

Published in final edited form as:

Curr Osteoporos Rep. 2014 December ; 12(4): 433–438. doi:10.1007/s11914-014-0235-y.

Aerobic Glycolysis in Osteoblasts

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Abstract

Osteoblasts, the chief bone-making cells in the body, are a focus of osteoporosis research. Although teriparatide, a synthetic fragment of the human parathyroid hormone (PTH), has been an effective bone anabolic drug, there remains a clinical need for additional therapeutics that safely stimulates osteoblast number and function. Work in the past several decades has provided unprecedented clarity about the roles of growth factors and transcription factors in regulating osteoblast differentiation and activity, but whether these factors may regulate cellular metabolism to influence cell fate and function has been largely unexplored. The past few years have witnessed a resurgence of interest in the cellular metabolism of osteoblasts, with the hope that elucidation of their metabolic profile may open new avenues for developing bone anabolic agents. Here we review the current understanding about glucose metabolism in osteoblasts.

Keywords

osteoblast; glucose metabolism; aerobic glycolysis; energy; bone formation

Introduction

Osteoblasts are the chief bone-making cells integral to skeletal health and disease in humans. They first appear in the embryo from mesodermal or neural crest cells, but are produced throughout life from mesenchymal progenitors during bone remodeling, remodeling and fracture healing¹. Osteoblasts engage in active bone formation for a limited time; subsequently the majority is believed to undergo apoptosis, whereas the remaining

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Conflict of Interest

E Esen and F Long both declare no conflicts of interest.

Human and Animal Rights and Informed Consent

All studies by the authors involving animal and/or human subjects were performed after approval by the appropriate institutional review boards. When required, written informed consent was obtained from all participants.

cells become either bone-lining cells on the bone surface, or osteocytes entombed in the bone matrix. In addition to making bone matrix, osteoblasts, together with osteocytes, also produce molecular signals to regulate osteoclastogenesis necessary for bone remodeling^{2,3}. Osteocytes are also known to regulate phosphate homeostasis through the secretion of Fgf23⁴. More recently, osteoblasts have been implicated in the regulation of systemic glucose metabolism in the mouse⁵. Thus, elucidating osteoblast identity and regulation is important not only for advancing skeletal biology but also for a better understanding of whole-body physiology.

Osteoblasts synthesize a large amount of extracellular matrix proteins, and therefore have a high demand for both energy and building materials. However, the bioenergetics and carbon source for osteoblasts are poorly understood. A number of studies on glucose metabolism in bone slices and isolated osteoblasts were conducted between the 1950's and 1980's. These studies indicated that aerobic glycolysis, i.e., production of lactate from glucose despite the presence of oxygen, was the main mode of glucose metabolism in osteoblasts. Moreover, the calcitropic hormone parathyroid hormone (PTH) was shown to stimulate the production of lactic acid from glucose, prompting researchers to hypothesize that increased production of lactic acid is responsible for more active bone resorption. Subsequent rejection of this hypothesis may have contributed to the loss of interest in aerobic glycolysis in osteoblasts during the ensuing decades. A resurgent interest in cellular metabolism in recent years has led to the appreciation that lactate-producing glycolysis may play an important role in osteoblast differentiation and function.

Cellular glucose metabolism

Glucose is a major energy and carbon source for mammalian cells. In most cells, glucose is transported via the GLUT family of facilitative transporters (GLUTs). This process does not require energy and can only transport glucose down a concentration gradient^{6,7}. Once inside the cell, glucose is phosphorylated to glucose-6-phosphate (G6P) by hexokinases, and is then either converted to glycogen for storage, or metabolized to produce ATP and intermediate metabolites for anabolic reactions. Glucose catabolism can follow multiple pathways, including glycolysis to produce pyruvate for further metabolism, entering pentose phosphate pathway (PPP), or fueling into hexosamine biosynthetic pathway (HBP)⁸ (Figure 1). Overall, glucose is not only a unique fuel that can produce ATP with or without oxygen, but also a critical source for building blocks necessary for biosynthesis in the cell.

