Int. J. Morphol.,
43(6):1934-1942, 2025.

Structural and Component Alterations in Intertrochanteric Crests of Femur in Induced Short- and Long- Term Diabetes

Alteraciones Estructurales y de Componentes en las Crestas Intertrocantéreas del Fémur en la Diabetes Inducida a Corto y Largo Plazo

Sasipa Pianrumluk[1]; Passara Lanlua[1]; Apichaya Niyomchan[1]; Kanokporn Plaengrit[2]; Sani Baimai[1] & Sirinush Sricharoenvej[1]

PIANRUMLUK, S.; LANLUA, P.; NIYOMCHAN, A.; PLAENGRIT, K.; BAIMAI, S. & SRICHAROENVEJ, S. Structural and component alterations in intertrochanteric crests of femur in induced short- and long-term diabetes. *Int. J. Morphol., 43(6)*:1934-1942, 2025.

SUMMARY: Individuals with type I diabetes mellitus have an increased risk of proximal femoral fracture. Moreover, low bone mineral density (BMD), altered serum levels of biomarkers of bone turnover and altered bone morphometry parameters, which affect bone strength, are observed in individuals with diabetes. Thus, this study aimed to compare the components of the intertrochanteric crest of the femur in terms of morphology, bone volume fraction (BV/TV), BMD, trabecular thickness (Tb.Th), and separation (Tb.Sp) by using microcomputed tomography, histomorphology, the determination of cell numbers, and the quantification of empty lacunae and collagen fibres via light microscopy in rats with both short- and long-term streptozotocin-induced diabetes. During both the early and late stages of diabetes, reductions in the femoral BV/TV, BMD, and Tb.Th values and an increase in the Tb.Sp value were observed; moreover, the numbers of osteoblasts and osteocytes decreased, but the numbers of empty lacunae and osteoclasts increased. Additionally, disorganized collagen fibres with lower density were observed in the two diabetes groups. The severity of these changes was more pronounced in the long-term diabetes model, along with thickening of blood vessels and an increased bone marrow adipocyte tissue. All of these changes lead to a decrease in bone quality and fragility, which might be related to an increased risk of femoral fractures in the diabetes groups. Thus, the current understanding of the bone damage that occurs during the development of diabetes might warrant careful consideration of bone strength during the early stages of the disease.

KEY WORDS: Diabetes mellitus; Femur; Bone cells; Microcomputed tomography.

INTRODUCTION

Diabetes mellitus (DM) is a disorder of carbohydrate metabolism characterized by hyperglycaemia. The prevalence of hip fracture is 3-6 times greater in diabetic patients than in nondiabetic patients, and the most common site of hip fracture is the intertrochanteric crest of the femur (Montagnani *et al*., 2011; Jackuliak & Payer, 2014). In humans and animals with diabetes, biomarkers of bone turnover in blood, morphometric parameters and bone density are evaluated. High blood glucose levels alter biomarkers, increasing bone resorption and suppressing bone formation; these changes cause a reduction in the volume of bone mineral density (BMD) and microarchitectural abnormalities in bone, ultimately decreasing bone strength and increasing the risk of fracture (Hie *et al*., 2007; Starup-Linde, 2013; Tsentidis *et al*., 2016). Typically, bone is composed of cells and a bone matrix. The cells involved in bone formation (osteoblastogenesis) are osteoblasts, whereas the cells involved in bone resorption (osteoclatogenesis) are osteoclasts. These cells are responsible for the repair and removal of damaged bone to maintain integrity and mineral homeostasis (Wongdee & Charoenphandhu, 2011). Osteoblasts synthesize and secrete bone matrix, such as collagen and ground substances. Osteocytes in lacunae are mature bone cells that differentiate from osteoblasts (Aubin, 2001). Osteoclasts are responsible for bone resorption during the bone remodelling process and for calcium homeostasis (Asagiri & Takayanagi, 2007). The other component, the bone matrix, contains organic and inorganic substances. The

[1] Department of Anatomy, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.
[2] Chulabhorn International College of Medicine, Thammasat University, Rungsit Campus, Pathumthani, Thailand.

Received: 2025-08-13 Accepted: 2025-10-08

organic matrix consists of collagen fibres in ground substances, which function to promote bone toughness and flexibility, whereas the inorganic matrix (bone minerals) supports the hardness of bone (Florencio-Silva *et al*., 2015). Although previous studies have examined changes in biomarkers of bone formation and resorption in patients with DM and correlated these changes with a greater risk of femoral fracture, the structural changes that occur in the bone composition, both in cells and in the bone matrix, are still not clear. This investigation was designed to examine the effects of both short- and long-term streptozotocin (STZ)-induced DM in rats on the intertrochanteric crest of the femur by using microcomputed tomography (microCT) to assess the bone volume fraction (BV/TV), evaluate the percentage of bone tissue within the total region of the bone and marrow cavity, and determine the BMD to quantitatively measure the minerals (mainly calcium). Additional parameters that were assessed included trabecular thickness (Tb.Th) to measure the thickness of individual trabeculae and trabecular separation (Tb.Sp) to determine the width of the spaces between trabeculae. Furthermore, structural characteristics, the number of cells (osteoblasts, osteocytes, osteoclasts), and the collagen fiber content of the organic matrix were analyzed by light microscopy. Understanding the changes that occur during early- and late-stage diabetes may help elucidate the possible pathogenic effects on femoral fractures in diabetes patients.

MATERIAL AND METHOD

Experimental animals and diabetes induction. Twenty-eight adult male Sprague-Dawley rats, aged 5-8 weeks and weighing 200-270 grams, were used. All the rats were obtained from the National Laboratory Animal Center, Mahidol University, Thailand, and the experiments were performed in compliance with the Guide for the Care and Use of Laboratory Animals. The experiments were approved by the Siriraj Animal Care and Use Committee, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand, with a certificate of approval number 007/2566. All of the procedures for animal preparation and diabetes induction were performed as described previously (Plaengrit *et al*., 2023; Baimai *et al*., 2024). The animals were randomly assigned to the STZ-induced diabetes (n=16; 7 short-term and 9 long-term DM) or control (n=12; 6 short-term and 6 long-term control) groups. At the end of the experiment (4 weeks for short-term and 24 weeks for long-term DM), the rats were sacrificed under deep anesthesia by halothane inhalation.

