**Title: Partial pressure of carbon dioxide in mechanical loaded tissue: The canary in the Cage singing in tune with the pressure ulcer mantra**

**[1]Peyman Mirtaheri, [2]Terje Gjøvaag, [3]Peter R. Worsley, [3*] Dan L. Bader**

**[1]Faculty of Technology, Art, and Design, Oslo and Akershus University College of Applied Sciences, Oslo, Norway**

**[2]Faculty of Health Sciences, Oslo and Akershus Univeristy College of Applied Sciences, Oslo, Norway**

**[3]Faculty of Health Sciences, University of Southampton, Southampton, UK**

Address Correspondence to Professor Dan L. Bader, Faculty of Health Sciences, University of Southampton, Southampton SO16 6YD. Email D.L.Bader@soton.ac.uk

***Abstract:***

Pressure Ulcers (PUs) can occur in any situations where people are subjected to prolonged mechanical loading. They can have devastating effects on the patients' well-being and in extreme conditions can prove fatal. In addition to traditional wisdom implicating mechanical-induced ischaemia, there is strong evidence that other mechanisms play a role in the cascade of events which can initiate the PU damage process at the cellular level. Some of these refer to a metabolic imbalance with compromised delivery of nutrients and accumulation of waste products associated with the cell niche. The approach of much research has focused on the measure of oxygen in compressed tissues as a means of predicting early damage. However, the present review adopting a hierarchical approach, using length scales ranging from cells through to human models, has revealed compelling evidence which highlights the importance of carbon dioxide levels and associated concentration of other metabolites, such as lactate and purines. The temporal profiles of these metabolites have been monitored in the various models subjected to periods of mechanical-induced loading where the localised cells have converted to anaerobic metabolism. They reveal threshold levels of carbon dioxide which might be indicative of early tissue damage during both mechanical-induced ischaemia and subsequent reperfusion and an appropriate sensor could be used in a similar manner to the long-standing "canary in a cage" method to detect toxic gases in enclosed mines.

**Keywords:**

Pressure ulcers, ischemia, $PCO_2$, perfusion, mechanical loaded tissues

## 1. Introduction

Years ago, working miners carried a canary in a canary cage as a simple but effective safety device. When the canary collapsed, it was an indication that the air was toxic and the miners had to rapidly leave the mine. The $CO_2$ in gastric tonometry has been referred to as the canary in the cage in an ischemic tissue (64). Can this canary sing a different tune for detection of pressure ulcers (PUs)?

The condition of pressure ulcers or decubitus (aka pressure sores, bed sores) represents a localized injury to skin and/or underlying tissue, usually over a bony prominence, as a result of prolonged mechanical loading in the form of pressure, or pressure in combination with shear (24). Pressure Ulcers (PUs) can occur in any situations where people are subjected to sustained mechanical loads, but are particularly common in subjects who are bedridden or confined to chairs for much of their waking day. Thus, common sites for tissue damage include the sacrum, heel and the ischial tuberosities. PUs have been traditionally associated with the elderly, particularly those who are malnourished and dehydrated with additional medical complications (28, 45, 56). However, PUs affect a wider age range including neonates and paediatrics nursed in intensive-care units, patients undergoing prolonged surgery and the Spinal Cord Injured (13). Accordingly, they represent a disabling chronic condition that has been universally implicated as both a Quality of Care and Patient Safety issue for individuals in hospital and community settings. Indeed when a pressure ulcer has developed, it can have devastating results for the patients' well-being and in extreme conditions can cause death (42).

The aetiopathogenesis of PUs has long been considered to involve the mechanically induced capillary occlusion, resulting in tissue ischemia with associated localised hypoxia. This mechanism will limit the delivery of vital nutrients, such as oxygen, to the cell niche. The resulting cell death would impede any remodelling processes and will result in the local

breakdown of soft tissues. This ischaemia-induced mechanism was supported by a number of seminal studies employing animal models (26, 33). However, in the last decade compelling evidence from different hierarchical levels have implicated other mechanisms in the development of pressure ulcers, namely, the blockage of lymphatics and altered interstitial fluid flow, ischaemia-reperfusion injury and cellular deformation (9, 34,47). Each of these mechanisms will result in the initiation of damage at a cellular level, associated with different tissue layers, namely skin, fat and muscle, overlying the bony prominences. A schematic indicating the potential inter-relationships between these mechanisms leading to cell damage is provided in Figure 1.

*Figure 1 should be inserted here*

A typical bioengineering approach to monitor individual risk factors for developing PUs has been to measure physical parameters at the loaded body-support interface e.g. supine subjects lying on a mattress. As an example, commercial interface pressure monitoring systems have been long established, with benefits of comparing support surfaces and/or providing feedback on the functional posture of individuals. However, it is well recognised that interface pressures do not inform clinicians to potential risk of tissue breakdown. For this, some measure of the effects of mechanical loading and time on the viability/ status of loaded tissues is needed. As an example, Laser Doppler flowmetry (LDF) can supply information about the location of the blood vessels and the magnitude of blood flow in the vessels. However, these measurements may not provide adequate information about the level of local tissue oxygenation. Routine measurements of transcutaneous gas tensions were developed over 30 years ago to monitor the respiration gases of neonate infants. These have been adapted to monitor oxygen ($T_CPO_2$) and carbon dioxide ($T_CPCO_2$) levels in loaded soft tissues in a number of studies on both healthy

subjects and those considered to be at high risk of developing pressure ulcers (1, 3, 4, 7, 8, 55, 57).

Although, the quantification of oxygen at cellular and tissue levels is well-established, the role of carbon dioxide in this context is still not clear. What is certain is that carbon dioxide is involved in three main processes that are essential for the survival of the organism, namely, i) Local blood transportation ii) Oxygen transportation and iii) Regulation of acid-base balance. Furthermore, an elevation of tissue carbon dioxide due to reduced circulation is based on two processes: an accumulation of tissue $PCO_2$ due to flow stagnation, and wash off processes in local tissue.

After an introduction to tissue carbon dioxide, we propose a hypothesis, based on hierarchical evidence derived from a diverse range of studies, that carbon dioxide could prove to be a reliable marker for early detection of pressure ulcers.

## 2. Brief History of $PCO_2$

Carbon dioxide was first discovered by a medical student, Joseph Black (17), who reported large quantities of a “fixed gas”, which was generated when chalk was heated or acidified. He observed that the “fixed air” was denser than air and did not support either flame or animal life. It is a colorless gas and does not have electrical dipole making it diamagnetic.

Carbon dioxide is generated in tissues, both as an end-product of the cellular respiratory process as a result of buffering protons with bicarbonates due to the metabolic acidosis following ischaemia (63). The carbon dioxide is soluble in water resulting to carbonic acid ($H_2CO_3$) which, itself, is converted into $H^+$ and $HCO_3^-$. The amount dissolved in the fluid phase is governed by Henry's Law, namely,

$$PCO_2 = K_H \cdot [CO_2] \qquad \text{Equation 1}$$

where $PCO_2$ is the partial pressure of carbon dioxide, often referred to as carbon dioxide tension (51), which increases with enhanced concentrations of carbon dioxide. The Henry's coefficient, $K_{H,}$ is dependent on the solubility of carbon dioxide in the tissue and its temperature (61). Although its value has been extensively estimated in blood, its value in tissue has not been fully established. The level of carbon dioxide expired from the body is about 4%, while in tissue the level of carbon dioxide is normally 1-2% higher (22, 47).

The presence of carbon dioxide helps the release of oxygen from hemoglobin. This process, known as the Bohr effect, is explained as the oxygen dissociation curves shift to the right implying that an increase in plasma carbon dioxide reduces the equilibrium of hemoglobin saturation. Increasing carbon dioxide may attach more molecules to hemoglobin to transport away the excess $CO_{2,}$ described as the Haldane effect (29). Thus, a several fold increase of $PCO_2$ in tissue above normal values, indicates both a stagnation of blood flow and a shift to anaerobic metabolism.

## 2. Determinants of $PCO_2$

It is believed that tissue $PCO_2$ is determined by the balance between arterial $PCO_2$, tissue blood flow and distribution, the mix of aerobic and anaerobic metabolism including lactate changes in tissue, and venous oxygen saturation, as indicated in Figure 2.

