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Orthopedic trauma-induced pulmonary injury in the obese Zucker rats

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Abstract

Objective—Obese subjects with orthopedic trauma exhibit increased inflammation and an increased risk of pulmonary edema. PGE₂ production is elevated during inflammation and associated with increased vascular permeability. We hypothesize that pulmonary edema in obesity following orthopedic trauma is due to elevated PGE₂ and resultant increases in pulmonary permeability.

Methods—Orthopedic trauma was induced in both hindlimbs in lean (LZ) and obese Zucker rats (OZ). On the following day, plasma IL-6 and PGE₂ levels, pulmonary edema, and pulmonary gas exchange capability were compared between groups: LZ, OZ, LZ with trauma (LZT), and OZ with trauma (OZT). Vascular permeability in isolated lungs was measured in LZ and OZ before and after application of PGE₂.

Results—As compared with the other groups, the OZT exhibited elevated plasma IL-6 and PGE₂ levels, increased lung wet/dry weight ratio and bronchoalveolar protein concentration, and an impaired pulmonary gas exchange. Indomethacin treatment normalized plasma PGE₂ levels and pulmonary edema. Basal pulmonary permeability in isolated lungs was higher in OZ than LZ, with a further increase in permeability following treatment with PGE₂.

Conclusions—These results suggest that pulmonary edema in OZ following orthopedic trauma is due to an elevated PGE₂ and resultant increases in pulmonary permeability.

Keywords

obesity; trauma; prostaglandin; inflammation; pulmonary edema

Introduction

Pulmonary complications such as acute respiratory distress syndrome (ARDS) are major post-injury causes of mortality in obese patients with orthopedic trauma (7, 14). Decades of clinical and animal research have demonstrated an increased alveolar capillary permeability

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and a resultant protein-rich edema in the alveoli as primary physiological abnormalities in the early stages of pulmonary failure (37, 49). However, the mechanisms responsible for the increased pulmonary vascular permeability and resultant pulmonary edema in obesity following orthopedic trauma are unclear.

Inflammatory stimuli (endotoxin, cytokines) cause a secondary production of arachidonic acid metabolites such as prostaglandin E₂ (PGE₂) by inducing cyclooxygenase (COX) and/or PGE₂ synthesis (25, 42). For example, elevated PGE₂ production in inflammatory circumstances has been demonstrated in both pulmonary vessels and airway smooth muscle (13, 34). PGE₂ can be synthesized at the sites of inflammation (30) and/or remote tissues in response to systemic stimuli (33). In addition, PGE₂ production can be elevated in the early stage of inflammation since increased activation of COX-2 and PGE₂ synthesis in vascular smooth muscle cells has been demonstrated within 24 hours after exposure to inflammatory cytokines (24). Obese patients with orthopedic trauma exhibit more pronounced inflammatory responses as compared with lean patients (3, 18). However, whether there is a secondary increase in PGE₂ synthesis leading to high risk of complications in obesity following orthopedic trauma is unknown.

Inflammatory mediators such as PGE₂, histamine, and bradykinin are known to increase vascular filtration and cause tissue edema, which are the key character during inflammatory responses. Pulmonary edema following PGE₂ treatment has been demonstrated in both clinical (21, 45) and animal studies (2, 19). These observations suggest a potential role of PGE₂ in mediating pulmonary edema under pathological conditions such as during orthopedic trauma. Indeed, obese patients with orthopedic trauma exhibit higher risks of pulmonary complications such as ARDS as compared with non-obese patients (8, 11). However the mechanisms responsible for the increased pulmonary complications are unclear. The current study will determine if there is increased production of PGE₂ following orthopedic trauma in the obese patients, which contributes to the development of pulmonary edema.

Obese Zucker rats (OZ) have been widely used as a model of obesity and metabolic syndrome. We have shown that, compared to lean Zuckers (LZ), OZ at 11–13 weeks of age exhibit severe obesity and insulin resistance with no changes in blood pressure (MAP) (51, 53). In addition, among all the inflammatory markers, circulating IL-6 levels display the highest correlations with the extent of trauma (46) and risk of remote organ dysfunction (15). Therefore, we chose circulating levels of IL-6 as an indicator for the orthopedic trauma-induced inflammation in the current study. We hypothesize that orthopedic trauma leads to increase in inflammation and a resultant production of PGE₂ in OZ, which increases pulmonary permeability and causes edema.

