The Time Effect of Pressure on Tissue Viability: Investigation Using an Experimental Rat Model

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An experimental rat model was used to investigate the time–pressure effect on tissue viability. External loading equivalent to 13.3 kPa (100 mm Hg) of pressure was applied to the greater trochanter and tibialis area of Sprague-Dawley rats using pneumatic indentors for duration of 6 hrs each day for 1 to 4 days. It was observed that postocclusive hyperemic responses were gradually increased at the trochanter throughout the 4 days of loading, whereas for the tibia there was a significant increase ($P = 0.04$) in postocclusive hyperemic flow between Days 2 and 3. In histologic evaluations, cutaneous tissue damage was observed at the trochanter area but not at the tibialis area after 2 consecutive days of load application. In contrast, degeneration of muscle cells characterized by numerous increases of nuclei occupying the central of the muscle fibers was observed after 2 days of load application at the tibialis. The situation was found to progress with time ($P = 0.17$). The presence of other histologic signs, including the internalization of peripherally located nuclei, replacement of muscle cells by fibrosis and adipose tissues, and the presence of pyknotic nuclei as well as karyorrhexis, confirmed that the affected tissues were damaged. These findings suggest that postocclusive hyperemia and the distress of tissues under loading could be closely related. Exp Biol Med 232:481–487, 2007

Key words: pressure ulcer; laser doppler flowmetry; trochanter; tibia; hyperemia; histological evaluation

Introduction

Pressure ulcers are common and serious complications of tissue damage developed in patients with diminished pain sensation and/or diminished mobility. Over the past several decades, researchers have attempted to seek answers to the etiology of the pressure ulcer. Unfortunately, our understanding so far is still not comprehensive. Studies on the pathophysiology of pressure ulcers found in the literature were centered on three main areas, including pressure, shear, and ischemia (1–3). Among these factors, prolonged pressure loading of vascularized tissues is generally regarded as the most important mechanical cause for pressure ulcer formation. Pressure ulcers can be initiated at the skin level or from deep tissues (i.e., muscle layer; Ref. 4). Among these two origins, the onset of deep pressure ulcers is of greater clinical concern, as there is no early sign of damage that can easily be observed at the skin level. It is generally accepted that tissues are at risk of injury to prolonged low pressure as well as momentary high pressure applications. By monitoring the interface pressure over the bony prominences of over 980 human subjects, Reswick and Rogers (5) found that the onset of pressure ulcers is pressure–time dependent, which can be characterized by an inverse parabolic curve.

In animal studies, similar findings also have been reported. Daniel *et al.* (6) and Nola and Vistnes (7) found that muscle tissue is more susceptible to mechanical loading than skin. Recent works by Bosboom *et al.* (8), Linder-Ganz and Gefen (9), Linder-Ganz *et al.* (10), and Stekelenburg *et al.* (11) also have similar findings. By pooling experimental data acquired at different magnitudes of pressure loadings and within 2 hrs of sustained compression, Linder-Ganz and Gefen (9) further suggested that a new sigmoid pressure–time curve can be established to represent cell death threshold for the skeletal muscles of rat.

Finding noninvasive ways to assess the viability of tissues is important for pressure ulcer prevention. The examination of postocclusive hyperemia, the repayment of a blood flow debt to tissues, has been used as a measure to reflect tissue injury (12, 13). Laser Doppler is a well-known

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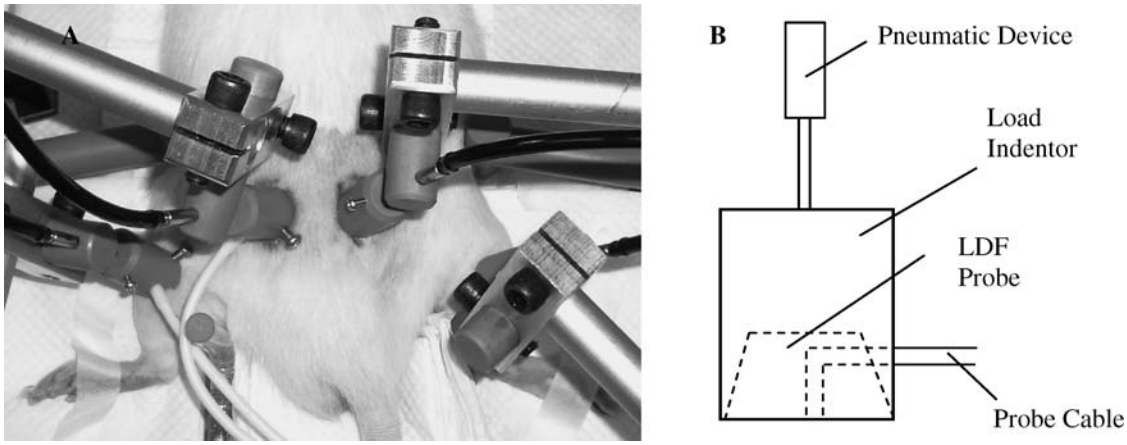


