Title: An evaluation of dermal microcirculatory occlusion under repeated mechanical loads: implication of lymphatic impairment in pressure ulcers

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Abstract:

Pressure ulcers represent a significant challenge to individuals with compromised mobility, affecting their quality of life and causing a substantive burden on healthcare providers. They are caused by prolonged mechanical loads deforming the underlying soft tissues. However, the thresholds that result in impairment of dermal microvascular and lymphatic vessels is unknown. The present study was designed to characterise the simultaneous response of microvascular and lymphatic structures under repeated mechanical loading.

The effects of two distinct loading/unloading cycles involving i) incremental loads of 30, 60 and 90mmHg and ii) repeated loads of 30, 30 and 30mmHg were evaluated on a cohort of able-bodied volunteers. Microvascular response involved the monitoring of transcutaneous gas tensions, while dermal lymphatic activity was estimated from Near Infrared Fluoroscopy Lymphatic Imaging. The respective responses were compared during each load cycle and thresholds for occlusion were explored.

During incremental loading, changes in microvascular response were dependent on the magnitudes, with the lowest pressure (30mmHg) resulting in a reduction in transcutaneous oxygen tension only, while the highest pressure affected both oxygen and carbon dioxide values indicative of ischemia in most participants (54%). By contrast, lymphatics revealed near total occlusion at the lowest pressure of 30mmHg. Although there were inter-subject differences in the microvascular and lymphatic responses, temporal trends consistently revealed partial or full impairment under load, with complete recovery during off-loading.

The study provided a simultaneous evaluation of microvascular and lymphatic response to repeated load cycles. The pressure required to cause local ischemia and lymphatic occlusion differed between individuals in the able-bodied cohort. This highlights the need for personalised care strategies, where patients are assessed and monitored depending on their individual tolerance to prolonged pressures.

1. Introduction:

Pressure ulcers (PUs) are defined as ”a localised 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” [1]. PUs represent a major burden to populations worldwide and have been attributed with the highest disability index in comparison to other dermatological conditions [2]. Despite increased awareness and interventions to improve the effectiveness of preventative strategies, the incidence in both the acute and community settings has remained unacceptably high, contributing to an estimated chronic wound care burden of £5 billion p.a. in the UK [3].

The sustained external pressure and shear forces experienced by immobile individuals in the lying and sitting postures can at result in internal tissue deformations, causing two major damage mechanisms [4]. First, ischaemic damage from the occlusion of blood and lymph vessels, which occurs at relatively low internal tissue strains. The resulting deficit of vital nutrients and accumulation of toxic metabolites lead to tissue damage, a process that can take several hours to develop [24]. Alternatively, direct cell damage resulting from high internal strains, which can occur within tens of minutes. Seminal research in the field identified an association between tissue damage and both the magnitude and duration of tissue deformations [5], which reflects that tissue damage is possible at high deformations for short periods of loading as well as at lower pressures applied for prolonged periods [6]. This relationship inevitably depends on the health status of the individual and their respective tissue tolerance, which is influenced by age, co-morbidities and nutritional status [7].

To date, an international team of bioengineers have investigated the aetiology of pressure ulcers creating a framework of understanding for the mechanisms, which lead to skin and subdermal tissue damage [7]. Such research encompasses a hierarchical approach, involving cell models, tissue engineered constructs and evaluations of specific sub-populations at risk of developing PUs [8]. Several studies have assessed the effects of large

strains in subdermal tissues involving muscle and fat, by evaluating the biomechanical and physiological response of the tissues employing imaging and biomarker analysis [9, 10]. However, this approach does not account for the damage mechanisms associated with the majority of PUs involving small tissue strains in superficial dermal tissues. Indeed, in this case, researchers have used biophysical and imaging techniques to assess the response of superficial skin tissues under representative loads [11-14]. These studies revealed that due to microvascular compromise, tissue ischemia can occur in both able-bodied and patient cohorts during periods of lying and sitting postures. In addition, specialised imaging techniques adopted in the host lab has enabled the quantification of dermal lymphatic vessel occlusion under applied loads [11, 12].

With the advent of state-of-the-art imaging and biophysical modalities, it is now possible to simultaneously assess microvascular and lymphatic changes during periods of loading and subsequent recovery. Therefore, the present study aimed to determine thresholds of dermal vessel occlusion and recovery following representative loading using a customised biophysical and imaging experimental design protocol.

