# Effects of Low Tidal Volume Ventilation in a Murine Model of Ventilator-induced Diaphragmatic Dysfunction

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# Introduction

- ✓ **Mechanical ventilation (MV)** is a life-saving procedure in many critically ill patients.

- ✓ Short period of MV has been associated with ventilator-induced lung injury even in healthy lungs.

***Wolthuis EK, et al., ANESTHESIOLOGY 2008***

## ✓ Controlled MV

- Maintains the diaphragm **at rest**
- Has been implicated in the development of **diaphragmatic dysfunction**
- Not only in animal models but also in humans.


Capdevila X, et al., Intensive Care Med 2003, Gayan-Ramirez G, et al., Intensive Care Med 2003
Jaber S, et al., Intensive Care Med 2005, Le Bourdelles G, et al., Am J Respir Crit Care Med 1994
Sassoon CS, et al., J Appl Physiol 2002, Yang L, et al., Am J Respir Crit Care Med 2002
Ramirez G: Crit Care 2010, Hussain SN, et al., Am J Respir Crit Care Med 2010
Jaber S, et al., Am J Respir Crit Care Med 2011, Levine S, et al., Am J Respir Crit Care Med 2011
Levine S, et al., N Engl J Med 2008

# Ventilator-Induced Diaphragmatic Dysfunction (VIDD)

- Play an important role in the **difficulties in weaning critically ill patients from the ventilator.**

Schultz MJ. ANESTHESIOLOGY 2010

- **Multiple mechanisms** appear to be involved in VIDD
  - increase of reactive oxygen species
  - mitochondrial dysfunction
  - inhibition of the insulin-like growth factor pathway
  - activation of different proteolytic systems, such as the calpains and caspase 3
    - Calpains and caspase 3 are ubiquitous nonlysosomal proteases, and their expression may be up-regulated early in the time course of VIDD.

Goll DE, et al. Physiol Rev 2003

Powers SK, et al. J Appl Physiol 2007

- ✓ All of these mechanisms culminate in
  - ➢ muscle atrophy
  - ➢ injury
  - ➢ loss of diaphragmatic force-generating capacity

Jaber S, et al. Am J Respir Crit Care Med 2011

Jaber S, et al. Crit Care 2011

Petrof BJ, et al. Curr Opin Crit Care 2010

Tobin MJ, et al. Ann Intern Med 2010

# VIDD according to Tidal Volume

- ✓ **Low tidal volume ventilation (LTVV)**

- ➢ Important concept of **lung protective strategy** from ventilator associated lung injury

- ➢ However, there was **no evidence** that LTVV has protective effect **on diaphragm**, especially on ventilator induced diaphragmatic dysfunction.

# Study Objectives

- ✓ **To evaluate the effects of LTVV** in murine VIDD model by exploring both histologic and the main protease pathway after MV

# MATERIALS AND METHODS

# Materials and methods

Healthy male C57/BL6 mice (10-12 weeks, 25-30g)

1) Higher tidal volume MV for 6 h (**HTV group**, n=6)
   - TV: 10 ul/mg BW in HTV
2) Lower tidal volume MV for 6 h (**LTV group**, n=6)
   - TV: 6 ul/mg BW in LTV
3) Controls (Control group, n=6).

- ✓ Anesthesia:
  - IP inj of pentobarbital sodium (50mg/kg body weight)
- ✓ Tracheostomy:
  - 22-gauge angiocatheter was inserted
- ✓ Hemodynamic stability
  - 0.05ml RL solution IP every hour
- ✓ Other general care:
  - bladder expression
  - ocular lubrication
  - passive limb movement

# Experimental protocol for MV

- ✓ **Small animal ventilator**

  : Flexivent®, SCIREQ Inc, Canada

- ✓ **Ventilator setting:**

  **CMV mode**

  FiO2: 0.21

  RR: 150 rpm

  PEEP: 3-4 cm H2O

  Tidal volume

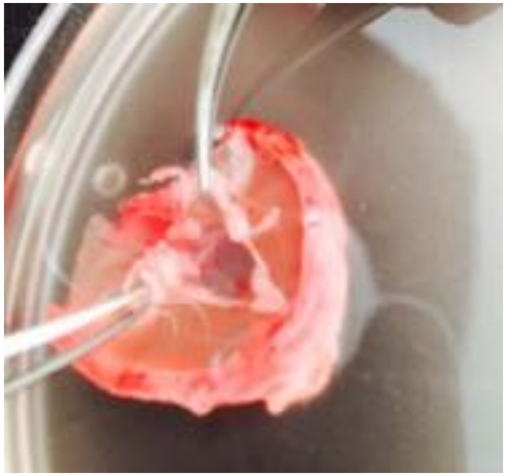
  - **10ul/mg BW in HTV**
  - **6ul/mg BW in LTV**