Tissue preparation for the bone microarchitectural study and histological analysis. After sacrifice, all the animals were perfused with Bouin's solution to preserve the tissues, as described previously (Plaengrit *et al*., 2023; Baimai *et al*., 2024).Then, whole femur bones were subsequently collected from the rats in each group and imaged by using microCT imager (SkyScan 1173; Bruker Company, Kontich, Belgium) in the cross section at the intertrochanteric crest, which is the most common site of femoral fracture (Wu *et al*., 2019). Moreover, structural parameters were assessed by measuring the BV/TV (%), BMD (g/cm3), Tb.Th (mm), and Tb.Sp (mm) using CT Analyser Version 1.18.9.0. The data are presented as the mean ± standard error of the mean (SEM).

For the histological examination of bone tissues, the femurs were carefully excised into small pieces and decalcified. The samples were processed via the conventional histological method (Plaengrit *et al*., 2023), serially sectioned to a thickness of 6 μm, stained with haematoxylin and eosin (H&E) and Masson's trichrome, imaged and photographed under a light microscope (Axiostar plus, Jena, Germany) connected to a digital camera (Axiocam MRc, Jena, Germany). The numbers of osteoblasts, osteocytes, osteoclasts, and empty lacunae in the cortical bone of the femur were counted in images of H&E-stained sections at 40x magnification (resolution 100 pixels/inch, 5000×6667 pixels). A total of 50 sections from each group were analyzed, including 3 areas per section, and every fifth section was included to avoid counting the same cells in multiple sections. The number of each cell type was reported as the number per 141x188 μm^2 area of the femur. For the collagen density measurements, the tissue sections from all the animal groups were stained with Masson's trichrome. The percentage of collagen staining relative to the total tissue area in 3 areas from each of the 20 tissue sections was subsequently quantified at 40x magnification (resolution 100 pixels/inch, 5000×6667 pixels), and the results were subsequently analysed with ImageJ software (National Institutes of Health and Laboratory for Optical and Computational Instrumentation, University of Wisconsin, Wisconsin, USA).

Statistical analysis. The quantitative data from each group are expressed as the mean ± standard error of the mean (SEM). The analytics software SPSS Statistics version 18.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The BV/TV, BMD, Tb.Th, Tb.Sp, number of cells, number of empty lacunae and collagen contents were analyzed via an independent-samples t-test. A value of $p<0.05$ was considered to indicate a statistically significant difference.

RESULTS

Bone microarchitectures of the intertrochanteric crest in the femur. After microCT reconstruction, 3D images of the cortical and trabecular bones from rats with either short- or long-term STZ-induced diabetes were qualitatively and quantitatively evaluated, and the results were compared with

those of age-matched control rats (Fig. 1). In cross sections of intertrochanteric crests in the femur, cortical bone was clearly observed around the trabecular bone. The thickening of the cortical bones from rats with either short- and especially long-term DM seemed to decrease with increased trabecular space (Figs. 1A-1D). Furthermore, the significant decreases in the BV/TV were observed in both short- and long-term diabetes groups compared with the control groups (Fig. 1E). Compared with that of age-matched control rats, the BMD of short- and long-term DM model rats was significantly lower (Fig. 1F). Moreover, a significant reduction in the Tb.Th was noted in bones from the long-term diabetes group compared with those from the control group (Fig. 1G). In both the short- and long-term diabetes groups, the Tb.Sp in the trabecular bones was greater than that in the age-matched control groups (Fig. 1H).

