Accelerated Features of Age-Related Bone Loss in Zmpste24 Metalloproteinase-Deficient Bone Mice

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Age-related bone loss is associated with changes in bone cellularity, which include marrow fat infiltration and decreasing levels of osteoblastogenesis. The mechanisms that explain these changes remain unclear. Although nuclear lamina alterations occur in premature aging syndromes that include changes in body fat and severe osteoporosis, the role of proteins of the nuclear lamina in age-related bone loss remains unknown. Using the Zmpste24-null progeroid mice (Zmpste24⁺⁻), which exhibit nuclear lamina defects and accumulate unprocessed prelamin A, we identified several alterations in bone cellularity in vivo. We found that defective prelamin A processing induced accelerated features of age-related bone loss including lower osteoblast and osteocyte numbers and higher levels of marrow adipogenesis. In summary, processing of prelamin A could become a new approach to regulate osteoblastogenesis and bone turnover and thus for the prevention and treatment of senile osteoporosis.

Key Words: Lamin A/C—Osteoporosis—Mesenchymal stem cells—Osteoblastogenesis—Adipogenesis.
cellular mechanisms that explain this decrease in bone mass remain unknown. In addition, the elucidation of the potential mechanisms that explain this bone loss in a model of progeria mice could provide new potential anabolic therapeutic targets for senile osteoporosis.

Our results demonstrate that Zmpste24−/− mice are osteopenic with low bone turnover and concomitant levels of marrow fat, which are the hallmarks of age-related bone loss. Taken together, these data confirm previous in vitro data on the role of proteins of the nuclear envelope in MSC differentiation and opens the path for the development of anabolic drugs that maintain the bone mass through the regulation of prelamin A processing in MSC.

METHODS

Animals
Zmpste24−/− mice were kindly provided by Dr. Carlos Lopez-Otin from University of Oviedo (9). A colony of Zmpste24−/− mice and their corresponding wild-type (WT+/-) controls was developed at the Lady Davis Institute for Medical Research (McGill University). Genotype was verified by real-time polymerase chain reaction (RT-PCR). Male and female mice were housed in cages in a limited access room. Animal husbandry adhered to Canadian Council on Animal Care Standards. All protocols were approved by the McGill University Health Center Animal Care Utilization Committee. Mice (male and female) were killed at 3 months of age. Both side tibiae and femora were obtained for further analysis. All experiments and measurements were repeated at least three times.

Quantitative Radiological Imaging
Micro-computed tomography (Micro-CT) was performed, using a modification of previously published methods (20), on the left femur after removal of soft tissues and overnight fixation in 4% paraformaldehyde. A Skyscan 1172 instrument (Skyscan, Antwerp, Belgium) equipped with a 1.3 Mp camera was used to capture two-dimensional (2D) serial cross-sections, which were used to reconstruct three-dimensional images for the quantification of the volume of bone in the distal metaphysis. Bone microarchitecture measurements were assessed using a set of calibrated phantoms purchased from Skyscan.

Histology and Histomorphometry
For histomorphometric analyses, the left femur was fixed overnight in 4% paraformaldehyde, rinsed in three changes of phosphate-buffered saline (PBS), and embedded in polymethyl methacrylate (MMA) or a mixture of 50% MMA and 50% glycol methacrylate (GMA). Serial 4- to 6-μm sections of MMA-embedded tissues were left unstained or stained with von Kossa, whereas 4-μm MMA-GMA sections were stained for alkaline phosphatase (ALP; osteoblasts), toluidine blue (osteocytes), and tartrate resistance acid phosphatase (TRAP; osteoclasts) activity as described previously (20). Images were captured using a Leica DMR microscope (Leica Microsystems, Richmond Hill, Ontario, Canada) equipped with a Retiga 1300 camera (Qimaging, Burnaby, British Columbia, Canada) and the primary histomorphometric data obtained using Bioquant Nova Prime image analysis software (Bioquant Image Analysis Corp., Nashville, TN). Nomenclature and abbreviations conform to those recommended by the American Society for Bone and Mineral Research (21).

For marrow fat analysis, the right femur was cleaned of soft tissue, fixed for 16 hours in 4% paraformaldehyde, rinsed thoroughly in PBS, decalcified in 10% ethylenediaminetetraacetic acid (EDTA), and processed for paraffin embedding. Serial 4-μm sections were cut on a modified Leica RM 2155 rotary microtome (Leica Microsystems). Fat volume (FV) was quantified using von Kossa-stained sections as previously described (22).

