REVIEW

The Key Role of the Blood Supply to Bone

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The importance of the vascular supply for bone is well-known to orthopaedists but is still rather overlooked within the wider field of skeletal research. Blood supplies oxygen, nutrients and regulatory factors to tissues, as well as removing metabolic waste products such as carbon dioxide and acid. Bone receives up to about 10% of cardiac output, and this blood supply permits a much higher degree of cellularity, remodelling and repair than is possible in cartilage, which is avascular. The blood supply to bone is delivered to the endosteal cavity by nutrient arteries, then flows through marrow sinusoids before exiting via numerous small vessels that ramify through the cortex. The marrow cavity affords a range of vascular niches that are thought to regulate the growth and differentiation of hematopoietic and stromal cells, in part via gradients of oxygen tension. The quality of vascular supply to bone tends to decline with age and may be compromised in common pathological settings, including diabetes, anaemias, chronic airway diseases and immobility, as well as by tumours. Reductions in vascular supply are associated with bone loss. This may be due in part to the direct effects of hypoxia, which blocks osteoblast function and bone formation but causes reciprocal increases in osteoclastogenesis and bone resorption. Common regulatory factors such as parathyroid hormone or nitrates, both of which are potent vasodilators, might exert their osteogenic effects on bone via the vasculature. These observations suggest that the bone vasculature will be a fruitful area for future research.

Keywords: vasculature; skeleton; oxygen; hypoxia; osteoblast; osteoclast

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Introduction

The role of the blood supply in the pathophysiology of bone deserves wider attention outside the orthopaedic community. This brief review focuses on selected topics of particular current interest. For more general coverage of the bone vasculature, readers are referred to numerous excellent reviews (1–11).

Anatomy and physiology of the blood supply to bone

The channels and vessels in bone were observed by Van Leeuwenhoek and Havers in the late 17th century and were confirmed to be linked to blood supply by Albinus in the mid-18th century. The importance of the blood

supply for bone was clearly recognized by surgeons in the 19th century (1). In the 20th century advances in orthopedics gave strong impetus to detailed functional studies of the bone vasculature, with pioneering contributions from Trueta and colleagues (1-2). Key studies by Brookes and colleagues demonstrated that the main blood supply of healthy long bones was derived from the principal nutrient arteries, which penetrate the cortex and perfuse the medullary sinusoids, then exit via multiple small veins. The cortical bone is perfused by a mixture of arterial blood originating from the main nutrient arteries as well as from the separate, smaller periosteal arteries. Thus, the blood flow in the long bones, at least from young adults, is largely centrifugal (ie, radiating outward after delivery to the marrow cavity) (4, 12) (Figures 1 and 2).

Healthy bone requires a substantial blood flow to supply the requisite oxygen and nutrients, and to eliminate carbon dioxide, acid and other metabolic waste products. Estimates of the proportion of the cardiac output recei-

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ved directly by the skeleton range from about 5.5% to 11% (13–15). The rich perfusion of bone reflects not only the requirements of the bone cells (osteoblasts, osteocytes and osteoclasts), but also of the marrow (hematopoietic lineage cells, stromal cells and adipocytes), as well as endothelial cells. The long bones form from embryonic cartilage rudiments that are invaded by blood vessels and bone cells. The vascular supply of bone enables rapid growth, remodelling (including mechanical responsiveness) that is not possible in cartilage, which is essentially avascular. For a detailed consideration of the functional anatomy of the blood supply to bone during growth, maturity and regeneration, readers are referred to the comprehensive review of Wilson (2002) (7).

Effect of oxygen on bone cell function

It has long been recognized that bone growth (including endochondral ossification during development) and

repair occur in association with a rich vascular supply. Conversely, impairment of the blood supply is well-known to reduce growth and repair, cause bone loss and, ultimately, necrosis (2-3, 5, 7-8, 10-11, 16). These observations are clearly consistent with the role of the vasculature in supplying nutrients, minerals and regulatory factors to bone. In recent years the influence of oxygen tension and hypoxia on bone function has become a major research focus.

