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REVIEW

Wnt signaling and cellular metabolism in osteoblasts

Courtney M. Karner[1,2,3] · Fanxin Long[1,4]

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Abstract The adult human skeleton is a multifunctional organ undergoing continuous remodeling. Homeostasis of bone mass in a healthy adult requires an exquisite balance between bone resorption by osteoclasts and bone formation by osteoblasts; disturbance of such balance is the root cause for various bone disorders including osteoporosis. To develop effective and safe therapeutics to modulate bone formation, it is essential to elucidate the molecular mechanisms governing osteoblast differentiation and activity. Due to their specialized function in collagen synthesis and secretion, osteoblasts are expected to consume large amounts of nutrients. However, studies of bioenergetics and building blocks in osteoblasts have been lagging behind those of growth factors and transcription factors. Genetic studies in both humans and mice over the past 15 years have established Wnt signaling as a critical mechanism for stimulating osteoblast differentiation and activity. Importantly, recent studies have uncovered that Wnt signaling directly reprograms cellular metabolism by stimulating aerobic glycolysis, glutamine catabolism as well as fatty acid oxidation in osteoblast-lineage cells. Such findings therefore reveal an important regulatory axis between bone anabolic signals and cellular bioenergetics. A comprehensive understanding of osteoblast metabolism and its regulation is likely to reveal molecular targets for novel bone therapies.

Keywords Wnt · Metabolism · mTORC1 · mTORC2 · Glucose · Glutamine · Fatty acids · Osteoblast · Bone

✉ Fanxin Long
flong@wustl.edu

[1] Department of Orthopaedic Surgery, Washington University School of Medicine, St. Louis, MO 63131, USA

[2] Present Address: Department of Orthopaedic Surgery, Duke Orthopaedic, Cellular, Developmental and Genome Laboratories, Duke University School of Medicine, Durham, NC 27710, USA

[3] Department of Cell Biology, Duke University School of Medicine, Durham, NC 27710, USA

[4] Department of Developmental Biology, Washington University School of Medicine, St. Louis, MO 63131, USA

Introduction

The mammalian skeleton not only provides support and protection, but also performs endocrine functions. The homeostasis of adult bone mass under healthy conditions is maintained through the exquisite balance of bone resorption by osteoclasts and bone formation by osteoblasts. With aging or under pathological conditions bone resorption dominates over formation, resulting in osteopenia (low bone mass) or in the more severe cases, osteoporosis. Conversely, conditions that favor bone formation over resorption lead to high bone mass diseases such as sclerosteosis. Because osteoblasts are the chief cell type producing bone materials, elucidating the mechanisms that regulate osteoblast differentiation and activity is critical not only for understanding bone physiology but also for designing effective bone therapeutics. Extensive studies in the area during the past several decades have mostly focused on endocrine or paracrine signaling as well as transcriptional regulation [1, 2]. Those studies have uncovered the critical roles of growth factors such as Wnt proteins and transcription factors including Runx2, Osterix, and ATF4 during osteoblast differentiation [3–8]. However, relatively little is understood about how osteoblasts

fulfill their key function of active protein synthesis and matrix secretion, a process highly demanding not only in building blocks but also in energy [9]. Recent studies have discovered that the potent bone anabolic signal Wnt directly reprograms multiple aspects of cellular metabolism integral to osteoblast differentiation and activity. This review summarizes those recent advances.

Wnt signaling

Wnt proteins are a family of secreted glycoproteins that are critical regulators of osteoblast differentiation and activity in both mice and humans [10–16]. Wnt signals are transduced by a family of seven-pass transmembrane G-protein coupled receptors of the frizzled (Fzd) family and a co-receptor of the arrow/Lrp family (e.g., Lrp5 and Lrp6) or a Ryk or Ror transmembrane tyrosine kinase [17, 18]. The binding of a given Wnt to a Fzd receptor and coreceptor activates multiple distinct intracellular signaling cascades, historically divided into the canonical β-catenin-dependent pathway and noncanonical β-catenin-independent pathways [19]. The best characterized is the canonical Wnt pathway, which results in the stabilization and translocation of β-catenin into the nucleus. β-catenin (encoded by *Catnnb1*) is an important transcriptional co-activator that regulates gene transcription in response to Wnt signaling. Normally, in cells not exposed to ligand, cytoplasmic levels of β-catenin are kept low through interactions with the β-catenin destruction complex [20]. The binding of Wnt to a Fzd receptor complex results in phosphorylation of the Lrp co-receptors and recruitment and tethering of GSK-3β and Axin to the ligand-receptor complex. This complex is subsequently endocytosed and inhibited through sequestration into multivesicular endosomes resulting in the stabilization and accumulation of cytoplasmic β-catenin [21]. Stabilized β-catenin translocates into the nucleus and interacts with the Lymphoid-enhancing factor/T cell factor (Lef/Tcf) family of high mobility group (HMG)-type transcription factors to stimulate expression of target genes including *Lef1*, *Tcf7*, *Nkd2,* and *Axin2* [22–26]. Additionally, Wnt signaling can activate multiple signaling cascades independent of β-catenin. Here, Fzd seems to function more as a G-protein coupled receptor, activating intracellular cascades involving the GTPases Rho and Rac, the calcium calmodulin dependent kinase 2 (CaMK2), c-Jun N-terminal kinase (JNK) and p38, phospholipase-C, protein kinase C (PKC), protein kinase A (PKA), PI3 K/AKT, and mTOR [11, 27–33]. The pathway activated by a Wnt ligand is determined by many factors including specific ligand-receptor interactions, distinct receptor/co-receptor pairs, or the presence of intracellular proteins that regulate β-catenin activation [34–37]. Wnt signaling is also regulated by a number of secreted extracellular antagonists. These include Dickkopf (e.g., Dkk1 and Dkk2) and Sclerostin (Sost) proteins that bind to the extracellular domains of Lrp5 or Lrp6 and interfere with their interaction with Wnt proteins [38–41]. In addition, the secreted frizzled related proteins (sFRPs) bind directly to Wnt ligands and thus inhibit the formation of Wnt–Fzd complexes [42–44]. Overall, Wnt signaling is tightly controlled at multiple levels to ensure its proper activity during normal development and tissue homeostasis.

