Mechanical, hormonal and metabolic influences on blood vessels, blood flow and bone

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Abstract

Bone tissue is highly vascularized due to the various roles bone blood vessels play in bone and bone marrow function. For example, the vascular system is critical for bone development, maintenance and repair and provides O₂, nutrients, waste elimination, systemic hormones and precursor cells for bone remodeling. Further, bone blood vessels serve as egress and ingress routes for blood and immune cells to and from the bone marrow. It is becoming increasingly clear that the vascular and skeletal systems are intimately linked in metabolic regulation and physiological and pathological processes. This review examines how agents such as mechanical loading, parathyroid hormone, estrogen, vitamin D and calcitonin, all considered anabolic for bone, have tremendous impacts on the bone vasculature. In fact, these agents influence bone blood vessels prior to influencing bone. Further, data reveal strong associations between vasodilator capacity of bone blood vessels and trabecular bone volume, and poor associations between estrogen status and uterine mass and trabecular bone volume. Additionally, this review highlights the importance of the bone microcirculation, particularly the vascular endothelium and NO-mediated signaling, in the regulation of bone blood flow, bone interstitial fluid flow and pressure and the paracrine signaling of bone cells. Finally, the vascular endothelium as a mediator of bone health and disease is considered.

Key Words

- ▶ bone formation and resorption
- ▶ angiogenesis
- ▶ endothelium
- ▶ estrogen
- ▶ exercise

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Introduction

The vascular system is indispensable for the health and longevity of all biological tissues and organisms (North & Sinclair 2012). The demand for O₂, nutrients and waste elimination requires the foremost development of the vascular system during embryogenesis (Stocum 2006). In addition, regeneration of the vascular system is critical and ubiquitous for repair of all tissues (Stocum 2006). Therefore, a major requisite for biological activity is the vascular supply (Burkhardt *et al.* 1987). For years, bone biologists have focused their attention on the cellular and molecular functions of the skeleton in health and disease while often underappreciating the bone vascular network

(i.e., the organ system that enables the skeleton to form and function). As poignantly stated by a pioneer in bone biology, 'How can the methods of cell and molecular biology be deployed to study a biologic phenomenon that is manifested only in the intact organism?' (Parfitt 1994). While speaking of bone remodeling in the context of the activities of the bone basic multicellular unit (Parfitt 1994), this statement can be equally applied to the interactive and regulatory nature among the vascular system, bone and bone marrow.

Although gaining more attention in recent years, the importance of the vascular supply to the skeleton

has not undergone historical indifference (Lacey 1929, Brookes 1957, Brookes & Revell 1998). Another pioneer investigator stated that 'intraosseous blood flow gives life to bone, just as intracerebral blood flow gives life to the brain and coronary blood flow life to the heart' (Laroche 2002). Additionally, Brookes and Lloyd (1961) theorized that bone formation occurs only in relation to blood vessels, capillaries and sinusoids and that the structural development of the vascular pattern of mammalian bone marrow precedes and contributes significantly to the arrangement of bone trabeculae (Brookes & Lloyd 1961).

Besides providing O₂ and other essential nutrients critical for bone metabolism, bone blood vessels deliver systemic hormones and precursor cells for bone remodeling (Fujikawa *et al.* 1996, Eghbali-Fatourehchi *et al.* 2005), are integral components of bone basic multicellular units (Parfitt 1994, 1998), participate in hematopoiesis (Arai & Suda 2007) and are egress and ingress routes for blood and immune cells to and from the bone marrow (Mazo & von Andrian 1999, Nilsson *et al.* 2001, Martin *et al.* 2003). Further, bone development and maintenance are dependent upon the bone vascular network. For example, vascular ingrowth is necessary for endochondral and intramembranous bone formation, as reviewed by Kanczler and Oreffo (2008). Eventually, the skeletal system develops into an exceedingly vascularized tissue (Doll 2005). Throughout the longevity of the organism, the vascular supply is a constant necessity for the healthy maintenance of the skeleton.

The human skeleton contains over 200 bones, which are constantly undergoing modeling and remodeling to maintain bone strength (Clarke 2008). Modeling occurs as a result of the adaptation of bone in response to daily physiological stimuli, can occur via the independent activities of osteoblasts and osteoclasts (Clarke 2008) and declines with adulthood (Kobayashi *et al.* 2003). The maintenance of bone strength and mineral homeostasis occurs via remodeling, whereby localized pockets of old or damaged bone is renewed (Clarke 2008). Remodeling of bone occurs with bone basic multicellular units for cortical bone (Frost 1983b) and bone remodeling compartments for trabecular bone (Hauge *et al.* 2001). These units/compartments comprise bone-resorbing osteoclasts, bone-forming osteoblasts and a vascular capillary (Parfitt 1994, 1998, Melsen *et al.* 1995). The capillary of the bone basic multicellular unit is theorized to provide nutrients, O₂ and precursor cells to sites of bone remodeling. By default, the enhanced metabolism associated with bone modeling and remodeling augments blood flow.

Augmenting (or diminishing) tissue blood flow occurs by (1) the creation of new blood vessels via angiogenesis or arteriogenesis (or vascular rarefaction) and/or (2) by vasodilation (or vasoconstriction) of resistance arteries. Resistance arteries (i.e., arterioles and nutrient arteries) provide immediate regulation of tissue blood flow by altering blood vessel diameter with vascular smooth muscle contraction and relaxation, as reviewed by Hill and coworkers (Hill *et al.* 2001). Vascular smooth muscle contraction and relaxation are governed by systemic hormones, neural influences and locally produced factors designed to maintain tissue perfusion and blood pressure (Hill *et al.* 2001). There are several systemic hormones that control bone metabolism and the vasomotor activity of blood vessels. Neural control of the vasculature comes in the form of neurotransmitters and neuropeptides released by the nervous system (Bjurholm *et al.* 1988a,b, Hill & Elde 1991). Further, autocrine and paracrine factors are produced and released locally as a result of metabolism, perfusion pressure or shear stress acting on the vascular wall (Hill *et al.* 2001) and on bone cells as a result of interstitial fluid flow (Johnson *et al.* 1996).

Local environmental factors in bone modify blood vessel activity. In response to acute tissue hypoxia, local blood vessels increase tissue perfusion via enhanced vasodilator or diminished vasoconstrictor mechanisms. Following periods of chronic hypoxia, whereby vasomotor mechanisms to augment tissue perfusion are inadequate, enhanced blood flow can also result from angiogenesis (i.e., the formation of new from existing blood vessels). Another aspect to consider is the tissue hypoxia resulting from impaired vasomotor responses of local blood vessels due to pathologies within the vascular system. Figure 1 depicts the sequence of events following periods of acute or chronic hypoxia in bone. Further, the figure illustrates how dysfunction of the bone vascular network can lead to hypoxia within bone. While not as clinically immediate as occlusion of blood flow to the brain or heart, reduced or impeded blood flow to the skeleton manifests into several sub-clinical and clinical pathologies of bone and bone marrow. On the contrary, adequate delivery of nutrients, O₂, hormones, growth factors, cytokines and cells on a beat-to-beat basis allows for the proper functioning of bone and bone marrow on a daily basis. This review presents evidence of how the vascular system contributes to bone health and presents information on bone anabolic agents that also control bone blood vessels and bone blood flow. Further, this review may guide the reader into a systemic, integrative approach to bone metabolism in health and disease.

Figure 1

Hypoxia in bone. Acute hypoxia (seconds to minutes) will elicit rapid vasomotor responses (i.e., vasodilation and/or vasoconstriction) from bone arteries and arterioles to augmented blood flow and normalize the partial pressure of oxygen in the local environment. Chronic hypoxia (days to months) results from an inability of the arteries and arterioles to adequately augment tissue blood flow and oxygen. Under these circumstances, angiogenesis will ensue in order to meet the needs of the tissue. In regards to how the vascular system may impact bone health, dysfunction of the bone vasculature with advancing age and/or disease cause imbalances in arteriolar vasodilation and vasoconstriction and limit angiogenic capability. The result is diminished blood flow and oxygen delivery to bone. Under these circumstances, the etiology of hypoxia lies with the bone vascular network as opposed to with the enhanced metabolic activity of bone.

The microcirculation of bone

Similar to other circulatory beds, the circulation of bone comprises of (1) afferent vessels, (2) a microvascular network and (3) efferent vessels (Brookes & Revell 1998, McCarthy 2006). Capillaries and sinusoids are the sites of ionic exchange between the blood and bone and bone marrow cells (Brookes & Revell 1998). The arteries consist of the (1) metaphyseal, (2) epiphyseal, (3) principal nutrient artery (PNA) and (4) periosteal vessels (Brookes & Revell 1998). The metaphyseal, epiphyseal and periosteal vascular networks supply the regions of skeletal tissue from whence they obtain their names and the PNA supplies the diaphyseal marrow and inner 2/3rds of the diaphyseal cortex (Brookes & Revell 1998).

The PNA enters the cortex and branches into an ascending and descending limb, which runs down the center and traverses the length of the shaft, as reviewed by McCarthy (2006). Branches of the PNA spread out toward the endosteum and eventually ramify into sinuses or sinusoids, as reviewed by Gurkan and Akkus (2008). The collection of these sinuses or sinusoids journey back to the center of the shaft and into the principal nutrient vein, juxtaposed with the ascending and descending PNA, and exits the cortex through the principal nutrient foramen (Brookes & Revell 1998, Gurkan & Akkus 2008). Besides the principal nutrient vein, other routes of egress of blood include the epiphyseal, metaphyseal and cortical-periosteal veins (Brookes & Revell 1998).

Thus, the endosteal surface is replete with vessels and sinuses (Gurkan & Akkus 2008), which provides enhanced surface area for exchange and slower blood flow rates at these locations (Gurkan & Akkus 2008).

The vascularization of flat bones is similar to that in long bones; i.e., there is (1) a PNA, (2) numerous, smaller metaphyseal nutrients and (3) a profuse sinusoidal network in the marrow space with continued hematopoiesis (Brookes & Revell 1998). Similar to long bones, flat bones also have a periosteal vascular network, which provides the majority of perfusion (Brookes & Revell 1998). The importance of the periosteal network in membranous bones differs from that observed in young long bones, whereby the blood supply from this periosteal network is minimal. However, it is similar to aged long bones where increased reliance from the periosteal input is observed (Brookes & Revell 1998). Understanding the anatomical layout and functional properties of the bone circulation in relation to the cells of bone and bone marrow and elucidating the age- and disease-related alterations in vascular function under these contexts are important aspects to consider when examining the biological function of the skeletal system as a whole.

Ten-to-eleven and five percent of cardiac output in rats and humans, respectively, is dedicated to skeletal perfusion (Wootton *et al.* 1976, 1978, Charkes *et al.* 1979, Gross *et al.* 1979), such that bone blood flow is similar to resting blood flow to some skeletal muscles (Delp *et al.* 1999). Blood flow data collected via the

injection of radiolabelled microspheres into conscious rodents support this contention (Prisby *et al.* 2007). Femoral bone blood flows in young rats descended in the following manner: diaphyseal marrow, distal metaphysis, proximal metaphysis and diaphyseal cortex (Prisby *et al.* 2007). Regional blood flow differences in the diaphyseal marrow, distal metaphysis and proximal metaphysis were significantly diminished in old rats, while blood flow to the diaphyseal cortex remained unchanged (Prisby *et al.* 2007). Table 1 highlights the comparison between resting blood flows in the femur and skeletal muscles in rats (Delp *et al.* 1999, Prisby *et al.* 2007).

The attenuated femoral bone blood flow with advanced age corresponded with reduced endothelium-dependent vasodilation of the femoral PNA (Prisby *et al.* 2007). Blood flow in long bones are regionally dependent; i.e., hematopoietic marrow and the diaphyseal cortex have the highest and lowest blood flows, respectively (Davis & Wood 1997, Prisby *et al.* 2007) and regional differences in blood flow relate to the varied metabolic activities in the separate bone compartments (Brookes 1967). Protein synthesis is high during hematopoiesis and, therefore, high blood flows are expected in the marrow (Brookes 1967). Further, bone synthesis also requires considerable amounts of energy (Brookes 1967) and ionic exchange is augmented during bone growth (Leblond *et al.* 1950, Amprino & Murotti 1964) and coincides with higher blood flows (Brookes 1967). Thus, regional blood flows may serve as indicators of bone turnover and metabolism, and alterations in bone blood flow is a key influence on local bone accrual (Brookes 1967).

Mechanical loading

Wolff's Law characterizes how bone adapts to functionally withstand its mechanical environment (Wolff 1986). Conditions of chronically increased or decreased mechanical loading will augment (Westerlind *et al.* 1997, Dominguez *et al.* 2010) and diminish (Colleran *et al.* 2000, Prisby *et al.* 2015) bone mass, respectively. To initiate bone adaptation via mechanical loading, bone tissue must experience minimum strains of at least 800–2000 μ strain (Frost 1983a, Westerlind *et al.* 1997, Frost 2001). However, this threshold may be lower for some skeletal sites (Hsieh *et al.* 2001). Interestingly, *in vivo* experiments that altered intramedullary pressure and/or fluid flow without eliciting bone matrix strain have highlighted that bone accrual does occur (Kelly & Bronk 1990, Revell & Brookes 1994, Kwon *et al.* 2010, Qin *et al.* 2003). Thus, mechanical loading *per se* is not the direct stimulus for bone remodeling.

Bone tissue is highly fluidic and interstitial fluid flow is involved in the mechanically induced activation of bone cells (Cowin & Cardoso 2015). Mechanical loading of the skeleton creates pressure gradients that expels interstitial fluid from areas of bone that are compressed and reuptakes the interstitial fluid once the compression ends (Piekarski & Munro 1977). Since bone is a porous tissue composed of a collagen–hydroxapatite matrix with a lacunar–canalicular network (Beno *et al.* 2006), compression and tension serves to transport interstitial fluid throughout bone (Piekarski & Munro 1977). Leaky capillaries within the marrow cavity are the source of this

Table 1 Comparison of skeletal muscle and femoral bone regional blood flows in the rat.

Rat characteristic	Tissue	Blood flow (mL/min/100 g)	Reference
Male Sprague–Dawley rats (399–564 g)	Soleus	95	Delp <i>et al.</i> (1999)
	Plantaris	16	
	Gastrocnemius		
	Red	54	Prisby <i>et al.</i> (2007)
	Middle	18	
	White	10	
	Tibialis anterior		
	Red	59	
	White	25	
Male Fischer-344 rats (4–6 months, 341 g)	Extensor digitorum longus	16	
	Femoral diaphyseal marrow	39	Prisby <i>et al.</i> (2007)
	Distal femoral metaphysis	29	
	Proximal femoral metaphysis	26	
	Femoral diaphyseal cortex	13	
Male Fischer-344 rats (22–24 months, 411 g)	Femoral diaphyseal marrow	21	Prisby <i>et al.</i> (2007)
	Distal femoral metaphysis	20	
	Proximal femoral metaphysis	18	
	Femoral diaphyseal cortex	11	

Values represent means.

fluid flow (Montgomery *et al.* 1988), as these vessels are the final recipient of blood supplied from the nutrient arteries. Nutrient arteries also modulate bone intramedullary pressure through the circulatory supply (Lam *et al.* 2010). Shear forces acting upon bone cell surfaces, as a result of interstitial fluid flow, provide the stimulus for mechanical loading-induced activation of bone cells and based upon the stimulus (or lack thereof) activates bone anabolic or catabolic responses (Cowin & Cardoso 2015).

