA, PhD, Kieran P O'Dea, PhD, and Masao Takata, MD, PhD

Section of Anaesthetics, Pain Medicine and Intensive Care, Faculty of Medicine, Imperial College London, Chelsea & Westminster Hospital, London, UK

Abstract

Objective—Obesity has a complex impact on acute respiratory distress syndrome patients, being associated with increased likelihood of developing the syndrome, but reduced likelihood of dying. We propose that such observations are potentially explained by a model in which obesity influences the iatrogenic injury that occurs subsequent to intensive care admission. This study therefore investigated whether fat-feeding protected mice from ventilator-induced lung injury (VILI).

Design—In vivo study.

Setting—University research laboratory.

Subjects—Wildtype C57Bl/6 mice or TNF receptor 2 knockout mice, either fed a high fat diet for 12–14 weeks, or age-matched lean controls.

Interventions—Anesthetized mice were ventilated with injurious high tidal volume ventilation for periods up to 180 minutes.

Measurements and main results—Fat-fed mice showed clear attenuation of VILI in terms of respiratory mechanics, blood gases and pulmonary edema. Leukocyte recruitment and activation within the lungs were not significantly attenuated, nor were a host of circulating or intra-alveolar inflammatory cytokines. However, intra-alveolar matrix metalloproteinase (MMP) activity and levels of the MMP cleavage product sRAGE were significantly attenuated in fat-fed mice. This was associated with reduced stretch-induced CD147 expression on lung epithelial cells.

Conclusions—Consumption of a high fat diet protects mice from VILI in a manner independent of neutrophil recruitment, which we postulate instead arises through blunted upregulation of CD147 expression and subsequent activation of intra-alveolar MMPs. These findings may open avenues for therapeutic manipulation in ARDS, and could have implications for understanding the pathogenesis of lung disease in obese patients.

Address correspondence to: Dr Michael Wilson, Anaesthetics, Pain Medicine and Intensive Care, Imperial College London, Chelsea and Westminster Hospital, 369 Fulham Road, London SW10 9NH, UK. michael.wilson@imperial.ac.uk, Fax: +44 20 3315 5109, Tel: +44 20 3315 8292.

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Keywords

Acute respiratory distress syndrome; obesity; matrix metalloproteinases; mechanical ventilation; cytokines; inflammation

Introduction

Despite much research, mortality from acute respiratory distress syndrome (ARDS) remains high. Therapeutic options are limited to removal of identifiable insults and supportive treatment within the Intensive Care Unit. Mechanical ventilation forms the mainstay of support, although this has the potential to worsen patient outcome through the development of ventilator-induced lung injury (VILI).

Recent data reports the prevalence of obesity (body mass index (BMI) ≥ 30) in the United States as ~35%, and $>20\%$ in many Western countries (1, 2). Just as the impact of increased BMI on all-cause mortality remains the subject of debate (3), so too its impact within ARDS is uncertain. Studies of at-risk patients suggest that obesity increases the likelihood of developing ARDS (4, 5). Animal models have similarly concluded that obesity predisposes to ARDS, through mechanisms including exacerbated endothelial cell activation and dysfunctional neutrophil responses (6–11).

However, despite this, most clinical studies suggest that overweight/obese BMI is paradoxically associated with decreased mortality, or at least is not detrimental (12–16). Whether such a counterintuitive relationship truly exists for ARDS is inherently difficult to address clinically. Partly this is due to confounding factors such as differences in age and medication between cohorts (12, 15), and use of interventions that may acutely influence weight (e.g. administration of fluid boluses) (13). Such difficulties mean that identifying mechanisms to explain why obesity may be beneficial is likely only to be achieved within experimental settings.

We propose that the best explanation for previous clinical observations is that obesity has a beneficial effect on factors that influence mortality once ARDS is established, one of the most important of which is the impact of VILI (17). Supporting this possibility, it appears that increased BMI is particularly beneficial within ventilated, rather than non-ventilated patients (18). The current study is therefore designed to test the novel hypothesis that obesity induced by high fat feeding specifically attenuates VILI, using a mouse model.

Materials and Methods

Protocols were approved by the Ethical Review Board of Imperial College London, and carried out in accordance with the Animals (Scientific Procedures) Act 1986, UK. Male wildtype C57Bl/6 mice (Harlan, Bicester, UK), and TNF receptor 2 knockout mice (gift from Immunex, Thousand Oaks, CA) were used. Animals had free access to a high calorie/high fat diet (18.56 MJ/kg Atwater free energy (AFE), with 46% AFE from fat, 18% from protein and 36% from carbohydrate) or control diet (RM1; 13.75MJ/kg AFE, 7.4% fat, 17.5% protein, 75.1% carbohydrate), for 12–14 weeks, from 4 weeks of age.

