Analysis of sweat during soft tissue breakdown following pressure ischemia

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Abstract—This paper examines the nature of tissue metabolites collected in thermally induced sweat following the application of different loading regimes on the soft tissues of able-bodied subjects. Loading was produced by 1) external application on the forearm via both a tourniquet and a uniaxial indenter system, and 2) ischial support on a wheelchair and sacral support on an examination bed. In each case sweat pads were attached to the tissue areas of a group of able-bodied subjects and interface pressures were recorded. After a prescribed period, the pads were removed and a quantitative analysis of a range of metabolites was performed. Results indicated that tissues subjected to pressure ischemia produced a general increase in concentrations of lactate, chloride, urea, and urate associated with a decreased sweat rate. In the reperfusion phase, some of these metabolites returned to unloaded levels. It is proposed that specific metabolites may be used as an indicator of soft tissue damage.

Key words: pressure ischemia, pressure sores, soft tissue breakdown, sweat metabolism, thermal sweating.

INTRODUCTION

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 cause. 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. To survive, the cells must draw upon their stores of energy. The lymphatic drainage will also be impaired; thus, the breakdown products of metabolism accumulate within both the interstitial spaces and the cells. As energy stores diminish, the cellular processes begin to fail, ionic gradients across cellular membranes begin to dissipate and cell necrosis can occur.

The nature of the loading of the soft tissues is also an important determinant. It is well-known that body tissues can support high levels of hydrostatic pressure, with equal components in all directions, with no resulting tissue damage. This is well illustrated by deep sea divers, who are regularly exposed to hydrostatic pressures in excess of 100 kPa (750 mmHg) for prolonged periods with no ill effects on the soft tissues. This is because the external pressures that develop as the depth of the dive increases are balanced by the increasing pressures of the gases breathed, resulting in no pressure differential between the tissues and the applied external pressure (1). However, if the external pressures are applied nonuniformly, tissue distortion leading to localized tissue damage can result. This occurs in many
situations in which the body interfaces externally with load-supporting devices.

The soft tissues exhibit viscoelastic behavior; thus, the effects of loading will depend upon the rate and period of application as well as the magnitude of the load. The nature of the tissue recovery is determined by the resilience of the specific tissues and the tissue structures, including the blood and lymph vessels (2). Short-term loading generally produces elastic recovery on removal of the applied load, while long-term loading produces creep and requires a longer time for complete tissue recovery. It has been well established that tissue damage is often apparent following prolonged loading even at relatively low pressure intensities (3). Any significant period of loading will result in vascular and arterial occlusion. On load removal, there will be a period of increased blood flow through the tissues which had been ischemic. This phenomenon, termed reactive hyperemia, is a consequence of a local regulatory mechanism whereby the arterioles are dilated and the resistance to blood flow is reduced.

The microenvironment of the subject/support interface greatly affects the likelihood of tissue breakdown leading to the formation of a pressure sore. High moisture levels at the interface can be produced by incontinence or perspiration on impermeable cushion covers. This excess moisture can lead to maceration of the epidermis, a precursor of tissue breakdown which is accelerated by an alkaline environment.

There has been considerable speculation about the possible role of certain metabolites in tissue necrosis following insult of the soft tissues. The identity of the vasodilator agents remains unclear, although there are many possible candidates, such as histamine, adenosine, and prostaglandin-like substances, for the role. It has been shown that the nature of their release will be affected by the duration of ischemia (4). The role of oxygen-free radicals during reperfusion of ischemic tissues has also recently been discussed (5). The generation of these radicals is associated with the production of a series of metabolites, including urate and hypoxanthine.

Related Studies

The Oxford Pressure Monitor provides an accurate measure of the pressure distribution at the patient support interface (6). However, the measurement of interface pressure alone is not sufficient to alert the clinician to potential areas of tissue breakdown. For this, some measure of tissue damage is required which reflects an inadequate supply of nutrients or removal of metabolic products.