Bone cells react to the interstitial fluid flow and pressure in the bone marrow and the lacunar–canalicular network of bone, which creates shear stress along their surface membranes. Shear stress stimulates the release of factors such as nitric oxide (NO) and prostaglandins (e.g., PGE₂) by bone-derived cells and organ cultures (Johnson *et al.* 1996, McAllister & Frangos 1999, Zaman *et al.* 1999, McAllister *et al.* 2000). For example, under 12 dynes/cm² shear stress, the release of adenosine triphosphate (ATP), NO and PGE₂ from rat primary osteoblasts was significantly higher vs static control conditions (Li *et al.* 2016). These factors can modulate bone cellular activity. For example, NO aids in osteoblasts differentiation (Riancho *et al.* 1995, Hikiji *et al.* 1997) and inhibits bone resorption by osteoclasts (Kasten *et al.* 1994). Further, PGE₂ prevented bone loss associated with hind limb immobilization and augmented trabecular bone to values higher than age-matched, control animals (Akamine *et al.* 1992). In fact, augmented wall shear stress induced by fluid flow enhanced NO release from osteoblastic cells but mechanical strain did not elicit the same response (Smalt *et al.* 1997). Under *in vivo* conditions, unloaded turkey ulnae subjected to oscillatory intramedullary fluid pressure loading in the absence of bone matrix strain demonstrated bone accrual in the diaphysis (Qin *et al.* 2003). Delivery of oscillatory intramedullary fluid pressure at 3 Hz for 3 weeks without accompanying bone matrix deformation of the ulna, which generated intramedullary pressures between 50 and 90 mmHg, was shown to increase cross-sectional lumen area (+14%, statistically non-significant), arterial wall area (+46%) and wall thickness (+28%) of the nutrient artery (Lam *et al.* 2010). Bone adaptation under a similar fluid pressure loading protocol occurred at 4 weeks (Qin *et al.* 2003). Thus, the outward hypertrophic remodeling of the ulnar nutrient artery, which is consistent with chronically increased pressure and blood flow experienced by vessels (Langille 1995, Osol & Mandala 2009), preceded or occurred concomitantly with the initiation of bone adaptation (Lam *et al.* 2010).

When subjected to regular physical activity, bone adapts so that improvements in bone mineral density and

bone health are observed (Modlesky & Lewis 2002). It must be noted that alterations in hormonal and neural activity occur with exercise. Thus, the adaptations in bone and bone blood vessels with physical activity reflect these influences as well. However, given the context of this section of the article, these influences will not be addressed. So how may the bone vascular system contribute to the improvements in bone health associated with physical activity? During low- and moderate-intensity treadmill exercise in canines, mean arterial pressure increased ($P > 0.05$; 21% and 39%, respectively) and vascular resistances were augmented in the sternum, rib, ilium, inferior femoral epiphysis and femoral diaphysis (Gross *et al.* 1979). Blood flow to these various bones and bone regions was preserved, presumably as a result of augmented mean arterial pressure, despite the rise in bone vascular resistance (Gross *et al.* 1979). The ilium was the only bone to experience declines in blood flow with exercise and, overall, these data suggest that vasoconstriction occurred in the bone vasculature (Gross *et al.* 1979). In contrast, blood flow to various hind limb bones and bone regions was augmented with 5 min of treadmill exercise in sedentary young and old rats (Dominguez *et al.* 2010) and in young sedentary and exercise-trained rats (Stabley *et al.* 2014) vs resting conditions. Mean arterial pressure did not differ between the young sedentary and exercise-trained animals at rest and during exercise (Stabley *et al.* 2014). Only blood flow to the femoral diaphysis in the exercised-trained rats was significantly higher than the sedentary group (Stabley *et al.* 2014). In other words, the sedentary animals were able to augment regional bone blood flow during acute exercise to similar degrees as the exercise-trained rodents (Stabley *et al.* 2014).

Altering bone blood flow during acute exercise occurs immediately via changes in vasodilation and/or vasoconstriction of bone blood vessels. Following chronic exercise, changes in bone blood flow occur via modifications in the functional properties of bone blood vessels and/or angiogenesis. In addition to the aforementioned processes, a third mechanism by which to increase bone blood flow is through the exercise-induced augmentation in systemic blood pressure for a given bone vascular resistance. Age-related declines ($P = 0.06$) in bone volume (−22%) were demonstrated in old (22–24 months) vs young (4–6 months) rats (Prisby *et al.* 2007). Declines in bone volume corresponded with diminished endothelium-dependent vasodilation of the femoral PNA, reduced NO bioavailability for the PNA and reduced femoral bone blood flow vs the younger counterparts (Prisby *et al.* 2007). Following 10–12 weeks of treadmill

exercise training, bone volume and NO-mediated, vasodilator capacity of the femoral PNA were enhanced in both the young and old groups (Dominguez *et al.* 2010). Given the measured strain magnitudes of rat femora and measured strain during compression and tension of rat tibiae with treadmill running (Indrekvam *et al.* 1991, Rabkin *et al.* 2001), alterations in strain magnitude during this treadmill protocol (Dominguez *et al.* 2010) were presumably lower than the minimum threshold needed for bone adaptation (Frost 1983a, 2001, Westerlind *et al.* 1997). Thus, augmented femoral bone blood flow elicited by daily bouts of treadmill exercise served to enhance vasodilator function and presumably caused alterations in bone interstitial fluid flow and pressure as well (Dominguez *et al.* 2010). Additionally, when comparing hind limb bone blood flow between rest and an acute bout of treadmill exercise (5 min) in sedentary and exercise-trained (10–12 weeks of treadmill exercise) rats, exercise hyperemia occurred in 33% and 78% of the examined bone regions in the sedentary and exercised-trained animals, respectively (Stabley *et al.* 2014). The authors concluded that exercise training delivers blood to more regions of bone as opposed to enhancing blood flow capacity (Stabley *et al.* 2014).

In regards to angiogenesis as a means to augment blood flow to bone following chronic exercise training, blood vessel number in the tibial metaphysis of young rats was augmented by 19% vs sedentary animals following 2 weeks of treadmill exercise (Yao *et al.* 2004). Bone mineral density and bone volume were augmented following 4 and 5 weeks, respectively; i.e., 2–3 weeks subsequent to the rise in bone vascularity (Yao *et al.* 2004). Interestingly, when bone angiogenesis was inhibited with administration of an anti-vascular endothelial growth factor antibody in conjunction with treadmill exercise training, bone mineral density, bone volume and blood vessel number were comparable to the sedentary group (Yao *et al.* 2004); i.e., the inhibition of the vascular adaptation to exercise prohibited the adaptation to bone, even in the face of mechanical perturbation. Similarly, bone capillarity in aged, exercise-trained rats was augmented following 8 weeks of swimming in comparison to aged control animals (Viboolvorakul *et al.* 2009). The interesting aspect of this study is that the non-weight-bearing activity of swimming minimized the mechanical loads placed upon the skeleton in comparison to weight-bearing exercise such as treadmill running. While swimming, the skeleton would have only experienced the mechanical loads associated with muscle contraction; however, alterations in bone vascular density were still

observed. Unfortunately, no parameters were reported for potential alterations in bone (Viboolvorakul *et al.* 2009).

In conclusion, the vascular system provides blood supply to the skeleton, which delivers nutrients, O₂, precursor cells, etc. and removes wastes products produced as a result of metabolism. Through its regulation of interstitial fluid flow and pressure, bone blood vessels also stimulate the release of various factors from bone and bone marrow cells to regulate their activity. As a result of chronic mechanical perturbation, the vascular system can contribute to bone accrual by supplying larger areas of the skeleton with blood flow either through improved vasodilator capacity of bone blood vessels and/or via angiogenesis. Further, the adaptations in the vascular system may precede changes occurring in bone.

Parathyroid hormone and parathyroid hormone-related peptide

After production and release from the parathyroid glands, parathyroid hormone (PTH) 1–84 circulates systemically. Parathyroid hormone-related peptide (PTHrP) is produced locally and acts at the tissue level (Takahashi *et al.* 1995). The main role of PTH 1–84 is to maintain serum calcium homeostasis, and this occurs via bone resorption and calcium retention (Kousteni & Bilezikian 2008). Continual release of PTH 1–84 or administration of PTH 1–34 and PTHrP 1–36 caused declines in bone mineral density and bone formation and increased bone resorption (Hansen *et al.* 2010, Horwitz *et al.* 2011); however, when administered intermittently (e.g., one injection per day), PTH causes bone gain (Stewart *et al.* 2000, Prisby *et al.* 2011, 2013). Further, the anabolic actions of intermittent PTHrP administration have been explored (Amizuka *et al.* 1996, Stewart *et al.* 2000, Horwitz *et al.* 2003, Miao *et al.* 2005, de Castro *et al.* 2012) and are under clinical consideration (Leder *et al.* 2015).

The enhanced bone metabolism associated with PTH-induced bone accrual during intermittent administration must be accompanied by augmented bone blood flow. Thus, the role of the bone vascular network in facilitating bone accrual is of importance to understand. Charbon and coworkers were the first to demonstrate the vasodilator effects of PTH on the systemic vasculature (Charbon *et al.* 1968, Charbon 1969, Charbon & Hulstaert *et al.* 1974) and PTHrP is theorized to regulate vascular tone (Takahashi *et al.* 1995). Vasodilation of bone blood vessels occurs in the presence of PTH; e.g., vasodilation in isolated rat femoral PNAs was observed to increasing concentrations

of PTH 1–84, PTH 1–34 and PTHrP 1–34 (Benson *et al.* 2016). Additionally, bolus doses of PTH have produced a rise (Kapitola & Zák 2003, Gohin *et al.* 2016), a biphasic response (Boelkins *et al.* 1976) and no change (Driessens & Vanhoutte 1981) in blood flow to various hind limb bones. In regards to the chronic influences of intermittent PTH administration on bone blood vessels, the picture becomes more complex. As mentioned previously, blood flow can be augmented via the vasomotor activity of resistance vessels and via angiogenesis. Thus, PTH acts upon the functional and/or morphological properties of bone blood vessels.

Several reports in the literature have measured various bone vascular parameters following intermittent PTH administration to address these questions. Bone angiogenesis was not observed following 15 and 30 days of intermittent PTH 1–84 administration in young rats (Prisby *et al.* 2011). In fact, bone vessel density was lower in PTH-treated vs control animals (Prisby *et al.* 2011). Interestingly, the smallest bone marrow blood vessels in PTH-treated rats were closer to sites of new bone formation (Prisby *et al.* 2011), which is theorized to direct blood flow where needed the most and facilitate nutrient exchange between the vascular system and bone. Additionally, 15 days of intermittent PTH 1–84 administration enhanced endothelium-dependent vasodilation of the femoral PNA via an upregulation of NO signaling (Prisby *et al.* 2013). Thus, in addition to modifying the spatial location of bone marrow blood vessels (Prisby *et al.* 2011), PTH treatment modified the functional properties of the bone vasculature and enhanced the sensitivity of the vascular endothelium to agonists other than PTH (i.e., acetylcholine, ACh) (Prisby *et al.* 2013). Such endothelial cell modifications potentially enable bone blood vessels to enhance vasodilation in response to a myriad of locally released factors that mediate action through NO signaling and deliver more blood flow to bone. Further, this investigation demonstrated a relation between PTH-induced trabecular bone accrual and peak endothelium-dependent vasodilation, illustrating that PTH may influence bone mass via NO signaling in vascular endothelial cells (Prisby *et al.* 2013).

Enhanced blood flow following intermittent PTH administration has been documented. For example, post-menopausal women with osteoporosis experienced increased uptake of radiopharmaceutical technetium-99m methylene diphosphonate (^{99m}Tc -MDP) in the whole skeleton and to the calvarium, mandible, spine and upper and lower extremities following 18 months of teriparatide treatment (Moore *et al.* 2010). The presence of ^{99m}Tc -MDP

in the skeleton serves as a clinical indicator of bone blood flow (Blake *et al.* 2001). In a mouse model, trabecular bone volume in the distal femoral metaphysis and L5 vertebra and hind limb tissue perfusion were not altered following 4 weeks of intermittent PTH 1–34 administration (Gohin *et al.* 2016). Caution must be used in the interpretation of these data since the hind limb tissue perfusion measurements were not limited to the skeleton. Acute rises in bone perfusion (i.e., within 5–15 min) were observed in the whole hind limb as measured by laser Doppler imaging and in the cortical diaphysis as assessed by the augmented presence of procion red fluorescence following an injection of PTH (Gohin *et al.* 2016). Unfortunately, this measure was not obtained following the 4-week protocol when cortical bone volume and bone formation rate were enhanced (Gohin *et al.* 2016). The PTH-induced rise in hind limb tissue perfusion and enhanced cortical bone volume were blocked and blunted, respectively, with NO inhibition, highlighting the importance of this signaling cascade (Gohin *et al.* 2016). These data coincide with previous descriptions of the reliance on NO signaling for bone blood vessels (Prisby *et al.* 2013), bone blood flow (Kapitola & Zák 2003) and bone (Prisby *et al.* 2013) with PTH treatment. Fourteen days of intermittent administration of PTH 1–84 in mice augmented blood vessel number (+16% and +34%, respectively) and mean vessel volume (+70% and +45%, respectively) in the tibial metaphysis and diaphysis and increased tibial perfusion by 27% vs controls (Roche *et al.* 2014).

Interestingly, the two mouse studies highlight the independent influence of PTH on bone blood vessels and bone. Alterations in bone perfusion and bone vascular parameters were observed at time points when bone parameters were unaltered (Roche *et al.* 2014, Gohin *et al.* 2016). However, rapid bone cellular activities following an injection of PTH have been observed. Fifteen minutes following PTH (50 µg/kg) administration to 7-day-old postnatal rats, mast cells on bone surfaces underwent degranulation and by 1-h osteoclast numbers had risen (Nakamura *et al.* 1996). Additionally, the ruffled border and clear zone of the osteoclasts were augmented at 30 min and 30–90 min, respectively, in thyroparathyroidectomized rats following administration of 50 U of purified bovine PTH (Holtrop *et al.* 1979). Osteoclast cell size was augmented by 90 min (Holtrop *et al.* 1979). In contrast, rats with an intact thyroid and parathyroid glands demonstrated no change in osteoclast size or ruffled border area with PTH but the clear zone was augmented at minute 30 (Holtrop *et al.* 1979). Six hours following injection of parathyroid extract, osteoblasts demonstrated

numerous dense bodies and swollen mitochondria (Cameron *et al.* 1967). However, uncertainty exists as to whether these changes translate into physiological activity or were a result of toxicity from the large dose parathyroid extract (4 units per gram) (Cameron *et al.* 1967). Data regarding the kinetic activities of osteoblasts during earlier time points following PTH injection are lacking. Regardless, augmented bone perfusion is observed rapidly (i.e., within 5–15 min) following acute injections of PTH (Kapitola & Zák 2003, Roche *et al.* 2014, Gohin *et al.* 2016) as opposed to the 15-min-to-6 h delay for mast cells, osteoclasts and osteoblasts (Cameron *et al.* 1967, Holtrop *et al.* 1979, Nakamura *et al.* 1996), leaving one to speculate the involvement of bone blood vessels in the paracrine activation of bone cells.

Overall, PTH is a powerful stimulator of the bone vascular network in which it alters the spatial location of bone marrow blood vessels, enhances the vasodilator capacity of bone blood vessels, may elicit bone angiogenesis and augments bone blood flow, either after acute or chronic administration. It would be beneficial to ascertain whether intermittent PTH administration serves to deliver blood flow to more regions of bone tissue as reported following long-term (18 months) treatment in humans (Moore *et al.* 2010) and subsequent to chronic (10–12 weeks) exercise training in rats (Stabley *et al.* 2014). Additionally, data indicate independent actions of PTH on bone blood vessels and bone, whereby the effects may occur more rapidly in the vasculature. Finally, NO is an important regulator utilized by PTH to exert its actions on both blood vessels and bone.