Wnt signaling in bone

The importance of Wnt signaling during bone formation has been well documented [45]. The original discovery came from human genetic studies where inactivating mutations in the Wnt co-receptor *Lrp5* results in osteoporosis pseudoglioma syndrome while gain-of-function mutations causes osteosclerosis [15, 46, 47]. Moreover, mutations in either coding or regulatory sequences of *Sost* cause high bone mass in sclerosteosis or Van Buchem disease, respectively [48–50]. Subsequent genome-wide association studies in humans strongly support a role for Wnt signaling regulating bone mineral density (BMD) [51–54]. More recently, exome sequencing in humans identified multiple mutations in *Wnt1* associated with early onset osteoporosis and osteogenesis imperfecta [55, 56]. In addition, missense mutations in *Wnt16* are associated with decreased forearm and hip BMD and increased fracture risk [57].

In keeping with those findings in humans, genetic studies in mice have established a causal relationship between Wnt signaling and bone formation. Mice lacking *Lrp5* either globally or in osteoblasts are characterized by osteopenia whereas expression of mutant *Lrp5* alleles associated with human high-bone-mass syndromes increases bone mass in mice [58–60]. Activation of Wnt signaling through deletion of *Sfrp1, Sost* or a single allele of *Dkk1* increases osteoblast number and activity [61–63]. In addition, multiple Wnts, including Wnt1, Wnt7b, Wnt10b, and Wnt16 and the Frizzled receptors Fzd7 and Fzd9, have been shown to regulate bone formation [10, 11, 57, 64–66]. Moreover, targeted deletion of *Gpr177* (also known as Wntless—*Wls*, required for the secretion of all Wnt ligands) inhibits bone formation in mice [67, 68]. It is worth noting that the bone phenotypes resulting from loss of individual Wnt ligands or Fzd receptors are less severe than those reported in *Gpr177* knockout mice, indicating significant functional redundancy among Wnt ligands and Fzd receptors. This viewpoint is consistent with the fact that osteoblast-lineage cells express multiple Wnt ligands during development [13, 69].

Genetic studies in mice have highlighted the importance of β-catenin in mediating Wnt signaling in bone formation. Multiple studies demonstrate that β-catenin is required for bone formation and acts at multiple stages of osteoblast differentiation to regulate both osteoblast and osteoclasts [12, 13, 16, 70–74]. The direct target genes of β-catenin during osteoblast differentiation are not fully elucidated, but β-catenin together with Tcf1 has been shown to stimulate *Runx2* transcription directly [75]. In mature osteoblasts, *Opg,* encoding an anti-osteoclastogenic factor, is known to be a direct target of β-catenin [71]. While the importance of β-catenin in bone is well established, the contribution of β-catenin-independent Wnt signaling to bone formation is becoming increasingly clear. For example, Wnt7b can stimulate osteoblast differentiation through activation of PKCdelta [11]. Multiple Wnt proteins also activate the serine threonine kinase mammalian target of rapamycin complex 1 (mTORC1) which promotes protein synthesis and bone formation [29, 76, 77]. The stimulatory effect of mTORC1 in bone formation is further supported by genetic studies that either abolish or enhance mTORC1 activity in the mouse [78, 79]. In addition, Wnt activates mTORC2 that is required for the optimal bone accrual in response to mechanical loading or an anti-sclerostin neutralizing antibody [80–82]. Because the mTOR pathways are central to nutrient sensing and metabolic regulation, Wnt signaling has emerged as an important mechanism for modulating cellular metabolism in osteoblasts.

Glucose metabolism in osteoblasts

Glucose is the primary energy source for most mammalian cell types. Glucose is transported into the cell via the Glut family of facilitative glucose transporters. The Gluts transport glucose down a concentration gradient independent of ATP [83, 84]. Inside the cell, glucose is phosphorylated by hexokinase (Hk) to form glucose-6-phosphate (G6P). G6P can be converted to glycogen for storage or metabolized by disparate pathways including hexosamine biosynthetic pathway (HBP), pentose phosphate pathway (PPP), and glycolysis [85]. The HBP is used to produce uridine diphosphate N-acetylglucosamine (UDPGlcNAc) for protein glycosylation. The PPP is important to generate NADPH and ribose-5-phosphate important for nucleotide synthesis. Glycolysis occurs in the cytosol and produces 2 molecules of pyruvate, 2 ATP, and 2 reducing equivalents in the form of NADH per glucose molecule. Pyruvate can be converted into lactate by the enzyme lactate dehydrogenase (Ldh) independent of oxygen. This reaction regenerates oxidized NAD (NAD^+) that is necessary for further glycolysis. Alternatively, pyruvate can be decarboxylated to form acetyl-CoA by the enzyme pyruvate dehydrogenase (Pdh). Pyruvate oxidation in the tricarboxylic acid (TCA) cycle produces the most ATP per glucose molecule through oxidative phosphorylation (OXPHOS). Importantly, TCA intermediates are often extracted from the cycle (cataplerosis) and used for lipid and amino acid biosynthesis, redox regulation, and epigenetic regulation of gene expression [86–88]. The TCA cycle intermediates are replenished through metabolism of amino acids or fatty acids, a process known as anaplerosis. Thus, glucose is not only an important energy source but also a critical provider of building blocks for biosynthetic reactions.

Glucose is an important nutrient for osteoblasts. Osteoblasts express the glucose transporter Glut1 and rapidly consume glucose in response to a variety of signals [81, 89–94]. Recent work has revealed a feed-forward mechanism between Glut1 and Runx2 expression, highlighting a critical role for glucose metabolism in osteoblast differentiation [93]. Mature osteoblasts are known to possess numerous mitochondria and exhibit active OXPHOS [95–97]. Interestingly, however, aerobic glycolysis appears to be the dominant mode of glucose utilization in osteoblasts. For example, early studies using both bone slices and isolated osteoblasts reveal that osteoblasts rapidly take up glucose and metabolize it primarily into lactate [98–101]. More recent studies have confirmed aerobic glycolysis as a predominant mode of glucose metabolism in primary calvarial osteoblasts despite increased OXPHOS as the cells further differentiate to form mineralized nodules in response to ascorbic acid and β-glycerophosphate [96, 102]. Functionally, stimulating glycolysis through activation of Hif1α signaling in preosteoblasts increases bone formation in vivo, indicating that reprogramming glucose metabolism is sufficient to promote osteoblast differentiation [103]. Moreover, Hif1α activation has been shown to promote bone healing partly through reprogramming of glucose metabolism, highlighting the clinical implications of understanding metabolic regulation in osteoblasts [104]. Recent studies have demonstrated that aerobic glycolysis is directly stimulated in response to osteogenic signals such as PTH and Wnt [81, 105]. For example, Wnt rapidly increases Glut1 and Hk2 protein levels to increase glucose consumption. Wnt further promotes aerobic glycolysis by upregulating Ldha and Pdk1 to favor lactate over acetyl-CoA production from pyruvate (Fig. 1). Consistent with these observations, osteoblasts from mice expressing the human *Lrp5* high bone mass mutation show increased glucose consumption and expression of glycolytic enzymes. Conversely, *Lrp5*$^{-/-}$ mice have decreased glycolytic enzyme expression and lower serum lactate levels. Mechanistically, Wnt can induce glycolysis independent of β-catenin activity, instead through mTORC2 signaling [81]. It should be noted that β-catenin has been shown to induce transcription of *Pdk1* in