Mouse model of ventilator-induced lung injury

To explore the influence of diet on susceptibility to ventilation, a 'one-hit model' of VILI induced by very high tidal volume (V_T) was used (19, 20). Mice were anesthetized (80-100mg/kg ketamine and 8-10mg/kg xylazine) and ventilated using a custom-made volume-controlled ventilator (19). During surgical preparation for carotid artery cannulation, animals were ventilated with low stretch (V_T 200-250 μ l), positive end-expiratory pressure (PEEP) 3cmH₂O, using 100% O₂. Lung recruitment was performed by sustained inflation (30cmH₂O, 5 seconds) to standardise lung volume history. V_T was then increased to produce a standardised peak inspiratory pressure (PIP) of 40cmH₂O (19, 20), which thereafter was maintained constant throughout. PEEP remained at 3cmH₂O, and O₂ supplemented with 4% CO₂ was used to avoid hypocapnia. In some experiments animals remained on low V_T settings, with recruitments performed every 30 minutes, to act as a control group. Anesthesia was maintained by intraperitoneal ketamine (1mg) and xylazine (0.1mg) every 25-30 minutes. While fat-fed animals required marginally more anesthetic for induction, over the entire protocol the total amount given relative to body weight was not different. Ventilation was continued for predetermined times up to 180 minutes, or until arterial blood pressure (BP) dropped below 50mmHg as a surrogate marker of mortality if this occurred first (20).

Sample collection

Animals were terminated by exsanguination. Lavage fluid neutrophils were evaluated by differential cytology on CytoSpin slides. Lavage protein was determined using a colorimetric assay (Bio-Rad, Hemel Hempstead, UK). CXCL1, Interleukin-6 (IL-6), and soluble Receptor for Advanced Glycation End-products (sRAGE) in lavage fluid, and plasma soluble TNF receptors were determined by ELISA (R&D systems, Abingdon, UK). Lavage fluid TNF was determined by high sensitivity ELISA (eBioscience, Altrincham, UK). Matrix metalloproteinase (MMP) activity was determined by fluorometric assay (abcam, Cambridge, UK). Other plasma biomarkers were determined by FlowCytomix assay (eBioscience).

Flow cytometry

Lung samples were processed for flow cytometry using previously validated methods. In brief, single cell suspensions were produced by mechanical disruption of lungs followed by sieving through a 40 μ m filter. Samples for leukocyte quantification were stained with fluorophore-conjugated antibodies against F4/80, CD11b, NK1.1, Gr-1 (Biolegend, Bar Hill, UK), and Ly-6C (BD Biosciences, Oxford, UK), and counting beads (Invitrogen, Paisley, UK) were added (21, 22). In other experiments epithelial cells were identified using previously reported methods (23), by staining using antibodies against CD45, T1 α , (both Biolegend), CD31 and EpCAM, along with CD147 or isotype-matched control (all eBioscience). Samples were run on a CyAn ADP flow cytometer (Beckman Coulter, High Wycombe, UK) and analysis was performed using FlowJo software (Tree Star, Ashland, OR).

Statistical Analysis

Data were analysed using SPSS v.20 (IBM, New York, NY). The assumption of normality of residuals was assessed by QQ plot and Shapiro-Wilk test, and homogeneity of variance evaluated by Levene's test. Statistical analyses were made using ANOVA, Student's t-tests (following data transformation if required), or Mann-Whitney U-tests. Data are presented as mean $\pm$ SD or median with interquartile range. Statistical significance was defined as $p < 0.05$.

Results

Effect of fat feeding on VILI

Fat-fed mice had substantially greater total body weight (41.0 ± 3.9 vs 33.3 ± 4.1 g; $N = 22-24$; $p < 0.0001$), and a higher percentage of body weight comprised of epididymal and perirenal fat deposits (% fat pad:body weight $6.5 \pm 1.7\%$ vs $3.2 \pm 0.8\%$; $p < 0.0001$) compared to age/strain-matched lean controls, confirming altered body composition.

In initial experiments wildtype animals were ventilated with high V_T for 180 minutes, or until they met the mortality surrogate. Injurious ventilation, standardised with a starting PIP of $40 \text{ cmH}_2\text{O}$, produced a similar V_T in the two groups of animals (1010 ± 80 vs. $1050 \pm 80 \mu\text{l}$ in fat-fed and control animals; $N = 11-12$). Crucially, respiratory system elastance (33.7 ± 3.7 vs. $31.4 \pm 2.8 \text{ cmH}_2\text{O/ml}$) and resistance (0.88 ± 0.07 vs. $0.86 \pm 0.09 \text{ cmH}_2\text{O/ml.s}^{-1}$) were not different between lean and fat-fed animals at the onset of ventilation.

