Establishing predictive indicators for the status of loaded soft tissues

SARAH L. KNIGHT,¹ RICHARD P. TAYLOR,² ADRIAN A. POLLIACK,² AND DAN L. BADER³

¹I.R.C. in Biomedical Materials, Queen Mary, University of London, London E1 4NS; and
²Department of Clinical Biochemistry, John Radcliffe Hospital, Oxford OX3 9DU, United Kingdom

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Knights, Sarah L., Richard P. Taylor, Adrian A. Polliack, and Dan L. Bader. Establishing predictive indicators for the status of loaded soft tissues. J Appl Physiol 90: 2231–2237, 2001.—Two complementary techniques were employed to assess the soft tissue response to applied pressure. The noninvasive methods involve the simultaneous measurement of the local tensions of oxygen and carbon dioxide (tcPO₂ and tcPCO₂) and the collection and subsequent analysis of sweat collected from the sacrum, a common site for the development of pressure sores. All tests were performed on able-bodied subjects. Results have indicated that oxygen levels (tcPO₂) were lowered in soft tissues subjected to applied pressures of between 40 (5.3 kPa) and 120 mmHg (16.0 kPa). At the higher pressures, this decrease was generally associated with an increase in carbon dioxide levels (tcPCO₂) well above the normal basal levels of 45 mmHg (6 kPa). There were also considerable increases, in some cases up to twofold, in the concentrations of both sweat lactate and urea at the loaded site compared with the unloaded control. By comparing selected parameters, a threshold value for the loaded tcPO₂ was identified, representing a reduction of ~60% from unloaded values. Above this threshold, there was a significant relationship between this parameter and the loaded/unloaded concentration ratios for both sweat metabolites. These parameters may prove useful in identifying those subjects whose soft tissue may be compromised during periods of pressure ischemia.

transcutaneous gas tensions; sweat lactate; sweat urea; soft tissue status; applied pressures; ischemia; pressure sores

THE BREAKDOWN OF SOFT TISSUE leading to the development of pressure sores has implications in terms of both the overall health of an individual and the overall resources required for health care. Although the cause of the condition is multifactorial, it is well established that prolonged-pressure ischemia will affect the viability of soft tissues, leading to their eventual breakdown. There are a host of external factors, generally physical and biochemical in nature, that contribute to the development of tissue breakdown and the formation of pressure sores. However, the presence of pressures applied normally at the interface between the soft tissues and the patient support must be considered as an initiating factor. When prolonged pressure is applied to the skin, the underlying blood vessels are partially or totally occluded, and oxygen and other nutrients are not delivered at a rate sufficient to satisfy the metabolic demands of the tissue. The lymphatic and venous drainage will also be impaired and thus the breakdown products of metabolism accumulate within both the interstitial spaces and the cells (14). As energy stores diminish, there is an increasing possibility of failure of some of the cellular processes and dissipation of ionic gradients across cellular membranes, resulting in cell damage. In the able-bodied subject, regular movement relieves these pressures at local tissue areas and there follows a period of increased blood flow after vascular occlusion, termed reactive hyperemia. However, many immobile and disabled subjects are less able to relieve pressures, and their tissues are often compromised. This makes them a prime group for the development of pressure sores.

Over the last decade, a series of techniques has been proposed to indicate the viability, or status, of soft tissues subjected to periods of loading. These include laser Doppler fluxmetry (19), reflective spectrophotometry (11), and tissue oxygenation. The latter technique has proved an accurate and repeatable method to investigate the effects of loads on tissue viability (15). The early work was extended by Bader and co-workers (1–3) utilizing a transcutaneous electrochemical sensor to examine the effects of a range of loading regimens, including prolonged and repetitive loading, on the local oxygen (tcPO₂) and carbon dioxide (tcPCO₂) tensions, at sites adjacent to bony prominences. The group has also used this technique in a clinical setting to investigate the performance of prescribed support cushions in subacute spinal cord-injured subjects (4). This study employed an assessment criterion for tissue viability based on the percentage of time at which the tcPO₂ and tcPCO₂ values were within acceptable levels. Clear relationships were indicated between depressed levels of tcPO₂ and elevated levels of tcPCO₂ at associated high values of interface pressure (4). However, there are still no clear guidelines as to the precise relationship between compromised tissue gas levels for a set time period and the onset of tissue breakdown.

