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**Effect of hypercapnia on respiratory and peripheral skeletal muscle loss during critical illness – A pilot study**

Paul Twose MSc BSc MCSP

<sup>1</sup>Clinical Specialist Physiotherapist – Critical Care

Physiotherapy Department, University Hospital of Wales, Heath Park, Cardiff, CF14 4XW, UK

<sup>2</sup>Honorary Lecturer

School of Healthcare Sciences, Cardiff University, Cardiff, CF14 4XN, UK

[Paul.Twose@wales.nhs.uk](mailto:Paul.Twose@wales.nhs.uk)

Dr. Una Jones PhD MSc BSc MCSP

Senior Lecturer

School of Healthcare Sciences, Cardiff University, Cardiff, CF14 4XN, UK

[JonesUF@cardiff.ac.uk](mailto:JonesUF@cardiff.ac.uk)

Dr. Matt Wise BA MA MB BChir MRCP DPhil

Consultant Intensivist

Critical Care, University Hospital of Wales, Heath Park, Cardiff, CF14 4XW, UK

[mattwise@doctors.org.uk](mailto:mattwise@doctors.org.uk)

## **INTRODUCTION:**

It is well recognised that critical illness contributes to development of muscle weakness (ICU-AW) [1] with evidence that atrophy is caused by increased breakdown and decreased synthesis of muscle protein [2-5]. This may be secondary to inflammation, immobilisation, changes in endocrine stress responses, nutritional deficit and denervation, yet none of the above show direct correlation with ICU-AW [5].

Skeletal muscle loss occurs early and rapidly during the first week of critical illness and is more severe among those with multi-organ failure compared with single organ failure [6]. For critically ill patients with Acute Respiratory Distress Syndrome (ARDS) muscle weakness is the single greatest determinant of functional outcome [6]; the weakness impeding rehabilitation and recovery due to prolonged inactivity, a catabolic state and reduced nutritional uptake [7]. Similarly, impaired diaphragm contractility has profound long-term sequelae resulting in prolonged periods of mechanical ventilation, delayed weaning and potential ongoing respiratory compromise [8].

Low tidal volume (LTV) ventilation is associated with a reduced risk for developing ventilator induced lung injury with or without ARDS [9]. Meta-analyses have demonstrated fewer pulmonary infections, less atelectasis and shorter length of stay with targeted lung volumes [10]. As such, LTV ventilation or 'lung protective ventilation' has become common place in critical care [11]. However, an accepted side-effect of LTV is 'permissive' hypercapnia.

Little is known about the influence of hypercapnia on muscle wasting in humans but animal models have shown that severe acute acidosis can protect the contractility of the diaphragm [12]. A recent study using a healthy animal model demonstrated that moderate prolonged

hypercapnia (defined as PaCO<sub>2</sub> between 7.3kPa and 9.3kPa for 72 hours) protected against ventilator induced diaphragmatic dysfunction (VIDD) [12], this is possibly as a consequence of an anti-inflammatory effect mediated by preventing oxidative stress and NF-kappa B activation [13, 14, 15]. It remains unknown if this effect occurs in humans, and whether hypercapnia could prevent both diaphragmatic and peripheral skeletal muscle wasting in critically ill patients.

The aim of this pilot study was therefore to investigate the effect of hypercapnia on changes in respiratory and peripheral skeletal muscle in critically ill patients receiving mechanical ventilation.

## **METHODS**

### **Study Design**

A prospective observational pilot study to measure change in respiratory muscle (diaphragm) thickness and peripheral skeletal muscle (quadriceps rectus femoris cross-sectional area (RF<sub>CSA</sub>)) in three non-matched groups of invasively mechanically ventilated patients with differing levels of arterial carbon dioxide.

### **Ethical approval**

Ethical approval was obtained from Wales Research Ethics Committee – REC3 (14/WA/0054). Written informed consent was provided directly from patients or their personal consultee (with retrospective patient consent obtained once capacity restored).

## **Participants**

Adult ( $\geq 18$  years of age) patients admitted to a 32-bedded tertiary referral critical care unit and receiving a mandatory mode of invasive mechanical ventilation, were eligible for the study.