Methods

Animals

Male LZ and OZ (11–13 wk) were acquired from Harlan Laboratories and the mean bodyweight was 324 ± 6 g for LZ and 516 ± 12 g for OZ. The experimental protocols for this study were approved by the Institutional Animal Care and Use Committee at the University of Mississippi Medical Center and were carried out according to both the “Guide for the Care and Use of Laboratory Animals” from the National Institutes of Health and the guidelines of the Animal Welfare Act. All the rats were housed 2–3 animals per cage at 22°C (12-h light-dark cycle) with free access to food and water.

Animal model of orthopedic trauma—About 90% of patients with severe trauma suffer long bone fractures along with an associated soft-tissue injury (14). According to the

guidelines set forth in the Animal Welfare Act and the “Guide for the Care and Use of Laboratory Animals”, experiments involving animals with bone fracture should be treated with a post-trauma fixation surgery. However, in the clinical scenarios the surgical repair of an orthopedic trauma has been reported to exacerbate the initiate trauma and increase the progression of multiple organ failure or mortality (30, 35). In addition, experimental animals with bone fracture are expected to be treated with appropriate sedatives, analgesics, anti-inflammatories, or antibiotics to relieve chronic pain, distress or infections, which would no doubt affect the trauma-induced inflammatory response. Therefore, there are limitations in using an animal model of orthopedic trauma in which the bone is broken, followed by surgical repair. Kobbe et al first demonstrated in mice that an injection of bone components followed by soft tissue injury could mimic long bone fracture and result in inflammatory responses and pulmonary injury (26), similar with clinical observations. In the current study we developed a similar protocol to mimic bilateral femur fracture in lean and obese Zucker rats by injecting bone components followed by soft tissue injury on both hindlimbs.

11–13 week old LZ and OZ (bone donors) were anesthetized using pentobarbital (50mg/kg, ip) and both femur and tibia bones were harvested under sterile condition. The bone along with marrow was crushed with a mortar and pestle and homogenized in PBS (2g/5ml). Animals (bone donors) were euthanized with overdose of pentobarbital after the collection of bone components. Another group of LZ and OZ (bone recipients, 11–13 wk) were anesthetized and subjected to a soft tissue injury on both hindlimbs by crushing the middle section of the muscle group behind femur bone with a Kelly clamp (5½", 14cm) at the first notch for 30 s, followed by the sterile injection of bone components suspension (1.5 ml/leg) into the injured muscles using a 18-gauge needle. After the treatment, the animals are able to move freely and show no sign of distress or infection.

Four animal groups were used in the current study: LZ, OZ, LZ with orthopedic trauma (LZT), and OZ with orthopedic trauma (OZT). Clinical evidence indicates peak levels of inflammation (31) as well as increased risk of multiple organ failure (22) within three days following trauma. In addition, there is evidence that elevated plasma cytokines and liver injury are found within 6 hours following bilateral femur bone fracture in mice (27). Therefore, in the current study all of the experiments were performed on the first day following orthopedic trauma.

Measurement of plasma interleukin-6, PGE₂ and pulmonary edema

One day after orthopedic trauma, blood samples were collected from animals (LZ, OZ, LZT, OZT, and OZT treated with indomethacin) following decapitation. Plasma interleukin-6 (IL-6) and PGE₂ levels were measured with ELISA system (R&D Systems).

Measurement of pulmonary edema

Five groups of animals were used for the measurement of pulmonary edema: LZ, OZ, LZT, OZT, and OZT treated with the COX inhibitor indomethacin (ip. 5mg/kg, per 6–8 hours) immediately following the orthopedic trauma. Lungs were removed from the rats following decapitation and stored at 60°C for ~a week until a stable weight was achieved. The lung wet weight-to-dry weight ratios were calculated as an index of edema, which was calculated by using formula: (Wet weight / dry weight) × 100%. The interpretation of wet/dry weight of lung can be complicated by the inclusion of blood in the wet lung weight. Therefore, in the current study the lungs were collected from decapitated rats with a minimal amount of residual intravascular blood. In another set of experiment, LZ, OZ, LZT, and OZT were anesthetized with pentobarbital. The lungs were lavaged via trachea tube with 4 ml of cold saline and 3 ml of bronchoalveolar lavage fluid (BALF) was withdrawn and centrifuged at a

low speed ($1,000 \times g$, 15 min). The total protein concentration in the supernate was measured (Bio-Rad).