Serum Biochemistry and Bone Biomarkers
Serum levels of calcium were determined at the Rodent Diagnostics Lab (McGill University, Montréal, Quebec, Canada) using routine automated techniques. Bone biomarkers, calcitropic, and sex hormones were analyzed at the Centre for Bone and Periodontal Research using commercial assays for serum C-telopeptides (C-Tx; Nordic Bioscience, Herlev, Denmark), 17β-estradiol and testosterone (IBL Immuno-Biological, Hamburg, Germany), osteocalcin (OCN; ImmunoDiagnostics Systems Ltd, Fountain Hills, AZ), parathyroid hormone (PTH; Immunotopics Inc., San Clemente, CA), and 25(OH)-vitamin D (ImmunoDiagnostics Systems Ltd).

Ex vivo Cultures of Bone Marrow Cells
To establish adherent bone marrow cultures, bone marrow cells were obtained and induced to differentiate into osteoblast as previously described (23). Briefly, both tibiae from 3-month-old Zmpste24−/− and WT+/- (n = 10 per group) were flushed using a 21-gauge needle attached to a 10-mL syringe filled with Dulbecco’s modified Eagle’s Medium (DMEM; GIBCO BRL, Gaithersburg, MD). Cells from both tibiae were filtered through a cell strainer with 70-μm nylon mesh (BD Bioscience, Bedford, MA) and then combined to produce a volume of 2 mL containing approximately 10^7 cells/mL. Six-well plate cultures were then established in triplicate, with each well containing a 100-μL aliquot of cell suspension combined with 4 mL of fresh minimal essential medium. The cells were incubated in MSC growth media at 37°C with 5% humidified CO2 and isolated by their adherence to tissue culture plastic. Medium was aspirated and replaced with fresh medium to remove nonadherent cells every 2–3 days. The adherent MSC were grown to approximately 80% confluence for about 7 days defined as MSC at passage 0, harvested with 0.25% trypsin and 1 mM EDTA for 5 minutes at 37°C,
diluted 1:3 in MSC growth media, plated, and grown to confluence for further expansion. After second and third passages, MSC were used for subsequent experiments.

To induce differentiation, a total of $10^4$ cells were diluted in osteogenic medium (prepared with DMEM, 10% fetal calf serum [FCS], 0.2 mM dexamethasone, 10 mmol/L β-glycerol phosphate, and 50 μg/mL ascorbic acid) and plated in six-well plates. Media was aspirated and replaced with fresh osteogenic medium every 3 days. After 21 days in culture, cells were washed with PBS, ethanol fixed, stained for ALP, and counterstained with hematoxylin (Sigma, Oakville, Ontario, Canada). The colonies with more than 10% of cells staining positive for ALP were considered as colony-forming units–osteoblasts (CFU-OB).

For adipocyte differentiation, cells were obtained and plated as previously described. After passage 3, $10^4$ MSC were diluted in adipogenic medium (prepared with DMEM, 4.5 g/L glucose, 1 μM dexamethasone, 0.2 mM indomethacin, 1.7 μM insulin, 0.5 mM 3-isobutyl-1-methylxanthine, 10% FCS, 0.05 U/mL penicillin, and 0.05 μg/mL streptomycin) and plated in six-well plates for 3 days. To promote adipogenic phenotype, cells were then incubated for 3 days in adipogenesis maintenance medium (DMEM, 4.5 g/L glucose, 1.7 μM insulin, 10% fetal bovine serum, 0.05 U/mL penicillin, and 0.05 μg/mL streptomycin) and then switched to induction media again. After 3 cycles of induction or maintenance (Day 18), media was aspirated and cells were fixed and adipocytes visualized by staining with Oil Red O and counterstaining with hematoxylin. Colonies with more than 10% of cells staining positive for Oil Red O were considered as CFU–adipocytes (CFU-AD).