Estrogen

Estrogen regulates bone metabolism in females and males and bone accrual during puberty is driven by 17 β -estradiol (Khalid & Krum 2016). The maintenance of bone mineral density is also dependent upon estrogen, particularly 17 β -estradiol, that interacts with the estrogen receptors α and β (Khalid & Krum 2016). Several mechanisms by which estrogen serves to build or protect bone mass have been suggested. For example, estrogen induced osteoclast apoptosis (Kameda *et al.* 1997, Kousteni *et al.* 2002). Additionally, the effects of estrogen on bone formation includes increased protein content for the estrogen receptors α and β , osteoprotegerin (i.e., the decoy receptor for receptor activator of nuclear factor K-B ligand (RANKL)) and RANKL (Bord *et al.* 2003). Estrogen also inhibits the

apoptosis of osteoblasts and osteocytes (Kousteni *et al.* 2001). Estrogen deficiency is linked with low bone mass (Nilas & Christiansen 1987), as plasma levels of estrogen correspond with low bone mass or bone loss (Johnston *et al.* 1985, Wronski *et al.* 1989). Following menopause, the rapid phase of bone loss occurs within 5–10 years; however, bone loss and fracture are mitigated by estrogen replacement (Ettinger *et al.* 1985).

Estrogen receptors are present on endothelial cells (Brandi *et al.* 1993) and, therefore, the influence of estrogen deficiency on bone blood vessels must be considered in regards to bone metabolism. The role of the vascular system in relation to bone homeostasis, as dictated by estrogen, can be deciphered by examining the effects of estrogen loss and replacement. Estrogen deficiency influences the vasomotor activity of bone blood vessels and/or alters their quantity in bone. Negative alterations in one or both will ultimately affect the delivery of blood to the skeleton. Bone loss with estrogen deficiency has been examined by several investigators who sought to determine the associated bone vascular consequences; however, the data have provided mixed results. In some investigations, estrogen deficiency via ovariectomy (OVX) served to (1) diminished trabecular bone volume and area, bone mineral density, bone biomechanical properties, erythropoietic marrow area, vascular density, vascular volume, immunohistochemical expression of vascular endothelial cell growth factor (VEGF) in bone, and bone perfusion and (2) increased the area of sinusoidal capillaries and marrow fat cell area (Laroche *et al.* 1996, Griffith *et al.* 2010, Ding *et al.* 2011). The area of sinusoids in the marrow coincided with bone resorption ($r=+0.46$), trabecular bone volume ($r=-0.54$) and trabecular number ($r=-0.60$) (Laroche *et al.* 1996). Interestingly, 15 days following OVX in mice, trabecular bone volume was not diminished but tibial bone perfusion and vascular density and volume in the marrow were reduced (Roche *et al.* 2014). Treatment with bisphosphonates prevented bone loss (Laroche *et al.* 1996) and attenuated the declines in bone perfusion (Roche *et al.* 2014) with OVX, but did not alter bone vascular density, volume and blood vessel size (Laroche *et al.* 1996, Roche *et al.* 2014). In contrast, OVX and orchidectomy (i.e., castration) in rats demonstrated increased bone blood flow (Kapitola & Kubícková 1990, Egrise *et al.* 1992, Kapitola *et al.* 1994) with enhanced bone turnover and bone loss (Kapitola & Kubícková 1990, Egrise *et al.* 1992, Kapitola *et al.* 1994) and diminished bone density and ash weight (Kapitola & Kubícková 1990, Kapitola *et al.* 1994) in young rats.

Sequentially, the physiological events following OVX demonstrate that alterations in bone vascular parameters occur more rapidly than bone parameters. For example, mRNA expression of VEGF 120 and 164 (–15% and –19%, respectively), endothelial and inducible NO synthase (–38% and –34%, respectively), and fibroblast growth factor-2 (–18%) in the proximal tibial metaphysis were reduced 3 days following OVX (Mekraldi *et al.* 2003). Declines in bone blood vessel number occurred at 7 days and reduced trabecular bone volume did not occur until day 14 (Mekraldi *et al.* 2003). Vascular endothelial growth factor and fibroblast growth factor-2 are angiogenic factors and NO synthase is responsible for the production of NO. Similarly, vascular density and volume were reduced by ~13–15%, bone vascular resistance augmented by ~36% and tibial perfusion reduced by 28% vs sham-operated controls following 15 days of OVX in C57BL/6 mice; i.e., at time points when trabecular bone volume and cortical thickness in the tibia were unaltered (Roche *et al.* 2013, 2014). Collectively, these data suggest that estrogen deficiency influences the bone vascular system prior to impacting bone.

Data regarding enhanced vasoconstrictor and diminished vasodilator activity of bone blood vessels support the results in rats and mice demonstrating declines in bone perfusion with estrogen loss (Griffith *et al.* 2010, Roche *et al.* 2014). Six weeks after bilateral OVX in rabbits, force development in femoral metaphyseal arteries was significantly enhanced following stimulation with norepinephrine and endothelin-1, suggesting increased vasoconstrictor responsiveness of these vessels. However, no changes in force development were observed in diaphyseal arteries of the femur (Hansen *et al.* 2001). Additionally, OVX did not alter vasodilator responsiveness to ACh or substance P in metaphyseal and diaphyseal arteries (Hansen *et al.* 2001), indicating that vasodilator mechanisms are unaltered. Unfortunately, no bone parameters were reported.

In addition, OVX in young (4–6 months) rats reduced ACh-mediated, endothelium-dependent vasodilation of the femoral PNA and reduced femoral trabecular bone volume (Prisby *et al.* 2012). Endothelium-dependent vasodilator capacity (Prisby *et al.* 2012) and blood flow responses (Kapitola *et al.* 1997) following OVX were related to the ability of endothelial cells to regulate vasodilation via NO-mediated signaling. Similar to the bone data in OVX mice (Roche *et al.* 2013, 2014), trabecular bone volume and endothelium-dependent vasodilation in old (22–24 months) rats were not altered by OVX (Prisby *et al.* 2012). Strong relations were established, however, between peak endothelium-dependent vasodilation of the femoral

PNA and trabecular bone volume in both young and old animals (Prisby *et al.* 2012). In other words, rats with high vasodilator capacity also demonstrated high trabecular bone volume in the distal femur (Fig. 2). Interestingly, animals with low circulating estrogen (i.e., young OVX, old intact and old OVX) displayed poor associations between trabecular bone volume and plasma estrogen levels and uterine mass, highlighting that circulating estrogen has a minimal effect on trabecular bone mass in these groups (Figs 3 and 4A and B). However, strong relations were observed between peak endothelium-dependent vasodilation and trabecular bone volume in these low estrogen animals (Prisby *et al.* 2012). Thus, bone vascular function, as mediated by endothelial NO signaling, is coupled more to bone volume than estrogen status (Figs 3 and 4C).

The importance of NO-mediated vasodilation with the loss of estrogen is also highlighted in an investigation examining vasomotor responsiveness in large conduit vessels (i.e., aorta and femoral artery). Following 8 weeks of OVX, ACh-mediated, endothelium-dependent relaxation was impaired and phenylephrine-induced contraction was augmented in OVX vs control rats (Griffith *et al.* 2010). Thus, large conduit vessels external to bone also demonstrate reduced relaxation and enhanced contraction with estrogen deficiency, illustrating the global effects of this hormone. The differences in responsiveness to phenylephrine between OVX and control rats disappeared in the presence of the NO synthase inhibitor, L-NAME and no differences were observed between the groups in endothelium-independent relaxation to the NO donor, sodium nitroprusside (Griffith *et al.* 2010). Even though conduit vessels (i.e., aorta and femoral artery) were examined in this investigation, results correspond with bone blood vessel data demonstrating a reliance on NO-mediated, endothelium-dependent vasodilation and no change in vascular smooth muscle cell responsiveness with OVX (Prisby *et al.* 2012). Further, these data demonstrate that the effects of estrogen deficiency are systemic and not localized to bone blood vessels. Thus, bone loss with estrogen deficiency may result directly from bone cellular dysfunction or from dysfunction within the vascular system.

Estrogen replacement with OVX reverses the changes in bone and vascular parameters. For example, estrogen repletion with OVX maintained (young rats) and augmented (old rats) endothelium-dependent vasodilation and trabecular bone volume (Prisby *et al.* 2012). Correspondingly, administration of 17 β -estradiol to OVX animals restored the observed declines in

Figure 2

Regression analyses between peak endothelium-dependent vasodilation (%) of the rat femoral PNA and trabecular bone volume (%) of the distal femur in (A) young and (B) old ovariectomized (OVX) and estrogen-replaced (E2) Fischer-344 rats. The regression analyses were $P < 0.001$ and $P < 0.001$ in (A) young and (B) old rats, respectively. Values are means $\pm$ s.e. Adapted, under the terms of the Creative Commons Attribution License, from [Prisby et al. \(2012\)](#).

mRNA expression of VEGF 120 and 164, bone blood vessel number and bone volume in the proximal tibial metaphysis ([Mekraldi et al. 2003](#)). When given in supraphysiological doses, massive bone formation occurred in the epiphysis, metaphysis and diaphysis of tibiae and femora from immature female and male mice; yet, the vascular pattern within the bone was unaltered ([Brookes & Lloyd 1961](#)). These data suggest that estrogen repletion enhances the vasodilator capacity of bone blood vessels. However, administration of estradiol benzoate in intact and OVX rats diminished blood flow to the tibia and enhanced bone density and ash weight in the OVX group only ([Kapitola & Kubicková 1990](#)).

Figure 3

Scattergrams illustrating the relation between (A) plasma estrogen, (B) uterine mass and (C) peak endothelium-dependent vasodilation of the rat femoral PNA. Bone volume (BV/TV, %) was not correlated to (A) plasma estrogen nor (B) uterine mass in young Fischer-344 rats with low estrogen status (i.e., ovariectomized (OVX)). However, BV/TV and (C) peak vasodilation of the femoral PNA were significantly ($P < 0.001$) and positively related. Adapted, under the terms of the Creative Commons Attribution License, from [Prisby et al. \(2012\)](#).

Figure 4

Scattergrams illustrating the relation between (A) plasma estrogen, (B) uterine mass and (C) peak endothelium-dependent vasodilation of the rat femoral PNA. Bone volume (BV/TV, %) was not correlated to (A) plasma estrogen nor (B) uterine mass in old Fischer-344 rats with low estrogen status (i.e., ovariectomized (OVX) and old age). However, BV/TV and (C) peak vasodilation of the femoral PNA were significantly ($P < 0.001$) and positively related. Adapted, under the terms of the Creative Commons Attribution License, from [Prisby et al. \(2012\)](#).

Given the totality of data with estrogen deficiency and repletion, a clear picture does not emerge in regards to the direction of influence estrogen has on bone blood flow and bone vascular responses. However, the data seem to indicate that bone vascular changes occur at a rapid rate as opposed to the delayed responses in bone.

Vitamin D

Normal development and the maintenance of the skeleton is reliant upon vitamin D ($25(\text{OH})\text{D}_3$), such that the active form, $1\alpha,25(\text{OH})_2\text{D}_3$, is involved in calcium regulation and bone homeostasis via endocrine and autocrine functions ([Siddiqui & Partridge 2016](#)). In addition to vitamin D levels being associated with bone mineral density ([Bischoff-Ferrari et al. 2004](#)), $1\alpha,25(\text{OH})_2\text{D}_3$ is a treatment for post-menopausal osteoporosis ([Dong et al. 2013](#)). For example, calcitriol, a compound of vitamin D, augmented trabecular bone mass and cortical thickness in sham-operated and OVX rats ([Faugere 1986](#)).

Among other functions, vitamin D is theorized to regulate vascular tone ([Dalan et al. 2014](#)) and vitamin D receptors (VDRs) are present on endothelial and smooth muscle cells ([Andrukhova et al. 2014](#)). Knockout of the VDR receptor in young mice reduced NO bioavailability and NO synthase 3 gene expression and protein content ([Andrukhova et al. 2014](#)). The enzyme ($25(\text{OH})\text{D}_3$ - 1α -hydroxylase) that converts circulating, inactive $25(\text{OH})\text{D}_3$ to active $1\alpha,25(\text{OH})_2\text{D}_3$ is present in human renal endothelial cells, post-capillary venules from lymphoid tissue and in human umbilical vein endothelial cells ([Zehnder et al. 2002](#)). Further, $1\alpha,25(\text{OH})_2\text{D}_3$ transcriptionally regulates endothelial NO synthase ([Andrukhova et al. 2014](#)), providing evidence for an autocrine effect on vasodilation.

In humans, brachial flow-mediated dilation (i.e., a measure of NO-related endothelial cell function) was diminished in vitamin D-deficient, type 2 diabetic patients ([Yiu et al. 2011](#)). However, supplementation with vitamin D to improve endothelial cell function has produced equivocal results. Investigations in humans and animals have reported improved ([Harris et al. 2011](#), [Dong et al. 2013](#)) or no change in ([Gepner et al. 2012](#), [Yiu et al. 2013](#)) endothelium-dependent vasodilation with vitamin D supplementation. Given the dual role of vitamin D on the vascular and skeletal systems, it is plausible that this hormone would influence bone blood vessels and regulate bone blood flow. To date, examination of acute or chronic effects of vitamin D on bone vascular function has not been conducted.

Calcitonin and calcitonin gene-related peptide

Calcitonin is a hormone produced in the thyroid gland and, due to its anti-resorptive actions, is FDA approved for the treatment of osteoporosis and diseases with high bone turnover (Naot & Cornish 2008, Khosla 2011). Calcitonin gene-related peptide (CGRP) is produced as a result of alternative splicing of the calcitonin gene at the tissue level (Siddiqui & Partridge 2016) and is released from nerve terminals (Hill & Elde 1988). Both peptides bind to the CT receptor (Inzerillo *et al.* 2002). While not observed on osteoblasts (Naot & Cornish 2008), the CT receptor is present on osteoclasts (Ikegame *et al.* 1995) and perhaps osteocytes (Gooi *et al.* 2010). Calcitonin was shown to inhibit apoptosis of osteoblasts and osteocytes (Plotkin *et al.* 1999), demonstrating a potential indirect influence on bone formation. Calcitonin gene-related peptide activates CT receptors on osteoclasts (Zaidi *et al.* 1987) to reduce their activity and diminish bone resorption (Zaidi *et al.* 1987). The net effect enhances osteogenesis, mineralization and calcium uptake into bone (Bernard & Shih 1990, Ancill *et al.* 1991). However, mice deficient in α CGRP or calcitonin demonstrated low and high bone masses, respectively (Schinke *et al.* 2004) and overexpression of osteoblastic CGRP in mice augmented trabecular bone density and volume in the femoral metaphysis (Ballica *et al.* 1999). Calcitonin gene-related peptide is localized in nerve fibers (Hill & Elde 1988) observed between trabeculae in the epiphysis and at the epiphyseal plate (Shih & Bernard 1997). These nerve fibers are also located in Haversian and Volkmann canals (Shih & Bernard 1997); i.e., the channels for blood vessels in the cortical diaphysis. Thus, CGRP is uniquely positioned to regulate vasomotor activity.

Bone resistance arteries are responsive to the same factors that induce bone accrual and remodeling. For example, data confirm that CGRP elicits vasodilation in rat femoral PNAs, whereby modest vasodilation (33%) occurred to increasing concentrations (RD Prisby, unpublished observations). Similarly, relaxation to CGRP was observed in pre-constricted arterial segments from cancellous bone of pigs (Lundgaard *et al.* 1997). However, not all species are responsive, such that bone vascular resistance in rabbits was unaltered by CGRP (Vendéghe *et al.* 2010). Further, tibial (–33%) and femoral (–34%) marrow flow velocities and systemic blood pressure (–26%) were reduced following administration of CGRP (Vendéghe *et al.* 2010). The authors concluded that since marrow vascular resistance remained unchanged, the declines in

bone perfusion may have resulted from the shunting of blood from bone to other tissue beds (e.g., muscle, skin and fat) undergoing active vasodilation (Vendéghe *et al.* 2010). Given the alterations in bone cellular activity in the presence of CGRP (Hohmann *et al.* 1983, Zaidi *et al.* 1987, Bernard & Shih 1990, Zheng *et al.* 2016), its ability to induce vasodilation (RD Prisby, unpublished observations; Lundgaard *et al.* 1997) at the level of the resistance vessel is suggestive of a role in regulating bone blood flow.

