

Short-term intermittent PTH 1–34 administration and bone marrow blood vessel ossification in Mature and Middle-Aged C57BL/6 mice



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ABSTRACT

Intermittent parathyroid hormone (PTH) administration augments bone and progressive bone marrow blood vessel (BMBV) ossification occurs with advancing age. Since intermittent PTH administration augments bone, it may also serve to increase BMBV ossification. We assessed the influence of 5- and 10-days of intermittent PTH 1–34 administration on trabecular and cortical bone and BMBV ossification in mature (6–8 mon; $n = 30$) and middle-aged (10–12 mon; $n = 30$) male and female C57BL/6 mice. Mice were divided accordingly: control (CON) and 5-days (5dPTH) and 10-days (10dPTH) of PTH. Mice were given PBS (50 μ l) or PTH 1–34 (43 μ g/kg/d) for 5- and 10-consecutive days. Trabecular bone microarchitecture (i.e., BV/TV [%], Tb.Th [μ m], Tb.N [$1/\text{mm}$], and Tb.Sp [μ m]) was assessed in the distal femoral metaphysis and cortical bone parameters (i.e., Ct.Th [μ m] and CSMI [mm^4]) at the femoral mid-shaft. BMBV ossification (i.e., ossified vessel volume [OsVV, %] and ossified vessel thickness [OsV.Th, μ m]) was assessed in the medullary cavity of the femoral shaft. All parameters were determined by μ CT. At this sample size, no gender-related differences were observed so female and male data were pooled. There were no main effects nor interactions for trabecular microarchitecture and Ct.Th. However, CSMI was larger ($p < 0.05$) in Middle-Age vs. Mature and larger ($p < 0.05$) in CON and 10dPTH vs. 5dPTH. OsVV tended ($p = 0.057$) to be higher ($0.18 \pm 0.04\%$ vs. $0.09 \pm 0.02\%$, respectively) and OsV.Th was higher ($p < 0.05$; $17.4 \pm 1.6 \mu\text{m}$ vs. $12.1 \pm 1.4 \mu\text{m}$, respectively) in Middle-Aged vs. Mature mice. OsVV was not altered, but ossified vessels tended ($p = 0.08$) to be thicker in 10dPTH ($17.6 \pm 2.0 \mu\text{m}$) vs. CON ($12.5 \pm 1.7 \mu\text{m}$). No interactions were observed for OsVV and OsV.Th. In conclusion, this is the first report of ossified BMBV in C57BL/6 mice. The increased OsV.Th in Middle-Aged mice coincides with previous reports of increased OsVV in aged rats. The tendency of augmented OsV.Th in 10dPTH suggests that this treatment may ultimately impair the patency of bone marrow blood vessels.

1. Introduction

The vascular system is crucial for the optimal functioning of bone and bone marrow. Blood vessels deliver O_2 , nutrients and systemic hormones to these tissues and remove waste products (Brookes and Revell, 1998; McCarthy, 2006; Sparks et al., 2017). Additionally, immune cells (Goldsby et al., 2000) and precursor cells involved in bone remodeling are produced in the marrow and blood vessels are responsible for the transport of these cells (Eghbali-Fatourehchi et al., 2005; Fujikawa et al., 1996). Thus, blood vessels are fundamental for bone remodeling (Jilka, 2003; Parfitt, 2000; Sims and Martin, 2014) and, in regards to hematopoiesis, play a role in stem cell niches (Sacchetti et al., 2007; Kiel et al., 2005).

Given the various roles of the bone vascular system in the day-to-day functioning of a healthy skeleton, it has been theorized that

dysfunction in the bone vascular network is highly associated with dysfunction in bone and bone marrow (Bloomfield et al., 2002; Collier et al., 2000; Dominguez et al., 2010; Griffith et al., 2005; Prisby et al., 2007). For example, diminished bone blood flow or perfusion (Bloomfield et al., 2002; Dominguez et al., 2010; Griffith et al., 2005; Prisby et al., 2007; Griffith et al., 2008) and impaired vasodilator function of bone blood vessels (Dominguez et al., 2010; Prisby et al., 2007; Prisby et al., 2008) are associated with reduced skeletal mass in rats and humans (Dominguez et al., 2010; Griffith et al., 2005; Prisby et al., 2007; Griffith et al., 2008). Further, age-related rarefaction of blood vessels coincides with bone loss and augmented bone marrow adiposity (Burkhardt et al., 1987; Prisby, 2014). In a rat aging model, we recently described a novel pathology whereby bone marrow blood vessels (BMBV) progressively and theoretically convert into bone (i.e., BMBV ossification) with advancing age (Prisby, 2014). Ossified blood

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Table 1
Trabecular and cortical bone parameters following short-term intermittent PTH 1–34 administration in Mature and Middle-Aged mice.

Trabecular bone						
	Mature CON	Mature 5dPTH	Mature 10dPTH	Middle-Aged CON	Middle-Aged 5dPTH	Middle-Aged 10dPTH
BV/TV (%)	2.2 ± 1.1	2.5 ± 1.0	2.2 ± 0.8	2.3 ± 0.9	3.7 ± 1.2	4.0 ± 1.0
Tb.Th (µm)	27 ± 3	26 ± 2	26 ± 2	28 ± 2	27 ± 4	29 ± 2
Tb.N /mm	0.7 ± 0.3	0.9 ± 0.3	0.7 ± 0.2	0.7 ± 0.2	1.1 ± 0.3	1.2 ± 0.3
Tb.Sp (µm)	22,227.4 ± 12,679.5	4225.5 ± 1850.6	4923.0 ± 2593.1	3987.4 ± 1482.4	22,649.9 ± 16,864.9	5810.4 ± 4636.8