Isolated lung Preparation

On the first day following orthopedic trauma, LZ, OZ, LZT, and OZT were anesthetized with pentobarbital (65mg/kg, i.p.) and lungs and hearts were isolated by using established procedures (40). Lungs were ventilated via trachea tube by using a Harvard positive-pressure rodent ventilator (model #680) at a frequency of 55 breaths/min and a tidal volume of 2.5 ml with a warmed and humidified room air. Peak inspiratory and positive end-expiratory pressures were set at 9 cmH₂O and 3 cmH₂O, respectively. After a median sternotomy, heparin (100 U in 0.1ml) was injected into the right ventricle. The pulmonary artery was perfused at 0.8 ml/min by a microprocessor pump drive (Minipuls 2) through a water-jacketed Radnoti bubble trap maintained at 38°C before entering the pulmonary circulation. The perfusate PSS contained (in mM) 129.8 NaCl, 5.4 KCl, 0.83 MgSO₄, 19 NaHCO₃, 1.8 CaCl₂, and 5.5 glucose with 4% bovine serum albumin (Sigma). Then the left ventricle was cannulated and the heart and lungs were removed and suspended on a force-displacement transducer (Grass FT03) above a humidified chamber maintained at 38°C. Perfusion rate was gradually increased to 30 ml/min/kg body weight for LZ. The perfusion rate for OZ was determined by the “ideal body weight” which was calculated: (OZ lung weight / LZ mean lung weight) $\times$ LZ mean bodyweight. After the lungs were washed with ~30ml of perfusate, the perfusate reservoir was elevated until venous pressure (Pv) was 3.5 mmHg (*zone 3* condition). Pulmonary arterial pressure (Pa) and venous pressure (Pv) was measured with WinDaq system (DataQ Instrument).

Vascular permeability in isolated lung

To determine pulmonary vascular permeability, K_{fc} (capillary filtration coefficient) and fluid flux was assessed using methodology similar to that previously described (40). After the 30-min equilibration period, baseline Pc was determined by double occlusion on arterial and venous sides. Pv was rapidly increased by 5 mmHg for a period of 15 min. The increase in lung weight in the first five minutes was due to vascular recruitment and distension, whereas the increase in weight in the following 10 minutes represented transcapillary filtration (1). At the end of the 15-min period, Pc was measured again. After the experiments the lungs were stored at 60°C for ~a week until a stable weight is achieved. K_{fc} was calculated by using following formula: $K_{fc} = \Delta W / \Delta P_c / \text{Dry weight}$. ΔW is the delta change of lung weight from the 5- to 15-min time points, and ΔP_c is the delta change of Pc from the basal to the 15th minute after elevation of Pv. We assumed that changes in interstitial oncotic and hydrostatic pressures were minimal during the measurement period.

In another set of experiments, lungs were isolated from LZ and OZ to determine the effect of PGE₂ on pulmonary permeability. After the baseline Pc was determined, PGE₂ was added into the perfusate 5 minutes before the increase in Pv, yielding a final concentration of 35 ng/ml (10^{-7} μ M). After a 15-min period, Pc was measured and the K_{fc} was calculated as previously described. The vascular resistance (R) was calculated by (Pa - Pv)/flow, where Pa is the arterial pressure.

Pulmonary gas exchange ability

To determine if the pulmonary gas exchange ability is altered due to a cyclooxygenase-dependent elevation of PGE₂, five groups of animals were used for these experiments: LZ, OZ, LZT, OZT, and OZT treated with the COX inhibitor indomethacin (ip. 5mg/kg, per 6–8 hours) immediately following the orthopedic trauma. This dose has been shown effective in a previous study in rats (4). One day after orthopedic trauma, the animals were anesthetized and the right spinotrapezius muscle was prepared for the experimental observation as

previously described (20, 52). The trachea was intubated with the animals spontaneously breathing a gas mixture containing 30% oxygen and 70% nitrogen. The spinotrapezius muscle was continuously superfused with a PSS aerated with a 5% CO₂, 95% N₂ gas mixture (pH=7.4, 35 °C) to ensure the oxygen was mainly supplied by the blood. Superfusate flow was maintained at 4–6 ml/min to minimize equilibration with atmospheric O₂. Animals were allowed to stabilize under this condition for 15–30 minutes after completion of the surgical procedure and a segment of arcade arteriole (3rd – 4th order) was chosen for the following study.