Glycolysis is the predominant route for cellular glucose utilization. The process occurs in the cytosol and breaks down one molecule of glucose into two molecules of pyruvate. Pyruvate can be converted to lactate by the enzyme lactate dehydrogenase (LDH) in the cytosol. This reaction regenerates NAD⁺ necessary for further glycolysis, and can occur with or without oxygen. LDH is a tetrameric enzyme consisting of subunits A, B or C expressed from different genes. The A and B subunits are expressed ubiquitously and can form five different tetrameric enzyme forms, namely LDH1-5, through different combinations. On the other hand, the C subunit is specific to the testis and sperms⁹. An alternative fate for pyruvate is to participate in the tricarboxylic acid (TCA) cycle (also known as Krebs cycle) within the matrix of mitochondria. This process produces the most

ATP per glucose molecule but requires molecular oxygen. The predominant route for mitochondrial pyruvate to enter the TCA cycle is through decarboxylation by pyruvate dehydrogenase complex (PDH) to form acetyl-coA. This entry point is tightly regulated as elevated levels of ATP and pyruvate dehydrogenase kinase (PDK) can inactivate PDH. Acetyl-coA enters the TCA cycle by condensing with oxaloacetate to form citrate, which then sequentially releases two CO₂ molecules to regenerate oxaloacetate. The TCA cycle not only produces ATP through oxidative phosphorylation, but also provides intermediate molecules (e.g., citrate) that exit mitochondria to participate in biosynthesis (e.g., fatty acid synthesis) elsewhere in the cell.

During glycolysis, a small portion of the intermediates is diverted into HBP and PPP. 5–30% of glucose is estimated to enter PPP that is the source of NADPH and ribose-5-phosphate (R5P). Whereas NADPH provides the reducing equivalents for fatty acid and cholesterol synthesis, R5P is the precursor for nucleotide synthesis. PPP starts with an oxidative phase including the rate-limiting, irreversible dehydrogenation of G6P by glucose 6-phosphate dehydrogenase (G6PD). This is followed by further oxidation and decarboxylation into ribose-5 phosphate (R5P). In the non-oxidative phase, PPP yields fructose 6-phosphate and glyceraldehyde 3-phosphate, both of which can enter the glycolytic pathway^{10,11}. Approximately 2–5% of cellular glucose is metabolized through HBP. Here, fructose-6-phosphate together with glutamine is catalyzed by GFPT1 (also known as GFAT) to form glucosamine-6-phosphate in the very first and rate-limiting step. Glucosamine-6-phosphate is further used to generate uridine diphospho-N-acetylglucosamine (UDP-GlcNAc), which is a precursor necessary for protein glycosylation¹². Overall, PPP and HBP consume a relatively small percentage of cellular glucose but provide critical intermediates for various anabolic reactions. The extent to which glycolytic intermediates are diverted to PPP or HBP is determined by the biosynthetic needs of the cell.

Aerobic glycolysis in osteoblasts

Members of the GLUT glucose transporter family are expressed in osteoblast-lineage cells. GLUT1 and GLUT3 were detected in an osteosarcoma cell line, UMR 106-01^{13,14}. There is also evidence for the expression of GLUT1 in rodent osteoblastic cells (PyMS)¹⁵. Further investigation is necessary to characterize the specific expression pattern and function of each GLUT protein in osteoblasts *in vivo*.