Figure 1. (A) Experimental setup for pressure loading over the trochanter area and tibias area. (B) Schematics of indentor with incorporated probe (diameter = 14 mm).

technique used to measure blood flow in small blood vessels. Attempts have been made by researchers (14, 15) to quantify the postocclusive hyperemic effects of tissues. Several parameters, including the magnitude of peak hyperemic flow, the half-life time of the hyperemic episode, and the total hyperemia experienced by the affected area, have been used to quantify the hyperemic response. However, the actual damage experienced by affected tissues was not measured. Spectrum analysis of laser Doppler flowmetry (LDF) signal also has been conducted to study the development pressure ulcer (16, 21). This analysis technique has given us new insight to the hemodynamic changes of the compressed tissue. Unfortunately, the histologic changes of the affected tissue and its relationship with the LDF signal are still unclear. Therefore, to gain better insight on the effect of external loading on tissue, this study aims to investigate the postocclusive hyperemic response of tissue subjected to different loading durations and compare the morphologic changes in tissues using an experimental rat model.

Materials and Methods

In this study, a total of 45 male Sprague-Dawley rats weighing between 400 g and 500 g were used. The rat was selected because the reconstruction of the loaded muscle requires a small animal (17–20). These rats were divided into five groups: 24-hr, 48-hr, 72-hr, 96-hr, and 168-hr groups. Within each group, these rats were further subdivided into experimental and control groups, each having seven and two rats, respectively. All rats were fed a standard nutrient diet with no supplement of any extra vitamins. The Animal Ethics Subcommittee of the investigators' institution reviewed and approved the procedures of this study.

Before applying loading, rats were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) injected intraperitoneally, and one third of this dose was used for maintenance of the level of anesthesia during experiments. The sufficiency of the level of anesthesia was determined by a moustache dithering test. Hair of the loading sites was carefully shaved without damage to the skin at least 1 day before the experiment to prevent any disturbance of skin blood flow.

Experimental Setup. In the experimental group, rats were subjected to a static pressure loading using pneumatic indentors. Pressure of 13.3 kPa (100 mm Hg) was delivered to an area of 1.5 cm^2 at both sides over trochanter and tibialis anterior regions (Fig. 1A), such that subcutaneous and muscular tissues were compressed between indentor and underlying bony structure. The edges of indentors were curved to avoid high-stress concentration. An LDF (DRT4; Moor Instruments, Axminster, UK) with a contact probe (DP1T/7-V2) was used to monitor the blood flow of the loading site (Fig. 1B). The contact probe was incorporated in the indentor for real-time measurements.

The loading duration was 6 hrs each day for 1 to 4 consecutive days, according to their assigned grouping. Skin

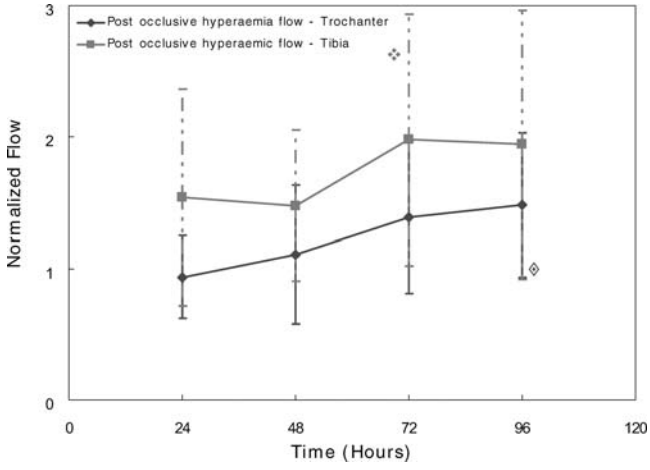


Figure 2. Postocclusive hyperemic response of tissues at the trochanter and tibia areas during the 4 days of loading (statistically significant ◈ $P = 0.02$ between Days 1 and 4). ❖ $P = 0.04$ between Days 2 and 3, and difference.

