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Skin perfusion responses to surface pressure-induced ischemia: Implication for the developing pressure ulcer

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Abstract--This study describes alterations in skin perfusion in response to step increases in surface pressure, before and after long-term (5 hr) exposure to pressure-induced ischemia. A provocative test was developed in which surface pressure was increased in increments of 3.7 mmHg until perfusion reached an apparent minimum by a computer-controlled plunger that included a force cell, a laser Doppler flowmeter to determine perfusion, and a thermistor to monitor skin temperature. Force was applied to the greater trochanters of adult male fuzzy rats. Skin perfusion (n=7) initially increased with low levels of surface pressure (up to 13.9±1.9 mmHg) and then decreased with further increases in pressure, reaching minimum (zero) perfusion at 58.2±3.64 mmHg. After pressure release, reactive hyperemia (3× normal) was observed, with levels returning to normal within 15-30 min. The provocative test was then applied after a 5-hr ischemic episode (produced by 92 mmHg) and 3 hr of recovery. A comparison of responses between stressed and unstressed skin revealed: elevated (63%) control perfusion levels; loss of the initial increase in perfusion with low levels of increasing pressure; a depression (45%) in the hyperemic response with delayed recovery time; and a decrease (54%) in amplitude of low frequency (<1 Hz) rhythms in skin perfusion. Skin surface temperature gradually increased both during the control period and the period of incremental increases in surface pressure (total ΔT=3.3 °C). The results suggest a compromised vasodilator mechanism(s). The provocative test developed in this study may have clinical potential for assessing tissue viability in early pressure ulcer development.

Key words: *animal model, decubitus, ischemia/reperfusion, pressure ulcer, skin perfusion, surface pressure.*

INTRODUCTION

Pressure ulcers are defined by the National Pressure Ulcer Advisory Panel as areas of unrelieved pressure over a region of the skin resulting in ischemia, cell death, and tissue necrosis (1). Clinically, they most often appear in the tissues covering bony prominences. Despite many advances in medicine, surgery, and nursing care over the years, pressure ulcers remain a significant and recurrent health care problem (2,3), with major impact on such important patient populations as the elderly and individuals with impaired mobility.

Static external pressure exerted against the skin may impair the microcirculation, the lymphatic circulation, and the interstitial transport processes, thereby leading to skin breakdown and tissue damage (3). Although surface pressure is known to be a primary risk factor for developing pressure ulcers, the relationship between the magnitude of surface pressure and skin perfusion in the development of pressure ulcers remains unclear. The present study focuses on these relationships.

Experiments were designed to serve two purposes: to develop a provocative test in which skin perfusion was measured as a function of increasing, incremental steps (30 s application/step) of skin surface pressure (Part A), and to apply this test to determine the extent to which skin perfusion is altered by the application of long-term (5 hr) surface pressure-induced ischemia (Part B).

METHODS

Subjects

Seven adult, male fuzzy rats (12-14 mo, mean body weight 482.9±21.1 g, hypotrichotic) were used to develop the provocative skin test (Part A) and six adult, male fuzzy rats (12-14 mo, mean body weight 488.5±23.4 g) were studied to determine the skin's perfusion response to prolonged (5 hr) surface pressure (Part B). Each rat was anesthetized by intraperitoneal delivery of a combination of acetylpromazine (2.25 mg/kg) and ketamine (112.5 mg/kg; Miles, Inc., Shawnee Mission, KS). Additional reduced doses (acetylpromazine at 1.5 mg/kg; ketamine at 75 mg/kg) were supplied at appropriate intervals during the study to maintain the proper plane of anesthesia and the insensate state of the animal. Skin surface pressure was applied to the hip over the greater trochanter (4), a common site for the development of pressure ulcers in humans. The care and handling of the animals followed the guidelines of the Institutional Animal Care and Use Committee (IACUC) of the University of Kentucky.

Apparatus

The apparatus for the present study has been modified from previous use (4). Animals anesthetized in accord with IACUC protocol were placed in a custom-made saddle and restraining device (Dupont, Foam Design, Inc., Lexington, KY). and positioned to align the skin plunger over their greater trochanters.

The skin plunger shown in **Figure 1** is composed of a force transducer (load cell, A.L. Design, Inc., Buffalo, NY), a thermistor (Yellow Springs Instrument Co., Inc., Yellow Springs, OH), and a laser Doppler flowmeter (PeriFlux by Perimed, Smithtown, NY). The force transducer was used to measure the normal force. The laser Doppler flowmeter extended into the center of the plunger, flush with the surface, to measure skin perfusion (0.5 to 1 mm depth) as surface pressure was applied. Three signals from the flowmeter were acquired: 1) perfusion or flux, 2) red cell velocity, and 3) number of red cells (concentration). A true zero reading for perfusion cannot be achieved during complete vessel occlusion because of the small movements of muscle cells, vessel walls, and proteins within the blood or interstitial space. The lowest value of the flowmeter signal when flow is stopped is termed "biological zero" and referred to as "zero perfusion." A thermistor probe, flush with the surface of the plunger, was used to measure skin temperature during the application of surface pressure.

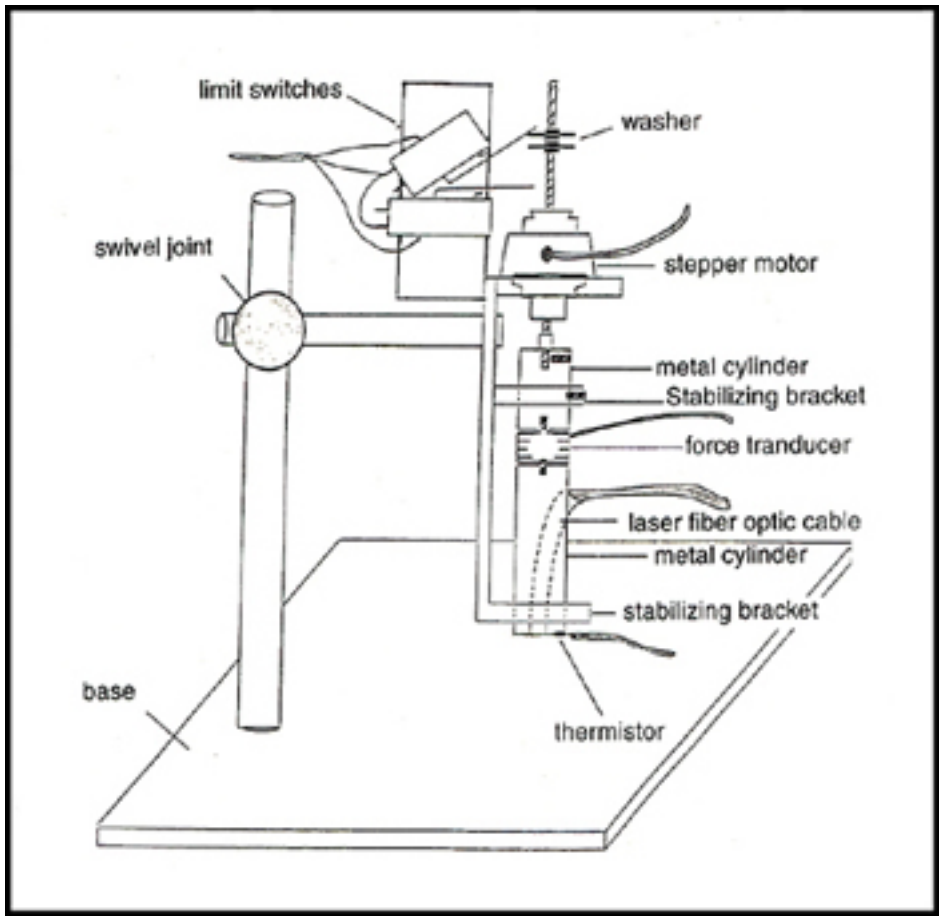


Figure 1.
Detailed diagram of computer-controlled skin plunger system.