Hypoxia occurs when the blood supply to tissues is reduced or disrupted. Oxygen tension (pO_2) in arterial blood is about 12.64 kPa (12%); in venous and capillary blood it is about 5.32 kPa (5%), approximately a quarter of that in atmospheric air. In normal tissues, median interstitial pO_2 values range from about 3–9% (17). Measurements of bone marrow aspirates from normal human volunteer donors produced mean pO_2 values of 6.6% (18). Cellular oxygen concentrations are normally maintained within narrow physiological ranges. Cells respond

Figure 1 Schematic diagram showing general arrangement of the vascular supply to healthy adult bone. The main blood supply is derived from one or more nutrient arteries, which penetrate to the medulla and connect to the smaller periosteal arterial supply to enable perfusion of cortical bone. The arterial branches drain into arterio-venous sinuses in the medulla that support hematopoietic and stromal cells. Blood exits the medullary cavity via multiple small veins that penetrate the cortex. Thus, perfusion is predominantly centrifugal, at least in young adult bone.

Figure 2 Bone vasculature. A: Light micrograph showing a thin-walled blood vessel (white arrows) penetrating lamellar cortical bone of adult human phalanx; longitudinal section, image width 1.5 mm. B: Transverse section of human phalanx (ground with carbon black) showing Haversian systems with central blood vessel canals; image width 2 mm. C: MicroCT scan of a sagittal section (2-D) of adult mouse tibia infused *ex vivo* with a radiopaque contrast agent showing the main medullary vessel (highlighted in red); image width 3.5 mm. D: 3-D rendering of the vascular network and sinuses of the same tibia, rescanned by microCT after decalcifying the bone.

to changes in pO_2 via the oxygen-dependent degradation of hypoxia-inducible transcription factors (HIFs). In the presence of oxygen, HIF-1 α , and the closely related HIF-2 α , are targeted by prolyl hydroxylases, which utilize molecular oxygen (with ascorbate as a co-factor) to selectively hydroxylate two conserved proline residues, which effectively tag HIF- α for polyubiquitination and

proteasomal degradation. In the absence of sufficient oxygen, the prolyl hydroxylases are inactive and the proline residues on HIF- α remain unmodified; HIF- α is stabilized and heterodimerises with its transcription partner, HIF- β . The HIF heterodimer binds hypoxia response elements in promoter sequences of target genes, initiating transcription of hypoxia-regulated genes

involved in a variety of cellular processes including angiogenesis (of which vascular endothelial growth factor, VEGF, is a key mediator), energy metabolism, cell proliferation/survival and pH control (19-20).

Experiments to test the direct effects of pO_2 on osteoclast function yielded surprising results. Hypoxia was shown to strongly stimulate the number and size of osteoclasts formed in cultures of mouse marrow or human peripheral blood mononuclear cells, resulting in large increases in resorption pit formation. Optimal osteoclast formation occurred in 1-2% O_2 (with infrequent re-oxygenation), but osteoclastogenesis and resorption were elevated even in cultures gassed with 0.2% O_2 (17, 21-23). Conversely, hyperoxygemia decreases osteoclast formation and function (24). These responses are consistent with the known stimulatory action of hypoxia on other cells of the monocyte-macrophage lineage, and the adaptation of such cells to function in harsh, oxygen-deprived environments (17). Hypoxia is also associated with tissue acidosis, and reduced ambient pH is a well-known requirement for activation of mature osteoclasts (17) (Figure 3).