## 1. Lung Function Measurement

- In 3 groups
- **Forced oscillation technique in FlexiVent system**
  - Airway resistence [Rn]
  - Tissue damping (resistance)[G]
  - Tissue elasticity [H]

## 2. Blood collection & Tissue Collection (Diaphragm)

- By thoracotomy and laparotomy



# 3. Measurement of Diaphragm contractile Properties

- ✓ **The force-frequency relationship**

  **:Enhanced contractility at higher rates of stimulation**

  - at ***10, 20, 30, 50, 60, 80, 100, 120 Hz***
  - for ***600 ms***
  - with 1 min between the stimulation trains.

- ✓ **The fatigablity assessment**
  - measured by loss of force to repeated stimuli
  - ***over 10 min***
  - ***with 30 Hz***
  - ***300 ms*** duration

## 4. Measurement of inflammation and atrophy

- ✓ Stained by **Hematoxylin and Eosin**
- ✓ The complete area was evaluated by a blinded investigator for **invasion of neutrophils and lymphocyte**

## 5. Measurement of the sarcolemmal injury

- ✓ Exposed to a **fluorescent tracer dye**
  - **Procion orange**; Sigma, St. Louis, MO

# 6. Biochemical Evaluation

- ✓ Total levels of **calpains 1 and 2**
- ✓ **Immonoblot analysis** was performed.
  - **Calpain 1** : ab 49652 1:1,000 dilution in 3% BSA/TBST, 80KDa; Abcam, Cambridge, MA
  - **Calpain 2** : ab39165 1:1000 dilution with TBST containing 5% skim-milk, 76 KDa; Abcam, Cambridge, MA

# Statistical Analysis

- Data are presented as mean ± standard deviation.
- Kruskal-Wallis test
  - additional analysis between 2 groups was performed using Mann-Whitney test.
- Repeated measure of ANOVA.
  - Differences in muscle contractility force-frequency response and muscle contractility for fatigability between groups
- Significance was established at $P<0.05$.
- Statistical analysis was performed using SPSS 21.0.

# RESULTS

# Animal characteristics

**Table 1.** Animal characteristics of all 3 groups and mean peak inspiratory pressure (PIP), positive end expiratory pressure (PEEP), and tidal volume (TV) in ventilated groups (HTV and LTV).

| | HTV (n=6) | LTV (n=6) | Control (n=6) | P value |
|---|---|---|---|---|
| Body weight (g) | 22.0 ± 0.9 | 21.8 ± 1.1 | 23.6 ± 2.1 | 0.273 |
| Ventilator | | 3.2 | | |
| PIP | 11.0 ± 0.9 | 8.9 ± 0.6 | - | 0.006 |
| PEEP | 2.5 ± 1.1 | 2.9 ± 0.03 | - | 0.873 |
| TV | 0.2 ±0.007 | 0.12 ± 0.003 | - | 0.005 |

Data are represented as mean ± SE.

HTV = high tidal volume; LTV = low tidal volume; PIP = peak inspiratory pressure; PEEP – positive end expiratory pressure; TV = tidal volume.

# Arterial blood gas analyses

| | PH | $PaO_2$ (mmHg) | $PaCO_2$ (mmHg) | $HCO_3^-$ (mM) |
|---|---|---|---|---|
| HTV (n=6) | 7.4 ± 0.1 | 65.2 ± 19.7 | 21.6 ± 4.4 | 20.0 ± 5.0 |
| LTV (n=6) | 7.4 ± 0.1 | 84.5 ± 11.6 | 21.0 ± 2.3 | 22.6 ± 0.9 |
| Control (n=6) | 7.4 ± 0.2 | 92.4 ± 25.3 | 19.7 ± 5.9 | 26.1 ± 3.2 |

Data are represented as mean ± SE.