**Semi-Quantitative RT-PCR**

Bone marrow cells were flushed and isolated as previously described. Total RNA was isolated from MSC using Trizol reagent (Invitrogen, Carlsbad, CA). Total RNA (1 μg) was reverse transcribed with either random hexamers or oligo-dT using Qiagen One Step RT-PCR Enzyme Mix (Qiagen, Valencia, CA). The resulting complementary DNA was amplified by 35 PCR cycles with an annealing temperature of 58°C. Primers for specific detection of messenger RNA (mRNA) expression included the following: Zmpste24 (F: 5′-AGTCCGATCAGAGTTGGTG-3′ and R: 5′-GGGGAAGGGAGTGAGCTGAA-3′), runt-related transcription factor 2 (Runx2; F: 5′-ACAGGAAAGTGGG-3′ and R: 5′-GGGACTAGTCACTGAAAC-3′), osteopontin (OPN;F: 5′-AGGTCCTCATCGAGCTGAC-3′ and R: 5′-TGACCCAGATCCTATAGCC-3′); bone sialoprotein II (BSPII; F: 5′-GGTCCCTTGACCTGCTC-3′ and R: 5′-GGGAGGCGTACTTCCTCAG-3′); OCN (F: 5′-ACTTGCGAGGGCAAGAGAG-3′ and R: 5′-CTGACCTCAGATGCGCA-3′); CCAAT enhancer-binding protein-α (C/EBPα; F: 5′-AGGTTTGGCCAAGAATCAGA-3′ and R: 5′-GGGTGAGTATGGAGATG-3′), and peroxisome proliferator-activated receptor gamma 2 (PPARγ2; F: 5′-CCAAACAGCTTGCTTCTCG-3′ and R: 5′-CGAGTCTGTGGGATGAAAC-3′). Amplification of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as control. The expected sizes of the amplification products were between 400 and 800 bp. Amplified products were analyzed by 2% agarose gel electrophoresis. The signals were quantified by densitometry (Bio-Rad laboratories, Hercules, CA) and normalized according to GAPDH density.

**Western Blot Analysis**

For Western blot analysis, 20 μg of total protein were loaded on polyacrylamide gels and separated by standard sodium dodecyl sulfate–polyacrylamide gel electrophoresis. To control for differences in gel migration, exposure time, antibody incubation, etc., samples were run on the same gels and transferred to the same polyvinylidene fluoride membranes (Amersham, Little Chalfont, UK). Blots were blocked overnight in 2.5% nonfat dried milk and probed with antibodies directed against Zmpste24, Runx2, OPN, BSPII, OCN, C/EBPα, and PPARγ2 (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA). Positive controls were included in all experiments as provided by the manufacturer (Santa Cruz Biotechnology), to confirm antibody specificity. Secondary antibodies conjugated to horseradish peroxidase were from Sigma (1:5000). Antigen–antibody complexes were detected by chemiluminescence using a kit of reagents from ECL (Amersham), and blots were exposed to high-performance chemiluminescence film (Amersham). Films were scanned and the optical density of each specific complex was detected by chemiluminescence using a kit of reagents from ECL (Amersham), and blots were exposed to high-performance chemiluminescence film (Amersham). Results are expressed as OD/mm²/100 μg of total protein. Relative intensity of the samples was determined comparing the protein of interest in the mutant mice using the values of WT as controls (100%). Values are reported as the average of samples obtained from 8 to 10 mice.

**Statistical Analysis**

Results are expressed as means ± SD and differences between groups of mice were determined by the Student’s t test and the Mann–Whitney test. A p value of ≤0.05 was considered statistically significant.

**Results**

**Accumulation of Unprocessed Prelamin A Affects Bone Mass and Reduces Bone Quality**

Using a model of Zmpste24-null progeroid mice (Zmpste24+/−) (8), we analyzed whether accumulation of unprocessed prelamin A has an effect on bone mass and microarchitecture and whether gender differences are found. Figure 1 shows a qualitative (A–E) and quantitative (F–H) decline in bone mass and architecture in both male and
female Zmpste24−/− mice compared with WT controls. Three-dimensional reconstruction (A–D) and 2D sagittal histological sections (E) of the distal femur showed a significant loss in trabecular and cortical bone between the Zmpste24−/− mice compared with the WT +/+ controls. At 3 months of age, histological and micro-CT measurements of distal femur in Zmpste24−/− mice revealed a significant decrease in bone volume/tissue volume (BV/TV; F), trabecular thickness (Tb.Th; G), and trabecular number (Tb.N.; H) compared with their WT +/+ littermates (p < .001).

