ACTA ABP BIOCHIMICA POLONICA

Vol. 70, No 4/2023
951–954
https://doi.org/10.18388/abp.2020_6893

Regular paper

# The relationship between EMG high frequency and low frequency band amplitude changes correlates with tissue inorganic phosphate levels

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**Assessing inorganic phosphate levels seems crucial in deciphering the biochemical state of organisms or tissues. The concentration of inorganic phosphate in blood is an order of magnitude smaller than in tissues and, on top of that, it is dynamically used to fill temporary gaps in tissues. This is the reason blood inorganic phosphate level is considered a poor proxy for tissue levels. Therefore, tissue biopsy seems to be the dominant method when assessing inorganic phosphate levels for instance in muscles. In this study, we attempted to derive a non-invasive biomarker for phosphate tissue levels. We analyzed surface electromyography signals taken during 31P spectroscopy of leg muscles in five adult pigs. We induced hypophosphatemia *via* 20 minutes-long hyperventilation. It turned out that the proportion of the amplitude of the low frequency band and the high frequency band is significantly ($p$=0.002) correlated with the relative phosphate levels. The electromyographic signal did not correlate significantly with pCO2 levels in the blood, suggesting that the changes in the signal are a result of inorganic phosphate levels, not hyperventilation. The results might lead to the development of a real-time phosphate fluctuations measurement procedure.**

**Keywords**: EMG, phosphate, biomarker, MRI, hyperventilation, hypocapnia

**Received**: 20 June, 2023; revised: 07 August, 2023; accepted: 13 September, 2023; available on-line: 18 October, 2023

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**Acknowledgements of Financial Support**: This research was funded by The National Centre for Research and Development, grant number LIDER/028/565/L-5/13/NCBR/2014.

## INTRODUCTION

Disturbance in phosphate levels is an electrolyte disorder associated with many diseases, but hypophosphatemia is more common than hyperphosphatemia. Hypophosphatemia is typically diagnosed when serum phosphate levels drop below 2.0 mg/dl. The most common causes of hypophosphatemia are infection, refeeding, and Fanconi syndrome (Saito *et al.*, 2014). It also manifests as a complication in critically ill patients in the postoperative phase, hypothermia, or trauma (Geerse *et al.*, 2010). Symptoms of hypophosphatemia are potentially life-threatening but at the same time nonspecific - it includes muscle weakness, arrhythmias, respiratory failure, hypercalciuria, and others (Assadi, 2010). The disturbance in phosphate levels is associated with increased morbidity (Felsenfeld & Levine, 2012; Sin *et al.*, 2021).

The relationship between phosphate levels in compartments is tightly regulated and complicated. As only 1% of total phosphate in the body is in the extracellular compartment, the changes of concentration in serum are dynamic and a poor indicator of total-body phosphorus level (Felsenfeld & Levine, 2012). Routine phosphate monitoring is uncommon in patients admitted to intensive care units (ICU), and hypophosphatemia is often not corrected (Berger *et al.*, 2021). 31P spectroscopy is rarely a viable solution due to equipment requirements.

As hypophosphatemia is associated with disturbances in muscle function and ATP synthesis (Pesta *et al.*, 2016), the aim of this study was to determine if muscle activity could be a non-invasive, real-time indicator of phosphate level. The studies performed on cows showed that phosphate deprivation could induce abnormalities in muscle activity that can be detected by electromyography, but there is no data about transient and immediate effects, which could have clinical implications in human studies (Pesta *et al.*, 2016; Grünberg *et al.*, 2019, 2015). The approach of combining 31P with surface EMG for studying muscle disorders and fatigue has been used many times since 1993 (Roy, 1993; Giannesini *et al.*, 2003; Rzanny *et al.*, 2006). But again, experiments using this approach did not investigate fast fluctuations of inorganic phosphate in healthy and unfatigued muscle. Our study fills this gap and might lead to the development of a novel phosphate biomarker.

