Glucose metabolism in skeletal cells

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ABSTRACT

The mammalian skeleton is integral to whole body physiology with a multitude of functions beyond mechanical support and locomotion, including support of hematopoiesis, mineral homeostasis and potentially other endocrine roles. Formation of the skeleton begins in the embryo and mostly from a cartilage template that is ultimately replaced by bone through endochondral ossification. Skeletal development and maturation continue after birth in most species and last into the second decade of postnatal life in humans. In the mature skeleton, articular cartilage lining the synovial joint surfaces is vital for bodily movement and damages to the cartilage are a hallmark of osteoarthritis. The mature bone tissue undergoes continuous remodeling initiated with bone resorption by osteoclasts and completed with bone formation from osteoblasts. In a healthy state, the exquisite balance between bone resorption and formation is responsible for maintaining a stable bone mass and structural integrity, while meeting the physiological needs for minerals via controlled release from bone. Disruption of the balance in favor of bone resorption is the root cause for osteoporosis. Whereas osteoclasts pump molar quantities of hydrochloric acid to dissolve the bone minerals in a process requiring ATP hydrolysis, osteoblasts build bone mass by synthesizing and secreting copious amounts of bone matrix proteins. Thus, both osteoclasts and osteoblasts engage in energy-intensive activities to fulfill their physiological functions, but the bioenergetics of those and other skeletal cell types are not well understood. Nonetheless, the past ten years have witnessed a resurgence of interest in studies of skeletal cell metabolism, resulting in an unprecedented understanding of energy substrate utilization and its role in cell fate and activity regulation. The present review attempts to synthesize the current findings of glucose metabolism in chondrocytes, osteoblasts and osteoclasts. Advances with the other relevant cell types including skeletal stem cells and marrow adipocytes will not be discussed here as they have been extensively reviewed recently by others (van Gastel and Carmeliet, 2021). Elucidation of the bioenergetic mechanisms in the skeletal cells is likely to open new avenues for developing additional safe and effective bone therapies.

1. A brief introduction to glucose metabolism

Glucose is not only a major bioenergetic substrate but also a critical carbon source for biosynthesis in mammalian cells (Karner and Long, 2018). Glucose is transported across the plasma membrane in most cells by the Glut family of facilitative transporters encoded by the solute carrier family 2A (Slc2a) genes (Augustin, 2010). Glucose entry into the cell through the Glut proteins is driven by a concentration gradient of glucose and does not require energy. Phosphorylation by members of the hexokinase family prevents glucose from exiting the cell; the resultant glucose-6-phosphate (G6P) is either converted to glycogen for storage, or shunted through the pentose phosphate pathway (PPP) for nucleotide synthesis, or in most cases catabolized through the core glycolysis pathway (Fig. 1). Glycolysis of each glucose molecule generates two reduced nicotinamide adenine dinucleotide (NADH) and a net gain of two ATP, culminating in the production of two pyruvates. Upon transport into mitochondria, pyruvate is further oxidized through the TCA cycle to produce ATP via oxidative phosphorylation (OXPHOS). Alternatively, pyruvate can be converted to lactate in the cytosol with regeneration of NAD⁺ from NADH to support further glycolysis. Complete oxidation in the mitochondria extracts much more energy from glucose than does the conversion to lactate (>30 vs 2 ATP per glucose molecule), but the lactate pathway consumes glucose at a faster pace and produces energy without the need for oxygen. Recent studies have shown that the flux rate of glycolysis in mammalian cell lines are controlled at four key steps including glucose and lactate transport as well as hexokinase and phosphofructokinase activities (Tanner et al., 2018). Although a traditional view holds that normal somatic cells...
metabolize glucose mainly through mitochondrial respiration when oxygen is not limiting, multiple cell types including endothelial cells, astrocytes and osteoblasts are known to convert glucose mainly to lactate under aerobic conditions, in a phenomenon known as aerobic glycolysis (Rohlena et al., 2018; Barros et al., 2021; Esen and Long, 2014).

