High Strain Rate Tissue Deformation

A Theory on the Mechanical Etiology of Diabetic Foot Ulcerations

ADAM S. LANDSMAN, DPM, PhD *
DAVID F. MEANEY, PhD †
ROBERT S. CARGILL, II, PhD ‡
EDWARD J. MACARAK, PhD §
LAWRENCE E. THIBAULT, DSc ‖

Foot ulcerations are one of the most common and dangerous complications associated with chronic diabetes mellitus. Many studies have focused on neuropathy, in conjunction with elevated ground reactive forces, as the principal cause of these ulcerations. The authors discuss the mechanical cause of diabetic ulcerations at the cellular level. It is hypothesized that increased rate of tissue deformation associated with foot slap secondary to progressive motor neuropathy is the actual culprit, and not the magnitude of local pressure applied. The authors present a cellular model that shows that high rates of tissue deformation may result in elevated intracellular calcium concentrations, which may lead to cellular death, while comparable loads gradually applied do not. Furthermore, there is no significant difference in the response observed at 5 psi and 10 psi. Based on these findings, it is hypothesized that techniques such as ankle foot orthoses, which control the velocity of foot strike, may be useful in treating diabetic foot ulcerations.

One of the most common and most serious complications associated with chronic diabetes mellitus is amputations of the lower extremity. Of the 80,000 amputations performed on persons with diabetes annually, it has been estimated that 84% of these patients ini-tially present with a common foot ulceration.[1-4]

In part, the high rate of amputations for people with diabetes is caused by a lack of knowledge regarding the prevention and management of foot ulcers.[5] Early recognition and effective treatment is needed to reach the US Department of Health's goal for the year 2000 of a 40% reduction in amputation rates among patients with diabetes.[6] Although a significant number of these foot ulcerations will heal when treated with a comprehensive approach that includes techniques for relieving weight from the ulcerated area, treatment of infection, and restoration of arterial perfusion, these methods are extremely labor- and cost-intensive, and do not prevent reccurrence of the ulcers once the patient returns to normal ambulation.[7, 8]

*Director of Research, Associate Professor of Medicine, and Assistant Professor of Surgery, Dr. William M. Scholl College of Podiatric Medicine, 1001 N Dearborn St, Chicago, IL 60610; Research Fellow, Department of Bioengineering, University of Pennsylvania, Philadelphia.

†Assistant professor, Department of Bioengineering, University of Pennsylvania, Philadelphia.

‡Submitted during fellowship, Department of Bioengineering, University of Pennsylvania, Philadelphia.

§Associate Professor of Anatomy and Histology, School of Dental Medicine; Adjunct professor, Department of Bioengineering, University of Pennsylvania, Philadelphia.

‖Professor, Department of Bioengineering, University of Pennsylvania, Philadelphia.

Because the initiating mechanical causes of the ulcer may not be addressed, a pattern of continual ulceration, immobilization, hospitalization, surgery, physical therapy, and reconstruction of custom-molded shoes and insoles is seen. Eventually, because of a lack of patient compliance, uncontrolled infection, formation of scar tissue, or progression of vascular disease, the ulcer can no longer be closed, and amputation becomes inevitable.

Numerous mechanisms have been suggested as the causative factor in the formation of foot ulcerations in individuals with diabetes. Skin ulcerations on the plantar surface of the foot do not usually occur initially because of vascular disease, even though this is still often believed to be the case by many physicians and patients.[5, 9, 10] Clearly, vascular supply plays some role in all wound healing; however, foot ulcerations typically first appear when the blood supply is adequate for healing.

Significant scientific evidence indicates that mechanical forces applied to the tissue surface will somehow result in ulcerations. Brand et al[11-15] demonstrated that there was a mechanical etiology involved in the formation of ulcers in neuropathic patients with Hansen's disease. They found that prolonged repetitive loads to the foot resulted in ulcer formation. To redistribute peak pressures away from ulcer sites, they recommended custom shoes with prescription insoles, and special casts (total contact casts) that were found to be effective at immobilizing the lower leg and redistributing the peak pressures away from the ulcer site. After a prolonged period of protected ambulation, the ulcers closed.

In 1975, Stokes et al[16] reported on the incidence of diabetic foot ulcerations in a retrospective study. They found that ulcers typically occurred in regions of highest plantar pressure, and inferred that diabetic foot ulcers had a mechanical etiology. Drawing on parallels between their work and that of Brand et al[11-15], Stokes et al realized that in both chronic diabetes and leprosy, there appeared to be an association between local pressures and ulceration. Also, they noted that in both cases, neuropathy usually was present and preceded the presentation of plantar ulcers.

Clinical studies have also demonstrated a relationship between moderate repetitive stress and plantar pedal ulcerations in patients with diabetes mellitus. Duckworth et al[17] used a pressure-sensitive mat to demonstrate that during ambulation, ground reactive peak pressures greater than 10 kg/cm² frequently occur in the region of a plantar ulcer. Others have identified significantly different pressure thresholds in the region of a plantar ulcer, or in a preulcerative site.[15] Similarly, there is no consensus on the load duration required to cause ulcerations. However, no studies have demonstrated a direct mechanism whereby ulcerations are produced in response to a specific, characteristic mechanical load.