Trabecular bone						
	Age		Treatment		Interaction	
	F-ratio	p-Value	F-ratio	p-Value	F-ratio	p-Value
BV/TV	1.492	0.228	0.486	0.618	0.328	0.722
Tb.Th	0.927	0.340	0.457	0.636	0.373	0.691
Tb.N	0.553	0.461	0.170	0.844	0.116	0.890
Tb.Sp	0.002	0.961	0.522	0.596	2.241	0.117

Cortical bone						
	Mature CON	Mature 5dPTH	Mature 10dPTH	Middle-Aged CON	Middle-Aged 5dPTH	Middle-Aged 10dPTH
Ct.Th (µm)	172 ± 5	175 ± 7	182 ± 5	174 ± 6	163 ± 19	180 ± 4

Cortical bone						
	Age		Treatment		Interaction	
	F-ratio	p-Value	F-ratio	p-Value	F-ratio	p-Value
Ct.Th	0.175	0.677	0.712	0.495	0.226	0.798

Values are mean ± SE. No statistical differences were observed.

vessels were also present in amputated long bones from elderly patients with arteriosclerotic vascular disease and peripheral vascular disease with cellulitis (Prisby, 2014). These findings confirm the translation of this disease to the human condition.

Ossification of BMBV is characterized by osteocyte lacunae on the abluminal surface and may result from a transition of vascular endothelial and/or smooth muscle cells into osteogenic phenotypes (Prisby, 2014). Additionally, the etiologies of this disease may be attributable to the bone marrow microenvironment and the release of pro-inflammatory cytokines from the resident cells (Lee et al., 2018). Regardless of the etiologies, ossified BMBV lose vasomotor function (i.e., vasodilator and vasoconstrictor activity) and have reduced or abolished patency, resulting in “microvascular dead space” within bone (Prisby, 2014). “Microvascular dead space” presumably contributes to the age-related declines in skeletal blood flow or perfusion (Bloomfield et al., 2002; Dominguez et al., 2010; Griffith et al., 2005; Prisby et al., 2007; Griffith et al., 2008) and reversal in direction of skeletal blood flow (Prisby, 2017), potentially impacting delivery of nutrients, systemic hormones and precursor cells to bone and bone marrow (Colleran et al., 2000; Prisby et al., 2007; Prisby, 2014). Such a progressive pathology may ultimately contribute to the diminished bone mass observed with advanced age (Bloomfield et al., 2002; Dominguez et al., 2010; Prisby et al., 2007).

Intermittent parathyroid hormone (PTH) administration is osteogenic and a well-known anabolic agent used to treat conditions of low bone mass (Esbrit et al., 2000; Henriksen et al., 2009). Intermittent PTH administration has regulatory effects on bone cellular communications and remodeling (Henriksen et al., 2009; Nishida et al., 1994). For example, intermittent PTH administration augmented osteoblast differentiation and activated bone lining cells (Dobnig and Turner, 1995), in addition to reducing osteoblast apoptosis (Jilka et al., 1999). While the effects of intermittent PTH administration on bone have been well

documented, PTH also elicits vasodilation of blood vessels in various organs (Pang et al., 1985; Pang et al., 1980; Nickols et al., 1986), including the skeleton (Prisby et al., 2013; Benson et al., 2016). This physiological effect may be particularly important for the bone vascular network in terms of enhanced blood flow delivery during the augmented bone metabolism induced by intermittent PTH administration. In fact, augmented bone volume following 15 days of intermittent PTH 1–84 administration in young rats was accompanied by enhanced endothelium-dependent vasodilation of the femoral principal nutrient artery (PNA) (Prisby et al., 2013); i.e., the primary conduit for blood flow to long bones (Brookes and Revell, 1998). Additionally, intermittent PTH administration was demonstrated to augment bone vascular density (i.e., blood vessel number) and skeletal perfusion (Roche et al., 2014; Moore et al., 2012). In contrast, bone vascular density was lower in PTH-treated vs. control rats following 15- and 30-days of intermittent PTH 1–84 administration (Prisby et al., 2011).

The totality of data suggests a beneficial physiological outcome for several bone vascular parameters (i.e., vasodilator capacity of blood vessels, blood flow and angiogenesis) following intermittent PTH administration that would facilitate bone accrual. However, given that bone marrow blood vessels progressively and theoretically convert into bone and that intermittent PTH administration is effective at eliciting bone formation, it may also, and unfortunately, promote or exacerbate BMBV ossification. The long-term consequences of such a result would serve to further reduce the ability of bone blood vessels to deliver flow to the aging skeleton. Since BMBV ossification has been demonstrated in an aging rat model and in human amputated long bones (Prisby, 2014), we hypothesized that BMBV ossification would be observed in Mature and Middle-Aged C57BL/6 mice and that it would be more severe in the Middle-Aged animals. Additionally, previous investigations revealed that physiological stimuli influence the bone vascular system prior to impacting bone (Roche et al., 2014; Gohin et al., 2016).