In contrast, hypoxia has profound inhibitory effects on osteoblasts. Ambient pO_2 levels below 2% result in near-complete abolition of bone formation by cultured primary osteoblasts (25) (Figure 3). The inhibition of osteogenesis in hypoxia was due to reductions in both growth and differentiation of osteoblasts, with inhibition of cell proliferation, collagen production and alkaline phosphatase. The inhibitory effect of hypoxia on collagen production may involve decreased expression and activity of the oxygen-dependent enzymes, prolyl 4-hydroxylase (a member of the same enzyme family as the oxygen-dependent prolyl hydroxylases that act upon HIF α) and lysyl oxidase, which are required for post-translational modification of collagen molecules. Interestingly, chronic hypoxia (unlike anoxia) does not increase cell death in osteoblasts, but rather induces a reversible state of quiescence (25). These studies also indicated that high (supraphysiological) pO_2 levels are osteogenic and support the notion that bone formation is dependent on a rich vascular supply.

Vascular niches in bone

The fine anatomy of the vasculature in bone requires specialist techniques to study histologically, not only because of the technical difficulties presented by the bone itself, but also because the vessels and sinusoids are mostly very delicate, with very thin walls, or consisting only of endothelial cells (26). The marrow is a site for haematopoiesis, as well as acting as a reserve for stromal

cells that could differentiate into osteoblasts, fibroblasts and the adipocytes that make up the bulk of its volume. Although the mean pO_2 in healthy marrow may be in the range of 6-7% (18), mathematical models predict that pO_2 may fall as low as 1% in the microenvironments most distant from capillaries (27). In the absence of clear experimental evidence, such hypoxic sites are postulated to provide niches favourable to the maintenance of undifferentiated haematopoietic stem cells (20, 28). Although experimental evidence shows that hypoxia actually stimulates osteoclast formation, at least from mixed marrow or circulating mononuclear cells (17, 22), this concept is consistent with the maintenance of quiescent, undifferentiated cells of the stromal lineage (17, 25, 29-30).

Another vascular microenvironment that has attracted attention in recent years is the 'bone remodeling compartment' or 'canopy', first described by Hauge in 2001. This canopy is formed by flattened, osteoblast-like cells at an early differentiation stage, similar to bone lining cells; it is proposed to be a site in which bone resorption formation take place in regulated ('coupled') manner (31-33). A related concept was also put forward by Parfitt (6). Such compartments, although technically difficult (or impossible) to access for real-time physiological analysis, could serve to provide optimal conditions, including pO_2 and pH, for bone resorption and bone formation (17, 32-33).

Role of vasculature in bone regeneration and fracture healing

Orthopaedic surgeons have long appreciated the role of the blood supply in bone growth and healing (2-3). The trauma of a fracture or other major bone injury also damages the blood supply, resulting in local hypoxia, which may be maintained by the subsequent inflammation (34). In rabbits, for example, pO_2 in the fracture hematoma is <1% (35) and in the medullary cavity following osteotomy between about 1-3% (36). The HIF- α pathway, which is activated in hypoxia, is reported to be a key mechanism for coupling bone growth to angiogenesis, via increased expression of VEGF, the major angiogenic cytokine expressed by hypoxic osteoblasts. Mice selectively overexpressing HIF α in their osteoblasts had high levels of VEGF expression and extremely dense, highly vascularised bones (37-38). These mice also produced more bone in response to tibial osteotomy and distraction osteogenesis, whereas mice lacking HIF-1 α in osteoblasts had impaired VEGF-dependent angiogenesis and bone healing (39). The role of HIFs in osteogenesis may be quite complex, however. A recent report indi-

cates that HIF-1 α may also activate expression of sclerostin (the key bone-specific inhibitor of Wnt signaling) in osteoblasts, thus potentially reducing osteogenesis (40). The VEGF homologue, PlGF (placental growth factor), which acts through the VEGF receptor, also appears to play a significant role in promoting fracture healing (34). VEGF stimulates the regrowth of blood vessels into the injury site, so that oxygen and nutrient levels can begin to return to normal values.