2. Methods

2.1 Participants

Participants between the ages of 18 and 65 years were recruited via poster advertisement. Ethics Approval for the study was granted by the Local Institutional Committee at the University of Southampton (REC ID: 19378). Each participant was provided with an information sheet, which detailed any risks associated with the protocol. Exclusion criteria included a history of skin damage or disease and contraindications for fluorophore injections.

2.2 Methodology

The experiments were performed in an environmentally controlled laboratory, with an ambient temperature of 22±1ºC and relative humidity of 42±6%. For each participant, the dominant arm was positioned on a foam-based structure at the level of the heart, designed to ensure that the arm remained as still as possible during testing (Figure 1). This position ensured minimal contribution of passive mechanisms to lymphatic clearance and reduced potential movement artefacts during imaging.

Indocyanine green (ICG) injections of 50µL 0.05%w/v at a shallow intradermal depth were administrated to delineate dermal lymphatic vessels. Single injections were delivered into the two inter-digital spaces between the thumb and the second finger by a registered practitioner (PW), constituting a total micro-dose of 0.05mg. To encourage rapid uptake of ICG into lymphatic vessels, each participant was instructed to clench their fists 20 times following injection. Dermal lymphatic video sequences were captured using a Near Infrared Fluoroscopy Lymphatic Imaging (NIRFLI) system, with a commercial camera (Fluorbeam R 800) and associated software (Fluobeam v3.1.1, Fluopotics, France). The system incorporates an integrated laser (780 nm) and CCD sensor, with appropriate filters to isolate fluorescence of ICG (peak 830 nm). Frame acquisition was captured at frequencies of between 3-4Hz, individually optimised to maximise detection of vessels and minimise greyscale saturation. The imaging system was used initially to identify ante-cubital delineation of lymphatic vessels, to provide a suitable focal point for loading on an active dermal lymphatic vessel.

A transcutaneous gas monitor (TCM5, Radiometer, Denmark) with a combined gas electrode set at a local temperature of 43.5ºC was then attached to the selected test site. A special 3D printed case was placed on top of the electrode to produce a curved loading surface and minimise stress concentrations at the edges of the indenter. The electrode was calibrated and attached to the skin in an unloaded state for 20 minutes. Following the

equilibrium phase, a baseline lymphatic video sequence was recorded for 20 minutes to accommodate the effects of local heating induced by the electrode.

2.3 Loading Protocol

To investigate the effects of repeated loading on lymphatic activity and transcutaneous gas tensions, two distinct loading regimes were employed. The first involved incremental repeating loading with pressures of 30mmHg, 60mmHg and 90mmHg applied to the test site with the 38mm diameter indenter (Figure 1). Each load was maintained for a 20 minute period followed by 20 minutes of unloading (recovery phase). During these loading and unloading periods, video sequences with the NIRFLI were recorded. In addition, transcutaneous tissue gas tensions (T_cPO_2 and T_cPCO_2) were continuously monitored at 0.5Hz, with changes in loading conditions identified at each interval.

On a separate day separated by a minimum of 48 hours, six participants returned to the laboratory for a further test session. This involved three repeated loads each at a constant pressure of 30mmHg applied to the identical test site. Identical time periods, each of 20 minutes, were employed for loading and unloading, with continuous monitoring of transcutaneous gas data and NIRFLI capture at each test condition. This was performed to assess the accumulative effects of repetitive loading, in comparison to that of the incremental pressures, previously described.