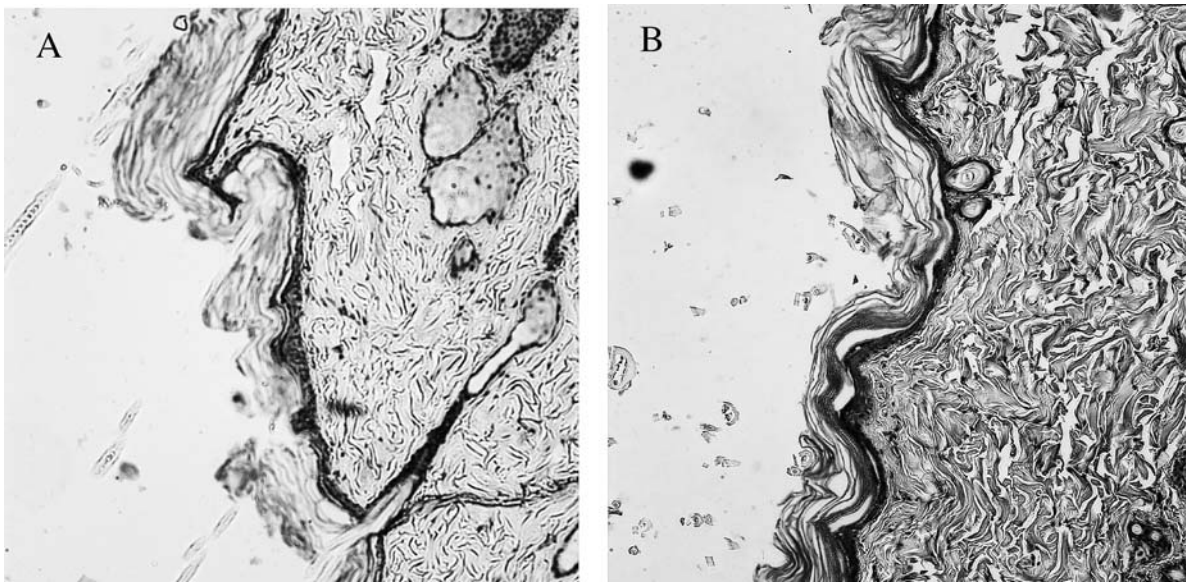


Figure 3. Photomicrograph of skin at the trochanter area of control rat (A) and skin at the trochanter area of the 48-hr group (B) exposed to 100 mm Hg pressure loading for 6 hrs/day with 2 consecutive days; the 72-hr, 96-hr, and 168-hr groups also showed similar results. Skin damage was characterized in (B) as thickening of keratin layer and accumulation of fluid within the epidermis (hematoxylin and eosin; magnification: ×200).

blood flow was measured for 10 mins prior to loading to determine the resting flow condition. Subsequent measurements were taken for the same period of time at the end of each hour during the loading period and prior to the end of the sixth hour. Then, postocclusive hyperemic flow was measured for a period of 20 mins. Tissue samples were taken in areas under pressure indentors 18 hrs after the last pressure loading to investigate the time effect of loading. For the 168-hr group, the rats were exposed to 4 consecutive days of loading followed 3 untreated days, and tissue samples also were taken the same as for other groups. For the control group, no loading was applied, but the rats were subjected to the same general anesthetic condition.

Histologic Analysis. All rats were killed by overdose of mixture of ketamin and xylazine. The interested tissue samples (i.e., at the trochanter and tibialis areas) were excised directly under the sites of compression. All samples were fixed in neutral buffered formalin (10%) for 22–24 hrs followed by a series of alcohol solution dehydration, and were embedded in paraffin. Sectioning of the samples was