Despite the incomplete understanding of glucose transporters, numerous studies have demonstrated active glycolysis in osteoblasts. The initial studies with bone slices in the early 1960's showed that bone not only consumed a large amount of glucose, but also produced lactate as the major end product while the TCA cycle played a minor role even in aerobic conditions^{16,17}. It was estimated that >80% of glucose consumed by bone was converted to lactate¹⁶. Providing excess amino acid had no effect on the amount of cellular CO₂ or lactate production from glucose¹⁸. The metabolic features of bone slices were later confirmed in osteoblasts enzymatically digested from calvaria¹⁹. More recently, calvarial osteoblastic cells were shown to utilize both aerobic glycolysis and oxidative phosphorylation during differentiation *in vitro*, but relied more on the former than the latter when they became

mature osteoblasts²⁰. Moreover, forced activation of glycolysis by overexpressing a stabilized form of HIF1 α led to more osteoblasts and a higher bone mass in the mouse²¹. Overall, osteoblasts use glucose as a major nutrient and metabolize it mostly to lactate even in the presence of oxygen. This process is known as aerobic glycolysis.

The aerobic glycolysis in osteoblasts is akin to the Warburg effect observed in cancer cells²². In cancer cells, aerobic glycolysis has been postulated to provide the necessary glycolytic intermediates to support uncontrolled proliferation, but this is unlikely to be the case for normal somatic cells such as osteoblasts^{23,24}. It is possible that aerobic glycolysis is necessary to fulfill the specific metabolic needs of osteoblasts. For instance, osteoblasts have been long known to produce and secrete a large amount of citrate^{25,26}. Citrate in bone was recently shown to be critical for the structure of the apatite nanocrystal, providing stability, strength, and resistance to fracture^{27–29}. The mechanism for the high citrate output by osteoblasts is not fully understood, but may be partly due to a relatively low activity of isocitrate dehydrogenase (IDH) which converts isocitrate to α -ketoglutarate for the continuation of the TCA cycle²⁵. In addition, the essential trace element zinc is known to attenuate the activity of aconitase, the enzyme responsible for isomerizing citrate to isocitrate, in prostate epithelial cells³⁰. Although a similar role has not been demonstrated in osteoblasts, zinc is abundant in bone and mice lacking a zinc transporter, ZNT5, manifest poor growth and decreased bone density due to impairment of osteoblast maturation^{31,32}. Moreover, zinc uptake and the level of ZIP1 zinc transporter were shown to increase during osteoblast differentiation, and overexpression ZIP1 enhanced osteoblastogenesis *in vitro*³³. If indeed a high level of citrate accumulates in the mitochondria of osteoblasts, it may suppress pyruvate entering the TCA cycle and promote the conversion to lactate.

Regulation of osteoblast glycolysis by PTH

Intermittent use of parathyroid hormone (PTH) or its N-terminal fragment known as teriparatide effectively promotes bone formation, but the mechanism underlying such anabolic function is incompletely understood. Interestingly, long before PTH was used as a bone anabolic agent, it was shown to stimulate aerobic glycolysis in osteoblasts. Experiments with metaphyseal bone explants of the long bones showed that injections of parathyroid extracts prior to sacrifice of the mice increased lactate production by the explants in culture under aerobic conditions³⁴. This effect was only observed in the presence but not absence of glucose, and estradiol did not have the same effect. Later, PTH was shown to increase lactate production by calvarium explants^{35,36}. More recently, PTH was reported to induce glucose uptake in rat osteoblastic cells (PyMS)³⁷. Overall, PTH promotes lactate-producing glycolysis in osteoblasts, but the mechanism for such regulation is unclear. Moreover, whether increased aerobic glycolysis contributes to the bone anabolic effect of PTH has not been explored.