Brand et al[15] attempted to show a direct correlation with an animal model developed for testing the theory that elevated, repetitive loads would result in ulceration. An anesthetized laboratory rat was carefully supported and a 20 psi load was applied to the foot pad in a sinusoidal fashion using a rotating cam. The load was applied at a constant rate of 13.3. Hz (800 repetitions/min). Thermography was used to detect the local changes in surface temperature, indicating inflammation. The foot pad of the rat was loaded for a period of 28 days with one group experiencing 10,000 repetitions a day, while the other group experienced 8,000 repetitions a day (at the same rate), for five days, followed by two days without any experimental load applied per week.

Although animals in both groups ulcerated, animals in the first test group ulcerated more quickly than the animals in the second group. From this study, they concluded that damage to the skin resulted from accumulated injury, and that prolonged inflammation would result in the skin becoming more susceptible to ulceration, as shown by progressive histologic sections. Although the tests were conducted at a nonphysiologic loading rate, only the animal model in the study by Brand et al has demonstrated a direct link between mechanical loads and ulceration.

Role of Neuropathy in Diabetic Foot Ulcerations

Neuropathy and infection are the principal pathogenic factors in foot disease associated with diabetes.[5] Clinical studies have demonstrated that neuropathy is present in more than 80% of patients with diabetes who have foot ulcerations.[2, 9, 10] It is theorized that, as a result of advanced sensory neuropathy, the patient cannot perceive the existence of an offending focal pressure on the surface of the foot. Consequently, the patient does not compensate to avoid the offending stimulus, and a lesion develops. Although loss of protective sensation can result in local irritation, the more common cause of pedal ulcerations appears to be excessive, repetitive pressure at focal locations on the plantar surface of the foot.[5]

Motor neuropathy is also pronounced among patients with chronic diabetes. Typically, the loss of motor control occurs in a stocking or glove distribution, and affects distal and anterior compartment nerves earlier than proximal and posterior nerves, creating significant muscle imbalances.[18-20] Both intrinsic and extrinsic muscular control of the foot is affected, and this can result in clawing of the

toes, which leads to retrograde buckling of the digits and plantarflexion of the metatarsals.[19] When the metatarsals become plantarflexed, the metatarsal heads protrude against the plantar fat pad, resulting in prominences where ulcers typically occur.[21]

Anterior muscle group (specifically tibialis anterior, extensor hallucis longus, extensor digitorum longus) atrophy in the distal leg commonly occurs and has a profound effect on foot function.[20] This muscle group normally acts to decelerate the foot as it strikes the ground by counteracting the inertial and gravitational effects, while opposing the posterior muscle contraction. When strength in the anterior compartment of the leg is diminished, foot slap may result because of the unopposed action of the gastrocnemius soleus complex, producing alterations in the loading characteristics and subsequent ulceration.[20] This condition is further exacerbated by the preceding intrinsic musculature atrophy that leads to excessive prominence of the metatarsal heads, as described earlier. Thus, atrophy of the anterior muscle group results in accelerated impact of the forefoot with the ground.

Clinically, a correlation between anterior compartment motor neuropathy and the incidence of foot ulcerations has been suggested; however, no scientific data exist indicating the specific mechanical cause of the foot ulcerations formed.[20] The increase in velocity of foot strike associated with a dysfunctional anterior compartment muscle group will be shown to be a potentially critical factor in the formation of foot ulcerations.

Glycosylation of Soft Tissues

Soft tissue changes associated with diabetes may further enhance the development of ulcerations. Previous studies indicate that as the disease progresses, patients with diabetes demonstrate changes in the soft tissue mechanical properties, which may further predispose these patients to ulceration. Glycosylation of the tissues directly affects the collagen, causing excessive cross-linking between collagen strands, making it much stiffer.[22-24] Thus, because of the stiffening of these viscoelastic tissues, they are not able to deform as quickly in response to a given load, and should be more susceptible to splitting and cracking in response to rapid deformation associated with high velocity foot impact.[25]

These changes in the skin may further exacerbate the problem of accelerated loading of the foot associated with diabetic neuropathy and anterior compartment atrophy. The situation in the forefoot is aggravated even further by atrophy of the plantar fat pad associated with chronic diabetes.[18] These two conditions directly affect the resiliency of the skin to mechanical forces. Excessive callous formation associated with diabetes leads to thickening of the skin, making it even less malleable and, therefore, more susceptible to applied tensile and compressive loads at the skin surface.[2, 18]

Current Treatments for Foot Ulcerations in Persons With Diabetes

Current treatment regimens have focused on techniques for diminishing peak loads, rather then on addressing the loading rates. Despite the lack of solid evidence to clearly implicate peak pressures as the direct cause of these ulcerations, a tremendous amount of research has demonstrated that certain devices, which reportedly reduce peak pressures, are useful in healing the ulcers. Coincidentally, many of the most successful treatments also have the added effect of reducing tissue deformation rates and absorbing shock, by immobilizing the ankle joint and cushioning the impact between the foot and ground. Total contact casts have been used with success to close ulcerations, reportedly by redistributing plantar ground reactive forces to other areas of the foot, ankle, and leg.[4, 5, 20, 26, 27] However, total contact casts also immobilize the ankle joint, thus preventing foot slap and reducing high loading rates by forcing the patient to walk only in an apropulsive manner.