Besides the core glycolysis pathway, several glycolytic intermediates are known to enter other metabolic routes with no direct production of ATP but nonetheless of critical importance. The PPP as mentioned earlier produces not only ribose 5-phosphate, the backbone of nucleic acids, but also nicotinamide adenine dinucleotide phosphate (NADPH) essential for both lipid synthesis and the redox balance through glutathione (GSH) and thioredoxin (TRX) (Fan et al., 2014) (Fig. 1). Moreover, fructose-6-P (F6P) derived from G6P can enter the hexosamine biosynthesis pathway (HBP) to generate uridine diphosphate N-acetylglucosamine (UDP-GlcNAc) to be used for protein glycosylation (Bouche et al., 2004). Further downstream of the glycolysis pathway, fructose 1,6-bisphosphate (F1,6BP) is cleaved into two interconvertible three-carbon molecules glyceraldehyde 3-phosphate (G3P) and dihydroxyacetone phosphate (DHAP), the latter of which can be converted to glycerol 3-phosphate (G3P) to produce glycerol, the backbone of triglycerides and phospholipids. Finally, 3-phosphoglycerate (3PG) can be directed towards de novo synthesis of serine and subsequently glycine, a process integral to one-carbon metabolism that contributes to DNA and histone methylation, biosynthesis of purines and thymidine, as well as redox regulation (Ducker and Rabinowitz, 2017). Overall, besides energy production, glycolysis supplies critical intermediate metabolites for multiple biosynthetic pathways. Therefore, it stands to reason that the allocation of glucose among the various metabolic fates is entrained by the specific energy and biosynthesis needs of each cell.

2. Glucose metabolism in chondrocytes

Much of the mammalian skeleton is formed through an intermediate of cartilage template via endochondral bone development. The process begins with embryonic mesenchymal cells undergoing condensation to form a cartilage anlage wherein chondrocytes progress through proliferation and maturation culminating in hypertrophy, before the hypertrophic cartilage is replaced by nascent bony tissue (Long and Ornitz, 2013). Chondrocytes are unique among skeletal cells as they reside in the avascular cartilage and therefore have limited access to oxygen; those located at the interior of the tissue are particularly hypoxic as detected with a hypoxyprobe (Schipani et al., 2001). Genetic deletion of Hif1a has demonstrated that proper adaptation to hypoxia is essential for the survival of the interior hypoxic chondrocytes (Schipani et al., 2001). A critical aspect of the adaptive response appears to be active suppression of mitochondrial respiration as chondrocyte apoptosis is averted by simultaneous removal of the mitochondrial transcription factor A (Tfam), which is required for mitochondrial replication and function (Yao et al., 2019). However, a certain level of mitochondrial respiration may still be necessary in chondrocytes as uncontrolled stabilization of Hif1a following deletion of prolyl hydroxylase 2 (Phd2) caused an energy deficit associated with reduced glucose oxidation when the cells were cultured with atmospheric levels of oxygen (Stegen et al., 2019). Future studies are important to verify the findings in vivo and to uncover potential differences in the reliance on mitochondrial respiration between chondrocytes residing in the interior versus periphery of the cartilage.