*Figure 2 should be inserted here*

When an ischemic condition occurs, impaired blood flow decreases carbon dioxide clearance from tissues (31), thereby causing an increased oxygen release from haemoglobin and

producing additional carbon dioxide (63). However, as oxygen availability is finite when a diminished blood flow occurs, this accounts for only small increases in carbon dioxide and the majority of the excess carbon dioxide observed in ischaemic conditions is thought to derive from lactic acid, generated as a result of anaerobic metabolism (31). When this dissociates, hydrogen ions accumulate and, since intracellular bicarbonate levels are largely equivalent to blood plasma levels, intracellular hydrogen leads to the release of previously buffered carbon dioxide (63). Since carbon dioxide readily diffuses into the extracellular space, localised tissue acidosis may occur (63). These authors also suggest that tissue acidosis below a threshold may serve to protect cells by reducing the activity of enzymes involved in the generation of damaging substances.

## 3. Hierarchical Evidence of $PCO_2$ as an early indicator of PU formation

### *3.1 Cell-based Model systems*

Over the last 15 years, several *in vitro* models have been adopted to examine the effects of mechanical-induced cell damage. Such cell-based systems provide the opportunity for examining a number of output parameters in a controlled manner. Of particular relevance were the series of studies (19-21, 52), using a tissue engineered muscle model, termed bio-artificial muscles (BAMs), to examine the differential effects of compressive strain (up to 40%) and ischaemia on both cell apoptosis and necrosis. The results inferred that strain results in a gradual increase of damage over 22 hours, but there was no associated damage due to hypoxia (19). This may be explained by the oxygen conformance behaviour of the cells, resulting in a decrease in their energy demands with associated consumption of less oxygen under hypoxia

conditions, without decreasing their amount of energy present (ATP) (2). In addition, this behaviour is assisted by a switch to anaerobic metabolism with the associated increase of lactate in the culture medium.

These experiments were extended for culture periods up to 5 days to examine the effects, singly and combined, of glucose deprivation, pH change, lactic acid accumulation and deformation. An air tight box was designed containing four 6-wells plates with BAMs, which was flushed with gases to achieve either normoxic (20% $O_2$) or hypoxic (6 % $O_2$) conditions.

Figures 3a and 3b indicates the effect of low and high glucose levels on BAM performance at normoxic conditions. The effect of glucose deprivation in the absence of medium refreshment was significant from 24 hours and beyond. It was evident that after day 1 there was no glucose available to the cells in the low glucose medium (1g/L). Accordingly metabolism was limited with lactate production reaching a maximum at day 1 and thereafter remaining constant. The group exposed to high glucose medium revealed an increased lactate production up to day 3, thereafter remaining constant at approximately 23 mM (Figure 3b). This was associated with a reduction in pH from 7.4 to 6.5. It was clear that glucose deprivation represented a critical determinant of premature cell death (20).

Other findings revealed that BAMs subjected to deformation alone did not significantly change their glucose consumption (Figure 3c) or lactate production (Figure 3d) or cell death profile when compared to control samples ($p>0.05$ in all cases). By contrast, the hypoxic groups, with and without deformation, consumed significantly more glucose than the control group on days 1 and 2 ($p<0.01$) and exhibited an enhanced cell death profile and reduced pH over the 5 day

culture period (20). Additionally, the lactate release of the hypoxic group alone (Figure 3d) was significantly elevated compared to the control group ($p<0.01$).

*Figure 3 should be inserted here*

These collected studies reveal that deformation has a significant effect on cell death in 24 hour cultures. However, for extended culture periods, the hypoxia-induced elevated lactic acid production eventually exceeded the acid threshold, provided there was sufficient glucose present in the medium to continue metabolism. Thus, as long as the threshold levels for deformation or ischemia are not exceeded, the tissue samples may survive compression.

Other model systems provide compelling evidence, which suggest that lack of oxygen per se does not necessarily lead to cell damage. As an example, Hotter et al. (25) proposed that impaired oxygenation combined with an excess of carbon dioxide, termed hypercapnia, would influence cell apoptosis. The authors induced unilateral renal ischaemia in rats for thirty minutes, while monitoring intra-renal pH and computed $pCO_2$ values from these measurements. The resulting $pCO_2$ values, namely 18% and 30%, were subsequently reproduced *in vitro.* Following exposure and a subsequent return to normal culture conditions, selected experimental cultures, namely those exposed to hypoxia with concomitant hypercapnia, exhibited apoptotic activity, which was statistically higher ($p<0.05$) than both the control groups and the groups exposed to hypercapnia alone. The authors reaffirm that as $CO_2$ diffuses easily through cell membranes, its influence would be immediate in all intracellular compartments.

### *3.2 Animal Model Studies*

If $PCO_2$ provides an early marker for the detection of ischemia in muscle tissue, it must reflect the energy state of cells in tissue areas subjected to external mechanical loading. The question arises whether measuring $PCO_2$, when the local blood flow is reduced, will contain information about the lactate tissue acidosis and/or the breakdown of energy stores like ATP. If the energy stores in that tissue are already broken down, then the cells follow an irreversible pathway, which will restrict an effective treatment strategy.

It is well established that when the cell converts to anaerobic glycolysis, a considerable intracellular production of protons will ensue (11), a large proportion of which will be buffered by intracellular bicarbonates, forming $CO_2$ and water (10). If the blood flow is inadequate, the process results in an accumulation of $CO_2$ in tissue to values several fold higher than those due to oxidative phosphorylation alone. As muscle tissue is known to be resilient to anaerobic condition, it might be hypothesised that ATP would be maintained constant for a prolonged period due to the transfer of energy-rich phosphate groups from phosphocreatine (39). However, physiological and anatomical studies have reported that muscle can only tolerate ischemia for up to 4 hours, compared to much longer periods for fat (~13hours) and skin (~24hours) at normothermia (6). This indicates that the skeletal muscles overlying bony prominences may represent the tissues most vulnerable tissues to ischemia.

Lactate accumulation in muscle tissue as an index of accelerated glycolysis has long been appreciated, although studies to demonstrate its relationship to the partial pressure of carbon dioxide are limited. Of the few, a linear correlation was demonstrated between lactate and $PCO_2$ when porcine muscle was subjected to zero-flow conditions (37). The authors also demonstrated that after the onset of ischaemia, $PCO_2$ increased several fold when compared to

basal levels long before depletion of the energy stores, ATP, and phosphocreatine concentrations. A total depletion of these energy stores will result in a permanent irreversible injury to the cells and therefore monitoring $PCO_2$ could prove a promising marker of reversible tissue damage (38). Indeed carbon dioxide may diffuse out of the cells and be detected at the surface of the tissue, while measurements of lactate might prove problematic due to the collection of sufficient sample volume in a non-invasive manner (see later section).

It is recognised that if the zero-flow condition is induced, it will result in an associated decrease in the temperature of the tissue bed, a condition termed cold ischemia, which may accordingly decrease the measured $PCO_2$ according to Henry's law (Equation 1). At the same time, an decrease in temperature means that the tissue can tolerate ischemia for prolonged periods since the metabolism is lower and the energy supplies would be preserved (70). Therefore, it is logical to suggest that the tissue would tolerate ischemia during a combination of arterial and venous occlusion resulting in a zero flow condition. This was examined in a study using porcine muscle tissues (36), the results of which are illustrated in Figure 4, for both periods of ischaemia and reperfusion. It is evident that the relative increase in tissue carbon dioxide was almost identical in both arterial and venous stasis and the rate was fairly linear with lactate production. During reperfusion, a hyperaemic blood flow was evident in both states, while the blood flow increase was more significantly pronounced following arterial occlusion. In addition, the wash off process of $PCO_2$ was clearly more rapid following arterial occlusion in association with an increased removal of lactate from the tissue (Figure 4). However, both lactate concentrations and carbon dioxide tensions were still elevated after 30 minutes of reperfusion indicating that the tissues required more time to metabolise and wash-out the metabolites. It is therefore theoretically possible to differentiate between a venous and arterial occlusions based on the ratio changes of carbon dioxide after the perfusion is re-established (67).

