Temperature effects on surface pressure-induced changes in rat skin perfusion: Implications in pressure ulcer development

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ABSTRACT--The effect of varying local skin temperature on surface pressure-induced changes in skin perfusion and deformation was determined in hairless fuzzy rats (13.5 ± 3 mo, 474 ± 25 g). Skin surface pressure was applied by a computer-controlled plunger with corresponding skin deformation measured by a linear variable differential transformer while a laser Doppler flowmeter measured skin perfusion. In Protocol I, skin surface perfusion was measured without heating (control, $T=28°C$), with heating ($T=36°C$), for control (probe just touching skin, 3.7 mmHg), and at two different skin surface pressures, 18 mmHg and 73 mmHg. Heating caused perfusion to increase at control and 18 mmHg pressure, but not at 73 mmHg. In Protocol II, skin perfusion was measured with and without heating as in Protocol I, but this time skin surface pressure was increased from 3.7 to 62 mmHg in increments of 3.7 mmHg. For unheated skin, perfusion increased as skin surface pressure increased from 3.7 to 18 mmHg. Further increases in surface pressure caused a decrease in perfusion until zero perfusion was reached for pressures over 55 mmHg. Heating increased skin perfusion for surface pressures from 3.7 to 18 mmHg, but not for pressures greater than 18 mmHg. After the release of surface pressure, the reactive hyperemia peak of perfusion increased with heating. In Protocol III, where skin deformation (creep and relaxation) was measured during the application of 3.7 and 18 mmHg, heating caused the tissue to be stiffer, allowing less deformation. It was found that for surface pressures below 18 mmHg, increasing skin temperature significantly increased skin perfusion and tissue stiffness. The clinical significance of these findings may have relevance in evaluating temperature and

pressure effects on skin blood flow and deformation as well as the efficacy of using temperature as a therapeutic modality in the treatment of pressure ulcers.

INTRODUCTION

Development of pressure ulcers in the patient care setting is complicated and, at times, unavoidable. Once the ulcer forms, treatment options are often limited, very expensive, and have an adverse effect on the individual's quality of life. Identification of factors responsible for ulcer formation and propagation provides a sound basis for developing treatment strategies.

The etiology of pressure ulcers is multifactorial. Skin surface pressure (intensity and time of application), has been identified as a major risk factor and investigated (1-3). An inverse relationship between the intensity of the external pressure and the time required for ulcer formation has been demonstrated (4). Both normal and shear forces associated with skin surface pressure may cause damage by diminishing or completely eliminating circulation to vital tissue (5).

External temperature may also affect the rate of tissue damage caused by surface pressure. Generally, each increase of 1°C in skin temperature results in approximately a 10 percent increase in tissue metabolic requirements (6). In rat or man, increased temperature causes an exponential increase in perfusion. Increased perfusion in humans has been associated with an increase in core body temperature as well as in local skin temperature (7,8).

However, the combined effect of increased temperature and surface pressure on tissue perfusion and the resulting deformation of the tissue has not been addressed. Such knowledge is critical to understanding pressure ulcer development, because in practice, as the skin is covered at the site of increased surface pressure, temperature almost always increases as well. The present study is designed to test the hypotheses that heated skin in response to increased surface pressure will experience smaller decreases in tissue perfusion and consequently less deformation than unheated skin.

METHODS

Subjects
Male fuzzy rats were selected as test animals because they are essentially hairless and do not

require any pretreatment before testing. Salcido et al. (9) used fuzzy rats in previous studies on the histopathology of ulcer formation resulting from externally applied surface pressure. Our animals were housed, fed, and used in experiments according to the guidelines of the Institutional Animal Care and Use Committee (IACUC) at the University of Kentucky. Mature male rats (mean ages 13.5 ± 3 mo and mean weight 474 ± 25 g) were selected to minimize variability in skin due to time effects and to avoid the developmental processes associated with immature animals.

Experimental Apparatus

The apparatus in the present study has been modified from that previously used in our laboratory (9). After induction of anesthesia, rats were secured in a prone position, using a specially designed Plexiglas restrainer. This arrangement provided easy access to the skin overlying the greater trochanters where surface pressure was applied normal to the skin.

Skin surface pressure was applied by a plunger attached to a vertical rod by a swivel joint, allowing three-dimensional movement (**Figure 1**). The plunger consisted of a column containing a force transducer (Load Cell, A.L. Design, Inc., Buffalo, NY) at its center, capable of measuring applied forces up to 350 g (128 mmHg). The output from the force transducer was digitized and used as the feedback signal of the control system for maintaining selected levels of skin surface pressure. A computer-controlled stepper motor allowed us to program increases or decreases in skin surface pressure or to maintain a fixed level. Skin displacement was measured by a linear variable differential transformer (LVDT; G.L. Collins Corporation, Long Beach, CA), attached to the top of the force column.
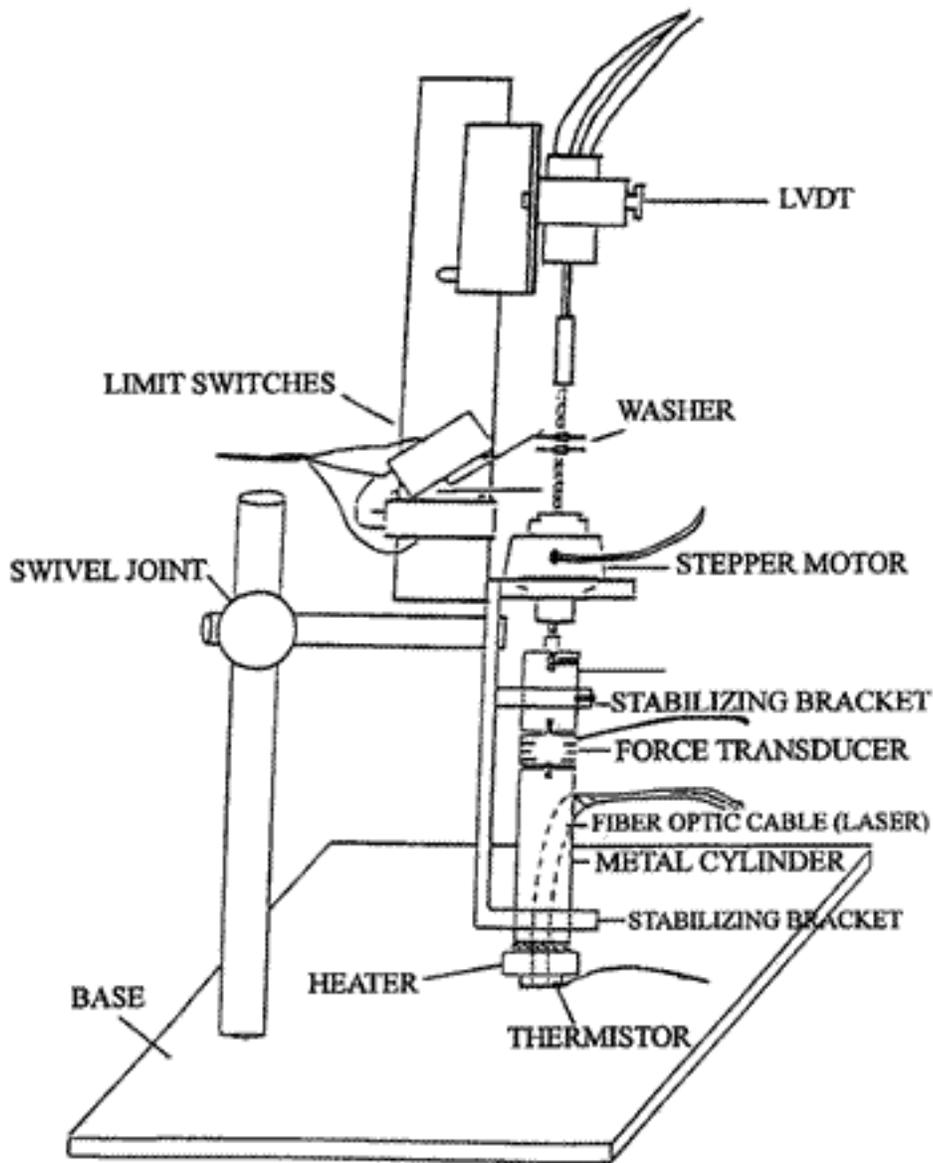
Figure 1.
Detailed schematic diagram of modified computer-controlled pressure column.

