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Glucose metabolism in bone

Courtney M. Karner^{a,*} and Fanxin Long^{b,*}

^aDepartment of Orthopaedic Surgery, Duke Orthopaedic Cellular, Developmental, and Genome Laboratories, Department of Cell Biology, Duke University School of Medicine, Durham, NC 27710, USA

^bDepartment of Orthopaedic Surgery, Department of Developmental Biology, Washington University School of Medicine, St Louis, MO 63131, USA

Abstract

The adult human skeleton is a multifunctional organ undergoing constant remodeling through the opposing activities of the bone-resorbing osteoclast and the bone-forming osteoblast. The exquisite balance between bone resorption and bone formation is responsible for bone homeostasis in healthy adults. However, evidence has emerged that such a balance is likely disrupted in diabetes where systemic glucose metabolism is dysregulated, resulting in increased bone frailty and osteoporotic fractures. These findings therefore underscore the significance of understanding the role and regulation of glucose metabolism in bone under both normal and pathological conditions. Recent studies have shed new light on the metabolic plasticity and the critical functions of glucose metabolism during osteoclast and osteoblast differentiation. Moreover, these studies have begun to identify intersections between glucose metabolism and the growth factors and transcription factors previously known to regulate osteoblasts and osteoclasts. Here we summarize the current knowledge in the nascent field, and suggest that a fundamental understanding of glucose metabolic pathways in the critical bone cell types may open new avenues for developing novel bone therapeutics.

Keywords

Glycolysis; Glucose; Metabolism; Oxidative phosphorylation (OXPHOS); Mitochondria; Osteoblast; Osteoclast; Bone; Osteoporosis; Diabetes

1. Introduction

Bone fractures associated with osteopenia and osteoporosis pose a major health threat especially among the aging population. Despite the recent progress in therapies such as denosumab and abaloparatide, additional safe and effective strategies are necessary to improve bone mass and quality to treat osteoporosis. Because osteoblasts and osteoclasts are the chief cell types regulating bone homeostasis, elucidating the mechanisms that regulate

*Corresponding authors. courtney.karner@duke.edu (C.M. Karner), flong@wustl.edu (F. Long).

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their 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 the regulation of cell-type-specific marker genes by endocrine or paracrine signals or transcriptional factors [1–4]. A critical aspect of cell differentiation is the acquisition of specific cellular functions, these including bone matrix deposition by the osteoblast or protease and acid secretion by the osteoclast. However, the bioenergetics in support of the cell-specific physiological activity is just beginning to be explored [5]. This review summarizes the current understanding about the role of glucose metabolism in osteoblasts and osteoclasts.

2. Glucose metabolism in mammalian cells

Glucose is a major energy source for most mammalian cell types. Upon transport into the cell *via* the Glut family of transporters down a concentration gradient independent of ATP, glucose is metabolized in the cytoplasm through glycolysis to produce two pyruvate molecules, 2 ATP, and two reducing equivalents in the form of nicotinamide adenine dinucleotide (NADH) [5–7] (Fig. 1). The first stage of glycolysis culminates in the generation of fructose 1,6-bisphosphate (F1,6 BP), which is then cleaved to form two interconvertible three carbon molecules glyceraldehyde 3-phosphate (G3P) and dihydroxyacetone phosphate (DHAP). During the final stage of glycolysis, G3P is eventually converted to pyruvate, producing ATP in the process. In addition to the core glycolysis pathway, glycolytic intermediates can enter other metabolic pathways in the cell. For example, G6P can be converted to glycogen for storage or be metabolized *via* the pentose phosphate pathway (PPP). The PPP is important for generating not only ribose 5-phosphate, the backbone of nucleic acids, but also the reducing equivalent nicotinamide adenine dinucleotide phosphate (NADPH) essential for reductive biosynthetic reactions and for redox homeostasis *via* glutathione (GSH) and thioredoxin (TRX) [8]. In addition, Fructose-6-P (F6P) derived from G6P can enter the hexosamine biosynthetic pathway (HBP) which produces uridine diphosphate *N*-acetylglucosamine (UDPGlcNAc) for protein glycosylation. Finally, 3-Phosphoglycerate (3PG) can be metabolized *via* the serine biosynthetic pathway to produce serine and subsequently glycine, a process integral to one-carbon metabolism required for methylation of DNA and histones, NADPH production as well as GSH biosynthesis [8–10]. Overall, glycolysis produces numerous intermediate metabolites critical for various biosynthetic pathways.