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An alternative method of assessing tissue status is to examine the metabolite levels in soft tissue areas subjected to pressure ischemia. These metabolites can be transferred via the sweat glands and can be collected at the skin surface. Products arising from the metabolism of the sweat glands themselves may contribute to the components collected from the sweat. A few such studies have been reported (10, 16, 17). The latter studies collected thermally induced sweat by absorption on thin pads attached to the skin surface. This collection system provided minimal distortion and proved ideal for use at a loaded tissue support interface. The authors reported the potential usefulness of monitoring changes in sweat metabolites such as lactate, urea, and urate in soft tissue areas that had been subjected to prolonged loading. In a recent study, the potential of this technique as a clinical tool was demonstrated with a group of debilitated subjects (17).

The present study employs two complementary techniques to assess the soft tissue response to different applied pressures on a group of able-bodied subjects to establish baseline data. The methods involve the simultaneous measurement of the local tcPo$_2$ and tcPCO$_2$ and the collection and subsequent analysis of sweat collected from the sacrum, a common site for the development of pressure sores. Adjacent loaded and unloaded sites were tested to allow for between-subject variation. Several parameters were selected from each of the techniques, and their interrelationships were examined.

MATERIALS AND METHODS

In total, 14 healthy volunteers were used in the study. The mean age of the nine male volunteers was 26.7 yr (range 23–41 yr), and that of the five female volunteers was 27.6 yr (range 22–36 yr). All subjects gave informed consent to their participation in the protocol approved by the local research ethical committee. The experiments were carried out in a physiological chamber controlled at a temperature of 36°C and a humidity of 35%.

Subjects entered the chamber and adopted a prone position on a standard hospital mattress on a King’s fund hospital bed. A loading gantry with a fixed frame was attached to the bed, which contained a freely moveable vertical column, at the base of which was attached a 40-mm Perspex indenter, as shown in Fig. 1. The indenter, a similar design to that previously described (1), incorporated a gas tension electrode at its center surrounded by a solid annular area over which a sweat pad could be attached.

An acclimatization period of 20 min was required for each patient to establish a constant sweat rate (5) at the elevated room temperature. During this period, a combined oxygen and carbon dioxide tension electrode (model D841, Radiometer A/S, Copenhagen, Denmark), connected to a monitor (TCM3, Radiometer A/S), was placed within an indenter and attached to a relatively flat skin surface of the sacrum. A separate indenter, incorporating a second electrode, was attached to an adjacent control site. Both electrodes had been previously calibrated using gas containing 20% O$_2$ and 5% CO$_2$ in nitrogen. The temperature control system of each monitor was set at 44.5°C.

After the equilibration period, sweat pads were applied to the skin around both electrodes. Each sweat pad consisted of an annular piece of filter paper (Whatman Chromatography Ch3, Whatman Paper, Maidstone, UK) with an inner and outer diameter of 12 and 42 mm, respectively, covered with an impermeable membrane of acetate sheeting to prevent evaporation during the collection period. This arrangement was attached to the skin using strips of Blenderm tape (3M Healthcare, Loughborough, UK).

Loads were applied to the experimental test site via the indenter and bed-mounted loading jig. Three nominal pressures were selected as being representative of the physiological range for interface pressures, namely 40, 80, and 120 mmHg (5.3, 10.7, and 16.0 kPa). The control site remained unloaded. Gas tensions were measured continuously at both sites during the test period.

The sweat was collected at both sites over periods of between 30 and 60 min. After the prescribed period, each pad was removed and sealed in a preweighed bottle, which was reweighed to obtain the net sweat rate by difference. The samples were subsequently stored at −20°C before chemical analysis.