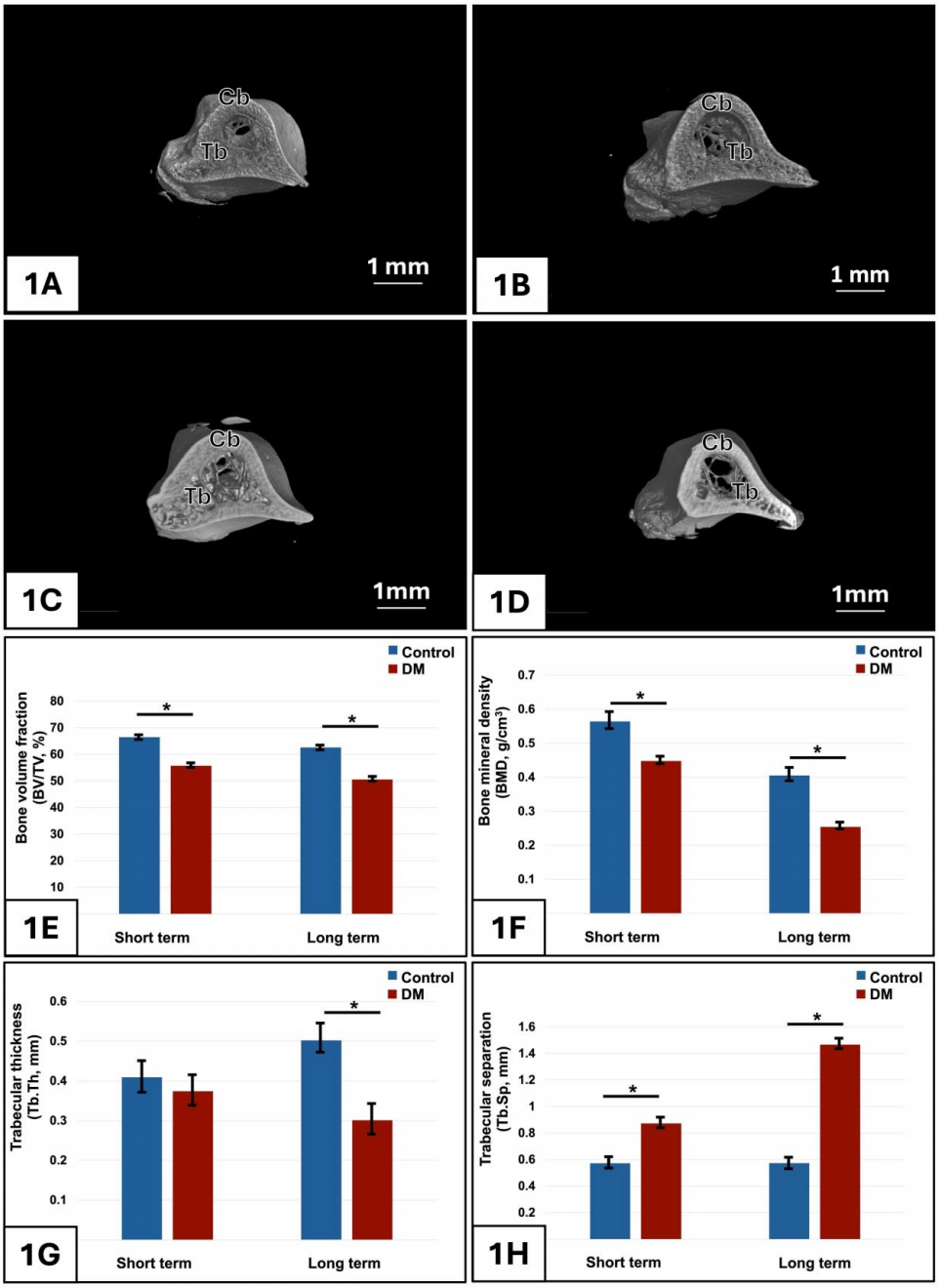

Fig. 1. 3D images of cross sections of the intertrochanteric crests of the femur in the short-term control (1A), short-term DM (1B), long-term control (1C), and long-term DM (1D) groups. Cortical bone (Cb) and trabecular bone (Tb). Comparisons of the BV/TV (1E), BMD (1F), Tb.Th (1G), and Tb.Sp (1H) of the controls and diabetes groups were performed over both short and long periods. *p-value < 0.05.

Histopathological analysis of the intertrochanteric crest in the femur. At higher magnification, normal osteoblasts were cuboidal or polyhedral cells with a single eccentric nucleus on the bone surface (Figs. 2A, 2C). The disappearance of osteoblasts was observed in the short-term diabetes group, and a marked loss of osteoblasts was observed in the long-term DM group (Figs. 2B, 2D). Significant reductions in osteoblast number were observed in both the short-term (2.80±0.28) and long-term (0.35±0.11) diabetes groups, compared with the age-matched control groups (8.75±0.30 for short-term and 6.20±0.21 for long-term, respectively) (Fig. 2E).