In regards to calcitonin, the vasoconstrictor and vasodilator influences on the bone vasculature is complex. For example, calcitonin elicited dose-dependent increases in perfusion pressure, perhaps via direct actions on vascular smooth muscle cells to induce vasoconstriction (Driessens & Vanhoutte 1981) and calcitonin administered for 7 days and 7 weeks diminished skeletal blood flow in patients with Paget's disease (Wootton *et al.* 1978). In contrast, calcitonin administered for 30 days had no effect in reducing the elevated tibial and femoral bone blood flow induced by paraplegia in rats; however, it did augment femoral blood flow in intact control animals (Schoutens *et al.* 1988). Correspondingly, the paraplegic rats treated for 30 days with calcitonin demonstrated augmented tibial bone volume, periosteal apposition rate and calcium accretion in comparison to paraplegic rats with no treatment (Schoutens *et al.* 1988).

Taken together, the vasomotor effects of calcitonin on the bone vascular system is difficult at this point to ascertain. The vasoconstrictor influence of calcitonin on the bone vascular system (Wootton *et al.* 1978, Driessens & Vanhoutte 1981) appears divergent from its role as a treatment for osteoporosis. Further, CGRP released from nerve terminals or produced locally may provide vasodilator cues and stimulate bone formation. The data are divergent, however, and additional work is warranted.

Metabolic byproducts

Mechanical and hormonal factors stimulate the release of VEGF from osteoblasts to ultimately augment blood flow and the delivery of O₂, nutrients and precursor cells for bone formation. For example, osteoblast-derived VEGF-initiated repair of small bone defects via angiogenesis and inflammation (Hu & Olsen 2016) and, along with Hif-1 α and Hif-2 α , VEGF was reduced in the distal femur of OVX mice (Zhao *et al.* 2012). Further, VEGF was augmented at the tibial growth plate in juvenile mice with administration of 1 α ,25(OH)₂D₃ (Lin *et al.* 2002) and its variants were

produced by low and high mechanical deformations in human osteoblasts (Faure *et al.* 2008). Thus, under the control of bone anabolic agents, bone cells are capable of enhancing blood flow via angiogenesis. What should also be considered is the regulation of bone blood flow, not via angiogenesis, but by enhanced vasodilation or reduced vasoconstriction of nearby blood vessels as a result of bone cellular metabolism.

Metabolic vasodilation is necessary for delivering blood to tissues such as skeletal muscle and the heart (Rowell 1993, Barvitenko *et al.* 2013), particularly in the face of increased metabolism. In reference to dynamically active skeletal muscle, many factors released into the interstitial space produce vasomotor activity of the nearby vasculature. These factors (e.g., hydrogen ions, carbon dioxide, adenosine nucleotides, potassium, phosphate ions, lactate, intermediates of the tricarboxylic acid cycle, vasoactive peptides, etc.), in addition to changes in the partial pressure of oxygen (pO_2), directly and indirectly stimulate vasodilation (Rowell 1993). They represent the various means by which metabolism serves to augment tissue perfusion. The effects produced on the vasculature by these factors can occur independently; however, collectively they have a far stronger influence (Rowell 1993).

Similar to skeletal muscle, which increases its metabolic activity through movement, the metabolic activity of bone cells is augmented during cellular differentiation, and during processes such as bone resorption and formation. Bone cells produce many of the metabolic byproducts observed in active skeletal muscle (e.g., carbon dioxide, lactate, hydrogen ions, phosphate ions, adenosine diphosphate (ADP) and citric acid) (Borle *et al.* 1960a, Karner & Long 2016). For example, differentiating osteoblasts rely heavily upon glucose utilization (Esen *et al.* 2013, 2015) and produce lactate as a result (Felix *et al.* 1978). During osteoclastogenesis, mitochondrial mass within the cell is high and the formation of osteoclasts correlates to elevated rates of oxidative phosphorylation, as reviewed by Lemma and coworkers (2016). Yet, during bone resorption, glycolysis is the predominant pathway (Lemma *et al.* 2016). In addition, bone cells have the capacity to utilize amino and fatty acids (Karner & Long 2016). Fatty acids undergo β -oxidation when they enter the cell, producing acetyl-coA for the tricarboxylic acid cycle (Karner & Long 2016). Since metabolic activity is continual in bone and bone marrow cells, we must underscore how these factors released during cellular differentiation and bone formation and resorption contribute to the local regulation of bone blood flow.

The regulation of metabolic activity during bone formation and resorption is heavily dependent upon the pO_2 at the bone surface and the production of acids, primarily lactate, that allows for continued bone degradation (Borle *et al.* 1960b). Bone marrow pO_2 is ~7% in normal, healthy individuals (Harrison *et al.* 2002). Altering pO_2 to mimic physiological and pathophysiological conditions has revealed cell responsiveness to these changes. For example, reducing pO_2 to 2% in a 7-day mouse marrow culture augmented the number (+3.2-fold) and activity (+21-fold) of osteoclasts (Arnett *et al.* 2003). Additionally, bone nodule formation by osteoblasts was reduced (–10-fold) and almost completely inhibited as a result of moderate (1–2% O_2) and severe (0.2% O_2) hypoxia, respectively (Utting *et al.* 2006). Reduced pO_2 is a powerful stimulator for vasodilation in other vascular beds (Spilk *et al.* 2013) and no doubt produces similar responses in the bone vascular system. In order to rapidly restore bone pO_2 , vasodilation of bone blood vessels would be the foremost response. Reduced pO_2 is usually accompanied by an acidic environment in bone. The production of acids, lactate in particular, during enhanced metabolism serves to lower bone pH. *In vitro*, resorption pit area on cortical bone slices was increased (+14-fold) with a 0.6 decline in pH of the cell culture medium (Arnett & Dempster 1986) and reducing pH from 7.08 to 6.82 increased resorption of dentine disks by osteoclasts (Hoebertz *et al.* 2001). Reduced pO_2 and pH in bone enhances metabolic activity in osteoclasts and, with augmented metabolic activity, a rise in bone blood flow is requisite.

Systemic alterations in blood gases and pH were demonstrated to regulate bone blood flow. For example, when hypercapnia (i.e., carbon dioxide retention; 65 ± 1 mmHg) and acidosis (7.14 ± 0.02 units) were introduced to anesthetized canines, blood flow to the sternum, rib and femoral marrow were augmented 166, 67 and 233%, respectively (Gross *et al.* 1979). Further, reduced pH or increased partial pressure of carbon dioxide in bone (Brookes 1966, Brookes & Revell 1998) was theorized as the stimuli for bone accrual following long-term femoral vein ligation (Brookes 1966, Brookes & Singh 1972). Additional metabolic byproducts elicit changes in bone cell metabolism and vascular responses. For example, extracellular ADP and ATP produced significant increases in pit formation with acidified cell culture medium and induced the formation of osteoclasts on dentine and ivory disks; however, adenosine and adenosine monophosphate (AMP) had

no effect (Morrison *et al.* 1998, Hoebertz *et al.* 2001). Introduction of adenosine into the brachial vein of conscious dogs reduced vascular resistance and augmented blood flow to the sternum by ~37%; however, the adenosine also reduced arterial pressure by 29% (Gross *et al.* 1979).

While not all metabolic byproducts have been examined, data illustrate how alterations in their concentrations stimulate osteoclasts to resorb bone and induce vasodilation of the local vasculature to augment blood flow. As mentioned previously, enhanced bone blood flow augments bone interstitial fluid flow and pressure to stimulate the release of paracrine and autocrine factors from bone cells. Additionally, augmented shear stress on vascular endothelial cells elicit release of paracrine cues. Thus, we can begin to appreciate the integrative nature of the vascular and skeletal systems, whereby augmented metabolism in the form of catabolic activity may quickly lead to bone anabolism by participation of the blood vessel at the core of bone basic multicellular unit or bone remodeling compartment (Fig. 5). The close proximity of

blood vessels to osteoclasts and osteoblasts allows for this intimate bi-communication.

The vascular endothelium

As presented in the previous section, early studies speculated that bone modulates the blood flow that it receives via the production of metabolic byproducts (Davis 1992). We now know bone cells release a myriad of factors that modulate blood vessel function (Alam *et al.* 1992, Delany & Canalis 1997, Ishida *et al.* 2002, Brandi & Collin-Osdoby 2006, Li *et al.* 2016). What is not greatly appreciated is the reciprocal relation whereby bone blood vessels release factors (e.g., insulin-like growth factor-1, endothelin-1, prostacyclin, RANKL, interleukin-11, PGE₂, NO, ATP, ADP, AMP, adenosine, etc.) that stimulate bone cellular activity (Alam *et al.* 1992, Fiorelli *et al.* 1994, Shinozuka *et al.* 1994, Zhang *et al.* 1998, Kage *et al.* 1999, Ishida *et al.* 2002, Brandi & Collin-Osdoby 2006, Gonzalez *et al.* 2015, Wen *et al.* 2015, Gutterman *et al.* 2016).

Figure 5

A sequential scenario by which metabolic activity leads to enhanced vasodilation of the surrounding arterioles and arteries. The schematic represents the bone remodeling compartment with trabecular bone containing osteocytes and the neighboring marrow occupied by osteoclasts, osteoblasts, bone lining cells and the vascular network (i.e., arteriole, capillary and venule). Panel A serves to orient the reader to the various cells and structures within this schematic. Vascular smooth muscle cells were omitted from the arteriole and venule for simplicity, thus, the cells being depicted are vascular endothelial cells. Panel B depicts osteoclasts actively resorbing bone (1). During the course of enhanced metabolism, the osteoclasts release factors (e.g., carbon dioxide (CO₂), hydrogen ion (H⁺), phosphate (PO₄), ADP, lactate, etc.) that diffuse to the arteriole and initiate metabolic vasodilation (2). The metabolic vasodilation of the arteriole causes ascending vasodilation of the feed and conduit arteries upstream (not depicted). This process is called Conducted Vasodilation and ensures a rise in blood flow to the surrounding tissue undergoing metabolism. Panel C illustrates how vasodilation and the subsequent rise in blood flow (3) augments filtration and pressure from the capillary (4) into the bone interstitial space (5). The increased bone interstitial pressure and fluid flow generate shear stress on bone cells (Panel D, 6). Shear-mediated release of PGE₂ and NO from osteoblasts (7) serves to enhanced osteoblast activity and reduced osteoclast activity, slowing down bone degradation and ramping up bone formation. In addition, enhanced blood flow as a result of conducted vasodilation augments shear stress on vascular endothelial cells (Panel E, 8). As a result, vascular endothelial cells release factors (e.g., NO, PGE₂, prostacyclin (PGI₂)) that diffuse into the bone interstitial space to stimulate osteoblast activity and inhibit osteoclast activity (9). The theories of vascular contribution to enhanced bone formation were previously put forth by the laboratory of Michael Delp (Colleran *et al.* 2000). Note that these processes do not have to begin with osteoclast activity. For example, circulating factors that induce vasodilation in the bone vascular network will serve to commence similar processes.

In order to meet the metabolic needs of various tissues with an adequate supply of blood, the arterial microcirculation regulates vascular resistance to control tissue blood flow, as reviewed by [Guterman *et al.* \(2016\)](#). Only ~7% of body volume is occupied by the microcirculation; however, it is precisely distributed to provide nutrient delivery and remove waste products from every cell ([Guterman *et al.* 2016](#)). Optimal microcirculatory function maintains tissue health because it provides blood flow, is the largest paracrine producer in the body, and as a result, modulates the function of the surrounding parenchyma ([Guterman *et al.* 2016](#)). When factors are released from the endothelium, they travel in an abluminal direction to vascular smooth muscle cells to elicit a physiological response (e.g., hypertrophy, fibrosis or vasomotor activity) ([Guterman *et al.* 2016](#)). Additionally, these factors pass beyond the vascular smooth muscle

cell layer in arteries and easily escape the endothelial cell layer of capillaries, eventually infiltrating the surrounding tissue ([Guterman *et al.* 2016](#)). Thus, vascular endothelial and smooth muscle cells provide paracrine cues to the underlying parenchyma ([Guterman *et al.* 2016](#)).

Endothelial cells have the ability to release ATP ([Vizi *et al.* 1992](#), [Shinozuka *et al.* 1994](#)). As discussed in the previous section, ATP and ADP influence osteoclast activity ([Morrison *et al.* 1998](#), [Hoebertz *et al.* 2001](#)) and adenosine reduced vascular resistance and increased blood flow to the sternum ([Gross *et al.* 1979](#)). Cortical blood vessel- and osteocyte-derived endothelial NO synthase was augmented rapidly after fracture in rat tibiae and was assumed to contribute to early increases in blood flow to the fracture site ([Corbett *et al.* 1999](#)). Targeted disruption of the NO gene reduced bone mineral density and bone volume, cortical thickness, predicted biomechanical

Figure 6
μCT imaging of young and old rat femora. Panels represent cross-sections of the mid-diaphysis and frontal and sagittal views from (A) young (4–6 months) and (B) old (22–24 months) male, Fischer-344 rats, respectively. The arrows denote ossified blood vessels evident in the diaphyseal marrow of the old femur. Femora were imaged with non-contrast CT using a MicroCAT II CT scanner (Imtek Inc.; Siemens), yielding reconstructed voxels of 103 × 103 × 103 μm. X-rays were generated at 80 kVp and 500 mA.

strength and bone formation rate in 6- to 9-week-old endothelial NO synthase knockout mice (Aguirre *et al.* 2001, Armour *et al.* 2001); however, these changes were corrected or not observed by 12 weeks (Aguirre *et al.* 2001, Sabanai *et al.* 2008). These data highlight the importance of NO in early bone development.

In addition to NO, vascular endothelial cells release endothelium-dependent hyperpolarizing factors, which cause vasodilation and enhances blood flow in numerous tissues (Barvitenko *et al.* 2013). Prostaglandin E₂ released from endothelial cells may serve to augment bone mass (Akamine *et al.* 1992) and insulin-like growth factors, also released from blood vessels, are important for bone growth and development (Baker *et al.* 1993). Several other chemokines, matrix molecules, cytokines and growth factors released by blood vessels may regulate a variety of bone cellular activities (e.g., recruitment, proliferation, differentiation, etc.) necessary for function and survival (Brandi & Collin-Osdoby 2006). How these various factors contribute to the optimal (and perhaps sub-optimal) functioning of bone and bone marrow cells is too vast for discussion here. However, the reader is directed to an excellent review on the topic (Brandi & Collin-Osdoby 2006). At any rate, the microcirculation could be considered a bone anabolic agent that modulates the functioning of bone cells as well as being a regulator of bone blood flow.

Dysfunction of the microcirculation has been suspected in a variety of disease states (Gutterman *et al.* 2016). As endothelial cells are intricately important for bone and bone marrow function, it is important to consider how pathology of the vascular system may impact these two other organs. Age- and disease-related vascular dysfunction occurs in bone (Prisby *et al.* 2007, Prisby *et al.* 2008, 2012, 2015, Stabley *et al.* 2015) as it does in other tissue beds (Lesniewski *et al.* 2008, 2011, Kang *et al.* 2009, Park *et al.* 2012). For example, a recently identified capillary subtype, found in mouse bone marrow and theorized to regulate angiogenesis, declines in advanced age (Kusumbe *et al.* 2014). Further, arteriosclerotic lesions were observed in arteries from human femora, with disease progression being about 10 years more advanced than what was observed in similar arteries outside of the skeleton (Ramseier 1962). Additionally, the progressive ossification of bone marrow blood vessels (i.e., the presumed conversion of blood vessels into bone; Fig. 6) with advancing age leads to 'microvascular dead space' (Prisby 2014). 'Microvascular dead space' has been defined as a loss of vasomotor

function (i.e., vasodilation and vasoconstriction) and patency in bone marrow blood vessels (Prisby 2014) and presumably has dramatic impacts on the delivery of bone blood flow, bone interstitial fluid flow and pressure and release of paracrine factors from the vascular endothelium. In addition, progressive ossification of bone marrow blood vessels most likely contributes to and precipitates the centripetal blood flow direction in long bones observed in advanced age, as opposed to the centrifugal direction observed in youth (Fig. 7; Prisby 2014). Thus, when considering age- and disease-related pathologies of bone, the independent pathologies of the vascular system must be considered. In other words, pathologies that originate, develop independent from the skeleton and cause dysfunction in the vascular system may proceed and contribute to developing osteopathology. Given the interconnectedness of bone blood vessels and bone, these physiological and pathological concepts may not be so easily separable.