  A heater (Peritemp: Perimed, Piscataway, NJ), designed to raise the skin surface temperature locally, was attached near the distal portion of the metal cylinder in contact with the surface of the skin (see **Figure 1**). Its temperature was measured by a thermistor probe contained within its body and displayed on the LCD screen of the heater control unit. The actual skin surface temperature was measured by a separate thermistor probe attached to the bottom surface of the cylinder in contact with the skin surface during the experimental procedures.

  Skin perfusion was measured by a laser Doppler flowmeter (LDF; Periflux: Perimed). The LDF provided three measurements: the concentration of moving blood cells (CMBC), mean velocity of moving particles (primarily blood cells), and the product of both, referred to as the perfusion signal. The fiber optic probe of the LDF was inserted into the center of the distal portion of the lower metal cylinder of the pressure column until the tip was flush with the surface at the end of the column in contact with the skin.

Skin perfusion, surface temperature, surface pressure, and skin surface displacement were monitored and recorded by a PC using the DATAQ data acquisition software package (DATAQ Instruments, Inc., Akron, OH). A strip chart recorder (Servoger 124, Norma Goerz Instruments, Elk Grove Village, IL) was used to monitor the output from the skin surface thermistor, LDF, and force transducer during the experiment.

Experimental Protocols

Initially, skin surface temperature during control was increased in 2°C steps to measure mean and spectral changes in perfusion in order to determine appropriate data collection lengths and analysis techniques (see **Spectral Analysis** below). In the next experiment (Protocol I) conducted on a separate day, skin surface perfusion was measured without heating (control, T=28°C) and with heating (T=36°C) and at two different skin surface pressures, 18 mmHg (50 g) and 73 mmHg (200 g). During preliminary studies it was found that 3.7 mmHg (10 g force) caused just enough pressure to maintain good contact between the probe and the skin surface, resulting in an accurate signal. Therefore, this contact force was used during control. A temperature of 36°C was chosen to produce moderate heating of the skin. Control periods were 10 min long. Surface force was then applied for 3 min, followed by another control period of 10-min duration, after which the next force level was applied for 3 min. A total of eight animals were tested.

To better define skin responses to surface force and temperature, another experiment (Protocol II) was conducted. Skin perfusion and displacement were measured with and without heating as in Protocol I; however, skin surface pressure was increased in 10-g (3.7 mmHg) increments (3-min steps) from 10 to 170 g (62 mmHg).

To further investigate skin deformation (creep and relaxation) Protocol III was employed to measure displacement for longer periods of applied surface pressure. In these experiments, force was initially held constant at 10 g (3.7 mmHg, control) for 15 min, then increased to 50 g (18 mmHg) and held constant for 30 min and finally reduced to 10 g force for a 30- to 90-min recovery period. This force sequence was applied with and without heating as in Protocol I. The potential influence of the sequence of application was checked by performing some experiments without heating first, then with heating, and vice versa.

Spectral Analysis

In order to observe the temporal features (oscillations) of skin perfusion resulting from vasomotion, power spectrum analysis (Welch transforms) of the LDF signal was performed on 3-min data segments. Total spectral power and power in three frequency ranges (low, 0-1 Hz), (mid, >1-2.5 Hz) and (high, >2.5-5 Hz) were determined from skin perfusion, velocity, and CMBC. In rats, vasomotion is generally associated with power in the lowest frequency range (10).

Statistical Analysis

The Statview statistical package was used to perform an ANOVA-2 factor analysis for repeated measures in order to test for significant differences in skin perfusion and skin displacement due to changes in skin temperature. Results were expressed as the mean ± the standard error of the mean and were considered significant at p-values ≤0.05. Interaction between the two factors was also measured. The evidence of a strong interaction between the two factors would suggest that

temperature had a significant overall effect on perfusion and/or displacement.

   If results showed that there was a strong overall effect of temperature on perfusion, then the mean perfusion (P_m) with and without heating at each pressure level was calculated and the difference due to heating compared using the t-test for related values. The results were taken to be significant at a p-value ≤ 0.05.

   Similarly, for displacement experiments, if a strong overall affect of temperature on displacement was found, then t-tests were performed to determine if the difference in skin displacement due to heating was significant at each individual pressure level. Again the results were taken to be significant at a p-value ≤ 0.05.