In keeping with the role of Hif1a in stimulating glycolysis, evidence supports glucose as a main energy substrate for chondrocytes. Embryonic deletion of Glut1 (official gene name Slc2a1) in chondrocytes causes severe skeletal dysplasia, owing to marked suppression of cell proliferation, matrix protein production as well as hypertrophy (Lee et al., 2018). It is worth noting that deletion of Glut1 in the limb mesenchymal precursors did not impair the size of the initial cartilage anlagen, thus highlighting the increased dependence on Glut1 by chondrocytes during the subsequent growth phase. Interestingly, Bmp signaling, long known to control chondrocyte proliferation and maturation in the developing cartilage, stimulates glycolysis in chondrocytes through upregulation of Glut1 downstream of mTORC1 and Hif1a signaling, underscoring the interplay between growth factor signaling and metabolic regulation in development (Lee et al., 2018). Similarly, in postnatal mice, the dependence of cartilage growth on Igf2 has been linked to the growth factor's role in modulating glucose metabolism in chondrocytes (Uchimura et al., 2017). Specifically, the impaired growth of metatarsal cartilage in postnatal Igf2-null mice is associated with increased glycolysis and oxidative phosphorylation. The mechanism for the apparent hypermetabolism to cause growth retardation is not clear, but it may reflect the need for balancing the bioenergetic versus biosynthetic needs to achieve normal cellular physiology. In this regard, a recent study has demonstrated the importance of de novo serine synthesis, branching off the core glycolysis pathway, in supporting chondrocyte proliferation (Stegen et al., 2022). As discussed earlier, the glycolytic intermediate 3-phosphoglycerate (3PG) can be diverted to

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**Fig. 1.** A schematic for major pathways of glucose metabolism in mammalian cells. PPP: Pentose phosphate pathway. HBP: hexosamine biosynthesis pathway. Mitochondrion is depicted as an oval.
produce serine and subsequently glycolate, thus contributing one-carbon units to folate metabolism that produces purines and thymidine necessary for DNA replication. Deletion of phosphoglycerate dehydrogenase (PHGDH), the rate-limiting enzyme for diverting 3PG to the serine synthesis pathway, impaired longitudinal bone growth in postnatal mice due to suppression of chondrocyte proliferation, presumably due to insufficient nucleotides (Stegen et al., 2022). Of note, disruption of the serine synthesis pathway also reduced glucose entering the pentose phosphate pathway and the TCA cycle, thus highlighting the independence of the various metabolic fates of glucose. Overall, the studies underscore the importance of glucose as both an energy substrate and a carbon source for biosynthesis in chondrocytes, and further indicate that proper allocation of the different metabolic fates is critical for ensuring the proper cellular functions.

Proper glucose metabolism has also been long implicated in the health of articular cartilage. Multiple members of the Glut family, including Glut1 and Glut3, are expressed by the articular chondrocytes (Mobasher et al., 2002). Energy production in articular chondrocytes is predominantly through glycolysis with lactate as the end product, even under aerobic conditions (Otte, 1991). Nevertheless, oxygen is necessary to sustain aerobic glycolysis as anoxia (≤1 % O2) suppresses the process through a negative Pasteur effect (Lee and Urban, 1997). The role of oxygen here is independent of OXPHOS, but instead is mediated by physiological oxidants produced through the mitochondrial electron transport chain and necessary for maintaining redox balance in the cell (Martin et al., 2012; Lee and Urban, 2002). Glucose metabolism in articular cartilage has been shown to respond to both anabolic and catabolic signals at least partly through upregulation of Glut1 expression (Shikhman et al., 2001; Shikhman et al., 2004). Interestingly, in primary cultures of human articular chondrocytes isolated from osteoarthritic patients, Tgfβ1 greatly stimulated aerobic glycolysis but Bmp2 enhanced glucose oxidation in the mitochondria without increasing overall glucose consumption (Wang et al., 2018). The Bmp2 effect here is distinct from that observed in murine growth plate chondrocytes where it stimulates glucose consumption through upregulation of Glut1 (Lee et al., 2018). Although the different effects might merely reflect the impact of osteoarthritis, it could potentially indicate distinct metabolic responses to Bmp signaling between growth plate and articular chondrocytes.