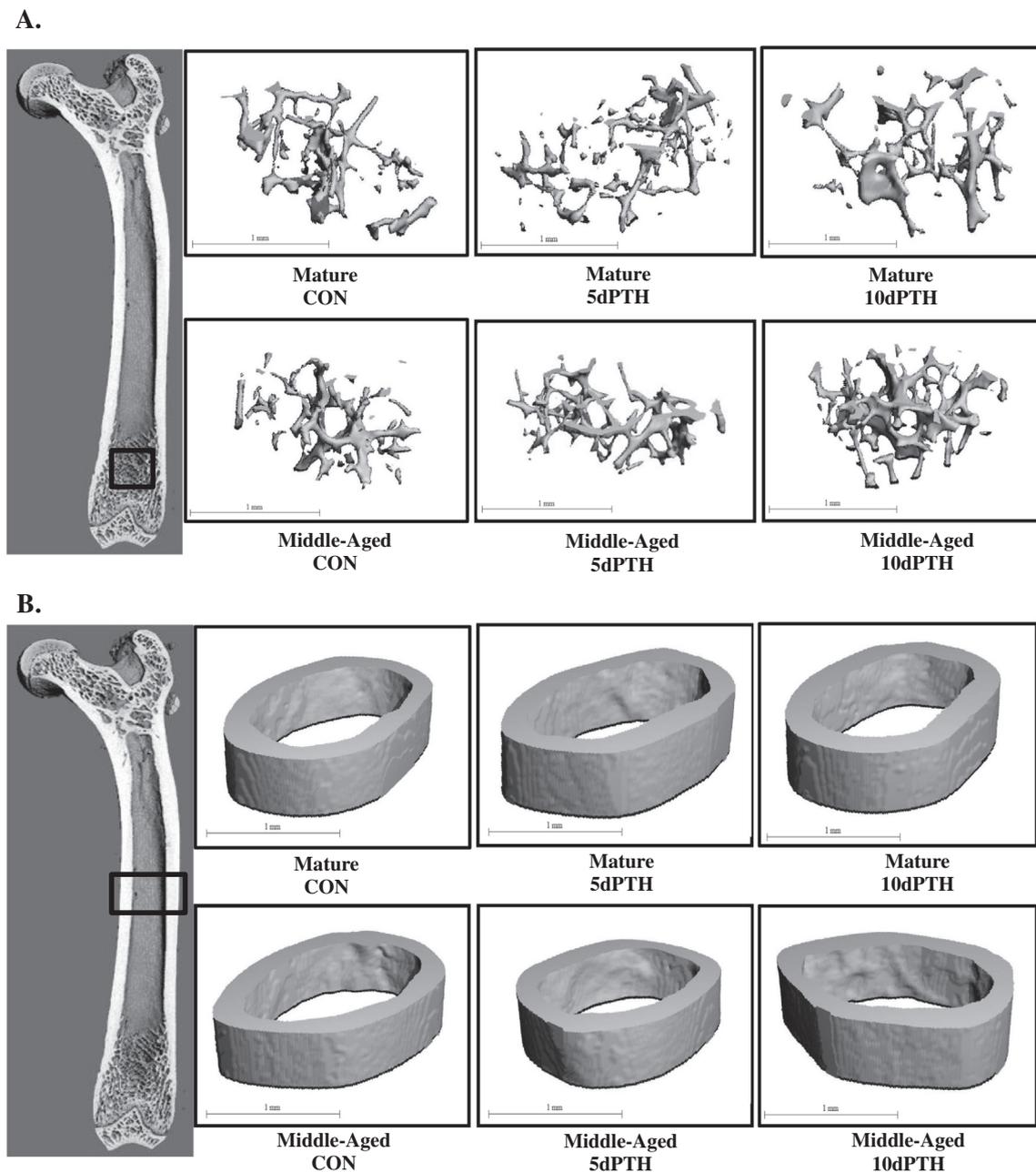


Fig. 1. Representative μ CT images of (A) trabecular bone microarchitecture and (B) cortical thickness in Mature and Middle-Aged CON, 5dPTH, and 10dPTH mice. The black boxes overlaying the secondary spongiosa (Fig. 1A) and cortical midshaft (Fig. 1B) represent the regions of analyses and correspond with the representative images provided for each group. There were no significant differences.

Thus, we chose short duration protocols (i.e., 5 and 10 days) to capture these sequences of events. We hypothesize that short-term intermittent PTH 1–34 administration would not be of sufficient duration to alter trabecular and cortical bone parameters but would enhance BMBV ossification in Mature and Middle-Aged C57BL/6 mice.

2. Materials and methods

All animal experiments were conducted and performed according to the protocols approved by University of Delaware and University of Texas at Arlington Institutional Animal Care and Use Committees and conform to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85–23, revised 1996). Mature (6–8 month-old) and Middle-Aged (10–12 month-old) female and male C57BL/6 mice were obtained from

a mouse colony at the University of Delaware. They were housed in standard cages in a temperature- ($23 \pm 2^\circ\text{C}$) and light-controlled (12 h/12 h light/dark) room. Tap water and regular mouse chow were provided ad libitum.

2.1. Intermittent parathyroid hormone administration and sample preparation

Mature and Middle-Aged mice were matched according to age and body mass and randomly assigned to following six groups: 1) Mature control (Mature CON; $n = 10$); 2) Mature with five days of intermittent PTH 1–34 administration (Mature 5dPTH; $n = 10$); 3) Mature with ten days of intermittent PTH 1–34 administration (Mature 10dPTH; $n = 10$); 4) Middle-Aged CON (Middle-Aged CON; $n = 10$); 5) Middle-Aged 5dPTH ($n = 10$); and 6) Middle-Aged 10dPTH ($n = 10$). Thus,