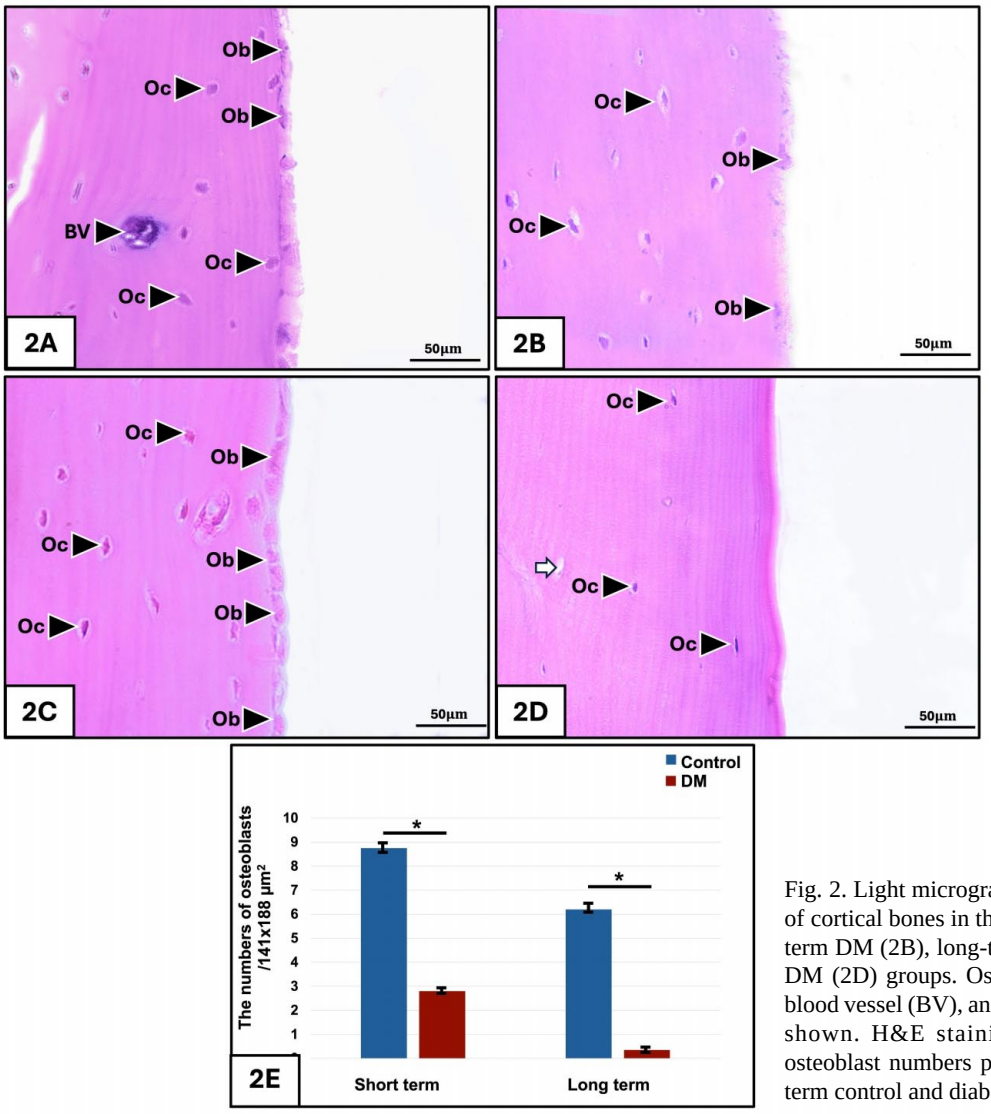

Fig. 2. Light micrographs of osteoblasts at the surface of cortical bones in the short-term control (2A), short-term DM (2B), long-term control (2C), and long-term DM (2D) groups. Osteoblasts (Ob), osteocytes (Oc), blood vessel (BV), and empty lacuna (white arrow) are shown. H&E staining. Quantitative analysis of osteoblast numbers per area (2E) in short- and long-term control and diabetic rats. *p-value < 0.05.

Normally, osteocytes are the most abundant cells in the bone within the lacunae. Osteocytes were visible as almond-shaped cells with dendritic processes that contacted the cell processes of neighbouring osteocytes (Figs. 3A, 3C, and the insets). Under short-term diabetic conditions, the osteocytes became flat, decreased in size, and presented darkly stained nuclei (Fig. 3B); however, the control osteocytes were larger and presented more lightly stained nuclei (Fig. 3A). In animals with long-term diabetes, osteocytes with markedly pyknotic nuclei and a loss of cytoplasmic dendritic processes (inset of Fig. 3D), were found in disorganized osteons and thickened blood vessel walls (Fig. 3D). The number of osteocytes was significantly lower in both the short-term (11.40±0.61) and long-term (1.60±0.18) diabetes groups than in the age-matched control groups (19.70±0.61 for short-term and 17.70±0.53 for long-term) (Fig. 3E). Moreover, empty lacunae without osteocytes were observed, as evidenced by significant increases in the numbers of empty lacunae in both the short-term (2.90±0.34) and long-term (6.15±0.24) DM groups compared with the corresponding control groups (0.45±0.11 and 0.70±0.18, respectively) (Fig. 3F).

