Comparison between a weight compression and a magnet compression for experimental pressure ulcers in the rat.  
Histological studies and effects of anesthesia*

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Summary. To develop an experimental model and evaluate the effects of the magnitude and duration of pressure, the rat abdominal wall (25 × 20 mm) was subjected to compression either by a weight or by two magnets. In the weight compression tests, a steel plate was inserted under anesthesia into the rat peritoneal cavity, and the abdominal wall was compressed in situ between the underlying steel plate and a weight placed on the abdominal wall. This method resulted in moderate changes in the subcutaneous connective tissue and muscle at 100 mmHg (13.3 kPa) for 4 h, while some muscle damage was observed at 50 mmHg (6.7 kPa) for 4 h and at 100 mmHg for 2 or 3 h. In the magnet pinching tests, a magnet was inserted into the peritoneal cavity, and another magnet overlaid on the skin. Then the abdominal wall was compressed by the two magnets with or without anesthesia. The compression without anesthesia produced significant edema and injuries of the abdominal wall at 50 mmHg for 4 h and at 100 mmHg for 3 or 4 h, while the injuries incurred at 100 mmHg for 2 h were mild. Susceptibility to pressure was high in the muscle, moderate in the subcutaneous connective tissue, and low in the skin. The compression with anesthesia produced significantly milder injuries than those under anesthesia. These findings indicate that the difference in the extent of injuries between the weight compression and magnet compression models are clearly attributable to the pentobarbital anesthesia induced during the compression. Results therefore show that experimental pressure ulcers should be examined in a waking condition and that magnet compression is a useful model for studying the pathogenesis of pressure ulcers.

Introduction

Pressure ulcers (pressure sores, decubitus sores) are major problems for aged and/or disabled people who are unable to change the position of their bodies. Pressure ulcers often develop in patients with spinal cord injury, cerebral vascular disease, bone fracture, chronic obstructive lung disease, and diabetes mellitus. They often develop at the sacral region, the greater femoral trochanter, and the ischial tuberosities (Kosiak, 1959). Various hypotheses have been proposed concerning the development and progression of pressure ulcers. One major cause of pressure ulcers is ischemia (Kosiak, 1959; Daniel et al., 1981) due to prolonged pressure over a bony prominence. Pressure applied to a body surface leads to a decrease or interruption of blood flow in the skin, the subcutaneous connective tissue, and muscle. An estimated threshold of 35 mmHg of pressure has been set for capillary occlusion (Daniel et al., 1981). Blood flow in areas of skin compressed by 50 mmHg of pressure is reported to decline to an average of 20% of the control value (Peirce et al., 2000). When such ischemic conditions continue over a critical period of time (e.g., 2 h; Daniel et al., 1981), it eventually leads to necrosis.
and breakdown of the skin. Tissue viability depends on both the magnitude and duration of pressure. An inverse relationship exists between these parameters required to produce a pressure ulcer (Kosiak, 1959; Daniel et al., 1981; Linder-Ganz et al., 2006). Another plausible cause of pressure ulcers is ischemia-reperfusion injury (Peirce et al., 2000; Reid et al., 2004; Cordeiro et al., 2005, Şener et al., 2006). These two factors, i.e., prolonged ischemia and ischemia-reperfusion, are generally considered the most important causes for the development of pressure ulcers. However, the mechanisms of development and progression of pressure ulcers are not fully understood.