Transcutaneous gas monitoring has proved an accurate and repeatable method for investigating the effects of prolonged loading on the viability of tissues overlying bony prominences on a mixed group of debilitated subjects, who were considered to be particularly prone to the development of pressure sores (7). Results indicated a wide range of integrated pressure and time which the soft tissues will tolerate. The applied pressures to produce, for example, a 50 percent reduction in unloaded resting value of transcutaneous oxygen tension (TcPO2) ranged from 3.0 kPa (22 mmHg) to 12.2 kPa (92 mmHg). This emphasized the individual nature of the tissue response, which should be determined before clinical guidelines of safe pressure levels can be established. The level of TcPO2 gives some idea of tissue viability, but there is still no absolute evidence that prolonged periods below a specific threshold level of TcPO2 will inevitably lead to tissue damage.

Very little work has been reported on the analysis of sweat under ischemic conditions. Of the few reported studies, Van Heyningen and Weiner (8) examined the effects of ischemia on sweat composition and demonstrated a decreased sweat rate following tourniquet ischemia of the arm. They also indicated that prolonged arterial occlusion resulted in an increase in the lactate and urea concentrations. It is worthy of note that the absolute levels of these metabolites measured in sweat were an order of magnitude greater than the comparative levels generally observed in blood. In an important comparative study, Ferguson-Pell and Hagisawa (9) demonstrated the feasibility of measuring lactate and sodium concentrations in sweat collected locally from the volar aspect of the forearm, using electrochemical stimulation techniques. Elevated levels of sweat lactate were recorded during local tissue indentation which returned to basal levels following load removal in a group of able-bodied subjects. However, the system employed, the Macroduct sweat collector, uses an artificial means of inducing localized sweat via the introduction of pilocarpine nitrate using iontophoresis and its bulk size pre-
cludes its use at the loaded body support interface. Also, the volume of sweat collected in this study was relatively low with mean values not exceeding 40 μl.

The sweat glands are simple tubular glands, whose major functions are to control temperature and conductivity by secretion of water and electrolytes (10). Sweat is a hypotonic solution of sodium and chloride ions in water, together with other constituents including lactate, urea, and potassium. The presence of these metabolites accounts for about 95 percent of the osmotically active substances in sweat (8). Sweat is a true secretion and not simply a filtrate. It is controlled by mechanisms that are present in almost all levels of the nervous system. The hypothalamus is the primary center for temperature regulation of the body (11). However, there is evidence that local cutaneous thermoreceptors are the initial reflex response to exercise and may trigger the hypothalamus to act on the pituitary gland which, in turn, stimulates the sweat glands.

Objectives

This study describes two separate investigations to assess the sweat collected at the soft tissues of able-bodied subjects both during and after periods of pressure ischemia. In the first, the effects of two different loading regimes on forearm tissues were determined. The second study involved monitoring subjects who were supported either sitting in a wheelchair or lying supine on an examination bed, which provided loading at the ischial tuberosity and the sacral tissues, respectively. Sweat was analyzed for potential biochemical markers of compromised tissue viability in the loaded tissues.

METHODS AND MATERIALS

Sweat collection was achieved with cellulose chromatography paper (Whatman Paper Ltd., Maidstone, UK), which is known to be inert in contact with moist skin. For each test, a sweat pad of 5,000 mm² was used, an area which could retain a maximum sweat volume of 1 ml. Skin preparation of the test site involved washing the skin with soap and rinsing with distilled water. Once the site was completely dry, the sweat pad was placed over the test site and folded over several times to achieve a localized collection area of typically 1,600 mm². In order to minimize evaporation from the sweat pad, a 50 μm-thick transparent polypropylene sheet (455 SCB 50 Shorko, Cortauils Films, Swindon, UK) of larger dimensions than the sweat pad was placed over it. The polypropylene sheet was hydrophobic in nature and thus, neither reacted with nor retained any of the collected sweat. The sheet was sealed to the skin with surgical tape (Blenderm Surgical Tape, 3M Health Care Ltd., Loughborough, UK). This tape is transparent, occlusive to moisture vapor transmission and hypoallergenic to skin.