HTV = high tidal volume; LTV = low tidal volume

No significant differences were observed between groups.

**NS**

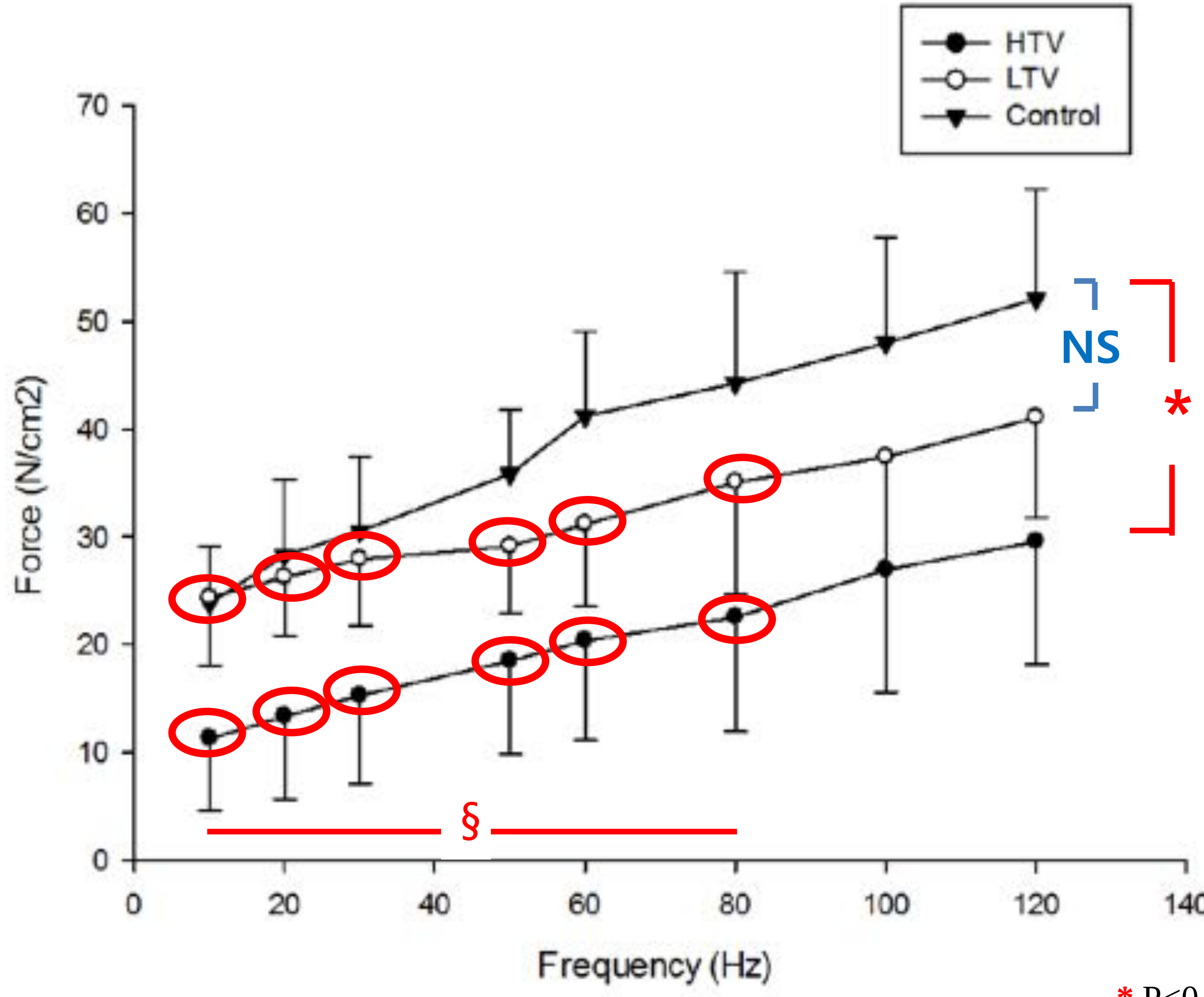
# Invasive Lung Function Measurement by Forced oscillation technique (FlexiVent)

| | Airway resistance (RN), cmH2O.s/ml | Tissue Damping (G), cm H2O/ml | Tissue Elasticity (H), cm H2O/ml | P value |
|---|---|---|---|---|
| HTV (n=6) | 0.37±0.09 | 4.48 ± 1.15 | 33.1 ± 5.79 | 0.343 |
| LTV (n=6) | 0.38 ± 0.05 | 5.10± 0.71 | 39.2 ± 4.95 | 0.158 |
| Control (n=6) | 0.32 ± 0.07 | 4.25 ± 0.77 | 31.5 ± 7.16 | 0.087 |