## MATERIALS AND METHODS

### Major protocol

The experiments were approved by the II Local Ethical Committee on Animal Testing in Warsaw, Poland (permit number: 20/2015 from 23 April 2015) on behalf of the National Ethical Committees on Animal Testing. Three preliminary experiments were conducted: two animals under normo- and hyperventilation were performed outside the MRI scanner chamber and one in the MRI chamber under normoventylation (animal 0). In the main part of this study, four female healthy piglets (animals 1–4) with an average body weight of 20 kg and an average age of 2 months were first sedated with azaperone

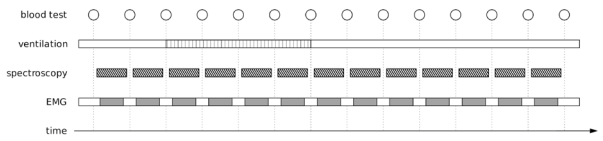


**Figure 1. Schematic of the main experiment - dashed and filled rectangles indicate which fragments of data were taken into further analysis.**
Additionally, the range of hyperventilation and moments of blood gasometry were shown as vertical lines and circles respectively. Each spectroscopy measurement lasted 4 min 26 seconds. The time between measurements slightly varied but the mean time between blood tests was 5 min.

(Stresnil, 3 mg/kg body weight (b.wt.), Janssen Pharmaceutica, Turnhoutseweg, Belgium). Each animal was then orotracheally intubated with an endotracheal tube and mechanically ventilated with room air. The respiration rate was set to zero (free respiration) during normoventylation and 20/min during hyperventilation. To evaluate muscle activity, the surface EMG electrodes were placed on the trapezius and triceps (front leg), and biceps femoris (rear leg) muscles. During the experiment, a swine was placed inside the MRI scanner. Each measurement lasted 4 min 26 seconds. In four cases, between the measurements, the pig's blood was drawn to evaluate gasometry. After the first two control scans, the next four were made in a hyperventilated state, followed by another seven scans under normoventylation. A schematic of the main experiment is shown in Fig. 1. The well-being of animals was closely monitored by an anesthesiologist and in case of suspected risk to animal health, the experiment was terminated. In addition to experiments with $^{31}$P spectroscopy, EMG measurements for two other animals upon normo- and hyperventilation were performed outside of the MRI scanner chamber.

### $^{31}$P MRS data analysis

$^{31}$P-MRS data was acquired using an MR750w 3T MRI scanner (GE Healthcare, USA) with $^{31}$P/$^{1}$H surface coil (Rapid Biomedical, Germany). Data was acquired with parameters: TR 1s, Navg 256, spectral bandwidth 5kHz, acquisition time 102.4 ms, slice thickness 40 mm. No shimming was applied for the $^{31}$P-MRS acquisition. Data were acquired before, during, and after hyperventilation. Localization was done through coil sensitivity. The coil was located on the biceps femoris. Raw data were quantified with jMRUI software (Naressi *et al.*, 2001; Stefan *et al.*, 2009) using the AMARES algorithm (Vanhamme *et al.*, 1997) according to published procedures and parametrization (de Graaf, 2019). Signal location and assignment were done for each spectrum individually to account for pH-induced changes in the spectra.

### Electromyography data analysis

The electromyography record was running continuously throughout the whole experiment. Because between spectroscopy measurements the pigs were touched by a technician for blood extraction, the analysis of electromyography measurements has been made only on fragments of record that were recorded during spectroscopy. The first part of the analysis was made using the "signal" library from Python 3. The "spectrogram" function was used to create a signal spectrogram which then has been converted into dB. To extract the spectroscopy parts of the signal the sum of signal power in the 210 dB – 249 dB range has been taken and then the fragments where the summed signal power went above the average for the known spectroscopy time – 4 minutes and 26 seconds – were chosen. Further signal analysis has been done using the R language "psd" library. The signal was further cleaned up, a spectrogram was created, and then the relation between high and low frequency bands was calculated according to the equation:

This approach to the analysis of EMG signals was already used in clinical research (Allison & Fujiwara, 2002; Badier *et al.*, 1993; Krogh-Lund & Jørgensen, 1993).