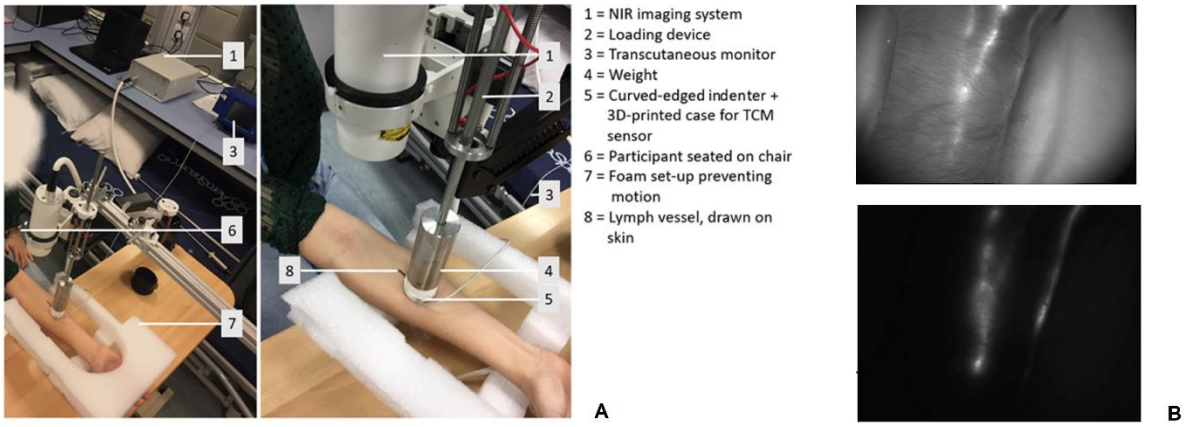


Figure 1. A) Test setup involving the dominant arm of participants resting on a foam surface. The indenter has a specially designed interface to house the transcutaneous gas electrode and the lymphatic imaging was conducted proximal to the loading site. B) Image of delineated dermal lymphatic image using ICG injection and near infra-red imaging.

2.3 Data and Statistical Analysis

Robust parameters of lymphatic function from the imaging sequences were identified using a customised software application (Matlab, Mathworks, USA), described in previous publications [12, 13]. To review briefly, the features were established using a droplet morphometry and velocimetry (DMV) tracking approach. Here image subtraction, binary conversion and centroid tracking provided the basis to identify and measure each transient lymph packet event captured within the 20 minute video sequences. Lymph packets are related with contractile propulsion events, associated with the lymph “pump” [12], where lymph fluid transitions toward collecting nodes. Each transient event was analysed to provide x and y coordinates of the centroid of the packet, whereby the resultant displacements could be estimated. An Extended Kalman Filter (EKF) was applied to ensure the centroid axes from distinct packets were isolated between the video frames. The primary output parameter involved the frequency of transient lymph packets. Participant data sets were presented to describe transient lymph behaviour across each of the test phases involving both loading

and unloading periods. Data regarding transient lymphatic events were non-parametric in distribution and appropriate descriptors of median and interquartile ranges were used. Subsequent analysis of the effects of incremental pressures (30, 60 or 90mmHg) and repeated pressures (30mmHg, 30mmHg and 30mmHg) were conducted using a Friedman test. Comparisons between post-loading data and baseline values were conducted using Wilcoxon signed-rank tests.

The transcutaneous gas data were normalised to baseline unloaded values, then categorised according to the established characteristic responses during the applied loads [14]; Category 1 - minimal changes in both T_cPO_2 and T_cPCO_2 values, Category 2 - >25% decrease in T_cPO_2 with minimal change inT_cPCO_2 and Category 3 - >25% decrease in T_cPO_2 associated with a >25% increase inT_cPCO_2. The resultant categorical responses are described as a percentage of the number of participants. Oxygen deficit was estimated by calculating the integral between T_cPO_2 values and time, with respect to the basal T_cPO_2 levels.

3. Results

3.1 Participants

A total of twelve participants (7 male, 5 female) were recruited with a mean age of 26 years (range 22-37 years), mean height of 1.66±0.11m, mean weight of 65.3±10.8kg and their corresponding BMI was 21.15 ±3.4kg/m^2. These were subjected to the incremental loading protocol (30, 60, 90mmHg). In addition, six participants (3 male, 3 female) were subsequently subjected to repeated constant loading (30, 30, 30mmHg). Their mean height was 1.71±0.11m, mean weight was 68.3±8.3kg and their corresponding BMI was 23.2 ±4.1kg/m^2.

3.2 Effects of Incremental Pressures of 30, 60 and 90mmHg

At baseline, the number of lymph packages revealed a large inter-subject variation, with a median value of n=9 transient events (quartile range 6-12). During the incremental loading regimen, the number of transient lymphatic events reduced during the loading periods but then increased in the subsequent recovery periods. Indeed, for each pressure, there were very few cases of transient through flow events, with median values of between 2-3 (Figure 2). During the subsequent recovery periods, the number of transient events increased from baseline, reflecting a full recovery. There were no significant differences ($p>0.05$) between the number of transient lymphatic packets at each of incremental loading periods.