The general pattern of bone cell activity following fracture or osteotomy is broadly consistent with the known responses of osteoblasts and osteoclasts to changes in pO₂: the early hypoxic phase favours osteoclast recruitment, whilst inhibiting osteoblasts (which may survive locally in a quiescent state), whereas revascularisation will progressively favour osteoblast function (proliferation, differentiation and bone formation). Osteoblast precursors could also move into developing and fractured bones along with invading blood

vessels (41). Bone microdamage, induced by fatigue loading, has also been shown to increase local vascularity and blood perfusion, probably as a repair mechanism to reconstruct a disrupted lacuno-canalicular network (42). It is worth noting that application of early or delayed functional loading has been shown to respectively inhibit or stimulate neovascular growth in a rat model of large bone defect regeneration (43). This suggests that biomechanical stimulation could modulate vascular growth and remodelling during bone repair, partially overriding the normal sequence of cellular responses described above.

The role of angiogenesis in bone metabolism and blood perfusion

Bone perfusion by oxygen and nutrients is the product of the vascular tone, which regulates blood flow as discussed above, and the density of the vessels. The densi-

Figure 3 Schematic diagram summarising the key effects of oxygen on bone cell function. Osteoblast function (proliferation, differentiation and collagen production) are inhibited in hypoxia; osteoblasts enter a quiescent state. In contrast, hypoxia stimulates the formation of osteoclasts from mononuclear precursor cells (in the presence of RANKL and M-CSF), resulting in increased bone resorption. The negative impact of hypoxia is exacerbated by the accompanying acidosis, which blocks matrix mineralisation and stimulates mature osteoclasts to resorb.

ty and in general the structure of the vascular bed depends on the regulation of local angiogenic and vasculogenic factors. Angiogenesis has been studied much more extensively in relation to the endochondral ossification process and bone repair; readers are referred to excellent recent reviews on this topic (10-11, 44-45).

The pivotal factor linking angiogenesis to bone remodelling is VEGF, which, when overexpressed in cells of the osteoblast lineage *in vivo*, leads to a high bone mass phenotype associated to marrow fibrosis and increased number of blood vessels (46). These effects appear to be secondary, at least in part, to activation of Wnt/ β -catenin signaling in osteoblasts. This is consistent with a role of Wnt-1 signaling in the angiogenic response, which ameliorated blood perfusion in ischaemic limbs (47). Conversely, mice that lack VEGF in osteoprogenitors display a reduced bone mass phenotype with increased bone marrow fat (48).

HIF-1 α is the most important transcription factor regulating VEGF production by bone cells in response to hypoxia (34, 49). Importantly, VEGF expression, as expected, is increased in mutant bones lacking VHL, which increases activated HIF-1 α activity in cells of the osteoblast lineage (37). Therefore, it is reasonable to hypothesize that VEGF could be responsible for the increased number and/or size of blood vessels in these mutants (19), although this possibility has still to be experimentally tested.

It is reported that bone mass accrual induced by intermittent PTH in mice is not accompanied by an angiogenic response in the bone marrow vascular network, although blood vessels were observed to relocate closer to bone formation sites. The osteogenic effect was blunted by blocking VEGF (50). It is not clear from these studies whether VEGF was required for the relocation of marrow vessels (50), or to mediate the vasorelaxant effect of PTH (51).

Pathological changes to bone blood supply associated with aging or systemic disorders

The general association between ageing, vascular dysfunction and bone loss was not widely recognised until quite recently (8, 10, 52). The oxygen consumption of human bone declines with age, accompanied by decreased vascular conductance (53). Doppler ultrasound measurements showed that femoral artery blood flow was about 30% lower in old men (average age 64 years), compared with young men (average age 28 years), whereas vascular resistance was about 50% higher (54). Elderly women with osteoporosis are reported to have

reduced femoral blood flow, measured by magnetic resonance perfusion imaging, in association with increased marrow fat compared with non-osteoporotic subjects (55). Decreased femoral blood flow, measured using radiolabelled microspheres, has also been reported in aged male rats, correlating with reduced bone strength (56) and reduced endothelium-dependent vasodilation (57). In female rats, magnetic resonance imaging showed that vertebral blood flow was reduced 8 weeks after ovariectomy (a model for post-menopausal osteoporosis), alongside decreased bone mineral density (58).