The glycolytic end product pyruvate can be either converted to lactate or further oxidized in the TCA cycle. Pyruvate conversion into lactate is catalyzed by the enzyme lactate dehydrogenase (LDH) independent of oxygen and regenerates the oxidized NAD (NAD⁺) 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 through a process known as cataplerosis to support lipid and amino acid biosynthesis, redox regulation and epigenetic regulation of gene expression [11–13]. Thus, glucose is not only an important energy source but also a critical provider of intermediate metabolites necessary for supporting cellular homeostasis and function.

3. Glucose metabolism in osteoblasts

The bone-forming osteoblasts differentiate through a series of stages characterized by the expression of distinct transcription factors and marker genes [2]. Originated from the mesenchymal progenitors, early osteoblast precursors express the transcription factor Runx2 whereas the more specified preosteoblasts express osterix (*Osx* or *Sp7*) (Fig. 2A). Upon further differentiation, the mature osteoblasts activate Atf4 and upregulate the expression of collagen I as well as other matrix proteins including integrin binding sialoprotein (Ibsp) and osteocalcin (Bglap). Whereas most osteoblasts are believed to undergo apoptosis, some become entombed in the bone matrix and further differentiate to osteocytes. Although much has been learned about the transcriptional regulation of osteoblast differentiation, relatively little is understood how cellular metabolism is modulated to accommodate the active biosynthetic activity of mature osteoblasts.

Glucose has been long recognized as an important nutrient for the osteoblast lineages, as indicated by the early studies of either bone explants or isolated osteoblastic cells [14–16]. Consistent with their glucose consumption, the osteoblast-lineage cells have been shown to express several Glut family transporters. For instance, the rat bone-derived osteoblastic PyMS cells were reported to express *Glut1*, *Glut3* but not *Glut4* [17]. A more recent study, however, showed that Glut4 was also detectable in primary cultures of murine calvarial preosteoblasts and was up-regulated upon further differentiation towards osteoblasts *in vitro*, whereas Glut1 and Glut3 expression stayed relatively constant [18]. Furthermore, Glut4 appeared to be responsible for the insulin-induced glucose uptake in osteoblasts even though its deletion did not impair bone formation in the mouse [18]. On the other hand, Glut1, expressed at the highest level among the Gluts in the osteoblast lineage, has been shown to engage in a feed-forward mechanism with Runx2 to determine not only the onset of osteoblast differentiation during embryogenesis but also the extent of bone formation throughout life [19]. Future genetic studies are necessary to determine the role of Glut3 and any potential functional overlap with Glut1 in the osteoblast lineage.

Osteoblasts appear to metabolize glucose mostly into lactate even in the presence of sufficient oxygen, a process known as aerobic glycolysis [20]. The phenomenon was documented initially in bone explants and later in isolated osteoblasts [16,21–23]. More recent studies have confirmed an increase in aerobic glycolysis in primary calvarial preosteoblasts when they further differentiate in response to ascorbic acid and β -glycerophosphate [24,25]. Aerobic glycolysis occurs despite the fact that mature osteoblasts possess numerous mitochondria and exhibit active OXPHOS [24,26,27]. Thus, other nutrients besides glucose, such as fatty acids and glutamine as implicated by recent studies, may be fueling the mitochondrial respiration in mature osteoblasts [28,29].

Accumulating evidence has indicated that glycolysis in osteoblast-lineage cells is directly stimulated by various bone anabolic signals. Long before parathyroid hormone (PTH) signaling was harnessed clinically to promote bone formation in osteoporotic patients, it was historically shown to stimulate glucose consumption and lactate production in bone explants [16,30,31]. More recently, a mechanistic study has demonstrated that PTH signaling enhances aerobic glycolysis indirectly through the transcriptional induction of Igf1 that in