Fig. 1. Loading gantry used to apply pressure to the sacrum of a prone subject.
**Biochemical analysis.** The collected sweat was eluted from the filter paper by adding five times the volume of distilled water. The tubes were centrifuged and the eluate was collected from the conical portion of the tube after removal of the paper and perforated disk. The diluted sweat samples were quantitatively analyzed by use of established methods for serum and urine analysis, all of which required modification for the low analyte concentrations or low sweat volumes (22). All measurements were made in duplicate. For both analytes, all samples from each individual in an experiment were analyzed in the same run to minimize analysis error. Lactate and urea were measured by enzymatic assays. Recovery of both analytes was close to 100%, despite a minimum eluate volume of only 0.6 ml. Excellent linearity was reported for both methods (22).

**Data analysis.** There was a range of possible variables that could be used to describe the continuous recordings of gas tensions at both loaded and unloaded sites. With respect to oxygen tensions, the median values were estimated for each prescribed period at both sites. It is well established that there is a wide variation in the unloaded tcPO2 values both at different body sites of the same individuals and between individuals. Thus a normalized parameter, the reduction in median tcPO2 as a percentage of the median unloaded values, was also determined for each individual in a similar manner to that described in a previous study (2).

Arterial carbon dioxide tension in normal healthy soft tissues is in the range of 36–44 mmHg (4.8–5.9 kPa). Our laboratory has previously derived a parameter for carbon dioxide based on the time at which this normal range was exceeded (4). In the present study, the threshold value was set at 50 mmHg (6.7 kPa), and the estimated parameter was the percentage time for which the tcPCO2 levels exceeded this value during the test period.

In addition to absolute values of sweat metabolite concentrations, a ratio of the concentration at both loaded and unloaded tissue sites was also estimated for each of the two metabolites. This approach permitted each individual to act as their own control, thus minimizing intersubject variability.

**RESULTS**

**Effects of applied pressure.** The gas tensions at the control sacral site were found to be within the normal range of between 51 and 90 mmHg (6.8–12.0 kPa) and were unaffected by the loading at the adjacent experimental site. For all applied pressures, the tcPO2 tensions decreased rapidly within the first few minutes and then fluctuated about a constant value in a similar manner to that described previously (1, 3). The median values of tcPO2 tensions at the experimental site generally decreased with applied loading, as summarized in Table 1.

There appeared to be a series of responses to applied load involving changes in carbon dioxide tensions. These could be conveniently divided into three distinct modes, as illustrated in Fig. 2, and described as **mode 1**, minimal change in tcPCO2 from a steady-state value between 36 and 44 mmHg; **mode 2**, some elevation in tcPCO2, reaching a steady-state value of between 50 and 60 mmHg during the loading period; and **mode 3**, a monotonic increase in tcPCO2 with time, reaching values in excess of 80 mmHg. This response was only evident when the equivalent nominal pressures at the sacrum were 80 mmHg or above.

Changes in the selected tcPCO2 parameter with applied pressure are summarized in Table 1. Sweat lactate and urea concentrations generally increased during the loaded period compared with unloaded control values, with increases in median values indicated in Table 1.

**Comparison between selected parameters.** Figure 3 illustrates the relationship between the percentage reduction in tcPO2 and the percentage time at which tcPCO2 exceeds 50 mmHg, equivalent to **modes 2** and **3**. Close examination of these data reveals some distinct trends, based upon an approximate threshold level of 60% reduction in tcPO2 from the unloaded value. Above this threshold value, the tcPCO2 values exceeded 50 mmHg for a significant proportion of the loaded period for 20 out of the 22 cases. By contrast, for cases in which this threshold level for tcPO2 was not exceeded, the associated values of tcPCO2 were consistently below 50 mmHg, corresponding to **mode 1** (Fig. 2).

The mean values of sweat rate were 3.25 ± 1.25 and 3.00 ± 1.22 µl/min for all the subjects at unloaded and loaded sites, respectively. The differences were not statistically significant. The absolute sweat metabolite concentration of lactate at both test sites as a function of the estimated median values of tcPO2 are illustrated in Fig. 4. The graph strongly suggests an inverse relationship between the selected parameters. At the experimental loaded sites, there were elevated concentrations of sweat lactate and urea associated with low median tcPO2 values. A similar relationship was evident between sweat urea and the median value of tcPO2. The sweat lactate and urea data were also examined in ratio form, loaded compared with unloaded values, against percentage reduction in tcPO2 for each individual subject in Figs. 5 and 6, respectively. On the basis of a threshold value of a 60% reduction in tcPO2, as observed in Fig. 3, the data were divided. Below this value, there was a relatively small variation in the ratio values, with a range from 0.89 to 1.36 and a mean value of 1.10 ± 0.16 and 1.14 ± 0.16 for lactate and urea, respectively. There was little obvious trend be-