An important contributor to reduced systemic blood flow in the elderly is the increased vascular stiffness and resistance due to calcification of the muscle walls of large vessels. There is a well-known, reciprocal relationship between mineral in bone and mineral deposition in major blood vessels (59-61), which can be viewed as a kind of 'vicious cycle'. Decreased perfusion of bone will tend to lead to decreased medullary pO₂, which, as noted above, will favor increased osteoclastic resorption and reduced bone formation. The resulting chronic net efflux of calcium from the bones into the blood supply might, in turn, be expected to favor mineralisation of vessel walls. *Ex-vivo* studies of human long bones have suggested that, with age, decreasing medullary perfusion may to some extent be compensated by an increased periosteal blood supply (5). The responses of bone cells to changes in pO₂ may also help to explain why the diameter of long bones slowly increases with age, albeit accompanied by thinner cortices (i.e., due to increased endosteal resorption, plus increased periosteal formation).

Essential hypertension is a key reversible risk factor in cardiovascular disease, which is strongly associated with aging (62). Hypertension has been shown to correlate with reduction in BMD in women (63-65) and animals (66) and has been proposed as a risk factor for osteoporotic fractures (67). The stiffening of the blood vessels characterising hypertension is caused by several factors, including imbalance of osteotropic hormones and endothelial dysfunction with reduced NO bioavailability and impaired vasodilation (61). Antihypertensive drugs have hemodynamic and physiologic effects that attenuate these vascular disease processes, but are class-specific (e.g., anticoagulants, β -blockers, and calcium channel blockers) and have provided generally beneficial effects to bone mass (68-71).

A number of common systemic disorders can potentially impair vascular perfusion of bone, with associated bone loss. These include diabetes (61, 72-73), chronic obstructive pulmonary diseases (74-76) and anaemias

(77-78). Smoking, which delivers the vasoconstrictor, nicotine, is also strongly associated with bone loss (79-80). The role of disuse or decreased mechanical loading in bone loss related to reduced perfusion is discussed in the following section.

Mechanoadaptation of bone and the blood supply

The beneficial effect of exercise on maintaining bone homeostasis with aging has been shown to be associated with alterations in endothelium-dependent vasodilatation and structure of rat skeletal muscle arterioles, which, in turn, affect limb and bone perfusion (81). Increased bone vessel function (NO-mediated vasodilation of bone resistance arteries) has been associated with increased trabecular bone volume induced by endurance training in rats (82). Besides increasing vessel function, exercise also causes an elevation in the number and density of intraosseous vessels in rats, which has been attributed to an elevation in the angiogenic factor VEGF (83).

Conversely, disuse-induced bone loss caused by bed-rest (84), hindlimb unloading (85), neurectomy and spinal cord injury (86), have been associated with altered indexes of bone blood perfusion. Although the adaptation of bone to mechanical strain (beyond or below customary levels) seems to clearly involve changes in bone perfusion and vessel function, the physical and molecular mechanisms linking are not fully understood. Given the key role of Wnt/beta-catenin signaling on mechanoadaptation and osteogenesis (87), further investigation is needed to relate this important pathway to bone blood flow.

Elevation of the intramedullary pressure (IMP) by intramedullary fluid pumps (88-90), or by dynamic skeletal muscle stimulation (91), can strongly affect bone mass and quality, even independent of direct strain applied to bone. This phenomenon is also observable after venous occlusion (92-94), hyperhydration (95), microgravity (85, 96) and hypertension (97).