Skin perfusion, temperature, and pressure data were collected (125 samples/s) by computer using DATAQ software (DATAQ Instruments, Inc., Akron, OH). The force signal was also used

for feedback control of the stepper motor, resulting in the maintenance of desired levels of skin surface pressure. The stepper motor controller was turned off when perfusion measurements were taken, to minimize motion-induced artifact in the skin perfusion signal.

Core body temperature of each rat was monitored throughout the experiment by a rectal temperature probe (Yellow Springs Instruments, Yellow Springs, OH).

Procedure

Part A: Development of the Provocative Test

A pilot study was performed to establish the protocol for the provocative test by optimizing the control period for assessing skin perfusion. Skin perfusion often varies rhythmically with time (vasomotion), and therefore a data record length should be sufficient to include at least two or more cycles of the lowest frequency for analysis. The mean value and the frequency content of the signal are both important parameters in characterizing perfusion. To determine the appropriate data record length during control, skin perfusion was measured initially for 30 min to establish stable flow (5). Mean skin perfusion and perfusion variance (frequency content, spectral analysis by the Welch technique) were calculated from 30-min data records. Spectral analysis provides information about the different rhythms that make up skin perfusion. Spectral plots, which show "power" at given frequencies, represent the contribution of each frequency to the whole signal. The higher the value of power at a given frequency, the greater the amplitude of the rhythm at that frequency. The data record length was then divided into shorter time periods (e.g., 20, 15, 10, 6, 3, and 2 min) and analyzed to determine the mean values and the frequency content of each time period. Eight 30-min data records, acquired four times on different days for each of two adult male rats, were analyzed. As 10 min was the shortest record length that provided values consistent with those obtained for the total 30-min period, the 10-min control period was adopted for the remainder of the study.

After the control data record length was determined, the experimental protocol shown in **Figure 2** (bottom) was designed to determine the relationship between skin perfusion and incremental increases in surface pressure. Two identical tests on the same animal were repeated approximately 1 hr apart and used to determine any residual influence of test 1 on test 2. A control surface pressure of 3.7 mmHg (10 g) was applied to ensure the stability and uniformity of skin contact required for accurate laser Doppler flowmeter readings. After the 10-min control period, pressure was incrementally increased in steps of 3.7 mmHg while measuring skin perfusion (the duration of the steps is discussed below). Pressure was increased until zero perfusion occurred (i.e., minimum skin perfusion value maintained). Pressure was then rapidly (within 5 s) decreased to control pressure (3.7 mmHg) and the recovery perfusion was measured for 15 min. The plunger was completely removed from the skin to allow the tissue to further decompress for a time determined by direct visualization of the tissue (30 min to 1 hr). The plunger was then placed on the tissue again for the start of test 2, which used the same protocol as test 1. This experimental protocol was conducted four times on four different days (separated by at least two days) on each of seven rats.

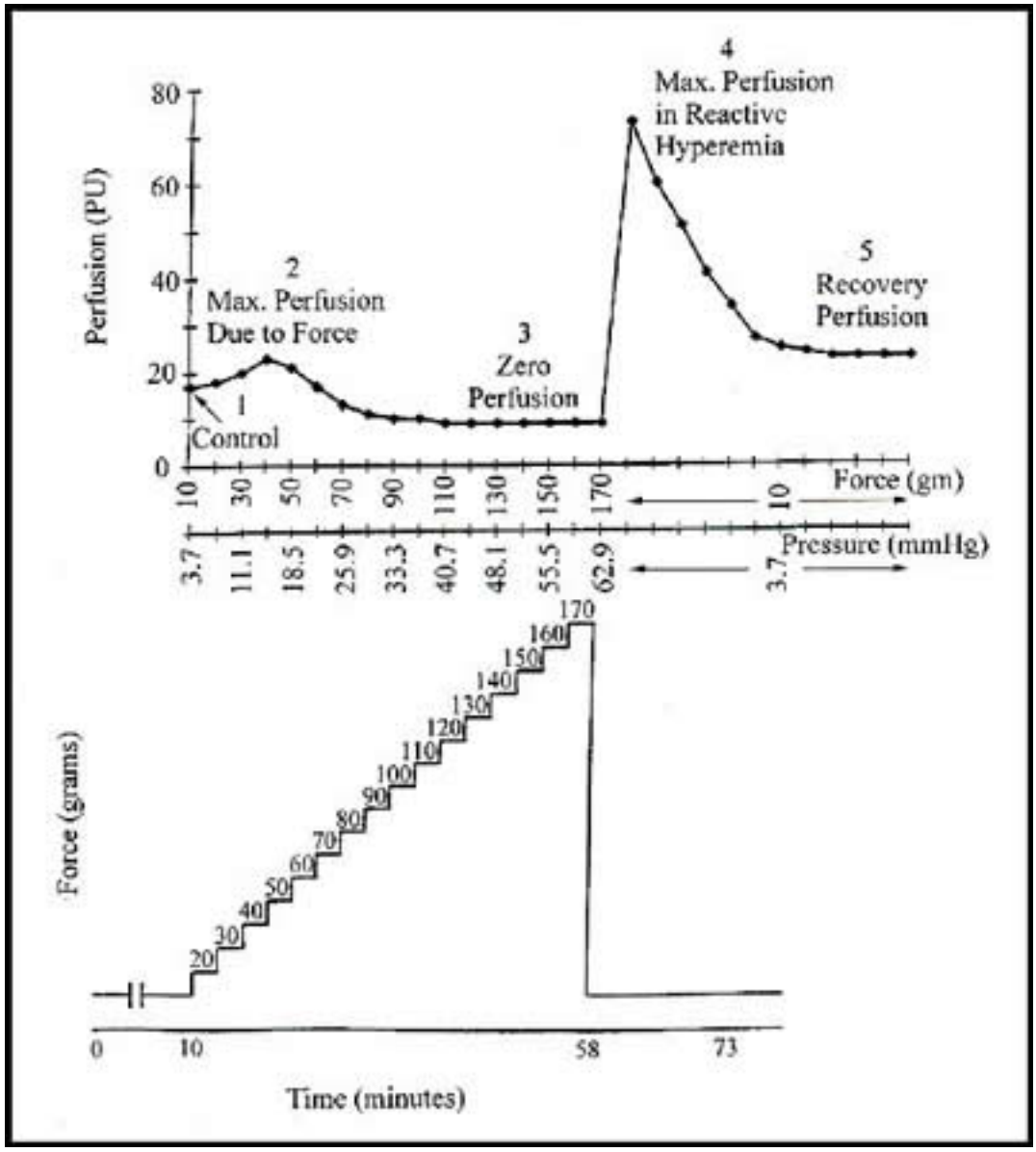


Figure 2.
Typical perfusion response for Stages 1-5 (top) to the 3-min steps experimental protocol (bottom).