*Figure 4 to be inserted here*

If the carbon dioxide is a linear function of lactate, why not just measure lactate as an indicator for ischemia? A study on various organs and tissues including muscle in a porcine model (66), revealed a significant accumulation of carbon dioxide under aerobic metabolism, in contrast to metabolic parameters of ischaemia e.g. lactate and glycerol, which remained low. As blood flow declines, more oxygen is extracted from haemoglobin to maintain a balance between oxygen utilization and $CO_2$ generation in tissues. With decreased blood flow, more $CO_2$ is consequently added to each unit volume of blood and $PCO_2$ will increase in venous effluent blood as well as in tissues (16, 59, 60). Based on dual line regression analyses for oxygen threshold, a critical transition point between aerobic and anaerobic metabolism was proposed. The calculated threshold level for muscle tissue was about 9.3kPa (69.8 mmHg), which corresponded to a lactate concentration of 2.1mM. The ratio of $PCO_2$ over time changed from 0.61 to 3.7kPa/h. This demonstrated that the $PCO_2$ was increasing several fold when tissue metabolism changed from aerobic to anaerobic state. In addition, one could also show significant increase above zero even when the tissue was still in aerobic metabolism but the perfusion was reduced (66).

As discussed with respect to cell model systems, tissue damage may not only be due to the haemodynamic origins but also deformation of the cells per se. This was demonstrated in series of studies in which the tibialis anterior muscle of a rat tibia, was either subjected to a mechanical deformation or an ischemic insult alone. These studies revealed that:

i) Irreversible damage, consisting of gross tissue necrosis, due to large deformations occurred at an earlier stage than pressure-induced ischaemia (62). This occurred within 2 hours, which is below the threshold for the onset of skeletal muscle necrosis (6).

ii) Loading for as short as 10 min can cause small levels of muscle damage (41).

iii) Above a strain threshold value, the accumulation of deformation-induced damage corresponded to areas exposed to increasing mechanical shear strains (14)

iv) As the loading period extends to 4 hours, both ischemia and reperfusion increasingly contribute to the damage process (40).

### *3.3 Human Studies*

When testing human subjects, there is a need for non-invasive measurement techniques applied on or at least close to the skin surface. In the case of measuring transcutaneous gas tensions, the values contain information from skin tissues in addition to the muscles, fat, connective tissue and circulating vessels. These measurements have been regularly employed to measure gas tensions in loaded soft tissues in a range of subject groups. Most studies have focused on examining the range of interface pressures and time needed to reduce threshold levels of oxygen, below values considered to compromise the viability of soft tissues (3-4). However, a few studies have examined the interplay of $T_CPO_2$ and $T_CPCO_2$ in loaded tissues. As an example, in a prospective study of wheelchair-bound spinal cord injury (SCI) subjects, the gas tensions at the loaded ischial tuberosities were examined (7). Results indicated that, in many cases, subjects revealed a progressive improvement in tissue viability after injury, as exemplified by small reduction in $T_CPO_2$, during load-bearing, which returned to unloaded levels during a period of pressure relief. The associated $T_CPCO_2$ levels remained within the normal range of 4.8-6.4kPa (36-48mmHg) throughout the assessment period (12). However, a

small proportion of SCI subjects, typically those with low level lesions and flaccid paralysis, demonstrated significant reductions in $T_CPO_2$ with an associated increase of $T_CPCO_2$ in excess of the normal range. The authors suggested that it was this latter group, who are at potential risk of developing PUs and thus require effective support cushions with strict adherence to a pressure relief regime. The authors also proposed that carbon dioxide levels can control vascular tone in acute SCI subjects (7).

In a separate study the viability of tissues in elderly patients undergoing orthopaedic surgery was examined at interface pressure representative of values experienced on the operating tables (3). Findings demonstrated that the $T_CPO_2$ fell below critical low levels, defined as 2.7 kPa (20.3mmHg) which were often associated with significant increases in $T_CPCO_2$ levels. The latter response indicated an impairment of vascular drainage. It also highlighted the inadequacy of support surfaces used on operating tables for surgeries, such as fixation of femoral neck fractures, particularly for high risk sick elderly patients (1, 3-4, 7, 8, 55, 57).

There have been a number of studies involving the physiological response of skin tissues to a range of support surfaces (15, 43-44, 53-54). For example, the performance of a prototype alternating pressure air mattress (APAM) was recently evaluated, in terms of its ability to maintain skin viability in a group of 12 healthy volunteers lying in a supine position (15). The mattress included a sacral section supported with alternating low pressure (ALP), with internal pressures values adjusted to subject morphology and BMI, by means of an in-built pressure sensor. Internal mattress pressures and transcutaneous gas tensions at the sacrum and a control site, the scapula, were monitored. Interface pressures were also measured. The skin response to alternating support pressures could most conveniently be divided into three distinct categories, labelled Category 1-3, as presented schematically in Figure 5.

*Figure 5 should be inserted here*

In the majority of test conditions the internal support produced sacral $T_cPO_2$ values which provided adequate viability, either remaining similar to those at the control site (Category 1) or fluctuating in concert with the cycles of the alternating pressure (Category 2). The associated $T_cPCO_2$ levels remained within the normal range for both categories (12). However, in a few cases, particularly when the head of bed was raised ($\geq 45^o$), there was compromise to the skin viability at the sacrum, as reflected in depressed $T_cPO_2$ levels associated with an elevation of $T_cPCO_2$ levels above the normal range (Category 3 in Figure 5). In all cases, interface pressures at the sacrum rarely exceeded 8kPa (60mmHg). It is evident that the prototype mattress could not ensure maintenance of skin viability if a patient was nursed on a mattress with an elevated head of bed angle.

The physiological response was also examined in a group of able-bodied volunteers subjected to intermittent loading at ischial tuberosities during periods of loading and unloading in the sitting posture (Figure 6, unpublished data). The majority of the able bodied volunteers demonstrated a Category 2 response during the loading phase, characterised by a decrease in $T_cPO_2$ levels (Figure 6 left graph). However, in a few cases, a Category 3 response was evident with a marked increase in $T_cPCO_2$ levels (Figure 6 right graph). Both these responses were reversible during the unloaded phases with both gas tensions returning to basal ranges.

*Figure 6: should be inserted here*

In a separate study the status of loaded tissues was monitored, using a combination of physical sensors and sweat biomarkers, at the sacrum of able-bodied volunteers. A range of parameters were estimated from the separate measurements techniques. Results indicated that $T_CPO_2$ levels were progressively reduced when the sacral were subjected to applied pressures of between 5.3kPa (40mmHg) and 16.0kPa (120mmHg). At the higher pressures, this decrease was generally associated with an increase in carbon dioxide above basal levels (12). Close examination of the data revealed a threshold value for loaded $T_CPO_2$, equivalent to a reduction of 60% from unloaded median values, which could be correlated with changes in other physiological parameters. As an example, it was observed that above this threshold, the corresponding $T_cPCO_2$ values were generally in excess of 6.7kPa (50 mmHg) for a significant proportion of the loading period, as indicated in Figure 7. This response is identical to Category 3 response in Figure 5.

The concentrations of both sweat lactate and urea increased considerably as a result of loading. The lactate ratio, loaded compared with unloaded values, were compared to the percentage reduction in $T_CPO_2$ for each individual as illustrated in Figure 7. It was evident that below the threshold value for $T_CPO_2$, there was a relatively small variation, with a mean value of $1.10 \pm 0.16$. By contrast, above this threshold value, lactate ratios regularly exceeded 1.40. Indeed, when the data above this threshold were analysed, the resulting linear model for sweat lactate ($y = 0.023x - 0.33$; $r = 0.58$) was found to be statistically significant ($p<0.01$).

*Figure 7 should be inserted here*

Close examination of the relationship between the lactate ratio and percentage time at which $T_cPCO_2$ was elevated, revealed the presence of two distinct clusters of data**.** Indeed there were

some subjects who exhibited metabolite ratios greater than unity, in association with $T_cPCO_2$ values that did not exceed 6.7kPa (50mmHg) for any of the loaded period. By contrast, other subjects revealed a value for the carbon dioxide parameter which exceeded 37%, equivalent to a Category 3 response, associated with the lactate ratios well in excess of unity. In the latter cases, both sweat lactate and $T_cPCO_2$ may be useful as markers of tissue viability or status as a direct consequence of tissue ischemia (32).

