Biophysical Effects of Water and Synthetic Urine on Skin

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Abstract

Objective: Pressure ulcers often occur at sites subjected to combined pressure and wetness. Though skin wetness is a risk factor to be avoided, the mechanisms and effects of wetness vs. urine constituents on skin breakdown is unclear. We tested the hypothesis that wetness reduces skin hardness and thereby increases vulnerability of underlying blood vessels to pressure induced flow reductions.

Design: Pads saturated with water alone or with the main chemical constituents of urine (S-urine) were applied to forearm skin for 5.5 hours. Skin hardness (durometer), blood flow change caused by 60 mmHg of pressure (laser-Doppler), erythema and temperature were compared among dry, water and s-urine test sites.

Participants: Ten healthy females

Setting: Research Center

Main Results: S-urine and water caused significant reductions in initial hardness and caused greater initial perfusion decreases during pressure load compared with dry sites. Skin temperature and erythema were less at wet exposed sites.

Conclusions: Findings are *consistent with* the concept that sustained skin wetness increases vulnerability to pressure induced blood flow reduction. Effect appears mainly dependent on wetness but urine constituents may exacerbate effects. *Also, wetness related skin cooling might also play a role.* In the healthy subjects studied, the blood flow decrease was not sustained due to perfusion recovery under load. However, in patients in whom this recovery mechanism is compromised, skin wetness would likely have more sustained and ominous effects. Thus, measures to diminish skin exposure to wetness in such patients, whatever the wetness source, would seem to be an important consideration in a multifaceted strategy to reduce the risk of pressure ulcers.

<u>Introduction</u>

Common sites for pressure ulcers are at skin areas not only subjected to pressure but also exposed to increased wetness from perspiration and urine.[1-2] The role of wetness per se compared with the composition of wetting substances in the skin breakdown process is unclear. It has be stated that skin integrity is compromised by dampness, somewhat independently of the source of the wetness.[3] With respect to the impacts of urine, this view is consistent with findings reported for the role of urinary ammonia in infant diaper dermatitis.[4] However, experimental animal work[1] and some pediatric findings[5] suggest interactions between urine and fecal enzymes that promote skin damage. Because this process depends on breakdown of urinary urea, such an effect would seem to depend on urine presence. Some of the main extrinsic factors involved in diaper dermatitis and projections as to their role in adult perineal skin injury have recently been summarized.[2] These include skin wetness, urine and its components, feces and its components and skin pH. *No doubt there are interactions among these quantities, and others such as skin temperature[6] and other, as yet unknown factors.* However, it is known that mere wetting of skin (water or urine) increases skin permeability to noxious substances.[7-8]. This altered skin barrier function likely plays some role in the breakdown process.

An aspect not previously addressed is the issue of if and how skin wetness may affect mechanical properties of skin and underlying tissue. A possible mechanical linkage between skin wetness and pressure effects is suggested from a near 90 year-old observation that pressure required to compress nailfold capillaries is reduced when skin is moist rather than dry.[9] This phenomena may be attributed to a reduction in skin stiffness of moist skin and thus less "elastic" skin resistance to oppose the pressure load. A natural extension of this early observation is that such a process may render moist tissue blood perfusion more vulnerable to pressure induced blood flow deficits. Whether such effects would depend on skin wetness alone or wetting agent constituents is unknown. Thus the primary aim of the present work was to investigate the relative effects of water vs. a water solution containing the main chemical constituents of urine, on skin tissue hardness, erythema, temperature and blood perfusion changes caused by a standardized local pressure load. This was done by applying water and synthetic urine soaked pads to volar forearm skin for ~ 5.5 hours, and then measuring and comparing each skin biophysical parameter in a sample of 10 healthy female volunteers.

Methods

Subjects

Ten female volunteer subjects (28.8 ± 2.8 years) selected from the School of Pharmacy, participated after signing an Institutional Review Board approved informed consent form. To be accepted for participation, volunteers needed to be at least 21 years of age and in generally good health. Subjects would not qualify for participation if any of the following were true: 1)a history of arm vascular abnormalities or disease, 2)taking any medication or substance affecting blood vessels, 3)pregnant or possibly pregnant 4)known allergies to any substance 5)open skin area on their arm 5)diabetes. *No attempt was made to test these subjects on a constant day of their menstrual cycle*.