Impaired glucose metabolism has been linked with the progression of posttraumatic osteoarthritis (OA) in surgery-induced murine models. Glut1 protein levels in the articular chondrocytes declined with the progression of OA, and postnatal deletion of Glut1 in the superficial layers of articular cartilage with Prg4-CreER<sup>T2</sup> exacerbated OA phenotypes including chondrocyte apoptosis, loss of cartilage matrix and cartilage degradation (Li et al., 2022). Importantly, forced expression of Glut1 in the articular chondrocytes improved the OA outcomes. Likewise, postnatal deletion of Glut1 with Acan-CreER<sup>T2</sup> which targeted all chondrocytes reduced articular cellularity and proteoglycan production, eventually causing spontaneous fibrosis in the articular cartilage (Wang et al., 2021). Interestingly, disruption of glucose metabolism in the chondrocytes enhanced glutamine metabolism leading to overhydroxylation of collagen resistant to protease degradation (Wang et al., 2021). A similar increase in glutamine metabolism was observed in growth plate chondrocytes when glycolysis was enhanced upon deletion of Phd2 (Stegen et al., 2019). These results underline the importance of proper glucose metabolism in chondrocytes as its disruption leads to apparent metabolic maladaptation.

3. Glucose metabolism in osteoblasts

Studies in the 1960s have already identified glucose as a major nutrient for either bone explants or isolated osteoblastic cells (Cohn and Forscher, 1962; Peck et al., 1964; Borle et al., 1960). The early studies further documented that osteoblasts either in tissue explants or in cell culture following isolation metabolized glucose mainly to lactate even under aerobic conditions, in a phenomenon known as aerobic glycolysis (Cohn and Forscher, 1962; Borle et al., 1960; Neuman et al., 1978; Felix et al., 1978). More recent studies have not only confirmed aerobic glycolysis in primary calvarial preosteoblast cultures but also demonstrated a further increase in glycolysis flux following osteoblast differentiation in response to ascorbic acid and β-glycerophosphate (Komarova et al., 2000; Guntur et al., 2014; Lee et al., 2020). By one estimation based on extracellular flux assays with Seahorse bioanalyzer, mature osteoblasts derived from in vitro differentiation produced approximately 80 % of energy from aerobic glycolysis (Lee et al., 2020). Carbon tracing with stable isotope in vivo confirmed lactate as the predominant fate for glucose in the cortical bone of murine long bones (Lee et al., 2020). Studies of osteoblast differentiation in cultures of human mesenchymal stem cells (MSC) have yielded variable results. It should be noted that the use of the term MSC has been confusing and is best to be avoided in the future as the cells used in most studies are heterogeneous with unproven stemness. The term is used here only to be consistent with the original studies cited here. In one study, bone marrow-derived MSC was shown to increase oxidative phosphorylation without an obvious change in glycolysis after 14 days of osteoblast differentiation (Shum et al., 2016). Another study using MSC isolated from human adipose tissues reported a significant decrease in oxygen consumption following 21 days of osteoblast differentiation (Morganti et al., 2020). The discrepancy could reflect the dynamic nature of metabolic changes associated with osteoblast differentiation from MSC. In particular, the data is consistent with a transient increase in oxidative phosphorylation at an intermediate stage but more reliance on glycolysis by mature osteoblasts. Consistent with this notion, electron microscopy studies have reported that “condensed mitochondria”, believed to be active in oxidative phosphorylation, transiently increased with osteoblast differentiation of bone marrow stromal cells in diffusion chambers implanted in vivo (Passi-Even et al., 1993). Finally, the role of the mitochondria in osteoblasts extends beyond direct ATP production via OXPHOS as the malate-aspartate shuttle is likely important to sustain the NAD+/NADH balance necessary for continued glycolysis (Lee et al., 2020). Future studies may reveal additional coupling mechanisms between glycolysis and mitochondrial respiration in osteoblasts.