In three separate experiments, perfusion/pressure response curves were determined for the 3-min steps (total test time of 90 min), for the 1-min steps, and for the 30-s steps in an effort to optimize total test time. It was found that the 30-s steps at 10 g force would produce response curves equivalent to those using 3-min steps. Therefore, the 30-sec step protocol (total test time of 35 min) was adopted for the remainder of the study (Part B).

Part B: Application of the Provocative Test

The provocative test was then applied to determine the effect of 5 hr of pressure-induced ischemia at 92 mmHg on the skin perfusion/surface pressure relationship. The experimental procedure for Part B was as follows. The provocative test described in Part A was performed initially on normal skin. The test included a control period (3.7 mmHg for 10 min), stepwise increases (30-s increments) in skin surface pressure, and a recovery period. After a steady-state perfusion level was established in the recovery period, skin surface pressure of 92 mmHg (250 g) was applied to the same tissue for 5 hr to produce ischemia (zero perfusion). Skin perfusion

remained at biological zero during this time. At the end of 5 hr, the skin surface pressure was reduced to zero (plunger not touching the tissue), allowing the tissue to recover or decompress for a time determined by visual inspection. When the tissue indentation disappeared (3 hr), the skin plunger replaced on the identical area of tissue to repeat another provocative test (n=6 rats). Sham experiments were conducted on an additional four rats in which the skin perfusion/surface pressure responses were determined after 8 hr of anesthesia without induced ischemia.

Statistical Analysis

In Part A, an ANOVA-2 factor variance method for repeated measures (STAT-VIEW software package) was performed to test for significant differences in skin perfusion and temperature due to changes in skin pressure between test 1 and 2 for each of the seven rats exposed to repeated (n=4) tests. The results were expressed as mean±standard error of the mean, and taken to be significant at p values ≤ 0.05.

Mean perfusion over time was calculated at each stage (i.e., control perfusion, maximum perfusion due to force, zero perfusion, maximum perfusion in reactive hyperemia, and recovery perfusion) for each rat. Each rat was exposed to the protocol (i.e., test 1 and 2) on four different days. The four values at each stage were averaged to provide one value/rat/stage for each test. A comparison of mean (n=7) perfusion for all rats at the different stages was performed using the t-test for related measures. A similar comparison using the t-test for related measures was performed to test significant differences between tests 1 and 2 of the skin surface pressure data for each of the seven rats.

In Part B, as in Part A, an ANOVA-2 factor variance method for repeated measures was performed to test for significant differences in perfusion and temperature parameters due to changes in skin pressure between the normal and stressed skin for each of the six rats. A comparison of mean perfusion between the normal and stressed skin at each surface pressure level was performed using the t-test for related measures.

RESULTS

Part A: Development of the Provocative Test

Perfusion Response to Step Increases in Skin Surface Pressure

Experiments (n=7 animals) in which skin surface pressure was increased in 3-min steps were conducted twice in the day separated by approximately 1 hr. Control periods were 10 min, and recovery periods lasted 15 min. Each rat was tested four times, with each test separated by at least 2 days.

A typical response (one rat) is shown in **Figure 2** (top). The perfusion response was divided into five stages. Perfusion as measured during the 10-min control period was identified as Stage 1. As surface pressure increased in 3.7 mmHg increments, there was an initial increase in perfusion followed by decreasing perfusion as surface pressure continued to increase. Maximum perfusion during this part of the provocative test was designated as Stage 2. The minimum value (zero

perfusion) was designated Stage 3 as defined by at least three successive pressure increments with little or no change in the perfusion. The smallest value of surface pressure associated with this area of flat response was chosen as the zero perfusion value. After zero perfusion was established, the surface pressure was rapidly decreased to the control value (3.7 mmHg), resulting in a reactive hyperemic response. Maximum perfusion during reactive hyperemia was identified as Stage 4. The return of skin perfusion to a steady-state level after a recovery period was designated Stage 5.

Figure 3 shows the perfusion responses of seven animals to 3-min step increases in skin surface pressure, averaged over four experiments, each consisting of test 1 and test 2 separated by approximately 1 hr. As the perfusion responses were not significantly different between test 1 and 2, their results were pooled and averaged in order to evaluate stage differences statistically. The increase in perfusion in Stage 2 was significantly different from control ($p \leq 0.01$) and Stage 3 ($p \leq 0.001$). After pressure relief, Stage 4 occurred, with perfusion rapidly increasing to almost three times control levels ($p \leq 0.001$) and then gradually returning close to control levels. Recovery perfusion (at the end of 15 minutes) was not significantly different from control levels. The average value of skin surface pressure that caused zero perfusion was 58.2±3.64 mmHg).

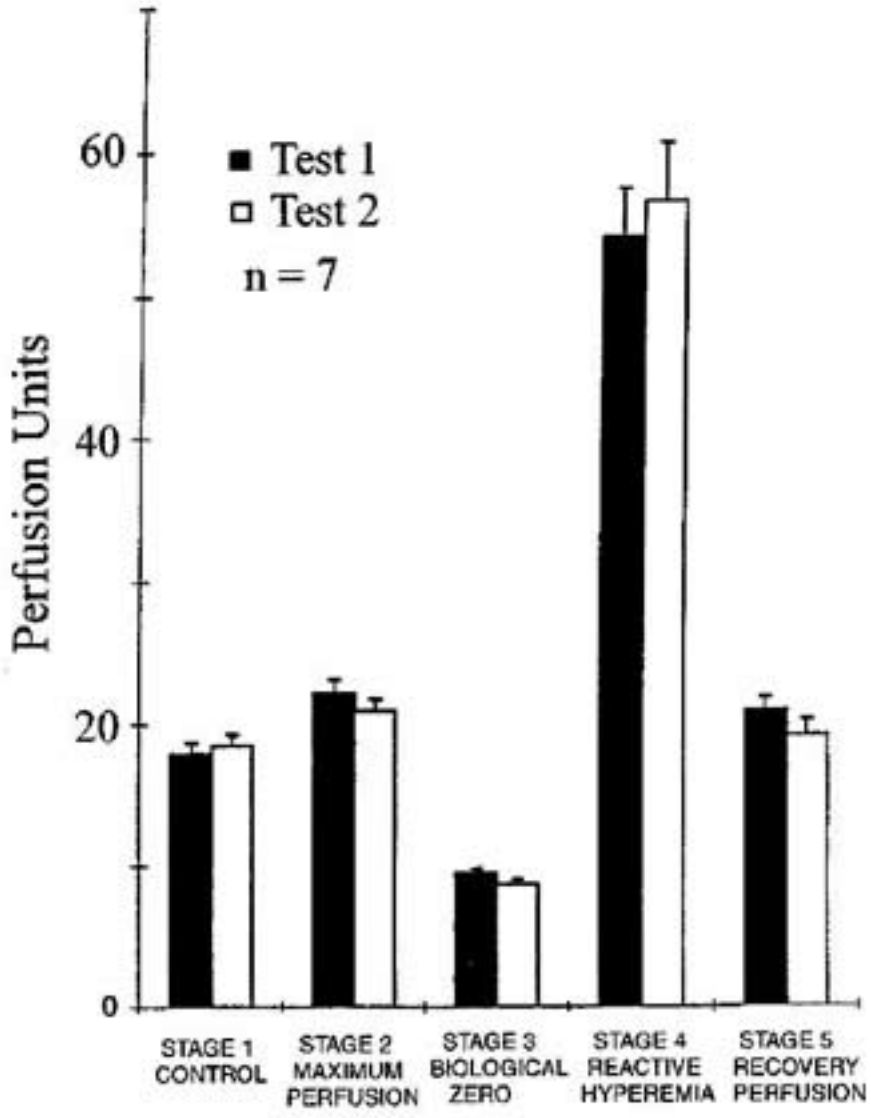


Figure 3.