Before the tests, each subject was acclimatized in an assessment room heated to a controlled temperature of 30°C for a 15-minute period; then the sweat collection pads were attached. This temperature was maintained for the duration of the test to facilitate thermal sweating of each subject.

Effects of Different Loading Regimes on Tissues of the Forearm

This series of tests required the subject to remain seated for the duration of the trial with both arms supported on a bead-filled support cushion, resting on a table at the same approximate level as the heart. A sweat pad was attached to the volar aspect of each forearm when the subject began sweating. Two distinct loading regimes were applied to the soft tissues:

1. Ischemic loading was achieved by applying a sphygmomanometer cuff around the biceps of the left arm, which was then inflated to a pressure of 20 kPa (150 mmHg). Sweat collection pads were applied to the volar aspect of both forearms with the right arm as control. Sweat was collected from control arms for 30 minutes, but on the experimental arm, the pain and discomfort in the forearm caused by the cuff could be tolerated for only 10 minutes, at which time the cuff and sweat pad were removed. Upon removal, the sweat pads were inserted immediately into separate airtight containers.

2. Uniaxial loading was applied via a rigid indenter positioned immediately beneath a loading pan which was incorporated in a balanced beam counterbalanced at the other end by a moveable weight (Figure 1). The 33 mm indenter was curved at its edges to minimize the effects of high stresses at the periphery of the indenter/soft tissue contact area (2). Careful alignment of the experimental system ensured that the loading was perpendicular to the skin surface, thereby avoiding significant shear forces, directly above the experimental sweat pad.
Figure 1.
Uniaxial loading of tissues by using an indenter on the subject’s forearm.

The pressure at the interface between indenter and sweat pad was measured for each applied load using one cell of the Oxford Pressure Monitor (Talley Medical Equipment Ltd., Romsey, UK). Weights of 1 kg each were incrementally placed in the loading pan, until the interface pressure reached a value of approximately 20 kPa (150 mmHg). An applied pressure of this magnitude for a total test period of 30 minutes was considered to be sufficient to establish localized tissue ischemia (9). None of the subjects complained of any discomfort during the test. After 30 minutes, the indenter on the experimental arm was removed and both sweat pads were immediately removed and inserted into separate containers.

A subsequent test involved the collection of sweat during the postischemic or reperfusion period. On removal of the indenter, the existing sweat pad on the experimental arm was replaced by a fresh pad located over the test site. After a further 10 minutes, the second pad was removed to obtain sweat secreted during this reperfusion phase.

Analysis was restricted to the measurement of lactate and chloride levels in the collected sweat. Both sets of measurements were performed on six able-bodied subjects (5 males and 1 female), aged 22 to 26 years, with no underlying medical problems.

Effects of Wheelchair Sitting and Supine Lying on Soft Tissues

Initially, subjects lay prone on a standard examination bed with a sweat pad attached either over the sacral or ischial tissues. After approximately 40 minutes, sufficient sweat had been collected at the test sites. The pad was then removed and inserted into a separate container and a fresh pad was attached. The subjects then underwent one of two separate experimental test procedures as described below.

1. The subject was seated directly on the canvas of a standard wheelchair with sweat pads attached to the ischium, as shown in Figure 2. The interface pressure distribution across the ischium was continuously measured by the Oxford Pressure Monitor. This support was maintained for a period of 55 minutes—sufficient time to create minimal ischemia. Upon completion of this stage, the sweat pad was removed and replaced by a fresh pad, and the subject reassumed the prone position. After approximately 25 minutes, sufficient sweat had been collected at the test site during this reperfusion stage. The pad was then removed and placed in a container and the test was terminated.

2. The subject attained a supine position on the examination bed (Figure 3) with a sweat pad attached to the loaded sacrum. The interface pressure distribution across the sacral area was continu-
A subject lying supine on an examination bed, with loading of the sacral tissues.