RESULTS

   A typical recording demonstrating the effect of temperature on control perfusion (i.e., 3.7 mmHg) is shown at the top of **Figure 2.** Oscillations around a mean value were observed, both of which were affected by temperature. As skin temperature increased from 28 (unheated condition) to 36°C, there was approximately a 50 percent increase in P_m. The total spectral power of the perfusion signal, (**Figure 2,** bottom) shows a major peak in the low frequency range. An increase in skin surface temperature from 28 to 34°C resulted in skin flow oscillations with higher amplitude and lower frequency.
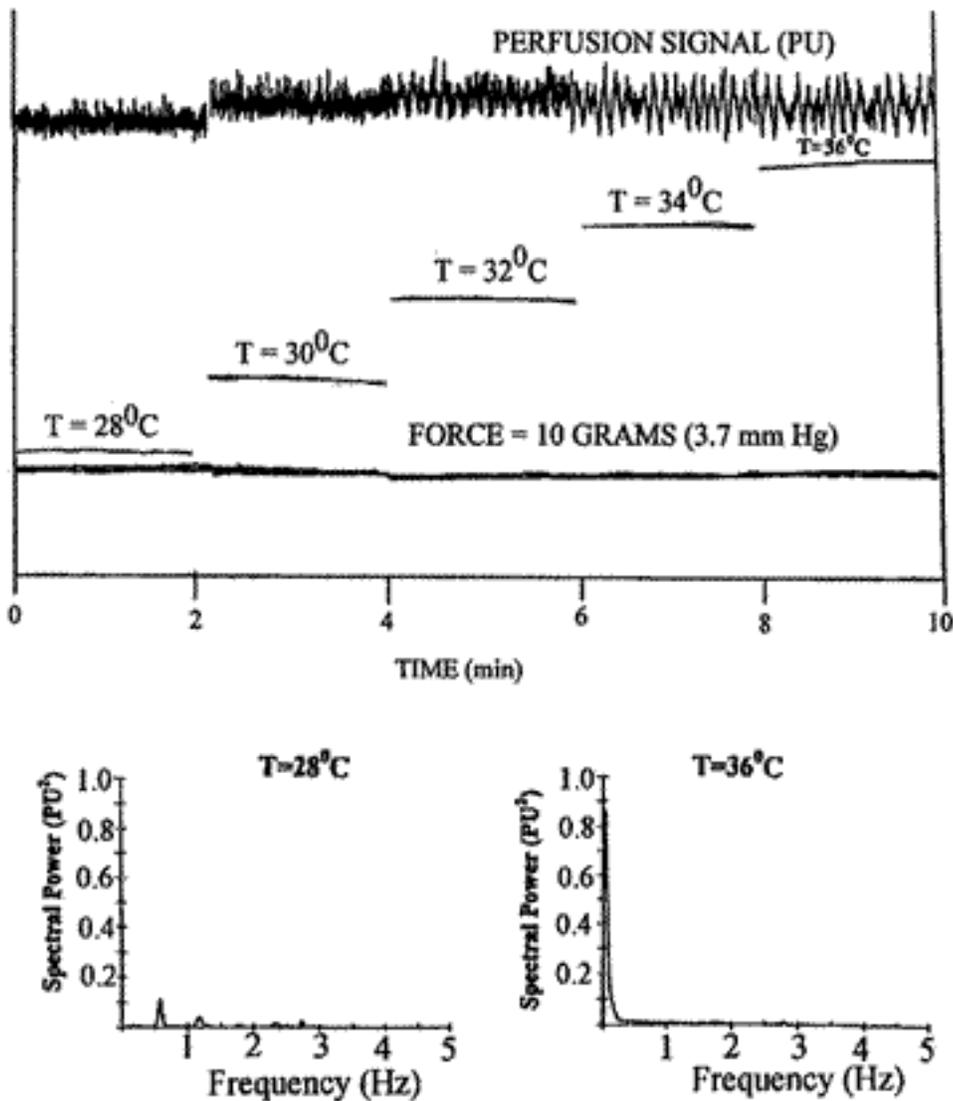
Figure 2.
Top: Changes in perfusion signal with 2°C step increases in temperature at control. Bottom: The corresponding power spectral analysis at 28°C (without heating) and at 36°C (with heating). PU=Perfusion Units.

Data for two-degree step increases in temperature during control (illustrated in **Figure 2,** composite plot in **Figure 3,** n=5), showed that P_m increased from 28 to 30°C and remained essentially unchanged from 30 to 36°C. However, spectral power in the low frequency (vasomotion) range did not change between 28 and 30°C, but increased 620 percent from 28 to 32°C and 2100 percent from 28 to 34°C. Spectral power remained the same from 34 to 36°C. An increase in skin surface temperature from 28 to 34°C resulted in a doubling of perfusion oscillations. However, an increase in temperature from 34 to 36°C resulted in little change in the amplitude or the mean value of perfusion oscillations.
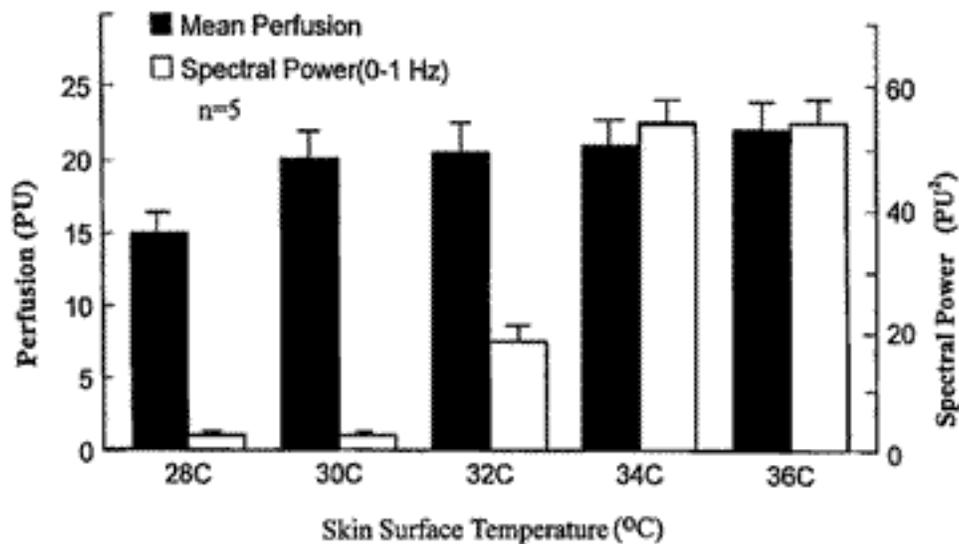
Figure 3.
Mean perfusion and spectral power in the low frequency range (0-1 Hz) with 2°C step-increases in temperature. Surface pressure was constant at 3.7 mmHg (i.e., control conditions).

Pooled data (Protocol I) from eight animals showing P_m at control (3.7 mmHg) and during 18 and 73 mmHg of surface pressure are presented in **Table 1.** Data are from experiments with (36° C) and without (28°C) heating. Both control perfusion and perfusion at a pressure of 18 mmHg (50 g) were significantly increased by raising skin temperature 8°C (p<0.05). Control perfusion increased 14 percent and perfusion at 18 mmHg increased 20 percent. However, heating at 73 mmHg (200 g) did not significantly change P_m.

Table 1.