Comparison of mean perfusion (Stages 1-5) for tests 1 and 2.

Skin surface temperature (data not shown; n=7) gradually increased (28.6±0.27 to 31.5±0.12 °C) from the beginning of the control period through the pressure application phase (attainment of biological zero). After pressure release, the temperature decreased about 1 °C as perfusion recovered. The only significant difference in temperature between tests 1 and 2 occurred during the control period ($p \leq 0.05$); however, control perfusion was not significantly different between the two runs. The animal's core temperature remained unchanged (37.6±0.2 °C).

Optimization of the time required for completion of the provocative test resulted in a reduction of the initial 3-min step to 1 min and finally down to 30 s while maintaining the reproducibility of the data. The control (10-min) and recovery (15-min) periods were unchanged. Because no significant differences in perfusion within stages between the 3-min, 1-min, and 30-s steps were observed, the 30-sec step was adopted. The total time required for the provocative test was then 33.5 min (10-min control, 8.5-min steps, 15-min recovery). The 30-sec protocol was used in Part B to evaluate long-term pressure-induced ischemia.

Part B: Application of the Provocative Test

Skin Perfusion Responses before and after 5 Hours of Surface Pressure-induced Ischemia

Figure 4 shows a composite plot (n=6) of perfusion versus pressure consisting of the 30-s step protocol applied before and after the application of a constant surface pressure of 91.6 mmHg for 5 hr (followed by approximately 3 hr of tissue decompression). Unstressed skin manifested the same five Stages described earlier. However, there were major differences in perfusion response of stressed skin during several of these stages. First, control perfusion for stressed skin was 63 percent greater than for unstressed skin. Second, the small increase in perfusion with initial applications of increasing pressure did not occur. Third, a modified hyperemic response occurred in which maximal flow values decreased by 45 percent from unstressed values. Fourth, the recovery perfusion values remained elevated for a longer time.

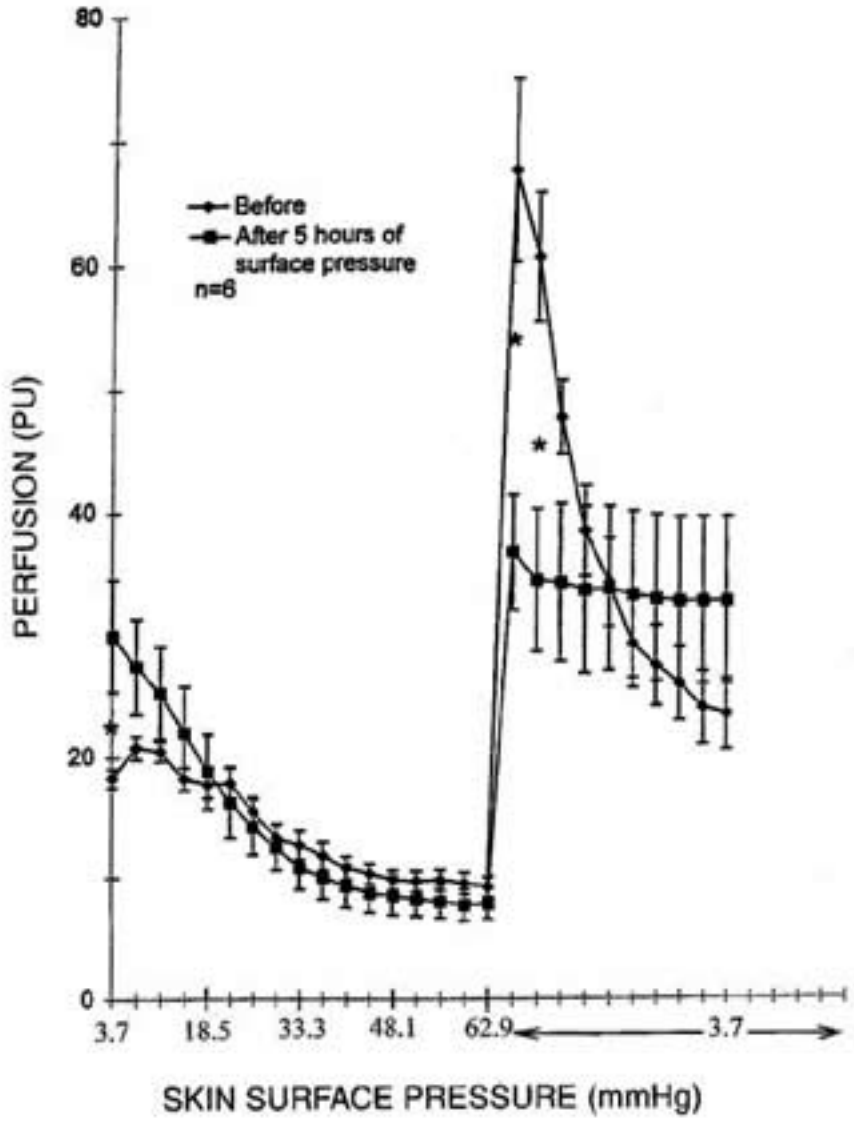
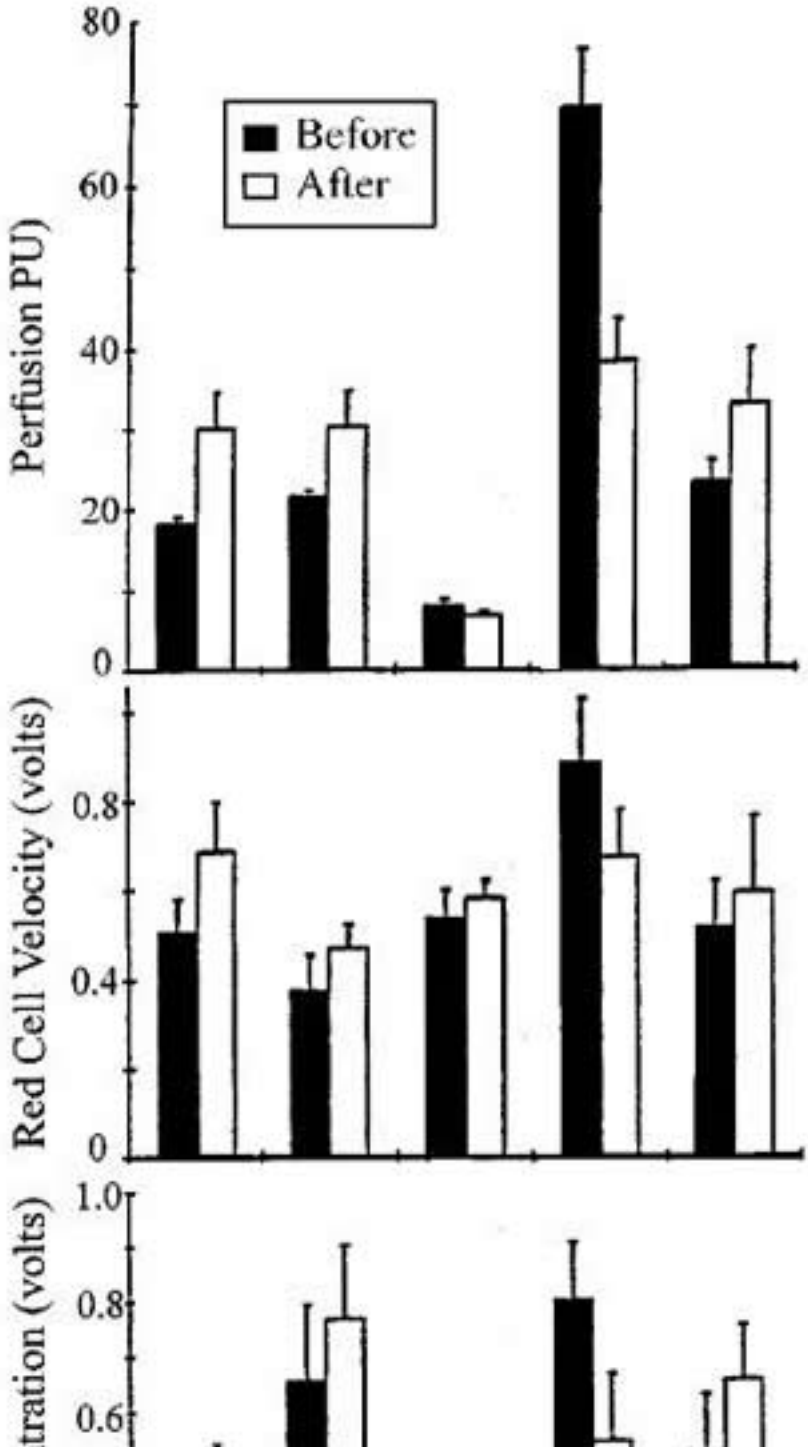