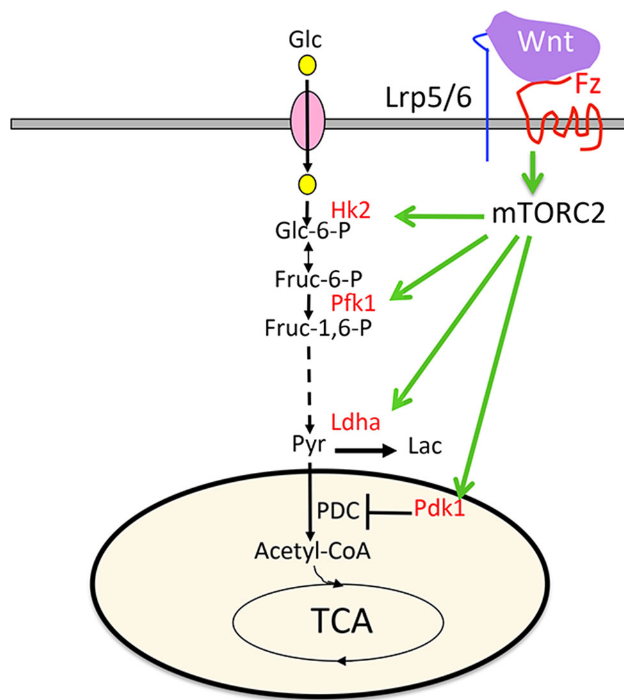

Fig. 1 Wnt signaling promotes aerobic glycolysis through mTORC2 activation. Wnt signaling through Frizzled (Fz) and Lrp5/6 induces mTORC2 activity downstream of PI3K-Rac1 signaling whereas mTORC2 activation acutely increases the protein abundance of metabolic enzymes (in *red*) without changing their mRNA levels. *Glc* glucose, *Glc-6-P* glucose 6-phosphate, *Fruc-6-P* fructose 6-phosphate, *Fruc-1,6-P* fructose 1,6-bisphosphate, *Pyr* pyruvate, *Lac* lactate, *Hk2* hexokinase 2, *Pfk1* phosphofructokinase 1, *Ldha* lactate dehydrogenase A, *Pdk1* pyruvate dehydrogenase kinase 1, *PDC* pyruvate dehydrogenase complex, *TCA* tricarboxylic acid cycle. See original reference for details [81]

cancer cells, indicating an additional mechanism for Wnt to stimulate aerobic glycolysis [105]. Direct inhibition of mTORC2 signaling by deletion of *Rictor* reduced both physiological bone formation and Wnt-induced bone formation in response to an anti-sclerostin neutralizing antibody [80, 107]. It is important to note that increased aerobic glycolysis contributes to Wnt-induced osteoblast differentiation in vitro as Ldha or Pdk1 knockdown impaired induction of osteoblast differentiation marker genes [81]. Likewise, dichloroacetate, which inhibits Pdk1 and promotes glucose metabolism through the TCA cycle, reduced bone formation in vivo in response to Hif1a overexpression or anabolic PTH treatment [91, 103]. Overall, both Wnt and PTH stimulate aerobic glycolysis as a mechanism to promote bone anabolism.

The reasons for osteoblasts to prefer aerobic glycolysis are not fully understood at present. From the bioenergetics viewpoint, aerobic glycolysis is a less efficient means of producing ATP compared to metabolism through the TCA cycle and OXPHOS. Cancer cells display a similar metabolic reprogramming, which is postulated to provide nucleotides, amino acids, and lipids needed to support cell division [106]. Mature osteoblasts, however, generally exhibit little proliferation in vivo [107]. Increased aerobic glycolysis may help reducing reactive oxygen species or generate more amino acids to support protein synthesis in osteoblasts. Moreover, glycolytic changes could directly exert epigenetic regulation to influence osteoblast differentiation. We have recently demonstrated a link between increased aerobic glycolysis and gene suppression in response to Wnt. Increased aerobic glycolysis limits the amount of citrate exiting the TCA cycle, resulting in decreased nuclear levels of both citrate and acetyl-CoA. This leads to a large scale decrease in histone acetylation and suppression of adipogenic or chondrogenic transcription factors, thus favoring osteogenic differentiation over the alternative fates in the multipotent progenitors [108]. Further studies are warranted to elucidate the full mechanism whereby aerobic glycolysis promotes the osteoblast phenotype.

Amino acid metabolism in osteoblasts

Amino acids are not only the building block of proteins, but also an important energy source. The cellular amino acid pool is derived from multiple sources including import of extracellular amino acids, degradation of intracellular protein, and de novo synthesis. Based on their mode of catabolism, amino acids can be categorized as glucogenic or ketogenic or both. Whereas ketogenic amino acids are broken down into acetyl-coA or acetoacetate, glucogenic amino acids are broken down into either pyruvate, or different TCA intermediates including oxaloacetate, α-ketoglutarate, fumarate, and succinyl-coA. Thus, amino acids can contribute directly to ATP production via the TCA cycle and OXPHOS. Indeed, amino acid catabolism through the TCA cycle is required in many contexts including cancer cell proliferation, pluripotent progenitor maintenance, and differentiation [109–113].

Initial studies in bone explants and calvarial osteoblasts focused on amino acid uptake. These studies defined multiple transport systems in bone, including system A, system L, and system ASC, and suggested differences in amino acid transport between adult and fetal bone [114–119]. Amino acid uptake is regulated in osteoblasts, being stimulated by cAMP and various growth factors and hormones [118–124]. More recent studies have implicated individual amino acid transporters in bone biology. For example, the cystine/glutamate antiporter xCT normally suppresses osteoblast differentiation likely through decrease of glutathione production [125–127]. Amino acid uptake is also regulated transcriptionally by the transcription factor Atf4, a critical transcription factor that can be activated by unfolded protein

in the endoplasmic reticulum (ER) or amino acid depletion [7, 128, 129]. Atf4 stimulates osteoblast differentiation in part through increasing amino acid import to support collagen synthesis [130–132]. The importance of amino acid import is highlighted by the observation that a high protein diet or amino acid supplementation corrects differentiation defects and bone loss in *Atf4*$^{-/-}$ osteoblasts [130]. Besides direct contribution to protein translation, amino acids also act as a growth signal to activate mTORC1, a critical regulator of osteoblast differentiation and bone formation [29, 76, 79, 133–138]. Thus, amino acids regulate bone formation through multiple mechanisms.