Figure 3.

Biochemical Analysis

Following the trial, the 30 ml plastic containers (Universal, Sterilin Ltd., Hounslow, UK) were reweighed to obtain the net sweat weight by difference, and the samples subsequently stored at -20°C prior to chemical analysis. The collected sweat was eluted by adding five times the volume of distilled water. The diluted sweat samples were quantitatively analyzed using established methods for serum and urine analysis, all of which required modification for the low analyte concentrations or sweat volumes available. Lactate, urate, urea, and chloride assays were performed on a Cobas FARA automated centrifugal analyzer (Roche Diagnostic Systems, Welwyn Garden City, UK). For each analyte, all samples from each patient in an experiment were analyzed in the same analytical run to minimize analysis error.

Lactate was measured by a lactate dehydrogenase spectrophotometric method from NADH production at 340 nm (12). Urate was measured by a uricase method (Sigma Chemical Co. Ltd., Poole, UK) by measurement of allantoin production at 292 nm. Urea was measured by a urease method as the decrease in absorbance at 340 nm (13). Reagents were from Technicon (Bayer Diagnostics Ltd., Basingstoke, UK). Chloride was measured by the mercuric thiocyanate method (14) with reagents from Nycomed (UK) Ltd, Birmingham, UK.

Sodium and potassium were measured using a Corning 455 flame photometer (Ciba Corning Diagnostics Ltd., Halstead, UK) with lithium internal standard. The filter paper eluate was centrifuged and aspirated directly without further dilution.

Within-run imprecisions, as estimated from the coefficient of variation, at the specified concentrations were as follows:

- Lactate 1.9% at 4.90 mmol/L, n = 21;
- Urea 2.3% at 1.98 mmol/L, n = 21;
- Sodium 2.0% at 4.79 mmol/L, n = 22;
- Urate 8.6% at 24.0 μmol/L, n = 21;
- Chloride 0.7% at 4.22 mmol/L, n = 21;
- Potassium 4.3% at 0.81 mmol/L, n = 22

Data Analysis

A mean value and a standard deviation were calculated for each set of data. In the first study, statistical analysis was performed to examine any differences in the levels of lactate and chloride found in sweat when comparing sample values between the three distinct collection periods. For each subject, a paired Student’s t-test was employed. For the second study, involving six metabolites measured in sweat, an unpaired Student’s t-test was employed. The criterion level of significance was set at P ≤ 0.05.

RESULTS

Effects of Different Loading Regimes on Tissues of the Forearm

A comparison of the effects of hydrostatic loading with uniaxial loading is presented in Figure 4.
Ischemic loading produced a statistically significant increase in chloride levels compared to control values, but no difference between lactate levels. By comparison, uniaxial loading produced a statistically significant increase in lactate levels compared to control samples, but no difference between chloride levels. Figure 5 indicates the levels of lactate and chloride during the three distinct collection periods of the indenter trial. Following the removal of uniaxial loading, the lactate levels decreased rapidly toward basal levels, while the chloride levels appeared to increase only slightly during this reperfusion phase. A summary of the findings with appropriate statistical test results are presented in Table 1. It can be seen that the sweat rate decreased during the ischemic phase for both hydrostatic and uniaxial loading, although the decrease was only significant for the latter case. Table 1 also compares present findings with the results of previous work related to hydrostatic (8), and uniaxial loading (9).