Histochemical and Biochemical Analyses of Cellular Activity

To determine the rate of bone turnover and changes in bone cell numbers in the different groups of mice, Figure 2 shows sections of undecalcified bone stained with ALP to identify osteoblasts (purple, A), toluidine blue to visualize trabecular osteocytes (blue, D), and TRAP to identify osteoclasts (red, F). Quantification of cell numbers after normalization with bone surface (B, E, and G) revealed a significant reduction in osteoblast and osteocytes numbers (B and E) in both male and female Zmpste24−/− mice compared with their WT +/+ littermates (p < .001). A similar reduction in osteoclast numbers (G) was noted in both male and female Zmpste24−/− mice compared with their WT +/+ littermates (p < .001).

Furthermore, to determine whether the reduction in cell numbers seen in Zmpste24−/− mice correlated with circulating levels of bone biomarkers similar to those used in the clinical setting, serum levels of OCN (bone formation) and C-Tx (bone resorption) were measured. Serum OCN (Figure 2C) was significantly higher at 3 months of age in WT +/+ control mice compared with Zmpste24−/− mice.

Figure 1. Changes in bone architecture in male and female Zmpste24−/− mice. (A–D) Micro-computed tomography analysis of the distal femur of 3-month-old Zmpste24−/− mice and wild-type (WT +/+ ) littermates. Representative two-dimensional reconstructions, obtained with a 0.9 degree rotation between frames on a Skyscan 1072 instrument, are shown for male and female Zmpste24−/− (B and D) and WT +/+ (A and C) mice. The right upper panels are representative of the area just below the growth plate, whereas the right lower panels show the cross-sectional (cortical) bone structure. Zmpste24−/− mice exhibited profound thinning of cortical bone, a reduction in plate-like structures, and a lack of trabecular connectivity. These changes correlated with von Kossa staining (E). Quantitation of bone parameters (F–H) further exemplified a decrease in bone quality (volume/trabecular volume [BV/TV]) (F), trabecular thickness (Tb.Th, (G), and number (Tb.N.; H) in the mutant femora compared with the WT +/+ littermate controls. Results are expressed as the mean ± SD of eight independent analyses per group. Significantly different from control; *p < .001.
ACCELERATED BONE LOSS IN PROGERIA MICE

1019

(p < .01), correlating with lower levels of osteoblast numbers found in the histochemical analysis. In addition, Figure 2H shows a significant decrease in serum C-Tx in Zmpste24−/− mice compared with WT+/+ mice. In addition, the number of Ost was significantly reduced in Zmpste24−/− mice compared with WT+/+ mice (E). Scale bar represents 50 μm for A and F and 25 μm for D. Micrographs are representative of those from five different mice of each genotype. *p < .01 and **p < .001. (C and H) Changes in bone cellularity correlated with changes in serum biochemical markers of bone formation (C, OCN: osteocalcin) and bone resorption (H, C-Tx: C-telopeptides) in Zmpste24−/− mice compared with WT+/+ mice. *p < .01

Figure 2. Changes in bone cellularity of 3-month-old Zmpste24−/− mice. Sections of plastic embedded tibiae from Zmpste24−/− and wild-type (WT+/+) were stained sequentially for alkaline phosphatase (ALP) (osteoblasts, OB) (A, arrows, magnification x20), toluidine blue (osteocytes, Ost) (B, arrows, magnification x40), and tartrate resistance acid phosphatase (TRAP) (osteoclasts, OC) (F, arrows, magnification x20). A significant decrease in the number of ALP expressing OB (B) and OC showing TRAP enzyme activity (E) was seen in Zmpste24−/− mice compared with WT+/+ mice. In addition, the number of Ost was significantly reduced in Zmpste24−/− mice compared with WT+/+ mice (E). Scale bar represents 50 μm for A and F and 25 μm for D. Micrographs are representative of those from five different mice of each genotype. *p < .01 and **p < .001. (C and H) Changes in bone cellularity correlated with changes in serum biochemical markers of bone formation (C, OCN: osteocalcin) and bone resorption (H, C-Tx: C-telopeptides) in Zmpste24−/− mice compared with WT+/+ mice. *p < .01

(p < .01), correlating with lower levels of osteoblast numbers found in the histochemical analysis. In addition, Figure 2H shows a significant decrease in serum C-Tx in Zmpste24−/− mice compared with their WT+/+ controls (p < .01), also correlating with the lower levels of osteoclast numbers found in the histochemical analysis.