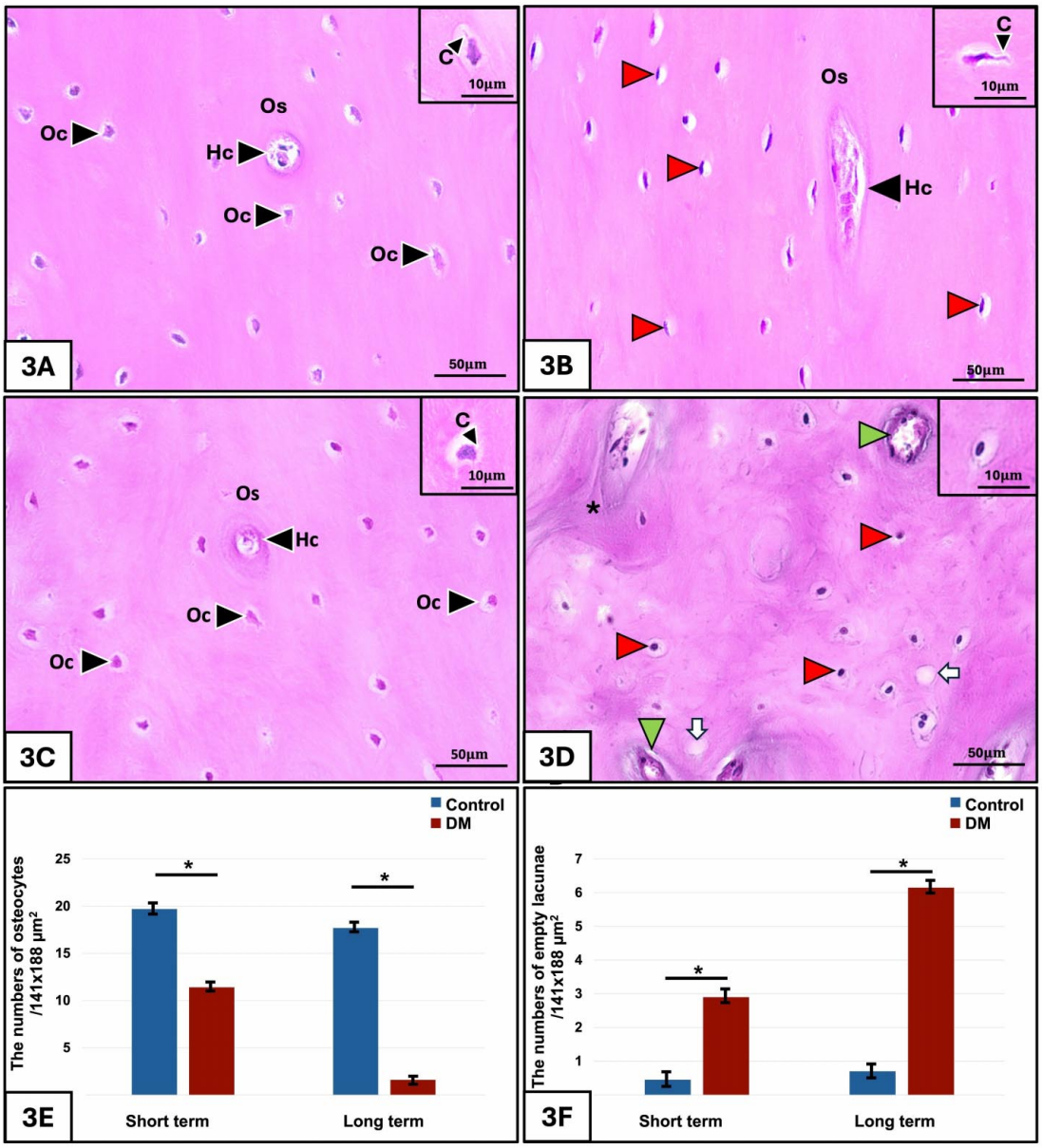

Fig. 3. Light micrographs of osteocytes in the cortical bones of the short-term control (3A), short-term DM (3B), long-term control (3C), and long-term DM (3D) groups. Osteocytes (Oc), Haversian canals (Hc), osteon (Os), osteocyte with pyknotic nucleus (red arrowhead), disorganization of the osteon (black asterisk), empty lacuna (white arrow), and thick wall of blood vessel (green arrowhead) are shown. Inset: a high magnification image of an osteocyte with a cytoplasmic process (c). H&E staining. Comparisons of the numbers of osteocytes per area (3E) and empty lacunae per area (3F) in the short- and long-term control and DM groups. *p-value < 0.05.

Normal osteoclasts or bone-destroying cells are large multinucleated cells that were found adjacent to the bone surface in the red bone marrow (Figs. 4A, 4C). In the short-term diabetes group, the histologic features of osteoclasts seemed to be the same as those in the control group (Figs. 4A-4B). Moreover, increases in the size and number of these cells, which are distinctive hypernucleated giant cells, were clearly observed in the long-term diabetes group. In addition, white adipocytes were found among haematopoietic stem cells from the long-term diabetes group compared with those from the age-matched control group (Figs. 4C-4D). After the number of osteoclasts was determined, significant increases in the number of cells were observed in the short-term DM group (2.1±0.18) compared with the corresponding control group (0.65±0.15), as well as in the long-term DM group (4.7±0.28) compared with the corresponding control group (0.9±0.31) (Fig. 4E).

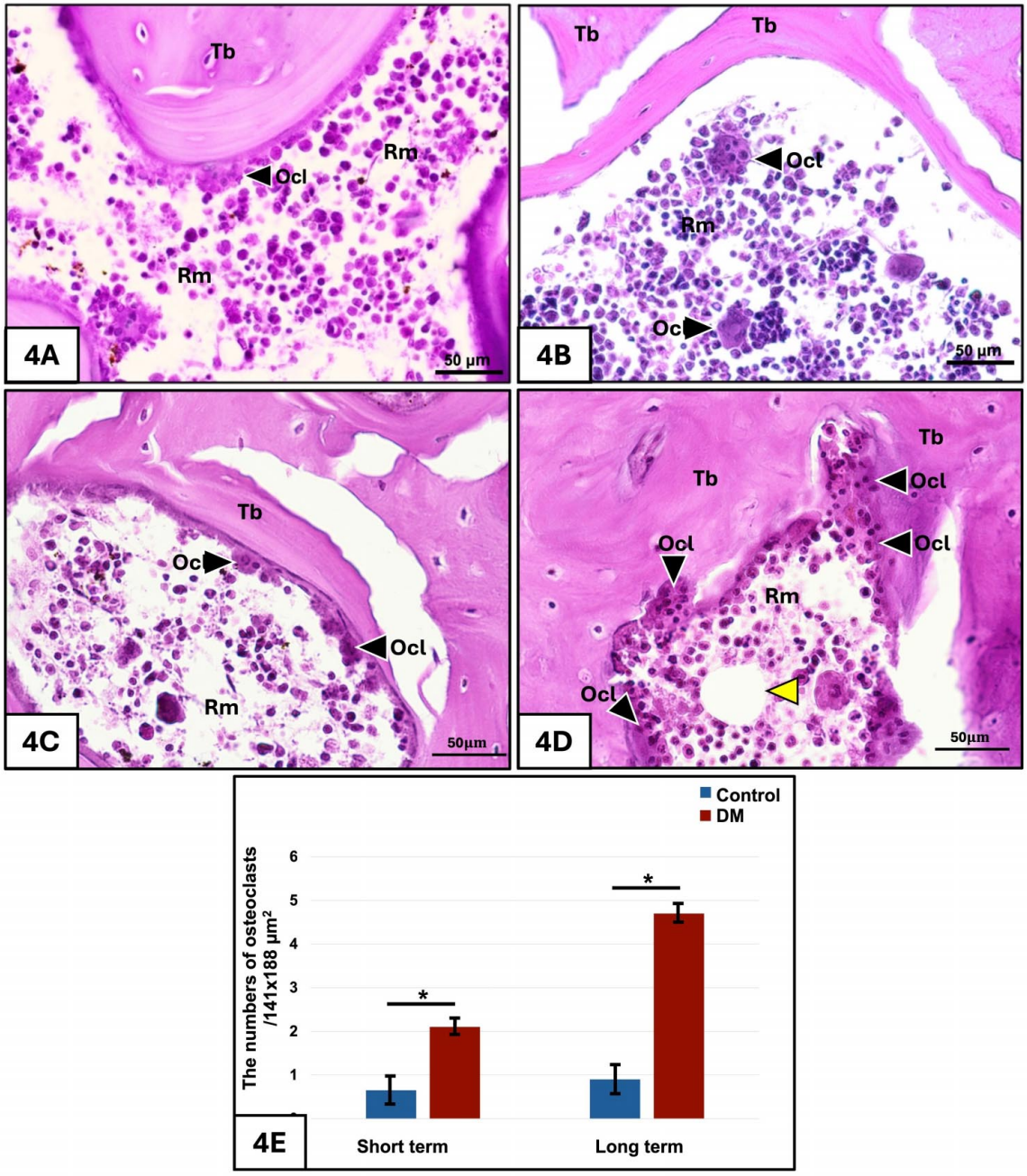

Fig. 4. Light micrographs of osteoclasts in the bone marrow of cortical bones in the short-term control (4A), short-term DM (4B), long-term control (4C), and long-term DM (4D) groups. Osteoclasts (Ocl), trabeculae (Tb), red bone marrow (Rm), and white adipocytes (yellow arrowhead) are shown. H&E staining. Comparisons of the numbers of osteoclasts per area (4E) in the short- and long-term control and diabetic groups. *p-value < 0.05.