Two groups of patients were initially identified with a further retrospective grouping of patients during analysis. Group 1 consisted of patients admitted with acute brain injury (ABI). This group was included as patients with brain injury normally ventilated to a  $\text{PaCO}_2$  4.5 - 5.0kPa and on similar medication to those admitted with severe respiratory failure (e.g. sedation, use of paralyzing agents). The remaining included patients had no diagnosis of head injury. At the end of the study this group of non head injury patients were retrospectively categorised into 'Normocapnic' [Group 2] ( $\text{PaCO}_2$  4.6 – 5.9kPa) or 'Hypercapnic' [Group 3] ( $\geq 6$ kpa) groups according to average  $\text{PaCO}_2$  during the study period (calculated from daily blood gases).

Exclusion criteria included expected deterioration leading to death within 24 hours, pre-existing muscle or neuromuscular disease, or chronic respiratory disease.

## **Sample Size**

No previous studies provided sufficient data for a sample size calculation to be carried out. A convenience sample of 30 was used to ensure 10 participants in each group. This was deemed feasible within the funding period of the study.

## **Protocol**

Ultrasonographic measurements of  $\text{RF}_{\text{CSA}}$  and diaphragm thickness were completed using a Sonosite X-Porte<sup>®</sup> ultrasound device (Fujifilm<sup>™</sup>) based on previously described techniques [5,

7, 16]. Subjects were positioned semi-supine with the legs rested in passive extension. Use of excess ultrasound gel facilitated image quality, and the visual feedback was used to obtain the smallest cross-sectional area / diaphragm thickness in each image. Minimal compression was applied to avoid compression of the underlying muscle.

Measurements of  $RF_{CSA}$  and diaphragm thickness commenced within the first 24 hours of initiation of mechanical ventilation (classified as 'baseline'), and were repeated on Days 3, 5, 7, and 10 or until discontinuation of mechanical ventilation (whichever occurred first).

For the diaphragm, a 13-6MHz rectangular transducer (L25xp, Sonosite S Series Ultrasound, Hitchin, UK) was placed in the right mid-auxiliary line between the 8<sup>th</sup> and 9<sup>th</sup> rib. Confirmation of imaging the diaphragm was made through direct visualisation of a contraction or passive stretching. All images were obtained at end-expiration.

$RF_{CSA}$  was determined using a 5-2MHz curvilinear transducer (C60xp, Sonosite S Series Ultrasound, Hitchin, UK). Distance from the anterior superior iliac spine (ASIS) to the superior patellar border was measured and a point marked on the thigh, at two-thirds of this distance. The transducer was placed perpendicular to the long axis of the thigh on the superior aspect.

Ultrasound images were analysed using Image J software (Image J, U.S. National Institutes of Health, Maryland USA, <http://rsb.info.nih.gov/ij/>). For both the diaphragm and  $RF_{CSA}$ , three images were obtained at each time point. An average of three measurements (all within a 10% variance) was calculated from each image and then an overall average was then calculated for the three images captured at each time point to provide a single figure for diaphragm thickness and  $RF_{CSA}$ .

## **Statistical analysis**

Demographic data was analysed using descriptive statistics. Inter-group baseline differences were analysed using either one-way anova with poc hoc bonferonni tests or Kruskall-Wallis for non-parametric data.

Changes in diaphragm thickness and RFCSA were calculated by percentage change from baseline (within 24 hours of initiating mechanical ventilation) to each subsequent assessment point (Days 3, 5, 7 and 10). To assess the degree of change in muscle thickness / cross sectional area, all data were assessed for normality using histograms. Descriptive analysis included mean and standard deviations, followed by multilevel linear regression analysis, accounting for the correlated nature of repeated measurements within individuals. Pearson's correlation was used to assess correlation between relative change in RF<sub>CSA</sub> and diaphragm thickness at each time point during mechanical ventilation.

Statistical analysis was completed using SPSS<sup>®</sup> Version 20 (SPSS, Inc. Chicago, Ill) and Stata Version 13.0. A p-value of  $\leq 0.05$  was considered statistically significant.

## **RESULTS**

### **Participants**

Twenty-six patients were recruited between April and November 2014, of which 9 were categorised as Group 1, 7 as Group 2 and 10 as Group 3. Baseline characteristics for the patients according to group are reported in table 1. As expected inter-group analysis demonstrated significant variation in average partial pressure of carbon dioxide ( $p=0.000$ ), with the hypercapnic group having significantly higher levels. The hypercapnic group also had significantly higher APACHE II scores ( $F=4.410$ ;  $p=0.024$ , post hoc group 1 & 3  $p=0.022$ ), and lower numbers of organs failing on admission ( $H=15.030$ ;  $p=0.001$ ) when compared to both

the normocapnic (U=17.000; p=0.037) and acquired brain injury groups (U=4.000; p<0.001).