Interstitial fluid flow across the lacuno-canalicular porosity, driven by dynamic mechanical strain of the poroelastic bone matrix and oscillating elevation in the IMP, is considered the most likely stimulus for osteocytic mechanosensing (98). Although, it has long been known that IMP pressure is about one fourth of the systemic arterial pressure and IMP depends on the total blood flow entering the bone and the total blood flow leaving it and has a pulsatile regime synchronous with the arterial blood pressure and respiration (99). Theoretical (100) and experimental (101) results suggest that local or systemic

vascular pressure itself does not enhance solute transport within the bone lacuno-canalicular porosity. This, however, does not exclude that the changes in bone perfusion may either interact with the osteocytic mechanoadaptive response indirectly, by altering the composition of the milieu of the bone interstitial fluid (which is an ultrafiltrate of blood), or by changing the mechanical environment of the bone marrow, thereby stimulating the bone cells in direct contact with the marrow, rather than the osteocytes (99). This is supported by the recent finding that elevation in IMP is able to stimulate bone adaptation also without the contribution of osteocytes, which were genetically ablated (88).

Whether and how changes in mechanical loading and/or IMP may affect bone blood circulation and the tone of the intramedullary blood vessel is not clear (99), but it is likely that muscle contraction plays a role through the modulation of vascular resistance regulation the flow of blood exiting bone (102-103). Further research in this area is required to understand whether skeletal blood perfusion and mechanoadaptation to exercise-induced bone strain may induce synergistic effects on bone formation.

Physiological and pharmacological regulators of bone perfusion

A number of seminal studies dating from the late 1970s on the perfusion pressure of the nutrient artery in the tibia of dogs have demonstrated that the vascular bed of bone actively responds to vasoconstrictors and vasodilators. These factors included NO (104), agonists and antagonists of the alpha and beta adrenergic receptors (15, 105-107) and modulators of the cAMP or cGMP signaling (104), which are present in endothelial and smooth muscle cells (eg, acetylcholine, adrenaline, methoxamine, alpha-blockers, propranolol, isoproterenol, dibutyl cyclic AMP, 8-bromo-cyclic GMP), and have the ability to regulate systemic blood pressure and/or cardiac output.

However, only recently has the relationship between blood flow regulators and bone metabolism been demonstrated. Clinical and experimental observations appear to support a general model in which factors that decrease systemic blood pressure and increase blood perfusion induce beneficial effects on bone mass and vice versa (ie, vasoactive agents, which increase vascular tone and decrease blood flow are associated with bone loss). The best known endogenous modulator of the vascular tone is Nitric Oxide (NO), which is essential for the maintenance of a functional cardiovascular system (108). Aging has been shown to reduce endothelium-

dependent vasodilation, and NO bioavailability in rats, which was associated with reduced skeletal blood flow (57). Hind limb blood flow in rodents has been shown to depend largely on NO and is modulated to a lesser extent by the endothelium-derived hyperpolarising factor (109). There is strong evidence that supplementation of NO donors such as nitroglycerine (110–114) and isosorbide mononitrate (114–115) increase bone mass in animals and osteoporotic patients, although there are also some conflicting studies in humans (116–117). Whether the osteoanabolic action of NO in bone is mediated indirectly by endothelium-controlled blood perfusion, or whether it also has significant direct actions on bone cells, is still not known. The effects of NO on bone cell function are not very clear-cut: biphasic effects on the function of both osteoblasts and osteoclasts have been reported (118). The role of nitric oxide synthases in bone also appears to require further clarification. Recent studies indicate that endothelial nitric oxide synthase (eNOS), previously thought to be important for NO production in bone, may not be required for the responses of bone and bone cells to mechanical loading (119–121). Thus, other forms of NOS expressed by bone and vascular cells (neural NOS and inducible NOS) might play significant roles in NO synthesis.