$$EMG_{score} = \Sigma_{150-400Hz}(amplitude)/\Sigma_{1-49Hz}(amplitude)$$

### Gasometry data analysis

Between $^{31}$P-MRS measurements, blood samples were collected and further using a critical points analyzer RAPIDPoint 500 (Siemens, Erlangen, Germany). The measured parameters were: pH, $pCO_2$ (mmHg), $p0_2$ (mmHg), $cHCO^{3-}$(mmol/L), BE (ecf) (mmol/L), $cSO_2$ (%), $Na^+$ (mmol/L), $K^+$ (mmol/L), $Ca^{++}$(mmol/L), $Cl^-$ (mmol/L), $cTCO_2$ (mmol/L), Anion gap (mmol/L), Anion gap $K^+$ (mmol/L), Hct (%), cHgb (g/dL), BE (b) (mmol/L), Glucose (mg/dL), Lactate (mmol/L), Creatinine (mg/dL) (Supplementary Table 1 at https://ojs.ptbioch.edu.pl/index.php/abp/).

## RESULTS

### Hyperventilation induces hypophosphatemia in most animals

Four hyperventilated animals were put in an MRI chamber. Two measurements were done with normoventilation and then animals were hyperventilated for 4 consecutive scans (see Materials and Methods for exact protocol). Analysis of the $^{31}$P-MRS signal indicates that inorganic phosphate content in muscles drops by as much as 30% after 5 steps of the experiment (about 20 minutes of hyperventilation) (Fig. 2). Not all cases had a drop in phosphate levels following hyperventilation, but when blood gasometry analysis was performed, hyperventilation robustly induces a drop in pCO2 levels (Supplementary Fig. 1 at https://ojs.ptbioch.edu.pl/index.php/abp/).

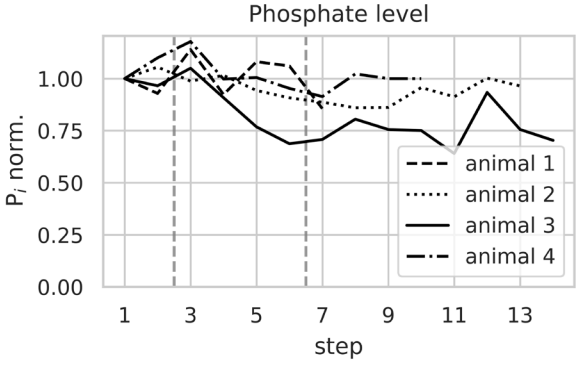


**Figure 2. Inorganic phosphate levels in muscles before, during and after hyperventilation.**
The total number of points per animal depended on its condition – some experiments were decided to finish earlier. Phosphate levels are normalized against the first measurement.

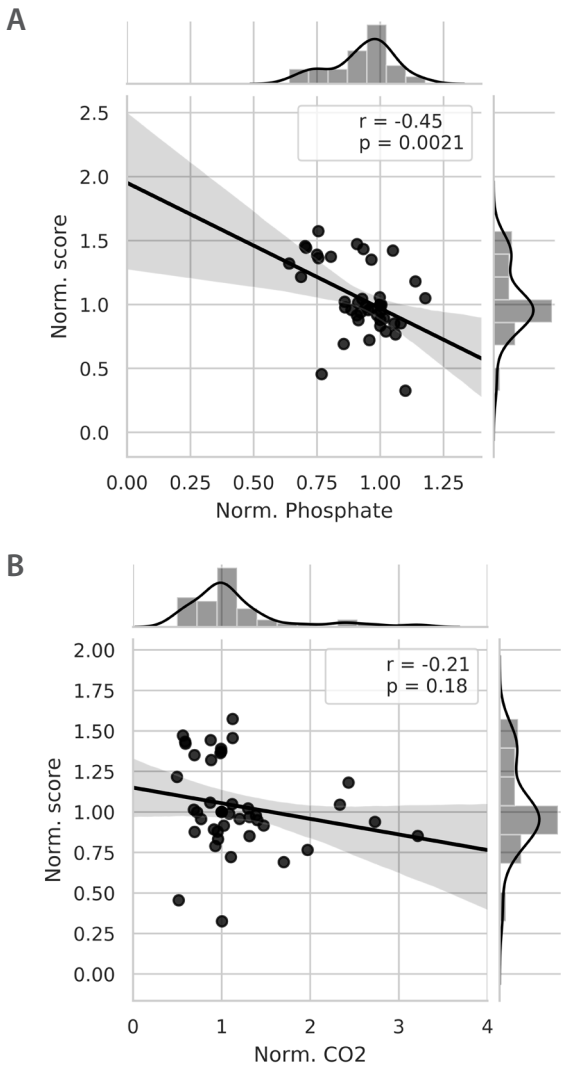


**Figure 3. Correlation between EMG score (see Materials and Methods) and phosphate levels (panel A) and pCO2 (panel B). Values on all axes were normalized against the first measurement per animal.**