All other inter-group baseline differences were non-significant.

Table 1: Demographic Data for Each Group

	<b>Group 1 ABI (n=9)</b>	<b>Group 2 Normocapnic (n=7)</b>	<b>Group 3 Hypercapnic (n=10)</b>	
Male : Female	6 : 3	4 : 3	7 : 3	p = 0.861
Age (years)	50.8 (18.1)	53.0 (29.5)	51.8 (16.7)	p = 0.979
BMI (kg/m <sup>2</sup> )	24.5 (2.8)	23.5 (3.4)	28.7 (7.7)	p = 0.119
APACHE II	9.4 (96.3 – 12.6)	11.7 (7.0 – 16.4)	15.4 (12.4 – 18.4)	<b>p = 0.024</b>
Number of organs failing in first 24 hours (Based on SOFA Score)	3 (3 - 4)	3 (2 – 3)	2 (1 – 3)	<b>p = 0.001</b>
Days of sedation	7 (7 – 14)	7 (6 – 12)	8 (5 – 7)	p = 0.499
Duration of mechanical ventilation (days)	11 (8 – 14)	10 (7 – 22)	8 (5 – 15.75)	p = 0.754
Critical Care Length of Stay (level 3 & 2) (days)	11 (8 – 16)	13 (11 – 24)	10 (7 – 19)	p = 0.512
Average PaCO <sub>2</sub> during study period (kPa)	5.1 (0.4)	5.6 (0.4)	7.1 (0.8)	<b>p &lt; 0.001</b>

BMI: Body Mass Index; APACHE II: Acute Physiology and Chronic Health Evaluation Version 2; ABI: Acquired Brain Injury; SOFA: Sequential Organ Failure Assessment;

Average PaCO<sub>2</sub> calculated from admission to day 14 or extubation (whichever the sooner)

Results reported as mean (SD) or Median (IQR)

### Changes in diaphragm thickness

Differences from baseline for the whole cohort (n=26) were: day 3 -0.44% (9.0) n=25; day 5 -2.3% (7.9) n=22; day 7 -5.8% (9.5) n=17; and day 10 -6.5% (6.7) n=7 (see figure 1). Significant differences were observed between baseline to day 7 (p=0.029) and baseline to day 10 (p<0.001). Inter-group comparisons for diaphragm thickness were non-significant at each time point (p=0.132) (see table 2).