Procedure

Subjects were asked to arrive at the laboratory wearing short sleeves. Upon arrival, four 1.5 inch diameter pads were placed on their volar forearms and each covered with a water impermeable transparent dressing. Two of the pads were saturated with 6 ml of fluid, one being plain water and the other synthetic urine (s-urine). The proximal edge of these wet pads was placed about 4 cm distal to the antecubital space. A dry control pad was placed distal to the wet pad on each arm *such that its proximal edge was about 2.5 cm from the distal edge of the wet pad*. Subjects were then excused to attend classes. They returned about 5 hours later for testing. Pads were removed in random order and biophysical measurements were then done at each test site. Overall, pads were left in place for 325 ± 15 minutes (mean ± sem).

Synthetic Urine

S-urine formulations have been used for many applications with several variants of its constituents.[10-13] The formulation used in the present study is derived from these and specifically has the following constituents in grams per liter of sterile water: Urea 25 g, Sodium Chloride 9 g, Disodium hydrogen orthophosphate, anhydrous 2.5g, Ammonium Chloride 3 g, Creatinine 2g , sodium sulfite, hydrated 3g. The S-urine solution has a pH of 7.8.

Skin Blood Perfusion Measurements

After pad removal, a laser-Doppler probe (Vasamedics, Minneapolis MN, standard right angle) was taped to the center of the test site. Principles of operation have been previously described.[14-15] Briefly, the probe transmits a low intensity laser light signal into the skin to a depth of about 1 mm and the reflected light is used to measure local blood perfusion via the laser-Doppler method. The Doppler-shifted signal contains information about the speed and number density of moving red blood cells in a tissue region to a depth of about 1-2 mm. Speed and number density information is processed to yield a parameter - perfusion - that is proportional to blood flow.

Perfusion was measured and recorded continuously at each site, in sequential order, for 15 minutes divided into three five-minute intervals. The 1st interval was baseline preload, the 2nd was during tissue loading and the 3rd interval was after load removal. These three intervals are illustrated in **figure 1**. Blood perfusion in each interval is, for convenience, expressed as the interval's flow integral calculated by the product the perfusion x time. *These data were processed off-line*. Average laser-Doppler perfusion in perfusion units (p.u.) can be obtained by dividing the flow integral by 300 seconds.

Tissue Loading

The tissue was loaded *for five minutes* with a weight of 258 grams placed onto the laser-Doppler probe *(diameter=2.0 cm). The weight was a solid brass rod that had its contact cross-section machined to fit directly onto the probe. The rod lied flat against the portion of the probe in contact with the skin and was near self-supporting during the 5-minute loading interval. The experimenter, via light finger pressure, manually minimized any slight movements away from its vertical (90°) position.* This weight was chosen to produce an average skin pressure of 60 mmHg. The skin surface pressure level, *using the above support technique,* was confirmed by measurements of the interface pressure using a pressure measurement system. Based on previous work[16-17] using a similar loading procedure it was reasoned that if a substantially larger pressure were used then differential effects due to wetness may be masked. Typically when loads in excess of arterial pressure are used, flow is reduced to a biological zero value as illustrated in **figure 2**. Thus the pressure load used was standardized to 60 mmHg.

Tissue Hardness

Hardness was measured with a handheld durometer (Rex Gauge Co., Model 1600 - OO, Buffalo Grove IL). The device weighs about six ounces and records hardness in relation to the deformation resistance encountered by a small 3/32" spherical spring-loaded indentor that is in contact with the skin and coupled to a calibrated analog meter with a range from 0 to 100 points. The higher the reading, the harder the material under test. Durometers are calibrated according to engineering standards (ASTM D2240) and the type used in the present study (Type OO) is optimal for human skin work. *Units of measure are durometer points and are dimensionless.* Because of a process called "creep" whereby skin and underlying tissue deform slowly with time during loading, the initial skin hardness reading is

usually the highest value and some reduction in hardness is recorded with time. Previous work has shown that most or all of the change is complete in less than 30 seconds in normal arm.[18] and this was confirmed in our hands in preliminary tests. In the present work, the hardness measurement was made over a full minute, and the initial and final hardness measurements separately analyzed. A similar durometer device has been used to measure skin hardness of patients with sclerederma.[19] Tissue hardness was measured at each test site prior to pad placement and subsequently after pad removal.