Concerning pressure ulcers, medical history and health status differ considerably between patients. Furthermore, numerous factors are involved in the production of pressure ulcers (Stekelenburg et al., 2005) including the magnitude of pressure, exposure time, shear force on the skin, age, nutrition (Cordeiro et al., 2005), chronic disease, and medication. These non-uniform factors make it difficult to identify the causes of pressure ulcers in clinical situations. In addition, the sampling of tissues and other specimens from patients present ethical problems. The heterogeneous backgrounds of patients have hampered an appropriate assessment of procedures for the prevention and treatment of pressure ulcers. Therefore, animal models of pressure ulcers produced under controlled and precise experimental conditions are necessary. In fact, various animal models have been developed. In early experiments, heavy weights and pressure applicators (e.g., indenters) were used. This required the animals to be anesthetized during experiments to sedate and immobilize them (Kosiak, 1959; Daniel et al., 1981; Bosboom et al., 2001, 2003; Linder-Ganz and Gefen, 2004, Kawai et al., 2005; Linder-Ganz et al., 2006; Stekelenburg et al., 2007). On the other hand, Peirce et al. (2000), Reid et al. (2004), Şener et al. (2006) and Sisco et al. (2007) compressed skin using magnets in waking conditions. They implanted a magnet or a steel plate in the subcutaneous connective tissue of the back and caused compression by applying a magnet to the skin. However, the pressure on the tissue may have gradually increased due to thinning of the skin and subcutaneous connective tissue that was sandwiched between the magnet and implanted steel plate/magnet. Other than these methods mentioned above, both cultured muscles (Gawlitta et al., 2007) and artificial skin (Bronneberg et al., 2007) have been studied in vitro.

To establish a better animal experimental model of pressure ulcers and evaluate the effects of the magnitude and duration of pressure, we compared two models, i.e., a weight compression and a magnet compression,
by observing histological changes up to 1 w after compression.

**Materials and Methods**

A total of 113 male Wistar rats (280 ± 24 g) aged 8 and 9 weeks were used. According to the clinically relevant pressure, pressures of 50 (6.7 kPa) and 100 mmHg (13.3 kPa) were selected for compression (Swain, 2005; Stekelenburg et al., 2005). We chose the rat abdominal wall as the material and compressed it at a constant pressure using a weight (lead ingot) or two magnets. The present experimental procedures were approved by the Committee of Research Facilities for Laboratory Animal Science, Natural Science Center for Basic Research and Development, Hiroshima University.

**Weight compression with pentobarbital anesthesia**

Lead ingots (25 mm in length × 20 mm in width and 60 or 120 mm in height corresponding to 50 and 100 mmHg of pressure, respectively) were manufactured. Each weight, with a flat and smooth base, was stood on a flat horizontal surface (an adjustable balance table for immunostaining) and had a level meter attached to its top to properly indicate the horizontal plane.

The rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (an initial dose 50 mg/kg body weight) and laid on a thermo-regulated heating pad in a supine position. Abdominal wall hair was shaved with an electric clipper. The following landmarks were marked on the skin with a felt pen and a ruler: the xiphoid process, the costal margin, the midline, the area to be compressed (i.e., a rectangle of 25 mm in length × 20 mm in width, 5-mm caudal to the xiphoid process), and a right-to-left incision line (about 25 mm) 60 mm caudal from the xiphoid process (Fig. 1a). After disinfection, the lower abdominal wall was incised, and a sterilized steel plate (25 mm in width × 2 mm in thickness × 190 mm in length with a siliconized surface) was inserted into the peritoneal cavity until its tip nearly reached the xiphoid process (Fig. 1a). The steel plate had its height adjusted so as not to produce tension in the abdominal wall as this could interfere with the blood flow of the abdominal wall. The wound in the lower abdomen was covered during compression with a sterilized cotton sheet soaked with saline. The other end of the steel plate was secured with a stand, and a weight was placed on the abdominal wall, thus causing ischemia (Fig. 1a, 2a). While monitoring the level meter on top of the weight, the setting angle of the steel plate was adjusted to keep the weight standing at the correct angle on the abdominal wall to avoid any sliding since the subcutaneous connective tissue is easily movable (Kawamata et al., 2003). To protect the rat during compression, a case was hung over the weight without touching it in case it fell. Early in the experiments, the weights sometimes slipped during the compression; these rats were not used for further processing. Four needles were then placed at the corners of the weight during compression. Owing to this modification, we later found out that the precise pressures on the abdominal wall were 53 and 102 mmHg. However, we refer to these pressures as 50 and 100 mmHg, respectively, judging that probably this would not influence the results and because similar minor pressure increases were also observed in the magnet compression (Table 1). The rats were subjected to one of the following compression conditions: 1) 50 mmHg for 4 h; 2) 100 mmHg for 2 h; 3) 100 mmHg for 3 h; or 4) 100 mmHg for 4 h. Three rats were used for each treatment. During compression, additional sodium pentobarbital (1/4 of initial dose) was intraperitoneally administered as necessary (Vaculín and Rokyta, 2004). Before the cessation of compression, the 4 points of the abdominal wall corresponding to the 4 corners of the weight were tattooed with India ink using a needle for identification at sampling. After suturing the lower abdominal incision, the animals were returned to their cages and reared with free access to water and food. All animals behaved normally and remained without peritonitis or other problems. The rats were euthanized via an overinhalation of diethyl ether for sampling at 12 h, 1 day, 3 days, and 1 w after the start of compression.