Glucose entry into osteoblast lineage cells is mainly mediated by members of the Glut family. Glut1, 3 and 4 have been reported in osteoblastic cell lines or primary osteoblast cultures (Zoidis et al., 2011; Li et al., 2016; Wei et al., 2015). Recent RNA-seq experiments in murine calvarial preosteoblast cultures confirmed Glut1 as the predominant transporter but also uncovered the expression of additional members including Glut8 and Glut10 before and after osteoblast differentiation (Lee et al., 2020). Besides immediate fueling of glycolysis, the imported glucose can also be converted to glycogen in osteoblasts for later use. Indeed, studies with electron microscopy identified in preosteoblasts prominent glycogen granules whose size was later reduced in mature osteoblasts, presumably due to increased glycogenolysis in support of the energetic or biosynthetic needs (Schajowicz and Cabrini, 1958). Genetic knockout studies have implicated Glut1 in promoting osteoblast differentiation by increasing Runx2 protein levels in the mouse embryo, but another study shows that Glut1 is dispensable for osteoblast differentiation even though it is required for the optimal mineralizing activity of osteoblasts (Lee et al., 2018; Wei et al., 2015). Glut4 has been reported to mediate insulin-stimulated glucose uptake by osteoblasts in vitro, but it is dispensable for bone development and homeostasis in the mouse (Li et al., 2016). Deliberation of individual contributions of Glut1 and Glut4 proteins is complicated not only by functional redundancy among the family members, but also by metabolic plasticity that could involve rebalancing of the glucose metabolic fates (e.g., glycolysis versus TCA metabolism), or the use of alternative energy substrates such as fatty acids or amino acids.

Glucose metabolism in osteoblast-lineage cells is stimulated by bone anabolic signals. Long before teriparatide (a synthetic fragment of human parathyroid hormone, i.e., PTH) was used as a bone anabolic
therapy in osteoporotic patients, PTH was shown to stimulate glucose consumption and lactate production in bone explants (Borle et al., 1960; Neuman et al., 1978; Felix et al., 1978; Rodan et al., 1978). More recently, a mechanistic study in MC3T3-E1 cells demonstrated that PTH enhanced aerobic glycolysis through transcriptional induction of Igf1 that in turn activated mTORC2 to increase the abundance of glycolytic enzymes (Esen et al., 2015) (Fig. 2). PTH also increased glucose flux into the PPP but reduced the flux of glucose-derived pyruvate entering the TCA cycle. Somewhat paradoxically, PTH stimulated the overall activity of OXPHOS, indicating that alternative substrates such as fatty acids or amino acids might be fueling mitochondrial respiration in response to PTH. Importantly, suppression of aerobic glycolysis with dichloroacetate (DCA) that increases pyruvate entry to the TCA cycle notably dampened the bone anabolic effect of intermittent PTH, thus functionally linking glycolysis with bone formation (Esen et al., 2015). Further supporting the coupling between glycolysis and bone anabolism, overexpression of Hif1a, which normally accumulates in response to hypoxia to activate glycolysis through transcriptional stimulation, increased bone formation in the mouse and the effect was independent of increased vascularization but was reversed upon reduction of glycolysis by DCA (Regan, 2014) (Fig. 2). Similarly, increased stabilization of Hif1a by deletion of Vhl markedly increased bone formation in the mouse (Dirckx et al., 2018). More recently, nitric oxide signaling has been shown to stimulate bone anabolism through activation of aerobic glycolysis in osteoblast (Jin et al., 2021). Insulin, an anabolic hormone, has been shown to increase glucose uptake not only in osteoblast cultures, but also in murine bones according to PET/CT imaging in vivo (Zoch et al., 2016; Hahn et al., 1988). However, the precise effects of insulin on the metabolic fates of glucose in osteoblasts remain to be elucidated. Overall, emerging evidence supports that increased bone anabolism is coupled with activation of glycolysis in osteoblast-lineage cells.