In a theoretical model, it was predicted that the time for the removal of lactic acid from previously ischemic tissues was greater than that necessary for re-oxygenation as a result of reactive hyperaemia (27). This reaffirms the proposition that oxygen may only represent one of a range of markers involved in tissue recovery. Indeed, it can be speculated that both carbon dioxide and lactate are critical in tissue recovery and in the control of related physiological responses, particularly when the skin is exposed to alternating pressures (4).

As previously discussed, ischaemia is followed by a complex biochemical response when the blood supply is re-established and this may result in additional injury to the tissue (23). During ischaemia-reperfusion (I/R), one aspect of biochemical changes involves the irreversible loss of high-energy phosphate (ATP) (Figure 1). In addition, an important mechanism is triggered with the influx of molecular oxygen during reperfusion, which can lead to the formation of unstable and reactive oxygen-derived free radicals, or superoxides. Their presence can cause tissue damage by initiating an inflammatory cascade, resulting in microvascular dysfunction and cell apoptosis. There is considerable evidence in the literature that I/R is associated with purine metabolism in particular, some of its terminal products, which may directly produce cell injury (18). Such purines include allantoin, hypoxanthine, inosine, uric add and xanthine (Figure 1).

Such a hypothesis was examined in a cohort study using sweat biomarkers (5). Sweat was collected initially in an unloaded period and subsequently during four separate 30 minute periods (two loading followed by two reperfusion periods). The results as presented in Figure 8 report the median biomarker ratios of loading compared to unloaded values. It can be seen that for both first and second ischaemic periods, all biomarker ratios were well above unity and, in some cases, exceeded a value of 4.0.

*Figure 8 should be inserted here*

During the first recovery period, the ratio values for xanthine, hypoxanthine and uric acid all remained above unity suggesting that the 30-min period was not sufficient for adequate recovery from the ischaemic insult, although lactate returned to basal levels. It was also noted that the high concentrations of uric acid in previously ischaemic tissue implied the further formation of free radicals, which have been implicated in tissue damage (46, 68). This implies that the sweat purines provide additional information on tissue status to that available from sweat lactate alone. During extended reperfusion, the decrease in hypoxanthine ratio to unity could indicate that the purine metabolism had effectively returned to basal levels.

## 4. Detection Methods for $PCO_2$

Currently there is no single sensor, which fully matches the requirements of a monitoring system for $PCO_2$, specifically for the early detyection of pressure ulcers. The traditional method of monitoring blood perfusion, namely laser doppler flowmetery (LDF) with a parameter in the form of arbitrary units, is well established in both clinical and physiological investigations of blood microcrculation (50,69). However, its output does not reflect the state of the cells and, as

such, does not provide robust markers, paticularly to detect damage during the reperfusion phase. Other potential methods, such as doppler ultrasound flowmetry (35,49) and bioimpedance (48) are limited similarly and, in addition, are sensitive to movements of the probe and the geomerty changes caused by deformation of the tissue.

The well documented monitoring of transcutaneous gase tensions, including $T_cPCO_2$, has proved useful in assessing the relative changes in partial pressures as a result of applied loading to the skin (Figure 6). However, the method is highly dependent on heating the skin in order to lower the solubility of blood gases in tissue. This inevitably increases the metabolic activities, with the potential of causing additional damage to the tissue.

The near infrared spectroscopy (NIRS) (61) method has evolved since the time it was only considered as a trancutaneous monitoring method for tissue oxygenation (30). Nonetheless, the technique is still limited due to movement artefacts, finite measurements area and large costs for routine use in a clinical setting (58).

To interrogate the internal state of the tissues, a minimally invasive technique, microdialysis, may be worthy of consideration (65). It represents a diffusion-based separation method that allows analytes to freely diffuse across a hollow fibre semi-permeable dialysis membrane. This minimally invasive sampling technique has been widely used for *in vivo* biochemical collection from fluid perfused through the tissue. It is currently being used by the authors to interrogate the biomarker changes within loaded tissues. Micro-dialysis might prove valuable as a “gold standard” against which simple “paper-based” systems could be evaluated. Ultimately an ideal indicator of PU risk will carry information about the condition of the cells as a direct representation of the integrity of skin in both loaded and unloaded conditions.

## 5. Conclusions

As pressure ulcers represent a major burden to both individuals and health services, there is a need for a robust detector of tissue damage. Such an indicator for PUs should detect ischemia when any tissue damge is reversible. Based on the current knowledge from cellular, animal and human models, $PCO_2$ does indeed prove to represent, such an indicator both in the ischaemic and reperfusion phases, the latter of which can involve oxygen radical damage. Specifically, animal studies have indicated that the temporal profile of $PCO_2$ can indicate the effectiveness of the wash off processes and perhaps estimate the amount of damage to the tissue. Future challenges involve the development of technological solutions to measure the PCO2 in affected tissue continuously and non-invasively without interfering the metabolism or perfusion of the tissue. Therefore, further research is needed to find “clinically friendly” methods to measure carbon dioxide in tissue.

## LIST OF FIGURES

**Figure 1:** A schematic indicating the mechanical–induced changes in the mechanisms associated with blood and lymph flow which can lead to cell damage (Based on 34,47)

**Figure 2:** Deteminents of tissue carbon dioxide

**Figure 3:** Effects of glucose deprivation on temporal profiles over a 5 day culture period for (a and c) glucose consumption, (b and d) lactate production, for the following experimental conditions (a) and (b) - high glucose (4.5 g/L) and low glucose (1.0 g/L); (c ) and (d) - normoxic undeformed control, hypoxic undeformed, normoxic deformed and hypoxic deformed each at high glucose.

**Figure 4:** The percentage changes in partial pressure of carbon dioxide in porcine muscle tissue as a function of lactate during arterial and venous occlusion and subsequent reperfusion (Based on 36).

**Figure 5:** Schematic representation of three distinct categories of skin response at the sacrum when subjected to alternating pressures provided by a support mattress. Continuous lines represent $T_cPO_2$ responses and dashed lines represent $T_cPCO_2$ responses (Based on 15).

**Figure 6:** Temporal Responses of gas tensions at the ischial tuberosities of two able bodied subjects subjected to periods of unloading and loading in a seated posture.

**Figure 7:** Relationships between both the parameter reflecting compromise levels of $T_cPCO_2$ (black diamonds)and the ratio of sweat lactate concentrations (grey triangles) and the percentage reduction in median $T_cPO_2$ values as a result of sacral loading on able-bodied volunteers. The linear model represents the latter relationship above the threshold value (dashed line) (Based on 32)

**Figure 8:** Median sweat concentrations ratios on the sacrum for able-bodied volunteers during two separate collection periods of mechanical-induced ischaemia followed by two periods of reperfusion (Based on 5)

**References:**


1. Andreozzi, G.M., et al., *Transcutaneous PCO2 level as an index of tissue resistance to ischemia.* Angiology. 46(12): 1097-102, 1995.
2. Arthur, P.G., J.J. Giles, and C.M. Wakeford, *Protein synthesis during oxygen conformance and severe hypoxia in the mouse muscle cell line C2C12.* Biochim Biophys Acta. 1475(1): 83-9, 2000.
3. Bader, D. and S. White, *Monitoring soft tissue viability during orthopaedic surgical procedures.* Age and Ageing. 27: 217-21, 1998.
4. Bader, D.L., *The recovery characteristics of soft tissues following repeated loading.* J Rehabil Res Dev. 27(2): 141-50, 1990.
5. Bader, D.L., et al., *Biochemical status of soft tissue subjected to substained pressure.* Pressure Ulcer research Current and future Perspectives. (Bader, DL. et al. eds)(Springer-Verlag:Berling): 109-128, 2005.
6. Blaisdell, F.W., *The pathophysiology of skeletal muscle ischemia and the reperfusion syndrome: a review.* Cardiovasc Surg. 10(6): 620-30, 2002.
7. Bogie, K.M., I. Nuseibeh, and D.L. Bader, *Early progressive changes in tissue viability in the seated spinal cord injured subject.* Paraplegia. 33(3): 141-7, 1995.
8. Bogie, K.M., I. Nuseibeh, and D.L. Bader, *Transcutaneous gas tensions in the sacrum during the acute phase of spinal cord injury.* Proc Inst Mech Eng H. 206(1): 1-6, 1992.
9. Bouten, C.V., et al., *The etiology of pressure ulcers: skin deep or muscle bound?* Arch Phys Med Rehabil. 84(4): 616-9, 2003.
10. Breton, S., *The cellular Phyiology of Carbonic Anhydrases.* JOP. J Pancreas (online). 2(4 Suppl): 159-164, 2001.