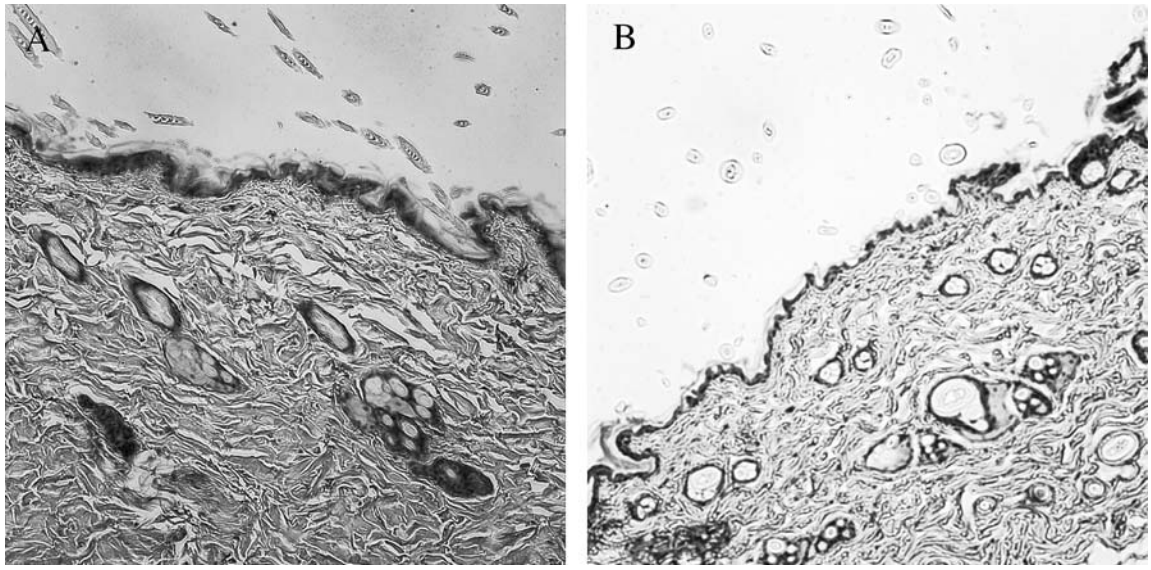
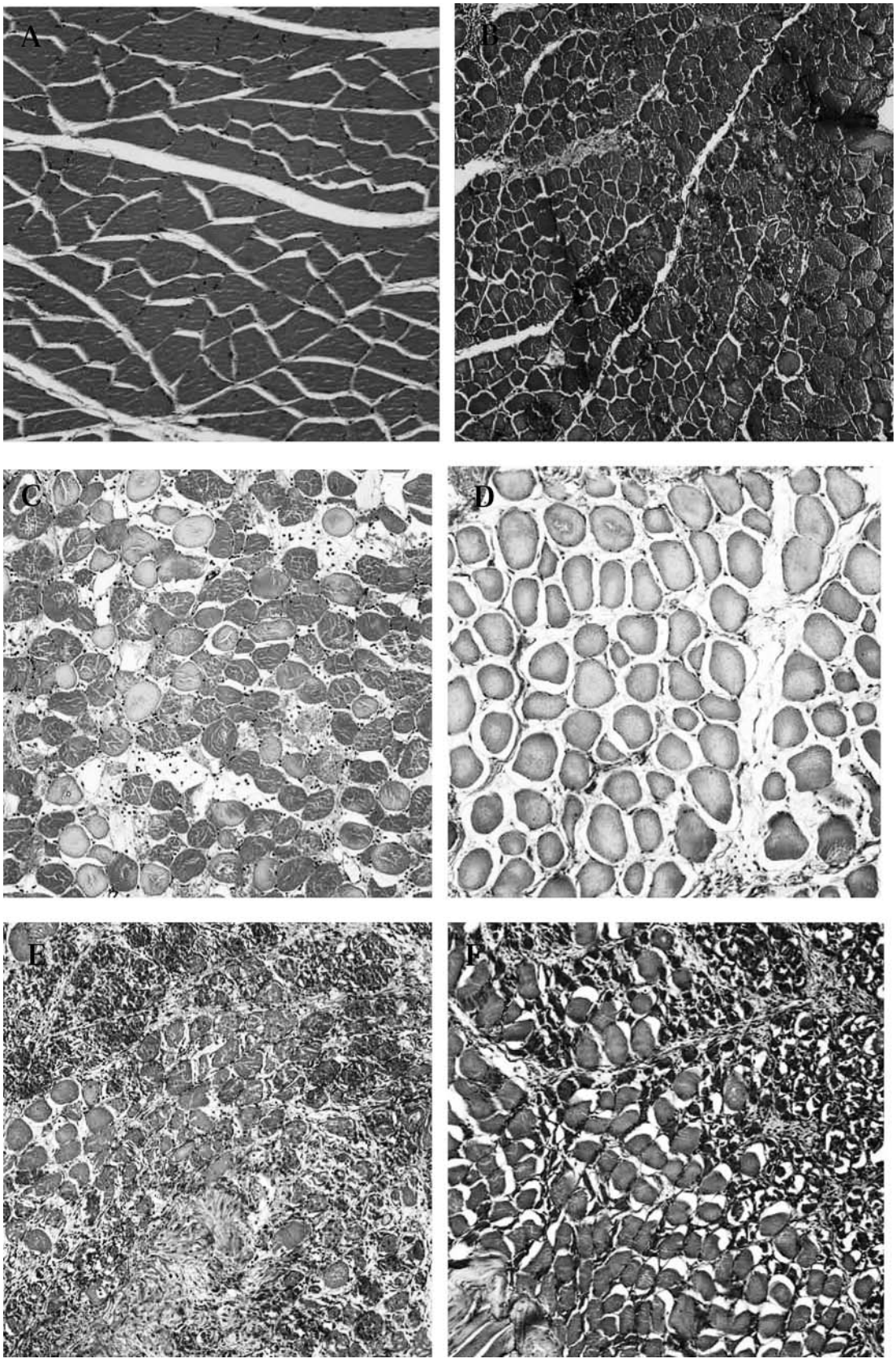


