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The Emerging Role of Glucose Metabolism in Cartilage Development

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Abstract

Purpose of Review—Proper cartilage development is critical to bone formation during endochondral ossification. This review highlights the current understanding of various aspects of glucose metabolism in chondrocytes during cartilage development.

Recent Findings—Recent studies indicate that chondrocytes transdifferentiate into osteoblasts and bone marrow stromal cells during endochondral ossification. In cartilage development, signaling molecules, including IGF2 and BMP2, tightly control glucose uptake and utilization in a stage-specific manner. Perturbation of glucose metabolism alters the course of chondrocyte maturation, suggesting a key role for glucose metabolism during endochondral ossification.

Summary—During prenatal and postnatal growth, chondrocytes experience bursts of nutrient availability and energy expenditure, which demand sophisticated control of the glucose-dependent processes of cartilage matrix production, cell proliferation, and hypertrophy. Investigating the regulation of glucose metabolism may therefore lead to a unifying mechanism for signaling events in cartilage development and provide insight into causes of skeletal growth abnormalities.

Keywords

Cartilage development; Glucose transporters; Glycolysis; Oxidative phosphorylation; Pentose phosphate pathway; Glycogen

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Conflict of Interest Judith M. Hollander and Li Zeng declare that they have no conflict of interest.

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Endochondral Ossification Is a Continuous Process of Cartilage and Bone Formation

The skeleton of vertebrates plays a critical role in supporting the body and facilitating movement. Most bones, including long bones and the irregular vertebral bones, form by the process of endochondral ossification, in which a cartilage template (or anlage) is replaced by mineralized bone tissue [1–3]. Within the anlage lie chondrocytes at different developmental stages (Fig. 1A). At the end of the bone, or the epiphysis, is the resting zone, which provides a reserve of cells for proliferation and differentiation [4, 5]. Proliferating chondrocytes form distinct columns along the long axis of the bone and express the master transcriptional regulator, *Sox9*, as well as the signature cartilage matrix proteins aggrecan and collagen II. As these cells exit the cell cycle, they enter the prehypertrophic phase. This is quickly followed by hypertrophy, during which chondrocytes increase in size and express another key transcription factor (*Runx2*) and the cartilage matrix protein collagen X.

For many years, it was believed that chondrocytes die at the end of the hypertrophic stage [6, 7]. However, recent lineage tracing studies demonstrate that an estimated 20% to 80% of chondrocytes actually transdifferentiate into osteoblasts and osteocytes as part of a single, continuous developmental process in both the long bone and the mandibular condyle [4, 7–13]. Furthermore, these hypertrophic chondrocytes can also differentiate into marrow stromal and reticular cells, but not into bone marrow adipocytes [4]. This transdifferentiation process is additionally replicated in the fracture callus and in cartilage grafts, where bone formation in the recipient was contributed by cells derived from the donor graft [7, 11, 14]. Other studies have shown both bone morphogenetic protein receptor 1a (*BMPR1a*) and β -catenin to be critical for hypertrophic chondrocyte contribution to bone formation [8, 15]. Ablation of β -catenin function in collagen X-positive hypertrophic chondrocytes in mice directly decreased the number of chondrocyte-derived osteoblasts, while increased activity increased osteoblastic cells and decreased osteoclast number [15]. It is interesting to note that hypertrophic chondrocytes during fracture healing express the well-known pluripotency genes *Sox2*, *Oct4*, and *Nanog*, and that conditional knockout of *Sox2* prevents the transition of chondrocytes to bone cells, suggesting a progenitor-like intermediate between chondrocytes and osteoblasts [14, 16, 17]. With the transition of hypertrophic cells to osteoblasts, the cartilage of the hypertrophic zone calcifies and is subsequently destroyed, giving rise to mineralized bone matrix. This occurs first at the primary ossification center in the middle (diaphysis) of the anlage. Shortly after birth, a secondary ossification center forms at the epiphysis, thus restricting developing chondrocytes between two ossification centers.