11. Brooks, G.A., *What does glycolysis make and why is it important?* J Appl Physiol 108(6): 1450-1, 2010.

12. Campbell, E.J., C. Dickenson, and J. Slater, *Clinical Physiology*. 4.edition ed. 1974: Blackwell Oxford.

13. Carbone, L.D., et al., *Morbidity following lower extremity fractures in men with spinal cord injury.* Osteoporos Int. 24(8): 2261-7, 2013.

14. Ceelen, K.K., et al., *Compression-induced damage and internal tissue strains are related.* J Biomech. 41(16): 3399-404, 2008.

15. Chai, C.Y. and D.L. Bader, *The physiological response of skin tissues to alternating support pressures in able-bodied subjects.* J Mech Behav Biomed Mater. 28: 427-35, 2013.

16. Dill, D.B., J.H. Talbott, and W.V. Consolanzio, *Blood as physicochemical system.* J.Biol.Chem. 118: 649-666, 1937.

17. Foregger, R., *Joseph Black and the identification of carbon dioxide.* Anesthesiology. 18(2): 257-64, 1957.

18. Fox, K.A., J.E. Saffitz, and P.B. Corr, *Pathophysiology of myocardial reperfusion.* Cardiol Clin. 5(1): 31-48, 1987.

19. Gawlitta, D., et al., *The influence of serum-free culture conditions on skeletal muscle differentiation in a tissue-engineered model.* Tissue Eng Part A. 14(1): 161-71, 2008.

20. Gawlitta, D., et al., *The relative contributions of compression and hypoxia to development of muscle tissue damage: an in vitro study.* Ann Biomed Eng. 35(2): 273-84, 2007.

21. Gawlitta, D., et al., *Temporal differences in the influence of ischemic factors and deformation on the metabolism of engineered skeletal muscle.* J Appl Physiol (1985). 103(2): 464-73, 2007.

563 22. Gjovaag, T., et al., *Assessment of aerobic capacity and walking economy of unilateral*
564 *transfemoral amputees.* Prosthet Orthot Int. 38(2): 140-7, 2014.
565 23. Granger, D.N. and R.J. Korthuis, *Physiologic mechanisms of postischemic tissue*
566 *injury.* Annu Rev Physiol. 57: 311-32, 1995.

567 24. Guideline on Prevention and Treatment for Pressure Ulcers: Clinical Practice
568 Guideline. European Pressure Advisory Panel/ National Pressure Advisory Panel,
569 Quick reference guide, 2014

570 25. Hotter, G., L. Palacios, and A. Sola, *Low O2 and high CO2 in LLC-PK1 cells culture*
571 *mimics renal ischemia-induced apoptosis.* Lab Invest. 84(2): 213-20, 2004.

572 26. Husain, T., *An experimental study of some pressure effects on tissues, with reference*
573 *to the bed-sore problem.* J Pathol Bacteriol. 66(2): 347-58, 1953.
574 27. Hyman, W.A. and R.S. Artigue, *Oxygen and lactic acid transport in skeletal muscle:*
575 *effect of reactive hyperemia.* Ann Biomed Eng. 5(3): 260-72, 1977.
576 28. Iizaka, S., et al., *The impact of malnutrition and nutrition-related factors on the*
577 *development and severity of pressure ulcers in older patients receiving home care.*
578 Clin Nutr. 29(1): 47-53, 2010.
579 29. Jensen, F.B., *Red blood cell pH, the Bohr effect, and other oxygenation-linked*
580 *phenomena in blood O2 and CO2 transport.* Acta Physiol Scand. 182(3): 215-27,
581 2004.
582 30. Jobsis, F.F., *Noninvasive, infrared monitoring of cerebral and myocardial oxygen*
583 *sufficiency and circulatory parameters.* Science. 198(4323): 1264-7, 1977.
584 31. Johnson, B.A. and M.H. Weil, *Redefining ischemia due to circulatory failure as dual*
585 *defects of oxygen deficits and of carbon dioxide excesses.* Crit Care Med. 19(11):
586 1432-8, 1991.

32. Knight, S.L., et al., *Establishing predictive indicators for the status of loaded soft tissues.* J Appl Physiol . 90(6): 2231-7, 2001.

33. Kosiak, M., *Etiology of decubitus ulcers.* Arch Phys Med Rehabil. 42: 19-29, 1961.

34. Krouskop TA. A synthesis of the factors that contribute to pressure sore formation. Med Hypotheses. 11(2):255-67, 1983

35. Kubota, K., et al., *Evaluation of the intratumoral vasculature of hepatocellular carcinoma by power doppler sonography: advantages and disadvantages versus conventional color doppler sonography.* Abdom Imaging. 25(2): 172-8, 2000.

36. Kvarstein, G., et al., *Tissue carbon dioxide tension: A putative specific indicator of ischemia in porcine latissimus dorsi flaps.* Plastic and reconstructive surgery 112(7): 1825-1831, 2004.

37. Kvarstein, G., P. Mirtaheri, and T.I. Tønnessen, *Detection of ischemia by PCO2 before adenosine triphosphate declines in skeletal muscle.* Critical Care Medicine. 32(1): 232-237, 2004.

38. Lefer, A.M., J.C. Daw, and R.M. Berne, *Cardiac and skeletal muscle metabolic energy stores in hemorrhagic shock.* Am J Physiol. 216(3): 483-6, 1969.

39. Lippmann, F., *Metabolic generation and utilization of phosphate bond energy.* Adv Enxymol. 1: 99-162, 1941.

40. Loerakker, S., et al., *Ischemia-reperfusion injury in rat skeletal muscle assessed with T2-weighted and dynamic contrast-enhanced MRI.* Magn Reson Med. 66(2): 528-37, 2011.

41. Loerakker, S., et al., *Temporal effects of mechanical loading on deformation-induced damage in skeletal muscle tissue.* Ann Biomed Eng. 38(8): 2577-87, 2010.

42. Lyder, C.H., *Pressure ulcer prevention and management.* JAMA. 289(2): 223-6, 2003.

43. Mayrovitz, H.N. and N. Sims, *Effects of different cyclic pressurization and relief patterns on heel skin blood perfusion.* Adv Skin Wound Care. 15(4): 158-64, 2002.

44. Mayrovitz, H.N., N. Sims, and M.C. Taylor, *Sacral skin blood perfusion: a factor in pressure ulcers?* Ostomy Wound Manage. 48(6): 34-8, 40-2, 2002.

45. Maziere, S., P. Couturier, and G. Gavazzi, *Impact of functional status on the onset of nosocomial infections in an acute care for elders unit.* J Nutr Health Aging. 17(10): 903-7, 2013.

46. McCord, J.M., *Oxygen-derived free radicals in postischemic tissue injury.* N Engl J Med. 312(3): 159-63, 1985.

47. Miller GE & Seale JL (1981) Lymphatic clearance during compressive loading. Lymphology 14: 161-166

48. Mirtaheri, P., *A novel biomedical sensor for early detection of organ ischemia*, in *Departement of Physyics & Rikshospitalet Univeristy Hospital*. Univeristy of Oslo: Unipub2005

49. Nelson, T.R. and D.H. Pretorius, *The Doppler signal: where does it come from and what does it mean?* AJR Am J Roentgenol. 151(3): 439-47, 1988.

50. Nilsson, G.E., T. Tenland, and P.A. Oberg, *Evaluation of a laser Doppler flowmeter for measurement of tissue blood flow.* IEEE Trans Biomed Eng. 27(10): 597-604, 1980.