Figure 4.
Comparison of mean skin perfusion vs. surface pressure before and after a 5-hr ischemic event (*=significance at $p \leq 0.05$, n=6).

An ANOVA-2 factor analysis for repeated measures showed that the average perfusion level of all stages was significantly different ($p \leq 0.0001$) in stressed and unstressed skin. Therefore, perfusion levels at individual surface pressures in stressed and unstressed skin were compared using the t-test. For stressed skin, control perfusion (at 3.7 mmHg) and recovery perfusion (at 3.7 mmHg) were significantly higher and the peak reactive hyperemic response was significantly depressed. Results from the sham experiments (i.e., a provocative test before and after 8 hr of anesthesia only with no surface pressure-induced ischemia) revealed that control responses were maintained for all five stages (data not shown), indicating that the statistically significant changes reported above resulted from the long-term application of pressure. There were no significant differences in skin surface temperature (data not shown) during control, at zero perfusion, or during recovery between the stressed and unstressed skin.

The perfusion signal is the product of red cell velocity and concentration (number of red cells).

Figure 5 provides comparison of the skin perfusion, red blood cell velocities, and red cell concentrations during the five stages of the provocative test before and after pressure-induced ischemia (n=6). During the initial increases in skin surface pressure (Stage 2), mean red cell velocity was significantly decreased from control (Stage 1, $p \leq 0.025$) in both stressed and unstressed skin. Because no secondary peak occurred in Stage 2 for stressed skin, the comparison was made using values that occurred at the same surface pressure. During zero perfusion (Stage 3) and recovery perfusion (Stage 5) the mean red cell velocities were not significantly different from their respective controls ($p \leq 0.1$). Mean red cell velocity during the reactive hyperemic stage was significantly increased over control in unstressed skin, but not in stressed skin.



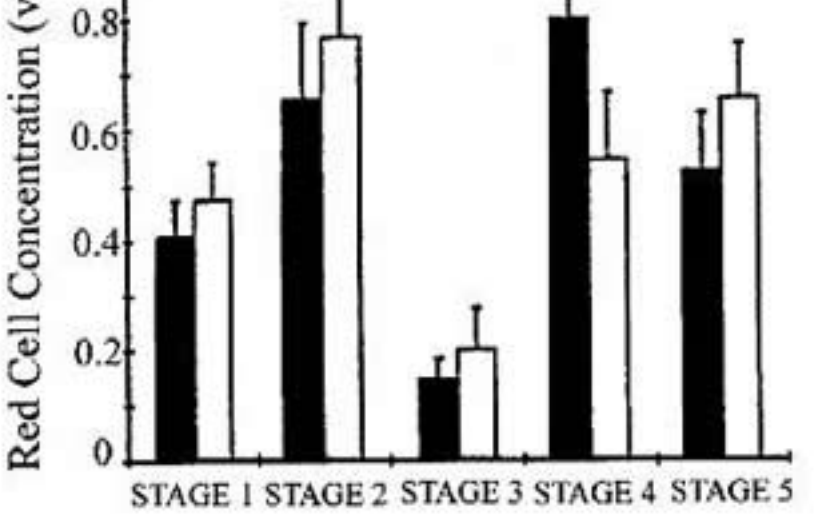


Figure 5.
Comparison of skin perfusion, red cell concentration, and red cell velocity during Stages 1-5, before and after 5 hrs of pressure-induced ischemia and 3 hr of tissue decompression (n=6). Stage 1, control period; Stage 2, period of maximum perfusion during force application; Stage 3, zero perfusion; Stage 4, reactive hyperemia; Stage 5, recovery phase.

Corresponding data (n=6) for red cell concentration appear at the bottom of **Figure 5.** During Stage 4 (reactive hyperemia) there were significant differences in the mean red cell concentrations in stressed and unstressed skin that were not observed in Stages 1, 2, 3, and 5. In both stressed and unstressed skin, red cell concentrations during the initial increases in skin surface pressure (Stage 2) were significantly increased from control (Stage 1) and were significantly decreased during zero perfusion (Stage 3, $p \leq 0.001$). After the release of skin surface pressure, red cell concentrations during reactive hyperemia (Stage 4) were significantly increased from control (Stage 1) for unstressed skin, but not for stressed skin ($p \leq 0.1$). During recovery (Stage 5), mean red cell concentration of unstressed skin was not significantly different from control ($p \leq 0.1$), but for stressed skin it was significantly greater.

Perfusion Rhythms before and after 5 Hours of Surface Pressure-induced Ischemia

It is possible that blood flow rhythms may be different after the ischemic episode. Factors that may account for--or at least contribute to--these changes include vasomotion associated with thermoregulation, respiration, and heart rate.