To determine the pulmonary gas exchange ability, we changed the inspired gas so that the rats breathed 100% O₂ for ~ 5 minutes and the vascular diameters were measured. Since the superfusion solution was equilibrated with gases containing 0% O₂, any vascular responses are associated with an increase in blood PO₂. Therefore, any vasoconstrictor response to high [O₂] inhalation could be considered as an indirect index of the pulmonary gas exchange ability.

We also performed control experiments to determine the inherent vasoconstrictor capability in response to 21% oxygen-superfused PSS. The LZT, OZT, and OZT treated with indomethacin spontaneously breathed the gas mixture containing 30% oxygen balance 70% nitrogen and the superfusion PSS on spinotrapezius muscle was equilibrated with a 21% O₂, 5% CO₂ for ~ 5 minutes until a peak vasoconstrictor response was achieved. At the end of experiment, 100 μM of adenosine (ADO) plus 100 μM of sodium nitroprusside (SNP) was used to achieve the maximal diameter.

Data analysis and statistical methods

The data was compared between groups using two-way ANOVA and where significant main effects occurred, individual groups were compared using the Holm-Sidak method. All the data are presented as means ± SE. A probability of $P < 0.05$ was accepted as statistically significant for all comparisons.

Results

1. Plasma IL-6 and PGE₂ levels

Figure 1a and 1b represent the plasma IL-6 and PGE₂ levels in each animal group, respectively. OZ exhibited higher basal levels of circulating IL-6 as compared with the LZ. Orthopedic trauma further increased the IL-6 levels in OZ with no effect in the LZ. The PGE₂ levels were similar between LZ, OZ, and LZT but were significantly higher in the OZT. Treatment with indomethacin decreased the PGE₂ levels in OZT to the levels similar with the other groups.

2. Bronchoalveolar protein concentration and pulmonary edema

The bronchoalveolar protein concentrations and the ratio of wet/dry lung weight in LZ, OZ, LZT, and OZT are represented in Figure 2a and 2b, respectively. The bronchoalveolar protein concentrations were similar between LZ, OZ, and LZT but were significantly increased in the OZT (Figure 2a). The wet/dry lung weight ratio was not different between LZ and OZ. Orthopedic trauma resulted in a significant increase in the wet/ dry weight ratio in OZT with no effect in LZT. In addition, the elevated wet/dry weight ratio in the OZT was normalized following the treatment with indomethacin (Figure 2b).

3. Vascular permeability in isolated lung

The pulmonary vascular permeability and vascular resistance in isolated lungs are presented in Figure 3a and 3b, respectively. Figure 3a shows that the basal vascular permeability is

significantly higher in the OZ as compared with LZ. Orthopedic trauma did not alter the permeability in isolated lungs from either LZT or OZT. Treatment with PGE₂ increased the vascular permeability (Figure 3a) without altering the resistance in both LZ and OZ (Figure 3b). The delta symbols in Figure 3a represent the difference between the mean pulmonary permeability before and after treatment with PGE₂ in LZ or OZ. The addition of PGE₂ to the perfusate resulted in a larger increase in the absolute value of pulmonary permeability in OZ as compared with LZ. Note that we used a higher concentration of PGE₂ (35 ng/ml) (19) than the plasma concentration observed in OZT and this the PGE₂ treatment lasted for only 15 minutes in an attempt to determine the acute response to elevated PGE₂.

4. Pulmonary gas exchange ability

The spinotrapezius arteriolar basal and maximal diameters (ADO plus SNP) for each group are shown in Table 1. The maximal diameters were not different among all the groups. OZT exhibited an increased basal arteriolar diameter as compared with the LZ, OZ, and LZT. However, the basal diameters of indomethacin-treated OZT were not different from the LZ, OZ, and LZT.