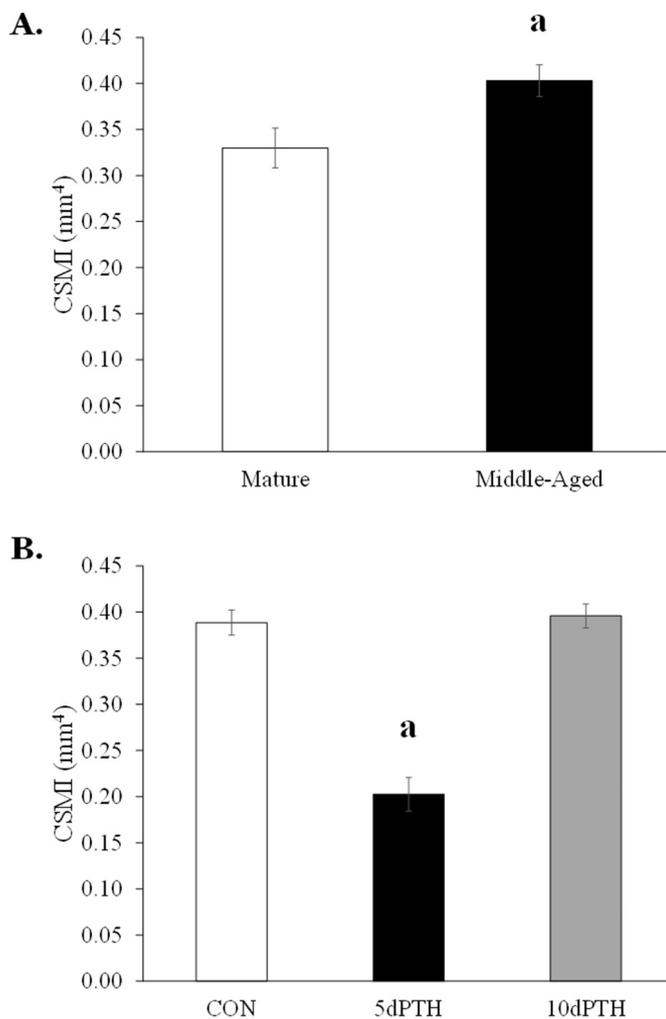


Fig. 2. A) Main effects of age on cross-sectional moment of inertia. B) Main effects for treatment on cross-sectional moment of inertia. Values represent means \pm S.E. ^adenotes a significant difference ($p < 0.05$) vs. the other group (s). CSMI = cross-sectional moment of inertia.

mean body masses at the start of the experiment were similar among respective age groups. According to treatment, mice received subcutaneous injections of either 43 $\mu\text{g}/\text{kg}/\text{day}$ of PTH 1–34 (ProSpec, East Brunswick, NJ) for five and ten consecutive days or 50 $\mu\text{l}/\text{day}$ of phosphate buffered saline as a vehicle for ten consecutive days. Five and ten days of intermittent PTH 1–34 administration in mice is equivalent to approximately six and twelve months of treatment, respectively, in clinical populations (Dutta and Sengupta, 2016). A dose of 43 $\mu\text{g}/\text{kg}/\text{day}$ of PTH 1–34 is molecularly equivalent to 100 $\mu\text{g}/\text{kg}/\text{day}$ of PTH 1–84, which shows bone anabolic efficacy in human clinical trials (Verhaar and Lems, 2009). Also, similar doses of PTH 1–34 (40 $\mu\text{g}/\text{kg}/\text{day}$) have been shown to improve bone healing (Silva et al., 2015) and bone mass (Sugiyama et al., 2008). PTH and the vehicle were administered at the same time each morning. After completion of the protocol, all mice were anesthetized by inhalation of isoflurane (2.5% to O_2 balance). Mice were sacrificed via myocardial removal and left femora were collected. The femora were cleaned and fixed overnight in 4% paraformaldehyde at 4 $^{\circ}\text{C}$. Femora were subsequently stored in 70% ethanol at -20°C until scanning by micro-computed tomography.

2.2. Micro-computed tomography (μCT) scans and analyses

Scans were performed using a high-resolution μCT 35 (Scanco Medical, Brüttisellen, Switzerland). Femora were scanned at a

resolution of 10 μm at 55 kVp. Trabecular bone microarchitecture was determined from 60 slices in the distal femoral metaphysis, beginning 600 μm superior to an anatomically defined region of the growth plate. The following trabecular bone microarchitectural parameters were calculated: bone volume/total volume ratio (BV/TV, %), trabecular thickness (Tb.Th, μm), trabecular number (Tb.N, /mm) and trabecular separation (Tb.Sp, μm). Cortical bone parameters were determined from 50 slices at the femoral mid-shaft and the following parameters were calculated: cortical thickness (Ct.Th, μm) and cross-sectional moment of inertia (CSMI, mm^4). In addition, the femoral shaft was analyzed to assess ossified BMBV in the marrow space. Care was taken so that trabecular and cortical bone was not included in this analysis. Thus, the analysis began where trabecular bone disappeared at the end of the secondary spongiosa in the proximal metaphysis and continued just prior to the beginning of the secondary spongiosa at the distal metaphysis. In addition, the medullary cavity was contoured so that bone from the cortical shell was not included in the analysis. The following parameters were calculated: ossified vessel volume (OsVV, %) and ossified vessel thickness (OsV.Th, μm).

2.3. Isolation of ossified bone marrow blood vessels from the medullary cavity of a mouse femoral diaphysis

A femur from a male mouse in the Middle-Aged, 5dPTH group was used to isolate ossified bone marrow blood vessels from the medullary cavity. The distal end of the femur was cut with a rotary tool and a diamond cutting disc. Microsurgical forceps were used to pull the marrow from the femoral shaft. The marrow was placed in a petri dish and a stereomicroscope was utilized to isolate ossified bone marrow blood vessels. Phosphate buffered saline was used to wash away bone marrow cells from the isolated ossified vessels. The vessels were then transferred to a microscope slide, placed under a stereomicroscope and light microscope equipped with cameras to obtain images.

2.4. Statistical analysis

Data were analyzed by two-way ANOVA with SPSS statistical software (version 24; IBM, Armonk, NY) to determine significant main effects (i.e. age and treatment) and interactions for the following: trabecular bone microarchitecture, cortical bone parameters and ossified BMBV parameters. One-way ANOVA was used to examine differences in body mass. Student-Newman-Keuls (SNK) post-hoc tests were performed to assess group differences. The significance level was set at $p \leq 0.05$. Tendencies for significant differences ($p \leq 0.10$) are reported. Data are expressed as a mean \pm standard error (M \pm SE).