turn signals through mTORC2 to elevate various glycolytic enzymes [32] (Fig. 2B). Importantly, diminution of aerobic glycolysis with dichloacetate (DCA) that increases pyruvate entry to the TCA cycle notably suppressed the bone anabolic effect of PTH, thus demonstrating a functional link between glycolysis and bone formation [32]. In further support of the connection, stimulating glycolysis through overexpression of Hif1 α known to induce the transcription of multiple glycolytic genes, increased bone formation *in vivo*, and the effect was effectively reversed by DCA [33] (Fig. 2B). Besides PTH, the bone anabolic Wnt proteins also promote aerobic glycolysis in osteoblast lineage cells [34]. In particular, Wnt3a-Lrp5 signaling acutely increased the levels of Glut1, Hk2, Ldha and Pdk1 downstream of mTORC2 and Akt activation while inducing osteoblast differentiation from the bone marrow mesenchymal progenitor cell line ST2 [35] (Fig. 2B). Whereas upregulation of Glut1 and Hk2 is expected to stimulate the overall rate of glycolysis, increased Ldha and Pdk1 would favor lactate production from pyruvate, thus altering the metabolic profile towards that of an osteoblast. In support of the role of mTORC2 in Wnt-induced bone formation, deletion of *Rictor* that is uniquely required for mTORC2 signaling not only reduced physiological bone formation but also blunted the anabolic effect of an anti-sclerostin therapy designed to boost Wnt interaction with the receptors [36,37]. Finally, Hedgehog (Hh) signaling, a critical inducer of the early steps of osteoblast differentiation, has been reported to stimulate aerobic glycolysis in both muscle and brown adipose tissue through a non-canonical mechanism [38,39]. Although a similar metabolic regulation by Hh has not been described during osteoblast differentiation, Hh signaling has been shown to induce Igf2 expression *via* Gli2, resulting in mTORC2 activation in the osteogenic progenitors [40]. Thus, it will be of interest to examine the potential relationship between Hh signaling and glycolysis in bone formation.

Currently, it remains unclear why osteoblasts have a propensity for aerobic glycolysis. Compared to metabolism through the TCA cycle and OXPHOS, aerobic glycolysis is a far less efficient means of producing ATP from glucose. However, aerobic glycolysis may be necessary in order for osteoblasts to generate sufficient intermediate metabolites to sustain active biosynthesis. In addition, aerobic glycolysis may be beneficial for offsetting reactive oxygen species (ROS) generated from OXPHOS as ROS was shown to favor adipogenesis from mesenchymal progenitors [41,42]. Another interesting possibility is that glycolytic changes could directly affect the levels of enzymatic cofactors (*e.g.*, α -ketoglutarate) or substrates (*e.g.*, acetyl-CoA) necessary for epigenetic modifications in differentiation [11,43]. For instance, increased aerobic glycolysis in response to Wnt signaling has been correlated with reduced nuclear levels of both citrate and acetyl-CoA, which could explain the large scale decrease in histone acetylation and suppression of adipogenic and chondrogenic transcription factors in mesenchymal progenitors [44]. However, further studies are warranted to elucidate the full mechanism whereby aerobic glycolysis promotes the osteoblast phenotype.

4. Glucose metabolism in osteoclasts

Osteoclasts are giant, multinucleated cells responsible for resorbing the bone matrix and maintaining mineral homeostasis. Osteoclasts form by differentiation and fusion of monocytic precursors of the macrophage lineage in response to macrophage colony

stimulating factor (M-CSF) and receptor activator of nuclear factor kappa B ligand (RANKL) [1,4]. During RANKL-induced osteoclast differentiation from murine bone marrow macrophages, both glycolysis and oxidative phosphorylation (OXPHOS) as well as lactate production were found to increase [45] (Fig. 3). The increase in OXPHOS is consistent with a substantial increase in the number, the size and the cristae abundance of mitochondria with osteoclast differentiation [46]. Interestingly, osteoclastogenesis appeared to be optimal with glucose at approximately 5 mM in the *in vitro* differentiation system, whereas high levels of glucose such as 20 mM were less effective even though cell proliferation was maximally stimulated. Furthermore, pyruvate was found to synergize with 5 mM glucose to stimulate osteoclast differentiation while reducing glucose consumption and lactate production, but inhibition of mitochondrial OXPHOS retarded osteoclastogenesis [45]. These results indicate that osteoclast differentiation is coupled with increased mitochondrial respiration that is at least partially fueled by the increased glycolysis. In support of the importance of glycolysis, depletion of *Ldha* or *Ldhb* was shown to reduce both glycolysis and mitochondrial respiration, resulting in suppression of osteoclastogenesis [47]. Remarkably however, limiting glycolysis by either omission of glucose in the culture media or knockdown of *Hif1a* did not impair osteoclastogenesis *in vitro*, indicating that alternative energy substrates were activated in those settings to support the differentiation process [48]. Indeed, glutamine was shown to be required for osteoclast differentiation, but whether it alone could compensate for the lack of glucose was not investigated [48]. Alternatively, pyruvate and fatty acids typically present in the culture medium may fuel OXPHOS to support osteoclast differentiation in the absence of glucose metabolism. Regardless of the exact fuel substrate, it is interesting that the compensatory mechanism was activated by glucose removal but apparently not by *Ldh* depletion. Future studies are necessary to decipher how fuel plasticity between glucose and the other substrates is regulated during osteoclastogenesis.