The vasoconstrictor response to inhalation of 100% O₂ in LZ and OZ with or without orthopedic trauma is shown in Figure 4a. Since the superfusion solution over the spinotrapezius muscle was equilibrated with gases containing 0% O₂ to ensure O₂ supply was only from the blood, the vasoconstrictor response to high O₂ inhalation could be considered due to the resultant increased arteriolar PaO₂ and used as an indirect index of the pulmonary gas exchange ability. The vasoconstrictor responses were similar between LZ, OZ, and LZT. However, the OZT exhibited a blunted hyperoxia-induced vasoconstriction, which was restored after treatment with indomethacin. The vasoconstrictor response to 21% O₂-equilibrated PSS in the spinotrapezius muscles could be considered as an index of inherent vascular sensitivity and activity to a direct exposure to hyperoxia. Figure 4b shows that increasing PO₂ in the superfusion PSS resulted in similar vasoconstrictor responses among all groups.

Discussion

The major findings of the current study are that: 1) following orthopedic trauma, the OZT but not LZT exhibited increased circulating IL-6 and PGE₂ levels; 2) OZT exhibited an increased bronchoalveolar protein concentration, pulmonary edema, and impaired gas exchange; 3) in isolated lungs OZ exhibited a higher basal level of pulmonary permeability than lean rats; 4) treatment with PGE₂ increased the pulmonary permeability in isolated lungs from both LZ and OZ, with a more significant response in the OZ; and 5) treatment with indomethacin in OZT prevented the increase in circulating PGE₂ and improved pulmonary edema and gas exchange as evidenced by the vasoconstrictor response to inhaled hyperoxia.

In the current study we did not induce bone fractures in the animals since doing so would require a post-injury fixation surgery according to the guidelines set forth in the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals". Also, the initiation of the inflammatory responses induced by orthopedic trauma may result in the increased sensitivity to additional surgery (5, 30). Indeed, post-injury surgery has been suggested as a 'second hit' phenomenon (5, 35) leading to exaggerated systemic inflammatory response, acute respiratory distress syndrome (ARDS) and multiple organ failure (35, 50). Therefore, in the current study we performed an injection of bone components along with soft tissue injury on both hindlimbs in the LZ and OZ to mimic bilateral femur fractures. This protocol was first used by Kobbe et al. in mice to study the relationship between inflammation and pulmonary injury (26), similar with the post-trauma

outcomes in clinical observations. In addition, Kobbe et al. also showed that the soft tissue injury with no bone components injection neither increased the cytokines levels nor induced pulmonary injury, similar with other studies demonstrating that soft tissue injury alone does not cause systemic inflammation (44) or remote organ failure (28).

The metabolic disorders in obesity are associated with chronic mild inflammation (33). Besides fatty acids and other lipid moieties, adipose tissue also expresses and releases inflammation-related adipokines, which are related to obesity-associated pathologies (48). We found a significant increase in circulating interleukin-6 (IL-6) levels in OZ as compared with LZ, suggesting a basal inflammatory state in this animal model of obesity. Similar results were reported in other studies that elevated circulating cytokines IL-6 and tumor necrosis factor- α are correlated with the adipose tissue mass in the OZ (47). The initial state of inflammation in obesity may increase sensitivity and vulnerability to stimuli such as trauma or infection (5), resulting in exaggerated inflammatory responses as reported in clinical observations (3, 18). The current study also shows that the OZ exhibited higher basal circulating IL-6 levels than the LZ, with orthopedic trauma further increasing the IL-6 in OZT but not in LZT (Figure 1a), similar with the clinical reports (18). Circulating IL-6 levels have been demonstrated as the early marker for orthopedic trauma with a close correlation with systemic inflammatory response scores (17). Among all the inflammatory markers, circulating IL-6 levels display the highest correlations with the extent of trauma (46) and risk of remote organ dysfunction (15). For these reasons, we chose circulating levels of IL-6 as an indicator for the orthopedic trauma-induced inflammation in the current study.