PIP remained stable for ~ 120 minutes in both groups of mice (Fig. 1A), after which it increased rapidly in lean animals, such that a number met the mortality surrogate from 150 minutes onwards. In contrast PIP did not increase substantially in fat-fed mice, all of which survived the full protocol (Fig. 1B). The final PIP was significantly lower in fat-fed mice (Fig. 1C). Deteriorations in respiratory system mechanics (Fig. 1D&E) and blood gases (Fig. 1F&G) were also attenuated with fat-feeding.

In lean mice VILI induced substantial alveolar-capillary barrier permeability (lavage fluid protein) and edema (lung wet:dry weight ratio), which were significantly attenuated by fat-feeding (Fig. 2A&B). Similarly, lavage fluid levels of the epithelial stress marker sRAGE were lower in fat-fed animals (Fig. 2C). We explored whether protection could be explained by decreased leukocyte recruitment, as occurs following obesity in other lung injury models (7–10). There was little intra-alveolar neutrophil infiltration ($< 3\%$ of leukocytes) in any animals after 2–3 hours ventilation, consistent with previous studies (20, 24). Hence we determined leukocyte accumulation within lung tissue by flow cytometry (21, 25). Substantial numbers of neutrophils and Gr-1^{high} 'inflammatory' monocytes were present following VILI, but fat feeding had little impact on either numbers (Fig. 2D&E) or activation of leukocytes, evaluated by surface CD11b expression (26) (Fig. 2F&G). Finally, we evaluated the general inflammatory milieu by determining pro- and anti-inflammatory mediators in plasma, as differences in circulating mediators have been reported between lean and obese ARDS patients (14). Most mediators were similar between groups (Supplemental Fig.1), apart from significantly higher levels of soluble TNFR2 in fat-fed mice.

Mechanistic investigations

To address whether upregulated soluble TNFR2 could explain the protection induced by fat feeding, we carried out experiments in TNFR2 knockout mice. Lean TNFR2 knockouts developed injury as anticipated, which was again significantly attenuated by fat-feeding (Fig. 3A-F). As with wildtypes, protection was not associated with reduced lung leukocytes (Fig. 3G&H).

To further explore possible causative factors, we returned to wildtype mice and evaluated various intra-alveolar mediators. In these experiments ventilation was terminated after 120 minutes, just before physiological lung injury was anticipated to develop in lean animals (according to Fig. 1A), to clarify whether mediators could be a cause rather than merely a consequence of subsequent injury. Consistent with this design, there was no injury at 120 minutes in any animal (Supplemental Fig. 2). Under these conditions, intra-alveolar IL-6, CXCL1 and TNF were present, but similar between lean and fat-fed mice (Fig. 4A-C). In contrast, lavage fluid MMP activity was significantly lower in fat-fed mice (Fig. 4D). In light of this we determined MMP activity within low V_T ventilated animals, which was similar in both groups.

Finally we determined expression of CD147, an upstream regulator of MMP activity, on lung epithelial cells by flow cytometry. Cell surface CD147 expression was initially studied in lean mice ventilated with high V_T for 1-2 hours. Expression tended to peak at 1 hour (Fig. 5A&B), so we compared 1 hour of high V_T or low V_T ventilation between lean and fat-fed animals. Stretch-induced upregulation of CD147 was significantly attenuated with fat-feeding (Fig. 5C&D).

Discussion

Obesity is implicated clinically with a greater susceptibility to development of, but lower subsequent mortality from, ARDS. We used mouse models to investigate whether this could be explained by an inherent protection from VILI. Our data show that fat-fed mice were protected from the physiological consequences of over-ventilation; animals displayed lower PIP, smaller deteriorations in respiratory system mechanics and gas exchange, and less alveolar-epithelial permeability.

In the current study we used V_T equivalent to ~31-33ml/kg to induce lung injury, much higher than would be utilised clinically. However, mouse lungs are much more compliant than human lungs (27), and such high V_T are necessary to induce inflammatory injury within healthy animals (20). It was vital for our hypothesis that mechanical stress/stretch induced by ventilation were matched between lean and fat-fed mice. Obesity is frequently associated with altered chest wall mechanics, making optimisation of ventilation protocols challenging. While these are clinically important considerations, the current study is not confused by such complications because our experiments were designed to ensure similar respiratory mechanics between groups. As mice have highly compliant rib cages and abdominal walls, chest wall elastance is minimal and respiratory system mechanics are effectively determined by lung mechanics under most circumstances (28). Although with extreme obesity in mice chest wall elastance can be increased, we deliberately used animals

before this became apparent. It was therefore possible to match ventilation between lean and fat-fed mice in terms of both PIP and V_T , meaning that lungs were exposed effectively to the same degree of mechanical stress (pressure) and resultant stretch (V_T) in both groups. Our data clearly demonstrated protection from this standardized mechanical lung insult in fat-fed mice.