Because of the pivotal roles of proliferation and hypertrophy in expanding the cartilage template, and the transdifferentiation of hypertrophic chondrocytes into osteoblasts and marrow stromal cells, the control of the pace of chondrocyte maturation ensures properly sized anlagen and sufficient progenitors for bone formation. Dysregulation of this process therefore leads to smaller templates and short stature, which is often associated with lower bone density and abnormal adult bone structure [18].

Multiple Signaling Pathways Regulate Cartilage Development

In developing cartilage, PTHrP and IHH function as a signaling loop, whereby chondrocytes and perichondrial cells at the end of the bone produce PTHrP to promote proliferation [19, 20]. Most recently, certain PTHrP-positive cells in the resting zone were found to be stem cells for growth plate chondrocytes, osteoblasts, and bone marrow stromal cells [4]. As chondrocytes proliferate and distance themselves from the PTHrP signal, they become prehypertrophic and produce IHH. In turn, IHH induces PTHrP expression, which feeds back into the proliferating zone to delay hypertrophy and maintain sufficient numbers of chondrocytes to lengthen the cartilage template [19, 21, 22]. IHH simultaneously promotes full hypertrophic differentiation of prehypertrophic chondrocytes in a PTHrP-independent manner [23].

In addition to PTHrP and IHH, other signaling molecules regulate bone development. BMPs, for example, directly regulate the PTHrP-IHH signaling loop by enhancing expression of IHH [24]. FGF2, on the other hand, acts upstream of IHH and negatively regulates chondrocyte proliferation and BMP activity [25]. Wnt family members also regulate the pace of cartilage development [26–29]. While the canonical Wnt pathway can inhibit the entire endochondral ossification process, the non-canonical pathways vary in that they can both promote hypertrophy and inhibit it [29–31]. Another molecule, transforming growth factor β (TGF β), acts in a PTHrP-dependent manner to inhibit hypertrophy, but in a PTHrP-independent manner to inhibit cartilage mineralization [32]. Insulin-like growth factor 1 (IGF1) is known to mediate the effects of growth hormone and to promote chondrocyte hypertrophy in parallel to the IHH pathway, while IGF2 was found to inhibit hypertrophy [33, 34]. The effect of IGFs may also be influenced by IGF-binding proteins [35, 36]. Additionally, biomechanical factors are known to regulate cartilage growth [37, 38]. The signals regulating chondrogenic differentiation are thus interconnected and highly complex (Fig. 1A) [39].

Despite the identification of these signaling events, it is still unclear how they control basic metabolism in chondrocytes to change cell behavior. During the long period of cartilage development from embryo to adolescence, chondrocytes experience bursts of both nutrient availability and energy expenditure, which demand sophisticated usage and control of resources. This important aspect of cartilage development is still largely elusive. The aim of this review is therefore to discuss the emerging role and regulation of glucose metabolism in growth plate development.

Overview of Glucose Metabolism

Cellular glucose metabolism initiates with the transport of glucose into cells (Fig. 1B). In the first committed step of metabolism, hexokinase uses adenosine triphosphate (ATP) to add a phosphate to glucose and form glucose 6-phosphate (G6P), which can enter a number of pathways. The vast majority of glucose will enter glycolysis to form pyruvate, producing 2 molecules of nicotinamide adenine dinucleotide (NAD⁺, NADH in its reduced form) and a net gain of 2 ATP. Pyruvate is either converted to lactate to regenerate NAD⁺ and complete the process of glycolysis or is shuttled into the mitochondria and converted into acetyl-CoA.