Because this possibility exists, the spectral content (frequency and power) of the control perfusion signal was determined. Typical spectral plots of the perfusion signals during control (10 min) for stressed (after 5 hr at 91.6 mmHg and 3 hr of tissue decompression) and unstressed skin are shown in **Figure 6.** Three frequency ranges were analyzed: low (<1 Hz), thought to be associated with the thermoregulatory system (6); mid (1-2.5 Hz), associated with the rat's respiration rate (as timed in the present study); and high (2.5-5 Hz) containing variations resulting from the rat's heart beat. Spectral analyses for normal and stressed skin are shown in **Figure 6.**

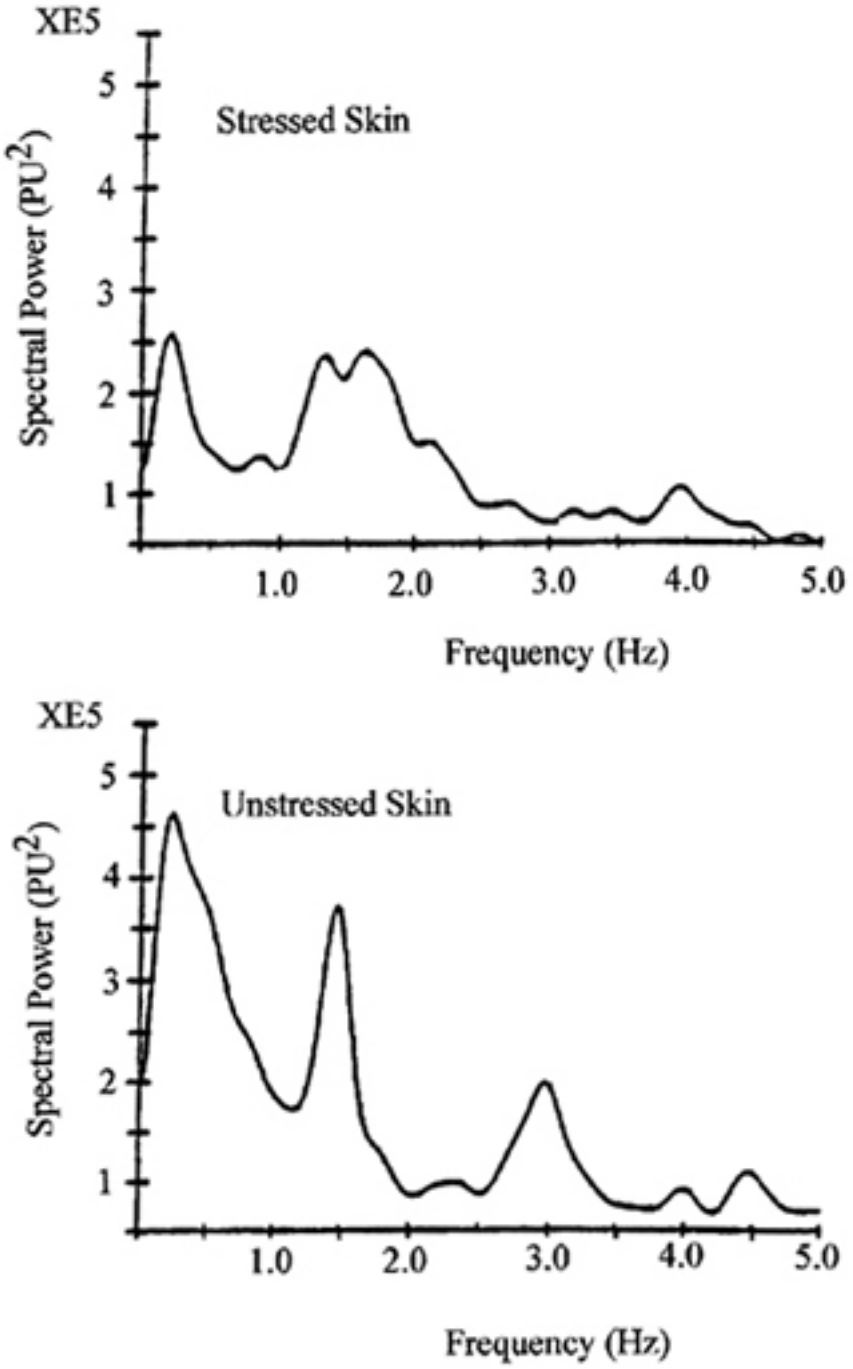


Figure 6.
Spectral analysis of control perfusion in unstressed (bottom) and stressed (top) skin.

Comparison of the average spectral power for the three frequency ranges for control perfusion for both stressed and unstressed skin is shown in **Figure 7.** The spectral power of perfusion in the low-frequency range significantly decreased by 54 percent after ischemic stress, but no significant differences were detected in the mid- or high-frequency ranges.

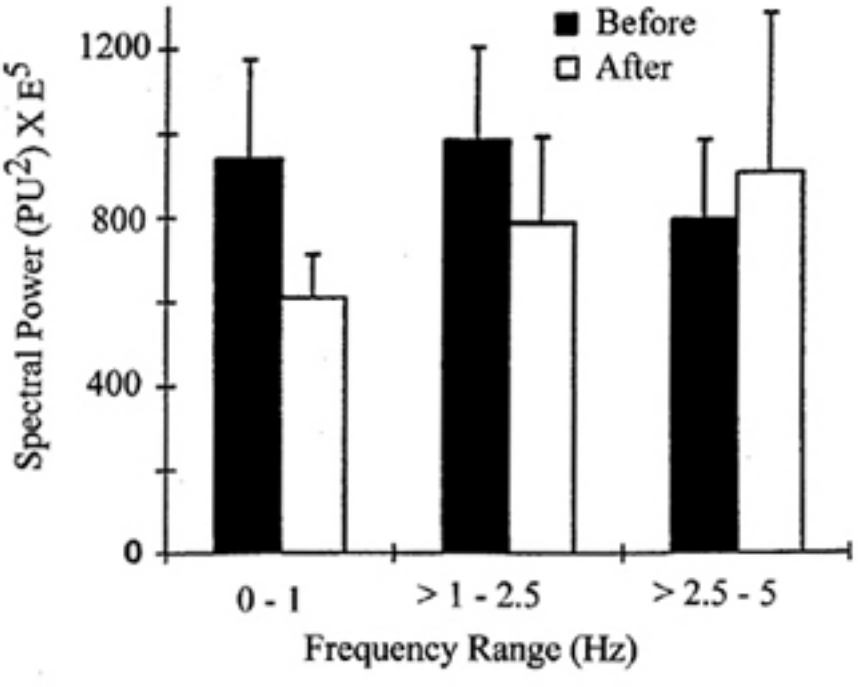


Figure 7.
Comparison of spectral power in three different frequency ranges before and after 5 hrs of pressure induced ischernia and 3 hr of tissue decompression (*= significance at p≤0.05; n=6).

DISCUSSION

The objective of the present study was to determine potential changes in the skin perfusion/ surface pressure relationship after 5 hr of surface pressure-induced ischemia. The major finding was that this perfusion/pressure response was significantly changed, probably resulting from functional microvascular alterations caused by the ischemic event.

A necessary part of the present study was the development of a provocative test sufficiently sensitive to indicate functional changes in skin perfusion without requiring a long recovery period. By using the optimized short protocol (30-s steps, 10-min control, and 15-min recovery), the frequency content of resting and recovery perfusion could still be estimated. One can determine the mean perfusion value, the shape of the perfusion/surface pressure response curve to be assessed, and the reproducibility of results when reapplied within a 1-hr period. The total time for the protocol could have been further reduced by decreasing the control and recovery periods, but not without sacrificing valuable information about changes in vasomotion.