51. Nunn, J.F., *Measurement of blood carbon dioxide tension.* Proc R Soc Med. 53: 180-2, 1960.

52. Oomens, C. and D. Bader, *Tissue Engineered Models: A valuable tool in pressure ulcer research.* Bioengineering Research of Chronic Wounds. 1: 249-262, 2009.

53. Rithalia, S.V., *Evaluation of alternating pressure air mattresses: one laboratory-based strategy.* J Tissue Viability. 14(2): 51-8, 2004.

54. Rithalia, S.V. and M. Gonsalkorale, *Quantification of pressure relief using interface pressure and tissue perfusion in alternating pressure air mattresses.* Arch Phys Med Rehabil. 81(10): 1364-9, 2000.

55. Rochat, M.C., et al., *Evaluation of skin viability in dogs, using transcutaneous carbon dioxide and sensor current monitoring.* Am J Vet Res. 54(3): 476-80, 1993.

56. Saleh, M., D. Anthony, and S. Parboteeah, *The impact of pressure ulcer risk assessment on patient outcomes among hospitalised patients.* J Clin Nurs. 18(13): 1923-9, 2009.

57. Salman, M., et al., *Measurement of critical lower limb tissue hypoxia by coupling chemical and optical techniques.* Clin Sci (Lond). 108(2): 159-65, 2005.

58. Scheeren, T.W., P. Schober, and L.A. Schwarte, *Monitoring tissue oxygenation by near infrared spectroscopy (NIRS): background and current applications.* J Clin Monit Comput. 26(4): 279-87, 2012.

59. Schlichtig, R. and S.A. Bowles, *Distinguishing between aerobic and anaerobic appearance of dissolved CO2 in intestine during low flow.* J Appl Physiol (1985). 76(6): 2443-51, 1994.

60. Schlichtig, R., N. Mehta, and T.J. Gayowski, *Tissue-arterial PCO2 difference is a better marker of ischemia than intramural pH (pHi) or arterial pH-pHi difference.* J Crit Care. 11(2): 51-6, 1996.

61. Soller, B.R., et al., *Investigation of muscle pH as an indicator of liver pH and injury from hemorrhagic shock.* J Surg Res. 114(2): 195-201, 2003.

62. Stekelenburg, A., et al., *Role of ischemia and deformation in the onset of compression-induced deep tissue injury: MRI-based studies in a rat model.* J Appl Physiol (1985). 102(5): 2002-11, 2007.

63. Tonnessen, T.I., *Biological basis for PCO2 as a detector of ischemia.* Acta Anaesthesiol Scand. 41(6): 659-69, 1997.

64. Tønnessen, T.I., *Are we able to interpret the different canary songs?* Acta anaesthesiologica scandinavica. 43: 691-694, 1999.

65. Ungerstedt, U., *Microdialysis--principles and applications for studies in animals and man.* J Intern Med. 230(4): 365-73, 1991.

66. Waelgaard, L., et al., *Tissue gas tensions and tissue metabolites for detection of organ hypoperfusion and ischemia.* Acta Anaesthesiol Scand. 56(2): 200-9, 2012.

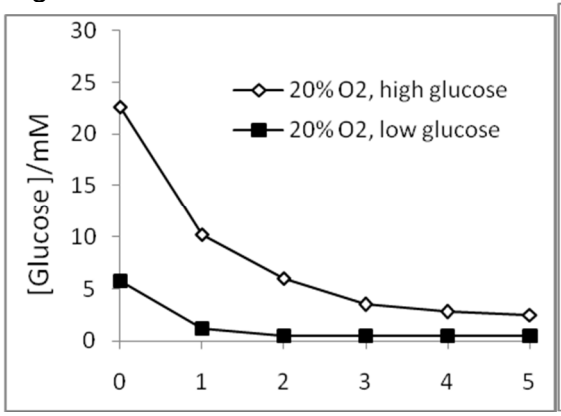
67. Walker, P.M., *Ischemia/reperfusion injury in skeletal muscle.* Ann Vasc Surg. 5(4): 399-402, 1991.

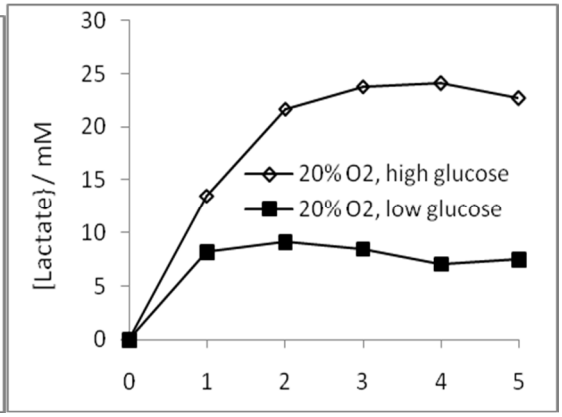
68. Walubo, A., P.J. Smith, and P.I. Folb, *Oxidative stress during antituberculous therapy in young and elderly patients.* Biomed Environ Sci. 8(2): 106-13, 1995.

69. Watkins, D. and G.A. Holloway, Jr., *An instrument to measure cutaneous blood flow using the Doppler shift of laser light.* IEEE Trans Biomed Eng. 25(1): 28-33, 1978.

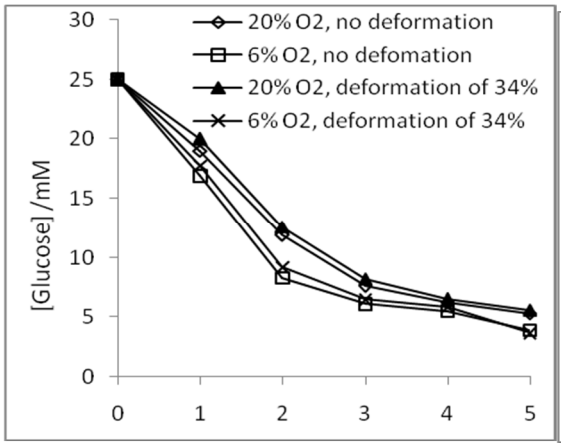
70. Wiedemann, D., et al., *Impact of cold ischemia on mitochondrial function in porcine hearts and blood vessels.* Int J Mol Sci. 14(11): 22042-51, 2013.

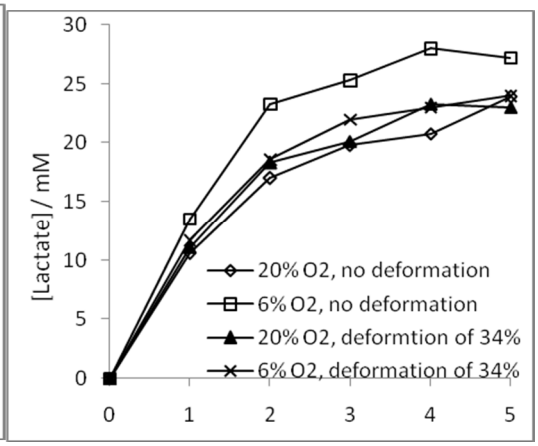
Fig 1.