**NS**

# Force-Frequency Relationship

## :Enhanced contractility at higher  rates of stimulation

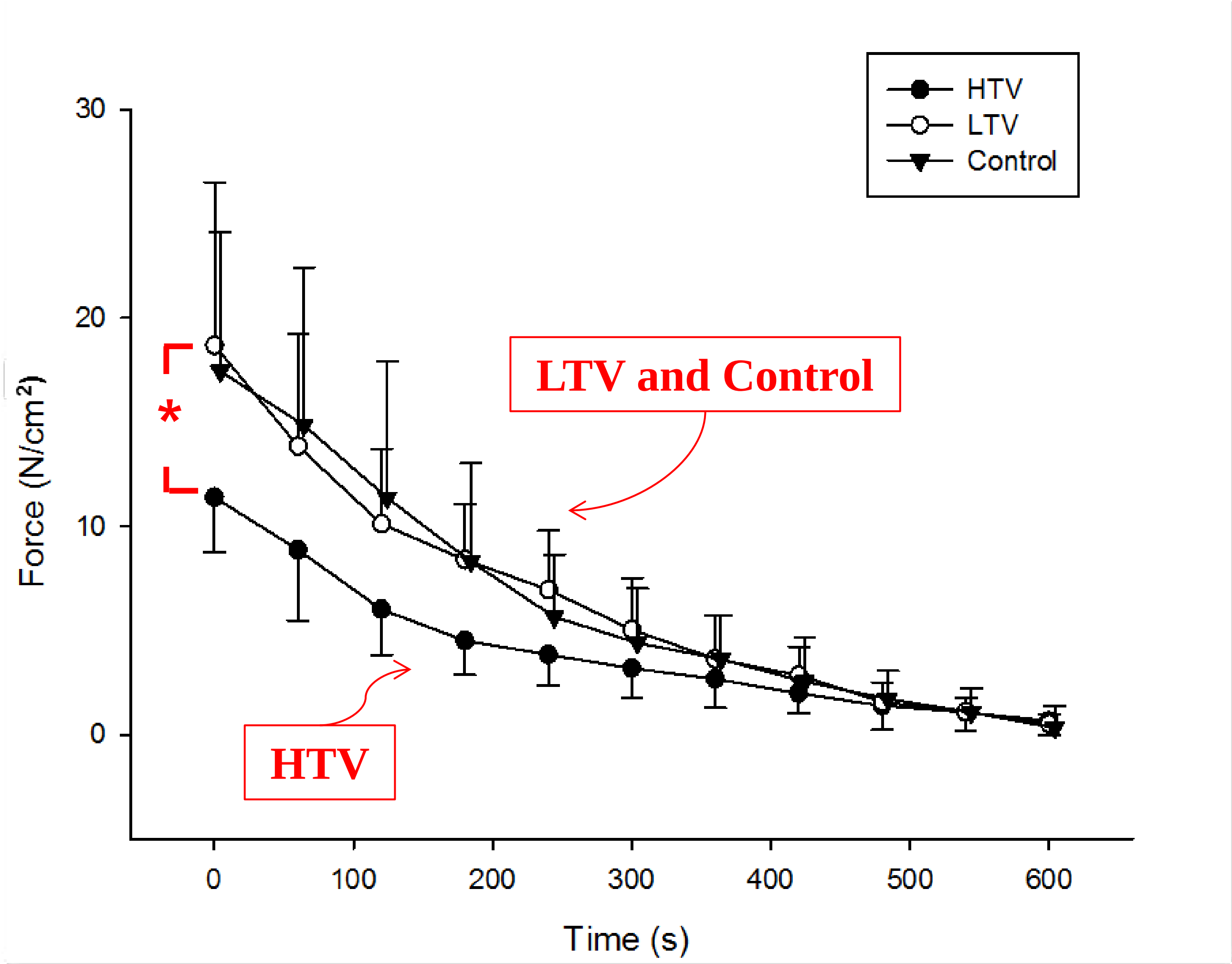