Figure 4. Photomicrograph of skin at the tibialis area of control rat (A) and skin at tibialis area of the 48-hr group (B) exposed to 100 mm Hg pressure loading for 6 hrs/day with 1 consecutive day; the 48-hr, 72-hr, 96-hr, and 168-hr groups also showed similar results. There was no visible different between experimental and control groups (hematoxylin and eosin; magnification: ×200).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carried out perpendicular to the skin in the direction of loading at a thickness of 5 μm. The samples were stained with hematoxylin and eosin to explore the viability of cells after delivering the selected time effect of static external loading.

Results

Blood Flow Changes During Loading. The result of the normalized perfusion expressed as a ratio of average flux at the time of measurement to the resting flux on Day 1 has been reported recently (21). In this paper, postocclusive hyperemic flow was expressed as a ratio of average flux during postocclusive hyperemia after loading of each day to the average resting flux of that day. Figure 2 shows the postocclusive reperfusion condition of the two loading sites. One-way repeated-measure ANOVA was used to evaluate the time effect of postocclusive hyperemia. It was found that postocclusive hyperemic flow was gradually increased at the trochanter throughout the 4 days of loading, whereas for the tibia there was a significant increase ($P = 0.04$) of postocclusive hyperemic flow between Days 2 and 3. In addition, with the same loading pressure it was observed that the reduction of average skin blood flow during loading at the tiabilis area was 27% more than at the trochanter area.

Morphologic Changes at Cutaneous Level of Trochanter Area. After application of 13.3 kPa (100 mm Hg) pressure for 6 hrs a day to trochanter, tissue damage was found at the subcutaneous level for the 48-hr, 72-hr, 96-hr, and 168-hr groups. The abnormalities of surface skin were characterized microscopically by keratin layer thickening and accumulation of fluid within the epidermis (Fig. 3). Contrarily, tissues exposed for 1 day (i.e., the 24-hr group) were indistinguishable from slides of control sets.

Morphological Changes at Cutaneous Level of Tibialis Area. Under the same pressure loading applied to the skin overlying the tibialis area, there was no observable difference between experimental and control groups, no matter which time groups the samples belonged to (Fig. 4).

Morphologic Changes at Muscular Levels. Disease of skeletal muscle cells was found after pressure loading for the 48-hr, 72-hr, 96-hr, and 168-hr groups, but not for the 24-hr and control groups (Fig. 5A). Histologically, the degenerative muscle cells were characterized by increasing numerous nuclei (Fig. 5B), and atrophic and hypertrophic muscle fibers with round contours were present (Fig. 5C). With abnormal variation in fiber size due to atrophy of some and hypertrophy of others, some muscle fibers were replaced by fibro-fatty tissue (Fig. 5D).

Apart from peripherally located nuclei of necrotic muscle fibers becoming internalized, there was destruction of muscle fibers with replacement of the muscle by fibrous tissue (Fig. 5E). In a severe case of necrosis, muscle atrophy affects groups of muscle fibers supplied by single motor units, in contrast to the haphazard pattern of atrophy seen at other groups (Fig. 5F). Further, pyknotic nuclei of necrotic cells also were found in specimens of the 96-hr group (Fig. 6).

One-way repeated-measure ANOVA was used to evaluate the time effect of muscle damage due to repetitive loading. By counting the number of nuclei within the muscle layer, it was found that the number of nuclei was significantly increased ($P = 0.03$) after 2 days of indentation, and increased progressively after Days 3 and 4. After removal of loading, the number of nuclei was increased further, thus indicating a further degeneration of the muscle cells. The findings are summarized in Figure 7.