The amino acid glutamine has emerged as an important regulator of osteoblasts. Glutamine is the most abundant free amino acid in circulation and is not only an important oxidative fuel, but also a precursor for the synthesis of non-essential amino acids, nucleotides, and the anti-oxidant glutathione. Initial studies in isolated calvaria and long bones demonstrated an active consumption and metabolism of glutamine [139]. Glutamine was later shown to be required in calvarial osteoblasts for matrix mineralization [140]. Decreased glutamine consumption by bone marrow stromal cells has been linked with impaired osteoblast differentiation associated with aging [141]. Moreover, increased glutathione production from glutamine in response to Hif1α has been shown to improve cell survival and bone repair in a critical-size tibial defect model [104]. In differentiating osteoblasts, glutamine anaplerosis fulfills part of the energetic requirement of bone formation in response to Wnt signaling. Wnt increases glutamine anaplerosis into the TCA cycle by rapidly increasing glutaminase (Gls) protein levels and activity (Fig. 2). Strikingly, increased glutamine anaplerosis reduces intracellular glutamine levels, leading to activation of Gcn2. This results in Atf4 activation which stimulates both the uptake and the de novo synthesis of amino acids to promote protein synthesis. Mechanistically, this cascade of events is dependent on mTORC1 activity downstream of Wnt. Importantly, pharmacological inhibition of Gls reduces bone formation in the *Lrp5*$^{A214V/+}$ high bone mass mouse model demonstrating a critical role for glutamine anaplerosis to support excessive bone anabolism [133]. Whether Gls and glutamine anaplerosis are required for physiological bone formation remains unknown as a systematic analysis of the bones is yet to be performed with the *Gls* knockout mice [142].

Fatty acid metabolism in osteoblasts

Lipids are another important carbon and energy source in mammalian cells. Lipids can be synthesized de novo or acquired either as free fatty acids that are taken up by cell

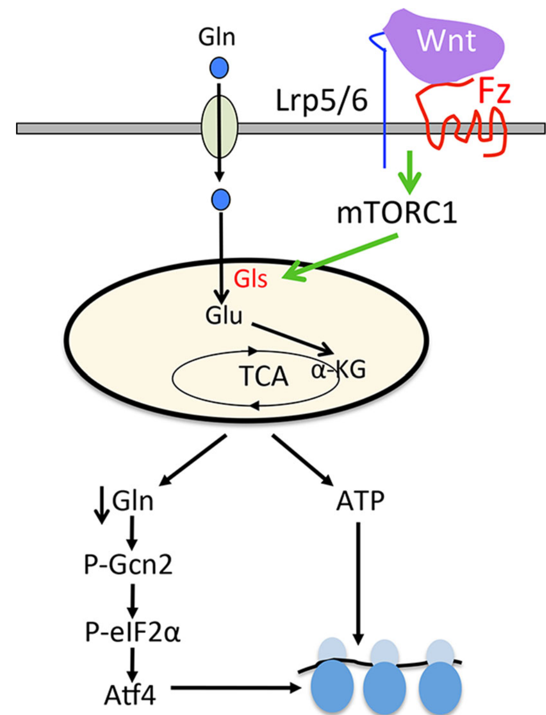

Fig. 2 Wnt signaling stimulates glutamine catabolism through the TCA cycle. Wnt signaling through Frizzled (Fz) and Lrp5/6 activates mTORC1 in a PI3K-Akt dependent manner; mTORC1 increases the protein abundance of glutaminase (Gls) and stimulates glutamine oxidation for ATP production. The increase in glutamine consumption results in lower intracellular glutamine levels that activate the Gcn2-Atf4 stress pathway and up-regulate the transcription of genes important for protein translation. *Gln* glutamine, *Glu* glutamate, *α-KG* α-ketoglutarate. See original reference for details [76, 77]

surface transporters or as lipoprotein particles bound by LDL receptor family members. Once transported into the cell, fatty acids can be metabolized in the mitochondrial matrix through β-oxidation that sequentially cleaves off two carbons as acetyl-coA that enters the TCA cycle. Fatty acids are first transported into the mitochondria by the carnitine shuttle. Here the rate limiting enzyme carnitine palmitoyltransferase 1 (CPT1) generates acyl-carnitine by transferring the acyl group of a long-chain fatty acyl-CoA to the hydroxyl group of carnitine. Acyl-carnitine is then shuttled inside the mitochondria in exchange for carnitine and converted back to acyl-CoA on the inner mitochondrial membrane by CPT2. Inside the mitochondrial matrix, acyl-coA can then undergo β-oxidation. Even chain fatty acids can be completely oxidized into acetyl-CoA whereas odd chain fatty acids ultimately form acetyl-CoA and succinyl-CoA which enter the TCA cycle. Complete oxidation of lipids by β-oxidation yields the most ATP per molecule compared to glucose or amino acids.

The role and regulation of lipid metabolism in osteoblasts is understudied. However, it has been reported that bone takes up the second highest amount of postprandial

lipoproteins behind liver in the mouse [143]. Moreover, lipid supplementation of serum-free medium was sufficient to support proliferation of osteoblastic cells in vitro [144]. Whether this requirement reflects an energetic or synthetic need for lipids was not clear, but another study estimated that fatty acid oxidation provided 40–80% of the energy derived from glucose in rat calvarial osteoblasts [145]. Furthermore, fatty acid oxidation increases during osteoblast differentiation in both murine and porcine models, implicating lipid metabolism in energy production [146, 147]. Interestingly, recent studies have implicated Wnt signaling in the regulation of lipid metabolism in bone. Osteoblasts lacking the Wnt co-receptor *Lrp5* exhibit decreased both expression of lipid metabolism genes and lipid oxidation. Conversely, expression of the $Lrp5^{G171V}$ allele or stimulation with the ligand Wnt10b increases lipid metabolism gene expression and stimulates lipid oxidation in bone. Mechanistically, the regulation by Wnt appears to be β-catenin dependent as GSK3β inhibition or β-catenin overexpression is sufficient to stimulate lipid oxidation in osteoblasts [147]. It appears that Wnt promotes fatty acid oxidation to fuel the TCA cycle and OXPHOS while also stimulating aerobic glycolysis and glutamine anaplerosis. Further studies are warranted to determine the physiological importance of lipid oxidation for osteoblast differentiation and bone formation in vivo.