Figure 7

Schematic representing centrifugal (young) and centripetal (old) blood flow supply in long bones (Brookes & Revell 1998). Centrifugal blood flow indicates that blood enters the long bone through the principal nutrient artery (PNA), flows into the middle of the marrow space, radiates to the endosteal network and exits primarily via the periosteal venular network (PVN). Blood is also capable of exiting via the principal nutrient vein (PNV). Progressive ossification of bone marrow blood vessels may contribute to and precipitate the transition of the blood supply from centrifugal in youth to centripetal in old age. By reducing or eliminating the patency of the bone marrow vasculature (i.e., the black X), the blood supply must predominately enter the long bone via the periosteal arterial network (PAN). Centripetal blood supply represents an abnormal blood flow pattern (Brookes 1973) and has been previously represented as a schematic by Thompson & Towler (2012).

Table 2 Effects of osteogenic agents on bone blood vessels.

	Outcome
Mechanical loading	
Acute	<p>↑ MAP, ↑ bone VR (Gross et al. 1979).</p> <p>↑ Blood flow to distal femur metaphysis and marrow (Dominguez et al. 2010).</p> <p>⇒ MAP, ↑ blood flow to tibia and femur bone regions and marrow, ↑ exercise hyperemia to varying bone regions (Stabley et al. 2014).</p>
Chronic	<p>↑ Blood vessel number and ⇒ blood vessel number with anti-VEGF antibody (Yao et al. 2004).</p> <p>↑ Bone capillarity (Viboolvorakul et al. 2009).</p> <p>↑ Endothelium-dependent vasodilation in PNA (Dominguez et al. 2010).</p>
PTH and PTHrP	
Acute	<p>↓, then ↑ in blood flow to various hind limb bones (Boelkins et al. 1976).</p> <p>⇒ Blood flow to isolated tibia (Driessens & Vanhoutte 1981).</p> <p>↑ Blood flow to tibia and distal femur (Kapitola & Zák 2003).</p> <p>↑ Vasodilation in PNA (Benson et al. 2016).</p> <p>↑ Whole hind limb perfusion, ↑ perfusion in cortical diaphysis (Gohin et al. 2016).</p>
Intermittent	<p>↑ Blood flow to whole skeleton, calvarium, mandible, spine, and upper and lower extremities (Moore et al. 2010).</p> <p>↓ Blood vessel number, relocation of smallest bone marrow blood vessels (Prisby et al. 2011).</p> <p>↑ Endothelium-dependent vasodilation in PNA (Prisby et al. 2013).</p> <p>↑ Blood vessel number and mean vessel volume, ↑ tibial perfusion (Roche et al. 2014).</p> <p>⇒ Whole hind limb tissue perfusion (Gohin et al. 2016).</p>
Estrogen	
Supplementation	<p>⇒ Vascular pattern in tibia and femur (Brookes & Lloyd 1961).</p> <p>↑ Tibia blood flow with OVX, ↓ tibia blood flow in Intact+E2 and OVX+E2 (Kapitola & Kubícková 1990).</p> <p>↑ VEGF 120 and 164 in proximal tibia metaphysis, ↑ blood vessel number (Mekraldi et al. 2003).</p> <p>⇒ and ↑ endothelium-dependent vasodilation in PNAs from young and old rats, respectively, ⇒ endothelium-independent vasodilation in PNA from young and old rats (Prisby et al. 2012).</p>
Estrogen loss	<p>↑ Femur blood flow at days 28–84 OVX (Egrise et al. 1992).</p> <p>↑ Tibia and distal femur blood flow with OVX (Kapitola et al. 1994).</p> <p>⇒ Femur blood flow, ↑ tibia and distal femur blood flow with OVX, ↓ tibia and distal femur blood flow in OVX and sham-operated rats with NO blocker (Kapitola et al. 1997).</p> <p>↑ Area of sinusoidal capillaries in marrow of L6 vertebra, ⇒ arterial capillaries (Laroche et al. 1996).</p> <p>↑ and ⇒ vasoconstriction in metaphyseal and diaphyseal arteries, respectively, from the femur, ⇒ vasodilation in metaphyseal and diaphyseal arteries (Hansen et al. 2001).</p> <p>↓ mRNA of VEGF 120 and 164, ↓ endothelial and inducible NO synthase, ↓ fibroblast growth factor-2 in proximal tibia metaphysis at day 3 OVX, ↓ blood vessel number at day 7 OVX (Mekraldi et al. 2003).</p> <p>↓ Endothelium-dependent relaxation and ↑ contraction in aorta and femoral arteries, ⇒ endothelium-independent relaxation in aorta and femoral artery, ↓ lumbar vertebral perfusion (Griffith et al. 2010).</p> <p>↓ and ⇒ endothelium-dependent vasodilation in PNAs from young and old OVX rats, respectively, ⇒ endothelium-independent vasodilation in PNAs from both groups (Prisby et al. 2012).</p> <p>↓ Bone marrow vascular density, ↑ tibia VR, ↓ tibia perfusion (Roche et al. 2013).</p> <p>↓ Bone marrow vascular density and volume, ⇒ vessel size, ↑ tibia VR and ↓ perfusion (Roche et al. 2014).</p>
Vitamin D	
Supplementation	<p>↑ Brachial flow-mediated dilation (Harris et al. 2011).</p> <p>⇒ Brachial flow-mediated dilation (Gepner et al. 2012, Yiu et al. 2013).</p> <p>↑ Relaxation of renal arteries from OVX rats with 12 h of calcitriol incubation, ↑ endothelium-dependent relaxation in renal arteries following supplementation in OVX rats (Dong et al. 2013).</p>
Deficiency	<p>↓ Brachial flow-mediated dilation (Yiu et al. 2011).</p> <p>↓ NO bioavailability and NO synthase 3 gene expression and protein content in VDR knockout mice (Andrukhova et al. 2014).</p>
Calcitonin and CGrP	
Administered doses	<p>↓ Skeletal blood flow (Wootton et al. 1978).</p> <p>↑ Perfusion pressure in isolated tibia (Driessens & Vanhoutte 1981).</p> <p>⇒ In elevated tibia and femur blood flow induced by paraplegia, ↑ femur blood flow in intact rats (Schoutens et al. 1988).</p> <p>↑ Relaxation in pre-constricted arterial segments (Lundgaard et al. 1997).</p> <p>⇒ Marrow VR, ↓ tibia and femur marrow flow velocities, ↓ MAP (Vendéghe et al. 2010).</p> <p>↑ Vasodilation in PNA (RD Prisby, unpublished observations).</p>

↑, increase; ↓, decrease; ⇒, no change; E2, estrogen supplementation; L6, lumbar 6; MAP, mean arterial pressure; NO, nitric oxide; OVX, ovariectomy; PNA, principal nutrient artery; VDR, vitamin D receptor knockout mice; VEGF, vascular endothelial growth factor; VR, vascular resistance.

Conclusion

Mechanical perturbations such as exercise and hormones (e.g., PTH and estrogen), classically recognized as bone anabolic agents, have profound influences on the bone vasculature. Evidence suggests that these stimuli trigger modifications in bone blood vessels more rapidly than in bone. Further, inhibition of the vascular adaptation with these stimuli prevents or attenuates bone accrual. Bone blood vessels may mediate changes through the NO signaling pathway, particularly for bone adaptation in response to advancing age, mechanical loading such as exercise, PTH and estrogen. Interestingly, bone mass was strongly associated with endothelium-dependent vasodilator capacity of bone blood vessels and poorly associated with serum estrogen concentrations. While it is evident that other hormones such as vitamin D and calcitonin influence both bone and blood vessels, present data are too scarce to decipher the interplay between the two systems. Table 2 provides a summary of how these osteogenic agents influence the bone vascular network.

The intimate bi-communication between bone blood vessels and bone is undeniable; however, these communications should not indicate mutual dependence between the systems. The vascular system can exist without bone, as evidenced by blood vessels in tissue beds outside of the skeleton. However, bone cannot form without the vascular system and healthy bone cannot exist without properly functioning blood vessels. Bone cells modulate vascular function via the release of growth factors and metabolic byproducts to enhance angiogenesis and bone blood flow. Yet, the *in vivo* modulation of bone cellular activity by neighboring blood vessels has not been thoroughly appreciated. For example, interstitial fluid flow and pressure is thought to be the mechanism by which mechanical loading imparts bone anabolic effects. Interstitial fluid flow and pressure are governed by the microcirculation. Besides controlling and directing blood flow, the microcirculation releases many factors involved in a variety of bone cellular activities. Thus, dysfunction of the microcirculation, which is known to occur with advancing age and malady, can contribute to bone and bone marrow pathology in a fashion independent of bone disease.

Declaration of interest

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review. The content presented in this manuscript is solely the responsibility of the author and

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References

- Aguirre J, Buttery L, O'Shaughnessy M, Afzal F, Fernandez de Marticorena I, Hukkanen M, Huang P, MacIntyre I & Polak J 2001 Endothelial nitric oxide synthase gene-deficient mice demonstrate marked retardation in postnatal bone formation, reduced bone volume, and defects in osteoblast maturation and activity. *American Journal of Pathology* **158** 247–257. (doi:10.1016/S0002-9440(10)63963-6)
- Akamine T, Jee WS, Ke HZ, Li XJ & Lin BY 1992 Prostaglandin E2 prevents bone loss and adds extra bone to immobilized distal femoral metaphysis in female rats. *Bone* **13** 11–22. (doi:10.1016/8756-3282(92)90356-2)
- Alam A, Gallagher A, Shankar V, Ghate MA, Datta HK, Huang CL, Moonga BS, Chambers TJ, Bloom SR & Zaidi M 1992 Endothelin inhibits osteoclastic bone resorption by a direct effect on cell motility: implications for the vascular control of bone resorption. *Endocrinology* **130** 3617–3624. (doi:10.1210/endo.130.6.1597159)
- Amizuka N, Karaplis AC, Henderson JE, Warshawsky H, Lipman ML, Matsuki Y, Ejiri S, Tanaka M, Izumi N, Ozawa H, *et al.* 1996 Haploinsufficiency of parathyroid hormone-related peptide (PTHrP) results in abnormal postnatal bone development. *Developmental Biology* **175** 166–176. (doi:10.1006/dbio.1996.0104)
- Amprino R & Marotti G 1964 A topographic quantitative study of bone formation and reconstruction. In *Proceedings of the First European Bone and Tooth Symposium*, pp 21–33. New York, NY, USA: Pergamon Press.
- Ancill A, Bascal ZA, Whitaker G & Dacke CG 1991 Calcitonin gene-related peptide promotes transient radiocalcium uptake into chick bone *in vivo*. *Experimental Physiology* **76** 143–146. (doi:10.1113/expphysiol.1991.sp003477)
- Andrukhova O, Slavic S, Zeitz U, Riesen SC, Heppelmann MS, Ambrisko TD, Markovic M, Kuebler WM & Erben RG 2014 Vitamin D is a regulator of endothelial nitric oxide synthase and arterial stiffness in mice. *Molecular Endocrinology* **28** 53–64. (doi:10.1210/me.2013-1252)
- Arai F & Suda T 2007 Maintenance of quiescent hematopoietic stem cells in the osteoblastic niche. *Annals of the New York Academy of Sciences* **1106** 41–53. (doi:10.1196/annals.1392.005)
- Armour K, Armour KJ, Gallagher ME, Gödecke A, Helfrich MH, Reid DM & Ralston SH 2001 Defective bone formation and anabolic response to exogenous estrogen in mice with targeted disruption of endothelial nitric oxide synthase. *Endocrinology* **142** 760–766. (doi:10.1210/endo.142.2.7977)
- Arnett T & Dempster DW 1986 Effect of pH on bone resorption by rat osteoclasts *in vitro*. *Endocrinology* **119** 119–124. (doi:10.1210/endo-119-1-119)
- Arnett T, Gibbons DC, Utting JC, Orriss IR, Hoebertz A, Rosendaal M & Meghji S 2003 Hypoxia is a major stimulator of osteoclast formation and bone resorption. *Journal of Cellular Physiology* **196** 2–8. (doi:10.1002/jcp.10321)
- Baker J, Liu JP, Robertson EJ & Efstratiadis A 1993 Role of insulin-like growth factors in embryonic and postnatal growth. *Cell* **75** 73–82. (doi:10.1016/S0092-8674(05)80085-6)
- Ballica R, Valentijn K, Khachatryan A, Guerder S, Kapadia S, Gundberg C, Gilligan J, Flavell RA & Vignery A 1999 Targeted expression of calcitonin gene-related peptide to osteoblasts increases bone density in mice. *Journal of Bone and Mineral Research* **14** 1067–1074. (doi:10.1359/jbmr.1999.14.7.1067)