### EMG signal correlates with inorganic phosphate but not with $pCO_2$ levels

The distribution of the collected EMG signal was symmetrical, and values ranged typically between -100mV and 100mV (Supplementary Fig. 2 at https://ojs.ptbioch.edu.pl/index.php/abp/). The ratio of the power spectrum between high and low frequencies was calculated for a window spanning 4 minutes during MRI acquisition (4 min 26 seconds). The window for EMG analysis was taken slightly smaller to remove artifacts caused by switching on/off of MRI scanning. Pearson correlation between this calculated ratio (called "normalized score") and normalized phosphate levels is negative and significant: R is –0.45, and the p-value is 0.002 (Fig. 3A). We made the same comparison between the score and normalized $pCO_2$ levels, and the Pearson test indicated an insignificant correlation (Fig. 3B). Detailed trajectories for each animal are in Supplemental Fig. 3 at at https://ojs.ptbioch.edu.pl/index.php/abp/.

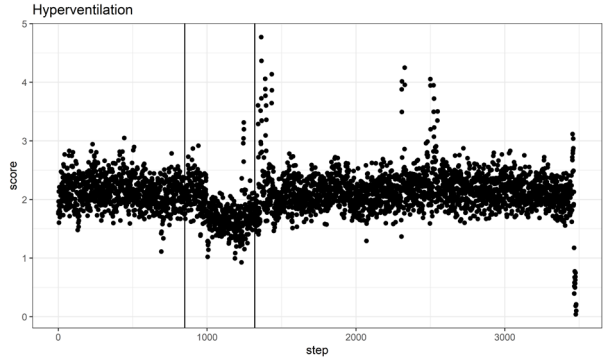


**Figure 4. Experiment with hyperventilation repeated outside of the MRI chamber.**
The start and end of hyperventilation are denoted as vertical lines. Dots are the EMG score calculated every 1s.

### Fast reaction to hyperventilation seen in EMG

The EMG signal collected during MRI scans is, despite shielding and usage of carbon electrodes, quite noisy. We repeated the hyperventilation experiment outside of the MRI chamber. Phosphate measurement wasn't possible but the goal was to obtain a clear picture of changes in EMG following hyperventilation. It turned out that the reaction of EMG to hyperventilation is pretty fast - the changes in the EMG spectrum can be observed tens of seconds after hyperventilation is started (Fig. 4). To remove the possibility that the interaction with a ventilating machine was the cause of EMG response, another animal was put on ventilator but with respiration rate set to 12 breaths. No significant changes in EMG score (other than artifacts from body movement) were detected (Supplemental Fig. 4 at at https://ojs.ptbioch.edu.pl/index.php/abp/).

## DISCUSSION

In this study, we presented the results of an attempt to derive a non-invasive biomarker of hypophosphatemia. We showed that hyperventilation induces hypophosphatemia and that the level of inorganic phosphate in muscles correlates with changes in the power spectrum density of a surface electromyographic signal. The observed correlation is significant despite the limited number of animals used for the research.

Animals appeared healthy, but we cannot rule out pre-existing conditions (one animal needed a longer recovery time after the experiment was concluded). This could potentially explain why, not in all cases, we were able to notice hypophosphatemia.

An interesting aspect of EMG response to hyperventilation is the time needed to elicit it. MRI scans affect the EMG signal, so we could not see when exactly the EMG started to drift. Experiments outside of the MRI chamber showed that a response is faster than 30 seconds. Existing hypotheses about how hyperventilation influences phosphate levels (O'Brien & Coberly, 2003) support a fast response rate. O'Brien and Coberly proposed a model where an increased rate of glycolysis and ATP production in response to respiratory alkalosis is responsible for a sudden drop in inorganic phosphate levels. However, this study doesn't provide evidence if that is the mechanism that occurs.

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