Wnt stimulates aerobic glycolysis during osteoblast differentiation

Although all major developmental signals have been shown to regulate osteoblast differentiation, their potential effects on cellular metabolism have been largely unexplored¹. Wnt signaling has emerged as a major promoter of bone accretion³⁸. Recently, Wnt proteins

were shown to reprogram cellular glucose metabolism during osteoblast differentiation³⁹. In particular, Wnt3a, 7b and 10b, each known to induce osteoblast differentiation, stimulated aerobic glycolysis in both cell lines and primary cultures of osteogenic cells. Mechanistically, Wnt3a rapidly increased the protein levels of several glycolytic enzymes independent of GSK3- β and β -catenin but downstream of mTORC2 and Akt. Suppressing the Wnt3a-induced glycolytic enzymes impaired osteoblast differentiation in vitro. Moreover, deletion of Lrp5 in mice reduced mTORC2 activity and the glycolytic enzymes in bone, concurrent with a decrease in postnatal bone mass. Conversely, mice harboring a high-bone-mass allele of Lrp5 expressed higher levels of glycolytic enzymes in bone. Thus, direct stimulation of aerobic glycolysis may contribute to bone anabolism in response to Wnt-Lrp5 signaling.

Regulation of osteoblasts by insulin and IGFs

Insulin is a critical hormone controlling whole-body glucose homeostasis. Type I diabetes mellitus, caused by insulin deficiency, has been linked with decreased bone density, increased risk as well as early onset for osteoporosis, increased fracture risk and poor fracture healing in human patients^{40–43}. Consistent with the clinical observations, studies in diabetic animal models revealed defects in osteoblast function associated with insulin deficiency^{44,45}. Delayed fracture healing in diabetic rats could be overcome by insulin delivery at the fracture site without affecting the systemic blood glucose level⁴⁶. Moreover, genetic deletion of insulin receptor (IR) in either mature osteoblasts or osteoprogenitors impaired bone formation in postnatal mice^{47–49}. These findings indicate that insulin likely functions as an anabolic signal in the osteoblast lineage. However, it is not known at present whether insulin may promote bone formation through reprogramming of cellular metabolism.

Insulin-like growth factors (IGF1 and 2) have also been implicated in bone formation⁵⁰. Whereas IGF2 mainly regulates fetal growth via both IGF1R and IR, IGF1 controls postnatal growth and homeostasis through IGF1 receptor (IGF1R)⁵¹. IGF1 is abundantly associated with the postnatal bone matrix and has been extensively studied as a bone anabolic signal. IGF1 knockout mice exhibit defects in bone formation, whereas overexpression of IGF1 in osteoblasts enhanced their activity^{52,53}. Removal of IGF1 at early stages of osteoblast differentiation produced a more severe defect in bone accretion⁵⁴. Similarly, deletion of IGF1R in either mature osteoblasts or osteoprogenitors impaired bone formation^{55,56}. There is evidence that reduced IGF signaling may relate to age-dependent bone loss. Both serum and bone-matrix levels of IGF1 appeared to decrease with aging in humans^{57,58}. Aging was also shown to impair IGF1R responsiveness to IGF1 in bone marrow stromal cells of mice⁵⁹. Moreover, injection of IGF1 together with IGF binding protein 3 (IGFBP3) ameliorated aging-related bone loss in rats⁶⁰. Thus, IGF signaling stimulates bone formation and plays important roles in bone homeostasis.

Apart from its importance in basal bone physiology, IGF1 signaling appears to mediate the bone anabolic effect of intermittent PTH. Removal of either IGF1 or IGF1R in osteoblasts essentially abolished the PTH response^{61–63}. The mechanism underlying the bone anabolic effect of IGF signaling is not fully understood. It is worth noting that like PTH, IGF1 also

induces glucose uptake in osteoblastic cells³⁷. Future studies are necessary to determine the role of glycolysis in bone anabolism induced by IGF signaling.