Glucose metabolism is regulated by developmental signals during osteoblast differentiation. In an in vitro osteoblast differentiation model, Wnt signaling through the Lrp5 co-receptor has been shown to stimulate aerobic glycolysis acutely through upregulation of key glycolytic enzymes downstream of mTORC2 and Akt activation (Esen et al., 2013) (Fig. 2). Importantly, in a mouse model where overexpression of Wnt7b in osteoblasts caused excessive bone formation, concurrent deletion of Glut1 did not impede normal bone accrual but eliminated the bone anabolic activity of Wnt7b, thus supporting the functional relevance of increased glycolysis to Wnt-induced bone formation (Chen et al., 2019).

More recently, Lrp4 has been reported to increase aerobic glycolysis and osteoblast differentiation through Wnt-β-catenin signaling (Yang et al., 2021). In support of the role of mTORC2, deletion of Rictor, an obligatory component of mTORC2, in the osteogenic mesenchyme blunted the bone anabolic effect of an anti-sclerostin therapy designed to boost Wnt signaling in postnatal mice (Chen et al., 2014a; Sun et al., 2016). Hedgehog (Hh) signaling has been shown to function upstream of Wnt signaling in promoting osteoblast differentiation during embryogenesis and in postnatal growing mice (Hu et al., 2005; Shi et al., 2017). As Hh signaling induces Igf2 expression to activate mTORC2 in osteogenic progenitors, it will be of interest to examine potential metabolic regulation by Hh during osteoblast differentiation (Shi et al., 2015). Contrary to Hh and Wnt signaling, Notch restricts osteoblast differentiation from the mesenchymal progenitors during embryonic development and postnatal growth (Hilton et al., 2008). Accordingly, Notch signaling via the canonical Rbpjk-dependent pathway suppresses glycolysis in the mesenchymal progenitors (Lee and Long, 2018) (Fig. 2). Whereas Notch2 deletion caused bone overgrowth associated with increased glycolysis, pharmacological reduction of glycolysis abrogated the excessive bone formation. Thus, developmental signals regulate osteoblast differentiation partly through the reprogramming of cellular glucose metabolism.

4. Glucose metabolism in osteoclasts

Osteoclast differentiation and function have been shown to depend critically on mitochondrial respiration. Rankl, a critical inducer of osteoclastogenesis, stimulates mitochondrial biogenesis both through upregulation of the Pparg coactivator Ppargc1b and via alternative NF-kb signaling in the macrophage precursors (Ishii et al., 2009; Zeng et al., 2015). In addition, Asxl2, an epigenetic regulator important of histone modifications, appears to act upstream of Pparg1b to control mitochondrial respiration. Rankl, a critical inducer of osteoclastogenesis, stimulates mitochondrial biogenesis during osteoclastogenesis (Izawa et al., 2015). In addition, Asxl2, an epigenetic regulator important of histone modifications, appears to act upstream of Pparg1b to control mitochondrial respiration. Rankl, a critical inducer of osteoclastogenesis, stimulates mitochondrial biogenesis during osteoclastogenesis (Izawa et al., 2015). In addition, Asxl2, an epigenetic regulator important of histone modifications, appears to act upstream of Pparg1b to control mitochondrial respiration. Rankl, a critical inducer of osteoclastogenesis, stimulates mitochondrial biogenesis during osteoclastogenesis (Izawa et al., 2015).

![Fig. 2. Regulation of glucose metabolism by oxygen levels and growth factors. Yellow oval denotes nucleus and grey oval denotes mitochondria. Grey arrows depict gene transcription in the nucleus. Effector genes or proteins are in green. NICD: Notch intracellular domain. Black solid lines with arrowheads indicate activation and those with flat heads denote inhibition.](image-url)
intracellular ATP levels and accelerated osteoclast apoptosis (Miyazaki et al., 2012). The studies therefore support mitochondrial biogenesis as an integral component of the osteoclastogenic program, and that mitochondrial respiration is necessary for the resorptive function of osteoclasts.