Closed cell foam insoles have also been used in certain cases. These devices are able to redistribute local peak pressures across the plantar surface of the foot as wound closure progresses, and decelerate the foot and dissipate shock as the foot strikes the ground.

Hypothesis

Based on the authors' review of the literature, it appears that no evidence directly links the formation of foot ulcers in people with diabetes solely to peak pressures. In response to this lack of information, a study was conducted in which the response of cells to specific and well controlled mechanical stimuli could be studied. Using this system, the effects of peak load magnitude and various loading rates to ascertain the mechanical cause of foot ulcers in patients with diabetes was studied, with the focus on the cellular level response to mechanical loads.

It is hypothesized that ulcer formation in diabetes is a result of high strain rate deformation, rather than a threshold peak load. Strain is defined as the deformation that results in response to an applied stress or load. It can be thought of as the ratio of the change in length to the initial length. Thus, a strain of 0.05 or 5% represents a 5% increase in the overall

length when compared with the initial length. Because strain represents the ratio of one length to another, the units cancel out, and it is reported as a dimensionless quantity, ie, inches/inches. Strain rate is the rate at which the strain occurs. Thus, a strain rate of 10 sec^{-1} means that a strain of 10 (the final length is 10 times the original length) would occur over a period of 1 sec.

It is further hypothesized that reduction of high strain rate deformation, rather than the reduction of peak loads, will lead to healing and minimize reccurrence of foot ulcerations in individuals with diabetes. This second hypothesis will be addressed in later studies.

It is important to understand whether peak loads or loading rates are the principal cause of ulcerations, as the treatment regimen could be significantly altered.

Materials and Methods

Cellular Model

As an initial attempt to understand the mechanical forces involved in ulcer formation, a cellular model was developed. Endothelial cells were selected as the cell model for many reasons. First, endothelial cells play a critical role as the monolayer lining the entire vascular system. They control the transport of nutrients and waste products into and out of the blood stream. In this capacity, these cells help maintain the integrity of the plantar foot tissues. Injury to these cells may predispose the plantar tissues to ulceration. Second, endothelial cells grow in a monolayer because of contact inhibition. This is essential for performing the sophisticated strain analysis described herein. Third, endothelial cells can be cloned and will reach senescence at a predictable level. Senescence, on the cellular level, is defined as the inability to continue to divide. Senescent cells can be used as a model for diabetes because of numerous similarities in structure, protein formation, and the progression of glycosylation at the cellular level.[22, 23, 28] Thus, it is believed that senescent and diabetic cells will respond similarly to mechanical forces.

Bovine aortic endothelial cells are harvested and cloned using the technique described by Macarak[29]. For this study, cells from primary cultures obtained from two fetal bovine aortas were used. From this initial harvest, cells were cloned and raised to senescence. Cells at the 30th population doubling level (ie, the number of divisions that the cell line has undergone) were also retained for comparison. The cells were confirmed to be endothelial cells by staining for the expression of factor VIII (von Willebrand's factor).

Experimental cell culture chambers were seeded at confluent density in modified M199 media with 16.6% fetal bovine serum, and transferred to an incubator with 5% CO_2 for 48-72 hr to allow the cells to attach firmly and spread on the quartz substrate in preparation for mechanical deformation studies.

Experimental Chamber

In order to study the response of endothelial cells to mechanical forces, a specialized cell culture chamber that uses hydrostatic pressure to deform the cells was selected.[30] The experimental chamber is shown in Figure 1. The chamber has three parts: a top, which consisted of a metal collar with a glass window; a bottom, which contained a thin quartz window; and a connector to join the top to the bottom, forming a water-tight chamber. Mounted in the connector was a portal for a pressure transducer, bleed valve, and pressure line.

The rate of change in chamber pressure and the magnitude of the peak pressure were carefully controlled using a custom controller (Landsman A: *Age-Dependent Response of Endothelial Cells to Mechanical Deformation*, PhD dissertation, University of Pennsylvania, 1992). Two modes of chamber pressurization were used in this study. Impulse loads occur when the chamber is pressurized and vented to the atmosphere in fractions of a second. Using this technique, the pressure could be increased from 0 to 10 psi (70 kPa) in 8 milliseconds (Fig. 2A). Ramp loads in which the chamber was pressurized from 0 to 10 psi (70 kPa) over 1.75 seconds could also be produced (Fig. 2B).