Figure 1: Percentage Change in Diaphragm Thickness from Baseline

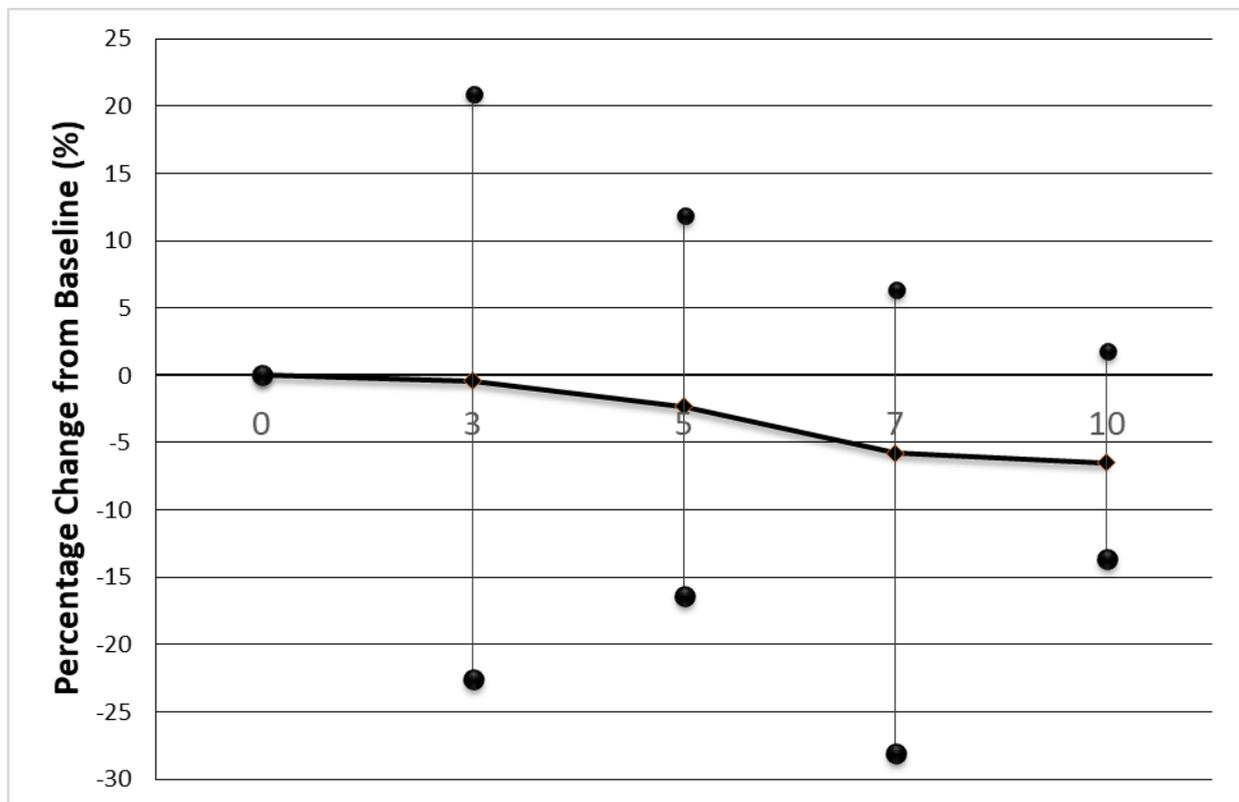


Table 2: Percentage Change in Diaphragm Thickness from Baseline for Each Group

	Group 1 ABI	Group 2 Normocapnic	Group 3 Hypercapnic
Day 3 % difference from baseline (sd)	-2.1 (6.6) (n=9)	0.5 (8.4) (n=7)	0.5 (11.9) (n=9)
Day 5 % difference from baseline (sd)	-1.1 (6.5) (n=9)	-4.3 (8.8) (n=6)	-2.2 (9.6) (n=7)
Day 7 % difference from baseline (sd)	-2.8 (5.3) (n=7)	-7.8 (4.8) (n=5)	-8.0 (16.4) (n=5)
Day 10 % difference from baseline (sd)	-0.2 (2.9) (n=2)	-11.6 (2.0) (n=4)	1.46 (n/a) (n=1)

ABI: Acquired Brain Injury

Between group comparisons for diaphragm thickness were non-significant at each time point ( $p > 0.05$ ).

### Changes in $RF_{CSA}$

Change in  $RF_{CSA}$  from baseline to Days 3, 5, 7, and 10 was calculated for all patients (figure 2).

Day 3 mean change from baseline was -4.0% (3.3) n=25; day 5 -7.0% (4.1) n=22; day 7 -10.5% (5.4) n=17; and day 10 -14.9% (8.2) n=8. Significant differences ( $p < 0.001$ ) were observed for all comparisons from baseline (baseline to day 3, 5, 7, and 10). Inter-group analysis showed no significant differences between the groups ( $p = 0.211$ ) (table 3).