While obesity in otherwise healthy individuals is characterised by chronic inflammation, plasma inflammatory biomarkers paradoxically decrease with rising BMI in ARDS patients (14), suggesting a blunted systemic response to the ‘challenge’ of ARDS. We evaluated this in our study, but found very few differences in circulating pro- and anti-inflammatory mediators apart from an increase in soluble TNFR2 in fat-fed animals. Plasma TNFR2 is similarly raised in obese humans (29), and theoretically could explain our findings as TNFR2 expression conveys protection during VILI (24). However, it was clear that fat-feeding remained protective in TNFR2 knockout mice, reiterating the beneficial effects of the dietary manipulation, but demonstrating that soluble TNFR2 is not necessary.

Many of the mechanistic studies exploring the influence of obesity on lung injury have focussed on leptin as a mediator (8, 10, 30). We therefore performed experiments in leptin-deficient Lep^{ob} mice to evaluate this possibility (Supplemental Fig. 3&4). Our findings were consistent with a role for leptin in VILI-mediated leukocyte recruitment, but we found no physiological protection. It is however dangerous to draw solid conclusions, as leptin plays an important role in postnatal lung development, such that Lep^{ob} mice have a reduced alveolar surface area (31), making standardised experiments between wildtype and Lep^{ob} mice very difficult. Hence, while leptin likely plays an important role in host defence (32), any involvement in VILI remains uncertain.

Unexpectedly, we found no significant attenuation in leukocyte recruitment in fat-fed animals, either wildtype or TNFR2 knockout. Changes in neutrophil recruitment and function have been proposed to explain the altered susceptibility of obese subjects to ARDS (33). Neutrophil dysfunction secondary to obesity is associated with impaired bacterial clearance and worse outcome in mouse models of pneumonia (8–10), while fat-feeding reduces neutrophil-mediated injury following inhaled LPS (7). However, from the current data it seems highly likely that within VILI, fat-feeding is protective through mechanisms independent of neutrophil recruitment. It remains possible that neutrophil functionality, such as reactive oxygen species production or NET formation was altered as we did not extensively investigate this possibility, although recent studies suggest that these are not impaired in fat-fed animals (34).

To explore alternative explanations, we determined various intra-alveolar mediators at an early point before any animals showed increased permeability. This is crucially important, because once barrier breakdown begins it becomes difficult to interpret whether differences in alveolar cytokine levels are a cause or a consequence of altered permeability between groups. Additionally, studying ‘end-point’ alveolar cytokines may be misleading, as lean animals developed injury more quickly and thus ventilation protocols often ended sooner. Given the aforementioned findings, we focussed specifically on mediators that have been reported to influence permeability/lung fluid balance independent of neutrophils (24, 35,

36), specifically CXCL1, TNF, IL6, and MMPs. Interestingly, short-term high fat nutrition reportedly limits haemorrhage (37, 38) or hemolysis-induced (39) organ damage, via activation of $\alpha 7$ -nicotinic acetylcholine receptors and attenuated TNF and IL-6 secretion. It was clear though that in our study there was no difference in TNF, IL-6 or CXCL1.

In stark contrast, intra-alveolar soluble MMP activity was significantly attenuated within fat-fed mice, due to reduction of stretch-induced MMP activation/secretion. Various MMPs have been shown to contribute to VILI (40–44). MMP2/9 activities may directly induce extracellular matrix degradation, and are also implicated in TGF β activation (45) which can influence lung fluid balance via epithelial sodium channels (46). MMP9 also plays an important role in ectodomain shedding of RAGE (47), so our finding of greatly reduced sRAGE levels within fat-fed mice further supports the likelihood that attenuated MMP activity had direct biological consequences. Attenuated MMP activation is likely related to our finding of abrogated CD147 upregulation following injurious ventilation in fat-fed mice. CD147 is an important regulator of expression, activation and secretion of multiple MMPs (48), and CD147 within epithelial and endothelial cells has been directly linked to ventilator-induced MMP activation (44, 49). We confirmed that epithelial cells from fat-fed mice were responsive to high stretch ventilation (via MAPkinase activation as seen in lean mice, Supplemental Fig. 5), implying that our findings are somewhat specific to CD147 upregulation. Perhaps surprisingly, despite CD147 having a known involvement (48) in many obesity-associated comorbidities (eg various cancers, atherosclerosis), this is seemingly the first time CD147 expression has been investigated in obesity per se.

While fat feeding is the de facto standard method for inducing obesity-like states in rodents (33), dietary fats can influence many biological functions (50). It therefore remains somewhat unclear whether dietary composition or obesity underlie the current findings. Future studies using alternative dietary models may provide greater clarity regarding this question (which however affects the majority of current animal studies of obesity), and give clues to the exact mechanism of protection. Clinical trials have investigated the potential of manipulating fatty acid profile in ARDS, primarily omega-3 fatty acid supplementation (51, 52), but with little benefit (53). The diets used currently did not differ substantially in omega-3 profile, although the high fat diet contained greater omega-6 content, primarily C18:2 linoleic acid. This is a precursor of pro-resolution lipoxins, although whether this is relevant is uncertain.