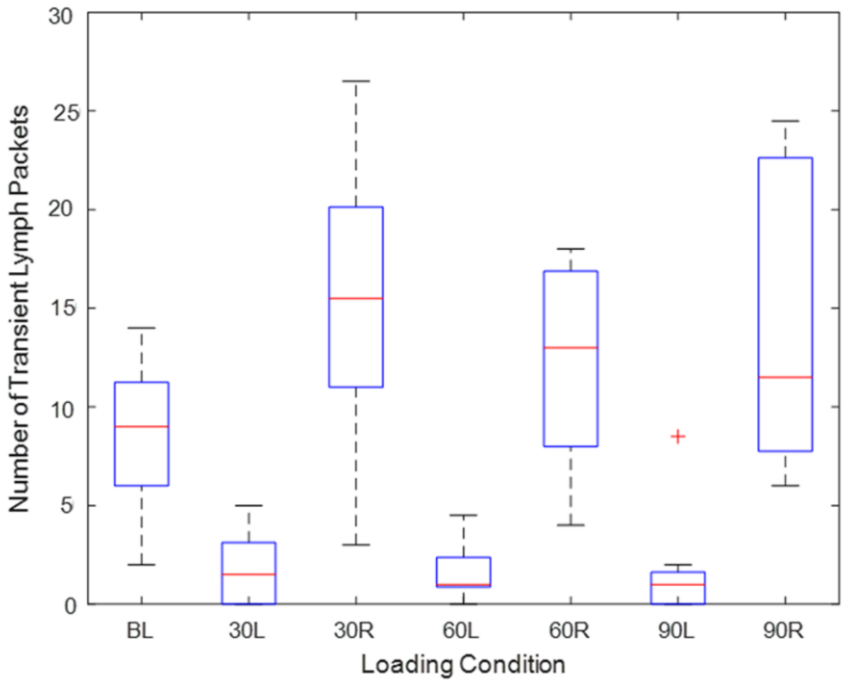
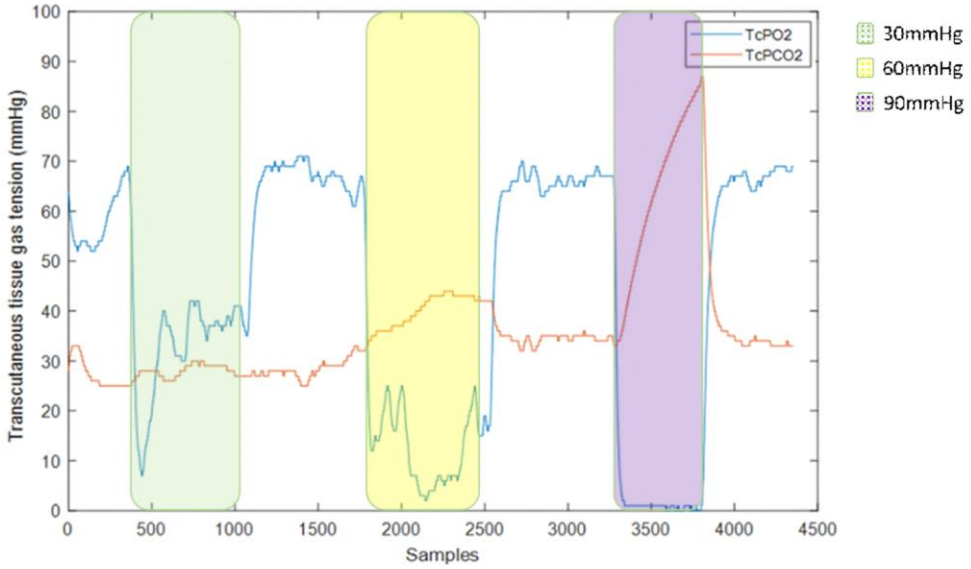


Figure 2. Number of transient lymphatic packets during the 20 minute loading (L) and recovery (R) cycles for incremental pressures of 30, 60 and 90mmHg.

There were contrasting changes in transcutaneous gas response depending on the magnitude of applied pressures (Figure 3). Indeed, for a pressure of 30mmHg, there was generally a reduction in T_cPO_2 and no corresponding changes in T_cPCO_2 i.e. 67% of participants exhibited a Category 2. With an increased pressure of 60mmHg, a number of individuals (44%) exhibited both a reduction in T_cPO_2 and an increase in T_cPCO_2, denoted by a Category 3 response. At the third load cycle corresponding to an applied pressure of 90mmHg, a Category 3 response was evident in over half of the participants (56%), indicative of an ischemic state in the underlying tissues. The temporal trend in the transcutaneous gas data reveals the cyclic changes in T_cPO_2 during periods of loading and recovery. Figure 3 provides a typical example for one participant (#P4), demonstrating that at the highest pressure (90mmHg) there is a sharp decline in T_cPO_2, to a point at which oxygen is depleted. The corresponding T_cPCO_2 values are observed to accumulate over this loaded period, reaching values of >80mmHg, representing a 100% increase from basal values (Figure 3). With the subsequent removal of load, full recovery to basal levels for both T_cPO_2 and T_cPCO_2 were observed.