(a) and (b)




(c ) and (d)

Fig 2

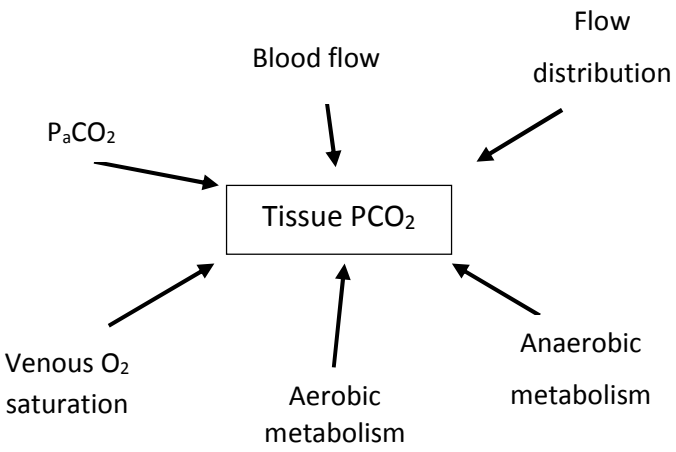
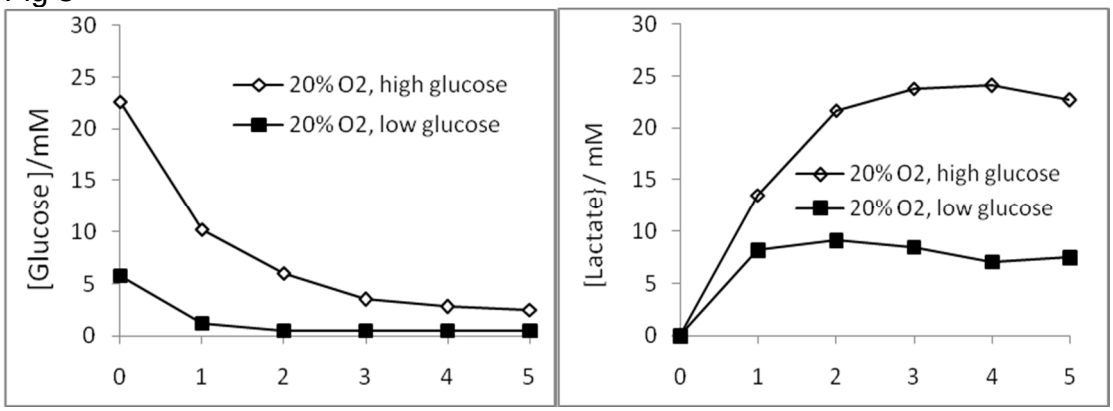
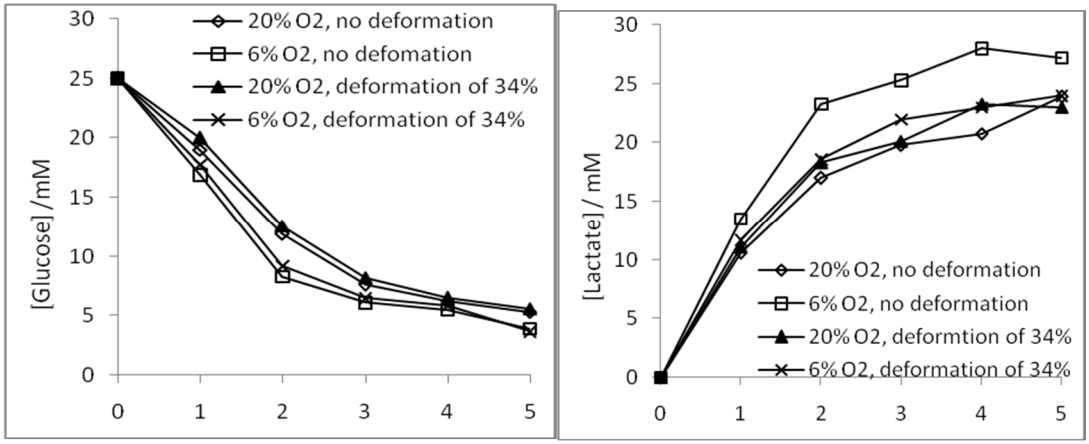

Flow
distribution
Blood flow
$P_aCO_2$
Tissue $PCO_2$
Venous $O_2$
saturation
Aerobic
metabolism
Anaerobic
metabolism

Fig 3




(a) and (b)




(c ) and (d)

**Fig 4**

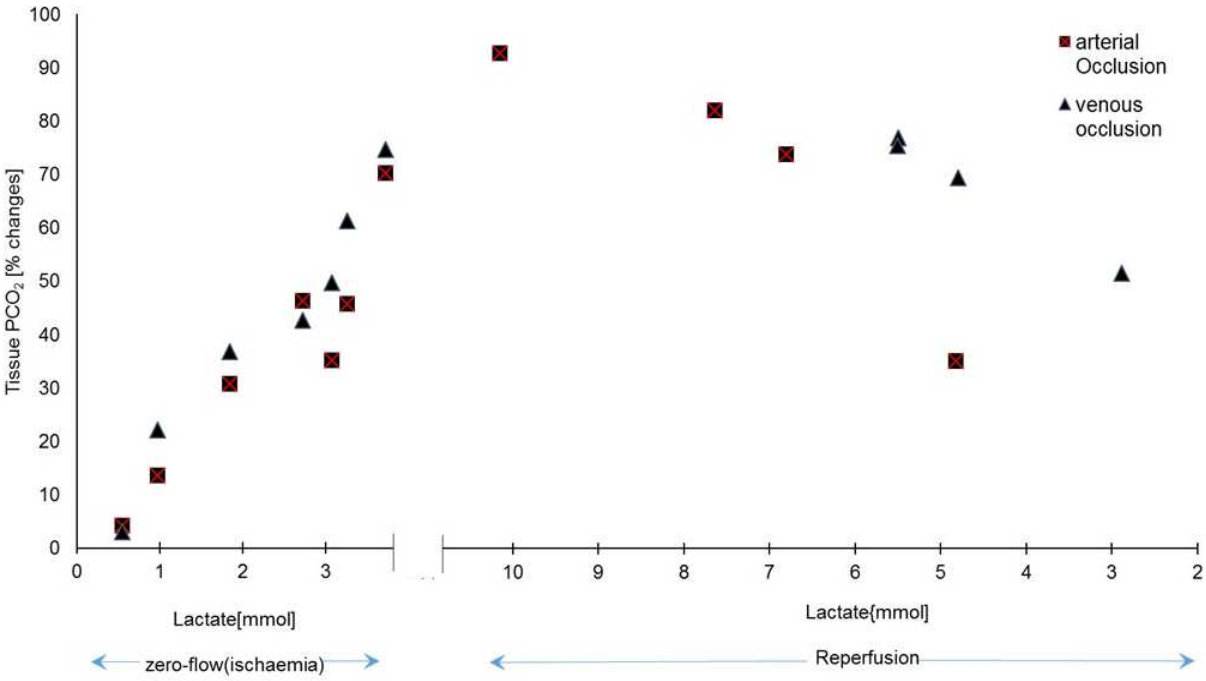

Tissue $PCO_2$ [% changes]
100
90
80
70
60
50
40
30
20
10
0
arterial Occlusion
venous occlusion
0
1
2
3
Lactate[mmol]
zero-flow(ischaemia)
10
9
8
7
6
5
4
3
2
Lactate{mmol]
Reperfusion

**Fig 5**

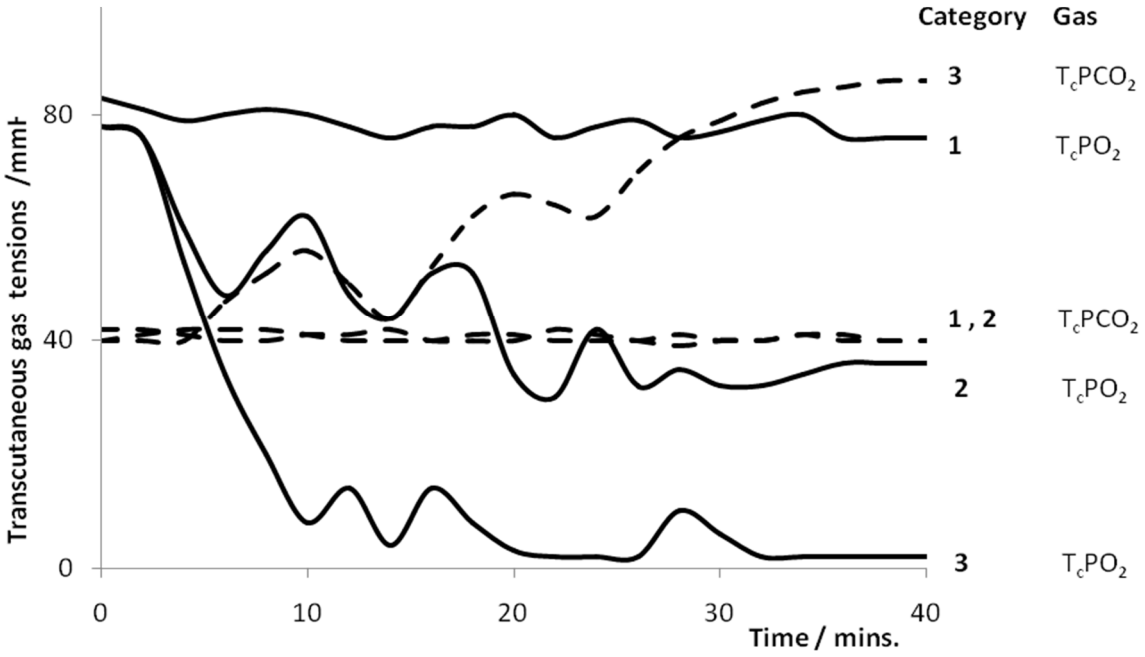

Category
Gas
3
$T_cPCO_2$
1
$T_cPO_2$
1 , 2
$T_cPCO_2$
2
$T_cPO_2$
3
$T_cPO_2$
Transcutaneous gas tensions /mmHg
80
40
0
0
10
20
30
40
Time / mins.