Conclusions

The current data demonstrate that high fat feeding protects mice from VILI, which we propose as an explanation for the clinical correlation between BMI and mortality from ARDS. This is seemingly not reliant on the obesity-related alterations in inflammatory cytokines and leukocyte accumulation that have been reported in other lung injury models. Instead, we uncovered a novel phenomenon whereby fat-feeding attenuates stretch-induced CD147 upregulation and activation of intra-alveolar MMPs. This finding may open avenues for therapeutic manipulation in ARDS, and could have implications for understanding the pathogenesis of lung disease in obese patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Flegal KM, Carroll MD, Kit BK, et al. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. *JAMA*. 2012; 307:491-7. [PubMed: 22253363]
2. [Accessed Nov 24 2016] Obesity Update 2014. Available at <http://www.oecd.org/els/health-systems/Obesity-Update-2014.pdf>
3. Flegal KM, Kit BK, Orpana H, et al. Association of all-cause mortality with overweight and obesity using standard body mass index categories: a systematic review and meta-analysis. *JAMA*. 2013; 309:71-82. [PubMed: 23280227]
4. Gong MN, Bajwa EK, Thompson BT, et al. Body mass index is associated with the development of acute respiratory distress syndrome. *Thorax*. 2010; 65:44-50. [PubMed: 19770169]
5. Anzueto A, Frutos-Vivar F, Esteban A, et al. Influence of body mass index on outcome of the mechanically ventilated patients. *Thorax*. 2011; 66:66-73. [PubMed: 20980246]
6. Shah D, Romero F, Duong M, et al. Obesity-induced adipokine imbalance impairs mouse pulmonary vascular endothelial function and primes the lung for injury. *Sci Rep*. 2015; 5:11362. [PubMed: 26068229]
7. Kordonowy LL, Burg E, Lenox CC, et al. Obesity is associated with neutrophil dysfunction and attenuation of murine acute lung injury. *Am J Respir Cell Mol Biol*. 2012; 47:120-7. [PubMed: 22427537]
8. Hsu A, Aronoff DM, Phipps J, et al. Leptin improves pulmonary bacterial clearance and survival in ob/ob mice during pneumococcal pneumonia. *Clin Exp Immunol*. 2007; 150:332-9. [PubMed: 17822444]
9. Mancuso P, Gottschalk A, Phare SM, et al. Leptin-deficient mice exhibit impaired host defense in Gram-negative pneumonia. *J Immunol*. 2002; 168:4018-24. [PubMed: 11937559]
10. Ubags ND, Vernooy JH, Burg E, et al. The role of leptin in the development of pulmonary neutrophilia in infection and acute lung injury. *Crit Care Med*. 2014; 42:e143-51. [PubMed: 24231757]
11. Wan T, Yuan G, Ren Y, et al. Diet-induced obese mice exhibit altered immune responses to acute lung injury induced by *Escherichia coli*. *Obesity (Silver Spring)*. 2016; 24:2101-10. [PubMed: 27558300]