3. Results

3.1. Mouse characteristics

Since no gender-related differences were observed (Supplementary Tables 1–4), female and male data were pooled and analyzed according to group. Body mass did not differ among groups (Mature CON, 31 ± 2 g; Mature 5dPTH, 32 ± 2 g; Mature 10dPTH, 30 ± 1 g; Middle-Aged CON, 30 ± 1 g; Middle-Aged 5dPTH, 31 ± 1 g; and Middle-Aged 10dPTH, 31 ± 1 g).

3.2. Effects of age and intermittent PTH administration on trabecular and cortical bone parameters

There were no main effects for age or treatment nor any significant interactions observed for the trabecular bone parameters. As anticipated, trabecular bone microarchitecture (i.e., BV/TV, Tb.N, Tb.Th, Tb.Sp) did not differ with PTH treatment (Table 1). Likewise, no main effects for age or treatment nor any significant interactions were observed for Ct.Th at the femoral mid-shaft (Table 1). Fig. 1 illustrates

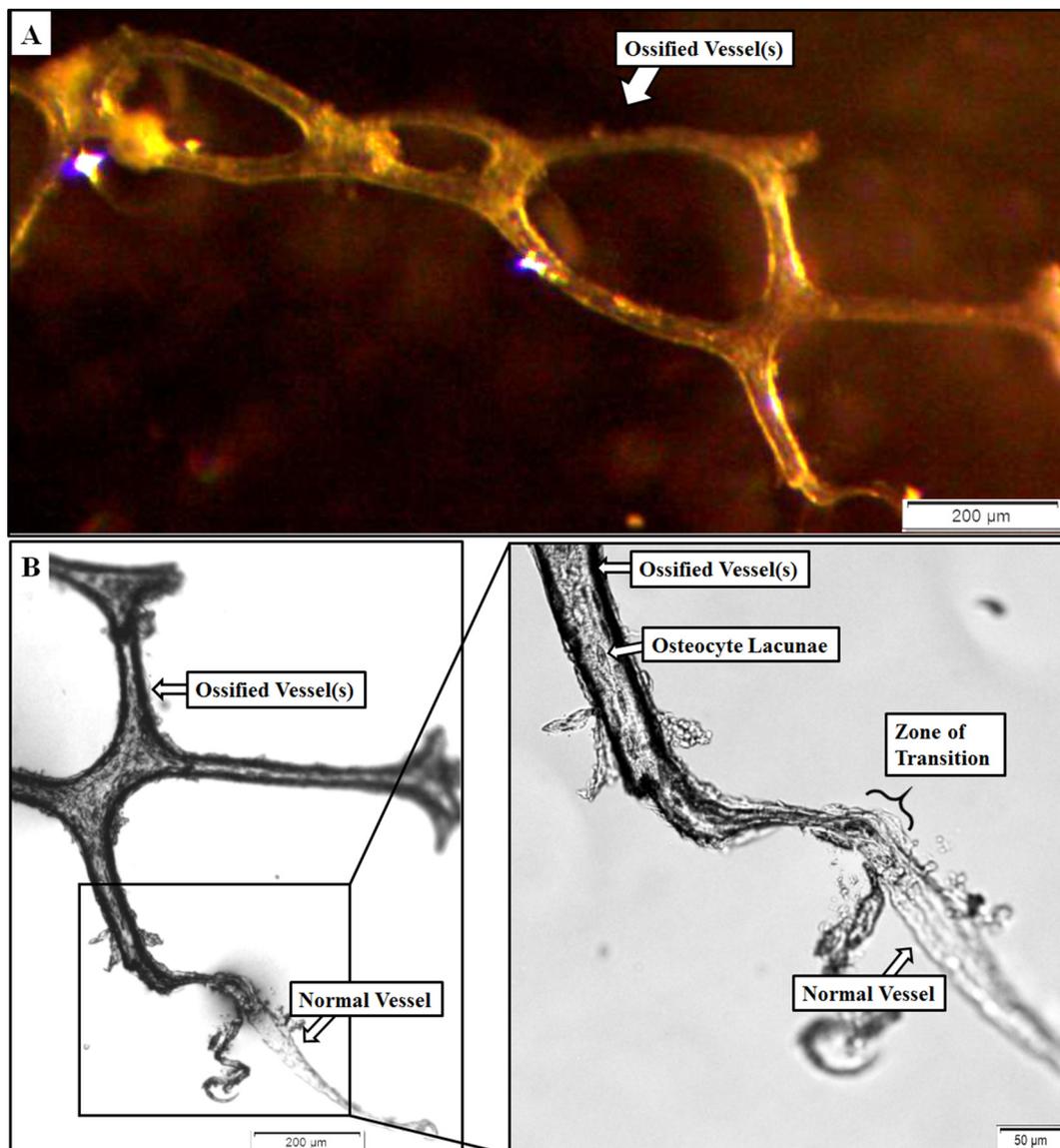


Fig. 3. A) Stereo- and B) light-microscopic images of an ossified bone marrow blood vessel(s) isolated from the medullary cavity of a Middle-Aged, 5dPTH-treated mouse. Osteocyte lacunae can be observed on the abluminal surface of the ossified bone marrow blood vessel(s). Additionally, it appears that the ossified bone marrow blood vessel(s) is “transitioning” into a normal blood vessel (magnified inset).

representative 3D μ CT reconstructions of trabecular bone micro-architecture in the secondary spongiosa of the distal femoral metaphysis and Ct.Th. The μ CT images of the entire femora are provided to illustrate the regions of analyses for the trabecular and cortical bone parameters. The black boxes overlaying the secondary spongiosa (Fig. 1A) and cortical midshaft (Fig. 1B) depict the regions of analyses and correspond to the representative μ CT images for each group. In regards to CSMI, significant main effects for age and treatment were observed (Fig. 2A and 2B, respectively), whereby CSMI was 22% higher ($p < 0.05$) in Middle-Aged vs. Mature and was lower ($p < 0.05$) in 5dPTH vs. CON and 10dPTH.