Summary

Here, we have highlighted data exploring the role and molecular regulation of cellular metabolism in osteoblasts by Wnt signaling. Recent evidence has indicated that Wnt signaling stimulates aerobic glycolysis, glutamine anaplerosis, and β-oxidation of fatty acids in osteoblast-lineage cells. It is likely that β-oxidation and glutamine anaplerosis contribute to ATP production via OXPHOS to sustain protein synthesis during bone formation. The mechanism through which aerobic glycolysis contributes to the osteoblast phenotype is likely multifaceted and certainly warrants further investigation. Similarly, future studies on the role of the various metabolic pathways during physiological bone formation are necessary. It is tempting to speculate that metabolic dysregulation at the cellular level might be involved in various bone pathologies such as ectopic ossification, vascular calcification, skeletal aging or diabetes-associated bone disorders. The potential link with diabetes is of particular clinical significance as patients with either type I or type II diabetes exhibit increased risk in bone fracture [148, 149]. Although the mechanisms for diabetes-related bone fragility are undoubtedly complex, pharmaceutical targeting of osteoblast metabolism may be a promising direction towards novel therapies to improve bone health in diabetic patients.

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References

1. Long F (2012) Building strong bones: molecular regulation of the osteoblast lineage. Nat Rev Mol Cell Biol 13(1):27–38
2. Karsenty G, Kronenberg HM, Settembre C (2009) Genetic control of bone formation. Annu Rev Cell Dev Biol 25:629–648
3. Ducy P et al (1997) Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation. Cell 89(5):747–754
4. Lee B et al (1997) Missense mutations abolishing DNA binding of the osteoblast-specific transcription factor OSF2/CBFA1 in cleidocranial dysplasia. Nat Genet 16(3):307–310
5. Komori T et al (1997) Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. Cell 89(5):755–764
6. Nakashima K et al (2002) The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. Cell 108(1):17–29
7. Yang X et al (2004) ATF4 is a substrate of RSK2 and an essential regulator of osteoblast biology; implication for Coffin–Lowry syndrome. Cell 117(3):387–398
8. Zhong Z, Ethen NJ, Williams BO (2014) WNT signaling in bone development and homeostasis. Wiley Interdiscip Rev Dev Biol 3(6):489–500
9. Buttgereit F, Brand MD (1995) A hierarchy of ATP-consuming processes in mammalian cells. Biochem J 312(Pt 1):163–167
10. Bennett CN et al (2005) Regulation of osteoblastogenesis and bone mass by Wnt10b. Proc Natl Acad Sci USA 102(9):3324–3329
11. Tu X et al (2007) Noncanonical Wnt signaling through G protein-Linked PKCdelta activation promotes bone formation. Dev Cell 12(1):113–127
12. Day TF et al (2005) Wnt/beta-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. Dev Cell 8(5):739–750
13. Hu H et al (2005) Sequential roles of Hedgehog and Wnt signaling in osteoblast development. Development 132(1):49–60
14. Babij P et al (2003) High bone mass in mice expressing a mutant LRP5 gene. J Bone Miner Res 18(6):960–974
15. Gong Y et al (2001) LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. Cell 107(4):513–523
16. Rodda SJ, McMahon AP (2006) Distinct roles for Hedgehog and canonical Wnt signaling in specification, differentiation and maintenance of osteoblast progenitors. Development 133(16):3231–3244
17. Lu W et al (2004) Mammalian Ryk is a Wnt coreceptor required for stimulation of neurite outgrowth. Cell 119(1):97–108
18. He X et al (2004) LDL receptor-related proteins 5 and 6 in Wnt/beta-catenin signaling: arrows point the way. Development 131(8):1663–1677
19. Logan CY, Nusse R (2004) The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol 20:781–810
20. Kimelman D, Xu W (2006) Beta-catenin destruction complex: insights and questions from a structural perspective. Oncogene 25(57):7482–7491