Skin Erythema and Temperature

Erythema was measured at each test site before pad placement and after pad removal using an erythema meter (Dia-Stron, LTD.). This device illuminates the skin with a tungsten-halogen white light via a fiberoptic cable and probe and then collects the diffusely reflected light from the skin. The amount of energy reflected at 546 nm, the peak absorption wavelength for hemoglobin, is inversely proportional to the amount of hemoglobin. A second reflected wavelength (671 nm) is used as a reference signal to compensate for skin tone, probe alignment and other factors. From the reflected energy at these two wavelengths, an erythema index is determined that is linearly related to the amount of hemoglobin concentration or degree of redness. Six erythema measurements were *made in rapid succession* within each test site. *These were done by placing the probe in gentle contact with the skin, recording the erythema index, removing the probe and then repeating the sequence five additional times. Each measurement was made at a different spot within the test site. The time to acquire all six measurements was about two minutes.* The average of these six measurements was used to represent the erythema index of each site. All sites were so measured before pad

placement and then after its removal. Skin temperature *at wet and dry sites was measured after bandage removal* with a fast response thermocouple in the central region of the site. *Temperature measurement was with a standard surface temperature probe with a one centimeter diameter surface contact area.*

Statistics

Statistical comparisons are based on the non-parametic Wilcoxon test for paired differences with a significance level of p<0.05. All data is presented as mean ± sem

Results

Effect of wetness on hardness

As shown in **figure 3**, skin exposure to both s-urine and water caused reductions (p<0.01) in initial tissue hardness of -4.0 ± 0.8 and -3.5 ± 0.9 *durometer points* respectively. A significant reduction was maintained after one minute of test time only in skin exposed to s-urine (-2.6 ± 0.6, p<0.01).

Effects of wetness on erythema

As shown in **figure 4**, skin exposure to both s-urine and water caused reductions (p<0.05) in skin erythema of -11.1 ± 5.6 and -18.3 ± 7.0 E.U. respectively. Differences between s-urine and water exposed sites were not significant. In contrast, after exposure there was a tendency (not significant) for erythema levels to have increased at each of the corresponding non-wetted control sites. For s-urine and water controls these changes were +9.8 ± 5.3 and +6.2 ± 3.6 E.U

Effects of wetness on temperature

Skin temperature comparisons, measured ~5 minutes after removal of dressings showed that both s-urine and water exposed site temperatures were reduced as compared to their corresponding control dry sites. For s-urine and water exposures the temperature differential was respectively

-1.2 ± 0.3 and -1.5 ± .3 °C p<0.01.

Effects of wetness on blood perfusion

Basal Preload Blood Perfusion

Prior to loading, there were no significant differences between wet and dry site average perfusions on either s-urine or water exposed arms. For the s-urine exposed arm, the 5-minute perfusion integral at the wet site was 30.4 ± 6.2 compared with 39.2 ± 11.4 at the dry control site. Corresponding values on the water exposed arm were 18.8 ± 3.8 and 22.6 ± 2.0.

Blood perfusion during the full loading interval

Considerable variability in the response to local pressure loading with 60 mmHg for 5-minutes was found as illustrated with representative responses **figure 5**. Based on the perfusion integral over the entire 5-minute load interval, the s-urine and water exposed sites showed an overall mean perfusion decrease of -5.1 ± 5.9 and -2.8 ± 2.5 (p.u. x sec) respectively as compared to the pre-load perfusion. Similar variability was found in each of the dry control sites during the 5-minute load interval. Overall (wet and dry sites), 60% of the responses to local pressure loading resulted in a perfusion integral increase during the loading interval and in the remaining 40% perfusion decreased. There was no detectible correlation between the amount of perfusion change produced by loading and either skin hardness or erythema changes caused by water or urine exposure.