Figure 2: Percentage Change in RFCSA from Baseline

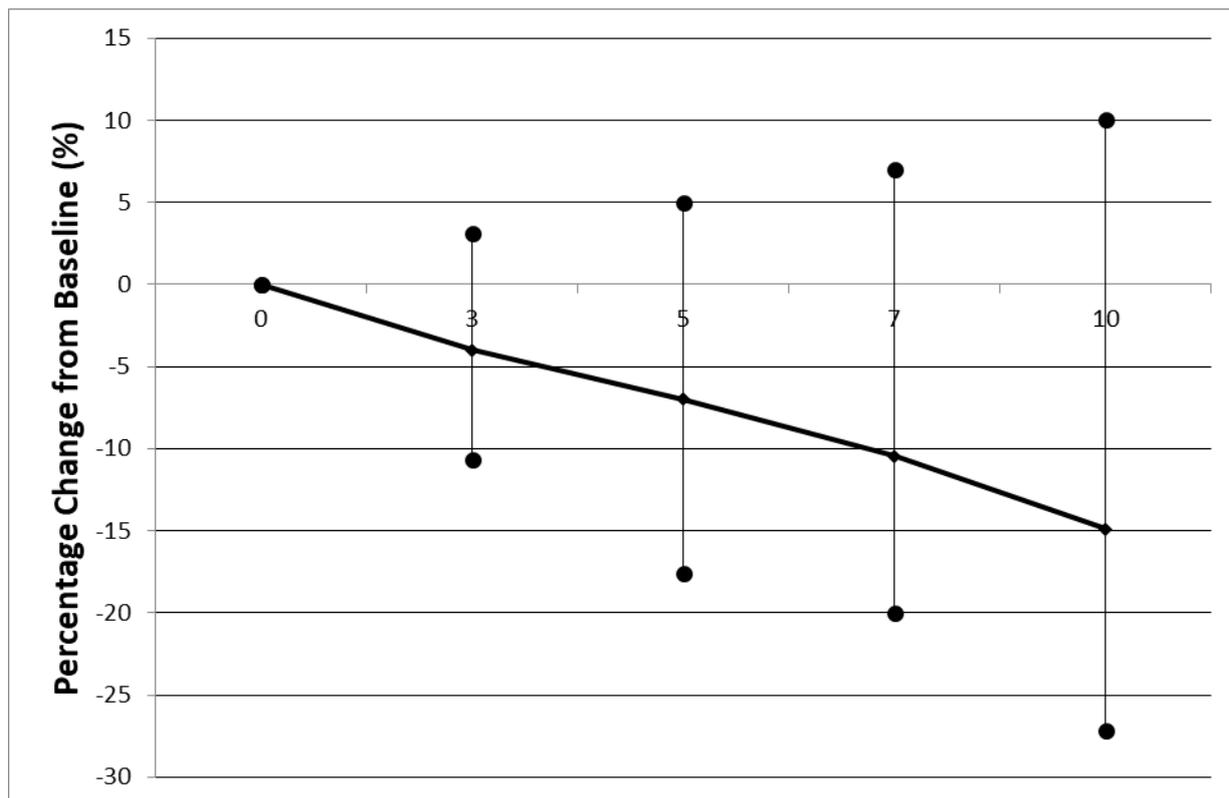


Table 3: Percentage Change in RFCSA from Baseline for Each Group

	Group 1 ABI	Group 2 Normocapnic	Group 3 Hypercapnic
Day 3 % difference from baseline (sd)	-3.7 (4.0) (n=9)	-4.9 (2.5) (n=7)	-3.6 (3.2) (n=9)
Day 5 % difference from baseline (sd)	-5.9 (2.3) (n=9)	-7.9 (4.3) (n=6)	-7.6 (5.7) (n=7)
Day 7 % difference from baseline (sd)	-12.4 (4.9) (n=7)	-7.2 (5.7) (n=5)	-11.0 (5.3) (n=5)
Day 10 % difference from baseline (sd)	-15.2 (1.2) (n=2)	-14.3 (8.2) (n=4)	-15.9 (16.1) (n=2)

ABI: Acquired Brain Injury

Between group comparisons for diaphragm thickness were non-significant at each time point ( $p > 0.05$ ).

## **Relationship between change in diaphragm thickness and RF cross-sectional area**

The relationship between percentage change in diaphragm thickness and RF<sub>CSA</sub> at all time points was analysed using bivariate Pearson product-moment correlation coefficient and was not statistically significant (day 3:  $r=-0.161$ ; day 5:  $r=-0.099$ ; day 7:  $r=0.002$ ; day 10:  $r=0.088$ ).

## **DISCUSSION**

This study demonstrated a significant decline in RF<sub>CSA</sub> from baseline to days 3, 5, 7 and 10 and in diaphragm thickness from baseline to days 7 and 10. There were no differences between the groups in either outcome measure, indicating that hypercapnia did not appear to influence respiratory and peripheral skeletal cross-sectional area in this small sample of people who were critically ill and receiving mechanical ventilation.

### **Change in diaphragm muscle mass**

In the cohort of patients recruited, there was an average decrease of approximately 6% in diaphragm thickness by day 10 (from baseline). This decrease was significant at day 7 when compared to baseline ( $p=0.029$ ).

The observed reduction in diaphragmatic thickness was substantially less than that reported by both Grosu et al [16] (6% per day of mechanical ventilation) and Schepens et al [17] (26% change from baseline at 72 hours). However, recently Goligher et al [18] examined changes in diaphragmatic thickness in a large multicentre cohort of 107 mechanically ventilated and 10 non-ventilated critically ill patients. In this cohort, there was a reduction of >10% thickness in 44% of patients, unchanged (<10%) in 44% of patients and increased (>10%) in 12% of patients. Similarly, Zambon et al [19] identified that rate of diaphragm thickness change was dependent on the mode of ventilation being delivered, with mandatory modes causing 7.5%

loss of thickness after 3 days, opposed to a 2.3% gain when only continuous positive pressure being utilised.