The observation that skin perfusion in unstressed skin initially increased with low levels of surface pressure is supported by previous studies (3,7). In humans, Schubert and Fagrell (3) reported an increase in skin perfusion with increased surface pressures up to 25-50 mmHg. They did not draw any specific conclusions related to this phenomenon. However, they did suggest that the greater frequency of pressure sores over the sacrum as compared to the gluteus region resulted

from differences in the regulation of microvascular flow in the two regions. Agache and Dupond used photoplethysmography on humans to assess indirectly the systolic-diastolic difference in skin blood volume while increasing external pressure was applied (7). This study found that the differential volume increased and then subsided progressively as pressure increased, reaching zero when external pressure equaled systolic pressure within the skin arterioles. Although the authors concluded that the systolic-diastolic differences corresponded to an external pressure equal to the arterial diastolic pressure, they did not attempt to explain the initial increase in differential volume with increasing surface pressure.

Several explanations could account for the observed increase in perfusion associated with low levels of increasing surface pressure. One explanation is that vasodilation of arterioles may have occurred in response to a compressive load, resulting in a decrease of the transmural pressure on the arterioles. Stacy et al. (8) have shown that decreased transmural pressure resulting from increased surface pressure caused vasodilation of the arterioles in the cheek pouch of hamsters. They also demonstrated that tissue suction, which leads to increased transmural pressure, produced vasoconstriction of the arterioles. This explanation is supported by the red cell concentration data of the present study. The increase in red cell concentration suggests increased vessel diameters and/or an increased number of perfused capillaries (reopening), both potentially a result of decreased arteriole resistance. The decreased red cell velocity multiplied by the greater effective cross-sectional area (as indicated by increased red cell mass) yielded the measured increase in perfusion.

Another explanation for this phenomenon may be that it is an artifact of the laser Doppler technique. For example, as skin surface pressure increased, the sample volume of the laser Doppler probe may change to include additional capillaries or arterioles as a direct result of skin compression, thereby artifactually illuminating a greater number of red blood cells. If the observed increase in red cell concentration were artifactual in nature, it should be present in both stressed and unstressed skin. However, it was not observed in stressed skin, which suggests a nonartifactual cause. For example, the vessels may have already been maximally dilated or the maximum number of vessels may have already dilated as a result of the pressure-induced ischemia. Our findings, along with those of Schubert and Fagrell (3), Stacy et al. (8), and Agache & Dupond (7), indicate that the initial increase in skin perfusion is most likely the result of vasodilatation in response to increased skin surface pressure.

In our model and in most natural situations, an unavoidable consequence of increased skin surface pressure is increased surface temperature. The gradual increase in local skin surface temperature during control as well as during step increases in surface pressure was probably due to the lack of heat convection to the air and the introduction of heat conduction resulting from contact of the skin plunger with the skin's surface. The increase in local skin surface temperature may have played a role in the initial increase in perfusion seen during Stage 2. It is known that increased skin temperature can cause increased perfusion (3). In unstressed skin, surface temperature increased to a maximum of 31.5 °C (ΔT=3.3 °C). This temperature change could not account for all of the increased skin perfusion observed (9). Arteriole dilation, which can be used for the diversion of heat from tissue to the skin surface in a process utilizing increased perfusion and resulting in elevated surface temperatures, may also be involved (10). The observed rise in skin surface temperature, however, was very gradual and did not seem to correlate well with the

rise in perfusion. A more likely explanation is that local changes in skin heat convection/ conduction accounted for the gradual rise in local temperature noted both during the control period and the testing phase. Core body temperature remained stable.

The decrease in skin perfusion with increasing surface pressure is primarily a result of matching local vessel internal pressure. However, changes in surface pressure may also contribute to changes within the skin matrix. Surface pressure in excess of internal pressure may be required to produce capillary closure and tissue ischemia (11). For example, increased surface pressure may cause changes within the matrix, including stiffness, internal pressure, proteoglycan concentration, and resistance to fluid movement (12), as well as the possibility of vasodilation or recruitment of small blood vessels. It is highly probable that the tissue was able to withstand the initial increases of compressive surface pressures during this study, while maintaining good perfusion because of: 1) the resistance or stiffness of the connective tissue (12), 2) an increased tissue internal pressure due to increased proteoglycan concentration resulting from the loss of water, and 3) vasodilation or recruitment of vessels. Eventually, the ability of these systems to compensate for increasing pressure loads was overcome, resulting in the progressive collapse of this balanced state of internal and external factors, and, ultimately, the complete restriction of blood flow. An average surface pressure of approximately 58 mmHg resulted in zero perfusion.

The large increase in the perfusion of unstressed skin after the release of surface pressure is the well documented reactive hyperemic response that results from vasodilatation and associated increases in blood flow. The recovery period following reactive hyperemia lasted about 15 to 30 min and resulted in the return of skin perfusion to approximately control levels. The recovery time required may be influenced not only by vasodilatation but also by the need for the collagen-proteoglycan matrix to regain lost fluid. Whether a reversal of the sequence of events described above is involved remains to be determined.

The recovery period was marked by a gradual decrease in skin surface temperature (approximately 0.7 °C/15 min). This decrease probably resulted from perfusion changes, because the skin plunger remained in contact with the skin during the recovery period and no external skin heat loss should have occurred.

When the provocative test was applied before and after the 5 hr of surface pressure-induced ischemia, four primary differences in the perfusion response were observed between stressed and unstressed skin. The first difference was that control perfusion in stressed skin during Stage 1 was always greater than in unstressed skin. This finding is consistent with a reported increase in resting fluxes (skin perfusion) in humans with leg ulcers (13). Sack et al. (14) observed a reduction in the number of perfused capillaries after 4 hr of complete muscle ischemia. However, in the present study, the increased control perfusion of stressed skin appears to be a result of vasodilatation or recruitment of vessels since the mean values of both red cell velocity and concentration were elevated.

The second difference was that perfusion during control was elevated for stressed skin as compared to unstressed skin. Also, as increasing steps of surface pressure were applied to the stressed skin perfusion decreased to zero without the initial increase (during Stage 2) seen in unstressed skin. The absence of this skin perfusion phenomena, normally seen with low levels of

increasing surface pressure, may result from vessels that were already maximally vasodilated as a result of 5 hr of ischemic exposure. With vasodilation already at maximal levels, skin perfusion would be expected to decrease immediately as the cross sectional area of the capillaries and arterioles decreased with increasing surface pressure. It is also possible that the vessels may have lost the ability to dilate to diameters equal to those for unstressed skin.

An important mechanism of vasodilatation involves the release of the endothelial relaxing factor (EDRF) nitric oxide (15). Nitric oxide released from endothelial cells is known to relax vessels and contribute to the vasodilatation of arterioles. Endothelial dysfunction has been shown to occur in postischemic tissues and is characterized by a marked reduction in endothelium-dependent relaxation (16,17), which could lead to an inability to vasodilate. Gidlof et al. (18) noted that the degree of endothelial damage was inversely related to the degree of vasodilatation and reactive hyperemia. In the present study it is possible that endothelial damage occurred in the vessels of stressed skin, leading to a loss of vasodilative capability at low levels of surface pressure during the provocative test and to the depressed reactive hyperemic response at the end of the test. Another contributing factor may be an increase in local vascular resistance as a result of the clogging and plugging of capillaries by leukocytes during the 5-hr ischemic episode. Hansell et al. (19) and Rochester et al. (20) have shown that this is possible. If leukocytes, platelets, and macrophages clogged the capillaries of stressed skin additional increases in perfusion over those of control may not have been possible.