Effects of Wheelchair Sitting and Supine Lying on Soft Tissues

The biochemical composition of sweat collected at tissue sites as a direct result of either sitting in a wheelchair or lying supine on an examination bed is illustrated in Figure 6 and Figure 7, respectively. These changes were associated with mean interface pressures of $7.6 \pm 1.2$ kPa ($57 \pm 9$ mmHg) at the ischium during wheelchair sitting, and $5.2 \pm 1.5$ kPa ($39 \pm 11$ mmHg) at the sacrum during support by the examination bed. In the wheelchair test, there was an increase in lactate and urea levels during ischemia, of 24 percent and 27 percent, respectively; these values returning toward basal levels during the reperfusion phase. There was an increase in both sodium and chloride levels during ischemia and this continued during the reperfusion phase to a total increase of 56 percent and 44 percent, respectively. Urate levels showed a statistically significant increase during tissue ischemia, but this increase was reduced during reperfusion. Potassium levels changed little throughout the three phases of the experiment. The study involving the supine position demonstrated similar increases in chloride, sodium, and urea, but no significant increases in lactate and urate were observed in the ischemic phase.

DISCUSSION

This study reports on the use of a simple, robust, inexpensive collection system, which provides a reliable and repeatable measurement of localized metabolite levels in sweat. It can also be used at the loaded interface between the soft tissues and the support surface without disturbing the surface characteristics. Thus, it has many of the design specifications of an ideal system discussed by Ferguson-Pell and Hagisawa (9). It also has the advantage of being sensitive to thermal sweating, as
Table 1.
Comparative results of sweat rate and sweat lactate under different loading regimes.

<table>
<thead>
<tr>
<th>Sweat Parameter in Collection Period</th>
<th>Sweat rate (µL/min)</th>
<th>Sweat lactate (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ischemic</td>
<td>Control</td>
</tr>
<tr>
<td>UNIAXIAL LOADING</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRESENT STUDY (30 min, 150 mm Hg)</td>
<td>Mean</td>
<td>9.1</td>
</tr>
<tr>
<td>forearm</td>
<td>SD</td>
<td>7.4</td>
</tr>
<tr>
<td>thermal to 30°C</td>
<td>n</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>--------</td>
</tr>
<tr>
<td>1600 mm²</td>
<td></td>
<td>--------</td>
</tr>
<tr>
<td>FERGUSON-PELL and HAGISAWA (9)</td>
<td>Mean</td>
<td>0.62</td>
</tr>
<tr>
<td>(30 min, 150 mm Hg)</td>
<td>SD</td>
<td>0.52</td>
</tr>
<tr>
<td>forearm</td>
<td>n</td>
<td>9</td>
</tr>
<tr>
<td>electrical stimulation</td>
<td>p</td>
<td>--------</td>
</tr>
<tr>
<td>500 mm²</td>
<td></td>
<td>--------</td>
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<tr>
<td>HYDROSTATIC LOADING</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRESENT STUDY (10 min, 150 mm Hg)</td>
<td>Mean</td>
<td>10.4</td>
</tr>
<tr>
<td>biceps</td>
<td>SD</td>
<td>2.5</td>
</tr>
<tr>
<td>thermal to 30°C</td>
<td>n</td>
<td>3</td>
</tr>
<tr>
<td>1600 mm²</td>
<td>p</td>
<td>--------</td>
</tr>
<tr>
<td>VAN HEYNINGEN and WEINER (8)</td>
<td>Mean</td>
<td>130</td>
</tr>
<tr>
<td>(25 min, 200 mm Hg)</td>
<td>SD</td>
<td>42</td>
</tr>
<tr>
<td>biceps</td>
<td>n</td>
<td>2</td>
</tr>
<tr>
<td>thermal to 32°C</td>
<td>p</td>
<td>--------</td>
</tr>
<tr>
<td>with continual exercise</td>
<td></td>
<td></td>
</tr>
<tr>
<td>whole arm</td>
<td></td>
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</tbody>
</table>

There were clear differences between the tissue responses to the two distinct loading regimes (Figure 4 and Table 1). Ischemic loading as a result of the sphygmomanometer cuff did not produce a significant increase in lactate concentration in sweat when compared to unloaded conditions. This would suggest that severe hypoxia, as a result of ischemia and subsequent accumulation of products of anaerobic metabolism, and typified by increased lactate levels (15), was not achieved. This may be explained by the opposed to sweat collected by iontophoresis or excessive exercise regimes.
lack of pressure differential between the internal tissues and the applied pressure. This would produce a uniform pressure on normal healthy tissues which, in itself, would not produce tissue distortion and subsequent damage. By comparison, lactate levels were significantly elevated in tissue under the uniaxial compression regime. This would strongly imply the achievement of tissue hypoxia and ischemia with associated tissue distortion.