**Table 1. Pressures at the start and end of magnet compression tests**

|                | 50  | 100 | 100 | 100 |
|----------------|-----|-----|-----|-----|
| Pressure at start (mmHg) | 50  | 100 | 100 | 100 |
| Duration of pressure (h)   | 4   | 2   | 3   | 4   |
| Pressure at end (mmHg)     | 51.7 ± 0.8 | 101.4 ± 0.8 | 102.3 ± 2.8 | 102.1 ± 1.0 |
| Number of animal           | 15  | 15  | 12  | 12  |
The rats were shortly anesthetized with diethyl ether, their abdominal wall hair was shaved and their abdomens marked with a felt pen and a ruler (Fig. 1a). A transverse incision was made in the lower abdomen. A disinfected thin neodymium magnet (25 × 20 × 2 mm, with a surface magnetic field strength of 2,100 Gauss, approximately 7.5 g, Seikou Sangyo, Ichikawa) was inserted into the peritoneal cavity. The incision was immediately sutured and the magnet was guided towards the area to be compressed (Fig. 1a, d). Then a strong neodymium magnet (25 × 20 × 5 mm, with a surface magnetic field strength of 3,100 Gauss, approximately 18.8 g, Seikou Sangyo) was applied on the skin. The rat abdominal wall was compressed by these two magnets at 50 (more precisely, 50-51) or 100 (100-101) mmHg. The pressure was adjusted by changing the total thickness of the intervening acrylic plates and papers between the rat skin and the strong magnet (Fig. 1c, 2b) in the following way. After insertion of a magnet into the peritoneal cavity and suturing of the abdominal incision, the rat was elevated in a prone position with its chest and legs held by an examiner. A strong magnet (25 × 20 × 5 mm), which had been secured to a plastic bottle containing water, was placed on top of papers, acrylic plates, and the skin. The weights of the cap and bottle were 340 and 680 g, which equates to 50 or 100 mmHg for 5 cm² (25 × 20 mm), respectively. When the magnet in the rat was unable to hold the bottle by magnetic attraction, the intervening papers (and acrylic plates) were decreased until the magnet could hold the bottle (Fig. 3a). Next, a heavier bottle (total weight 347 and 687 g which equates to 51 or 101 mmHg, respectively) was similarly tested. When the magnet in the rat could hold the heavier bottle, papers (and acrylic plates) were increased between the rat skin and the strong magnet until it could not (Fig. 3b). After adjustments using such procedures (Fig. 3a, b), the bottle was replaced with the same type of strong magnet without a bottle to induce ischemia (Fig. 2b). The distance between the rat skin and the strong magnet was several mm or more at 100 mmHg. The rats immediately recovered from the ether anesthesia and were reared in a cage individually during compression. The rats were subjected to one of the following compression conditions: 1) 50 mmHg for 4 h; 2) 100 mmHg for 2 h; 3) 100 mmHg for 3 h; or 4) 100 mmHg for 4 h. Three animals were used for each treatment. After compression, the rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg). The lower abdominal incision was reopened, the intraperitoneal magnet was removed,
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The abdominal wall was carefully sutured in sterilized conditions. The rats were allowed to survive with free access to water and food. The rats were sacrificed by an overinhalation of diethyl ether at 12 h, 1 day, 3 days, or 1 w after the start of compression. All animals behaved normally in their cages. However, when a magnet was placed for a few days in the peritoneal cavity in another experiment, gastrointestinal trouble occurred. This problem needs to be addressed in future studies.