Glucose is a major energy substrate for osteoclasts. Original evidence from chicken osteoclasts indicates that glucose instead of fatty acids or ketone bodies is the principle energy source fueling the resorptive activity (Williams et al., 1997). Glucose also stimulates both protein and mRNA levels of the vacuolar-type H+ -ATPases (V-ATPase) essential for the resorptive activity of the chicken osteoclasts (Larsen et al., 2002). Studies of murine osteoclasts differentiated from bone marrow precursors also support glucose as a critical substrate necessary for resorption (Taubmann et al., 2020; Indo et al., 2013). Likewise, human osteoclasts rely on glycolysis for their resorptive activity, as reducing the rate of glycolysis with galactose impairs collagen I degradation whereas suppression of the mitochondrial complex I with a non-cytotoxic dose of rotenone enhances osteoclast activity (Lamia et al., 2016).

The role of glucose metabolism in osteoclast differentiation is beginning to be unraveled. Both aerobic glycolysis andOXPHOS increase during murine osteoclast differentiation from bone marrow macrophages (Li et al., 2020; Ahn et al., 2016). An earlier study reported that omission of glucose in the culture media or knockdown of Hif1a did not impair osteoclast differentiation in vitro, but glycolysis could not be ruled out in those experiments as glycolgen could be used and the dependency on Hif1a might be limited in cells cultured with atmospheric oxygen (Indo et al., 2013). In support of a role for glycolysis, in vitro knockdown of lactate dehydrogenase Ldha or Ldhb in osteoclast progenitors suppressed osteoclast differentiation, even though mitochondrial respiration was also suppressed in that setting (Ahn et al., 2016).

On the other hand, deletion of Glut1 in osteoclast progenitors, diminished aerobic glycolysis without compromising OXPHOS, but nonetheless impaired osteoclast differentiation in vitro, thus further supporting the role of aerobic glycolysis (Li et al., 2020). Remarkably, the loss of Glut1 led to osteopetrosis thanks to fewer osteoclasts only in the female but not male mice (Li et al., 2016). The sexual dimorphism in Glut1 dependence of osteoclastogenesis in vivo is not understood at present and warrants future studies.

5. Interplay between bone and whole-body metabolism

The large bioenergetic needs of the skeleton have prompted interests in its potential role in systemic metabolism. Osteocalcin, a small non-collagenous bone matrix protein, has been proposed to function, in the uncarboxylated form, as a hormone to promote β-cell proliferation and insulin production to control glucose homeostasis (Lee et al., 2007). Furthermore, the hormonal osteocalcin engages in a feedforward loop wherein insulin signaling in osteoblasts promotes both the production of osteocalcin and the release of its bioactive form via bone resorption (Ferron et al., 2010; Fulzele et al., 2010). However, the model has been challenged by others who have reported no such hormonal function for osteocalcin in their independently produced osteocalcin-knockout mice (Diegel et al., 2020; Morishii et al., 2020). Thus, the physiological relevance of osteocalcin to glucose homeostasis remains uncertain at present, and further studies are necessary to resolve the controversy.

Beyond the potential endocrine function of bone, evidence has emerged that glucose consumption by bone could impact systemic glucose homeostasis. Deletion of Vhl in osteoblast lineage cells with OssCre not only increased glucose uptake and glycolysis in bone but also improved overall glucose handling in the mouse (Dirckx et al., 2018). Importantly, administration of DCA, a chemical inhibitor of glycolysis, normalized global glucose metabolism in the mutant mouse. However, as the treatment did not diminish the excessive bone accrual associated with heightened glycolysis, it remained uncertain whether the hypermetabolic osteoblasts were directly responsible for the systemic effect (Dirckx et al., 2018). In addition, deletion of Glut4 in osteoblasts with osteocalcin-Cre (Oc-Cre) led to hyperinsulinemia and impaired glucose clearance in the mouse (Li et al., 2016). However, the underlying mechanism was unclear as the deletion did not affect bone acquisition in the mutant mouse. Overall, additional studies are needed to establish a direct contribution of glucose disposal by bone to the control of global glucose homeostasis.