There were also differences between the chloride concentrations measured in sweat collected from tissues subjected to the two distinct loading regimes (Figure 4). The chloride levels in sweat are influenced by several factors, including sweat rate and mechanical stress. Thus, a decrease in sweat rate is associated with a decrease in sweat chloride levels (16). In addition, sweat rate, and therefore chloride concentration, has been shown to be directly correlated with skin temperature (16). Conversely, a stress gradient within the loaded tissues can drive the transport of osmotically active molecules, such as sodium chloride, from interstitial fluid into the lumen of the sweat coils (17). Therefore, loaded tissues would be expected to excrete larger amounts of chloride. This may explain the increase in chloride in tissues subjected to hydrostatic loading, where the sweat rate remained relatively unchanged from control values (Table 1). However, with uniaxial loading, the chloride levels remained unchanged from control values, which may be explained by the significantly decreased sweat rate during ischemia.

Figure 5 shows that lactate measured in samples from reperfused tissues had returned to basal levels after the period of tissue ischemia. This indicated that oxygenation of tissues induced a rapid return to aerobic respiration of tissues. With respect to chloride levels measured during reperfusion, their values were not significantly different from control values, where the sweat rate was unchanged, and from ischemic values, where the sweat rate was reduced. These findings reaffirm the suggestion that the level of sweat chloride is a function of both sweat rate and the mechanical state of the tissues.

The present results generally follow trends observed in previous studies (Table 1). The sweat rate decreased significantly during ischemia and returned to basal levels on reperfusion, in accord with previous reports. However, there are clear differences, for example, when comparing the abso-
olute sweat rates (8,9). These could be attributable to the means of sweat induction, area of sweat collection, and the fact that in the Van Heyningen and Weiner study subjects were tested during a period of physical exercise (8,9).

Both the wheelchair and bed support surfaces provided mean interface pressures at the ischium and sacrum respectively, which were representative of the clinical setting. The condition of pressure ischemia was more extreme in the case of wheelchair sitting. The metabolites measured showed different patterns of response to ischemia and reperfusion (Figure 6 and Figure 7). The trends in lactate and urea levels exhibited during the three phases were similar. The use of sweat lactate has recently been suggested as a good indicator in the evaluation of the severity of peripheral occlusive arterial disease and in assessing the efficiency of vasoactive drug treatment (15). The mechanisms underlying the changes in urea concentrations are unclear. It is known to be inversely related to sweat rate (18) and may, therefore, prove to be a useful additional indicator of tissue ischemia.

In addition, sodium and chloride values showed similar trends for both support surfaces. The complexity of the factors influencing chloride and sodium would render the interpretation of changes difficult in the assessment of tissue damage.

The only statistically significant difference involved the levels of sweat urate. Thus, there was a significant increase in sweat urate in the ischemic phase during wheelchair sitting. During ischemia, urate production may be increased as a result of degradative loss of high energy phosphate metabolites (19). There is also a link between urate and the production of oxygen free radicals in tissue damage (5). This may be relevant to the reperfusion period. Sweat urate may well prove an important marker in future investigations as an indicator of tissue damage.

There were no obvious differences in the response of soft tissues between the male and female subjects, although numbers were small in both groups. Previous studies have shown similar levels of sodium in thermal sweat (20) for both sexes, although changes in sweat rate as a result of aging have recently been reported (21).

Further work is now in progress using this methodology to analyze sweat samples collected from tissues subjected to varying amounts of pressure ischemia. This technique is also being employed to study the sweat characteristics of a range of subjects, during both surgical and rehabilitation procedures, who are at specific risk of developing pressure sores.

REFERENCES