- Barvitenko N, Aslam M, Filosa J, Matteucci E, Nikinmaa M, Pantaleo A, Saldanha C & Baskurt OK 2013 Tissue oxygen demand in regulation of the behavior of the cells in the vasculature. *Microcirculation* **20** 484–501. (doi:10.1111/micc.12052)
- Beno T, Yoon YJ, Cowin SC & Fritton SP 2006 Estimation of bone permeability using accurate microstructural measurements. *Journal of Biomechanics* **39** 2378–2387. (doi:10.1016/j.jbiomech.2005.08.005)
- Benson T, Menezes T, Campbell J, Bice A, Hood B & Prisby R 2016 Mechanisms of vasodilation to PTH 1–84, PTH 1–34, and PTHrP 1–34 in rat bone resistance arteries. *Osteoporosis International* **27** 1817–1826.
- Bernard G & Shih C 1990 The osteogenic stimulating effect of neuroactive calcitonin gene-related peptide. *Peptides* **11** 625–632. (doi:10.1016/0196-9781(90)90171-Z)
- Bischoff-Ferrari H, Dietrich T, Orav EJ & Dawson-Hughes B 2004 Positive association between 25-hydroxy vitamin D levels and bone mineral density: a population-based study of younger and older adults. *American Journal of Medicine* **116** 634–639. (doi:10.1016/j.amjmed.2003.12.029)
- Bjurholm A, Kreicbergs A, Brodin E & Schultzberg M 1988a Substance P- and CGRP-immunoreactive nerves in bone. *Peptides* **9** 165–171. (doi:10.1016/0196-9781(88)90023-X)
- Bjurholm A, Kreicbergs A, Terenius L, Goldstein M & Schultzberg M 1988b Neuropeptide Y, tyrosine hydroxylase- and vasoactive intestinal polypeptide-immunoreactive nerves in bone and surrounding tissues. *Journal of the Autonomic Nervous System* **25** 119–125. (doi:10.1016/0165-1838(88)90016-1)
- Blake G, Park-Holohan SJ, Cook GJ & Fogelman I 2001 Quantitative studies of bone with the use of 18F-fluoride and 99mTc-methylene diphosphonate. *Seminars in Nuclear Medicine* **31** 28–49. (doi:10.1053/snuc.2001.18742)
- Boelkins J, Mazurkiewicz M, Mazur PE & Mueller WJ 1976 Changes in blood flow to bones during the hypocalcemic and hypercalcemic phases of the response to parathyroid hormone. *Endocrinology* **98** 403–412. (doi:10.1210/endo-98-2-403)
- Bord S, Ireland DC, Beavan SR & Compston JE 2003 The effects of estrogen on osteoprotegerin, RANKL, and estrogen receptor expression in human osteoblasts. *Bone* **32** 136–141. (doi:10.1016/S8756-3282(02)00953-5)
- Borle A, Nichols N & Nichols G Jr 1960a Metabolic studies of bone in vitro. I. Normal bone. *Journal of Biological Chemistry* **235** 1206–1210.
- Borle A, Nichols N & Nichols G Jr 1960b Metabolic studies of bone in vitro. II. The metabolic patterns of accretion and resorption. *Journal of Biological Chemistry* **235** 1211–1214.
- Brandi ML & Collin-Osdoby P 2006 Vascular biology and the skeleton. *Journal of Bone and Mineral Research* **21** 183–192. (doi:10.1359/JBMR.050917)
- Brandi M, Crescioli C, Tanini A, Frediani U, Agnusdei D & Gennari C 1993 Bone endothelial cells as estrogen targets. *Calcified Tissue International* **53** 312–317. (doi:10.1007/BF01351835)
- Brookes M 1957 Femoral growth after occlusion of the principal nutrient canal in day-old rabbits. *Journal of Bone and Joint Surgery-British* **39-B** 563–571.
- Brookes M 1966 The vascular factor in osteoarthritis. *Surgery, Gynecology and Obstetrics* **123** 1255–1260.
- Brookes M 1967 Blood flow rates in compact and cancellous bone, and bone marrow. *Journal of Anatomy* **101** 533–541.
- Brookes M 1973 *La Circulation Osseuse Normale and Pathologique*. Paris, France: INSERM.
- Brookes M & Lloyd EG 1961 Marrow vascularization and oestrogen-induced endosteal bone formation in mice. *Journal of Anatomy* **95** 220–228.
- Brookes M & Revell WJ 1998 *Blood Supply of Bone: Scientific Aspects*. London, Great Britain: Springer-Verlag.
- Brookes M & Singh M 1972 Venous shunt in bone after ligation of the femoral vein. *Surgery, Gynecology and Obstetrics* **135** 85–88.
- Burkhardt R, Kettner G, Bohm W, Schmidmeier M, Schlag R, Frisch B, Mallmann B, Eisenmenger W & Gilg T 1987 Changes in trabecular bone, hematopoiesis and bone-marrow vessels in aplastic-anemia, primary osteoporosis, and old-age: a comparative histomorphometric study. *Bone* **8** 157–164. (doi:10.1016/8756-3282(87)90015-9)
- Cameron D, Paschall HA & Robinson RA 1967 Changes in the fine structure of bone cells after the administration of parathyroid extract. *Journal of Cell Biology* **33** 1–14. (doi:10.1083/jcb.33.1.1)
- Charbon G 1969 Vasodilator action of parathyroid hormone used as bioassay. *Archives Internationales de Pharmacodynamie et de Therapie* **178** 296–303.
- Charbon G & Hulstaert PF 1974 Augmentation of arterial hepatic and renal flow by extracted and synthetic parathyroid hormone. *Endocrinology* **95** 621–626. (doi:10.1210/endo-95-2-621)
- Charbon G, Brummer F & Reneman RS 1968 Diuretic and vascular action of parathyroid extracts in animals and man. *Archives Internationales de Pharmacodynamie et de Therapie* **171** 1–11.
- Charles ND, Brookes M & Makler PT 1979 Studies of skeletal tracer kinetics: evaluation of a five compartmental model of ¹⁸F fluoride. *Journal of Nuclear Medicine Technology* **20** 1150–1157.
- Clarke B 2008 Normal bone anatomy and physiology. *Clinical Journal of the American Society of Nephrology* **3** S131–S139. (doi:10.2215/CJN.04151206)
- Colleran PN, Wilkerson MK, Bloomfield SA, Suva LJ, Turner RT & Delp MD 2000 Alterations in skeletal perfusion with simulated microgravity: a possible mechanism for bone remodeling. *Journal of Applied Physiology* **89** 1046–1054.
- Corbett S, Hukkanen M, Batten J, McCarthy ID, Polak MJ & Hughes SP 1999 Nitric oxide in fracture repair. Differential localisation, expression and activity of nitric oxide synthases. *Journal of Bone and Joint Surgery-British* **81** 531–537. (doi:10.1302/0301-620X.81B3.8852)
- Cowin S & Cardoso L 2015 Blood and interstitial flow in the hierarchical pore space architecture of bone tissue. *Journal of Biomechanics* **48** 842–854. (doi:10.1016/j.jbiomech.2014.12.013)
- Dalan R, Liew H, Tan WKA, Chew DEK & Leow MK-S 2014 Vitamin D and the endothelium: basic, translational and clinical research updates. *IJC Metabolic and Endocrine* **4** 4–17. (doi:10.1016/j.ijcme.2014.06.003)
- Davis T 1992 Endothelial control of long bone vascular resistance. *Journal of Orthopaedic Research* **10** 344–349. (doi:10.1002/jor.1100100306)
- Davis TRC & Wood MB 1997 Bone blood flow. In *Microvascular Bone Reconstruction*, pp 13–17. Eds MB Wood & A Gilber. London, UK: The Livery House.
- de Castro L, Lozano D, Portal-Núñez S, Maycas M, De la Fuente M, Caeiro JR & Esbrit P 2012 Comparison of the skeletal effects induced by daily administration of PTHrP (1–36) and PTHrP (107–139) to ovariectomized mice. *Journal of Cellular Physiology* **227** 1752–1760. (doi:10.1002/jcp.22902)
- Delany A & Canalis E 1997 Growth factors and bone. In *Growth Factors and Cytokines in Health and Disease*, pp 127–155. Eds D LeRoith & C Bondy. Greenwich, CT, USA: JAI Press.
- Delp MD, Duan C, Ray CA & Armstrong RB 1999 Rat hindlimb muscle blood flow during level and downhill locomotion. *Journal of Applied Physiology* **86** 564–568.
- Ding W, Wei ZX & Liu JB 2011 Reduced local blood supply to the tibial metaphysis is associated with ovariectomy-induced osteoporosis in mice. *Connective Tissue Research* **52** 25–29. (doi:10.3109/03008201003783011)
- Doll B 2005 Developmental biology of the skeletal system. In *Bone Tissue Engineering*, pp 3–26. Eds J Hollinger, TA Einhorn, BA Doll & C Sfeir. Boca Raton, FL, USA: CRC Press.
- Dominguez JM 2nd, Prisby RD, Muller-Delp JM, Allen MR & Delp MD 2010 Increased nitric oxide-mediated vasodilation of bone resistance arteries is associated with increased trabecular bone volume after endurance training in rats. *Bone* **46** 813–819. (doi:10.1016/j.bone.2009.10.029)
- Dong J, Wong SL, Lau CW, Liu J, Wang YX, Dan He Z, Fai Ng C, Yu Chen Z, Yao X, Xu A, et al. 2013 Calcitriol restores renovascular

- function in estrogen-deficient rats through downregulation of cyclooxygenase-2 and the thromboxane-prostanoid receptor. *Kidney International* **84** 54–63. (doi:10.1038/ki.2013.12)
- Driessens M & Vanhoutte PM 1981 Effect of calcitonin, hydrocortisone, and parathyroid hormone on canine bone blood vessels. *American Journal of Physiology* **241** H91–H94.
- Eghbali-Fatourehchi G, Lamsam J, Fraser D, Nagel D, Riggs BL & Khosla S 2005 Circulating osteoblast-lineage cells in humans. *New England Journal of Medicine* **352** 1959–1966.
- Egrise D, Martin D, Neve P, Vienne A, Verhas M & Schoutens A 1992 Bone blood flow and in vitro proliferation of bone marrow and trabecular bone osteoblast-like cells in ovariectomized rats. *Calcified Tissue International* **50** 336–341. (doi:10.1007/BF00301631)
- Esen E, Chen J, Karner CM, Okunade AL, Patterson BW & Long F 2013 WNT-LRP5 signaling induces Warburg effect through mTORC2 activation during osteoblast differentiation. *Cell Metabolism* **17** 745–755. (doi:10.1016/j.cmet.2013.03.017)
- Esen E, Lee SY, Wice BM & Long F 2015 PTH promotes bone anabolism by stimulating aerobic glycolysis via IGF signaling. *Journal of Bone and Mineral Research* **30** 1959–1968. (doi:10.1002/jbmr.2714)
- Ettinger B, Genant HK & Cann CE 1985 Long-term estrogen replacement therapy prevents bone loss and fractures. *Annals of Internal Medicine* **102** 319–324. (doi:10.7326/0003-4819-102-3-319)
- Faugere M, Okamoto S, DeLuca HF & Malluche HH 1986 Calcitriol corrects bone loss induced by oophorectomy in rats. *American Journal of Physiology* **250** E35–E38.
- Faure C, Linossier MT, Malaval L, Lafage-Proust MH, Peyroche S, Vico L & Guignandon A 2008 Mechanical signals modulated vascular endothelial growth factor-A (VEGF-A) alternative splicing in osteoblastic cells through actin polymerisation. *Bone* **42** 1092–1101.
- Felix R, Neuman WF & Fleisch H 1978 Aerobic glycolysis in bone: lactic acid production by rat calvaria cells in culture. *American Journal of Physiology* **234** C51–C55.
- Fiorelli G, Orlando C, Benvenuti S, Franceschelli F, Bianchi S, Pioli P, Tanini A, Serio M, Bartucci F & Brandi ML 1994 Characterization, regulation, and function of specific cell membrane receptors for insulin-like growth factor I on bone endothelial cells. *Journal of Bone and Mineral Research* **9** 329–337. (doi:10.1002/jbmr.5650090307)
- Frost H 1983a A determinant of bone architecture. The minimum effective strain. *Clinical Orthopaedics and Related Research* **175** 286–292.
- Frost H 1983b The skeletal intermediary organization. *Metabolic Bone Disease and Related Research* **4** 281–290. (doi:10.1016/S0221-8747(83)80001-0)
- Frost H 2001 From Wolff's law to the Utah paradigm: insights about bone physiology and its clinical applications. *Anatomical Record* **262** 398–419. (doi:10.1002/ar.1049)
- Fujikawa Y, Quinn JM, Sabokbar A, McGee JO & Athanasou NA 1996 The human osteoclast precursor circulates in the monocyte fraction. *Endocrinology* **137** 4058–4060. (doi:10.1210/endo.137.9.8756585)
- Gepner A, Ramamurthy R, Krueger DC, Korcarz CE, Binkley N & Stein JH 2012 A prospective randomized controlled trial of the effects of vitamin D supplementation on cardiovascular disease risk. *PLoS ONE* **7** e36617. (doi:10.1371/journal.pone.0036617)
- Gohin S, Carrierio A, Chenu C, Pitsillides AA, Arnett TR & Marenzana M 2016 The anabolic action of intermittent parathyroid hormone on cortical bone depends partly on its ability to induce nitric oxide-mediated vasorelaxation in BALB/c mice. *Cell Biochemistry and Function* **34** 52–62. (doi:10.1002/cbf.3164)
- Gonzalez C, Rosas-Hernandez H, Jurado-Manzano B, Ramirez-Lee MA, Salazar-Garcia S, Martinez-Cuevas PP, Velarde-Salcedo AJ, Morales-Loredo H, Espinosa-Tanguma R, Ali SF, et al. 2015 The prolactin family hormones regulate vascular tone through NO and prostacyclin production in isolated rat aortic rings. *Acta Pharmacologica Sinica* **36** 572–586. (doi:10.1038/aps.2014.159)
- Gooi J, Pompolo S, Karsdal MA, Kulkarni NH, Kalajic I, McAhren SH, Han B, Onyia JE, Ho PW, Gillespie MT, et al. 2010 Calcitonin impairs the anabolic effect of PTH in young rats and stimulates expression of sclerostin by osteocytes. *Bone* **46** 1486–1497. (doi:10.1016/j.bone.2010.02.018)
- Griffith J, Wang YX, Zhou H, Kwong WH, Wong WT, Sun YL, Huang Y, Yeung DK, Qin L & Ahuja AT 2010 Reduced bone perfusion in osteoporosis: likely causes in an ovariectomy rat model. *Radiology* **254** 739–746. (doi:10.1148/radiol.09090608)
- Gross PM, Heistad DD & Marcus ML 1979 Neurohumoral regulation of blood flow to bones and marrow. *American Journal of Physiology: Heart and Circulatory Physiology* **237** H440–H448.
- Gurkan U & Akkus O 2008 The mechanical environment of bone marrow: a review. *Annals of Biomedical Engineering* **36** 1978–1991. (doi:10.1007/s10439-008-9577-x)
- Gutterman D, Chabowski DS, Kadlec AO, Durand MJ, Freed JK, Ait-Aissa K & Beyer AM 2016 The human microcirculation: regulation of flow and beyond. *Circulation Research* **118** 157–172. (doi:10.1161/CIRCRESAHA.115.305364)
- Hansen S, Beck Jensen JE, Rasmussen L, Hauge EM & Brixen K 2010 Effects on bone geometry, density, and microarchitecture in the distal radius but not the tibia in women with primary hyperparathyroidism: a case-control study using HR-pQCT. *Journal of Bone and Mineral Research* **25** 1941–1947. (doi:10.1002/jbmr.98)
- Hansen VB, Forman A, Lundgaard A, Aalkjær C, Skajaa K & Hansen ES 2001 Effects of oophorectomy on functional properties of resistance arteries isolated from the cancellous bone of the rabbit femur. *Journal of Orthopaedic Research* **19** 391–397. (doi:10.1016/S0736-0266(00)90026-3)
- Harris R, Pedersen-White J, Guo DH, Stallmann-Jorgensen IS, Keeton D, Huang Y, Shah Y, Zhu H & Dong Y 2011 Vitamin D3 supplementation for 16 weeks improves flow-mediated dilation in overweight African-American adults. *American Journal of Hypertension* **24** 557–562. (doi:10.1038/ajh.2011.12)
- Harrison J, Rameshwar P, Chang V & Bandari P 2002 Oxygen saturation in the bone marrow of healthy volunteers. *Blood* **99** 394. (doi:10.1182/blood.V99.1.394)
- Hauge EM, Qvesel D, Eriksen EF, Mosekilde L & Melsen F 2001 Cancellous bone remodeling occurs in specialized compartments lined by cells expressing osteoblastic markers. *Journal of Bone and Mineral Research* **16** 1575–1582. (doi:10.1359/jbmr.2001.16.9.1575)
- Hikiji H, Shin WS, Oida S, Takato T, Koizumi T & Toyo-Oka T 1997 Direct action of nitric oxide on osteoblastic differentiation. *FEBS Letters* **410** 238–242. (doi:10.1016/S0014-5793(97)00597-8)
- Hill E & Elde R 1988 Calcitonin gene-related peptide-immunoreactive nerve fibers in mandibular periosteum of rat: evidence for primary afferent origin. *Neuroscience Letters* **85** 172–178. (doi:10.1016/0304-3940(88)90347-3)
- Hill E & Elde R 1991 Distribution of CGRP-, VIP-, D beta H-, SP-, and NPY-immunoreactive nerves in the periosteum of the rat. *Cell and Tissue Research* **264** 469–480. (doi:10.1007/BF00319037)
- Hill C, Phillips JK & Sandow SL 2001 Heterogeneous control of blood flow amongst different vascular beds. *Medicinal Research Reviews* **21** 1–60. (doi:10.1002/1098-1128(200101)21:1<1::AID-MED1>3.0.CO;2-6)
- Hoebertz A, Meghji S, Burnstock G & Arnett TR 2001 Extracellular ADP is a powerful osteolytic agent: evidence for signaling through the P2Y(1) receptor on bone cells. *FASEB Journal* **15** 1139–1148. (doi:10.1096/fj.00-0395com)
- Hohmann E, Levine L & Tashjian AH Jr 1983 Vasoactive intestinal peptide stimulates bone resorption via a cyclic adenosine 3',5'-monophosphate-dependent mechanism. *Endocrinology* **112** 1233–1239. (doi:10.1210/endo-112-4-1233)
- Holtrop M, King GJ, Cox KA & Reit B 1979 Time-related changes in the ultrastructure of osteoclasts after injection of parathyroid hormone