Circulating Concentrations of Calciotropic and Sex Steroid Hormones in Zmpste24−/− Mice

To determine whether the changes in bone cellularity and microarchitecture were due to alterations in serum concentrations of calciotropic and sex steroids, we measured serum levels of PTH, 25-dihydroxy(OH)-vitamin D, calcium, estradiol, and testosterone in both male and female Zmpste24−/− and compared them with their WT+/+ controls. As shown in Table 1, there were no differences in serum levels of PTH, 25(OH)-vitamin D, or calcium between Zmpste24−/− and WT+/+ controls. As expected, levels of estradiol were higher in female than in male mice with no differences found between both groups. Similar results were found in testosterone levels where male have higher levels of testosterone without differences found between Zmpste24−/− and their WT+/+ controls.

Table 1. Circulating Concentrations of Calcium, Calciotropic (PTH and 25(OH)-vitamin D) and Sex Steroid Hormones in Zmpste24−/− Mice Versus WT+/+ Controls

<table>
<thead>
<tr>
<th>Assay</th>
<th>Male</th>
<th>Female</th>
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<tbody>
<tr>
<td></td>
<td>WT+/+</td>
<td>Zmpste24−/−</td>
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<tr>
<td>Ca^2+ (mmol/L)</td>
<td>2 ± 0.07</td>
<td>2.2 ± 0.08</td>
</tr>
<tr>
<td>25(OH)-vitamin D (nmol/L)</td>
<td>88 ± 13</td>
<td>107 ± 13</td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>56 ± 22</td>
<td>56 ± 13</td>
</tr>
<tr>
<td>Testosterone (ng/mL)</td>
<td>4.8 ± 0.2</td>
<td>5.2 ± 0.5</td>
</tr>
<tr>
<td>Estradiol (pg/mL)</td>
<td>2.6 ± 0.4</td>
<td>1.8 ± 0.4</td>
</tr>
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Note: NS = non significant; PTH = parathyroid hormone; WT+/+ = wild-type.
Accumulation of Unprocessed Prelamin A Affects Runx2 Expression Both at the Protein and at the mRNA Level

Runx2 is the most important transcription factor for osteoblastogenesis (24). Our previous in vitro studies have demonstrated that lack of lamin A/C affects Runx2 activity without affecting its protein expression (18), suggesting that proteins of the nuclear envelope play a role in the osteogenic transcription pathways. To assess whether accumulation of unprocessed prelamin A has an effect on Runx2 activity in vivo, we identified levels of Runx2 mRNA and protein expression in bone marrow of Zmpste24−/− mice.

Initially, the expression of Zmpste24 protein and mRNA was determined by Western blot (Figure 3A and C) and RT-PCR (Figure 3B and D), respectively. As expected, expression of Zmpste24 protein and mRNA were abolished in Zmpste24−/− mice compared with their wild-type (WT++) controls. **p < .001. (E-H) Low levels of Zmpste24 were associated with lower levels of expression of Runx2 at both protein (E and G) and mRNA (F and H) levels. For Western blot analysis, data from scanning densitometric analyses (C and G) are expressed as the ratio of the protein of interest in the mutant mice using the values of WT as controls (100%) representing the mean ± SD of triplicate determinations. **p < .001. For real-time polymerase chain reaction, data from scanning densitometric analyses (D and H) are expressed as the ratio of the gene of interest in the mutant mice using the values of WT as controls (100%) representing the mean ± SD of triplicate determinations. **p < .001. GAPDH, glyceraldehydes-3-phosphate dehydrogenase.
H; p < .001). This reduction was similar both in male and in female Zmpste24−/− mice.

Osteoblast Differentiation of MSC Is Reduced in Zmpste24 Progeria Mice Through Downregulation of Osteoblastogenesis Transcription Factors

Bone marrow progenitors were plated and then induced to differentiate into osteoblasts. After 3 weeks of treatment, the number of CFU-OB derived from Zmpste24−/− was significantly lower in both male and female mice compared with the ones derived from WT+/+ mice (Figure 4A and B).