**Controls**

The abdominal walls of 3 untreated normal rats taken from the same place as shown in Figure 1a were used as controls.

**Evaluation of the effects of anesthesia during compression on injuries**

When the magnitude and duration of pressure were the same, the injuries caused by the weight compression tests were considerably milder than the injuries caused by the magnet compression tests as described below. We therefore hypothesized that pentobarbital anesthesia during compression alleviates injuries. Further experiments were conducted for examination under two conditions, i.e., 50 mmHg for 4 h and 100 mmHg for 2 h, because these conditions showed larger differences in terms of tissue injuries between the weight and magnet compression methods than the other conditions (100 mmHg for 3 or 4 h).

The rat abdominal wall was compressed by two magnets with or without anesthesia. The conscious group was compressed when the animals were awake as described above. The anesthetized group was treated in the same way as the conscious group except that the rats were anesthetized with sodium pentobarbital (an initial dose 50 mg/kg with an additional dose of 12.5 mg/kg as necessary) during compression. Five animals from each group were sacrificed by an overinhalation of diethyl ether at 1 day (24 h) after the start of compression for evaluation of their injuries because this time point is appropriate for assessing tissue damage (Bosboom et al., 2001, 2003).

**Siliconization of steel plates and magnets**

To minimize adverse tissue responses caused by contact, the surfaces of the steel plates and inserted magnets were siliconized. The steel plates were coated with a 5% KF-99 (a siliconizing reagent kindly provided by Shin-Etsu Chemical Co., Ltd, Tokyo) solution in xylene, and the abdominal wall was carefully sutured in sterilized conditions. The rats were allowed to survive with free access to water and food. The rats were sacrificed by an overinhalation of diethyl ether at 12 h, 1 day, 3 days, or 1 w after the start of compression. All animals behaved normally in their cages. However, when a magnet was placed for a few days in the peritoneal cavity in another experiment, gastrointestinal trouble occurred. This problem needs to be addressed in future studies.

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**Preparation of specimens**

The rats were sacrificed at 12 h, 1 day, 3 days, or 1 w after the start of compression. The compressed portion (25 × 20 mm) of the abdominal wall was removed along with the surrounding uncompressed zones. In the weight compression tests, after removal of the abdominal wall, the India ink markings that were tattooed during compression were confirmed, and the skin marking and the muscle layer marking of the same corner of the compressed area were overlapped and pinned together to minimize shear between the skin and muscle layers. Then the specimens were trimmed and transversely bisected. In the magnet compression tests, tattooing was difficult because the underlying steel plate was lacking during the compression. Thus, after sacrifice by diethyl ether, the abdominal wall was pinned through the skin and muscle layers at the 4 corners of the compressed area which had been marked by a felt pen during compression to minimize shear between these layers. The removed specimens containing surrounding uncompressed zones were trimmed and transversely bisected.

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The upper (rostral) half of all specimens was adhered to a piece of balsa wood (1 mm thick) coated with 6% tragacanth gum jelly in such a manner that the mid portion of the compressed specimen could be cut transversely, perpendicular to the skin surface. The specimen and balsa wood were placed at a right angle on a cork disk (about 20 mm in diameter and several mm in thickness) and supported with 6% tragacanth gum jelly. The specimen and balsa wood were frozen together (Daitoku et al., 2007) in isopentane cooled by liquid nitrogen. Transverse sections (10 μm in thickness) were cut, stained with hematoxylin and eosin, and observed with an Olympus B51 light microscope (Olympus, Tokyo).