Blood perfusion reduction during first minute of loading

   To determine the early effects of pressure loading on blood perfusion which were reasoned to be unaffected by subsequent flow recovery, the minimum perfusion during the first minute of loading was compared with the minimum perfusion during the last minute prior to loading. These comparisons were done using absolute perfusion change and as a percentage of the preload values. Results showed that absolute and percentage reductions at s-urine exposed sites were significantly greater than for corresponding dry control sites. Thus for wet and dry sites respectively, the absolute flow reductions were -0.04 ± 0.009 and 0.01 ± 0.007 p.u. p<0.05 and for percentage reductions were -59.2± 5.2 and -21.1± 16.9%, p<0.01 .   Corresponding changes for water exposed skin (-0.03±0.01 p.u. and -49.0±12.1%) tended to be less but were not significantly different from s-urine. **Figure 6** graphically summarizes the difference in initial flow responses.

DISCUSSION

   Pressure ulcers are significant problems among some elderly bed-ridden persons and persons who are regaining their health following surgical procedures[20]. Common sites for some of these ulcers are at skin areas subjected to combined pressure and increased wetness from urine, perspiration and other sources. The main focus of the present study was the investigation of the possible role of skin wetness and wetting agent constituents on skin parameters that directly or indirectly affect the skin breakdown process. Specifically, it was hypothesized that sustained wetness would reduce tissue resistance to pressure loading thereby rendering underlying blood vessels more vulnerable to pressure induced blood flow deficits.

Hardness results showed that in fact skin wetness, maintained for about 5.5 hours, caused significant reductions in initial hardness of both s-urine and water exposed forearm skin and in final hardness for s-urine exposed skin. This finding supports the hypothesis that wet skin provides less support for pressure related deformation and suggests a possibly greater effect caused by s-urine. *As skin wetness was also associated with a slightly lower skin temperature after bandage removal than corresponding dry sites, it is unclear as to what role this reduced temperature may have played in the tissue hardness reduction. Recent work[6] has reported that skin stiffness is less at lower temperatures and this may have impacted the skin hardness measurements herein reported.*

In spite of the hardness reduction accompanying wetness, no consistent pattern of flow change occurred during the full 5-minutes of localized pressure loading. In about 60% of cases blood perfusion tended to increase during this interval usually, but not always, following an initial flow decrease. The physiological adaptive flow[21] increase during loading may have masked an initially greater flow deficit within wetted tissue. This possibility was tested by assessing early (<=1-min) effects. Results of this analysis showed that the initial perfusion decrease caused by pressure was in fact significantly greater in wet skin as compared with dry. Thus the hypothesis that reduced tissue hardness renders tissue perfusion more vulnerable to pressure loading is supported by this part of the findings although in the healthy subjects studied the flow reduction was not sustained.

The erythema reduction following the 5.5 hours of wetness exposure was somewhat similar, though less dramatic, than skin blanching-like effects of long duration water immersion of the digits. Erythema measurements showed a consistent decrease in average skin "redness" due to both s-urine and water exposure. Visual observations of wet exposed test sites most often revealed a

mottled non-uniform pattern of normal tone skin mixed with regions of relative blanch-like appearance. A consistently lower skin temperature of wet sites compared with dry control sites even up to 15 minutes after dressing removal was also found. The reduced temperature is likely in part related to the greater heat loss of the damp skin. However, in spite of the reduced temperature and erythema of the wet exposed sites, the preload blood perfusion of wet and dry sites were not significantly different. *However, a possible direct effect of the lowered skin temperature on blood perfusion regulation in response to the pressure load cannot be excluded as a contributing factor. Further work in this area is needed to more precisely estimate its effect.*