**Table 1. The effects of applied pressure on selected gas tension parameters**

<table>
<thead>
<tr>
<th>Applied Pressure, mmHg (kPa)</th>
<th>40 (5.3)</th>
<th>80 (10.7)</th>
<th>120 (16.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Reduction of unloaded tcPO2</td>
<td>44 [5,63]</td>
<td>81 [76,83]</td>
<td>77 [66,84]</td>
</tr>
<tr>
<td>% Time at which tcPCO2 exceeds 50 mmHg</td>
<td>0 [0,71]</td>
<td>83 [54,93]</td>
<td>90 [64,96]</td>
</tr>
<tr>
<td>Sweat metabolite concentrations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Increase in lactate</td>
<td>11 [0,21]</td>
<td>29 [4,74]</td>
<td>39 [18,75]</td>
</tr>
<tr>
<td>% Increase in urea</td>
<td>13 [0,37]</td>
<td>32 [1,67]</td>
<td>46 [32,75]</td>
</tr>
</tbody>
</table>

Values represent medians with interquartile ranges given in square brackets; n, number of tests.
tween either metabolite ratio and reduction in oxygen tension. By contrast, above this threshold value ratios regularly exceeded 1.40, particularly at large reductions compared with unloaded oxygen tensions. Indeed, when the data above this threshold were analyzed, the resulting linear models for both sweat metabolites were found to be statistically significant at the 1% level (Figs. 5 and 6).

The lactate and urea data in ratio form were also examined with respect to the percentage time at which $t_cP_{CO_2}$ exceeded 50 mmHg, as illustrated in Figs. 7 and 8, respectively. Two distinct clusters of data points are evident. For example, those subjects exhibiting values of the carbon dioxide parameter between 37 and 100%, equivalent to **modes 2** and **3**, generally produced metabolite ratios well in excess of 1.0, with maximum values up to 2.35. By contrast, there were some subjects who exhibited metabolite ratios greater than unity, in association with $t_cP_{CO_2}$ values that did not exceed 50 mmHg for any of the loaded period.

**DISCUSSION**

The present study confirms the feasibility of assessing the status of sacral tissues subjected to applied loading on a group of healthy volunteers. Two distinct measurement techniques were employed, neither of which can be regarded as a definitive measure of tissue status or viability. For example, the use of transcutaneous gas tensions involves the heating of skin tissues to values in excess of normal physiological temperatures to ensure maximum vasodilatation and reliable gas detection. Thus the gas tensions measured at the skin may not accurately measure the actual oxygen status of the underlying tissue, but will respond to
relative changes. The tcPO2 measurements were expressed as a percentage of the initial value in each experiment to allow for these characteristics and to minimize interindividual variations. The nature and cost of the commercial transcutaneous system also precludes its use as a general screening method for tissue viability. By contrast, the measurement of metabolite concentrations in sweat are simple and noninvasive, but, to date, only a few studies have been reported to examine their potential as markers of tissue status (10, 16, 17).

Current results support previous studies reporting a reduction in tcPO2 in soft tissues subjected to applied loading (1, 2, 15, 21). This finding is clearly related to the occlusion of the blood supply in the localized tissues. The results for tcPCO2 are less conclusive, particularly at relatively small applied pressures at which a mode 1 response (Fig. 2) is evident, involving stable tcPCO2 levels that are similar to basal values for soft tissues. However, at pressures in excess of 80 mmHg (10.7 kPa), the considerable accumulation of carbon dioxide in the localized tissues, often resulting in a mode 3 response, suggested an occlusion of both blood supply and lymphatic drainage. Indeed, in spinal cord-injured subjects with an impaired tissue response to applied load, tcPCO2 levels of this magnitude and above were measured during periods of wheelchair sitting (4).