3.3. Effects of age and intermittent PTH administration on bone marrow blood vessel ossification

As hypothesized, ossified BMBV were present in both Mature and Middle-Aged C57BL/6 mice. Fig. 3 depicts images of an ossified bone marrow blood vessel(s) isolated from the femoral shaft of a Middle-Aged mouse. The images were taken via stereomicroscopy (Fig. 3A) and light microscopy (Fig. 3B). Osteocyte lacunae can be observed on the

abluminal surface(s) of the ossified bone marrow blood vessel(s). In addition, the ossified bone marrow blood vessel appears to be transitioning into a normal blood vessel (Fig. 3B, magnified inset). Fig. 4 depicts representative μ CT images of ossified bone marrow blood vessels in the medullary cavity for each group. The μ CT image of the entire femur is provided to illustrate the region of analysis for the ossified bone marrow blood vessels. The black box overlaying the marrow cavity represents the region of analysis and corresponds to the representative images provided for each group. Of note, the region of analysis does not contain trabecular bone from the proximal or distal metaphyses nor bone from the cortical shell. Thus, the μ CT analyses represent ossified bone marrow blood vessels within the medullary cavity. No significant interactions were observed for the ossified vessel parameters (i.e., OsVV and OsV.Th); however, some main effects for age and treatment were found. OsVV tended ($p = 0.057$) to increase with advancing age (Fig. 5A) and ossified vessels were 44% thicker ($p < 0.05$) in Middle-Aged vs. Mature mice (Fig. 5B). Additionally, there were no significant differences in OsVV according to treatment (Fig. 5C). However, ossified vessels tended ($p = 0.08$) to be 41% thicker following 10 days of intermittent PTH administration (Fig. 5D).

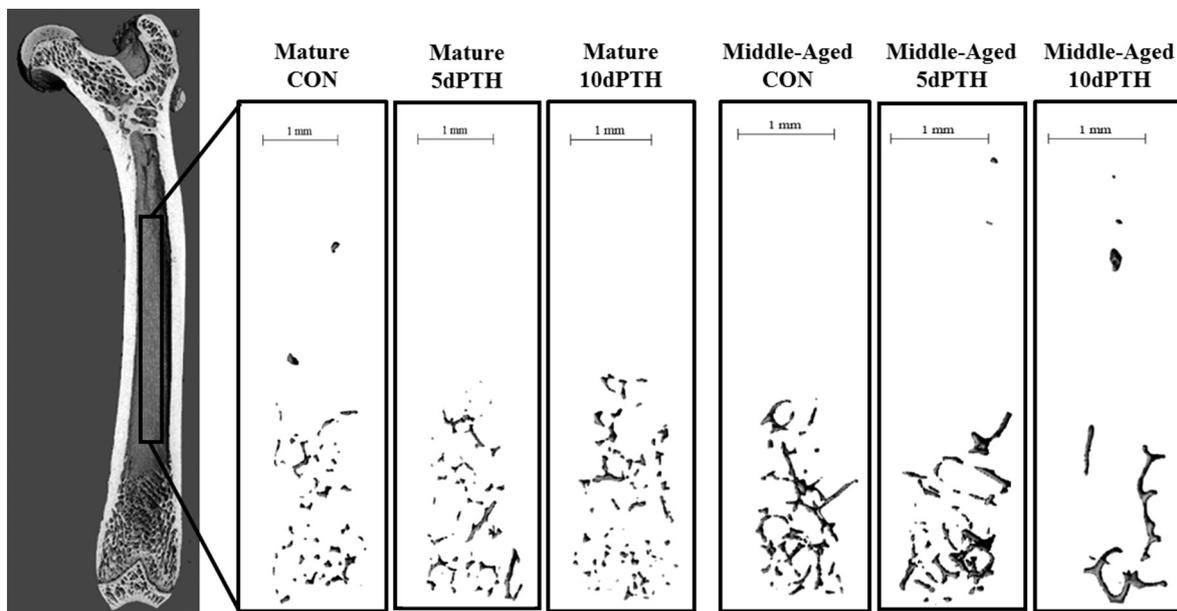


Fig. 4. Representative μ CT images of bone marrow blood vessel (BMBV) ossification in Mature and Middle-Aged CON, 5dPTH, and 10dPTH mice. The black box overlaying the marrow cavity depicts the region of analysis and corresponds with the representative images provided for each group. The region of analysis does not include trabecular bone from the proximal or distal metaphyses nor bone from the cortical shell. Thus, the analyses represent ossified bone marrow blood vessels in the medullary cavity.

4. Discussion

This investigation verified that ossified BMBV are present in Mature and Middle-Aged female and male C57BL/6 mice (Figs. 3 and 4), confirming that various species (i.e., mice, rats, and humans) develop this vascular pathology (current data and (Prisby, 2014)). In addition, ossified BMBV were thicker in Middle-Aged vs. Mature animals (Fig. 5), indicating bone accrual on these blood vessels as a function of advancing age. To our knowledge, these are the first data to report the presence of ossified BMBV in C57BL/6 mice.

These data confirm our previous reports of progressive ossification as a function of advancing age in rats (Prisby, 2014). Ossification of the bone vasculature, characterized by osteocyte lacunae and osteoid seams, presumably serves to enhance “microvascular dead space” (Prisby, 2014). “Microvascular dead space” indicates that ossified BMBV have reduced patency, are incapable of normal vasomotor activities (i.e., vasodilation and/or vasoconstriction), and thus may be deficient in the regulation of bone blood flow (Prisby, 2014). Further, ossified BMBV were observed in amputated long bones from elderly individuals (Prisby, 2014), highlighting the prevalence of this disease in humans. Even though not statistically significant ($p = 0.057$), OsVV doubled in a matter of months (i.e., from maturity to middle-age) and ossified vessels became thicker ($p < 0.05$) during this time frame. Both of these findings support previous claims of progressive ossification related to the aging process (Prisby, 2014).