Load	CAT1	CAT2	CAT3
BL (%)	100	0	0
30mmHg (%)	33	67	0
60mmHg (%)	0	56	44
90mmHg (%)	0	44	56

Figure 3. Transcutaneous gas response from one participant (#P4) during incremental pressures of 30, 60 and 90mmHg. The table below indicates the percentage categorical responses from the cohort (n=12).

3.2 Effects of Repeated Pressures of 30mmHg

A similar inter-subject variation was observed during the repeated loading test phase, as illustrated in Figure 4. The median number of transient lymph packets at baselines was n=5, with an inter-quartile range of 4-7. During the applied loading the number of transient events were reduced with median values ranging from 0-2 across each of the three loading cycles. During each unloading cycle, the lymphatic activity increased with median values of transient events ranging between 12-15. This represented a significant increase ($p<0.05$) of more

than two-fold compared with baseline values. The results were indicative of significant occlusion during loading and full recovery in the subsequent unloading periods, for each of the three loading cycles. There were no significant differences ($p>0.05$) between the number of transient lymphatic packets during each loading cycle.

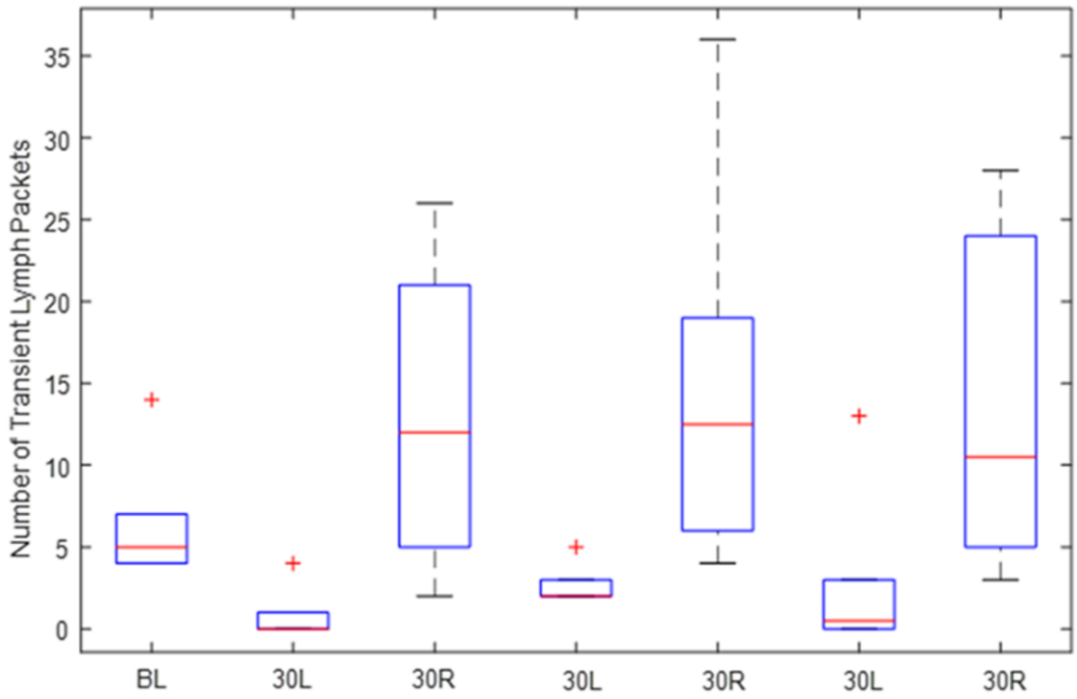
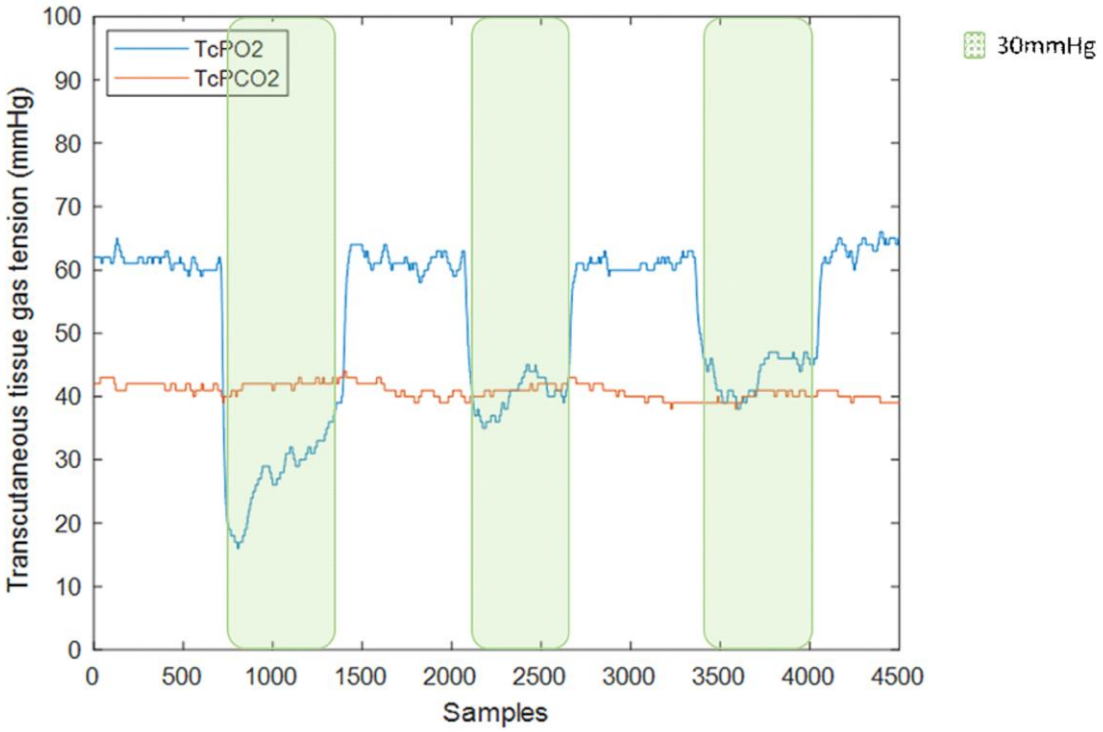