The other major organic component of the bone matrix is collagen fibres, which are normally tightly packaged and arranged parallel to each other surrounding the Haversian canals and forming osteons in compact bone (Figs. 5A, 5C). In both the short- and long-term diabetes groups, loosely packed collagen with more small spaces in the bone matrix, indicating the disorganization of collagen fibres, was observed (Figs. 5B, 5D). According to the results of the quantitative analysis of collagen density, there was a statistically significant reduction in both the short- and long-term diabetes groups compared with the age-matched control groups (Fig. 5E).

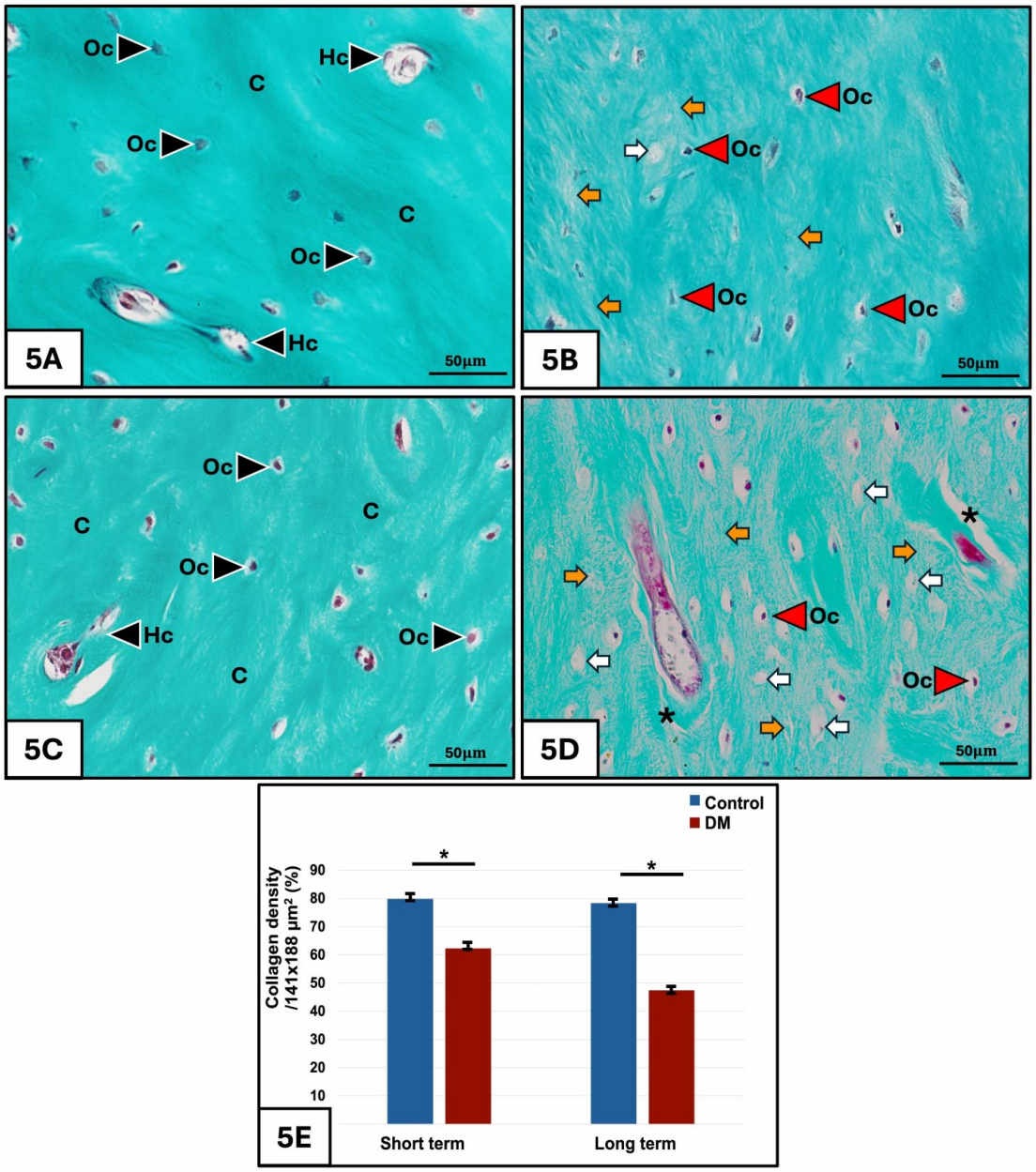

Fig. 5. Light micrographs of collagen staining with Masson's trichome of cortical bones in the short-term control (5A), short-term DM (5B), long-term control (5C), and long-term DM (5D) groups. Collagen fibres (C), osteocytes (Oc), Haversian canals (Hc), collagen fragmentation (orange arrow), osteocyte with pyknotic nucleus (red arrowhead), empty lacuna (white arrow), and large spaces between collagen fibres (black asterisk) are shown. Quantitative analysis of collagen density per area (5E) in the short- and long-term controls and DM groups. *p-value < 0.05.