Conclusions

The overall findings are consistent with the concept that sustained skin wetness increases the vulnerability of underlying blood vessels to pressure induced blood flow reduction. The effect appears mainly dependent on wetness per se *but a possible role of skin temperature reduction may also be involved. However, since near equal temperature reductions were caused by both water and S-urine, but only S-urine demonstrated a final significant reduction in hardness, it appears that temperature effects if present would only partially account for these findings.* The present results thus suggest that the S-urine constituents may exacerbate the effects. In the young healthy subjects studied, the effect on blood flow was not sustained on average due to blood flow adaptation/recovery under load. However, it is thought that in patients in whom this recovery mechanism is compromised, skin wetness would likely have more sustained and ominous effects. Thus, measures to diminish skin exposure to wetness in such patients, whatever the wetness source,

would seem to be an important consideration in a multifaceted strategy to reduce the risk of pressure ulcers.

<u>Acknowledgements</u>

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Figure Legends

Figure 1. _Blood perfusion assessment procedure_. Skin blood perfusion is continuously monitored for a 15 minute interval that is conveniently divided into three 5-minute sub-intervals. After the initial 5-minute preload baseline, the skin is loaded with a weight that causes a surface pressure of 60 mmHg. This pressure is kept in place for 5-minutes and then removed. Perfusion in each sub-interval is determined by integrating the perfusion over the entire 5-minutes (numbers shown in each sub-interval in figure). Average perfusion in perfusion units (p.u.) is obtained by dividing the perfusion interval by 300 seconds. To determine the initial changes in perfusion that are caused by the pressure load, the minimum perfusion within the first minute after loading is compared with the minimum perfusion within the last minute prior to loading.

Figure 2. _Example of perfusion response to a high pressure load_. If a high pressure is used to load the tissue, the skin blood perfusion is invariably reduced to a very low level that is called the biological zero. In the example shown, the blood flow pulsations that are in synchrony with the heart beat, are clearly seen before the 120 mmHg load is applied. During the pressure loading interval, these pulses are rapidly lost. When the pressure is released, there is usually a transient hyperemia, a return of the pulses and then a normalization of both pulsatile and mean blood perfusion during recovery.

Figure 3. _Skin Hardness Changes_. Initial reductions in hardness are shown expressed in standard hardness units as measured by the durometer. The changes in hardness were significantly (*) greater for wet exposed skin (s-urine and water) as compared with their corresponding dry control test sites (U-Dry and W-dry). S-urine exposed skin hardness remained significantly reduced after one minute.

Figure 4. _Skin Erythema Changes_. Both s-urine and water exposure resulted in a significant (*) reduction in test site erythema as compared with pre-exposure. In contrast, dry control sites had no significant change and in fact tended to have slightly higher erythema values.

Figure 5. _Variability in blood perfusion response to pressure loading_. These examples, from four different subjects, illustrate the variability in blood flow adaptation that occurs under pressure loading. The two subjects of the left panel demonstrated an actual progressive perfusion increase during the loading interval. The two subjects of the right panel demonstrated a large perfusion reduction that remained less than preload even at the end of the load interval. All responses shown are to the same scale.