- in young rats. *Calcified Tissue International* **27** 129–135. (doi:10.1007/BF02441175)
- Horwitz M, Tedesco MB, Gundberg C, Garcia-Ocana A & Stewart AF 2003 Short-term, high-dose parathyroid hormone-related protein as a skeletal anabolic agent for the treatment of postmenopausal osteoporosis. *Journal of Clinical Endocrinology and Metabolism* **88** 569–575. (doi:10.1210/jc.2002-021122)
- Horwitz M, Tedesco MB, Sereika SM, Prebehala L, Gundberg CM, Hollis BW, Bisello A, Garcia-Ocana A, Carneiro RM & Stewart AF 2011 A 7-day continuous infusion of PTH or PTHrP suppresses bone formation and uncouples bone turnover. *Journal of Bone and Mineral Research* **26** 2287–2297. (doi:10.1002/jbmr.415)
- Hsieh Y, Robling AG, Ambrosius WT, Burr DB & Turner CH 2001 Mechanical loading of diaphyseal bone in vivo: the strain threshold for an osteogenic response varies with location. *Journal of Bone and Mineral Research* **16** 2291–2297. (doi:10.1359/jbmr.2001.16.12.2291)
- Hu K & Olsen BR 2016 Osteoblast-derived VEGF regulates osteoblast differentiation and bone formation during bone repair. *Journal of Clinical Investigation* **126** 509–526. (doi:10.1172/JCI82585)
- Ikegame M, Rakopoulos M, Zhou H, Houssami S, Martin TJ, Moseley JM & Findlay DM 1995 Calcitonin receptor isoforms in mouse and rat osteoclasts. *Journal of Bone and Mineral Research* **10** 59–65. (doi:10.1002/jbmr.5650100110)
- Indrekvam K, Husby OS, Gjerdet NR, Engester LB & Langeland N 1991 Age-dependent mechanical properties of rat femur. Measured in vivo and in vitro. *Acta Orthopaedica Scandinavica* **62** 248–252. (doi:10.3109/17453679108993602)
- Inzerillo A, Zaidi M & Huang CL 2002 Calcitonin: the other thyroid hormone. *Thyroid* **12** 791–798. (doi:10.1089/105072502760339352)
- Ishida A, Fujita N, Kitazawa R & Tsuruo T 2002 Transforming growth factor-beta induces expression of receptor activator of NF-kappa B ligand in vascular endothelial cells derived from bone. *Journal of Biological Chemistry* **277** 26217–26224. (doi:10.1074/jbc.M111093200)
- Johnson DL, McAllister TN & Frangos JA. 1996 Fluid flow stimulates rapid and continuous release of nitric oxide in osteoblasts. *American Journal of Physiology* **271** E205–E208.
- Johnston C, Hui SL, Witt RM, Appledorn R, Baker RS & Longcope C 1985 Early menopausal changes in bone mass and sex steroids. *Journal of Clinical Endocrinology and Metabolism* **61** 905–911. (doi:10.1210/jcem-61-5-905)
- Kage K, Fujita N, Oh-hara T, Ogata E, Fujita T & Tsuruo T 1999 Basic fibroblast growth factor induces cyclooxygenase-2 expression in endothelial cells derived from bone. *Biochemical and Biophysical Research Communications* **254** 259–263. (doi:10.1006/bbrc.1998.9875)
- Kameda T, Mano H, Yuasa T, Mori Y, Miyazawa K, Shiokawa M, Nakamaru Y, Hiroi E, Hiura K, Kameda A, et al. 1997 Estrogen inhibits bone resorption by directly inducing apoptosis of the bone-resorbing osteoclasts. *Journal of Experimental Medicine* **186** 489–495. (doi:10.1084/jem.186.4.489)
- Kanczler JM & Oreffo RO 2008 Osteogenesis and angiogenesis: the potential for engineering bone. *European Cells and Materials* **15** 100–114. (doi:10.22203/eCM.v015a08)
- Kang L, Reyes RA & Muller-Delp JM 2009 Aging impairs flow-induced dilation in coronary arterioles: role of NO and H(2)O(2). *American Journal of Physiology: Heart and Circulatory Physiology* **297** H1087–1095. (doi:10.1152/ajpheart.00356.2009)
- Kapitola J & Kubícková J 1990 Estradiol benzoate decreases the blood flow through the tibia of female rats. *Experimental and Clinical Endocrinology* **96** 117–120. (doi:10.1055/s-0029-1210998)
- Kapitola J & Zák J 2003 Effect of parathormone on bone blood flow in rats – possible role of NO. *Šborník Lekarský* **104** 133–137.
- Kapitola J, Andrlé J & Kubícková J 1994 Possible participation of prostaglandins in the increase in the bone blood flow in oophorectomized female rats. *Experimental and Clinical Endocrinology* **102** 414–416. (doi:10.1055/s-0029-1211313)
- Kapitola J, Schreiber V, Andrlé J, Haas T & Kubícková J 1997 Possible participation of EDRF-NO in the hormonal regulation of bone blood flow in rats. *Physiological Research* **46** 15–20.
- Karner C & Long F 2016 Wnt signaling and cellular metabolism in osteoblasts. *Cellular and Molecular Life Sciences* **74** 1649–1657. (doi:10.1007/s00018-016-2425-5)
- Kasten TP, Collin-Osdoby P, Patel N, Osdoby P, Krukowski M, Misko TP, Settle SL, Currie MG & Nickols GA 1994 Potentiation of osteoclast bone-resorption activity by inhibition of nitric oxide synthase. *PNAS* **91** 3569–3573. (doi:10.1073/pnas.91.9.3569)
- Kelly P & Bronk JT 1990 Venous pressure and bone formation. *Microvascular Research* **39** 364–375. (doi:10.1016/0026-2862(90)90049-W)
- Khalid AB & Krum SA 2016 Estrogen receptors alpha and beta in bone. *Bone* **87** 130–135. (doi:10.1016/j.bone.2016.03.016)
- Khosla S 2011 Is nitroglycerin a novel and inexpensive treatment for osteoporosis? *JAMA* **305** 826–827. (doi:10.1001/jama.2011.191)
- Kobayashi S, Takahashi HE, Ito A, Saito N, Nawata M, Horiuchi H, Ohta H, Ito A, Iorio R, Yamamoto N, et al. 2003 Trabecular minimodeling in human iliac bone. *Bone* **32** 163–169. (doi:10.1016/S8756-3282(02)00947-X)
- Kousteni S & Bilezikian JP 2008 Cellular actions of parathyroid hormone. In *Principals of Bone Biology*, edn 3rd, pp 639–656. Eds JP Bilezikian, LG Raisz & TJ Martin. San Diego, CA, USA: Academic Press.
- Kousteni S, Bellido T, Plotkin LI, O'Brien CA, Bodenner DL, Han L, Han K, DiGregorio GB, Katzenellenbogen JA, Katzenellenbogen BS, et al. 2001 Nongenotropic, sex-nonspecific signaling through the estrogen or androgen receptors: dissociation from transcriptional activity. *Cell* **104** 719–730.
- Kousteni S, Chen JR, Bellido T, Han L, Ali AA, O'Brien CA, Plotkin L, Fu Q, Mancino AT, Wen Y, et al. 2002 Reversal of bone loss in mice by nongenotropic signaling of sex steroids. *Science* **298** 843–846. (doi:10.1126/science.1074935)
- Kusumbe A, Ramasamy SK & Adams RH 2014 Coupling of angiogenesis and osteogenesis by a specific vessel subtype in bone. *Nature* **507** 323–328. (doi:10.1038/nature13145)
- Kwon R, Meyas DR, Tang WJ & Frangos JA 2010 Microfluidic enhancement of intramedullary pressure increases interstitial fluid flow and inhibits bone loss in hindlimb suspended mice. *Journal of Bone and Mineral Research* **25** 1798–1807. (doi:10.1002/jbmr.74)
- Lacey J 1929 Non-union of fractures: an experimental study. *Annals of Surgery* **89** 813–848. (doi:10.1097/00000658-192906000-00002)
- Lam H, Brink P & Qin YX 2010 Skeletal nutrient vascular adaptation induced by external oscillatory intramedullary fluid pressure intervention. *Journal of Orthopaedic Surgery and Research* **5** 18. (doi:10.1186/1749-1799X-1185-1118)
- Langille B 1995 Blood flow-induced remodeling of the artery wall. In *Flow-Dependent Regulation of Vascular Function*, pp 277–299. Eds J Bevens, G Kaley & GM Rubanyi. New York, NY, USA: Springer.
- Laroche M 2002 Intraosseous circulation from physiology to disease. *Joint Bone Spine* **69** 262–269. (doi:10.1016/S1297-319X(02)00391-3)
- Laroche M, Barbier A, Ludot I, Vernhet C, Thiechart M, Viguier G & Mazieres B 1996 Effect of ovariectomy on intraosseous vascularization and bone remodelling in rats: action of tiludronate. *Osteoporosis International* **6** 127–129. (doi:10.1007/BF01623935)
- Leblond C, Wilkinson GW, Belanger LF & Robichon J 1950 Radio-autographic visualization of bone formation in the rat. *American Journal of Anatomy* **86** 289–341. (doi:10.1002/aja.1000860205)
- Leder B, O'Dea LS, Zanchetta JR, Kumar P, Banks K, McKay K, Lytle CR & Hattersley G 2015 Effects of abaloparatide, a human parathyroid hormone-related peptide analog, on bone mineral density in postmenopausal women with osteoporosis. *Journal of Clinical Endocrinology and Metabolism* **100** 697–706. (doi:10.1210/jc.2014-3718)
- Lemma S, Shoarina M, Porporato PE, Zini N, Sonveaux P, Di Pompo G, Baldini N & Avnet S 2016 Energy metabolism in osteoclast formation and activity. *International Journal of Biochemistry and Cell Biology* **79** 168–180. (doi:10.1016/j.biocel.2016.08.034)

- Lesniewski L, Donato AJ, Behnke BJ, Woodman CR, Laughlin MH, Ray CA & Delp MD 2008 Decreased NO signaling leads to enhanced vasoconstrictor responsiveness in skeletal muscle arterioles of the ZDF rat prior to overt diabetes and hypertension. *American Journal of Physiology: Heart and Circulatory Physiology* **294** H1840–H1850. (doi:10.1152/ajpheart.00692.2007)
- Lesniewski L, Durrant JR, Connell ML, Henson GD, Black AD, Donato AJ & Seals DR 2011 Aerobic exercise reverses arterial inflammation with aging in mice. *American Journal of Physiology: Heart and Circulatory Physiology* **301** H1025–H1032. (doi:10.1152/ajpheart.01276.2010)
- Li Y, Wang J, Xing J, Wang Y & Luo Y 2016 Surface chemistry regulates the sensitivity and tolerability of osteoblasts to various magnitudes of fluid shear stress. *Journal of Biomedical Materials Research Part A* **104** 2978–2991. (doi:10.1002/jbm.a.35848)
- Lin R, Amizuka N, Sasaki T, Aarts MM, Ozawa H, Goltzman D, Henderson JE & White JH 2002 1 α ,25-dihydroxyvitamin D3 promotes vascularization of the chondro-osseous junction by stimulating expression of vascular endothelial growth factor and matrix metalloproteinase 9. *Journal of Bone and Mineral Research* **17** 1604–1612. (doi:10.1359/jbmr.2002.17.9.1604)
- Lundgaard A, Aalkjaer C, Bjurholm A, Mulvany MJ & Hansen ES 1997 Vasoelaxation in isolated bone arteries. Vasoactive intestinal peptide, substance P, calcitonin gene-related peptide, and bradykinin studied in pigs. *Acta Orthopaedica Scandinavica* **68** 481–489. (doi:10.3109/17453679708996267)
- Martin C, Burdon PC, Bridger G, Gutierrez-Ramos JC, Williams TJ & Rankin SM 2003 Chemokines acting via CXCR2 and CXCR4 control the release of neutrophils from the bone marrow and their return following senescence. *Immunity* **19** 583–593. (doi:10.1016/S1074-7613(03)00263-2)
- Mazo I & von Andrian UH 1999 Adhesion and homing of blood-borne cells in bone marrow microvessels. *Journal of Leukocyte Biology* **66** 25–32.
- McAllister TN & Frangos JA 1999 Steady and transient fluid shear stress stimulate NO release in osteoblast through distinct biochemical pathways. *Journal of Bone and Mineral Research* **14** 930–936. (doi:10.1359/jbmr.1999.14.6.930)
- McAllister TN, Du T & Frangos JA 2000 Fluid shear stress stimulates prostaglandin and nitric oxide release in bone marrow-derived preosteoclast-like cells. *Biochemical and Biophysical Research Communications* **270** 643–648. (doi:10.1006/bbrc.2000.2467)
- McCarthy I 2006 The physiology of bone blood flow: a review. *Journal of Bone and Joint Surgery* **88** (Supplement 2) 4–9. (doi:10.2106/jbjs.f.00890)
- Mekraldi S, Lafage-Proust M-H, Bloomfield S, Alexandre C & Vico L 2003 Changes in vasoactive factors associated with altered vessel morphology in the tibial metaphysis during ovariectomy-induced bone loss in rats. *Bone* **32** 630–641. (doi:10.1016/S8756-3282(03)00099-1)
- Melsen F, Mosekilde L & Eriksen EF 1995 Spatial distribution of sinusoids in relation to remodeling sites: evidence for specialized sinusoidal structures associated with formative sites. *Journal of Bone and Mineral Research* **10** S209.
- Miao D, He B, Jiang Y, Kobayashi T, Sorocanu MA, Zhao J, Su H, Tong X, Amizuka N, Gupta A, et al. 2005 Osteoblast-derived PTHrP is a potent endogenous bone anabolic agent that modifies the therapeutic efficacy of administered PTH 1–34. *Journal of Clinical Investigation* **115** 2402–2411. (doi:10.1172/JCI24918)
- Modlesky C & Lewis RD 2002 Does exercise during growth have a long-term effect on bone health? *Exercise and Sport Sciences Reviews* **30** 171–176. (doi:10.1097/00003677-200210000-00006)
- Montgomery R, Sutker BD, Bronk JT, Smith SR & Kelly PJ 1988 Interstitial fluid flow in cortical bone. *Microvascular Research* **35** 295–307. (doi:10.1016/0026-2862(88)90084-2)
- Moore A, Blake GM, Taylor KA, Rana AE, Wong M, Chen P & Fogelman I 2010 Assessment of regional changes in skeletal metabolism following 3 and 18 months of teriparatide treatment. *Journal of Bone and Mineral Research* **25** 960–967.
- Morrison M, Turin L, King BF, Burnstock G & Arnett TR 1998 ATP is a potent stimulator of the activation and formation of rodent osteoclasts. *Journal of Physiology* **511** 495–500. (doi:10.1111/j.1469-7793.1998.495bh.x)
- Nakamura M, Kuroda H, Narita K & Endo Y 1996 Parathyroid hormone induces a rapid increase in the number of active osteoclasts by releasing histamine from mast cells. *Life Science* **58** 1861–1968. (doi:10.1016/0024-3205(96)00170-1)
- Naot D & Cornish J 2008 The role of peptides and receptors of the calcitonin family in the regulation of bone metabolism. *Bone* **43** 813–838. (doi:10.1016/j.bone.2008.07.003)
- Nilas L & Christiansen C 1987 Bone mass and its relationship to age and the menopause. *Journal of Clinical Endocrinology and Metabolism* **65** 697–702. (doi:10.1210/jcem-65-4-697)
- Nilsson SK, Johnston HM & Coverdale JA 2001 Spatial localization of transplanted hemopoietic stem cells: inferences for the localization of stem cell niches. *Blood* **97** 2293–2299. (doi:10.1182/blood.V97.8.2293)
- North B & Sinclair DA 2012 The intersection between aging and cardiovascular disease. *Circulation Research* **110** 1097–1108. (doi:10.1161/CIRCRESAHA.111.246876)
- Osol G & Mandala M 2009 Maternal uterine vascular remodeling during pregnancy. *Physiology* **24** 58–71. (doi:10.1152/physiol.00033.2008)
- Parfitt A 1994 Osteonal and hemi-osteonal remodeling: the spatial and temporal framework for signal traffic in adult human bone. *Journal of Cellular Biochemistry* **55** 273–286. (doi:10.1002/jcb.240550303)
- Parfitt AM 1998 Mini-review: osteoclast precursors as leukocytes: Importance of the area code. *Bone* **23** 491–494. (doi:10.1016/S8756-3282(98)00140-9)
- Park Y, Prisby RD, Behnke BJ, Dominguez JM, Lesniewski LA, Donato AJ, Muller-Delp J & Delp MD 2012 Effects of aging, TNF- α , and exercise training on angiotensin II-induced vasoconstriction of rat skeletal muscle arterioles. *Journal of Applied Physiology* **113** 1091–1100. (doi:10.1152/jappphysiol.00292.2012)
- Piekarski K & Munro M 1977 Transport mechanism operating between blood supply and osteocytes in long bones. *Nature* **269** 80–82. (doi:10.1038/269080a0)
- Plotkin L, Weinstein RS, Parfitt AM, Roberson PK, Manolagas SC & T Bellido 1999 Prevention of osteocyte and osteoblast apoptosis by bisphosphonates and calcitonin. *Journal of Clinical Investigation* **104** 1363–1374. (doi:10.1172/JCI6800)
- Prisby R 2014 Bone marrow blood vessel ossification and ‘microvascular dead space’ in rat and human long bone. *Bone* **64** 195–203. (doi:10.1016/j.bone.2014.03.041)
- Prisby RD, Ramsey MW, Behnke BJ, Dominguez JM, Donato AJ, Allen MR & Delp MD 2007 Aging reduces skeletal blood flow, endothelium-dependent vasodilation and nitric oxide bioavailability in rats. *Journal of Bone and Mineral Research* **22** 1280–1288. (doi:10.1359/jbmr.070415)
- Prisby RD, Muller-Delp J, Delp MD & Nurkiewicz TR 2008 Age, gender and hormonal status modulate the vascular toxicity of the diesel exhaust extract phenanthraquinone. *Journal of Toxicology and Environmental Health, Part A* **71** 464–470. (doi:10.1080/15287390701839349)
- Prisby R, Guignandon A, Vanden-Bossche A, Mac-Way F, Linossier MT, Thomas M, Laroche N, Malaval L, Langer M, Peter ZA, et al. 2011 Intermittent PTH (1–84) is osteoanabolic but not osteoangiogenic and relocates bone marrow blood vessels closer to bone-forming sites. *Journal of Bone and Mineral Research* **26** 2583–2596. (doi:10.1002/jbmr.459)
- Prisby RD, Dominguez JM 2nd, Muller-Delp J, Allen MR & Delp MD 2012 Aging and estrogen status: a possible endothelium-dependent vascular coupling mechanism in bone remodeling. *PLoS ONE* **7** e48564. (doi:10.1371/journal.pone.0048564)