Figure 4. Number of transient lymphatic packets during the 20 minute loading (L) and recovery (R) cycles for repeated pressures of 30mmHg.

Changes in transcutaneous gas data were also observed during the test period, with tissue gas deviations corresponding to loading and unloading phases, as illustrated with one participant (#4) (Figure 5). Indeed, there was a decrease in T_cPO_2 during 30mmHg loading with a recovery during the unloaded phases. In each cycle, oxygen was reduced while carbon dioxide remained at basal levels, indicative of a Category 2 response. This was consistent across the test participants, with full recovery of T_cPO_2 levels observed during each period of unloaded recovery (Figure 5).



Load	CAT1	CAT2	CAT3
BL (%)	100	0	0
30L1 (%)	17	83	0
30L2 (%)	0	100	0
30L3 (%)	0	100	0

Figure 5. Transcutaneous gas response from one participant (#P4) during repeated pressures of 30 mmHg. The table indicates the percentage categorical responses from the cohort (n=6).

3.4 Associations between lymphatic occlusion and transcutaneous changes

The effects of the incremental loading regime on both the perfusion, in the form of the oxygen debt parameter, and the lymphatic activity for two participants is illustrated in Figure

6. There is a clear increase in oxygen debt with a corresponding reduction in lymphatic activity during the periods of incremental loading. At each unloading cycle, full recovery of perfusion and an associated increase in lymphatic activity was observed (Figure 6a). These temporal trends were apparent in the vast majority of participants (n=10). In a small number of participants (n=2), the responses were less evident with minimal changes in both lymphatic activity and perfusion during the loading cycles (Figure 6b).