12. Morris AE, Stapleton RD, Rubenfeld GD, et al. The association between body mass index and clinical outcomes in acute lung injury. *Chest*. 2007; 131:342–8. [PubMed: 17296631]
13. O'Brien JM Jr, Phillips GS, Ali NA, et al. The association between body mass index, processes of care, and outcomes from mechanical ventilation: a prospective cohort study. *Crit Care Med*. 2012; 40:1456–63. [PubMed: 22430246]
14. Stapleton RD, Dixon AE, Parsons PE, et al. The association between BMI and plasma cytokine levels in patients with acute lung injury. *Chest*. 2010; 138:568–77. [PubMed: 20435656]
15. O'Brien JM Jr, Phillips GS, Ali NA, et al. Body mass index is independently associated with hospital mortality in mechanically ventilated adults with acute lung injury. *Crit Care Med*. 2006; 34:738–44. [PubMed: 16521268]
16. Sakr Y, Alhussami I, Nanchal R, et al. Being Overweight Is Associated With Greater Survival in ICU Patients: Results From the Intensive Care Over Nations Audit. *Crit Care Med*. 2015; 43:2623–32. [PubMed: 26427591]
17. Network ARDS. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. *N Engl J Med*. 2000; 342:1301–8. [PubMed: 10793162]
18. Sasabuchi Y, Yasunaga H, Matsui H, et al. The Dose-Response Relationship Between Body Mass Index and Mortality in Subjects Admitted to the ICU With and Without Mechanical Ventilation. *Respir Care*. 2015; 60:983–91. [PubMed: 25691767]
19. Wilson MR, Choudhury S, Goddard ME, et al. High tidal volume upregulates intrapulmonary cytokines in an in vivo mouse model of ventilator-induced lung injury. *J Appl Physiol*. 2003; 95:1385–93. [PubMed: 12807894]
20. Wilson MR, Patel BV, Takata M. Ventilation with “clinically relevant” high tidal volumes does not promote stretch-induced injury in the lungs of healthy mice. *Crit Care Med*. 2012; 40:2850–7. [PubMed: 22890257]
21. Bertok S, Wilson MR, Morley PJ, et al. Selective inhibition of intra-alveolar p55 TNF receptor attenuates ventilator-induced lung injury. *Thorax*. 2012; 67:244–51. [PubMed: 22156959]
22. Wakabayashi K, Wilson MR, Tatham KC, et al. Volutrauma, but not Atelectrauma, Induces Systemic Cytokine Production by Lung-Marginated Monocytes. *Crit Care Med*. 2014; 42:e49–57. [PubMed: 23963135]
23. Woods SJ, Waite AA, O'Dea KP, et al. Kinetic profiling of in vivo lung cellular inflammatory responses to mechanical ventilation. *Am J Physiol Lung Cell Mol Physiol*. 2015; 308:L912–21. [PubMed: 25770178]
24. Wilson MR, Goddard ME, O'Dea KP, et al. Differential roles of p55 and p75 tumor necrosis factor receptors on stretch-induced pulmonary edema in mice. *Am J Physiol Lung Cell Mol Physiol*. 2007; 293:L60–8. [PubMed: 17435079]
25. O'Dea KP, Young AJ, Yamamoto H, et al. Lung-marginated monocytes modulate pulmonary microvascular injury during early endotoxemia. *Am J Respir Crit Care Med*. 2005; 172:1119–27. [PubMed: 16081546]
26. Wilson MR, O'Dea KP, Zhang D, et al. Role of lung-marginated monocytes in an in vivo mouse model of ventilator-induced lung injury. *Am J Respir Crit Care Med*. 2009; 179:914–22. [PubMed: 19218195]
27. Zosky GR, Janosi TZ, Adamicza A, et al. The bimodal quasi-static and dynamic elastance of the murine lung. *J Appl Physiol*. 2008; 105:685–92. [PubMed: 18556435]
28. Tankersley CG, Rabold R, Mitzner W. Differential lung mechanics are genetically determined in inbred murine strains. *J Appl Physiol*. 1999; 86:1764–9. [PubMed: 10368335]
29. Cartier A, Cote M, Bergeron J, et al. Plasma soluble tumour necrosis factor-alpha receptor 2 is elevated in obesity: specific contribution of visceral adiposity. *Clin Endocrinol (Oxf)*. 2010; 72:349–57. [PubMed: 19650780]
30. Bellmeyer A, Martino JM, Chandel NS, et al. Leptin resistance protects mice from hyperoxia-induced acute lung injury. *Am J Respir Crit Care Med*. 2007; 175:587–94. [PubMed: 17185651]
31. Huang K, Rabold R, Abston E, et al. Effects of leptin deficiency on postnatal lung development in mice. *J Appl Physiol*. 2008; 105:249–59. [PubMed: 18467551]