While high levels of cytokines are associated with an increased risk of pulmonary edema, a causal relationship is not clear. It has been established that inflammation and elevated cytokines can increase the production of prostaglandin E_2 (PGE $_2$) via increasing cyclooxygenase (COX-2) and/or PGE $_2$ synthase activity (24, 25, and 42). The current study shows that the basal circulating levels of PGE $_2$ were not different between LZ and OZ despite an elevated IL-6 in the OZ (Figure 1b). These results raise the possibility that the chronic and moderate inflammation in OZ does not reach a threshold for an elevated PGE $_2$ production. However, increasing evidence shows that orthopedic trauma results in more profound inflammatory responses in obese individuals than in non-obese patients (3, 18). This increased inflammatory response may stimulate PGE $_2$ synthesis. For example, there is evidence that, as a key inflammatory mediator following orthopedic trauma, IL-6/IL-6 receptor complexes play an important role in increasing cyclooxygenase-2 function and subsequent PGE $_2$ formation (10). Therefore, the exaggerated inflammation in OZT could contribute to the increased production of PGE $_2$ with this production being inhibited following indomethacin treatment (Figure 1b). Additionally, we found that neither the IL-6 nor the PGE $_2$ levels were elevated in the LZT on the first day following the orthopedic trauma, whereas Kobbe et al. showed an elevation in the IL-6 levels in the wild type mice following the bone components injection (26). This inconsistency may be due to differences in animal models and/or experimental protocols. For example, in addition to a different dose of bone components for the injection between mice and rats, in Kobbe's study the blood was taken from unconscious animals 6 hours after the bone components injection, whereas in the current study the blood sample was taken from conscious animals 24 hours after the injection. Similar to the results in the current study Kobbe et al. reported in another study that there was only a transient increase in the circulating cytokines IL-6 and IL-1 β within 6 hours following bilateral femur fracture in wild type mice (27). These results suggest that the inflammatory responses in the lean animals tend to be localized to the injury sites and insufficient to induce a systemic production of PGE $_2$ (39). The mechanism(s) responsible for the prolonged systemic inflammation and its contribution to the elevated

PGE₂ in OZT needs to be determined in future studies. In the current study, we focused on the potential roles of elevated PGE₂ in mediating pulmonary edema in the OZT.

Pulmonary edema following prostaglandin E₂ (PGE₂) administration has been reported in both non-trauma humans (21, 45) and experimental animal models (2, 19). However, the mechanisms responsible for the PGE₂-induced pulmonary edema are not well defined. The vasodilator effect of PGE₂ has been reported to markedly potentiate the capillary leakage of plasma induced by histamine or cytokines (38). Downey et al. demonstrated that treatment with intrabronchial or intravenous PGE₂ facilitated pulmonary inflammation and bronchoalveolar leak, and these effects were mimicked by a vasodilator (nitroprusside) and attenuated by administration of a vasoconstrictor (angiotensin II) (12). These published results suggest a potential role of PGE₂ as a vasodilator in mediating pulmonary inflammation and edema. In the current study, OZT exhibited elevated circulating PGE₂ and larger basal arteriolar diameters in spinotrapezius muscle, with these abnormalities both prevented by indomethacin treatment (Table 1). These results suggest that the larger basal arteriolar diameter observed in OZT is associated with an elevated circulating COX-dependent vasodilator, such as PGE₂. A more recent study shows that PGE₂ increases pulmonary capillary permeability in isolated rat lung (19). Since the vasodilatory response is absent in isolated lungs, the PGE₂-induced increase in vascular permeability was independent of hemodynamic changes. Our results also showed that treatment with PGE₂ in the isolated lung from LZ and OZ increased the vascular permeability without affecting the vascular resistance (Figure 3). Together, these findings suggest that the elevated circulating PGE₂ facilitates pulmonary permeability via both hemodynamic-dependent and -independent mechanisms.

As a key mediator of pulmonary edema and early stage of ARDS, the increased capillary permeability in lungs leads to influx of protein-rich fluid into the alveolar space. We found increased bronchoalveolar protein concentrations and wet/ dry weight in the OZT, suggesting an orthopedic trauma-induced increase in pulmonary permeability and edema. Moreover, the vascular permeability in isolated lungs was not different between the OZ and OZT (Figure 3), suggesting an obligatory role for systemic factors such as circulating PGE₂ in mediating the elevated pulmonary permeability and edema following orthopedic trauma. Indeed, we found that the lung wet/dry weight ratio was normalized in the OZT following indomethacin treatment. However, it should be realized that, although the wet/dry weight has been used extensively as an index for pulmonary edema, the increased permeability and resultant extravasated protein may contribute to the total wet weight and affect the ratio value. For this reason, we measured the protein concentration in bronchoalveolar lavage fluid as well as the gas exchange capability, both of which could be altered by elevated pulmonary edema. In addition, we used a higher concentration of PGE₂ (35 ng/ml) to determine the permeability in isolated lung (19) than the plasma concentration in OZT, so that a significant alteration of permeability could be observed after a short period of time (15 minutes) following PGE₂ treatment. Therefore, to determine the physiological significance of elevated circulating PGE₂ in mediating the pulmonary edema in OZT, we compared the gas exchange capability between OZT with and without PGE₂ inhibition.