Figure 6. _Initial blood perfusion reduction due to pressure loading_
Pressure loading of 60 mmHg caused a significantly (*) greater decrease in skin blood perfusion in s-urine and water exposed skin as compared with dry control skin. The percentage reduction in skin blood perfusion for s-urine and water exposed skin did not significantly differ.
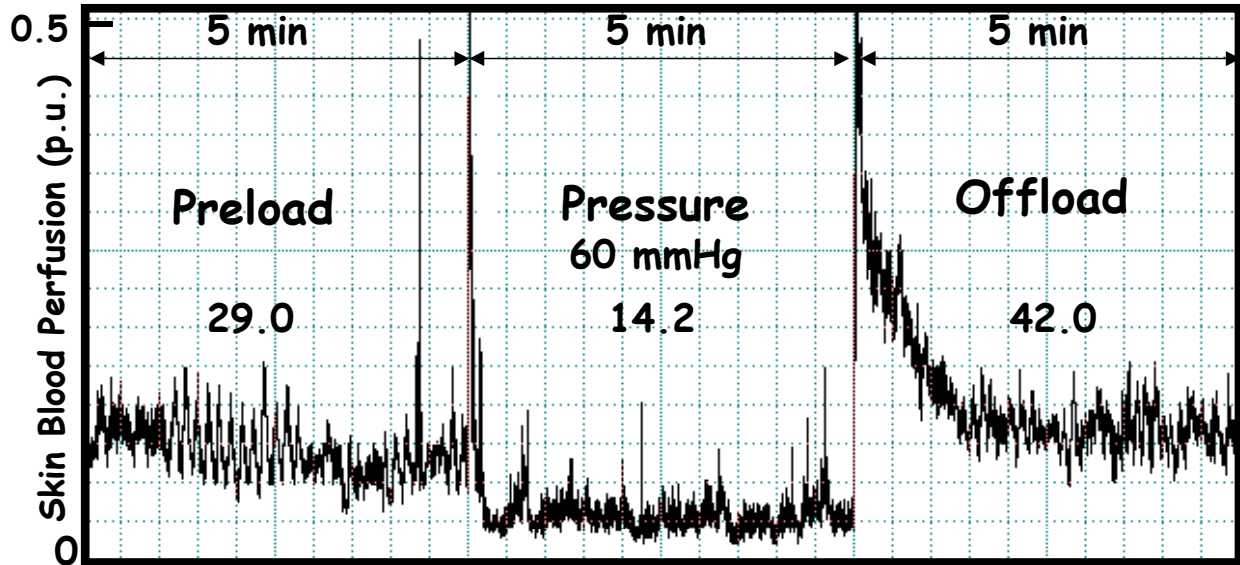
Figure 1. Blood Perfusion Protocol