The importance of mitochondrial respiration in osteoclasts has been demonstrated genetically. Disruption of mitochondrial complex I through deletion of *Ndufs4* impaired osteoclast differentiation and function resulting in osteopetrosis in the mouse [49]. Deletion of the mitochondrial transcription factor A (*Tfam*) with cathepsin K-Cre in the mouse reduced intracellular ATP levels in osteoclasts and accelerated their apoptosis [50]. Molecularly, *Rankl* has been shown to stimulate mitochondrial biogenesis *via* upregulation of the *Pparg* coactivator *Ppargc1b* during osteoclast differentiation [51]. Moreover, *Asxl2*, a regulator of histone methylation, appears to act upstream of *Pparg1b* to control mitochondrial biogenesis [52]. However, alternative NF- κ B signaling *via* *RelB* and *NIK* is also required for mitochondrial biogenesis in response to *Rankl*, and this requirement is likely independent of *Ppargc1b* [53]. Therefore, further studies are necessary to elucidate the precise mechanism for *Rankl* to activate mitochondrial biogenesis integral to the osteoclastogenic program.

Glucose appears to be a major nutrient for mature osteoclasts. Earlier studies with chicken osteoclasts demonstrated that glucose, instead of fatty acids or ketone bodies, was the principle energy source for bone resorption, and that attachment to the bone surface greatly simulated glucose consumption by osteoclasts [54]. Glucose was further shown in chicken osteoclasts to stimulate both protein and mRNA levels of the vacuolar-type H⁺-ATPases (V-

ATPase) essential for the resorptive activity of osteoclasts [55]. Similarly, limiting glucose metabolism by either omission of glucose in the culture media or knockdown of *Hif1a* reduced bone resorption by murine osteoclasts *in vitro* [48]. More recently, human osteoclasts were shown to rely on glycolysis for bone resorption, as reducing the rate of glycolysis with galactose significantly impaired collagen I degradation whereas suppression of the mitochondrial complex I with a non-cytotoxic dose of rotenone enhanced osteoclast activity [46]. Interestingly, immunostaining experiments revealed that some of the key glycolytic enzymes were localized to the actin ring of polarized osteoclasts attached to bone surface [46]. Therefore, in contrast to osteoclastogenesis that requires mitochondrial OXPHOS fueled by both glucose and potentially other substrates, the mature osteoclasts appear to rely heavily on active aerobic glycolysis to support bone resorption. Further studies however are necessary in this regard, as a study of osteoclasts in human osteophytes from osteoarthritis patients indicated active fatty acid oxidation and TCA metabolism *in situ* [56]. Whether these findings reflect the normal or a pathological state of metabolism in osteoclasts *in vivo* is not clear at present.

5. Clinical relevance

Systemic dysregulation of glucose metabolism due to the deficiency in either producing (type I) or responding to insulin (type II) is the hallmark of diabetes. Type I Diabetes in particular is associated with a variety of bone complications including reduced bone mineral density, increased fracture risk and poor fracture healing in both humans and rodent models [57,58]. Historically, the effects of diabetes on the skeleton have been primarily attributed to deleterious effects on osteoblasts and bone formation, but more recent data suggests that bone resorption is also affected [59–64]. The increased osteoclast numbers and bone resorption in several diabetic models may not be due to a direct effect of systemic glucose elevation as hyperglycemia has been shown to inhibit osteoclast differentiation and function *in vitro* [45,60,65–67]. Instead, increased osteoclastogenesis in diabetes could be secondary to hypoxia-induced acidosis in the bone marrow [68]. In addition, advanced glycation end products (AGEs) of lipids or proteins caused by hyperglycemia in diabetes may signal through the membrane RAGE to enhance bone resorption [69,70]. Mechanistically, AGEs dependent RAGE activation stimulates the generation of reactive oxygen species (ROS) that may stimulate osteoclastogenesis through RANKL signaling [71,72]. Increased ROS may also impair bone formation by inhibiting osteoblast differentiation and promoting apoptosis [73–76]. Despite the progress, a clear and mechanistic understanding of the deleterious effects of diabetes on bone mass and quality awaits further studies. In particular, it is not clear whether and how glucose metabolism in osteoblasts or osteoclasts is altered *in vivo* under diabetic conditions, and more importantly whether such potential changes contribute to the pathology in bone.