Perfusion, red cell velocity and concentration of moving blood cells (CMBC) at three different surface pressures without (28°C) and with heating (36°C).

Parameters	Force					
	3.7 mmHg (control)		**18 mmHg**		**73 mmHg**	
	28°C	**36°C**	**28°C**	**36°C**	**28°C**	**36°C**
Perfusion	15.8±0.86	20±1.87*	20.1±0.95	24.5±1.1*	10±0.75	9.9±0.53
Velocity	5±0.04	4.5±0.05*	4.2±0.04	4±0.03*	6.2±0.03	5.8±0.03*
CMBC	3.3±0.03	5.7±0.06*	4.5±0.03	5.7±0.04*	1.8±0.02	2.2±0.02*

*Significance $p \leq 0.05$.

In order to determine the relative contribution of changes in red cell velocity and red cell concentration to changes in perfusion, with and without heating, CMBC and velocity are presented in **Table 1** for control (3.7 mmHg), 18 mmHg and 73 mmHg surface pressures. For control, the significant increase in perfusion with heating was a direct result of increased CMBC, but not of red cell velocity. For a surface pressure of 18 mmHg, a similar significant increase in

perfusion occurred, again attributable to an increase in CMBC but not red cell velocity. However, at a pressure of 73 mmHg there was an increase in CMBC and a compensatory decrease in velocity due to heating, resulting in no statistically significant difference in perfusion.

Spectral power of skin perfusion (an indicator of oscillations in skin perfusion) for control (3.7 mmHg), 18 mm and 73 mmHg of surface pressure with (36°C) and without (28°C) heating are shown in **Table 2.** Total spectral power increased (p<0.05, n=8) with heating at control (3.7 mmHg) and at a surface pressure of 18 mmHg, but not at 73 mmHg (**Table 2**).

Table 2.

Spectral Power (PU^2) as a percent of total power at three different skin surface pressures without (28°C) and with heating (36°C). Frequency ranges include Low (0-1 Hz), Mid (>1-2.5 Hz), and High (>2.5-5 Hz).

Frequency			Force			
	3.7 mmHg (control)		18 mmHg		73 mmHg	
	28°C	36°C	28°C	36°C	28°C	36°C
Low	0.23±0.04	0.49±0.04*	0.24±0.04	0.38±0.05*	0.17±0.04	0.23±0.05
Medium	0.36±0.05	0.23±0.04*	0.28±0.05	0.27±0.04	0.44±0.04	0.49±0.03
High	0.41±0.03	0.27±0.04*	0.47±0.05	0.37±0.06*	0.38±0.05	0.28±0.03*
Total	1.3±0.05	3.2±0.05*	12.7±2.5	23±3.2*	9.3±2.3	11±2.4

*Significance p≤0.05.

A more detailed analysis of the total spectral power revealed that as skin temperature increased from 28 to 36°C, changes were found to be associated with specific frequency ranges of the total spectra. Therefore, total spectral power was divided into three frequency ranges: low (0-1.0 Hz), middle (> 1.0-2.5 Hz) and high (> 2.5-5.0 Hz) for further analysis. This approach revealed that as skin temperature increased from 28 to 36°C, spectral power increased (p≤0.05) in the low range at both control and 18 mmHg surface pressures. Data for ranges are presented as normalized spectral power (i.e., spectral power in a frequency range divided by total power). Normalization was used to avoid the possibility of the values of any one animal dominating the responses to surface pressure or temperature. Increased temperature caused a three-fold increase in spectral power of control perfusion, with the majority of change occurring in the low range (<1.0 Hz), the range in rats thought to be associated with vasomotion (10). Further analysis indicated that the peak in spectral power in the low range (0-1.0 Hz) significantly increased with heating at both control (3.7 mmHg) and at 18 mmHg, but did not occur at 73 mmHg.

To determine in more detail the effects of increasing pressure and temperature on skin perfusion (Protocol II), surface force was increased in 3.7 mmHg increments (3 min/ increment) up to 62 mmHg. The results of these experiments (n=8) are shown in **Figure 4.** Two factor, repeated measures ANOVA indicated significant pressure effect (p≤0.0001) as well as a significant pressure by temperature interaction (p≤0.005). P_m at 36°C was significantly greater (t-test, p

≤0.05) than at 28°C for control and for surface pressures of less than 18 mmHg. For skin pressure greater than 18 mmHg, P_m was not significantly changed by heating. The reactive hyperemia peak (~3.3 times control) at 36°C was greater (p≤0.05) than that at 28°C (~3.2 times control).
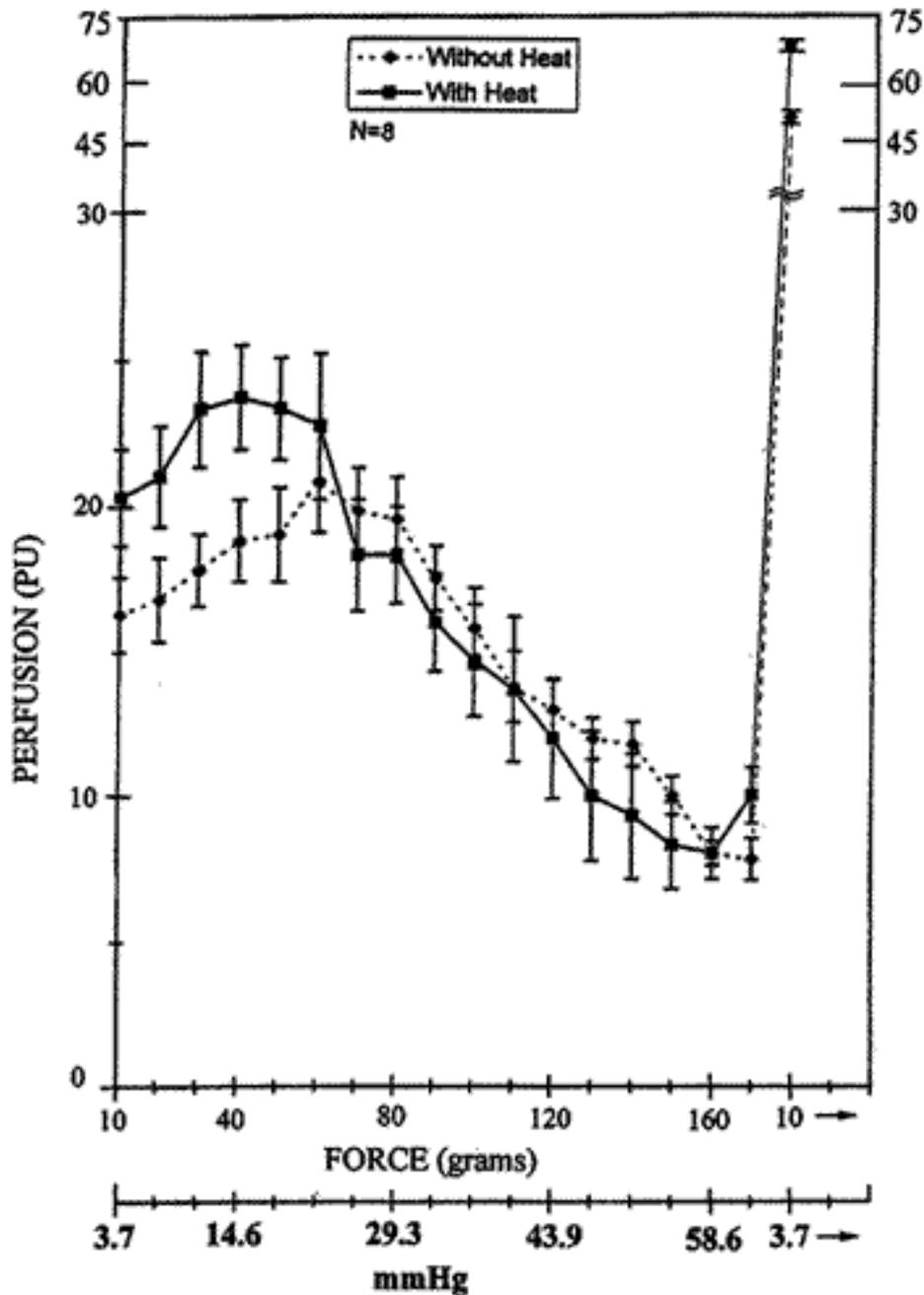