Discussion

In this study we employed a rat model to study the time effect of pressure on tissue viability. The main causes of pressure ulcer have been attributed to external unrelieved pressure, shear, and ischemia. Among these three, pressure loading remains as the primary factor of concern (3). From previous studies it was accepted that external pressure in excess of capillary pressure of 1.6 to 4.4 kPa (12 to 33 mm Hg; Ref. 22) applied over a sufficient period of time will cause ischemia followed by edema, inflammation, small vessel thrombosis, and will result in necrosis and ulceration (23). In the present study, pressure loading of 13.3 kPa (100 mm Hg) was applied to the trochanter and the tibialis areas. This applied pressure does not caused a complete occlusion of local skin blood flow. The decreased in blood flow was found to be 70% and 50% of the resting level at the trochanteric and tibialis areas, respectively. As noted in the LDF measurements, skin blood flow showed a decreasing trend toward the end of the loading period. The progressive reduction of blood flow at the loading site suggests that the buildup of flow resistance is time dependent, which is most likely due to the increase of deformation of the underlying tissues resulting from its viscoelastic characteristics. The magnitude of the postocclusive hyperemic response is usually interpreted as a reflection of the insult to which the affected tissues have been subjected. In this study, the

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Figure 5. Photomicrograph of muscle fibers at the tibialis area. (A) Normal muscle architecture of control rat shows closely packed polygonal muscle fiber profiles. There is little variation in muscle fiber size or shape. Cytoplasmic staining is uniform, and small, peripherally located nuclei are abundant. (B) Muscle fibers of the 48-hr group. Degeneration of muscle cells was characterized by increasingly numerous nuclei, which occupied the central of the muscle fibers. (C) In the 72-hr group, atrophic and hypertrophic muscle fibers with round contours are present. (D) In the 96-hr group, muscle fibers are replaced by fibro-fatty tissue, and abnormal variation in fiber size is due to the atrophy of some and the hypertrophy of others. (E) In the 96-hr group, muscle fibers become necrotic. Peripherally located nuclei of muscle fibers became internalized. There is destruction of muscle fibers with the replacement of the muscle by fibrous tissue. (F) In the 168-hr group, muscle fibers became a severe case of necrosis, where muscle atrophy affects groups of muscle fibers supplied by single motor units in contrast to the haphazard pattern of atrophy seen in other groups (hematoxylin and eosin; magnification: ×200).

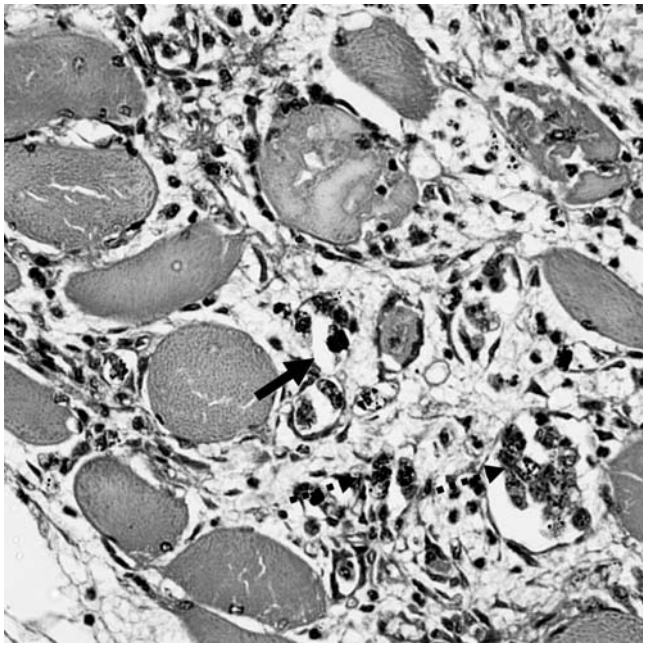

Figure 6. Pyknotic nuclei of necrotic cells (arrow) and karyorrhexis (broken arrow) can be observed in specimens from the 96-hr group (hematoxylin and eosin; magnification: ×400).