21. Taelman VF et al (2010) Wnt signaling requires sequestration of glycogen synthase kinase 3 inside multivesicular endosomes. Cell 143(7):1136–1148
22. Behrens J et al (1996) Functional interaction of beta-catenin with the transcription factor LEF-1. Nature 382(6592):638–642
23. Hovanes K et al (2001) Beta-catenin-sensitive isoforms of lymphoid enhancer factor-1 are selectively expressed in colon cancer. Nat Genet 28(1):53–57
24. Zeng W et al (2000) naked cuticle encodes an inducible antagonist of Wnt signalling. Nature 403(6771):789–795
25. Roose J et al (1999) Synergy between tumor suppressor APC and the beta-catenin-Tcf4 target Tcf1. Science 285(5435):1923–1926
26. Jho EH et al (2002) Wnt/beta-catenin/Tcf signaling induces the transcription of Axin2, a negative regulator of the signaling pathway. Mol Cell Biol 22(4):1172–1183
27. Chen AE, Ginty DD, Fan CM (2005) Protein kinase A signalling via CREB controls myogenesis induced by Wnt proteins. Nature 433(7023):317–322
28. Habas R, Dawid IB, He X (2003) Coactivation of Rac and Rho by Wnt/Frizzled signaling is required for vertebrate gastrulation. Genes Dev 17(2):295–309
29. Inoki K et al (2006) TSC2 integrates Wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth. Cell 126(5):955–968
30. Sheldahl LC et al (1999) Protein kinase C is differentially stimulated by Wnt and Frizzled homologs in a G-protein-dependent manner. Curr Biol 9(13):695–698
31. Sheldahl LC et al (2003) Dishevelled activates Ca^{2+} flux, PKC, and CamKII in vertebrate embryos. J Cell Biol 161(4):769–777
32. Wu X et al (2008) Rac1 activation controls nuclear localization of beta-catenin during canonical Wnt signaling. Cell 133(2):340–353
33. Yamanaka H et al (2002) JNK functions in the non-canonical Wnt pathway to regulate convergent extension movements in vertebrates. EMBO Rep 3(1):69–75
34. Hino S et al (2001) Inhibition of the Wnt signaling pathway by Idax, a novel Dvl-binding protein. Mol Cell Biol 21(1):330–342
35. Mikels AJ, Nusse R (2006) Purified Wnt5a protein activates or inhibits beta-catenin-TCF signaling depending on receptor context. PLoS Biol 4(4):e115
36. Simons M et al (2005) Inversin, the gene product mutated in nephronophthisis type II, functions as a molecular switch between Wnt signaling pathways. Nat Genet 37(5):537–543
37. van Amerongen R et al (2012) Wnt5a can both activate and repress Wnt/beta-catenin signaling during mouse embryonic development. Dev Biol 369(1):101–114
38. Mao B et al (2001) LDL-receptor-related protein 6 is a receptor for Dickkopf proteins. Nature 411(6835):321–325
39. Semenov MV et al (2001) Head inducer Dickkopf-1 is a ligand for Wnt coreceptor LRP6. Curr Biol 11(12):951–961
40. Semenov M, Tamai K, He X (2005) SOST is a ligand for LRP5/LRP6 and a Wnt signaling inhibitor. J Biol Chem 280(29):26770–26775
41. Bourhis E et al (2011) Wnt antagonists bind through a short peptide to the first beta-propeller domain of LRP5/6. Structure 19(10):1433–1442
42. Rattner A et al (1997) A family of secreted proteins contains homology to the cysteine-rich ligand-binding domain of frizzled receptors. Proc Natl Acad Sci USA 94(7):2859–2863
43. Finch PW et al (1997) Purification and molecular cloning of a secreted, Frizzled-related antagonist of Wnt action. Proc Natl Acad Sci USA 94(13):6770–6775
44. Leyns L et al (1997) Frzb-1 is a secreted antagonist of Wnt signaling expressed in the Spemann organizer. Cell 88(6):747–756
45. Maupin KA, Droscha CJ, Williams BO (2013) A comprehensive overview of skeletal phenotypes associated with alterations in Wnt/b-catenin signaling in humans and mice. Bone Res 1(1):27–71
46. Boyden LM et al (2002) High bone density due to a mutation in LDL-receptor-related protein 5. N Engl J Med 346(20):1513–1521
47. Little RD et al (2002) A mutation in the LDL receptor-related protein 5 gene results in the autosomal dominant high-bone-mass trait. Am J Hum Genet 70(1):11–19
48. van Bezooijen RL et al (2004) Sclerostin is an osteocyte-expressed negative regulator of bone formation, but not a classical BMP antagonist. J Exp Med 199(6):805–814
49. Brunkow ME et al (2001) Bone dysplasia sclerosteosis results from loss of the SOST gene product, a novel cystine knot-containing protein. Am J Hum Genet 68(3):577–589
50. Balemans W et al (2002) Identification of a 52 kb deletion downstream of the SOST gene in patients with van Buchem disease. J Med Genet 39(2):91–97
51. Richards JB et al (2008) Bone mineral density, osteoporosis, and osteoporotic fractures: a genome-wide association study. Lancet 371(9623):1505–1512
52. Rivadeneira F et al (2009) Twenty bone-mineral-density loci identified by large-scale meta-analysis of genome-wide association studies. Nat Genet 41(11):1199–1206
53. van Meurs JB et al (2008) Large-scale analysis of association between LRP5 and LRP6 variants and osteoporosis. JAMA 299(11):1277–1290
54. Estrada K et al (2012) Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture. Nat Genet 44(5):491–501
55. Fahiminiya S et al (2013) Mutations in WNT1 are a cause of osteogenesis imperfecta. J Med Genet 50(5):345–348
56. Laine CM et al (2013) WNT1 mutations in early-onset osteoporosis and osteogenesis imperfecta. N Engl J Med 368(19):1809–1816
57. Zheng HF et al (2012) WNT16 influences bone mineral density, cortical bone thickness, bone strength, and osteoporotic fracture risk. PLoS Genet 8(7):e1002745
58. Kato M et al (2002) Cbfa1-independent decrease in osteoblast proliferation, osteopenia, and persistent embryonic eye vascularization in mice deficient in Lrp5, a Wnt coreceptor. J Cell Biol 157(2):303–314
59. Riddle RC et al (2013) Lrp5 and Lrp6 exert overlapping functions in osteoblasts during postnatal bone acquisition. PLoS One 8(5):e63323
60. Cui Y et al (2011) Lrp5 functions in bone to regulate bone mass. Nat Med 17(6):684–691
61. Li X et al (2008) Targeted deletion of the sclerostin gene in mice results in increased bone formation and bone strength. J Bone Miner Res 23(6):860–869
62. Morvan F et al (2006) Deletion of a single allele of the Dkk1 gene leads to an increase in bone formation and bone mass. J Bone Miner Res 21(6):934–945
63. Bodine PV et al (2004) The Wnt antagonist secreted frizzled-related protein-1 is a negative regulator of trabecular bone formation in adult mice. Mol Endocrinol 18(5):1222–1237
64. Albers J et al (2013) Canonical Wnt signaling inhibits osteoclastogenesis independent of osteoprotegerin. J Cell Biol 200(4):537–549
65. Albers J et al (2011) Control of bone formation by the serpentine receptor Frizzled-9. J Cell Biol 192(6):1057–1072
66. Joeng KS et al (2014) The swaying mouse as a model of osteogenesis imperfecta caused by WNT1 mutations. Hum Mol Genet 23(15):4035–4042