Figure 4.
Skin perfusion with (36°C) and without (28°C) heat for step increases in skin surface pressures (Protocol II).

Figure 5 shows the relationship between perfusion, CMBC, and red cell velocity without heating, as the surface force was increased from 3.7 to 62 mmHg. Changes in CMBC paralleled the changes in perfusion, while changes in red cell velocity occurred in the opposite direction. With an increase in pressure above 26 mmHg, both perfusion and CMBC decreased while red cell

velocity increased in both heated and unheated tissue (**Figure 5** and **Table 1**). This series of events may have occurred as vessels were compressed in response to the larger force causing both perfusion and CMBC to decrease. A concurrent increase in red cell velocity implied that there may have been fewer red cells, but those remaining were moving more quickly through pressure constricted passages.
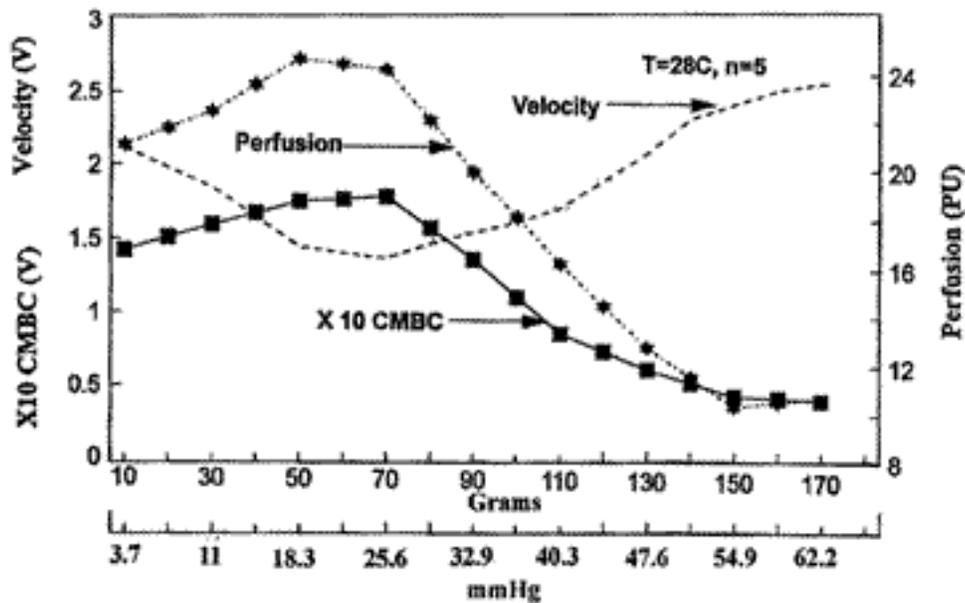

Figure 5.
Comparison of changes in perfusion, velocity and CMBC in response to pressure increases (protocol II).

Skin displacements associated with the pressure/temperature responses (Protocol II, n=5) are shown in **Figure 6.** The linear displacement noted with increasing force from 3.7 to 44 mmHg was followed by a slightly stiffer (decreased slope), linear displacement from 48 to 70 mmHg. The effect of heating was to increase stiffness for forces greater than 18 mmHg.
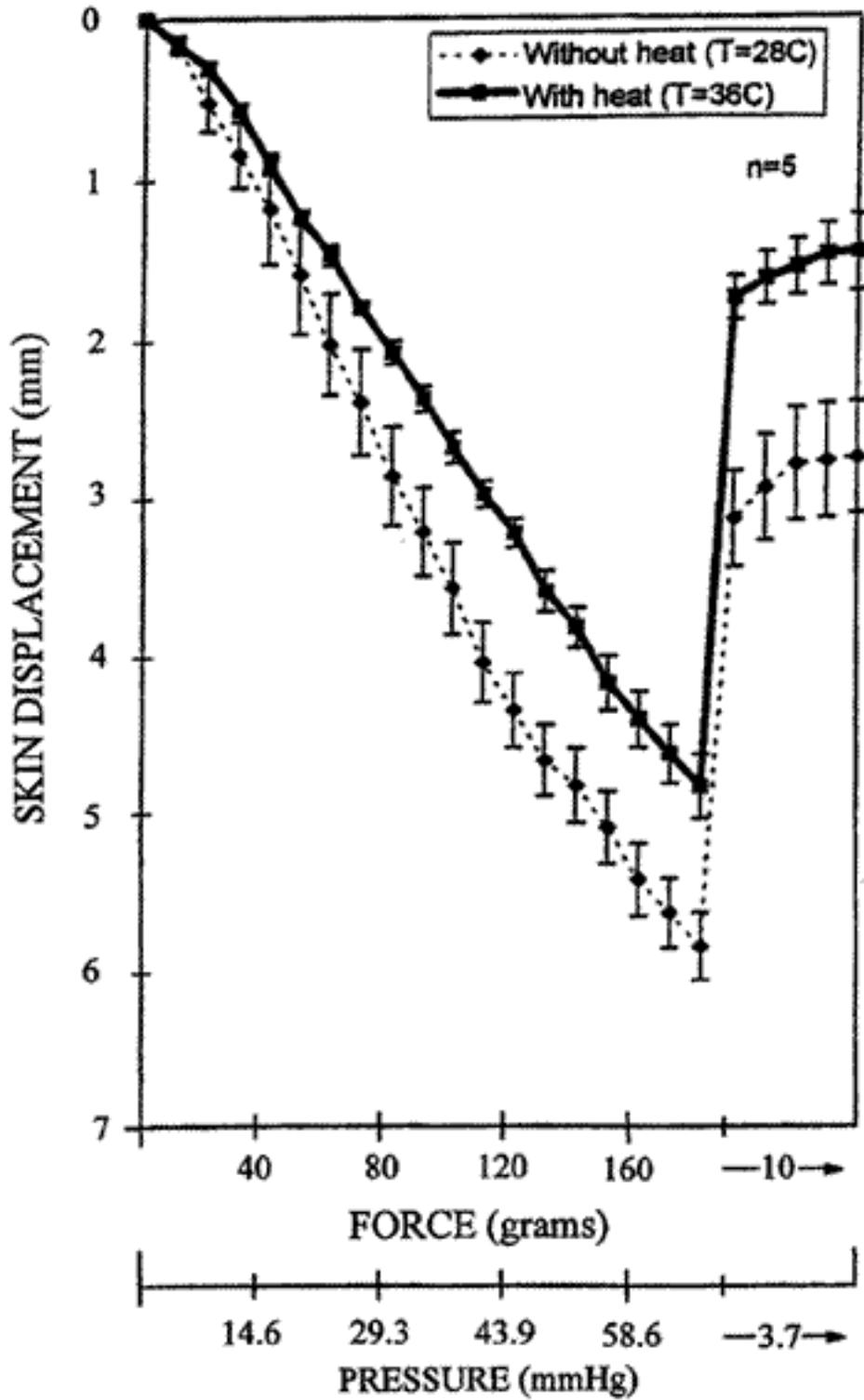
Figure 6.
Skin displacement with (36°C) and without (28°C) heat for step increases in skin surface pressures (Protocol II).