32. Ubags ND, Stapleton RD, Vernooij JH, et al. Hyperleptinemia is associated with impaired pulmonary host defense. *JCI Insight*. 2016; 1
33. Ubags ND, Burg E, Antkowiak M, et al. A Comparative Study of Lung Host Defense in Murine Obesity Models. Insights into Neutrophil Function. *Am J Respir Cell Mol Biol*. 2016; 55:188–200. [PubMed: 27128821]
34. Moorthy AN, Tan KB, Wang S, et al. Effect of High-Fat Diet on the Formation of Pulmonary Neutrophil Extracellular Traps during Influenza Pneumonia in BALB/c Mice. *Front Immunol*. 2016; 7:289. [PubMed: 27531997]
35. Reutershan J, Morris MA, Burcin TL, et al. Critical role of endothelial CXCR2 in LPS-induced neutrophil migration into the lung. *J Clin Invest*. 2006; 116:695–702. [PubMed: 16485040]
36. Gurkan OU, He C, Zielinski R, et al. Interleukin-6 mediates pulmonary vascular permeability in a two-hit model of ventilator-associated lung injury. *Exp Lung Res*. 2011; 37:575–84. [PubMed: 22044313]
37. Luyer MD, Greve JW, Hadfoune M, et al. Nutritional stimulation of cholecystokinin receptors inhibits inflammation via the vagus nerve. *J Exp Med*. 2005; 202:1023–9. [PubMed: 16216887]
38. de Haan JJ, Thuijls G, Lubbers T, et al. Protection against early intestinal compromise by lipid-rich enteral nutrition through cholecystokinin receptors. *Crit Care Med*. 2010; 38:1592–7. [PubMed: 20453642]
39. de Haan JJ, Windsant IV, Lubbers T, et al. Prevention of hemolysis-induced organ damage by nutritional activation of the vagal anti-inflammatory reflex*. *Crit Care Med*. 2013; 41:e361–7. [PubMed: 24105450]
40. Albaiceta GM, Gutierrez-Fernandez A, Garcia-Prieto E, et al. Absence or inhibition of matrix metalloproteinase-8 decreases ventilator-induced lung injury. *Am J Respir Cell Mol Biol*. 2010; 43:555–63. [PubMed: 19995943]
41. Villar J, Cabrera NE, Valladares F, et al. Activation of the Wnt/beta-catenin signaling pathway by mechanical ventilation is associated with ventilator-induced pulmonary fibrosis in healthy lungs. *PLoS One*. 2011; 6:e23914. [PubMed: 21935365]
42. Kim JH, Suk MH, Yoon DW, et al. Inhibition of matrix metalloproteinase-9 prevents neutrophilic inflammation in ventilator-induced lung injury. *Am J Physiol Lung Cell Mol Physiol*. 2006; 291:L580–7. [PubMed: 16698855]
43. Gonzalez-Lopez A, Astudillo A, Garcia-Prieto E, et al. Inflammation and matrix remodeling during repair of ventilator-induced lung injury. *Am J Physiol Lung Cell Mol Physiol*. 2011; 301:L500–9. [PubMed: 21743031]
44. Foda HD, Rollo EE, Drews M, et al. Ventilator-induced lung injury upregulates and activates gelatinases and EMMPRIN: attenuation by the synthetic matrix metalloproteinase inhibitor, Prinomastat (AG3340). *Am J Respir Cell Mol Biol*. 2001; 25:717–24. [PubMed: 11726397]
45. Tatler AL, Jenkins G. TGF-beta activation and lung fibrosis. *Proc Am Thorac Soc*. 2012; 9:130–6. [PubMed: 22802287]
46. Peters DM, Vadasz I, Wujak L, et al. TGF-beta directs trafficking of the epithelial sodium channel ENaC which has implications for ion and fluid transport in acute lung injury. *Proc Natl Acad Sci U S A*. 2014; 111:E374–83. [PubMed: 24324142]
47. Zhang L, Bukulin M, Kojro E, et al. Receptor for advanced glycation end products is subjected to protein ectodomain shedding by metalloproteinases. *J Biol Chem*. 2008; 283:35507–16. [PubMed: 18952609]
48. Grass GD, Toole BP. How, with whom and when: an overview of CD147-mediated regulatory networks influencing matrix metalloproteinase activity. *Biosci Rep*. 2016; 36:e00283.
49. Haseneen NA, Vaday GG, Zucker S, et al. Mechanical stretch induces MMP-2 release and activation in lung endothelium: role of EMMPRIN. *Am J Physiol Lung Cell Mol Physiol*. 2003; 284:L541–7. [PubMed: 12456388]
50. de Pablo MA, Alvarez de Cienfuegos G. Modulatory effects of dietary lipids on immune system functions. *Immunology and cell biology*. 2000; 78:31–9. [PubMed: 10651927]
51. Rice TW, Wheeler AP, Thompson BT, et al. Enteral omega-3 fatty acid, gamma-linolenic acid, and antioxidant supplementation in acute lung injury. *JAMA*. 2011; 306:1574–81. [PubMed: 21976613]

52. Stapleton RD, Martin TR, Weiss NS, et al. A phase II randomized placebo-controlled trial of omega-3 fatty acids for the treatment of acute lung injury. *Crit Care Med.* 2011; 39:1655–62. [PubMed: 21423000]
53. Zhu D, Zhang Y, Li S, et al. Enteral omega-3 fatty acid supplementation in adult patients with acute respiratory distress syndrome: a systematic review of randomized controlled trials with meta-analysis and trial sequential analysis. *Intensive Care Med.* 2014; 40:504–12. [PubMed: 24556914]

Figure 1.