The pattern of change in diaphragm thickness was inconsistent across the three groups. The percentage change from baseline to day 3 was greater in the ABI group than that in the other two groups, yet at days 5 and 7 the reverse was found. The relatively larger changes in the normocapnic and hypercapnic groups may account for the overall significant difference at day 7. Similarly the larger number of people being ventilated at Day 10 in the normocapnic group may account for the overall significant difference at day 10.

There are many potential causes for the differences in rate of diaphragm thinning within the current study and literature. The current study had a limited sample size especially when considering those assessed at day 10. Additionally, the data demonstrates large standard deviations suggesting a significant spread of results. Similarly, given the results of other studies [16], it may be hypothesised that weaning, ventilation mode or respiratory rate may be the major determinant or modifier of the rate of diaphragm thinning. As this was not controlled within the current study it is likely this may have had an effect especially when combined with the limited sample.

Although not assessed in the current study, previous studies [19] have demonstrated altered rates of diaphragm thinning or diaphragm dysfunction based on the mode of ventilation being utilised. When mandatory modes of ventilation, such as SIMV are utilised, the rate of atrophy is far greater (approximately 7.5%), with gradually reducing rates as ventilation support reduced. Indeed, continuous positive pressure ventilation resulted in an overall improvement in daily atrophy changes. It may also be argued that respiratory rate will also play a role, with higher rates of diaphragm activation may reduce likelihood of atrophy through a training effect.

Further study is still required to consolidate the understanding of the role of a variety of critical care interventions such as ventilator strategies and nutrition, and their potential effects on muscle physiology. This understanding is essential prior to universal implementation of hypercapnia or any other method in the prevention of ventilator induced diaphragmatic dysfunction.

### **Change in quadriceps rectus femoris**

The current study has demonstrated significant reductions in RF cross-sectional area during the first 10 days of critical illness, with statistically significant differences at each time point. By day 10 of critical illness patients, on average, had lost over 14.9% of their RF<sub>CSA</sub>. The observed changes in rectus femoris compare favourably with previous research. At day 7 the current study demonstrated a 10.5% reduction from baseline. Similarly, Puthuchery et al [7] recorded a 10.3% reduction. Of note, Puthuchery et al. [7] concluded among these critically ill patients, muscle wasting occurred early and rapidly during the first week of critical illness. Other research has also shown significant reductions in RF<sub>CSA</sub> over time within critical care [5]. Furthermore, Gruther et al. [5], suggested that the rate of loss could be described using a logarithmic function which is independent of other variables e.g. age or pathology. These findings may provide insights into skeletal muscle wasting in critical illness.

In keeping with diaphragm thickness, no between group differences were observed. However, Puthuchery et al. [7] also suggested that muscle wasting was more severe among those with multi-organ failure compared with single organ failure. Within the current study, the hypercapnic group had statistically higher APACHE II scores (when compared to ABI group), therefore based on Puthuchery et al [7], this group should have had the greatest rate of muscle mass loss. However, this did not occur, with the ABI group showing greater muscle

mass loss at day 7. These findings may in part be due to the small sample size and consequent variability in data, but could also be due to other factors such as hypercapnia. It may be possible to suggest that hypercapnia did indeed play a protective role by preventing high rates of muscle mass loss to a point of similarity with those with lower degrees of organ failure. Further investigation of this would require increased patient matching of numbers of organ failing and patient severity in an accurately powered study.

### **Comparing diaphragm and rectus femoris**

No previous research has compared changes in muscle mass for both RF<sub>CSA</sub> and the diaphragm simultaneously, although Dres et al [20] observed that 63% of patients undergoing a spontaneous breathing trial had diaphragm dysfunction whilst 34% had limb muscle weakness, and 21% had both. Within a COPD population, Caron et al. [21] reported that several studies have highlighted important structural and biochemical modifications in limb and respiratory muscles, and hence they may respond differently to inactivity.