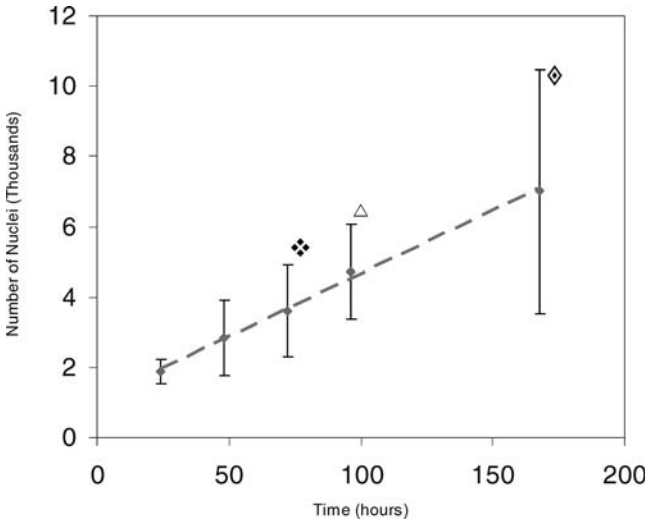

Figure 7. Time effect on muscle tissue degeneration (exemplified by the number of nuclei within the muscle layer). Statistically significant ❖$P = 0.03$ between Days 1 and 3; Days 1 and 4 as △$P = 0.01$ and ◈$P = 0.03$, difference well as Days 1 and 7.

postocclusive hyperemic response of the tissue at the trochanter area was found to be less rigorous than that of the tibialis area. This is likely due to the higher metabolic demand of the muscle tissues at the tibialis.

Upon loading, pressure is transmitted to the underlying bony structures by compressing the muscle together with the overlying skin. The kind of damage found in this study, consisting of keratin layer thickening, accumulation of fluid within the epidermis, and muscle fiber damage, resembles the tissue damage described previously by Nola *et al.* (7). At the skin level over the trochanteric region, epidermal breakdown and cellular infiltrate were observed in all studied groups, except the 24-hr group, after 6 hrs/day of load application of 13.3 kPa (100 mm Hg). The epidermal breakdown could be a result of a combined effect of pressure and shear stress at the indentor–tissue interface. With the same pressure loading applied to the skin overlying the tibialis area, no visible difference was observed among all the experimental groups and control groups. This seems to suggest that skin overlying the trochanter is more vulnerable to pressure induced damage compared with skin overlying muscle at the tibialis area (4, 6, 17).

For the muscle level, a recent study conducted by Linder-Ganz and Gefen (9) has shown that static compression of 35 and 70 kPa (263 and 525 mm Hg), but not compression of 11.5 kPa (86 mm Hg), at the gracilis muscle for 6 hrs could cause extensive necrotic death of muscle cells. Our results showed similar findings in which no muscle damage was found after compression of 13.3 kPa (100 mm Hg) for 6 hrs of pressure loading, but degeneration of muscle cells started at the tibialis area after 6 hrs a day for 2 consecutive days. This is in contrast of the finding by Bouten *et al.* (24), who reported that after pressure loading of 15 kPa for 2 hrs at the tibialis anterior muscle at approximately 30 degrees to horizontal, considerable muscle damage was revealed in most of the loaded muscles after the first day. This discrepancy is most likely the effect of a difference in loading site where the tissue and muscle thickness were different, and the direction of load application where shear stresses can be induced. Besides, the different histologic techniques used in different studies also may contribute to the discrepancy.

In clinical situations, complete removal of external loadings is the standard practice when pressure ulcers are suspected at a body site. It is believed that this could prevent the affected tissue from further damage. For the 168-hr group, in which the rats were subjected to 4 consecutive days of loading with 3 resting days, tissue recovery was not evident. Compared with the results of the 96-hr group, it showed the similar damage pattern at both subcutaneous and muscular levels. Although the difference in the number of nuclei found in the muscle level between the 96-hr and 168-hr groups was not statistically significant ($P = 0.17$), there was still a 48% increase in the number of nuclei in the 168-hr group. As cell necrosis also was evident in the 96-hr group, this confirms that tissue degeneration was present. Although the cause of this degeneration is not clear from this work, we suspect that this could be an effect of nitric oxide that triggered a damaging progress during prolonged loading (25). Further work is required to prove this hypothesis.

Conclusion. An experimental rat model was used to study the time effects of pressure on the cutaneous and muscular tissues and the related postocclusive hyperemic responses of tissues. Our results indicate that muscles are

more vulnerable to compressive loading than cutaneous tissues. The change in postocclusive hyperemic flow magnitude at the tiabilis between the 48-hr and 72-hr groups, together with the histopathologic signs found in the specimens suggested that postocclusive hyperemia and the distress of tissues under loading could be closely related.