Determination of Cellular Deformation

In order to determine the degree of cellular deformation resulting from the change in hydrostatic pressure, polystyrene microspheres (0.7 µm diameter) were attached to the cell surface using a previously described technique (Landsman A: *Age-Dependent Response of*
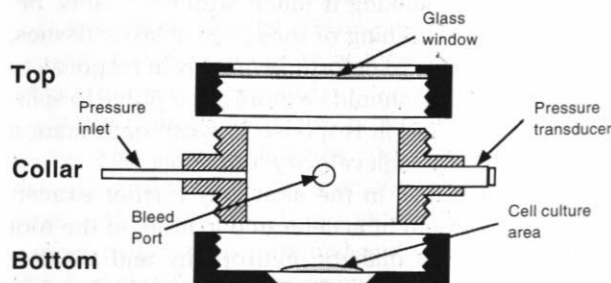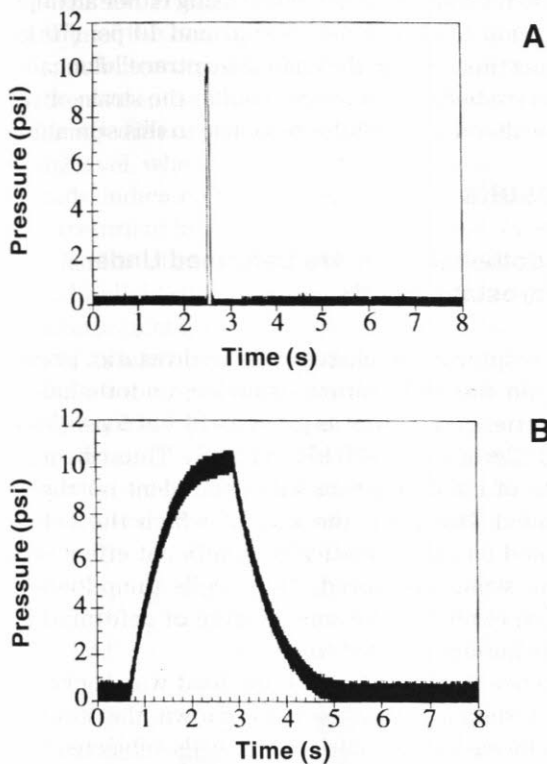

Figure 1. Experimental chamber.

Figure 2. High rates of chamber pressurization (to 10 psi) are used to create strain rates in excess of 10 sec^{-1}. Note that pressurization occurs here in a fraction of a second (A). Conversely, low rates of chamber pressurization (also to 10 psi) are used to create strain rates that are less than 0.1 sec^{-1}. Note that in this case, the same pressure magnitude was attained, but that loading of the cells took place over a period of several seconds (B).

Endothelial Cells to Mechanical Deformation, PhD dissertation, University of Pennsylvania, 1992).

Cells that have firmly attached within the cell culture chamber for 72 hr were removed from the incubator and the media were aspirated off. The cells were rinsed twice with phosphate buffered saline solution (pH 7.40) containing 5mM a-d(+)-glucose and 1mM calcium chloride at room temperature and 120 ml of a solution containing 10 ml of Fluoresbrite®[1] plain microspheres (0.7 mm diameter) per 1 ml of phosphate buffered saline solution was added to the cell culture chamber.

After rinsing away unattached beads, the chamber assembly was transferred to an inverted microscope. Cells were viewed with a combination of incandescent light to image the cell, and a xenon arc lamp with a fluorescein isothiocyanate filter set that was used to excite the fluorescent microspheres, making them visible on the cell surface (Fig. 3). Cells were photographed with a camera attached to the microscope. The position of the microspheres on the cell surface

®[1]Polysciences, Inc, Warrington, PA.

was recorded at atmospheric pressure, and with sustained loads of 5 psi (5 kPa) and 10 psi (70 kPa), as measured with the indwelling pressure transducer.

As the pressure increased in the chamber from 0 to 10 psi (70 kPa), the cell was deformed and the distance between the beads changed. This change was measured, and the resultant strain was determined. Strain magnitude was controlled by increasing or decreasing the hydrostatic pressure applied to the cells in the specialized chamber. By varying the amount of time required to achieve a specified level of strain, the strain rate could also be easily controlled.

Determination of Cellular Response To Load

In order to determine that injury, or some degree of biochemical response, occurred as a result of the mechanical stimulus, Fura-2 calcium binding dye was loaded into the cells.[31] This dye was used to determine the intracellular calcium concentration. A detailed description of the mechanism by which this dye functions can be found in the literature.[32] Intracellular calcium concentration was recorded prior to, during, and immediately following mechanical stimulus.