The effects of insulin signaling on glucose metabolism in osteoblasts or osteoclasts also warrant further studies. Osteoblasts express the insulin receptor (IR) but whether or not insulin stimulates glucose uptake in osteoblasts has been a matter of debate [18,19]. Nonetheless, IR deletion in osteoblasts recapitulates the bone phenotypes present in diabetic mouse models, namely reduced osteoblast numbers, bone formation and bone mass [77]. IR signaling deficiency in osteoblasts is likely relevant to both types of diabetes as emerging

evidence supports insulin resistance in osteoblasts in type II diabetes models [78,79]. In addition to low systemic insulin, type I diabetes is associated with decreased serum insulin-like growth factor-1 (IGF1) in both humans and rodent models [80,81]. As IGF1 signals through the IGF1 receptor (IGF1R) to directly stimulate osteoblast differentiation, matrix production and mineralization, IGF1 deficiency likely contributes to the bone formation deficit in diabetes [82–84]. Future studies are necessary to determine whether deficiency in IR or IGF1R signaling alters glucose metabolism in osteoblasts or osteoclasts to cause bone pathology associated with diabetes.

6. Conclusions

We have highlighted the major findings to date regarding the role and molecular regulation of glucose metabolism in osteoblasts and osteoclasts. A notable knowledge gap exists about the metabolic profile of osteocytes, the predominant cell type in bone. Although one might infer from the historical studies employing bone slices that osteocytes metabolize glucose mainly to lactate, a direct assessment of glucose metabolism in osteocytes is clearly necessary. Nonetheless, the existing literature supports a multifaceted role of glucose metabolism during physiological bone formation and bone resorption. The metabolic plasticity observed during osteoblast or osteoclast differentiation is particularly intriguing. Metabolic rewiring is likely to be integral to cell differentiation, but the mechanisms through which the metabolic changes contribute to the differentiated phenotype are undoubtedly complex and warrant further investigation. Genetic studies will be necessary to clarify the physiological roles of specific metabolic pathways in the various bone cell types. It is tempting to speculate that metabolic dysregulation at the cellular level might be involved in the bone pathology associated with diabetes. Although the mechanisms for diabetes-related bone fragility are undoubtedly complex, pharmaceutical targeting of cellular glucose metabolism may be a promising direction towards developing novel therapies to improve bone quality in diabetic patients.

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Fig. 1.

Glucose metabolism in mammalian cells. Protein abbreviations: GLUT – Glucose Transporter; HK – Hexokinase; PGI – Phosphoglucose Isomerase. PFK – Phosphofructokinase; TPI – Triose Phosphate Isomerase; GAPDH – Glyceraldehyde 3-phosphate Dehydrogenase; PGK – Phosphoglycerate Kinase; PGAM –Phosphoglycerate Mutase; ENO – Enolase; PKM – Pyruvate Kinase, Muscle; LDH – Lactate Dehydrogenase; PDH – Pyruvate Dehydrogenase; ACLY: ATP citrate lyase; MCT – Monocarboxylate Transporter. Metabolite abbreviations: G6P – Glucose 6-phosphate; F6P – Fructose 6-phosphate; F1,6BP – Fructose 1,6-bisphosphate; DHAP – Dihydroxyacetone Phosphate; G3P – Glyceraldehyde 3-phosphate; 1,3BPG – 1,3-Bisphosphoglycerate; 3PG – 3-Phosphoglycerate; 2PG – 2-Phosphoglycerate; PEP – Phosphoenolpyruvate.

Fig. 2.

Regulation of glucose metabolism in osteoblast lineage. (A) A schematic of osteoblast differentiation. Representative marker genes are denoted at specific stages. (B) Signal pathways regulating glucose metabolism during osteoblast differentiation. PTH – Parathyroid Hormone. PTH1R – Parathyroid Hormone 1 Receptor. IGF1 – Insulin Like Growth Factor 1. IGF1R – Insulin Like Growth Factor 1 Receptor. FZD – Frizzled. LRP5/6 – Low Density Lipoprotein Receptor-Related Protein 5 or 6. cAMP: Cyclic Adenosine Monophosphate. Hif1a - Hypoxia Inducible Factor 1a. GLUT1 – Glucose Transporter 1; HK2 – Hexokinase 2; LDHA – Lactate Dehydrogenase A; PDK1 – Pyruvate Dehydrogenase Kinase 1. Bent arrows denote gene transcription. Arrows and blocked arrows indicate stimulation and inhibition, respectively.

Fig. 3. Metabolic regulation during osteoclast differentiation. Both glycolysis and OXPHOS increase during osteoclastogenesis. MCSF: macrophage colony stimulating factor; RANKL: receptor activator of nuclear factor kappa-B ligand.