  To evaluate the effects of surface pressure and duration on tissue deformation, force was held constant while displacement and perfusion were measured over time (Protocol III, **Figure 7**). In that figure, time in minutes is plotted on the X-axis and displacement in millimeters on the Y-axis. As indicated by the X-axis, force was initially held constant at 3.7 mmHg (control) for 15

min, then increased to 18 mmHg, held constant for 36 min, and finally reduced to 3.7 mmHg for a 30 to 90-min recovery period. Even during control with only 3.7 mmHg pressure, there was a small but rapid displacement followed by a gradual creep. When pressure of 18 mmHg was applied, a rapid increase in displacement occurred, followed by a more gradual increase. When surface pressure was rapidly decreased to the control value, the skin abruptly recoiled and then slowly returned to its initial conformation.
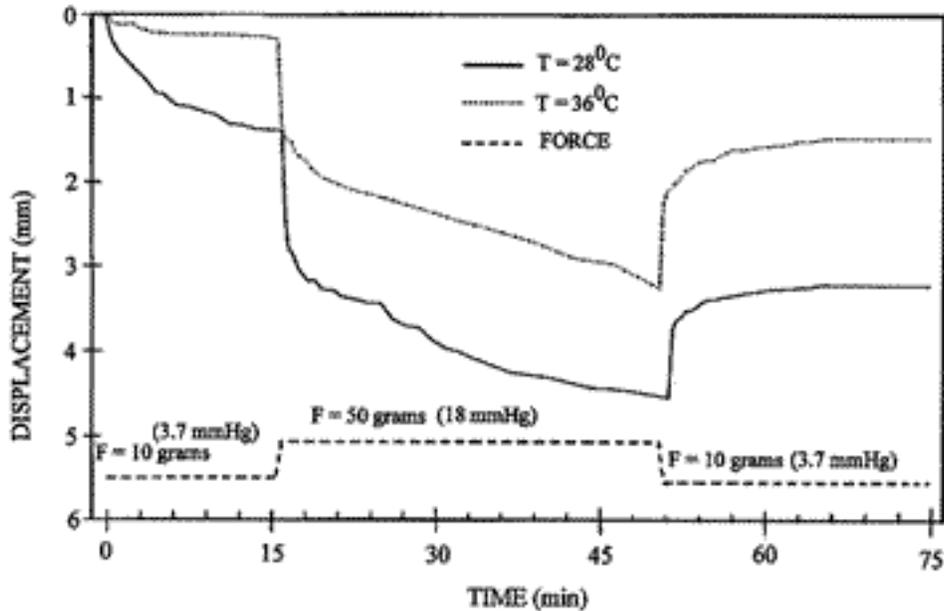

Figure 7.
Effects of temperature on displacement due to constant force (Protocol III).

The effect of increased skin temperature on displacement is also shown in **Figure 7.** A reduced rate of creep occurred at 36°C during control compared to the rate at 28°C. Skin heating produced a similar rate of creep for both temperatures when surface pressure was maintained at 18 mmHg. Both the creep associated with control pressure and the final displacement associated with 18 mmHg were significantly ($p \leq 0.05$) different with heating. After the force was released, skin recoil was always greater in heated tissue. These results were not affected by the order of the application of heat (i.e., whether the test was done initially with no heating followed by heating or vice versa).

DISCUSSION

Advantages of the Fuzzy Rat Animal Model
The fuzzy rat animal model provides several unique features for pressure ulcer or general skin studies. This strain of rat is essentially hypotrichotic (11) and therefore does not require depilation or prior treatment of the skin, to remove the fur (which might contribute to the formation of artifacts during pressure studies) present in the normal rat. However hair follicles, at reduced numbers, are present and manifest themselves on the skin surface as light fuzz, closely resembling

the human skin with light body hair present.

   The rat is relatively inexpensive in both initial cost and maintenance and therefore highly efficient from a cost standpoint and uniquely well suited for large experimental trials.

   More is known about the pharmacological effects, including the absorption, distribution, and metabolism of drugs with the rat than in any other species, including the human.

   The fuzzy rat computer-controlled animal model is well-defined (12) in terms of the presentation and application of pressure effects on both skin and muscle, especially in terms of the sequential histology (9). The model has been successfully used to evaluate drug intervention on pressure ulcer development (13).

   The microvascular circulation and thermal effects on blood perfusion in the rat and human have been shown to have key similarities (14). For example, the hairless plantar paw surface of the rat shows a high skin blood flow with a substantial response to thermal stimulation. This contrasts with the hair covered areas such as the back, where there is much lower basal flow and thermal response. These properties are similar to the differences seen in humans between skin sites which have a high density of arterioles and venules (AV areas) and sites with predominantly nutritive capillary perfusion. Therefore the conclusions drawn in this study on the rat have a high degree of probability of being successfully extended to human skin.