* P<0.05, HTV vs control

§ P<0.05, HTV vs LTV

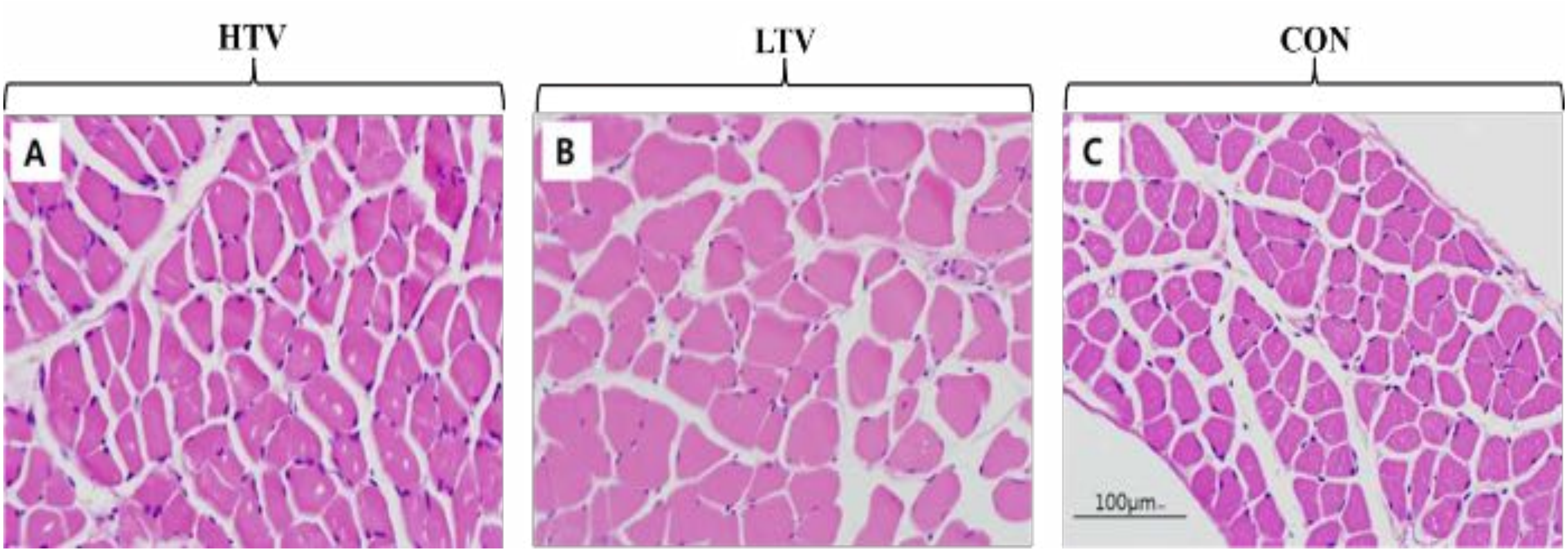
# Fatigability Assessment :