DISCUSSION

In this study, there were decreased osteoblast numbers in the early and late stages of diabetes, indicating reduced bone formation. Typically, new bone is created by osteoblasts, which are derived from osteoprogenitor cells via the wingless/integrated (Wnt)/b-catenin signalling pathway. In diabetes, excess reactive oxygen species (ROS) and proinflammatory cytokines disrupt signalling pathways in osteoprogenitors and osteoblasts to decrease osteoblastogenesis (Fowlkes *et al.*, 2011). In addition, hyperglycaemia induces osteoblasts to undergo programmed

cell death via pyroptosis (Li *et al*., 2023). Overall, osteoblastogenesis and osteogenesis were reduced, leading to a decrease in the number of osteoblasts, which caused decreases in the number of osteocyte and the degree of collagen production. In this study, a significantly decreased number of osteocytes with shrunken and dark basophilic nuclei but a significantly increased number of empty lacunae, were observed in both the short- and long-term diabetes groups. Under hyperglycaemic conditions, osteocytes, osteoblasts, and osteoclasts produce ROS to promote the release of proinflammatory cytokines, such as tumour necrosis factor alpha, that bind to protein on the cell membrane of osteocytes. This leads to the formation of apoptosomes in the cytoplasm of osteocytes to activate chromosomal DNA fragmentation and cytoskeletal degradation. Thus, osteocytes shrink and develop dark and basophilic nuclei, ultimately leading to osteocyte apoptosis (Burgos-Morón *et al*., 2019). Moreover, thickened capillary walls were observed in the bone matrix of diabetic rats. Under diabetic conditions, bones exhibit microvascular disorders, such as a thick capillary wall, which leads to decreased blood circulation to the bone. As a result, hypoxia occurs in the bone (Draghici *et al*., 2023), which was suggested to cause severe osteocyte apoptosis. Therefore, the number of osteocytes decreased, but the number of empty lacunae increased in the bones of the diabetic groups.

In terms of bone resorption, the numbers of osteoclasts increased, and collagen exhibited disorganization and decreased levels in both the short- and long-term diabetes groups. Under diabetic conditions, the presence of apoptotic osteocytes, insulin deficiency and ROS-induced proinflammatory cytokines leads to direct enhancement of osteoclastogenesis. Moreover, these proinflammatory cytokines stimulate monocytes and macrophages to differentiate into osteoclasts. As a result, the number of osteoclasts increased in both DM groups. Moreover, the activation of osteoclasts to increase the levels of hydrochloric acid and proteases resulted in increased bone resorption and degradation of collagen fibres in the DM groups (Boyce *et al*., 2009; Kalaitzoglou *et al*., 2016). In the late stage of diabetes, the white adipocyte number in the red bone marrow increased. Evidence shows that ROS due to hyperglycaemia induce an imbalance between the Wnt/b-catenin pathway and the peroxisome proliferator-activated receptor pathway in bone marrow stem cells, decrease osteoblast differentiation, and increase adipocyte differentiation (Tencerova *et al*., 2019). Moreover, hyperglycaemia activated the rat sarcoma protein/mitogen-activated protein kinase/ extracellular signal regulated protein kinase and c-Jun N-terminal kinase pathways, leading to adipocyte cell cycle progression (Lee *et al*., 2019; Rona *et al*., 2024). Finally, an increase in red bone marrow adipocyte proliferation occurred. Notably, white adipocytes also secrete proinflammatory cytokines and receptor activator of nuclear factor-kB ligand, promoting osteoclastogenesis and bone resorption (Li *et al*., 2019).

CONCLUSION

The intertrochanteric crests of the femur, the most common site of hip fracture, in both short- and long-term diabetic rats were examined. In the bone formation (osteoblastogenesis), the loss of osteoblasts caused decreases in the collagen density and number of osteocytes. Additionally, osteocytes underwent programmed cell death, as empty lacunae were observed. Furthermore, in the bone resorption (osteoclastogenesis), the number of osteoclasts, which degrade collagen fibres, was increased. Overall, diabetes leads to decreased bone formation but increased bone resorption. As a result, BV/TV, BMD, and Tb.Th were reduced within cortical bone, whereas Tb.Sp was increased. The structures and compositions of diabetic bone were altered, which led to thin bone and fragility. Thus, early examination of the bone strength of diabetic patients is suggested to prevent hip fracture.

ACKNOWLEDGEMENTS. This research was supported by Siriraj Graduate Scholarships and the Chalermphrakiat Grant, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand.

PIANRUMLUK, S.; LANLUA, P.; NIYOMCHAN, A.; PLAENGRIT, K.; BAIMAI, S. & SRICHAROENVEJ, S. Alteraciones estructurales y de componentes en las crestas intertrocantéreas del fémur en la diabetes inducida a corto y largo plazo. *Int. J. Morphol., 43(6)*:1934-1942, 2025.

RESUMEN: Las personas con diabetes mellitus tipo 1 presentan un mayor riesgo de fractura femoral proximal. Además, en las personas con diabetes se observa baja densidad mineral ósea (DMO), alteraciones en los niveles séricos de biomarcadores del recambio óseo y alteraciones en los parámetros morfométricos óseos, que afectan la resistencia ósea. Por lo tanto, este estudio tuvo como objetivo comparar los componentes de la cresta intertrocantérea del fémur en términos de morfología, fracción de volumen óseo (BV/TV), DMO, espesor trabecular (Tb.Th) y separación (Tb.Sp) mediante tomografía microcomputarizada, histomorfología, determinación del número de células y cuantificación de lagunas vacías y fibras de colágeno mediante microscopía óptica en ratas con diabetes inducida por estreptozotocina a corto y largo plazo. Durante las etapas temprana y tardía de la diabetes, se observaron reducciones en los valores de BV/TV femoral, DMO y Tb.Th y un aumento en el valor de Tb.Sp; además, el número de osteoblastos y osteocitos disminuyó, pero el número de lagunas vacías y osteoclastos aumentó. Además, se observaron fibras de colágeno desorganizadas con menor densidad en los dos grupos de diabetes. La gravedad de estos cambios fue más pronunciada en el modelo de diabetes a largo plazo, junto con

el engrosamiento de los vasos sanguíneos y el aumento del tejido adiposo en la médula ósea. Todos estos cambios conducen a una disminución de la calidad y la fragilidad ósea, lo que podría estar relacionado con un mayor riesgo de fracturas femorales en los grupos con diabetes. Por lo tanto, el conocimiento actual sobre el daño óseo que se produce durante el desarrollo de la diabetes podría justificar una cuidadosa consideración de la resistencia ósea durante las primeras etapas de la enfermedad.