Intracellular calcium is sequestered in organelles throughout the cell, including the endoplasmic reticulum. Additionally, the extracellular concentration of calcium is several orders of magnitude greater than the intracellular concentration when the cell is at homeostasis. It has been shown previously that in response to a mechanical stimulus, endothelial cells display a surge in the intracellular (cytoplasmic) calcium concentration (Winston FK: *The Modulation of Intracellular Free Calcium Concentration by Biaxial Extensional Strains of Bovine Pulmonary Artery Endothelial Cells*, PhD dissertation, University of Pennsylvania,

Figure 3. Bovine aortic endothelial cells seeded in the cell culture chamber. On the surface of each cell are polystyrene microspheres (white dots). Cell deformation can be determined by measuring the change in distance between these microspheres under hydrostatic loads.

1989; Landsman A: *Age-Dependent Response of Endothelial Cells to Mechanical Deformation*, PhD dissertation, University of Pennsylvania, 1992).[33]

A dye solution composed of 50 mg of Fura-2AM, dissolved in 10 ml of dimethyl sulfoxide containing 8% pluronic was diluted in 14 ml of phosphate buffered saline (pH 7.40) containing 5mM d-(+)-glucose and 1mM calcium chloride. The solution was permitted to equilibrate to room temperature in the dark. Cells were rinsed once with room temperature phosphate buffered saline without Fura-2AM. Next, 3 ml of the Fura-2AM solution was added to the cells set aside to take up the dye at room temperature in the dark for 40 to 45 min.

Forty-five minutes after the dye solution was added to the cell culture, the solution was suctioned off and the cells were rinsed twice with plain phosphate buffered saline and returned to the dark for an additional 10 min while covered in the phosphate buffered saline solution without dye. Next, the chamber was assembled, filled with phosphate buffered saline, and attached to the stage of an inverted microscope that was equipped with a stage heater that maintains a constant temperature of 7°C.

Cells loaded with dye were observed by exciting them with light at the 60 or 80 nm wavelengths. Emissions at 510 nm were captured with an SIT video camera (Fig. 4). The image was directed to an image processor and storage devices. The images were managed and calcium concentrations were determined from standard calibration curves using custom prototype software.

Using this system, images were collected at 15 Hz. The software package permits measurement of the calcium concentration within a single cell, as well as in a small portion of a single cell by demarcating an area of interest on the image of the cell.

Cloned bovine aortic endothelial cells from the 30th population doubling level and at senescence were mechanically deformed using either an impulse or ramp load to 5 psi (5 kPa) and 10 psi (70 kPa). Using this system, the change in intracellular calcium was studied to determine if either the strain or strain rate altered the cellular response to this stimulus.

Results

Endothelial Cells Are Deformed Under Hydrostatic Loads

In response to changes in hydrostatic pressure within the cell culture chamber, endothelial cells experienced strains as great as 14% at 5 psi (5 kPa), and 22% at 10 psi (70 kPa) (Fig. 5). Thus, the magnitude of cellular strain was dependent on the load applied. However, the rate at which the cell was loaded had no statistically significant effect on the peak strain measured. Thus, cells ramp-loaded to 10 psi exhibited the same degree of deformation as cells impulse-loaded to 10 psi.

Since the rate at which the load was applied and the resultant deformation are known, the strain rate can be easily calculated. Thus, cells subjected to impulse loads experienced strain rates of approximately 10 sec^{-1}, while ramp-loaded cells experienced the same level of deformation as the impulse-loaded cells, with a resultant strain rate of approximately 0.1 sec^{-1}. Senescent cells were found to exhibit the same magnitude of peak strain as their younger counterparts.

Effect of Strain and Strain Rate on Intracellular Calcium

Surges in intracellular calcium were observed in response to transient changes in hydrostatic pressure with high strain rate deformation (> 10 sec^{-1}) in approximately 20% of the cases, but were only observed < 3% of the time in response to low strain
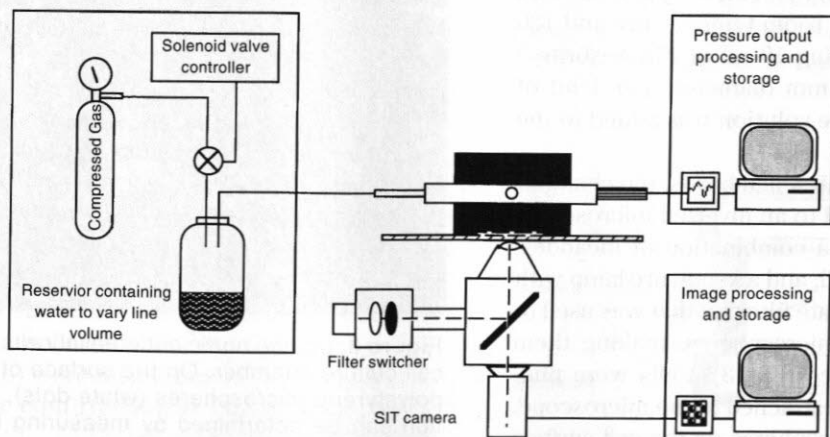

Figure 4. Experimental apparatus used for measuring changes in intracellular calcium.

rates (< 0.1 sec^{-1}) (Fig. 6). This study showed that the magnitude of the load applied to the cells was not a significant factor in producing calcium transients.