## Loss of absolute force production of the diaphragm during the fatigue test



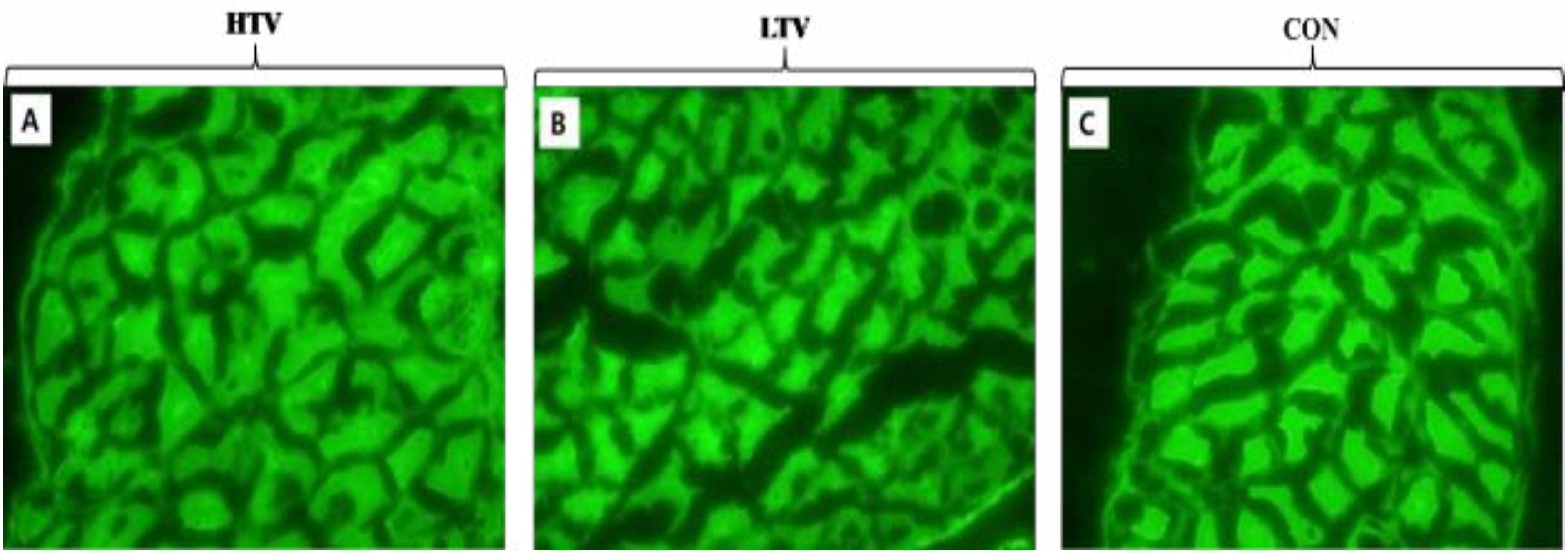

*P<0.05, HTV vs LTV and control groups.

# Histologic findings for inflammation in diaphragm



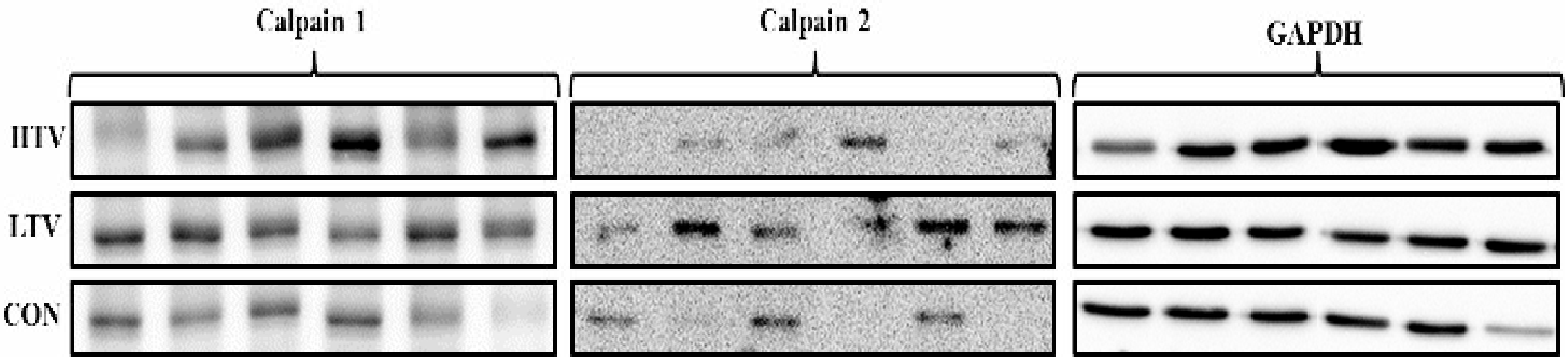

*No differences between groups*

# Quantification of the sarcolemmal injury in diaphragm




*No differences between groups*

# Expression of calpain isoforms in diaphragm




*No differences between groups*

# Conclusion

- ✓**Low tidal volume ventilation**
  - ✓**partially attenuates the development of VIDD** in murine model

- ✓Reducing ventilatory **support to only that level necessary for respiratory system recovery** while avoiding harm is just as true for VIDD as it is for VILI.