From a mechanistic approach, we determined whether this marked reduction in osteoblast differentiation was associated with changes in the expression of osteoblastogenesis transcription factors (24), both at mRNA (Figure 4C and D) and at protein (Figure 4E) levels. We found a significant reduction in the expression levels of mRNA and protein for OCN and BSPII in both male and female Zmpste24−/− mice compared with their WT+/+ controls (p < .001). In contrast, although levels of expression of OPN...
were significantly reduced both at protein and at mRNA levels in male Zmpste24−/− mice, no differences in expression were found between female Zmpste24−/− mice and their WT +/+ controls (Figure 4C and D).

Changes in Bone Marrow Fat and Ex vivo Adipogenesis in Zmpste24−/− Mice

Examination of bone marrow using von Kossa staining of undecalcified bone (Figure 5A and B) revealed a significant increase in bone marrow fat (FV/TV) in both male and female Zmpste24−/− mice compared with their WT +/+ littermates (p < .01). Zmpste24−/− female mice showed a significantly higher FV/TV than male mice (p < .01). In addition, the number of CFU-AD derived from Zmpste24−/− was significantly higher in both male and female Zmpste24−/− mice compared with their WT +/+ controls (p < .01).

Furthermore, to assess whether this increase in bone marrow fat and adipogenesis potential was associated with higher expression of transcription factors for adipogenesis, we quantified changes in mRNA expression of C/EBPα and PPARγ2, two factors required in adipocyte differentiation. As shown in Figure 5C and D, we found a significant higher expression in C/EBPα and PPARγ in both male and female Zmpste24−/− mice compared with their WT +/+ littermates (p < .001).

Discussion

The present study demonstrates that processing of prelamin A is required in osteoblast differentiation of MSC in vivo. Additionally, accumulation of unprocessed prelamin A in the absence of Zmpste24 activity facilitated the differentiation shift of MSC from osteoblastogenesis to adipogenesis, which is one of the hallmarks of age-related bone loss (2,3). Although previous studies have reported significant bone loss and spontaneous fractures in this mouse model (8), the underlying mechanisms that explain these findings and their potential significance for the understanding and treatment of age-related bone loss remain unknown.

The importance of proteins of the nuclear lamina in bone biology was suggested by the changes in bone mass seen in patients with HGPS (5,6). In addition, accumulation of un-farnesylated prelamin A has been associated with the typical features of progeria syndromes (14) and with alterations in
the differentiation of MSC during the aging process (16,25). However, despite increasing evidence suggesting that either accumulation of unprocessed prelamin A or lower levels of lamin A/C may play a role in normal and pathological aging, few studies have been pursued looking at their association with the cellular changes of aging bone.

A recent study has reported that levels of lamin A/C expression are decreased during normal aging in osteoblasts (26). This reduction in lamin A/C expression by mature osteoblasts could be associated with functional decline in osteoblast function seen in normal aging bone. From the osteoblast differentiation perspective, the role of proteins of the nuclear lamina in osteoblast differentiation has been previously assessed in vitro (18). Lamin A/C knockdown decreased osteoblast differentiation, whereas it facilitated adipogenic differentiation of MSC. Interestingly, absence of lamin A/C affected the activation of the Runx2 nuclear binding complex without affecting Runx2 expression, subsequently affecting the expression of osteoblastogenesis transcription factors.

In this study, we assessed whether the low bone mass and spontaneous fractures found in the Zmpste24−/− mice were due to the same mechanisms previously found in our experiments in vitro. In addition, we looked whether these abnormalities were similar in both sexes. Initially, we performed both micro-CT and histological analyses of bones of Zmpste24−/− mice at the age of 3 months. This time point was selected due to the short life span of the homozygous mouse and to previous reports that, after this age, Zmpste24−/− mice start suffering spontaneous fractures (8).