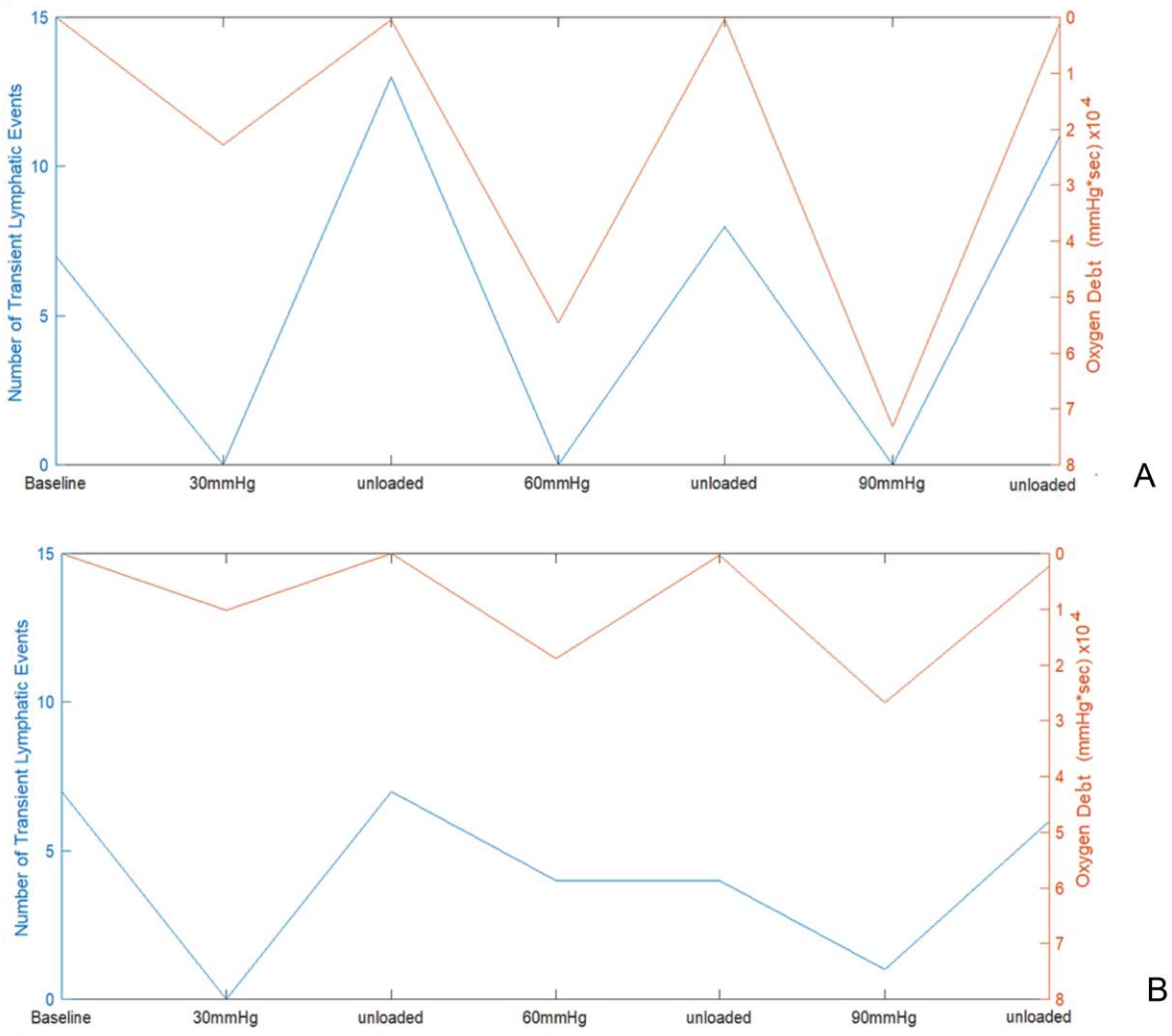


Figure 6. The profile of oxygen debt and the number of transient lymphatic events from two participants for each period of incremental loading and recovery. (a) #P6 and (b) #P5.

4. Discussion

This study represents for the first time a simultaneous evaluation of both dermal microvascular and lymphatic compromise as a direct result of mechanical loading of the skin. The combination of biophysical sensing and NIRFLI provided a unique opportunity to assess compromise to skin microcirculation, with a focus on two key mechanisms of pressure ulcer development, namely ischemia and impaired lymphatic drainage [8]. The results of this study on an able-bodied cohort revealed that even under relatively low pressures (30mmHg) the dermal lymphatic vessels were occluded with little or no transient events observed. By contrast, when subjected to repeated constant pressures, changes in the transcutaneous gas tensions generally revealed some reduction in partial pressures of oxygen associated with carbon dioxide remaining at basal values. The most significant changes in transcutaneous gas data were observed at the third cycle of incremental loading corresponding to a pressure of 90mmHg, where the majority of participants exhibited a large reduction in T_cPO_2 with an associated increase in T_cPCO_2, indicative of local ischemia.

The results of the study have demonstrated that an applied pressure of 30mmHg can compromise dermal lymphatic vessels. This pressure was smaller than the 60mmHg used in a previous study, which resulted in impaired valve function and backflow events in some of the able-bodied participants [12]. In both studies, however, there was considerable variability in both the basal lymphatic activity levels and the individual responses to mechanical loading. In addition, each study revealed a full recovery of lymphatic activity during the unloaded recovery phases (Figures 2 and 4). The present study design includes the advantage of imaging during the loading period, which was not available during the previous study [16]. With reference to the seminal animal study, which used radioisotope tracers to monitor the clearance of deeper lymphatic vessels, critical uniaxial pressures of between 60mmHg (8kPa) and 75mmHg (10kPa) were reported [15]. However, direct comparisons between this and the present study must be treated with caution due to the differences in lymphatic anatomy and physiology between species under investigation. It is

of note that lymphatic vessel function characterised in the present study may not be related to lymphatic capillary exchange occurring distally, although similar pressures were shown to cause distinct changes in interstitial flow, causing local halo patterns of ICG dispersion [11].