**Evaluation of injuries in the skin and subcutaneous connective tissue**

Transverse sections were photographed with a DP 70 digital camera (Olympus) and a B51 light microscope (Olympus). A montage image of each specimen was synthesized. The thickest point of the skin and subcutaneous connective tissue was determined, and 1-mm-long portions at both sides (total 2 mm) were selected for measurements using Photoshop version 6.0 (Adobe, San Jose, CA, USA) and then cut at both ends to make them a constant length (2 mm in tissue length) using the Image J computer software version 1.33u (National Institutes of Health, Bethesda, MD, USA). To calculate the thickness, the area from the epidermis to the surface of the epimysium in each sample was traced by the same operator on the montage image, trimmed, measured, and divided by its width (2 mm) using the computer software.

**Statistical analysis**

For analysis of the thickness of the skin and subcutaneous connective tissue, one section from each specimen was used. One way analysis of variance was conducted for a comparison of the groups. For comparison of the thickness with or without anesthesia by magnet compression, the data were analyzed with the Student's
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2) The skin muscle (panniculus carnosus); 2) the subcutaneous connective tissue; 3) the epimysium and abdominal muscles; and 4) the peritoneum (Fig. 4). The skin muscle was often lacking near the midline.

Weight compression tests with pentobarbital anesthesia

All abdominal organs seemed normal at specimen sampling except for occasional minute liver hemorrhages probably caused by the steel plate. Compression at 50 mmHg for 4 h resulted in almost normal skin and subcutaneous connective tissue, with focal muscle necrosis in deep regions (peritoneal side) of the muscle layers at 12 h, 1 day, and 3 days after the start of compression (Fig. 5). Sporadic muscle regeneration was recognized in muscle layers at 1 w.

Fig. 6. Sections of the abdominal wall after weight compression tests at 100 mmHg for 4 h. a: At 12 h. The skin and subcutaneous connective tissue are thicker compared with the control, and the muscle is apparently injured. b: At 1 day. The skin and subcutaneous connective tissue are thick. c: At 3 days. Considerable muscle necrosis (N) is present. d: At 1 w. Muscle regeneration is seen. Bars = 1 mm

$t$ test. Statistical significance was set at $P<0.05$ in all analyses. Values are means ± SD (standard deviation).

Results

A depression in the abdominal wall was observable after both weight and magnet compression tests. Varying degrees of edema were found at 12 h and 1 day after treatment, depending on the severity of compression. Neither discoloration nor necrosis were found at any time during the experiments.

Untreated normal control

The abdominal wall was composed of the following layers: 1) the skin, consisting of the epidermis, dermis, and skin muscle (panniculus carnosus); 2) the subcutaneous connective tissue; 3) the epimysium and abdominal muscles; and 4) the peritoneum (Fig. 4). The skin muscle was often lacking near the midline.
Compression at 100 mmHg for 2 h induced no recognizable change in the skin or the subcutaneous connective tissue, whereas mild edema and sporadic necrosis were found in the muscle layers at 12 h and 1 day. At 3 days and 1 w, the whole abdominal wall seemed normal. Compression at 100 mmHg for 3 h showed few changes in the skin or subcutaneous connective tissue; however, the muscle layers exhibited edema at 12 h and focal muscle necrosis at 1 day and 3 days. Sporadic phagocytotic images of necrotic muscle fibers were observed at 3 days, followed by regeneration at 1 w. Compression at 100 mmHg for 4 h resulted in edema of the skin and the subcutaneous connective tissue at 12 h and 1 day (Fig. 6). The edematous changes were more severe in the subcutaneous connective tissue than in the skin. At 3 days, the edema was mild, and the skin and subcutaneous connective tissue were nearly normal at 1 w. In the abdominal muscles, muscle necrosis was widely observed at 12 h and 1 day. At 3 days, phagocytotic images and regenerating muscle fibers were seen. At 1 w, numerous regenerated muscle fibers with central nuclei were observable (Fig. 7). The time course of the thickness of the skin and subcutaneous connective tissue are summarized in Figure 8.