67. Maruyama T, Jiang M, Hsu W (2013) Gpr177, a novel locus for bone mineral density and osteoporosis, regulates osteogenesis and chondrogenesis in skeletal development. J Bone Miner Res 28(5):1150–1159
68. Zhong Z et al (2012) Wntless functions in mature osteoblasts to regulate bone mass. Proc Natl Acad Sci USA 109(33):E2197–E2204
69. Witte F et al (2009) Comprehensive expression analysis of all Wnt genes and their major secreted antagonists during mouse limb development and cartilage differentiation. Gene Expr Patterns 9(4):215–223
70. Chen J, Long F (2013) beta-catenin promotes bone formation and suppresses bone resorption in postnatal growing mice. J Bone Miner Res 28(5):1160–1169
71. Glass DA 2nd et al (2005) Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation. Dev Cell 8(5):751–764
72. Hill TP et al (2005) Canonical Wnt/beta-catenin signaling prevents osteoblasts from differentiating into chondrocytes. Dev Cell 8(5):727–738
73. Holmen SL et al (2005) Essential role of beta-catenin in postnatal bone acquisition. J Biol Chem 280(22):21162–21168
74. Song L et al (2012) Loss of wnt/beta-catenin signaling causes cell fate shift of preosteoblasts from osteoblasts to adipocytes. J Bone Miner Res 27(11):2344–2358
75. Gaur T et al (2005) Canonical WNT signaling promotes osteogenesis by directly stimulating Runx2 gene expression. J Biol Chem 280(39):33132–33140
76. Chen J et al (2014) WNT7B promotes bone formation in part through mTORC1. PLoS Genet 10(1):e1004145
77. Karner CM et al (2015) Increased glutamine catabolism mediates bone anabolism in response to WNT signaling. J Clin Investig 125(2):551–562
78. Riddle RC et al (2014) Tsc2 is a molecular checkpoint controlling osteoblast development and glucose homeostasis. Mol Cell Biol 34(10):1850–1862
79. Chen J, Long F (2015) mTORC1 signaling promotes osteoblast differentiation from preosteoblasts. PLoS One 10(6):e0130627
80. Sun W et al (2016) Rictor is required for optimal bone accrual in response to anti-sclerostin therapy in the mouse. Bone 85:1–8
81. Esen E et al (2013) WNT-LRP5 signaling induces Warburg effect through mTORC2 activation during osteoblast differentiation. Cell Metab 17(5):745–755
82. Chen J et al (2015) mTORC2 signaling promotes skeletal growth and bone formation in mice. J Bone Miner Res 30:369–378
83. Bell GI et al (1993) Structure and function of mammalian facilitative sugar transporters. J Biol Chem 268(26):19161–19164
84. Mueckler M (1994) Facilitative glucose transporters. Eur J Biochem 219(3):713–725
85. Esen E, Long F (2014) Aerobic glycolysis in osteoblasts. Curr Osteoporos Rep 12(4):433–438
86. Tsukada Y et al (2006) Histone demethylation by a family of JmjC domain-containing proteins. Nature 439(7078):811–816
87. Tahiliani M et al (2009) Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science 324(5929):930–935
88. Wellen KE et al (2009) ATP-citrate lyase links cellular metabolism to histone acetylation. Science 324(5930):1076–1080
89. Brommage R, Neuman WF (1979) Mechanism of mobilization of bone mineral by 1,25-dihydroxyvitamin D3. Am J Physiol 237(2):E113–E120
90. Cohn DV, Forscher BK (1962) Effect of parathyroid extract on the oxidation in vitro of glucose and the production of 14CO-2 by bone and kidney. Biochim Biophys Acta 65:20–26
91. Esen E et al (2015) PTH promotes bone anabolism by stimulating aerobic glycolysis via IGF signaling. J Bone Miner Res 30(11):1959–1968
92. Schmid C, Steiner T, Froesch ER (1982) Parathormone promotes glycogen formation from [14C]glucose in cultured osteoblast-like cells. FEBS Lett 148(1):31–34
93. Wei J et al (2015) Glucose uptake and Runx2 synergize to orchestrate osteoblast differentiation and bone formation. Cell 161(7):1576–1591
94. Zoidis E, Ghirlanda-Keller C, Schmid C (2011) Stimulation of glucose transport in osteoblastic cells by parathyroid hormone and insulin-like growth factor I. Mol Cell Biochem 348(1–2):33–42
95. Passi-Even L, Gazit D, Bab I (1993) Ontogenesis of ultrastructural features during osteogenic differentiation in diffusion chamber cultures of marrow cells. J Bone Miner Res 8(5):589–595
96. Komarova SV, Ataullakhanov FI, Globus RK (2000) Bioenergetics and mitochondrial transmembrane potential during differentiation of cultured osteoblasts. Am J Physiol Cell Physiol 279(4):C1220–C1229
97. Klein BY et al (1993) Induction of osteoprogenitor cell differentiation in rat marrow stroma increases mitochondrial retention of rhodamine 123 in stromal cells. J Cell Biochem 53(3):190–197
98. Borle AB, Nichols N, Nichols G Jr (1960) Metabolic studies of bone in vitro. I. Normal bone. J Biol Chem 235:1206–1210
99. Cohn DV, Forscher BK (1962) Aerobic metabolism of glucose by bone. J Biol Chem 237:615–618
100. Felix R, Neuman WF, Fleisch H (1978) Aerobic glycolysis in bone: lactic acid production by rat calvaria cells in culture. Am J Physiol 234(1):C51–C55
101. Neuman WF, Neuman MW, Brommage R (1978) Aerobic glycolysis in bone: lactate production and gradients in calvaria. Am J Physiol 234(1):C41–C50
102. Guntur AR et al (2014) Bioenergetics during calvarial osteoblast differentiation reflect strain differences in bone mass. Endocrinology 155(5):1589–1595
103. Regan JN et al (2014) Up-regulation of glycolytic metabolism is required for HIF1alpha-driven bone formation. Proc Natl Acad Sci USA 111(23):8673–8678
104. Stegen S et al. (2016) HIF-1alpha promotes glutamine-mediated redox homeostasis and glycogen-dependent bioenergetics to support postimplantation bone cell survival. Cell Metab 23(2):265–279
105. Pate KT et al (2014) Wnt signaling directs a metabolic program of glycolysis and angiogenesis in colon cancer. EMBO J 33(13):1454–1473
106. Vander Heiden MG, Cantley LC, Thompson CB (2009) Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 324(5930):1029–1033
107. Owen M, Macpherson S (1963) Cell population kinetics of an osteogenic tissue. II. J Cell Biol 19:33–44
108. Karner CM et al (2016) Wnt signaling reduces nuclear acetyl-coA levels to suppress gene expression during osteoblast differentiation. J Biol Chem 291(25):13028–13039
109. DeBerardinis RJ et al (2007) Beyond aerobic glycolysis: transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis. Proc Natl Acad Sci USA 104(49):19345–19350
110. Green CR et al (2016) Branched-chain amino acid catabolism fuels adipocyte differentiation and lipogenesis. Nat Chem Biol 12(1):15–21
111. Shiraki N et al (2014) Methionine metabolism regulates maintenance and differentiation of human pluripotent stem cells. Cell Metab 19(5):780–794