Increased pulmonary permeability in OZT could lead to pulmonary edema along with an intrapulmonary shunt, with both resulting in impaired gas exchange ability and low oxygen levels in the arterial blood (PaO₂). An impaired oxygen delivery and tissue hypoxia have been demonstrated in obese patients with severe orthopedic trauma (3). To determine if there is impaired pulmonary gas exchange in OZT, we examined the vasoconstrictor response in the spinotrapezius during breathing 100% O₂. It has been demonstrated in both human and animal studies that high PaO₂-induced vasoconstriction is mediated by activation of endothelin A receptor (9, 23) and/or elevation of radical oxygen species (41, 43). Since

the superfusion solution flowing over the spinotrapezius muscle was equilibrated with gas containing 0% O₂ to ensure that O₂ supply was only from blood, the vasoconstrictor response to high [O₂] inhalation was due to a resultant elevation of PaO₂ and could be considered as an indirect index of pulmonary gas exchange ability. Following this protocol, we found an impaired gas exchange in the OZT as compared with the LZT and non-trauma animals. Moreover, treatment with indomethacin in OZT not only decreased the circulating PGE₂ but also normalized the vasoconstrictor response to high O₂ inhalation. These results suggest that the impaired pulmonary gas exchange in OZT is correlated with an overproduction of PGE₂ and most likely the secondary pulmonary edema. In addition, we performed control experiments showing that the vasoconstrictor responses to a direct increase in PO₂ in the superfusion solution were not different between LZT and OZT without or with indomethacin treatment. These results suggest that the inherent vascular sensitivity and response to hyperoxia was not different between phenotypes or altered by the treatment with indomethacin. However, it should be realized that the vasoconstrictor response to 100% O₂ inhalation may not be sensitive enough to detect the minor alterations in gas exchange capability. Therefore, the normalization of vasoconstrictor response does not necessarily mean normalized pulmonary edema or gas exchange. In addition, PGE₂ may not be the only factor to contribute pulmonary edema in OZT. For example, treatment with indomethacin could also decrease thromboxane production, which has been demonstrated to induce pulmonary edema in newborn lamb lungs (54). The current study was designed to determine the potential role of PGE₂ in mediating the pulmonary edema in OZT. Indeed, the isolated lung experiments provide additional support that increases in PGE₂ will increase pulmonary permeability and edema.

Another novel finding of the current study is the higher basal pulmonary permeability in the OZ as compared with the LZ. However, the mechanism(s) responsible for the difference is unknown. To our knowledge, only one study has provided indirect evidence for an elevated alveolar capillary leak in obese patients based on increased capillary blood volume but decreased membrane gas diffusion in lungs (31). Additionally, Frisbee demonstrated that the total endothelial surface area was decreased in OZ as compared with LZ (16), raising a possibility that the increased capillary filtration coefficient in OZ is due an increased hydraulic conductance or impaired alveolar-capillary barrier. Additionally, the elevated basal pulmonary permeability in OZ does not lead to edema in the OZ, suggesting that the increased basal pulmonary permeability in OZ does not exceed a “threshold” for *in vivo* edema. The present experiments do not directly address this issue but the increased capillary permeability in the lung can be compensated by a corresponding increase in reabsorption and clearance (6). There are two stages of pulmonary edema: interstitial edema and alveolar edema, whereas the alveolar edema is the consequence of a severe interstitial edema (interstitial space expanded by ~50%). Although the basal pulmonary permeability is shown increased in the OZ in the current study, a recent study demonstrates an increased basal alveolar edema clearance in the OZ (29). Moreover, several “safety factors” can prevent the formation of pulmonary interstitial edema in the OZ despite an increased basal vascular permeability. For example, when there is a net filtration across capillary beds, the net filtration pressure will be reduced due to an increased tissue hydrostatic pressure and decreased osmotic pressure. In addition, there will be a 5 – 10 fold increase in lymph flow, returning excess fluid to the circulation. These mechanisms will work to maintain a balance between filtration and absorption. However, this balance could be lost if there is a further increase in capillary permeability, such as in response to administration of PGE₂ or following orthopedic trauma. In addition, we found that treatment with PGE₂ in isolated lungs resulted in a larger increase in the absolute value of pulmonary permeability in OZ as compared with LZ, suggesting that the increased basal level of pulmonary permeability in OZ could actually facilitate the PGE₂-induced edema after orthopedic trauma.