Effect of Heating on Skin Blood Flow during Control (3.7 mmHg) Surface Pressure
   Mean skin perfusion during control increased significantly ($p \leq 0.05$) between 28 and 30°C and remained elevated at about the same level for temperatures from 32 to 36°C. The mechanisms responsible for these temperature-induced increases in perfusion are most likely multifactorial in nature. The response may be due to a combination of factors including: 1) temperature-mediated increased perfusion in opened vessels and/or opening of previously closed vessels (recruitment) (15); 2) the presence of temperature-sensitive precapillary sphincters with the capability to alter resistance to flow based on the ambient temperature (mediated by withdrawal of vasoconstrictor tone or by active vasodilatation; 16,17); 3) temperature-mediated increased capillary pressure which in turn led to increased perfusion (18,19); 4) temperature-mediated increased production of endothelial relaxation factor (EDRF), which led to increased perfusion (20); and 5) an increase in perfusion due to temperature resulting from changes in blood viscosity and the properties of red blood cell (RBC) membranes (21). A temperature increase could have led to decreased blood viscosity, by inhibiting rouleaux formation or by causing a more effective mixing of blood constituents. With increasing temperature, the RBC membrane may become more compliant (i.e., offers less resistance in passing through narrow capillaries) and may lead to increased perfusion due to increased RBC velocity and/or concentration.

   In the present study, heating to 36°C (control; **Table 1**) caused approximate increases of 25 percent in perfusion and 50 percent in RBC concentration, but a decrease of approximately 18 percent in RBC velocity. A decrease in RBC velocity was not observed in humans by Rendell (21). Rendell (21) and Richardson (22), also investigating humans, reported that heating produced an increase in perfusion in nonnutritive sites containing few arterio-venous anastomosis. This

increase in perfusion was attributed to recruitment of vessels as reflected in an increase in red cell concentration. Their findings are consistent with the increase in red cell concentration measured in the present study, but not with the decrease in velocity that we observed.

Vasomotion, as manifested by oscillations in skin perfusion, has been described by others (23, 24). Power in the low frequency (<1.0 Hz) range of perfusion in rats has been thought to be associated with vasomotion (10), while power between 1 and 3.5 Hz has been associated with respiration induced changes, and that between 3.5 and 5 Hz with the cardiac cycle. Evidence of vasomotion as reflected by oscillations in perfusion was also observed in the present study. In addition, we found indirect evidence that vasomotion was significantly affected by changes in temperature. Surface temperature greatly affected the frequency and amplitude of perfusion. At control, a temperature increase of as little as 4°C caused a change in the amplitude of the perfusion oscillations (**Figure 2**, top). Oscillations in flow characterized by low frequency (0.1-0.2 Hz) and large amplitudes became more predominant with increased temperature. A similar temperature mediated effect of increasing vasomotion frequency and amplitude was reported in man (25, 26) and in the bat (27). Also, Bernardi et al. (28) presented evidence in human skin that laser Doppler flow fluctuations at 0.1 Hz were markers of sympathetic activity to the circulation and suggested that fluctuations less than 0.1 Hz, as observed by others, were probably due to vasomotion related to thermoregulation. The overall effect of periodic changes in vessel diameter has been suggested by Funk et al. (29) to reduce resistance, thereby increasing perfusion for the same driving pressure. The responses presented in **Figure 3** between 28 and 30°C do not show the suggested correlation between increasing P_m and increasing spectral power in the low frequency range (an indicator of vasomotion). However, information about perfusion driving pressure in the present studies is unknown; therefore, we cannot confirm or deny Funk's analysis.

One possible explanation for the absence of temperature-mediated changes in spectral power at the lower temperature range may be that vascular smooth muscle is not very responsive in this temperature range (28 to 30°C), while at the high temperature range (34 to 36°C) it may already be maximally dilated with no further increase in vasomotion possible (i.e., no increase in spectral power in the low frequency range).

Surface Pressure-Induced Changes in Perfusion with Heating
This study showed that skin perfusion was significantly greater at 18 mmHg than at control pressure when skin temperature was elevated. Previous studies (30,31) have shown similar temperature induced elevations in blood flow; however, pressure effects were not investigated.

When surface pressure greater than 73 mmHg was applied, there was an overall reduction in perfusion relative to control. As additional pressure was transmitted to the skin, increased deformation and compression of vessels most likely occurred, which eventually produced complete occlusion. As the effective diameters of the capillaries and arterioles were diminished or totally occluded, perfusion decreased until zero perfusion was achieved.

The increased perfusion (**Table 1**) with heating (28 to 36°C) during control and 18 mmHg was associated with an increase in vasomotion as indicated by an increase in low frequency spectral power (**Table 2**). No increase in perfusion (**Table 1**) or vasomotion (**Table 2,** low frequency

content) with heating was seen at 73 mmHg. This observation was most likely a result of mechanical occlusion of vessels induced by high surface pressure.

In experiments where surface pressure was increased incrementally, skin perfusion initially increased with low levels (<18 mmHg) of surface pressure. This response was observed in a previous study using rats where temperature effects were not included (32). Schubert and Fagrell (3) observed a similar increase in perfusion with increasing pressure at 25 to 50 mmHg in human subjects.

Several plausible mechanisms for an increase in perfusion with increasing surface pressure (<18 mmHg) are:
1) One of the mechanisms involved in autoregulation is the myogenic response first described by Bayliss in 1902 (33). As the transmural pressure is decreased (i.e., external pressure is increased or intravascular pressure is decreased), the smooth muscle cells relax, leading to arteriolar vasodilatation. In this study, as the external pressure is increased from 3.7 to 18 mmHg, autoregulation (33) most likely produced arteriolar vasodilatation during increasing surface pressure (<18 mmHg) in order to maintain constant perfusion. However, the ability of tissue to maintain perfusion due to autoregulation in response to increasing pressure is limited. As external pressure is increased above 18 mmHg, some of the vessels may be mechanically occluded and autoregulation can no longer compensate for decreased levels of perfusion. Experimentally, we found that perfusion decreased as external pressure exceeded 18 mmHg.
2) The increased perfusion noted with increased surface pressure could be an artifact of tissue compression. The LDF, which measures perfusion at a depth of approximately 1 mm, may be viewing additional and/or different vessels in its measuring volume as surface pressure is increased. Therefore, with compression, flow in the deeper horizontal running arterioles may now be detected by the LDF. This may result in an increased perfusion signal from the increased red cell velocity and concentration in these larger vessels. However, increased red cell velocity with increased skin perfusion did not occur (**Table 1**) when pressure was increased from control to 18 mmHg, suggesting that artifacts of tissue compression were probably not the cause. Data from another study (32) further support this conclusion.