The changes in transcutaneous gas data revealed that moderate compromise was observed at 30mmHg, representing a Category 2 response. Repeating this pressure resulted in a similar response. By contrast, when pressure was incrementally increased, changes in both T_cPO_2 and T_cPCO_2 were observed (category 3), indicative of an ischemic state in over half the able-bodied cohort. Previous studies have shown that to achieve a 50% reduction in T_cPO_2 from unloaded resting value in an able-bodied cohort, applied pressures ranged from 22-92mmHg [16]. These findings highlight the individual nature of the tissue response, in terms of tolerance to mechanical-induced impairment of the microcirculation. This was also reported using biophysical measures to assess the physiological response to prolonged lying and sitting postures [14, 17, 18]. Here a proportion of individuals, typically 15-30% of the total, demonstrated ischemic responses during common postures i.e. supine, lateral lying and high sitting, with corresponding interface pressure values ranging between 30-90mmHg [8]. This could be due to differences in soft tissue structure and geometry, underlying changes in physiology, such as pre-clinical changes in microcirculation or nutrition factors [7].

It is clear that the blood and lymphatic vessels demonstrate different sensitivities to mechanical-induced occlusion, which could be attributed to their unique anatomy and physiology. For example, the lumen of lymphatic vessels is wider and more irregular than in blood vessels and under normal conditions, the lymphatic capillaries are maintained in a collapsed state [19]. By contrast, a relationship is known to exist between cutaneous mechano-sensitivity and vasodilation, referred to as pressure-induced vasodilation (PIV) [20]. When an external pressure is applied on the skin, the cutaneous microarteries vasodilate to prevent ischemia. The present study has shown that under relatively low pressures of 30mmHg, microcirculation remained functional with only small changes in

T_cPO_2, which often showed some recovery during loading indicative of PIV (Figure 3 and 5). However, at the higher loads of 60 and 90mmHg, there appears to be greater compromise in a number of individuals, with elevated T_cPCO_2 levels corresponding to complete microcirculation occlusion (Figure 3). The changes in microcirculation values should be put into the context of normative capillary pressures, which range from 10.5 to 22.5 mmHg at the apex of the capillary loop [21]. Hence, our loading regimes were above that of the normative values. However, PIV by vascular smooth muscle tone appears to compensate for loads up to a threshold between 60-90mmHg in most cases, a finding which has also been demonstrated in the microvascular response in animal models, e.g. threshold of 70mmHg in murine model [22]. Similar pressures have been reported to cause local ischemia and inflammation [23, 24], with an accumulation of metabolites associated with a change from aerobic to anaerobic cellular respiration [24, 25].

The present study clearly examines the response of a relatively small cohort of young able-bodied participants, limiting its generalisability to cohorts of patients at risk of pressure ulcers, such as those with co-morbidities including diabetes or the spinal cord injured. Demographic factors such as body fat, blood pressure, and hydration status may have influenced the individual responses. However, our sample of young healthy individuals within a limited BMI range (21.15 ±3.4kg/m2), provided limited scope to adopt any form of multifactorial analysis. The loading periods were restricted to 20 minutes, whereas in the clinical setting patients are supported in prolonged postures for periods in excess of 4 hours. The heated transcutaneous gas electrode could have influenced the underlying tissue physiology, although this condition was standardised across all test participants, ensuring both lymphatic and microcirculation measures reflected the relative changes in the vessel patency as a result of mechanical loading.

In clinical practice, carers and healthcare professionals advise vulnerable patients to regularly reposition to allow recovery of previously compromised tissues [1]. The present study has highlighted the importance of tissue recovery within an able-bodied cohort.

However, individuals with microvascular compromise and reduced tissue tolerance may require an extended period to recover [26]. In addition, the pressure required to cause local ischemia and lymphatic occlusion appears to differ between individuals, even within an able-bodied cohort. This highlights the need for personalised care strategies, where patients are assessed and monitored depending on their individual tolerance to prolonged postures. Further studies are required on specific patient sub-populations who may have underlying co-morbidities, which affect their microcirculation and the dermal tissue tolerance to applied loading.

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