Magnet compression tests without anesthesia during compression

The pressures at the start and the end of the compression by magnets are shown in Table 1. The pressures were changed only slightly.

Compression at 50 mmHg for 4 h resulted in edema
Fig. 8. Changes in the thickness of the skin and subcutaneous connective tissue after weight compression tests with anesthesia. After compression at 50 mmHg for 4 h, the skin and subcutaneous connective tissues were thickest at 1 day, while these tissues were thickest at 12 h in the 100 mmHg for 4 h treatment. At 100 mmHg, muscle injuries of an increasing degree were observed with increasing durations of pressure. Data are not significant relative to the control ($P > 0.05, n = 3$).

Compression at 100 mmHg for 2 h caused mild to moderate edema in the skin and subcutaneous connective tissue at 12 h and 1 day. Sporadic muscle necroses along the peritoneum were found at 12 h, with progression upward to the superficial muscle layer at 1 day. At 3 days, few abnormal changes were found in the skin or subcutaneous connective tissue, and focal necrotic muscle fibers were phagocytosed. At 1 w, the abdominal wall was almost normal except for the presence of regenerating muscle fibers.

Compression at 100 mmHg for 3 or 4 h caused severe edema in the skin and subcutaneous connective tissue at 12 h. It was noteworthy that both the skin and subcutaneous connective tissue were very thick and edematous and stained less intensely after compression at 100 mmHg for 4 h. Edema was the most severe at 12 h but was alleviated thereafter. At 3 days, the skin appeared normal, whereas the subcutaneous connective tissue was thick with numerous fibroblast-like cells. At 1 w, the subcutaneous connective tissue remained thick, and numerous fibroblast-like cells were found (Fig. 9). The muscle layers were necrotic at 12 h and 1 day. At 3 days, the necrotic muscle fibers were phagocytosed, and abundant muscle fibers with central nuclei were found at 1 w (Fig. 10). The time course of the thickness of the skin and subcutaneous connective tissue are summarized in Figure 11.

**Effects of pentobarbital anesthesia**

The skin and subcutaneous connective tissue were significantly thicker in the conscious group than in the anesthetized group after treatment with either 50 mmHg for 4 h or at 100 mmHg for 2 h (Fig. 12, 13). After compression with 50 mmHg for 4 h, muscle injuries were rather similar between the two groups. On the other hand, after compression with 100 mmHg for 2 h, most muscle layers were injured in the conscious group, whereas muscle damage was mild in the anesthetized group.
Fig. 9. Sections of the abdominal wall after magnet compression tests without anesthesia at 100 mmHg for 4 h. a: At 12 h. The skin and subcutaneous connective tissue, particularly the skin, are very thick. Muscle layers are also injured. b: At 1 day. The skin and subcutaneous connective tissue are thick. c: At 3 days. The skin seems normal. The subcutaneous connective tissue (asterisk) is thick and numerous fibroblast-like cells are seen. d: At 1 w. Numerous cells are observed in the thick subcutaneous connective tissue. e: At 1 w. The subcutaneous connective tissue is thick. f: Enlarged view of panel e. Numerous fibroblast-like cells are seen. Bars = 1 mm (a–c), 0.1 mm (f)
Discussion

Weight compression or magnet compression

The extent of injuries significantly differed between the two methods (Fig. 8, 11), and pentobarbital anesthesia was clearly demonstrated to alleviate pressure-induced injuries. The results of our experiment indicate that pentobarbital and possibly other general anesthetics should be avoided in experimental models of pressure ulcers. From this point of view, magnet compression, which does not require anesthesia, is a more useful and reliable method than a weight compression for studying pressure ulcers.