4.1. Effects of advancing age on the bone vascular network

Coupled with BMBV ossification, other vascular pathologies occur with advancing age that may have a tremendous impact on bone and bone marrow. For example, declines in trabecular bone volume and endothelium-dependent vasodilation of the femoral PNA were observed at 22–24 months vs. 4–6 months in male Fischer-344 rats (Dominguez et al., 2010; Prisby et al., 2007; Prisby et al., 2008). In addition, the loss of bone vascularity has been reported (Prisby, 2014; Viboolvorakul et al., 2009), which may coincide with diminishment of a capillary subtype theorized to regulate angiogenesis (Kusumbe et al., 2014). In addition, bone blood vessel rarefaction occurred between 4 and

7 months of age in 129sv/CD1 mice, coinciding with reduced trabecular bone volume (Roche et al., 2013). However, no changes in vascular density were observed in the C57BL/6 mice (Roche et al., 2013), highlighting potential strain-specific alterations. Bone vascular rarefaction with advancing age has also been reported in humans, whereby the number of arterial capillaries (per 100 mm² of tissue) declined from 10–20 to 30–50 years and the number of sinusoids (per 100 mm² of tissue) declined from 10–20 to 30–50 and again at > 70 years (Burkhardt et al., 1987). The declines in bone vascular density may partially reflect the progressive age-related increase in ossified bone marrow blood vessels. Since PTH influences the cardiovascular system and is often prescribed to the elderly, several authors have speculated beneficial alterations in bone blood vessels following its administration (Lee et al., 2018; Prisby et al., 2013; Roche et al., 2014; Prisby et al., 2011; Gohin et al., 2016). Thus, such therapies may serve to reverse the aforementioned age-related declines in the bone vascular network.

4.2. Effects of PTH on the bone vascular network

Both acute and intermittent PTH administration influence bone blood vessels. For example, vasodilation to cumulative doses of various PTH analogs (i.e., PTH 1–84, PTH 1–34 and PTHrP 1–34) was observed in the femoral PNA (Prisby et al., 2013; Benson et al., 2016), with vasodilation being most robust to PTHrP 1–34 (Benson et al., 2016). Further, single applications of PTH transiently augmented skeletal blood flow and perfusion (Gohin et al., 2016; Kapitola and Zák, 2003). When administered intermittently over several weeks or months, PTH augmented skeletal perfusion in mice and humans (Roche et al., 2014; Moore et al., 2010). Further, PTH 1–84 relocated the smallest bone marrow blood vessels closer to bone forming sites (Prisby et al., 2011). This spatial relocation presumably aided in directing blood flow to areas of bone undergoing remodeling and maximized nutrient exchange (Prisby et al., 2011). Additionally, 14 days of intermittent PTH 1–84 administration augmented bone vascular density profiles in hind limb long bones of mice (Roche et al., 2014); however, bone vascular density was lower in PTH-treated rats following 15 and 30 days of treatment (Prisby et al., 2011). Lastly, intermittent administration of PTH 1–84 and PTH 1–34 improved endothelium-dependent vasodilation in PNAs