Furthermore, the character of the response was significantly different in cells at the 30th population doubling level, when compared with senescent cells. This study indicated that senescent cells were less likely to return to homeostatic calcium levels following mechanical stimulus than the younger cells. This is crucial in light of the fact that failure to return to homeostatic calcium levels eventually leads to cellular death, as was observed in this study.[34]

Based on the results, it appears that high strain rate deformation produces surges in intracellular calcium, while low strain rates do not. Even more importantly, senescent cells are more prone to fatal injuries as a result of high strain rate deformation.
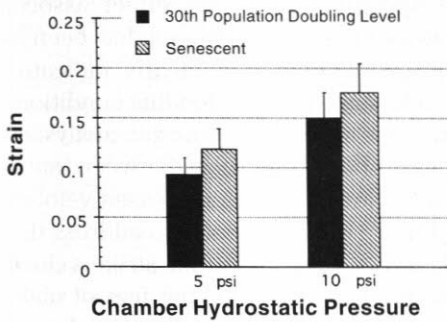
Figure 5. The amount of strain resulting from 5 psi and 10 psi hydrostatic pressure changes is shown for the 30th population doubling level and senescent cells. As the chamber pressure increases, the magnitude of deformation increases. Yet there was no significant correlation between the magnitude of deformation and the presence of intracellular calcium.
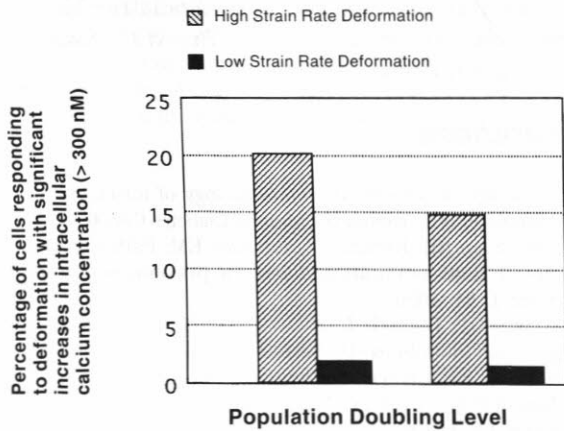
Figure 6. Cells at the 30th population doubling level and senescent cells produce surges in intracellular calcium in response to high strain rate deformation (> 10 sec^{-1}) but rarely in response to low strain rate deformation (< 0.10 sec^{-1}).

Conversely, neither the young nor senescent cells exhibited significant changes in intracellular calcium as a result of low strain rate deformation.

Discussion

The findings described herein indicate that senescent cells are more likely to display fatal surges in intracellular calcium as compared with their younger counterparts at the 30th population doubling level. This is interesting in the present context because of similarities between diabetic and senescent cells.[22, 23, 28] The implication of this work is that the rate of tissue deformation, rather than the magnitude of the peak load, is the critical factor in generating surges in intracellular calcium which are fatal to the cells. Cellular death resulting from uncontrolled surges in intracellular calcium could ultimately lead to ulcer formation.

If the cellular model of the response of these tissues to mechanical loads is an accurate reflection of the true clinical picture, then the strategy for treating diabetic foot ulcerations may change significantly. It has been reported that foot slap secondary to anterior compartment muscle atrophy in the lower leg may occur as the diabetic neuropathy progresses. As a result, the foot will strike the ground at an increased velocity because of the inability of the patient to decelerate the foot, and the unopposed action of the gastrocnemius soleus complex. The magnitude of the load has not changed since the weight of the foot remains more or less constant. However, loss of function of this muscle group results in the foot striking the ground at an increased speed, resulting in high strain rate deformation.

Brand's original animal model may unintentionally support the theory that increased deformation rates will lead to foot ulcerations. In each model, the foot pad of the animal was loaded at more than 13 Hz, in an effort to decrease the amount of time the animal had to be subjected to the stimulus, in order to simulate a specified number of steps. This is far in excess of normal physiologic loading rates. Even with a sinusoidal loading pattern, this loading rate is much more likely to simulate the loading of the patient with diabetes with anterior compartment motor neuropathy than the normal walking patient. In light of this fact, it is not surprising that all of the animals in Brand's study eventually ulcerated under this high loading rate.

If, in fact, high strain rate tissue deformation is the true mechanism, then treatments designed to decelerate the foot in a controlled fashion should be effective for reducing the propensity for ulceration formation, and help the ulcers to close. A simple treatment could be an ankle-foot orthosis that would help prevent the

foot from slapping the ground. Already there have been anecdotal reports from around the country which show this to be an effective treatment for closure and prevention of diabetic foot ulcers (Allan Banks, DPM, personal communication, 1995).

Furthermore, although total contact casting has been used to treat foot ulcers successfully by reportedly decreasing the peak loads over a particular ulcerated region, it is also clear that these casts have a secondary effect of immobilizing the ankle joint. This forces the subject to walk in an apropulsive manner, and reduces the velocity of foot strike.