In terms of temperature effects on ulcer development, a previous study has reported the results of decreasing temperature on the formation of deep ulcers after prolonged application of surface pressures on the dorsal surface of pig skin (34). Decreasing temperature was found to decrease the size of ulcers formed in response to a fixed surface pressure.

Surface Pressure-Induced Skin Deformation
In Protocol II, where incremental steps of increasing pressure (3.7 mmHg/3 min) were applied, changes in deformation were found to be linear (regression coefficient, r=0.90; see **Figure 6**). Similar results were shown by Sangeorzan et al. (35) for human skin subjected to external pressure. They reported that skin compression over the tibia as measured by a LVDT increased linearly with surface pressure from 0 to 20 mmHg and from 40 to 120 mmHg but at a different slope. This type of tissue deformation may be explained by the mechanics of water entry and removal from the tissue (36). **Figure 6** shows that the tissue only partially recovers after pressure release. The slow rate of tissue recovery may be explained by the observation that the rate of water entry into the tissue does not match the speed of its removal under pressure. Return to near

baseline levels may take more than 2 hrs (37). The rate of water absorption into the tissue following pressure release appears to be slower than its rate of removal under pressure.

In experiments to study viscoelastic characteristics of the skin and underlying tissue (Protocol III), application of the control pressure of 3.7 mmHg at 28°C, for 15 min, produced a gradual rate of deformation (creep), probably caused by a continuous removal of water from the interstitial spaces as mentioned above. The rapid application of 18 mmHg triggered an immediate deformation of the tissue, probably caused by a shape change in the relatively unrestricted (constant volume) tissue, rather than by the removal of water from the interstitium. The initial deformation was followed by a more gradual tissue creep over a 30-min period. At control pressure (3.7 mmHg), there was much less creep in heated skin than in unheated skin. For 18 mmHg after the initial rapid displacement, creep was similar between heated and unheated tissue. At the end of 51 min, the final displacement of heated skin was less than that of unheated skin. This difference occurred because of the different rates of creep during control (3.7 mmHg) which led to a greater initial displacement for unheated skin as compared to heated skin prior to the application of 18 mmHg surface pressure.

As surface pressure was decreased from 18 mmHg back to control (3.7 mmHg), there was a rapid decrease in elastic deformation (change of shape) followed by a gradual recovery. The recovery rate is initially rapid but quickly slows (**Figure 7**). This response may be a result of water movement into the interstitium with the release of pressure. The slower rate at which water returns to the interstitium may be partially caused by the structure of the lymphatics, which are in close proximity to other microvessels, within the tissue (38). With the application of pressure, some of the water in the interstitium is forced into the lymphatics and eventually into the central circulation. After release of external pressure, some fluid moves into the tissue from the adjacent areas, but fluid cannot move directly out of the lymphatics into the interstitium. A similar response for human skin subjected to a short periods of negative pressure (suction) was reported by Barel (39).

To determine the length of time required for tissue recovery, deformation was monitored for an extended time after pressure release. Residual deformation (0.5 mm) was still observed 3 hrs after pressure was released. A similar observation was made by Reddy (37) up to 6 hrs following pressure release.

The rate of displacement in the present study was probably due to decreased water permeability of tissue induced by tissue compaction (40). As water was removed from the interstitial space, the change in permeability may have resulted from an increasing affinity of protein molecules for the remaining water (as predicted by the Donnan Equilibrium Equation). Therefore, at ambient temperature, further removal of water became more difficult (41).

Raising skin temperature resulted in stiffer tissue that demonstrated less creep with added surface pressure. These temperature-induced differences may have been caused by decreased tissue permeability for interstitial fluid and/or changes in the microstructure of the interstitial matrix. With decreased permeability, less fluid was probably removed from the interstitium, thereby decreasing the rate of deformation (37). In cartilage, Mow et al. (41) concluded that compressive changes in the solid matrix resulted from exudation of the interstitial fluid. The

changes in skin deformation reported here and triggered by compression resulted from an interactive response of alterations in the flow of the interstitial fluid through the matrix and deformation of the matrix itself.

With respect to skin perfusion flowmotion, Bernardi et al. (28) presented evidence in sympathectomized patients that laser Doppler flow fluctuations were coherent with those obtained for blood pressure. Based on this evidence and additional data, they concluded that the frequency/amplitude content of skin blood flow, as determined by frequency analysis, reflects modifications induced by autonomic activity.

Clinically, our finding that a temperature-induced increase in perfusion may be of use in delaying the onset of skin destruction that often occurs during periods of prolonged pressure application to the skin. For example, debilitated patients confined to a nursing home bed are prone to the development of ulcers over the sacrum and ischium areas. Ulcers continue to occur in these patients with or without periodic shifting of the body to relieve skin pressure. Some of the commercially available air/water mattresses attempt to alleviate this problem by increasing and decreasing the pressure in the mattresses. By warming the fluid (air or water) several degrees in these mattresses, the blood supply to the tissues prone to develop ulcers could be increased, which may serve to decrease the chances of tissue destruction if proper dietary intake and utilization are maintained.

The beneficial temperature-induced effects of increased blood supply to the skin must be balanced against the increasing nutritional requirements of the tissue, which also occur. For each 1°C increase in temperature, the nutritional requirements of skin cells are raised 10 percent. The benefits of flushing toxins away from skin tissue must be weighed against the potential for oxidative damage and reperfusion injury. The data in the present study are consistent with the hypothesis that a small increase in temperature in the range of 1-5 degrees may be beneficial in preventing tissue breakdown and reduce the chances of pressure ulcer formation. However, Kokate (34) and Iaizzo (42) reported that in porcine skin reducing the surface interface temperature decreased the severity of damage due to pressure application. Clearly, additional studies are required to determine the effect of raising and lowering temperature during surface pressure-induced ulcer formation.

CONCLUSIONS

Two important external parameters known to influence the formation of pressure ulcers have been studied: skin surface pressure and increased temperature. The major conclusions of this study are:

1. There was a significant increase in perfusion with increased temperature at surface pressures below 50 mmHg, probably due to local auto regulatory mechanisms. Increased temperature also caused low frequency oscillations of arterioles to increase, suggesting possible increased vasomotion and decreased vascular resistance.

2. Increased temperature caused skin to become stiffer in response to increased surface pressure. At constant pressure, heated skin did not deform as much as unheated skin. Also with heating, tissue was stiffer and did not creep as much as without heating, possibly a result of heat-induced changes in tissue permeability of the interstitial matrix.

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