After 5 hr of surface pressure-induced ischemia and during the 3-hr tissue decompression period, a slight redness and swelling occurred in the local tissue in all six rats, suggesting an inflammatory response. An inflammatory reaction (21) may be associated with leukocyte-plugged capillaries. Constriction of vessels may occur soon after injury to stop blood leakage and then dilation may occur. Simultaneously, the endothelial cells lining the capillaries become increasingly activated and the capillaries become covered by adjacent leukocytes, erythrocytes, and platelets. With vasodilatation, leakage of plasma from capillaries can occur. If capillaries were damaged, the local lymphatic system may also be damaged, since it is even more fragile. All of these reactions--vasodilatation of capillaries, leakage of fluid into the extravascular space, and plugging of the lymphatic system--can provide the classic inflammatory signs: *rubor, tumor,* and *calor* (21). In the present study, the signs of redness and swelling disappeared toward the end of the 3-hr decompression period when the skin appeared to be normal. Although the external skin surface appeared normal, the microvasculature was probably still inflamed, as indicated by a reduced reactive hyperemia and a reduced index of vasomotion. Thus microcirculatory function had been altered.

The third difference between stressed and unstressed skin occurred in the reactive hyperemic response. The depressed reactive hyperemic response in stressed skin reported here is supported by a study of venous stasis ulcers by Jochmann et al. (13). They used laser Doppler flowmetry to evaluate the reactive hyperemic response of patients with leg ulcers and found that a reduced hyperemic response occurred after the release of occlusive skin surface pressure. Possible mechanisms that might explain this phenomenon were presented, including capillary damage, large reductions of perfused capillaries, endothelial dysfunction, and plugging of capillaries by leukocytes. These four mechanisms may contribute to or cause the depressed reactive hyperemic response seen in the present study.

Another potential mechanism relates to metabolic factors. It is known that vasodilatation of small arterioles during reactive hyperemia is both locally mediated by hypoxia, as well as by myogenic and metabolic factors (22). These factors are known to play a role in regulating the intensity of the reactive hyperemia (23). During ischemia, metabolic factors such as vascular prostaglandin and adenosine (24) are produced or released and have been shown to cause a reduction in the reactive hyperemic response. For whatever reason, it is clear from the present study that the ability of the tissue to provide the large reactive hyperemic response observed in the unstressed case was significantly impaired by the 5-hr ischemic exposure.

The fourth difference between stressed and unstressed skin occurred during the recovery response. Perfusion in stressed skin was elevated near the end of the recovery period compared to that of unstressed skin, most likely due to a continuation of the inflammatory response. Surface temperature of the stressed skin was higher than (Δ0.97 °C) that of unstressed skin during the recovery period. However, according to Wamsley (9) this temperature was not high enough to conclude that the local temperature caused the elevated skin perfusion, but rather that the slight increase in temperature probably reflected increased perfusion.

Further understanding of vasodilative mechanisms in the stressed skin can be obtained by determining changes in the rhythms of perfusion through spectral analysis. The three frequency ranges of skin perfusion in the present study were based on the work of Colantuoni et al. (6). They analyzed skin perfusion in rats using an autoregressive model and found spectra of skin perfusion of a similar nature. One frequency range corresponding to the fundamental frequency of vasomotion in terminal arterioles was at 0.15-0.23 Hz. The second frequency range coincided with respiratory rate (1-2.5 Hz), and the third coincided with heart rate (4.7-6.8 Hz). In this study, the amplitude of rhythms in the low frequency (<1 Hz) range of control perfusion consistently decreased after ischemic exposure. This decrease may be a result of the associated increased mean control perfusion of the stressed skin, but data from another study (25) in our laboratory indicate that when mean perfusion increased by increasing temperature, the amplitude of the rhythms also increased. Thus the decreased amplitudes of rhythmic perfusion in the low-frequency range after ischemic exposure suggest that there may have been impairment of vasodilator mechanisms in the terminal arterioles.

An interesting observation in the present study is that the surface pressure required to produce zero perfusion was the same for stressed and unstressed skin. Tissue deformation and eventual breakdown is a multifactorial process that includes the amount of subcutaneous tissue volume under pressure and the viscoelastic response of the tissue (3). The stressed skin is likely to be dehydrated after the ischemic exposure, and one might expect that the surface pressure causing zero perfusion would be different compared to unstressed skin. However, if the 3-hr tissue decompression period was sufficient for most of the interstitial fluid to return to the ischemic tissue site, the proteoglycan content may have sufficiently recovered to normal levels. If this occurred, one would expect little or no difference in the amount of pressure required to cause zero perfusion in stressed versus unstressed tissue. This observation (lack of a difference in skin pressure to produce zero perfusion) implies that the observed differences in the perfusion response between stressed and unstressed skin at the other stages were probably due to factors involved in maintaining the integrity of the skin and microcirculatory system (i.e., vasodilatation

of arterioles) and not to mechanical factors such as reorientation or reduction (crimping) of viable capillaries or the tissue matrix.

The general consensus has been that pressure ulcers result from conditions contributing to a reduction in blood flow. However, recent evidence (26) indicates that increases in skin blood flow may be just as important in determining whether or not a pressure ulcer actually occurs. The role of skin blood flow in pressure ulcer development in surgical patients provides evidence that increased blood flow may have a beneficial effect on ulcer outcome. Obviously, additional studies need to be conducted.

CONCLUSIONS

This study defines the relationship between skin perfusion and increasing surface pressure in the rat and alterations in this relationship after long-term ischemia. The short-term provocative test caused skin perfusion to initially increase with increasing surface pressure, reaching a maximum at about 14 mmHg, and then decreasing with further increases in pressure, reaching zero perfusion at about 58 mmHg. When surface pressure was released, perfusion reached values 3× control (reactive hyperemia) and then returned to normal control values after about 15-30 mins. This perfusion/surface pressure response of normal skin was significantly altered after a 5-hr ischemic episode (92 mmHg) and a 3-hr recovery period. Several differences were observed: 1) control perfusion in stressed skin was 63 percent greater than in unstressed skin. 2) As step increases in pressure were applied, perfusion in stressed skin decreased to zero without the initial increase seen in unstressed skin. 3) The reactive hyperemic response in stressed skin was 45 percent lower than in unstressed skin. 4) The amplitudes of rhythms (<1 Hz) in control perfusion were smaller for stressed skin than for unstressed skin. These results indicate that ischemic-induced changes in perfusion were probably due to alterations in vasodilator mechanisms. The provocative test developed for the present study appears to be sufficiently sensitive for use in studies designed to detect subtle changes in skin perfusion associated with the early formation of pressure ulcers. It may also be useful with appropriate blocking agents in further defining the regulation of skin blood flow in pressure ulcer development. Likewise, our provocative test may have potential in the clinical arena in determining whether an area of nonblanchable erythema (clinical stage 1 ulcer) heals or becomes a partial- or full-thickness pressure ulcer.

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