6. Future perspectives

The past ten years have yielded unprecedented insights into cellular metabolism of the skeletal cell types. While the present review focuses on chondrocytes, osteoblasts and osteoclasts, progress has also been made with marrow adipocytes and skeletal stem cells (van Gastel and Carmeliet, 2021). The detailed metabolic studies to date however are mostly limited to cell cultures so the results may not faithfully reflect the physiological metabolic profiles in vivo. Although recent studies have demonstrated the utility of in vivo stable isotope tracing in assessing the metabolic fates of labeled substrates in skeletal tissues, the method does not provide spatial resolution or reveal potential differences across different cell types within the tissue (Lee et al., 2020). The spatial resolution is particularly relevant to studies of cartilage which is typically avascular and relies on the diffusion of oxygen and nutrients through the matrix to sustain cellular metabolism. The uneven access to oxygen and the energy substrates is likely to impact the metabolic profiles of chondrocytes depending on their location (e.g., interior versus periphery) within the cartilage tissue. It follows then that genetic manipulations of a certain metabolic gene may not elicit the same metabolic response in all chondrocytes in a cartilage element. Technological advances such as those in single-cell metabolic profiling and nano-scale metabolic imaging will help meet the challenges in the field (Hartmann et al., 2021; Narendra and Steinhauser, 2020).

Despite their common reliance on glucose, articular versus growth plate chondrocytes are likely to exhibit differences in allocation of the substrate towards the various metabolic pathways. In contrast to growth plate chondrocytes that are destined to progress through proliferation and maturation before being replaced by bone, mature articular chondrocytes rarely proliferate but rather maintain their stable cellularity in a highly organized extracellular matrix throughout adulthood unless degenerative diseases strike (Goldring, 2012). However, the precise differences in glucose handling between the two types of chondrocytes remain to be elucidated.

It remains unclear at present why osteoblasts favor aerobic glycolysis even though it is far less efficient than OXPHOS in producing ATP from glucose. Aerobic glycolysis may be necessary in order for osteoblasts to generate sufficient intermediate metabolites to support biosynthesis but the specific needs remain to be determined. Glycolytic changes during osteoblast differentiation could alter the levels of enzymatic cofactors or substrates for epigenetic regulation of gene expression. For instance, increased aerobic glycolysis in response to Wnt signaling has been shown to reduce nuclear levels of both citrate and acetyl-CoA, leading to large scale decrease in histone acetylation and suppression of adipogenic and chondrogenic fates in mesenchymal progenitors (Kerner et al., 2016). More recently, aerobic glycolysis has been linked with increased histone lactylation during osteoblast differentiation in vitro (Nian et al., 2022). Finally, as mitochondria have historically been implicated in biological mineralization it will be of interest to explore the potential relationship between the mineralizing activity and reduced mitochondrial respiration in mature osteoblasts (Lehninger, 1977). In this scenario, aerobic glycolysis in osteoblasts would be an adaptive response to diminished OXPHOS coupled with mineralization, but future studies are necessary to test the hypothesis.

The role of energy metabolism in the skeleton and that of the whole body represents an exciting direction for future research. Most studies in the area to date rely on genetic tools that are believed to target skeletal-specific cell types such as osteoblasts. However, an increasing number of commonly used Cre lines have been shown to target unintended cell
types, including bone marrow stromal cells (BMSC) by Oss-Cre and Oc-Cre, marrow adipocytes by Osx-Cre, skeletal muscle by Dmp1-Cre and subcutaneous adipocytes by Prxl-Cre (Chen et al., 2014b; Zhang and Link, 2016; Lim et al., 2016; Sanchez-Gurmaches et al., 2015). Therefore, caution needs to be exercised in interpreting the global metabolic phenotypes based on the targeting specificity of mouse Cre lines.

**Declaration of competing interest**

None declared under financial, general, and institutional competing interests.

**Data availability**

No data was used for the research described in the article.

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