Each acetyl-CoA that enters the citric acid (TCA) cycle produces 3 molecules of NADH and 1 molecule of flavin adenine dinucleotide (FAD, FADH₂ in its reduced form). NADH and FADH₂ are oxidized by the electron transport chain (ETC) to NAD⁺ and FAD. The complexes (complexes I to IV) of the ETC use the energy of these molecules as they are transferred to pump protons from the mitochondrial matrix into the intermembrane space, generating a proton gradient. ATP synthase (complex V) uses the energy of protons traveling down that gradient and back into the mitochondrial matrix to produce ATP [40]. Oxidative phosphorylation gives a net yield of about 36 molecules of ATP per molecule of glucose. In addition to glycolysis and oxidative phosphorylation, some glucose may be converted to glycogen, for storage, and some may enter the pentose phosphate pathway (PPP), to make nucleotides (Fig. 1B) [40].

Glycolysis is the preferred method for ATP production by cells under hypoxic conditions, and thus is traditionally called anaerobic glycolysis. However, it is widely known that certain types of cells, such as cancer cells, use glycolysis even when there is an abundance of oxygen (the “Warburg effect” or “aerobic glycolysis”) [41]. The lack of blood vessels and low oxygen tension in the tissue environment, coupled with the high energy demand for production of glucose-derived matrix glycosaminoglycans and the difference in energy requirements for proliferation and hypertrophy, constitute a unique challenge for developing cartilage in balancing various aspects of glucose metabolism.

Glucose Uptake

Because cartilage is avascular, glucose diffuses into the growth plate via capillaries derived from the epiphyseal artery, located outside the cartilage template [42]. In mammalian cells, glucose uptake is dependent on facilitative glucose transporters (GLUTs), which allow for passive diffusion across the cell membrane, and sodium-coupled glucose transporters (SGLTs), which transport Na⁺ and glucose across the membrane using an electrochemical gradient. There are many GLUT family members, but not all transport glucose and not all are expressed in the developing cartilage. Among the GLUTs that are expressed in the developing cartilage are GLUTs 1–4, which transport glucose, and GLUTs 5 and 9, which transport fructose and urate, respectively [43••, 44, 45]. For a comprehensive review of the GLUT family members, please refer to [44]. As no members of the SGLT family have been reported to be expressed in growing cartilage, this review will focus on GLUT family members expressed in the growth plate.

GLUT1 is considered insulin-insensitive and responsible for basal glucose uptake. In the growth plate, it appears mainly in prehypertrophic chondrocytes and the upper hypertrophic zone. GLUT2 is a low-affinity transporter, while GLUT3 is a high-affinity transporter [46, 47]. Both GLUT2 and GLUT3 are expressed in the hypertrophic zone of the rat growth plate at postnatal day 7 (P7), but disappear by P28 [43••]. GLUT4 also has a high affinity for glucose, but unlike the GLUTs previously mentioned, it is responsive to insulin. It has been widely characterized in insulin-responsive tissues such as muscle and fat, but is now also known to be present in hypertrophic chondrocytes [43••]. GLUT4 mRNA expression is interestingly different: it is more strongly expressed in proliferating chondrocytes than in hypertrophic chondrocytes [48•]. The amount of glucose consumed in each zone of cartilage

has not been conclusively determined. Results from a short-term experiment with radiolabeled glucose indicated that proliferating chondrocytes took in less glucose than hypertrophic chondrocytes [43••]. Another study, however, showed that proliferating chondrocytes consumed more glucose than hypertrophic chondrocytes over time [49••]. Despite these opposite findings, studies thus far suggest that GLUT expression and glucose uptake are tightly controlled and dynamic during cartilage development (Table 1).

Both insulin and the IGFs play important roles in regulating glucose uptake and chondrocyte differentiation [50–52]. Using the acute diabetes mouse model of streptozotocin (STZ)-induced insulin deficiency, Maor and Karnieli showed that GLUT4, but not GLUT1, was significantly downregulated in the growth plate in this model, confirming that GLUT4 is insulin-responsive in chondrocytes [48•]. As expected, STZ-treated mice are smaller than their untreated counterparts. It is interesting to note that, although the insulin receptor (IR) is clearly expressed during proliferation, cartilage-specific knockout of this receptor did not lead to shorter bones, suggesting that insulin may also affect cartilage development indirectly [53]. Since IGF1 receptor (IGF1R) expression is induced in IR knockout mice, it is possible that some effects of insulin are mediated through the IGF1 signaling pathway [53].