PALABRAS CLAVE: Diabetes mellitus; Fémur; Células óseas; Tomografía microcomputarizada.

REFERENCES

Asagiri, M. & Takayanagi, H. The molecular understanding of osteoclast differentiation. *Bone, 40(2)*:251-64, 2007.

Aubin, J. E. Regulation of osteoblast formation and function. *Rev. Endocr. Metab. Disord., 2(1)*:81-94, 2001.

Baimai, S.; Sricharoenvej, S.; Lanlua, P. & Choompoo, N. Histopathology and ultrastructural alterations in gastric mucus-secreting cells in diabetic model rats. *Eur. J. Anat., 28(1)*:53-62, 2024.

Boyce, B. F.; Yao, Z. & Xing, L. Osteoclasts have multiple roles in bone in addition to bone resorption. *Crit. Rev. Eukaryot. Gene Expr., 19(3)*:171-80, 2009.

Burgos-Morón, E.; Abad-Jiménez, Z.; de Marañón, A. M.; Iannantuoni, F.; Escribano-López, I. & López-Domènech, S. Relationship between oxidative stress, ER stress, and inflammation in type 2 diabetes: the battle continues. *J. Clin. Med., 8(9)*:1385, 2019.

Draghici, A. E.; Zahedi, B.; Taylor, J. A.; Bouxsein, M. L. & Yu, E. W. Vascular deficits contributing to skeletal fragility in type 1 diabetes. *Front. Clin. Diabetes Healthc., 4*:1272804, 2023.

Florencio-Silva, R.; Rodrigues da Silva Sasso, G.; Sasso-Cerri, E.; Jesus Simões, M. & Cerri, P. S. Biology of bone tissue: structure, function, and factors that influence bone cells. *Biomed. Res. Int., 2015*:421746, 2015.

Fowlkes, J. L.; Bunn, R. C. & Thrailkill, K. M. Contributions of the insulin/insulin-like growth factor-1 axis to diabetic osteopathy. *J. Diabetes Metab., 1(3)*:1-14, 2011.

Hie, M.; Shimono, M.; Fujii, K. & Tsukamoto, I. Increased cathepsin K and tartrate-resistant acid phosphatase expression in bone of streptozotocin-induced diabetic rats. *Bone, 41(6)*:1045-50, 2007.

Jackuliak, P. & Payer, J. Osteoporosis, fractures, and diabetes. *Int. J. Endocrinol., 2014*:820615, 2014.

Kalaitzoglou, E.; Popescu, I.; Bunn, R. C.; Fowlkes, J. L. & Thrailkill, K. M. Effects of type 1 diabetes on osteoblasts, osteocytes, and osteoclasts. *Curr. Osteoporos. Rep., 14(6)*:310-9, 2016.

Lee, M. W.; Lee, M. & Oh, K. J. Adipose tissue-derived signatures for obesity and type 2 diabetes: adipokines, batokines and microRNAs. *J. Clin. Med., 8(6)*:854, 2019.

Li, Y.; Meng, Y. & Yu, X. The unique metabolic characteristics of bone marrow adipose tissue. *Front. Endocrinol. (Lausanne), 10*:69, 2019.

Li, Z.; Li, D.; Chen, R.; Gao, S.; Xu, Z. & Li, N. Cell death regulation: a new way for natural products to treat osteoporosis. *Pharmacol. Res., 187*:106635, 2023.

Montagnani, A.; Gonnelli, S.; Alessandri, M. & Nuti, R. Osteoporosis and risk of fracture in patients with diabetes: an update. *Aging Clin. Exp. Res., 23(2)*:84-90, 2011.

Plaengrit, K.; Lanlua, P.; Niyomchan, A. & Sricharoenvej, S. Uncontrolled diabetes with underlying structural changes in the atrioventricular node. *Int. J. Morphol., 41(4)*:1027-35, 2023.

Rona, G.; Miwatani-Minter, B.; Zhang, Q.; Goldberg, H. V.; Kerzhnerman, M. A.; Howard, J. B.; Simoneschi, D.; Lane, E.; Hobbs, J. W.; Sassani, E.; *et al*. D-type cyclins regulate DNA mismatch repair in the G1 and S phases of the cell cycle, maintaining genome stability. *bioRxiv, 84(7)*:1224-42, 2024.

Starup-Linde, J. Diabetes, biochemical markers of bone turnover, diabetes control, and bone. *Front. Endocrinol. (Lausanne), 4*:21, 2013.

Tencerova, M.; Okla, M. & Kassem, M. Insulin signaling in bone marrow adipocytes. *Curr. Osteoporos. Rep., 17(6)*:446-54, 2019.

Tsentidis, C.; Gourgiotis, D.; Kossiva, L.; Doulgeraki, A.; Marmarinos, A.; Galli-Tsinopoulou, A. Karavanaki, K. Higher levels of s-RANKL and osteoprotegerin in children and adolescents with type 1 diabetes mellitus may indicate increased osteoclast signaling and predisposition to lower bone mass: a multivariate cross-sectional analysis. *Osteoporos. Int., 27(4)*:1631-43, 2016.

Wongdee, K. & Charoenphandhu, N. Osteoporosis in diabetes mellitus: possible cellular and molecular mechanisms. *World J. Diabetes, 2(3)*:41-8, 2011.

Wu, S. C.; Rau, C. S.; Kuo, S. C. H.; Chien, P. C. & Hsieh, C. H. The influence of ageing on the incidence and site of trauma femoral fractures: a cross-sectional analysis. *BMCMusculoskelet. Disord., 20(1)*:413, 2019.

Corresponding author:
Passara Lanlua, Ph.D.
Department of Anatomy
Faculty of Medicine Siriraj Hospital
Mahidol University
2 Wanglang road
Bangkoknoi
Bangkok 10700
THAILAND

E-mail: passara.lan@mahidol.ac.th