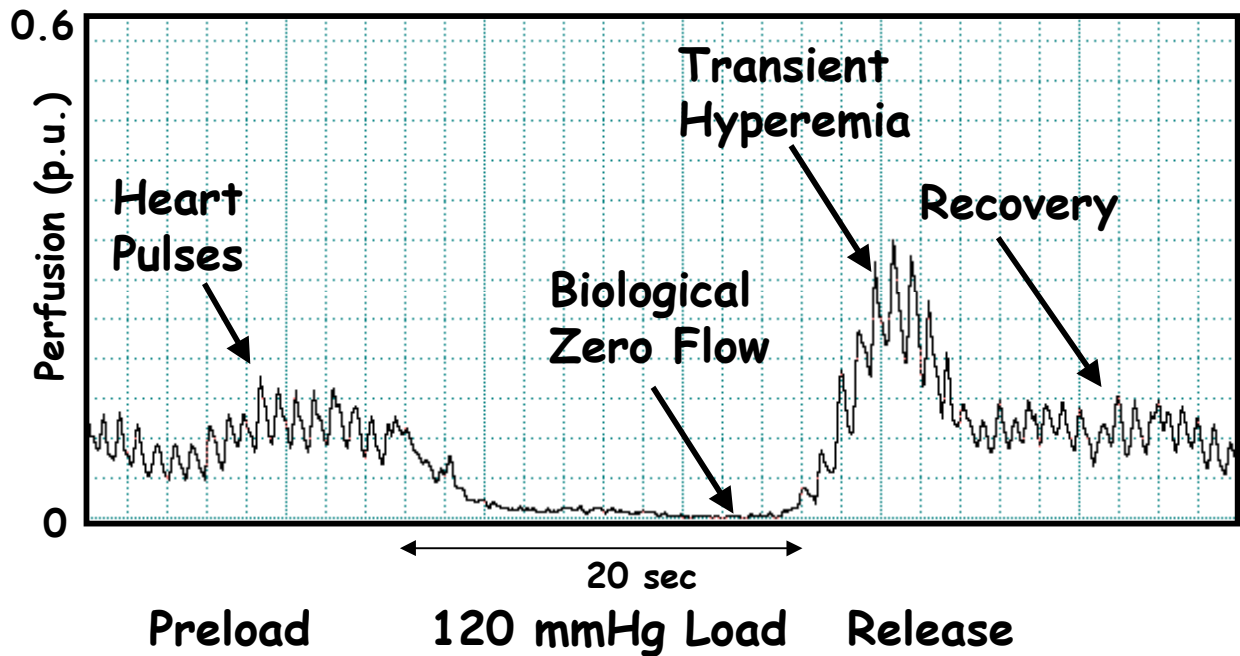

Figure 2. Example Response to High Pressure
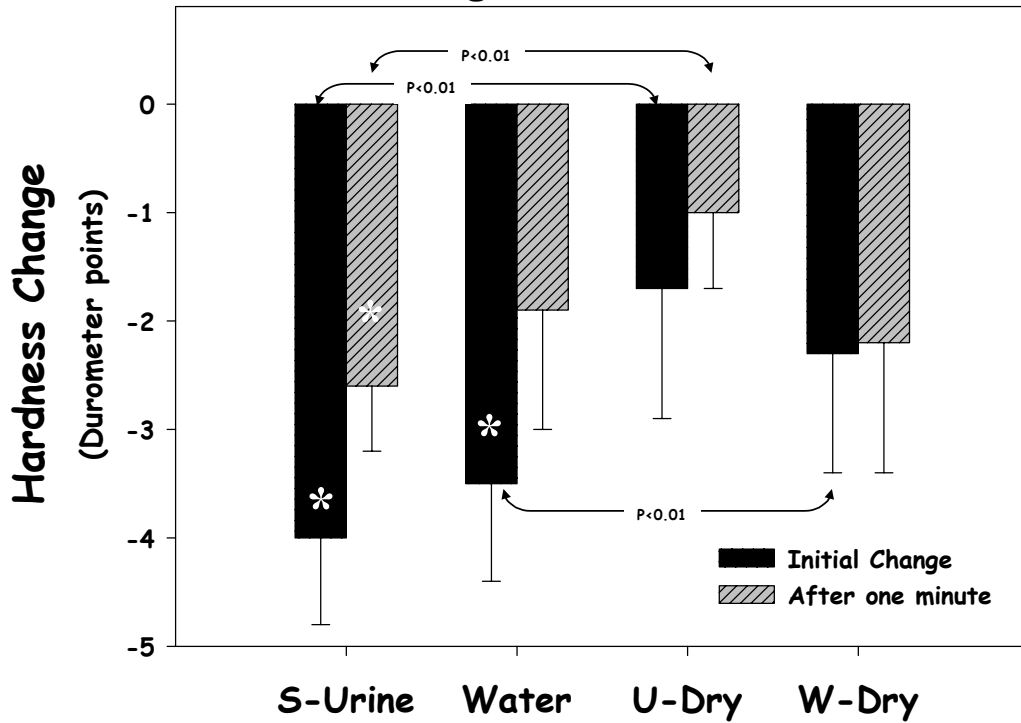
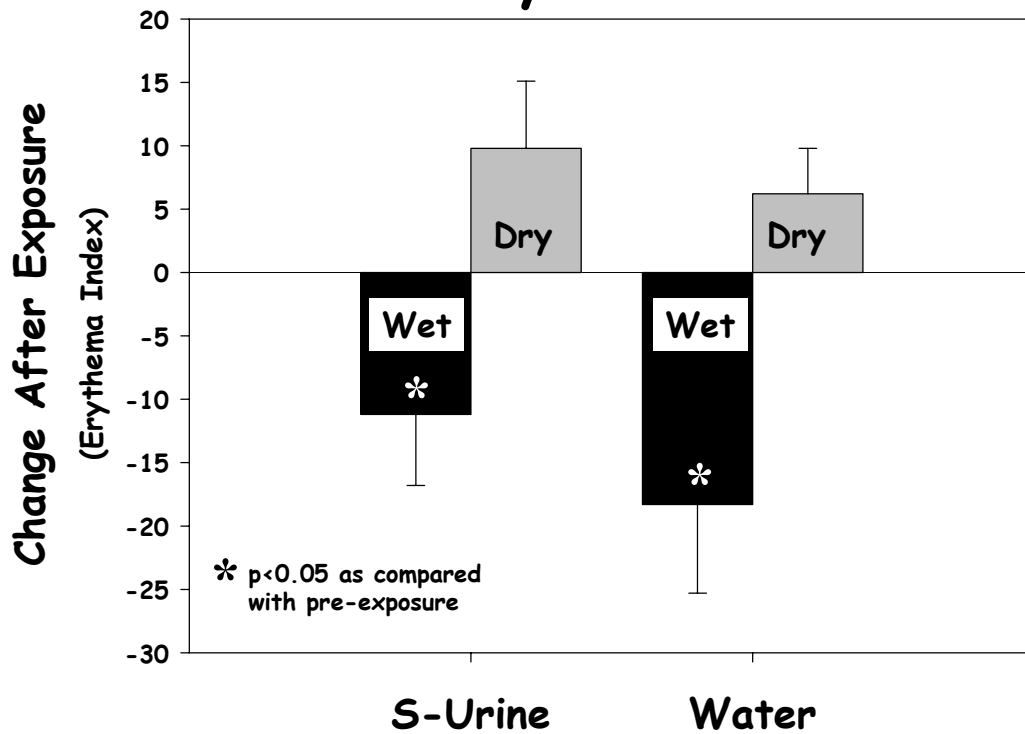## Figure 3. Hardness