IGF1 signaling functions primarily through IGF1R, and to a lesser extent, through IR [54, 55]. Knockout of IGF1R resulted in a shortened cartilage template, with a smaller hypertrophic zone and reduced GLUT4 expression, suggesting that IGF1R signaling is required for both hypertrophy and GLUT4 expression [56•]. Whether IGF1R KO affects glucose uptake in chondrocytes is not known, but this was the case in osteoblasts [50, 57]. IGF1 appears to affect glucose uptake in epiphyseal chondrocytes in a concentration-dependent way: in one study, IGF1 promoted glucose uptake only at a high concentration of 100 ng/mL, but in another study, it failed to promote glucose uptake at an even higher concentration of 1 µg/mL [58, 59]. IGF2 is also known to act through IGF1R to promote body growth, but can also bind to IR and IGF2R, though IGF2R is thought to act as a decoy receptor to sequester IGF2 [60–64].

Our laboratory has shown that the hypertrophic zone of *Igf2* null bones is larger in proportion to total bone length, suggesting a very different mechanism of action than that of IGF1 [34••]. Interestingly, despite the small size of the mice, *Igf2* null epiphyseal chondrocytes displayed increased glucose consumption [34••]. The results of our study and the STZ study by Maor and Karnieli imply that cartilage growth is negatively impacted by both too much and too little glucose consumption in chondrocytes. Although it is still not clear whether IGF2 regulates GLUT expression in this context, a recent study by Lee et al. revealed that BMP signaling could regulate GLUT1 expression [65••]. Additionally, *Glut1* null bones exhibited growth arrest and disorganization of the proliferating and hypertrophic zones, indicating an important role for GLUT1-mediated glucose metabolism in cartilage development [65••].

As the cartilage template grows, its center becomes increasingly hypoxic. This environment induces expression of hypoxia-inducible factor 1α (HIF1α), an important regulator of cartilage development [66, 67]. HIF1α knockout studies have shown that the expression of

this factor is required for *Glut1* expression, thus it will be interesting to examine how HIF1 α regulates glucose uptake [68]. Hypoxia may also induce expression of the glucose sensor AMP-activated kinase (AMPK) [69]. AMPK has been found to promote glucose uptake in muscle and inhibit catabolic events and osteoarthritis in articular chondrocytes [70–72]. However, it is still unclear whether AMPK is required for growth plate chondrocytes to regulate glucose uptake and whether it is controlled by IGFs or other signaling molecules in this process.

Glycolysis and Oxidative Phosphorylation

To determine whether chondrocytes use glycolysis or oxidative phosphorylation for ATP production, Rajpurohit et al. utilized the uncoupling reagent 2,4-dinitrophenol (DNP) [49••]. Uncoupling reagents separate electron transport from ATP synthesis, thus abolishing ATP production derived from oxidative phosphorylation [73]. When skin fibroblasts, a type of cell that primarily rely on oxidative phosphorylation for ATP, were treated with DNP, they exhibited the typical switch to glycolysis and lactate production [49••]. This caused an overall reduction in ATP, because glycolysis produces less ATP than oxidative phosphorylation [49••]. However, in epiphyseal chondrocytes, DNP did not increase lactate levels, a proxy measure of glycolysis, suggesting glycolysis already takes place at a high rate. Interestingly, DNP did substantially reduce ATP production, more so in embryonic chondrocytes than epiphyseal chondrocytes, suggesting that these chondrocytes still rely on oxidative phosphorylation to some extent for energy. Compared to embryonic chondrocytes, however, postnatal growth plate chondrocytes had lower mitochondrial function and were less dependent on oxidative phosphorylation for ATP production [49••].