1. Allman R. Pressure ulcer prevalence, incidence, risk factors and impact. Clin Geriatr Med 13:421–436, 1997.
2. Bouten CVC, Bader DL. Compressive strain-induced muscle cell damage in a model system. ASME Summer Bioengineering Conference, June 27–July 1, 2001. Snowbird, Utah. pp. 161–162, 2001.
3. Crenshaw RP, Vistnes LMA. A decade of pressure sore research: 1977–1987. J Rehab Res Dev 26:63–74, 1989.
4. Bliss MR. Aetiology of pressure sores. Rev Clin Gerontol 3:379–397, 1993.
5. Reswick JB, Rogers J. Experience at Rancho Los Amigos Hospital with devices and techniques to prevent pressure sores. In: Kenedi RM, Cowden JM, Scales JT, Eds. Bedsore Biomechanics. Baltimore, MD: University Park Press, pp301–310, 1976.
6. Daniel RK, Priest DL, Wheatley DC. Etiologic factors in pressure sores: an experimental model. Arch Phys Med Rehab 62:492–498, 1981.
7. Nola GT, Vistnes LM. Differential response of skin and muscle in the experimental production of pressure sores. Plast Reconstr Surg 66:728–733, 1980.
8. Bosboom EMH, Bouten CVC, Oomens CWJ, van Straaten HWM, Baaijens FPT, Kuipers H. Quantification and localisation of damage in rat muscles after controlled loading: a new approach to study the aetiology of presure sores. Med Eng Phys 23:195–200, 2001.
9. Linder-Ganz E, Gefen A. Mechanical compression-induced pressure sores in rat hindlimb: muscle stiffness, histology, and computational models. J Appl Physiol 96:2034–2049, 2004.
10. Linder-Ganz E, Engelberg S, Scheinowitz M, Gefen A. Pressure-time cell death threshold for albino rat skeletal muscles as related to pressure sore biomechanics, J Biomech 39:2725–2732, 2006.
11. Stekelenburg A, Oomens CWJ, Strijkers GJ, Nicolay K, Bader DL. Compression-induced deep tissue injury examined with magnetic resonance imaging and histology. J Appl Physiol 100:1946–1954, 2006.
12. Schubert v, Perbeck L, Schubert PA. Skin microcirculatory and thermal changes in elderly subjects with early stage of pressure sores. Clin Physiol 14:1–13, 1994.
13. Mayrovitz HN, Macdonald J, Smith JR. Blood perfusion hyperaemia in response to graded loading of human heels assessed by laser-doppler imaging. Clin Physiol 19:351–359, 1999.
14. Hagisawa S, Barbenel J, Kenedi R. Influence of age on postischaemia reactive hyperaemia, Clin Phys Physiol Meas 12:227–237, 1991.
15. Schubert V, Fagrell B. Evaluation of the dynamic cutaneous post-ischaemic hyperaemia and thermal response in elderly subjects and in area at risk of pressure sores, Clin Physiol 11:169–182, 1991.
16. Schubert V, Schubert PA, Breit G, Intaglietta M. Analysis of arterial flowmotion in spinal cord injuredand elderly subjects in an area at risk for the development of pressure sores, Paraplegia 33:387–397, 1995.
17. Kosiak M. Etiology of decubitus ulcer. Arch Phys Med Rehabil 42:19–29, 1961.
18. Husian T. An experimental study of some pressure effects on tissues, with reference to the bed-sore problem. J Pathol Bacteriol 66:347–385, 1953.
19. Salcido R, Donofrio JC, Fisher SB, LeGrand EK, Dickey K, Carney JM, Achosser R, Liang R. Histopathology of pressure ulcers as a result of sequential computer-controlled pressure sessions in a fuzzy rat model. Adv Wound Care 7:23–40, 1994.
20. Peirce SM, Skalak TC, Rodeheaver GT. Ischemia-reperfusion injury in chronic pressure ulcer formation: a skin model in the rat. Wound Repair Regen 8:68–76, 2000.
21. Li Z., Tam EWC, Kwan MPC, Mak AFT, Lo SCL, Leung MCP. Effects of prolonged surface pressure on the skin blood flowmotions in anaesthetized rats – an assessment by spectral analysis of laser Doppler flowmetry signals. Phys Med Biol 51:2681–2694, 2006
22. Landis EM. Micro-injection studies of capillary blood pressure in human skin. Heart 15:209–228, 1930.
23. Dinsdale SM. Decubitus ulcers in swine: Light and electron microscopy study of pathogenesis. Arch Phys Med Rehabil 54:51, 1973.
24. Bouten CV, Breuls RG, Peeters EA, Oomens CW, Baaijens FP. In vitro models to study compressive strain-induced muscle cell damage. Biorheology 40:383–388, 2003.
25. Reid RR, Sull AC, Mogford JE, Roy N, Mustoe TA. A novel murine model of cyclical cutaneous ischemia-reprefusion injury. J Surg Res 116:172–180, 2004.