Conclusion

Admittedly, the contention that endothelial cell deformation can be used to explain the pathologic mechanism of diabetic foot ulcerations is a tremendous leap of faith. However, this is an initial step to describing a complex system. Certain facts can be taken from this study to support a new theory on the cause of foot ulcerations:

1. Senescent (and by extension, possibly diabetic) tissues are more susceptible to mechanical trauma than are their normal counterparts.

2. Mechanical loading elicits a response on the cellular level that can be expressed by an increase in the intracellular calcium concentration.

3. Cells are more sensitive to the rate of deformation than they are to the magnitude of load used to create the deformation.

4. High rates of tissue deformation are shown to result in cellular death or injury, while low rates of tissue deformation are much less likely to produce lasting injury.

What about the supposition that senescent tissues behave similarly to diabetic tissues? If this is true, then why do people with diabetes ulcerate routinely, while nondiabetic elderly persons typically do not develop plantar ulcers? One requirement for ulceration may be high strain rate deformation, which occurs secondary to neuropathy and loss of anterior compartment muscle strength. This condition is not commonly associated with aging, *per se*, and may add further evidence that tissue deformation rate, and not peak pressure or biochemical changes associated with diabetes, are essential for ulceration.

Based on these findings, along with clinical reports indicating that elimination of foot slap may be effective in healing diabetic foot ulcers, it is reasonable to question whether peak ground reactive pressures are the principal cause of diabetic foot ulcers (Alan Banks, DPM, personal communication, 1995; Ronald Sage, DPM, Rodney Stuck, DPM, personal communication, 1995).

A good experimental study supposedly leaves more questions unanswered than when the study began. Many points suggested in this study require further assessment.

The cellular model proposed involves the use of endothelial cells, a key component of the vascular supply for the plantar tissues. Ideally, the work should be repeated with emphasis on fibroblasts to determine the effect of strain and strain rate on this cell line as well. Furthermore, although senescent cells are in many ways similar to those of patients with diabetes, they are not the same. Although cell culture techniques involving cells that come from type I diabetes are more complex, the work should be repeated with these cell lines to confirm that the hypothesized cellular responses exist in this cell line as well.

Much work must still be done to determine exactly the physiologic parameters of plantar loading. Although a specific physiologic effect associated with high rates of tissue deformation has been identified, there are no data that clearly indicate the physiologic relevance of these loading conditions.

Finally, there is a need for clinic gait analysis data that document the association between anterior compartment dysfunction and increased velocity of foot strike. Future studies must also address the relationship between increased foot strike velocities and the formation of ulcers on the feet of diabetic patients. This information is essential for determining the true significance of the study presented here.

References

1. MOST RS, SINNOCK P: The epidemiology of lower extremity amputations in diabetic individuals. Diabetes Care **6**: 87, 1983.

2. PECORARO RE, REIBER GE, BURGESS EM: Pathways to diabetic limb amputation: basis for prevention. Diabetes Care **1**: 51, 1990.

3. REIBER GE: Diabetic foot care: financial implications and practice guidelines. Diabetes Care **15**: 29, 1992.

4. SUSSMAN KE, REIBER G, ALBERT SF: The diabetic foot problem: a failed system of health care? Diabetes Res Clin Pract **17**: 1, 1992.

5. CAPUTO GM, CAVANAUGH PR, ULBRECHT JS, ET AL: Assessment and management of foot disease in patients with diabetes. N Engl J Med **33**: 854, 1994.

6. DEPARTMENT OF HEALTH AND HUMAN SERVICES: *Healthy People 2000: National Health Promotion and Disease Prevention Objectives*, Government Printing Office,

DHHS pub. no. 91-5021, Washington, DC, 1991.

7. MYERSON MS, PAPA J, EATON K, ET AL: The total-contact cast for management of neuropathic plantar ulceration of the foot. J Bone Joint Surg **74**: 261, 1992.

8. POMPOSELLI FB JR, JEPSEN SJ, GIBBONS GW, ET AL: Efficacy of the dorsal pedal bypass for limb salvage in diabetic patients: short-term observations. J Vasc Surg **11**: 745, 1990.

9. BOULTON AJM: The diabetic foot: neuropathic in aetiology? Diabet Med **7**: 852, 1990.

10. EDMONDS ME: "Experience in a Multidisciplinary Diabetic Foot Clinic," in *The Foot in Diabetics*, ed by H Connor, AJM Boulton, JD Ward, John Wiley & Sons, Chichester, United Kingdom, 1987.

11. BAUMAN JH, BRAND PW: Measurement of pressure between foot and shoe. Lancet **1**: 629, 1963.

12. BAUMAN J, GIRLING E, BRAND PW: Plantar pressures and trophic ulceration: an evaluation of footwear. J Bone Joint Surg **45B**: 652, 1963.