Time course of peak inspiratory pressure (PIP) change during high stretch ventilation (Panel A), showing that both lean control mice and high fat-fed mice displayed the same initial decrease in PIP, indicative of lung recruitment at very high tidal volumes. While PIP increased dramatically after 120 minutes in control mice, this was much less apparent in fat-fed animals. A number of animals were unable to complete the 180 minute protocol in the control group only, leading to variability in PIP as mice dropped out at later time points, and a significantly worse survival (Panel B). End PIP (Panel C), irrespective of the length of

ventilation, was significantly lower in fat-fed mice than controls. This was related to attenuation of changes in both respiratory system elastance (Panel D) and resistance (Panel E), determined by end-inflation occlusion. Arterial oxygenation was well maintained in both groups until 120 minutes, whereupon it fell more dramatically in the control group than in fat-fed mice (Panel F). End pO_2 values, representing either 180 minutes or the point at which mortality surrogates were met, were significantly higher in fat-fed animals. Arterial CO_2 was similarly well maintained in both groups until 120 minutes, after which it tended to increase in control animals as lung injury developed, but did not in fat-fed animals (Panel G). Data within panels A, D, E, F and G were normally distributed, and are shown as mean $\pm$ SD. Data within panel C were non-parametric, and thus are displayed as a box-whisker plot. N=11-12 / group (until 150 minutes for panel A, as animals dropped out from this point onwards). Panel B shows a Kaplan-Meier survival curve, with significance determined by logrank test. Data in panel C were evaluated by Mann-Whitney U-test, while data in Panels D-G were evaluated by t-test (end points only in panels F and G). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 2.

Alveolar-epithelial barrier permeability assessed by lavage fluid protein (A) and lung wet:dry weight ratio (B) was significantly decreased in fat-fed animals compared to lean controls. Similarly the epithelial stress marker soluble Receptor for Advanced Glycation End-products (sRAGE) in lavage fluid (Panel C) was attenuated in fat-fed mice. There was a tendency for the number of neutrophils (Panel D) and inflammatory Gr1^{high} monocytes (E) accumulated within the lungs to be reduced in fat-fed mice, but this was not statistically significant. Lung neutrophils (Panel F) and Gr1^{high} monocytes (G) showed no differences in

levels of the activation marker CD11b between groups. N=5 / group for panels B, F and G, and N=7 / group for panels A, C, D and E. Data in panel C are displayed as box-whisker plot and evaluated by Mann-Whitney U-test, while data in all other panels are displayed as mean $\pm$ SD and assessed by t-test. **p<0.01, ***p<0.001.

Figure 3.

High stretch ventilation experiments were carried out in lean and fat-fed TNF receptor 2 knockout mice. Feeding of a high fat diet led to similar attenuation of lung injury as seen with wildtypes, such that only 1/5 fat-fed animals displayed substantial injury, hence the very large variability in PIP towards the end of the experiment and the apparent decrease in PIP at 180 minutes as the only injured fat-fed animal dropped out of the analysis (Panel A). Survival (Panel B), final PIP (Panel C), pO₂ (panel D), pCO₂ (panel E) and lung wet:dry ratio (Panel F) were similarly attenuated with high fat feeding, all of which demonstrate that

the high fat diet-induced protection was not dependent on upregulation of soluble TNF receptor 2. As with wildtype mice, fat-diet induced protection was not mediated by decreased leukocyte infiltration as both neutrophils (Panel G) and inflammatory monocytes (Panel H) were not attenuated. Panels A, D, E, G and H are shown as mean $\pm$ SD, while panels C and F are displayed as box-whisker plots. N=5 / group for all data (until 120 minutes for panel A, as animals dropped out after this point), apart from panels G&H where N=4. Panel B shows a Kaplan-Meier survival curve, with significance determined by logrank test. Data in panels C and F were evaluated by Mann-Whitney U-test, while data in Panels D, E, G and H were evaluated by t-test (end points only in panels D and E). *p<0.05, **p<0.01, ***p<0.001.

Figure 4.

Lavage fluid Interleukin-6 (IL-6, Panel A), CXCL1 (Panel B) and tumour necrosis factor- α (TNF, Panel C) were no different following 120 minutes of injurious ventilation in lean or fat-fed mice. In contrast, net MMP activity was significantly lower in lavage fluid of fat-fed animals ventilated with high V_T (left part of Panel D). This was specifically due to attenuated stretch-induced upregulation, as MMP activity was not different in lean and obese mice ventilated with low stretch (right part of Panel D). N=6-7 for all data in panels A-C and 5-6 in Panel D. Data are displayed as mean $\pm$ SD for Panels A-C (statistical evaluation by t-test), and Box-whisker plots in Panel D (statistical evaluation by Mann-Whitney U-test).

**p<0.01.

Figure 5.

Surface expression (mean fluorescence intensity, MFI) of CD147 on type 1 (A) and type 2 (B) epithelial cells determined by flow cytometry. Within lean animals expression was increased on both cell types following 1 hour of high V_T ventilation. In a separate set of experiments, CD147 expression was compared after 1 hour of high or low V_T ventilation between lean and fat-fed mice (Panels C&D). Data for panels A&B are displayed as box-whisker plots, while data for panels C&D are displayed as mean $\pm$ SD, N=4-5 for each dataset. Statistical analysis for Panels C&D was carried out by ANOVA with Bonferroni correction. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.