Conclusions

Understanding the mechanisms governing osteoblast differentiation and function is key to developing novel bone anabolic therapeutics. Although transcriptional factors and developmental signals have been extensively studied over the past several decades, relatively little is known about the status of cellular metabolism during osteoblast differentiation. Future studies are necessary to define the metabolic phenotype of osteoblasts, including the energy and carbon sources as well as their metabolic fates. Work to date has mostly focused on glucose metabolism, but studies of fatty acids and amino acids are clearly necessary. In addition, to understand how osteoblasts achieve their specific metabolic profile, it is important to explore whether other developmental signals besides Wnt, or the known transcription factors reprogram cellular metabolism. The molecular basis for the effect of metabolic reprogramming on osteoblast differentiation needs to be elucidated. Finally, technical advances are necessary to monitor cellular metabolic changes in bone in vivo. Overall, a clear understanding of cellular metabolism in osteoblasts will not only advance basic biology, but may also provide mechanistic insights about bone frailty associated with metabolic diseases and aging.

Acknowledgments

Work in the Long lab was supported by NIH grants AR060456, AR055923 and DK065789.

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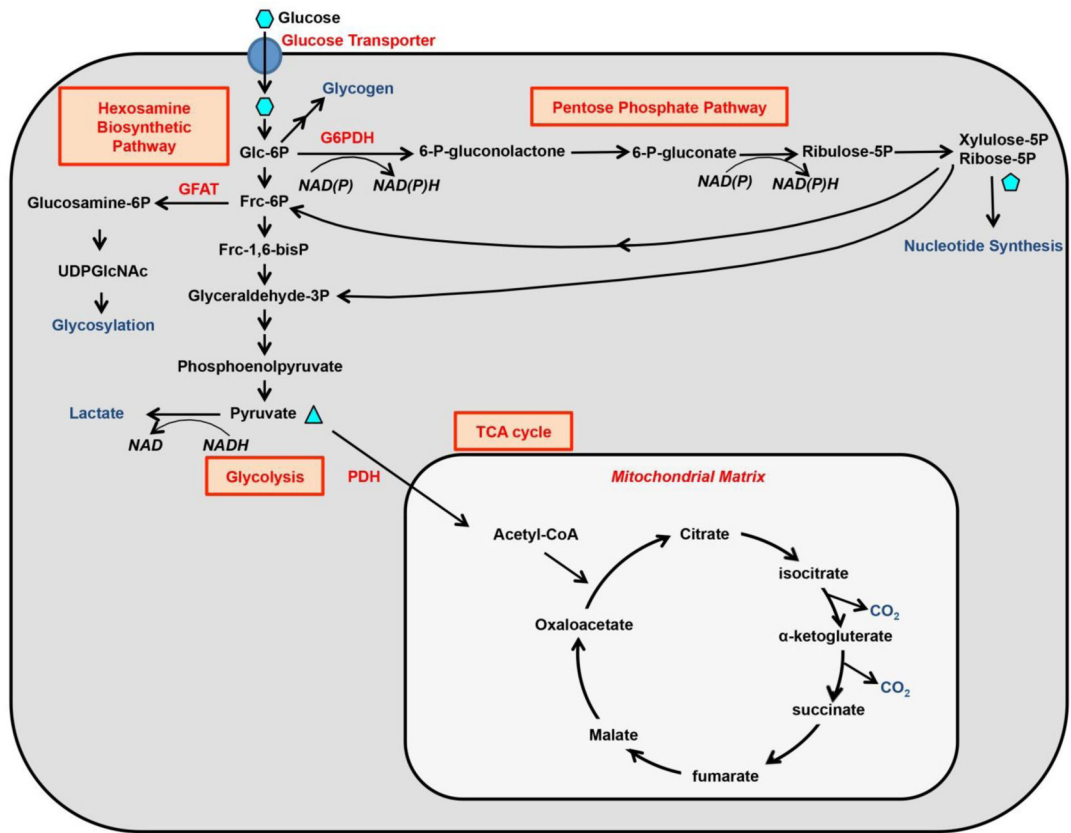


Figure 1. Metabolic fates of glucose in mammalian cells

G6PDH: Glucose-6-phosphate dehydrogenase. GFAT: Glutamine fructose-6-phosphate amidotransferase. PDH: Pyruvate dehydrogenase complex.