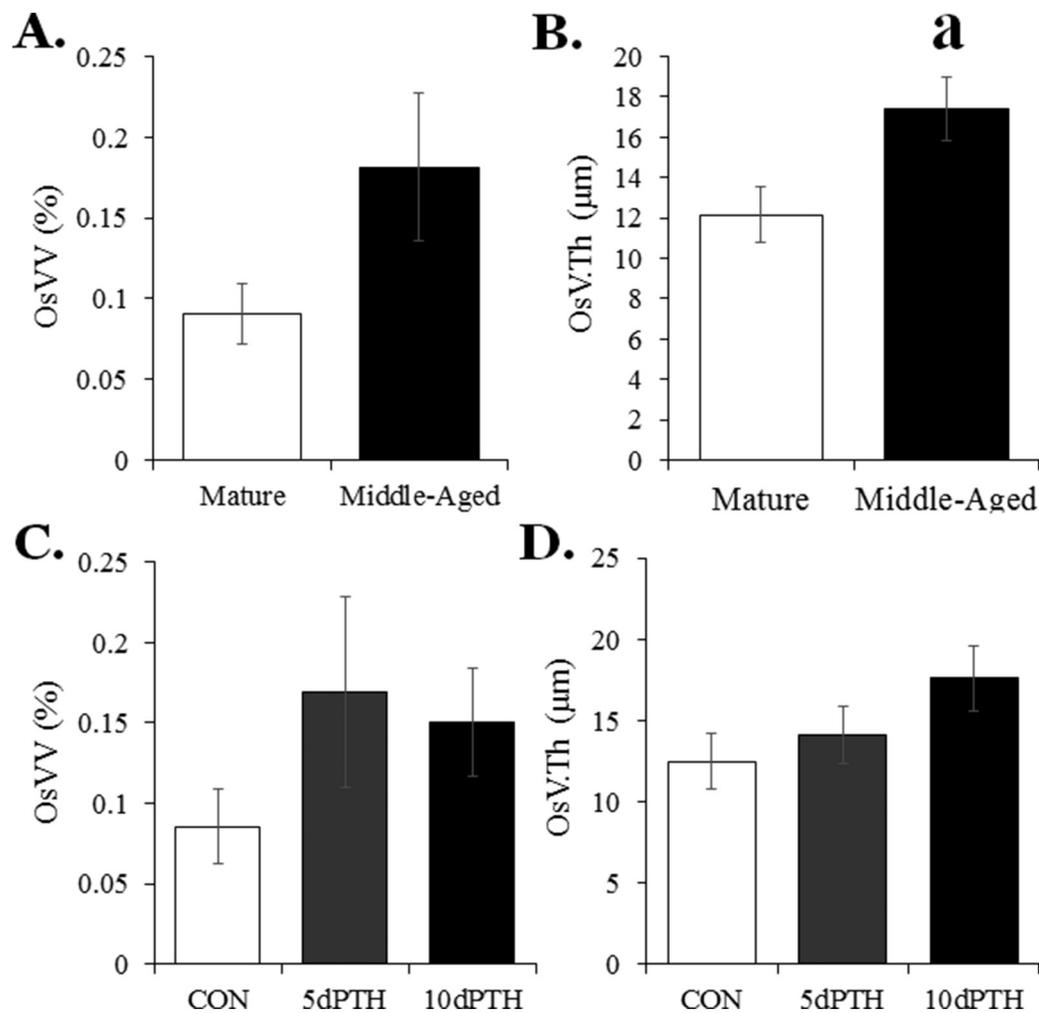


Fig. 5. Main effects of age (panels A & B) and treatment (panels C & D) on ossified bone marrow blood vessel (BMBV) parameters (i.e., OsVV and OsV.Th). (A) OsVV tended ($p = 0.057$) to be higher in Middle-Aged vs. Mature mice. (B) OsV.Th was higher ($p < 0.05$) in Middle-Aged mice vs. Mature mice. (C) OsVV was not altered with short-term (5- and 10-days) intermittent PTH 1–34 administration. (D) OsV.Th tended ($p = 0.08$) to be higher in 10dPTH vs. CON. Values are means \pm S.E. ^adenotes a significant difference ($p < 0.05$) vs. Mature.

from young and old rats, respectively (Lee et al., 2018; Prisby et al., 2013) and enhanced the marrow microenvironment in old (22–24 mon) rats (Lee et al., 2018). Overall, these studies highlight beneficial modifications to the bone vascular network with acute application or intermittent PTH administration for 14–30 days.

While the beneficial effects of PTH have been well documented in terms of enhancing the vasomotor properties of bone blood vessels and increasing bone vascular density and skeletal perfusion under certain circumstances, the potential effects of PTH on bone marrow blood vessel ossification deserve further attention. Intermittent PTH administration is bone anabolic (Prisby et al., 2013; Prisby et al., 2011; Greenspan et al., 2007; Lane et al., 1995); however, clinically-speaking, the treatment is of a limited duration (i.e., < 2 years) (Augustine and Horwitz, 2013). Thus, once treatment is arrested, what long-term consequences remain for the bone vascular system? In this investigation, intermittent PTH 1–34 administration tended to increase OsV.Th (Fig. 5) in as short as 10 days; i.e., at a time point when trabecular and cortical bone were unaltered. If bone marrow blood vessel ossification is enhanced with intermittent PTH administration, would this ultimately serve to impair blood vessel patency and bone blood flow and delivery? The question remains as to whether ossified blood vessels can transition back into a normal phenotype. These theories are important to address since the findings may prove particularly concerning for individuals who are prescribed and for physicians prescribing PTH

treatment for low bone mass.

4.3. Effects of age and short-term intermittent PTH administration on trabecular bone microarchitecture and cortical bone parameters

Trabecular bone microarchitecture (i.e., BV/TV, Tb.Th, Tb.N and Tb.Sp) and Ct.Th did not differ between the Mature and Middle-Aged groups; however, CSMI was higher in Middle-Aged vs. Mature mice. These data suggest increased radial growth from maturity to middle-age. Likewise, intermittent PTH 1–34 administration did not alter trabecular bone microarchitecture and Ct.Th. The lack of change in trabecular BV/TV, Tb.Th, Tb.N and Tb.Sp and Ct.Th no doubt reflects the short administration period (i.e., 5 and 10 days). This timeframe was chosen to examine the prompt effects of PTH administration on the bone vascular network as opposed to changes in bone. In contrast, 14 to 49 days of intermittent PTH administration coincides with trabecular and cortical bone accrual (Prisby et al., 2013; Prisby et al., 2011; Sugiyama et al., 2008; Iida-Klein et al., 2002); however, bone accrual is not always evident within these time frames (Gohin et al., 2016). Thus, the anabolic actions of intermittent PTH administration are wide-ranging and have been suspected to vary according to the strain of the animal (Gohin et al., 2016; Sugiyama et al., 2008). In addition, skeletal responses to PTH can be divergent from individual to individual as well (Rosen, 2004). Surprisingly, CSMI was reduced in 5dPTH vs. CON and

10PTH. The cross-sectional moment of inertia represents the mechanical resistance of the diaphysis to external forces. The current data suggests a spatial arrangement of bone in Middle-Aged, CON and 10dTPH that would be more resistant to loading; however, these interpretations are made with caution since other parameters (e.g., body mass, cortical porosity, bone mineral density, etc.) aid in determining fracture risk (Tu et al., 2015; Mayhew et al., 2005). In addition, a limitation of the current investigation results from the combination of both female and male mice in each group.

In conclusion, this is the first study to report the presence of ossified vessels in C57BL/6 mice. Current and previous (Prisby, 2014) results demonstrate that BMBV ossification is a pathology observed in rodent and human long bones. Additionally, 10 days of intermittent PTH 1–34 administration tended to increase the thickness of ossified BMBV, potentially exacerbating the pathology. Even though PTH is a treatment for osteoporosis, it may eventually impact the patency of BMBV by augmenting ossification and increasing the “microvascular dead space” in bone. While effective at bone anabolism, the long-term (i.e., following the arrest of treatment) consequences of intermittent PTH administration on the patency of bone marrow blood vessels may eventually be of concern.

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Competing interest

No author has a conflict of interest.

Author contribution

Authors' roles: Conception and design of the experiments: RP. Collection, assembly, analysis and interpretation of data: SL and RP. Drafting the article or revising it critically for important intellectual content: SL and RP.

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