In summary, the current study shows that orthopedic trauma leads to increases in circulating levels of IL-6 and PGE₂ in OZ. The data suggests that the increase in PGE₂ contributes to the development of protein-rich edema and impaired gas exchange. This study provides valuable information concerning pulmonary dysfunction and possible treatment options following orthopedic trauma in obese subjects. Future studies are needed to determine the mechanisms for exaggerated inflammatory responses and resultant elevation of PGE₂ in the obese patients with orthopedic trauma.

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1a

1b

Figure 1.

1a and 1b represents the plasma IL-6 and PGE₂ levels in each animal group, respectively. OZ exhibited higher basal levels of circulating IL-6 as compared with the LZ. Orthopedic trauma further increased the IL-6 levels in OZ with no effect in the LZ (* $P < 0.05$ vs. lean; + $P < 0.05$ vs. control within obese; $n = 5$ for each group). OZT exhibited a significant higher PGE₂ levels as compared with the other groups (+ $P < 0.01$ OZT vs. the other groups; $n = 6$ for LZ, $n = 4$ for OZ, $n = 5$ for LZT, OZT and OZT + indomethacin).

Figure 2.

2a and 2b represents the bronchoalveolar protein concentrations and the ratio of wet and dry lung weight, respectively. The bronchoalveolar protein concentrations (*P < 0.05 vs. OZ; n = 4 for each groups) or the wet and dry lung weight ratio (*P < 0.05 vs. OZ; n = 10 for each group) were similar between LZ, OZ, and LZT but were significantly increased in the OZT.

Figure 3.

a shows that the basal capillary permeability is significantly higher in the OZ as compared with LZ, with orthopedic trauma altering the permeability in neither LZT nor OZT. Treatment with PGE₂ increased the vascular permeability (**Figure 3a**) without altering the resistance in both LZ and OZ (**Figure 3b**) (* $P < 0.05$ PGE₂ vs. control or trauma; + $P < 0.01$ lean vs. obese; $n = 5$ for the all lean and obese within control and lean within trauma; $n = 4$ for lean and obese within PGE₂ and obese with trauma).

4a

4b

Figure 4.

a shows that breathing 100% O_2 resulted a blunted vasoconstriction in OZT, with treatment with indomethacin normalizing the vasoconstrictor response (* $P < 0.01$ vs. all the other groups; $n = 8$ for LZ; $n = 5$ for the other groups). **Figure 4b** shows that increasing $[O_2]$ in the PSS resulted in similar vasoconstrictor responses between groups ($n = 5$ for each group).

Table 1

The effects of orthopedic trauma and indomethacin on the arteriolar tone

Diameter (µm)	LZ	OZ	LZT	OZT	OZT + indomethacin
Basal	15 ± 1	15 ± 1	17 ± 2	22 ± 2 *	18 ± 1
Maximal	35 ± 3	34 ± 1	44 ± 4	37 ± 2	37 ± 4

Table 1 represents the effects of orthopedic trauma and indomethacin on the 3rd–4th order arcade arteriolar tone in the spinotrapezius muscle. The maximal diameters induced by SNP and ADO were not different among all the groups. OZT exhibited a larger basal arteriolar diameter as compared with the LZ, OZ, and LZT. However, there is no difference in the basal arteriolar diameters among LZ, OZ, LZT and indomethacin-treated OZT (* P < 0.05 vs. LZ, OZ, and LZT; n = 8 for LZ and n = 5 for the other groups).