Magnet compression as a model of compression ulcers

Magnets have been used for experimental models of pressure ulcers (Peirce et al., 2000; Reid et al., 2004; Sener et al., 2006; Saito et al., 2008). In these, a magnet or steel plate was implanted in the subcutaneous connective tissue and compressed by applying another magnet to the skin. Using this method, Peirce et al. (2000) reported that the skin blood flow declined to 20\% of the normal flow at 50 mmHg. As it is frequently employed, magnet compression has its advantages. However, magnet compression requires the following caution: 1) the initial pressure is usually not precisely measured; 2) pressure may increase during compression due to thinning of the skin and subcutaneous connective tissue, but this is
often not measured; 3) implanting a steel plate or magnet possibly interrupts the vascular network and affects blood flow to the skin, in particular when the magnet or steel plate is large.

In the present study, the pressures at the start and the end of compression were precisely measured using plastic bottles. The pressures were constant (Table 1) because the distance between the two magnets was large enough.
and the thinning of tissue thickness was almost negligible compared with the distance between the two magnets. Although the lower abdomen was incised, the vascular bed of the compressed abdominal area remained intact. The fact that specimens after compression at 100 mmHg for 2 h showed histological findings very similar to the normal controls indicates that incision alone is unlikely to affect histological observations. The abdominal wall is wide and roughly homogeneous in its layers and is therefore appropriate for quantitative assessment of the thickness of the skin and subcutaneous connective tissue (Fig. 8, 11).

**Effects of pentobarbital anesthesia during compression**

In previous studies using a weight or applicator, animals were anesthetized during compression for immobilization (Kosiak, 1959; Daniel et al., 1981; Linder-Ganz et al., 2006). The magnitudes and durations of pressure were often very large and clinically irrelevant. Kosiak (1959) examined pressures of 100-550 mmHg for 1-12 h. Daniel et al. (1981) investigated pressures of 500 mmHg for 4 h or 800 mmHg for 8 h. Kawai et al. (2005) compressed tissue with 500 g/cm² (368 mmHg) of pressure for 10 h for 2 consecutive days. In such experiments, it is possible that anesthesia alleviated the compression injuries. Other than pentobarbital as shown in this study, other general anesthetics possibly protect tissues against compression. This problem needs to be addressed.

**Injuries of the skin, subcutaneous connective tissue, and muscle in magnet compression**

Concerning the histological changes of the abdominal wall, increasing degrees of injuries were observed with increasing duration — and partly with increasing magnitude — of pressure applied to the tissue. These results favor the idea that ischemia is the main cause of pressure ulcers. Furthermore, differences in tolerance and response to pressure were demonstrated between the skin and the subcutaneous connective tissue. The degrees of susceptibility to pressure ranked in the order of the muscle, the subcutaneous connective tissue, and the skin — in agreement with Daniel et al. (1981) and Linder-Ganz et al. (2006). Muscle injuries were most frequently observed in the present study, perhaps due to the high metabolic demand of the muscle (Linder-Ganz et al., 2006). It is reported that pressures greater than 9 kPa (67 mmHg) applied for over 2 h consistently cause muscle cell death (Linder-Ganz et al., 2006) under anesthesia. The skin appears more resistant to pressure compared with the subcutaneous connective tissue because the skin swelled only after severe compression and returned to an almost normal state as early as at 3 days after treatment in the present study. Thus, attention must be paid to assess the severity of compression ulcers in clinical situations because muscle layers may be injured even when skin does not show any visible signs (Bosboom et al., 2003).