**Fig 6**

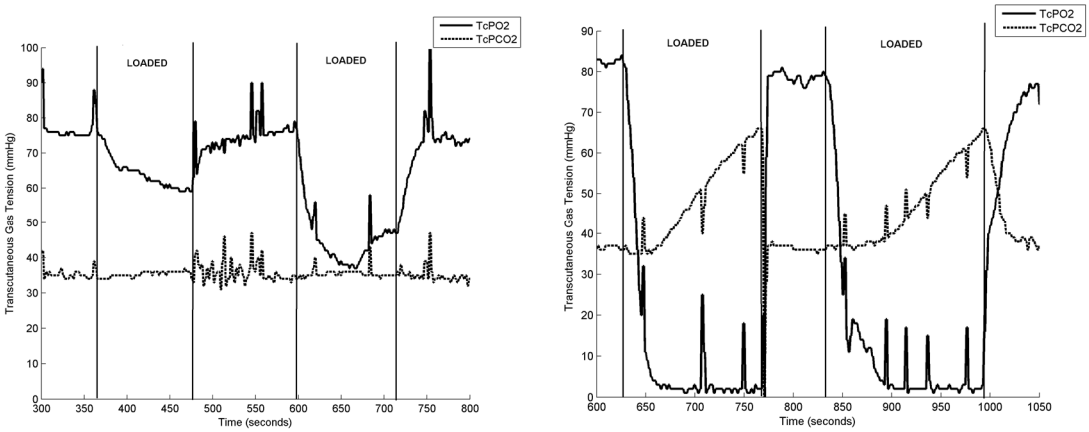
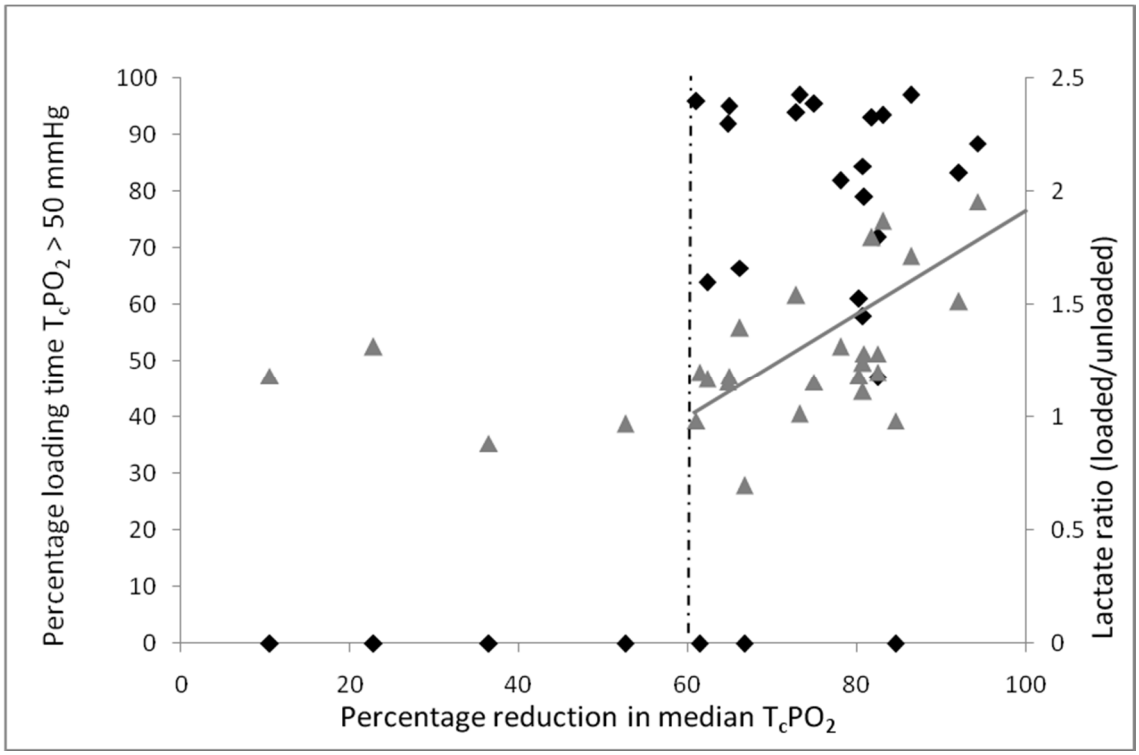
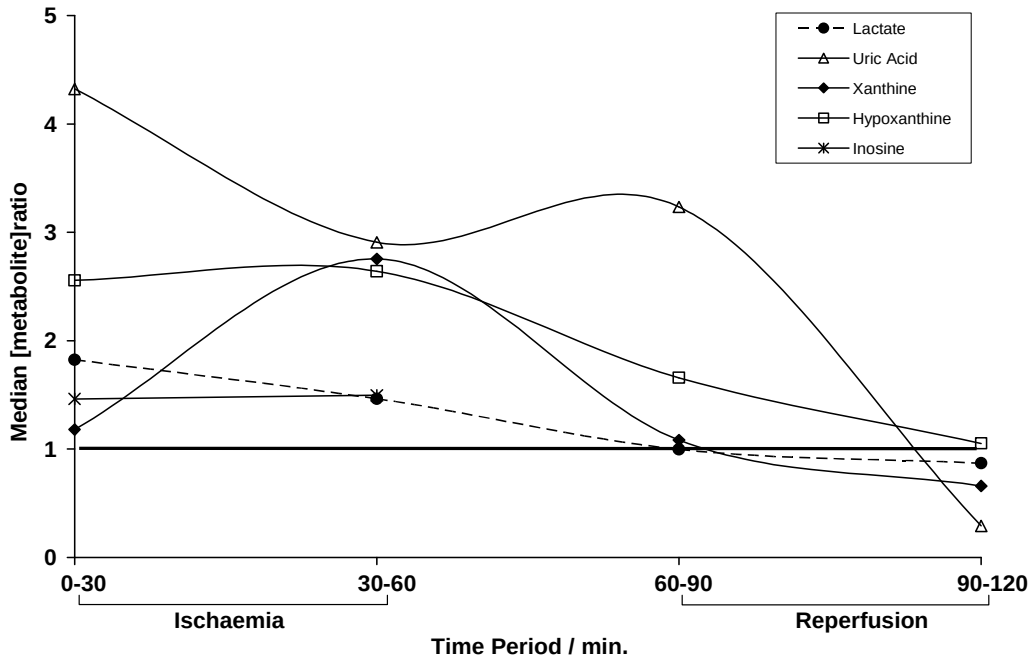

TcPO2
TcPCO2
LOADED
LOADED
Transcutaneous Gas Tension (mmHg)
Time (seconds)
TcPO2
TcPCO2
LOADED
LOADED
Transcutaneous Gas Tension (mmHg)
Time (seconds)

**Fig 7**


Percentage loading time $T_cPO_2$ > 50 mmHg
Lactate ratio (loaded/unloaded)
Percentage reduction in median $T_cPO_2$
0
10
20
30
40
50
60
70
80
90
100
0
0.5
1
1.5
2
2.5
0
20
40
60
80
100

821 **Fig 8**




822

823