Studies on the skeletal effects of other drugs used in the treatment of cardiovascular disease, including oral anticoagulants, beta-blockers, and calcium channel blockers, have yielded conflicting results (67–71, 122). Nevertheless, since drugs used to treat hypertension can increase systemic blood flow, further clinical and experimental research should be warranted into their potential beneficial effects on bone perfusion. It has been recently shown that phosphodiesterase 5 inhibitors, such as sildenafil, which are potent vasodilators used to treat erectile dysfunction, increase fracture healing in mice (123).

Vasoconstrictors, on the other hand, may have negative impacts on bone. For example, increased levels of endothelin (ET)-1, a potent endogenous vasoconstrictor linked to increased cardiovascular risk, are associated with the menopause and osteopenia (124–125). The powerful vasoconstrictor nicotine has been shown to inhibit bone regeneration in a rabbit model of distraction osteogenesis (126). Interestingly, this inhibitory effect of nicotine on bone formation and perfusion was associated with increased angiogenesis, which was unable to counteract the reduction in blood flow (127).

In addition to well known vasoactive agents, a number of other endogenous factors recognised as major regulators of bone mass have been shown to induce potent vasotonic effects. In line with this notion, factors which

increase bone formation such as PTH (128), PGE₂ (129) and estrogen (130–131) are potent vasodilators and increase bone blood flow, while factors that inhibit bone formation such as glucocorticoids also decrease vascularity and skeletal perfusion (132–133). It is currently not clear whether the vascular effects associated with these factors ultimately play a significant role in their actions on bone. This is an area of active current research.

It has been known since the 1920s that administration of exogenous PTH causes systemic hypotension and increased regional blood flow (130, 134–135). This effect is mediated by a potent vasorelaxant effect of PTH on vascular smooth muscle cells (SMC) (136); overexpression of PTH/PTHrP type 1 receptor in SMC reduces systemic blood pressure (137). PTH-induced vasorelaxation in bone blood vessels (*ex vivo*) has been linked with its osteoanabolic activity (51). A study by Prisby and colleagues showed that PTH caused dilation of bone arteries and that was partly mediated by VEGF (51), which also functions as a vasodilator, in addition to its better-known role in angiogenesis (138). Our own recent experimental data are in full agreement with these results. We have shown that blocking the vasorelaxant effect of PTH by an inhibitor of NO synthase (L-NAME) partially blunted its osteoanabolic action in mice (139).

A fascinating aspect of the mechanism of action of PTH and PGE₂ is the fact that while they both induce a potent anabolic effect on bone when dosed intermittently (eg, daily) (140–141), they become catabolic when administered by continuous slow release (142–144). At present, there is no clear experimental explanation for this time-dependent action. However, if acute vasorelaxation plays an important role in the mechanism of action of PGE₂ and PTH, it could be speculated that an adaptation similar to tachyphylaxis may take place in the cardiovascular system during continuous dosing, which may abolish the initial acute vasorelaxation response.

It is also well known that estrogen can boost blood flow (145–146). Deprivation of endogenous estrogen (130, 147–148) and synthetic estrogen supplementation (131, 149–150) have opposite potent vasoactive effects, although the relationship between the osteogenic and cardiovascular effects remains to be fully elucidated.

Conclusions

Considerable progress has been made towards better understanding the role of the vasculature in the pathophysiology of bone. Clearly, this will remain a fruitful topic for research because of the strong links to common bone disorders. Some key practical questions for

the future include: 1) What is the role of hypoxia and HIF in bone growth and repair? 2) To what extent do the osteoanabolic actions of agents such as PTH, Wnt proteins and nitric oxide donors depend on vascular responses? 3) Are the cardiovascular system and vascular tone involved in bone mass homeostasis? 4) To what extent do the osteocatabolic actions of glucocorticoids depend on vascular responses? A better understanding of the basic physiological mechanisms linking vascular function to bone metabolism has the potential to unravel new pharmacological approaches to activate bone growth and accelerate fracture healing.

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