In conclusion, compression by magnets is a more appropriate model than weight compression for simulating pressure ulcers. Although anatomical and physiological differences exist between rats and humans (Linder-Ganz et al., 2006), this model should be helpful for a better understanding of the pathogenesis and progression of compression ulcers and contribute to examining interventions for the prevention and treatment of pressure ulcers. However, compression was undertaken only once in our model, and skin necrosis was not produced. Perhaps a daily repetition of ischemia finally leads to necrosis of the skin and subcutaneous connective tissue. This remains a topic for further study.

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References

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 pressure sores. Med Eng Physics 23: 195-200 (2001).

Bosboom EMH, Bouten CVC, Oomens CWJ, Baaijens FPT, Nicolay K: Quantifying pressure sore-related muscle damage using high-resolution MRI. J Appl Physiol 95: 2235-2240 (2003).

Bronneberg D, Spiekstra SW, Cornelissen LH, Oomens CWJ, Gibbs S, Baaijens FRT, Bouten CVC: Cytokine and chemokine release upon prolonged mechanical loading of the epidermis. Exp Dermatol 16: 567-573 (2007).

Cordeiro MBC, Antonelli ÉJ, da Cunha DF, Júnior AAJ, Júnior VR, Vannucchi H: Oxidative stress and acute-phase response in patients with pressure sores. Nutrition 21: 901-907 (2005).

Daitoku D, Kurose T, Mori E, Hashimoto M, Kawamata S: Changes in the rat subcutaneous connective tissue after saline and histamine injection in relation to fluid storage and excretion. Arch Histol Cytol 70: 29-41 (2007).

Daniel RK, Priest DL, Wheatley DC: Etiologic factors in pressure sores: an experimental model. Arch Phys Med Rehabil 62: 492-498 (1981).

Gawlitta D, Li W, Oomens CWJ, Baaijens FPT, Bader DL, Bouten CVC: The relative contributions of compression and hypoxia to development of muscle tissue damage: an in vitro study. Ann Biomed Eng 35: 273-284 (2007).

Kawai K, Suzuki S, Tabata Y, Nishimura Y: Accelerated wound healing through the incorporation of basic fibroblast growth factor-impregnated gelatin microspheres into artificial dermis using a pressure-induced decubitus ulcer model in genetically diabetic mice. Br J Plast Surg 58: 1115-1123 (2005).

Kawamata S, Ozawa J, Hashimoto M, Kurose T, Shinohara H: Structure of the rat subcutaneous connective tissue in relation to its sliding mechanism. Arch Histol Cytol 66: 273-279 (2003).

Kosiak M: Etiology and pathology of ischemic ulcers. Arch Phys Med Rehabil 40: 62-69 (1959).

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).

Peirce SM, Skalak TC, Rodeheaver GT: Ischemia-reperfusion injury in chronic pressure ulcer formation: a skin model in the rat. Wound Rep Reg 8: 68-76 (2000).

Reid RR, Sull AC, Mogford JE, Roy N, Mustoe TA: A novel murine model of cyclical cutaneous ischemia-reperfusion injury. J Surg Res 116: 172-180 (2004).

Saito Y, Hasegawa M, Fujimoto M, Matsushita T, Horikawa M, Takenaka M, Ogawa F, Sugama J, Steele DA, Sato S, Takehara K: The loss of MCP-1 attenuates cutaneous ischemia-reperfusion injury in a mouse model of pressure ulcer. J Invest Dermatol 128: 1838-1851 (2008).

Stekelenburg A, Oomens C, Bader D: Compression-induced tissue damage: animal models. In: Pressure ulcer research. Current and future perspectives (Bader D, Bouten C, Colin D, Oomens C ed), Springer-Verlag, Heidelberg, 2005 (p. 187-204).

Swain Y: The measurement of interface pressure. In: Pressure ulcer research. Current and future perspectives (Bader D, Bouten C, Colin D, Oomens C ed), Springer-Verlag, Heidelberg, 2005 (p. 51-71).

Vaculín Š, Rokyta R: Effects of anesthesia and nociceptive stimulation in an experimental model of brachial plexus avulsion. Physiol Res 53: 209-214 (2004).