In the current study, there was no observed relationship between the degree of change for the two muscles and hence peripheral skeletal and respiratory muscle may to be affected differently during critical illness. For RF<sub>CSA</sub>, all groups demonstrated a similar pattern of muscle mass change, and similar day 10 outcomes (all groups between 14 and 16% reduction in RF<sub>CSA</sub>). In contrast, day 10 diaphragm thickness varied greatly between groups (all groups between 1.5% increase and 12% decrease). This supports the theory that skeletal and respiratory muscles respond differently and may be influenced by varied factors [16]. As previously identified, diaphragm thickness change may be influenced by ventilation modes and weaning strategies such as assisted spontaneous breathing. Use of such modes will result

in increased diaphragm activity as required to initiate inspiration and hence potentially encourage diaphragm thickening, whereas they will have no influence on quadriceps activity. Changes in quadriceps activity relies more on voluntary muscle contraction which is unlikely in the initial stages of critical illness whilst the patient remains sedated. This may be particularly apparent in the traumatic brain injury group which are likely to have required longer periods of sedation and an inability to produced voluntary quadriceps activity as a result of their neurological injury.

### **Limitations**

This study aimed to recruit a total of 30 patients (10 in each group) to understand the effects of hypercapnia on change in diaphragm and peripheral skeletal muscle during critical illness. A limited time-frame for recruitment meant the target recruitment was not met and hence the robustness and generalisability of the findings is reduced. Nonetheless these data could be used to power sample sizes in future research.

In the current study, baseline measurements were completed within 24 hours of admission to critical care. However, day of ICU admission may not accurately reflect the onset of critical illness [6]. Muscle loss may occur prior to ICU admission, albeit this is challenging to account for. Populations such as those patients admitted with trauma may represent the closest reflection of an acute, sudden insult where pre-admission muscle decline may not develop. This is an area for further consideration and research.

A single author (PT) was responsible for all ultrasound imaging throughout the study, albeit was not blinded to patient group allocation. The same author additionally analysed all

ultrasound images. Due to the pilot nature of the study the intra-observer reliability of the measurements was not analysed. Additionally, a standardised operating protocol was adopted for testing of both diaphragm and  $RF_{CSA}$ , including patient position, anatomical marking and the ultrasound scan procedure. However, absolute values of end-expiratory pressure were not measured. Variability in end-expiratory pressure (as a result of both internal and external PEEP) may have altered functional residual capacity and hence altered the resting position of the diaphragm [21]. Similarly differences in body somatotype and the presence of high intra-abdominal pressures may have affected diaphragm ultrasound thickness especially considering the limited sample size.

## **CONCLUSION**

In this pilot study, hypercapnia had no influence on total loss of diaphragm thickness and  $RF_{CSA}$ . Although further research is required correcting for influence of organ failure, severity of illness and ventilation mode. A significant reduction in muscle mass in rectus femoris cross sectional area was observed at all time points and significant reductions in diaphragm thickness at day 7. There was no correlation between the loss of diaphragm thickness and  $RF_{CSA}$ .

The authors declare that they have no competing interests

## **AUTHOR CONTRIBUTIONS**

PT participated in the design of the study, formulation of research protocol, ethical approval, data collection, statistical analysis and manuscript preparation. UJ participated in statistical analysis and manuscript preparation. MW participated in design of study, formulation of research protocol and ethical approval. All authors read and approved the final manuscript.

The authors acknowledge the contributions of Dr. David Gillespie (Cardiff University) for his assistance with statistical analysis. Additionally Dr. Bronwen Connolly (Guys and St Thomas' NHS Foundation Trust) provided training for the measurement of  $RF_{csa}$  and assisted with manuscript preparation.

#### **ABBREVIATIONS**

ICU-AW – Intensive care unit acquired weakness

ARDS – acute respiratory distress syndrome

VIDD – Ventilation induced diaphragmatic dysfunction

$PaCO_2$  – Partial pressure of arterial carbon dioxide

$RF_{csa}$  – Rectus femoris cross-sectional area

AB I – Acquired brain injury

ASIS – Anterior superior iliac spine

ICU – Intensive care unit

COPD – Chronic obstructive pulmonary disease

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## **FIGURES AND TABLES**

Figure 1: Percentage change in diaphragm thickness from baseline in all patients

Figure 2: Percentage change in RF cross-sectional area from baseline in all patients

Table 1: Demographic data for each group

Table 2: Average percentage change in diaphragm thickness from baseline for 3 groups

Table 3: Average percentage change in RF cross-sectional area from baseline for 3 groups

## **FUNDING SOURCES**

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