Initially, von Kossa staining and micro-CT demonstrated that bone mass is significantly lower in the Zmpste24−/− mice compared with WT control mice, leading to an osteopenic phenotype. This bone loss is reflected by a decrease in trabecular thickness and number, which were decreased to the same extent in both male and female Zmpste24−/− mice. Furthermore, to analyze the cellular mechanisms of bone loss in this model, we assessed the changes in bone cellularity in Zmpste24−/− mice compared with their WT littermates. We found that the number of osteoblasts and osteoclasts relative to the bone volume was significantly reduced in Zmpste24−/− mice compared with the WT controls. This reduction in cells number correlated with serum markers of osteoblastic (OCN) and osteoclastic (C-telopeptide) activities. No difference between male and female was found. Overall, these changes in bone cellularity were not associated with changes in serum levels of either calcium or calciotropic and sex steroid hormones.Taken together, these results demonstrate that accumulation of unprocessed prelamin A induces bone loss through a significant reduction in bone turnover. However, osteoblastic activity was more affected than osteoclastic activity, suggesting that processing of prelamin A may play a more important role in the regulation of osteoblast differentiation and function and that the reduction in osteoclast activity could be a consequence of lower number of osteoblast precursors which regulate osteoclast differentiation. Our findings correlate with a recent study looking at the effect of high levels of prelamin A on osteoclast activity in vitro (27). The investigators reported that the bone resorption activity of osteoclasts obtained in the presence of high prelamin A levels is lower with respect to control osteoclasts. Nevertheless, further in vivo studies looking at the role of proteins of the nuclear lamina in osteoclast differentiation and function will be required.

To further assess whether the reduction in osteoblast number was associated with alterations in Runx2 expression and activity, we quantified levels of Runx2 expression both at the protein and at the mRNA levels. We found that prelamin A accumulation induced a significant reduction in Runx2 at both protein and mRNA levels. Considering that Runx2 is an essential factor in osteoblastogenesis, we further assessed whether low levels of Runx2 in this model has an effect on osteoblast differentiation and on the expression of transcription factors downstream Runx2 activation (24,28). We found that MSC obtained from bone marrow in both male and female Zmpste24−/− mice lost their capacity to differentiate into osteoblasts and expressed lower levels of the osteoblastogenesis transcription factors OCN and BSPII. Interestingly, whereas levels of OPN expression remained stable in female Zmpste24−/− mice, male Zmpste24−/− mice showed lower levels of OPN expression suggesting that estrogens may play a protective effect to OPN expression in these mice. The mechanism involved in this gender-based difference should be assessed in further studies.

Furthermore, considering that osteocytes are the most abundant cell in bone, act as mechanosensors, and constitute the final fate of a proportion of osteoblasts (29), we looked at the effect that lack of Zmpste24 activity may have on osteocytes number in this model. We found a significant reduction in trabecular osteocyte number in Zmpste24−/− mice compared with their WT+/+ controls. More studies looking at the role of proteins of the nuclear lamina in osteocyte function and mechanotransduction are required.

Finally, a particularly striking finding of this study was that the significant bone loss in Zmpste24−/− mice was associated with higher levels of fat infiltration within the bone marrow. This evidence correlates with previous in vitro findings showing that lack of lamin A/C favors the differentiation of MSC into adipocytes (18). In fact, lack of lamin A/C and accumulation of unfarnesylated prelamin A have been associated with lipodystrophy or redistribution of fat to nonadipose tissues (30,31). In this study, the abnormally increased amount of bone marrow fat correlated with higher levels of expression of proadipogenic transcription factors in both male and female Zmpste24−/− mice. Considering that age-related bone loss constitutes a type of lipodystrophy where fat is redistributed into the bone marrow compartment (2), the regulation of the proteins of the lamina may play an important role in the prevention of this age-related phenomenon.

In summary, our findings provide new insights into the role of prelamin A processing in bone quality and MSC differentiation. Our findings allow us to postulate that prelamin A processing could be a determinant player in the pathogenesis...
of age-related bone loss. Considering that levels of lamin A/C expression in bone progressively decrease with aging, it is tempting to propose that age-related changes in the processing of proteins of the nuclear lamina are associated with the key features of senile osteoporosis, namely decreasing osteoblastogenesis, low bone turnover, and increasing adipogenesis. In addition, recent studies have demonstrated that some of the pathological findings associated with accumulation of unprocessed prelamin A could be reverted through the inhibition of prenylation (32,33). Overall, we conclude that the pharmacological modulation of prelamin A and lamin A/C activities in bone could offer a new potential target to prevent bone loss thus maintaining appropriate levels of bone formation, whereas decreasing fat infiltration within the bone marrow.

FUNDING
Grants from the Canadian Institutes of Health Research (2007031AP) and the Nepean Hospital Medical Research Foundation. G.D. holds a Fellowship from the University of Sydney Medical Research Foundation.

ACKNOWLEDGMENT
The authors would like to thank Mrs. Leigh Bambury for preparation of the manuscript.

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