112. Tonjes M et al (2013) BCAT1 promotes cell proliferation through amino acid catabolism in gliomas carrying wild-type IDH1. Nat Med 19(7):901–908
113. Wang J et al (2009) Dependence of mouse embryonic stem cells on threonine catabolism. Science 325(5939):435–439
114. Adamson LF, Ingbar SH (1967) Further studies of amino acid transport by embryonic chick bone. J Biol Chem 242(11):2646–2652
115. Finerman GA, Rosenberg LE (1966) Amino acid transport in bone. Evidence for separate transport systems for neutral amino and imino acids. J Biol Chem 241(7):1487–1493
116. Hahn TJ, Downing SJ, Phang JM (1969) Amino acid transport in adult diaphyseal bone: contrast with amino acid transport mechanisms in fetal membranous bone. Biochim Biophys Acta 183(1):194–203
117. Kim SG et al (2006) Differential expression and functional characterization of system L amino acid transporters in human normal osteoblast cells and osteogenic sarcoma cells. Anticancer Res 26(3A):1989–1996
118. Phang JM, Downing SJ (1973) Amino acid transport in bone: stimulation by cyclic AMP. Am J Physiol 224(1):191–196
119. Yee JA (1988) Effect of parathyroid hormone on amino acid transport by cultured neonatal mouse calvarial bone cells. J Bone Miner Res 3(2):211–218
120. Adamson LF, Ingbar SH (1967) Selective alteration by triiodothyronine of amino acid transport in embryonic bone. Endocrinology 81(6):1362–1371
121. Adamson LF, Ingbar SH (1967) Some properties of the stimulatory effect of thyroid hormones on amino acid transport by embryonic chick bone. Endocrinology 81(6):1372–1378
122. Baum BJ, Shteyer A (1987) Characteristics of a neutral amino acid transport system (system A) in osteoblastic rat osteosarcoma cells. Exp Cell Res 169(2):453–457
123. Hahn TJ, Downing SJ, Phang JM (1969) Insulin effect on amino acid transport in bone. Biochim Biophys Acta 184(3):675–677
124. Hahn TJ, Downing SJ, Phang JM (1971) Insulin effect on amino acid transport in bone: dependence on protein synthesis and Na^+. Am J Physiol 220(6):1717–1723
125. Takarada-Iemata M et al (2011) Glutamate preferentially suppresses osteoblastogenesis than adipogenesis through the cystine/glutamate antiporter in mesenchymal stem cells. J Cell Physiol 226(3):652–665
126. Uno K et al (2011) A negative correlation between expression profiles of runt-related transcription factor-2 and cystine/glutamate antiporter xCT subunit in ovariectomized mouse bone. J Pharmacol Sci 115(3):309–319
127. Uno K et al (2011) Negative regulation of osteoblastogenesis through downregulation of runt-related transcription factor-2 in osteoblastic MC3T3-E1 cells with stable overexpression of the cystine/glutamate antiporter xCT subunit. J Cell Physiol 226(11):2953–2964
128. Harding HP et al (2000) Regulated translation initiation controls stress-induced gene expression in mammalian cells. Mol Cell 6(5):1099–1108
129. Han J et al (2013) ER-stress-induced transcriptional regulation increases protein synthesis leading to cell death. Nat Cell Biol 15(5):481–490
130. Elefteriou F et al (2006) ATF4 mediation of NF1 functions in osteoblast reveals a nutritional basis for congenital skeletal dysplasiae. Cell Metab 4(6):441–451
131. Saito A et al (2011) Endoplasmic reticulum stress response mediated by the PERK-eIF2(alpha)-ATF4 pathway is involved in osteoblast differentiation induced by BMP2. J Biol Chem 286(6):4809–4818
132. Zhang P et al (2002) The PERK eukaryotic initiation factor 2 alpha kinase is required for the development of the skeletal system, postnatal growth, and the function and viability of the pancreas. Mol Cell Biol 22(11):3864–3874
133. Duran RV et al (2012) Glutaminolysis activates Rag-mTORC1 signaling. Mol Cell 47(3):349–358
134. Huang B et al (2015) mTORC1 prevents preosteoblast differentiation through the notch signaling pathway. PLoS Genet 11(8):e1005426
135. Jewell JL et al (2015) Metabolism. Differential regulation of mTORC1 by leucine and glutamine. Science 347(6218):194–198
136. Nicklin P et al (2009) Bidirectional transport of amino acids regulates mTOR and autophagy. Cell 136(3):521–534
137. Xian L et al (2012) Matrix IGF-1 maintains bone mass by activation of mTOR in mesenchymal stem cells. Nat Med 18(7):1095–1101
138. Zoncu R et al (2011) mTORC1 senses lysosomal amino acids through an inside-out mechanism that requires the vacuolar H(+)-ATPase. Science 334(6056):678–683
139. Biltz RM et al (1983) Glutamine metabolism in bone. Miner Electrolyte Metab 9(3):125–131
140. Brown PM, Hutchison JD, Crockett JC (2011) Absence of glutamine supplementation prevents differentiation of murine calvarial osteoblasts to a mineralizing phenotype. Calcif Tissue Int 89(6):472–482
141. Huang T et al (2016) Aging reduces an ERRalpha-directed mitochondrial glutaminase expression suppressing glutamine anaplerosis and osteogenic differentiation of mesenchymal stem cells. Stem Cells. doi:10.1002/stem.2470.
142. Masson J et al (2006) Mice lacking brain/kidney phosphate-activated glutaminase have impaired glutamatergic synaptic transmission, altered breathing, disorganized goal-directed behavior and die shortly after birth. J Neurosci 26(17):4660–4671
143. Niemeier A et al (2008) Uptake of postprandial lipoproteins into bone in vivo: impact on osteoblast function. Bone 43(2):230–237
144. Catherwood BD et al (1988) Growth of rat osteoblast-like cells in a lipid-enriched culture medium and regulation of function by parathyroid hormone and 1,25-dihydroxyvitamin D. J Bone Miner Res 3(4):431–438
145. Adamek G et al (1987) Fatty acid oxidation in bone tissue and bone cells in culture. Characterization and hormonal influences. Biochem J 248(1):129–137
146. Chiu KM et al (1999) Carnitine and dehydroepiandrosterone sulfate induce protein synthesis in porcine primary osteoblast-like cells. Calcif Tissue Int 64(6):527–533
147. Frey JL et al (2015) Wnt-Lrp5 signaling regulates fatty acid metabolism in the osteoblast. Mol Cell Biol 35(11):1979–1991
148. Napoli N et al (2016) Mechanisms of diabetes mellitus-induced bone fragility. Nat Rev Endocrinol. doi:10.1038/nrendo.2016.153
149. Lecka-Czernik B, Rosen CJ (2015) Energy excess, glucose utilization, and skeletal remodeling: new insights. J Bone Miner Res 30(8):1356–1361