Any degree of local occlusion of blood supply could lead to anaerobic respiration if oxygen supply does not meet demand. Lactate production would then rise as a consequence of increased glycolytic rate. There was some evidence for this occurring in loaded tissues, in which sweat lactate concentrations were elevated relative to sweat from unloaded tissue (Fig. 4). This relationship is similar to that previously reported in the calf tissues of patients with peripheral occlusive disease and ischemic skin changes (9), whose mean sweat lactate values were ~70% higher than those of a healthy asymptomatic group. In the present study, when external pressure was applied to the tissues, there was a clear relationship between the lactate concentration ratio and the percentage reduction in oxygen tension, coincident with the latter exceeding 60% (Fig. 5). A similar threshold for the accumulation of carbon dioxide was also observed (Fig. 3). Given that tissue tcPCO2 and sweat lactate reflect different aspects of tissue ischemia and yet both exhibited a threshold effect above a 60% reduction in tissue oxygen tension, this degree of reduction may be a critical level for the development of tissue damage. The data in Figs. 5 and 6, showing the relationship between lactate and urea ratios and tissue oxygen tension, were also divided at an assumed 60% reduction in tcPO2, and a broken-stick regression model was fitted to the data sets above and below to investigate this threshold point. The linear models intersected at values of 61% reduction in tcPO2 for lactate and 66% for urea, but it was not possible to derive meaningful confidence intervals for the intersection points with these data.
The degree of ischemia will clearly influence subtle changes in the metabolic parameters. For example, under conditions of mild ischemia associated with low applied pressures, elevated levels of tissue carbon dioxide may be released from the localized areas in the normal manner, thus resulting in tcPCO₂ values below 50 mmHg, i.e., a mode 1 response. However, the same conditions of mild ischemia may lead to an increased glycolytic rate in affected sweat glands, and the associated increase in localized production of lactate will result in sweat ratio values greater than unity. This may account for the moderately elevated lactate ratios at low tcPCO₂ in Fig. 7. In more severe conditions of ischemia, both sweat lactate and tcPCO₂ will be elevated.

The sweat urea concentrations followed similar trends to those observed for lactate. Sweat urea concentrations in unloaded tissues were greater than those associated with plasma urea and increased further with applied loading, resulting in ratio values in excess of unity (Fig. 6). Urea is generally regarded as freely diffusible across cell membranes and should therefore be observed at the same concentration in sweat as in plasma (18). This has been observed in some studies, particularly at high sweat rates (13, 24). However, sweat-to-plasma urea ratios greater than unity have been reported, for example, in sweat collected from an enclosed skin surface, with water reabsorption as a proposed mechanism (20). A low sweat rate, as observed in ischemic tissue (8, 16, 23) is also associated with elevated sweat urea concentrations (6). Whatever the mechanisms for these elevated levels, sweat urea has demonstrated a similar relationship with tcPO₂ to that of lactate.

There was no obvious difference in the response between genders in the 14 healthy subjects, although numbers were relatively small in both groups. Similar findings were reported in our laboratory’s previous study (16) and with a study measuring sodium levels in thermal sweat (5). The effect of aging will clearly influence sweat metabolism with, for example, a reported decrease in sweat rate and significant site variations with age (7). The latter observation may have implications in both sweat metabolite concentrations and the efficiency of sweat collection from aged subjects.

The present study strongly suggests that a reduction in tissue oxygen may be a critical factor in tissue metabolism, with evidence of marked increases in tissue tcPCO₂ and sweat lactate and urea beyond an apparent threshold reduction in tcPO₂ of 60%. Local tissue carbon dioxide and sweat lactate may be useful as markers of tissue viability or status as a direct consequence of tissue ischemia. 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 reoxygenation as a result of reactive hyperemia (12). Therefore, oxygen may be only one of a range of species involved in tissue recovery. In addition to lactate and urea, present studies are in progress involving other potential markers of tissue status such as sweat purines.

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Present address for S. L. Knight: Institute of Urology and Nephrology, Royal Free and University College Medical School, University College London, London W1P 7PN, UK.

Present address for A. A. Polliack: Los Amigos Research and Education Institute, Rancho Los Amigos Medical Center, Downey, CA 90242.

REFERENCES