13. BRAND PW, EBNER JD: Pressure sensitive devices for denervating hands and feet. J Bone Joint Surg **51A**: 109, 1969.

14. BRAND PW: "Repetitive Stress in the Development of Diabetic Foot Ulcers," in *The Diabetic Foot*, 4th Ed, ed by ME Levin, LW O'Neal, CV Mosby, St Louis, 1988.

15. BRAND PW: *Repetitive Stress on Insensitive Feet: The Pathology and Management of Plantar Ulceration in Neuropathic Feet*, US Public Health Service Hospital, Carville, LA, Social and Rehabilitation Service Grant No. RC 75 MPO, 1975.

16. STOKES IAF, FARIS IB, HUTTON WC; The neuropathic ulcer and loads on the foot in diabetic patients. Acta Orthop Scand **46**: 89, 1975.

17. DUCKWORTH T, BOULTON AJM, BETTS RR, ET AL: Plantar pressure measurements and the prevention of ulceration in the diabetic foot. J Bone Joint Surg **67B**: 79, 1985.

18. CAVANAGH PR, SIMONEAU GG, ULBRECHT JS: Ulceration, unsteadiness, and uncertainty: the biomechanical consequences of diabetes mellitus. J Biomech **26**: 23, 1993.

19. HABERSHAW G, DONOVAN JC: "Biomechanical Considerations of the Diabetic Foot," in *Management of Diabetic Foot Problems*, ed by GP Kozak, CS Hoar, JL Rowbotham, et al, WB Saunders, Philadelphia, 1984.

20. SCHOENHAUS HD, WERNICK E, COHEN RS: "Biomechanics of the Diabetic Foot," in *The High Risk Foot in Diabetes Mellitus*, ed by RG Frykberg, Churchill-Livingstone, New York, 1991.

21. CTERCTEKO GC, DHANENDRAN M, HUTTON WC, ET AL: Vertical forces acting on the feet of diabetic patients with neuropathic ulceration. Br J Surg **68**: 608, 1981.

22. BROWNLEE M: "Advanced Products of Nonenzymatic Glycosylation and the Pathogenesis of Diabetic Compli-cations,"

in *Diabetes Mellitus: Theory and Practice*, 4th Ed, ed by H Rifkin, D Porte, Elsevier Science, New York, 1990.

23. BROWNLEE M, CERAMI A, VLASSARA H: Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. N Engl J Med **18**: 115, 1988.

24. MONNIER VM: Nonenzymatic glycosylation of the Maillard reaction and the aging process. J Gerontol **45**: B105, 1990.

25. FLÜGGE W: *Viscoelasticity*, 2nd Ed, Springer-Verlag, New York, 1975.

26. MUELLER MJ, DIAMOND JE, SINACORE DR, ET AL: Total contact casting in treatment of diabetic plantar ulcers: controlled clinical trial. Diabetes Care **12**: 84, 1989.

27. NOVICK A, BIRKE JA, GRAHAM SL, ET AL: Effect of a walking splint and total contact casts on plantar forces. J Prosthet Orthot **3**: 168, 1991.

28. MAKITA Z, VLASSARA H, RAYFIELD E, ET AL: Hemoglobin-AGE: a circulating marker of advanced glycosylation. Science **258**: 651, 1992.

29. MACARAK EJ: "Endothelial Cells of the Lung," in *Models of Lung Disease-Microscopy and Structural Methods*, ed by J Gil, Marcel Dekker, New York, 1990.

30. LANDSMAN AS, THIBAULT LE, MEANEY DF, ET AL: "A New System to Study Mechanical Deformation and the Resultant Calcium Transients in Endothelial Cells," in *1992 Advances in Bioengineering*, Vol. 22, ed by MW Bidez, American Society of Mechanical Engineers, New York, 1992.

31. MORGAN BP, LUZIO JP, CAMPBELL AK: Intracellular Ca2+ and cell injury. Cell Calcium **7**: 99, 1986.

32. GRYNKIEWICZ G, POENIE M, TSIEN RY: "A New Generation of Ca 2+ Indicators With Greatly Improved Fluorescence Properties." J Biol Chem **260**: 3440, 1985.

33. SHEN J, LUSCINSKAS FW, CONNOLLY A ET AL: Fluid shear stress modulates cytosolic free calcium in vascular endothelial cells. Am J Physiol **262**: C84, 1992.

34. SCHANNE FAX, KANE AB, YOUNG EE, ET AL: Calcium dependence of toxic cell death: a final common pathway. Science **206**: 700, 1979.

Additional References

LEE JS, LU M, LEE VS, ET AL: Lower-extremity amputation: incidence, risk factors, and mortality in the Oklahoma indian diabetes study. Diabetes **42**: 876, 1993.

THIBAULT LE, LANDSMAN AS: "The Response of Aging Aortic Endothelium to Mechanical Stimulation in Cell Culture," ASME 1991 Biomechanics Symposium, ed by RL Spilker, MH Friedman, American Society of Mechanical Engineers, New York, c. 1991.