- Prisby R, Menezes T & Campbell J 2013 Vasodilation to PTH (1–84) in bone arteries is dependent upon the vascular endothelium and is mediated partially via VEGF signaling. *Bone* **54** 68–75. (doi:10.1016/j.bone.2013.01.028)
- Prisby R, Behnke BJ, Allen MR & Delp MD 2015 Effects of skeletal unloading on the vasomotor properties of the rat femur principal nutrient artery. *Journal of Applied Physiology* **118** 980–988. (doi:10.1152/jappphysiol.00576.2014)
- Qin Y, Kaplan T, Saldanha A & Rubin C 2003 Fluid pressure gradients, arising from oscillations in intramedullary pressure, is correlated with the formation of bone and inhibition of intracortical porosity. *Journal of Biomechanics* **36** 1427–1437. (doi:10.1016/S0021-9290(03)00127-1)
- Rabkin B, Szivek JA, Schonfeld JE & Halloran BP 2001 Long-term measurement of bone strain in vivo: the rat tibia. *Journal of Bone and Mineral Research* **58** 277–281. (doi:10.1002/1097-4636(2001)58:3<277::AID-JBM1017>3.0.CO;2-T)
- Ramseier E 1962 Arteriosclerotic lesions of the bone arteries. *Virchows Archiv* **336** 77–86. (doi:10.1007/BF00957640)
- Revell WJ & Brookes M 1994 Haemodynamic changes in the rat femur and tibia following femoral vein ligation. *Journal of Anatomy* **184** 625–633.
- Riancho JA, Salas E, Zarrabeitia MT, Olmos JM, Amado JA, Fernandez-Luna JL & Gonzalez-Macias J 1995 Expression and functional role of nitric oxide synthase in osteoblast-like cells. *Journal of Bone and Mineral Research* **10** 439–446. (doi:10.1002/jbmr.5650100315)
- Roche B, Vanden-Bossche A, Normand M, Malaval L, Vico L & Lafage-Proust MH 2013 Validated Laser Doppler protocol for measurement of mouse bone blood perfusion – response to age or ovariectomy differs with genetic background. *Bone* **55** 418–426. (doi:10.1016/j.bone.2013.03.022)
- Roche B, Vanden-Bossche A, Malaval L, Normand M, Jannot M, Chaux R, Vico L & Lafage-Proust MH 2014 Parathyroid hormone 1–84 targets bone vascular structure and perfusion in mice: impacts of its administration regimen and of ovariectomy. *Journal of Bone and Mineral Research* **29** 1608–1618. (doi:10.1002/jbmr.2191)
- Rowell LB 1993 Control of blood flow to dynamically active muscles. In *Human Cardiovascular Control*, pp 255–301. New York, NY, USA: Oxford University Press.
- Sabanai K, Tsutsui M, Sakai A, Hirasawa H, Tanaka S, Nakamura E, Tanimoto A, Sasaguri Y, Ito M, Shimokawa H, et al. 2008 Genetic disruption of all NO synthase isoforms enhances BMD and bone turnover in mice in vivo: involvement of the renin-angiotensin system. *Journal of Bone and Mineral Research* **23** 633–643. (doi:10.1359/jbmr.080107)
- Schinke T, Liese S, Priemel M, Haberland M, Schilling AF, Catala-Lehnen P, Blicharski D, Rueger JM, Gagel RF, Emeson RB, et al. 2004 Decreased bone formation and osteopenia in mice lacking alpha-calcitonin gene-related peptide. *Journal of Bone and Mineral Research* **19** 2049–2056. (doi:10.1359/jbmr.040915)
- Schoutens A, Verhas M, Dourov N, Bergmann P, Caulin F, Verschaeren A, Mone M & Heilporn A 1988 Bone loss and bone blood flow in paraplegic rats treated with calcitonin, diphosphonate, and indomethacin. *Calcified Tissue International* **42** 136–143. (doi:10.1007/BF02556346)
- Shih C & Bernard GW 1997 Neurogenic substance P stimulates osteogenesis in vitro. *Peptides* **18** 323–326. (doi:10.1016/S0196-9781(96)00280-X)
- Shinozuka K, Hashimoto M, Masumura S, Bjur RA, Westfall DP & Hattori K 1994 In vitro studies of release of adenine nucleotides and adenosine from rat vascular endothelium in response to alpha 1-adrenoceptor stimulation. *British Journal of Pharmacology* **113** 1203–1208. (doi:10.1111/j.1476-5381.1994.tb17125.x)
- Siddiqui JA & Partridge NC 2016 Physiological bone remodelling. Systemic regulation and growth factor involvement. *Physiology* **31** 233–245. (doi:10.1152/physiol.00061.2014)
- Smalt R, Mitchell FT, Howard RL & Chambers TJ 1997 Induction of NO and prostaglandin E2 in osteoblasts by wall-shear stress but not mechanical strain. *American Journal of Physiology* **273** E751–E758.
- Spilk S, Herr MD, Sinoway LI & Leuenberger UA 2013 Endothelium-derived hyperpolarizing factor contributes to hypoxia-induced skeletal muscle vasodilation in humans. *American Journal of Physiology: Heart and Circulatory Physiology* **305** H1639–H1645. (doi:10.1152/ajpheart.00073.2013)
- Stabley J, Moninga NC, Behnke BJ & Delp MD 2014 Exercise training augments regional bone and marrow blood flow during exercise. *Medicine and Science in Sports and Exercise* **46** 2107–2112. (doi:10.1249/MSS.0000000000000342)
- Stabley J, Prisby RD, Behnke BJ & Delp MD 2015 Type 2 diabetes alters bone and marrow blood flow and vascular control mechanisms in the ZDF rat. *Journal of Endocrinology* **225** 47–58. (doi:10.1530/JOE-14-0514)
- Stewart A, Cain RL, Burr DB, Jacob D, Turner CH & Hock JM 2000 Six-month daily administration of parathyroid hormone and parathyroid hormone-related protein peptides to adult ovariectomized rats markedly enhances bone mass and biomechanical properties: a comparison of human parathyroid hormone 1–34, parathyroid hormone-related protein 1–36, and SDZ-parathyroid hormone 893. *Journal of Bone and Mineral Research* **15** 1517–1525. (doi:10.1359/jbmr.2000.15.8.1517)
- Stocum DL 2006 *Regenerative Biology and Medicine*. Burlington, MA, USA: Academic Press.
- Takahashi K, Inoue D, Ando K, Matsumoto T, Ikeda K & Fujita T 1995 Parathyroid hormone-related peptide as a locally produced vasorelaxant: regulation of its mRNA by hypertension in rats. *Biochemical and Biophysical Research Communications* **208** 447–455. (doi:10.1006/bbrc.1995.1358)
- Thompson B & Towler DA 2012 Arterial calcification and bone physiology: role of the bone-vascular axis. *Nature Reviews Endocrinology* **8** 529–543. (doi:10.1038/nrendo.2012.36)
- Utting J, Robins SP, Brandao-Burch A, Orriss IR, Behar J & Arnett TR 2006 Hypoxia inhibits the growth, differentiation and bone-forming capacity of rat osteoblasts. *Experimental Cell Research* **312** 1693–1702. (doi:10.1016/j.yexcr.2006.02.007)
- Vendéghe Z, Melly A, Tóth B, Wolf K, Farkas T, Kádás I & Hamar J 2010 Calcitonin gene-related peptide, substance P, nitric oxide and epinephrine modulate bone marrow micro circulation of the rabbit tibia and femur. *Clinical Hemorheology and Microcirculation* **45** 9–17.
- Viboolvorakul S, Niimi H, Wongeak-in N, Eksakulkla S & Patumraj S 2009 Increased capillary vascularity in the femur of aged rats by exercise training. *Microvascular Research* **78** 459–463. (doi:10.1016/j.mvr.2009.07.003)
- Vizi E, Sperlág B & Baranyi M 1992 Evidence that ATP released from the postsynaptic site by noradrenaline, is involved in mechanical responses of guinea-pig vas deferens: cascade transmission. *Neuroscience* **50** 455–465. (doi:10.1016/0306-4522(92)90437-7)
- Wen Q, Lee KO, Sim SZ, Xu XG & Sim MK 2015 Des-aspartate-angiotensin I causes specific release of PGE2 and PG12 in HUVEC via the angiotensin AT1 receptor and biased agonism. *European Journal of Pharmacology* **768** 173–181. (doi:10.1016/j.ejphar.2015.10.051)
- Westerlind K, Wronski TJ, Ritman EL, Luo ZP, An KN, Bell NH & Turner RT 1997 Estrogen regulates the rate of bone turnover but bone balance in ovariectomized rats is modulated by prevailing mechanical strain. *PNAS* **94** 4199–4204. (doi:10.1073/pnas.94.8.4199)
- Wolff J 1986 *The Law of Bone Remodeling*. Berlin, Germany: Springer.
- Wootton R, Reeve J, Spellacy E & Tellez-Yudilevich M 1978 Skeletal blood flow in Paget's disease of bone and its response to calcitonin therapy. *Clinical Science and Molecular Medicine* **54** 69–74.
- Wootton R, Reeve J & Veall N 1976 The clinical measurement of skeletal blood flow. *Clinical Science and Molecular Medicine* **50** 261–268.
- Wronski T, Dann LM, Scott KS & Cintron M 1989 Long-term effects of ovariectomy and aging on the rat skeleton. *Calcified Tissue International* **45** 360–366. (doi:10.1007/BF02556007)
- Yao Z, Lafage-Proust MH, Plouët J, Bloomfield S, Alexandre C & Vico L 2004 Increase of both angiogenesis and bone mass in response to

- exercise depends on VEGF. *Journal of Bone and Mineral Research* **19** 1471–1480. (doi:10.1359/JBMR.040517)
- Yiu Y, Chan YH, Yiu KH, Siu CW, Li SW, Wong LY, Lee SW, Tam S, Wong EW, Cheung BM, *et al.* 2011 Vitamin D deficiency is associated with depletion of circulating endothelial progenitor cells and endothelial dysfunction in patients with type 2 diabetes. *Journal of Clinical Endocrinology and Metabolism* **96** E830–E835. (doi:10.1210/jc.2010-2212)
- Yiu Y, Yiu KH, Siu CW, Chan YH, Li SW, Wong LY, Lee SW, Tam S, Wong EW, Lau CP, *et al.* 2013 Randomized controlled trial of vitamin D supplement on endothelial function in patients with type 2 diabetes. *Atherosclerosis* **227** 140–146. (doi:10.1016/j.atherosclerosis.2012.12.013)
- Zaidi M, Chambers TJ, Gaines Das RE, Morris HR & MacIntyre I 1987 A direct action of human calcitonin gene-related peptide on isolated osteoclasts. *Journal of Endocrinology* **115** 511–518. (doi:10.1677/joe.0.1150511)
- Zaman G, Pitsillides AA, Rawlinson SC, Suswillo RF, Mosley JR, Cheng MZ, Platts LA, Hukkanen M, Polak JM & Lanyon LE 1999 Mechanical strain stimulates nitric oxide production by rapid activation of endothelial nitric oxide synthase in osteocytes. *Journal of Bone and Mineral Research* **14** 1123–1131. (doi:10.1359/jbmr.1999.14.7.1123)
- Zehnder D, Bland R, Chana RS, Wheeler DC, Howie AJ, Williams MC, Stewart PM & Hewison M 2002 Synthesis of 1,25-dihydroxyvitamin D3 by human endothelial cells is regulated by inflammatory cytokines: a novel autocrine determinant of vascular cell adhesion. *Journal of the American Society of Nephrology* **13** 621–629.
- Zhang Y, Fujita N, Oh-hara T, Morinaga Y, Nakagawa T, Yamada M & Tsuruo T 1998 Production of interleukin-11 in bone-derived endothelial cells and its role in the formation of osteolytic bone metastasis. *Oncogene* **16** 693–703. (doi:10.1038/sj.onc.1201581)
- Zhao Q, Shen X, Zhang W, Zhu G, Qi J & Deng L 2012 Mice with increased angiogenesis and osteogenesis due to conditional activation of HIF pathway in osteoblasts are protected from ovariectomy induced bone loss. *Bone* **50** 763–770. (doi:10.1016/j.bone.2011.12.003)
- Zheng X, Zhao ED, He JY, Zhang YH, Jiang SD & Jiang LS 2016 Inhibition of substance P signaling aggravates the bone loss in ovariectomy-induced osteoporosis. *Progress in Biophysics and Molecular Biology*. (doi:10.1016/j.pbiomolbio.2016.05.011)

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