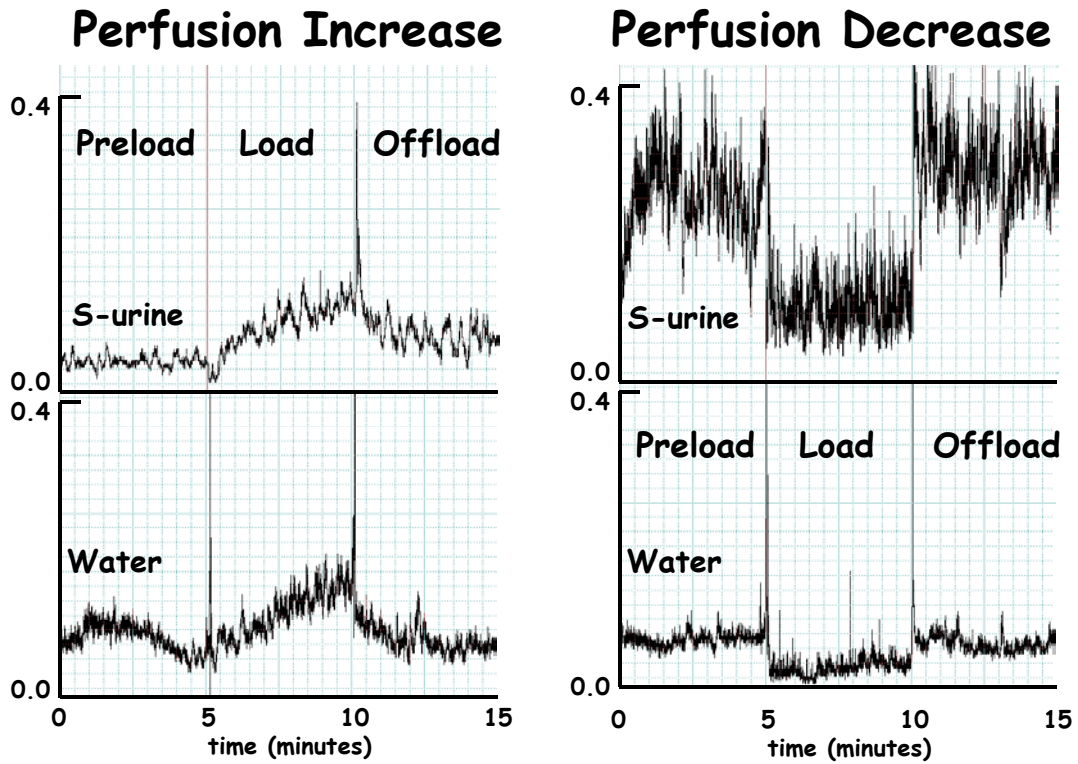## Figure 4 Erythema

Figure 5. Variable Response Examples

Perfusion Increase

Perfusion Decrease

Figure 6 Perfusion