Two shuttles facilitate transport of the electrons associated with NADH in the cytoplasm to the mitochondria, since NADH cannot diffuse across the mitochondrial membrane (Fig. 1B). The first is the malate-aspartate shuttle, in which malate is converted to oxaloacetate with electrons from NADH. Oxaloacetate crosses the mitochondrial membrane and is converted to aspartate, releasing the electrons to reform NADH [40]. In the second shuttle, the glycerol phosphate shuttle, the interconversion of glycerol-3-phosphate (Gly3P) and dihydroxyacetone phosphate (DHAP) by glycerol phosphate dehydrogenase, allows electron transport from cytoplasmic NADH into the mitochondria [40]. Brighton et al. discovered, strikingly, that glycerol phosphate dehydrogenase is inactive in chondrocytes, suggesting that chondrocytes may only depend on the malate-aspartate shuttle for this process, a notion that has yet to be verified [74].

Although epiphyseal chondrocytes as a whole rely heavily on glycolysis, they differ in glucose usage between zones of the growth plate. Using cartilage slices, Rajpurohit et al. found a lower lactate to glucose consumption ratio, and lower NADH production, in cartilage slices comprising proliferative chondrocytes than those comprising hypertrophic chondrocytes; furthermore, treatment with DNP significantly reduced ATP production in proliferating chondrocytes, but not in hypertrophic chondrocytes [49••]. These data suggest that proliferating chondrocytes partially use oxidative phosphorylation for their energy production, consistent with their higher mitochondrial membrane potential, and that hypertrophic chondrocytes are “uncoupled” from mitochondria [49••]. In fact, hypertrophic

chondrocytes express higher levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and lactate dehydrogenase (LDH), enzymes used in glycolysis and lactate production, as well as β -hydroxyacyl-CoA dehydrogenase (HOAD), the enzyme that converts fatty acids into acetyl-CoA to feed into the TCA cycle (Table 1) [75, 76]. The lower mitochondrial membrane potential in hypertrophic chondrocytes could be mediated by HIF1 α , which promotes glycolysis and inhibits oxidative phosphorylation via uncoupling proteins (UCPs) [67, 77]. This may also be related to the increase in inorganic phosphate associated with increased levels of alkaline phosphatase upon hypertrophy, as inorganic phosphate has been found to reduce mitochondrial activity through the ERK pathway [49, 78–80]. Since calcium efflux from mitochondria is upregulated by low pH, heightened glycolysis may be a way to prepare for calcification after hypertrophy [81, 82]. Whether low pH is the driving force for calcification remains to be determined, but this stage is certainly critical for subsequent bone formation, as osteoblasts are now known to derive from hypertrophic chondrocytes [4, 9–12].

Is ATP generation the sole function of mitochondria in chondrocytes? A key TCA cycle enzyme, succinate dehydrogenase (SDH), which converts succinate to fumarate, is highly active; in fact, it is more active in hypertrophic chondrocytes, implicating the presence of active mitochondria even though these cells do not use them for ATP production [76]. Mitochondrial uncoupling has been shown to reduce lactate production in hypertrophic chondrocytes, suggesting that glycolysis somehow relies on the mitochondria to achieve its full activity [49]. The answer to the above question is not clear. Mitochondrial activity may be required for redox balance in chondrocytes, perhaps via the malate-aspartate shuttle. In articular cartilage, which is also highly glycolytic, a similar phenomenon was found. Supplementation with oxidants (electron acceptors) was sufficient to elevate lactate production even under anoxic conditions when mitochondria could not undergo respiration, suggesting mitochondria in articular chondrocytes provide electron acceptors to sustain glycolysis [83, 84]. Whether this is also the case in growth plate chondrocytes remains to be investigated.

The balance between glycolysis and oxidative phosphorylation for ATP production is thus dynamic from embryonic to postnatal growth and from the proliferation phase to the hypertrophic phase. However, very little is known about the signals that control this intricate balance in growth plate chondrocytes. Recently, our group has discovered that IGF2 is a key regulator for redox balance in epiphyseal chondrocytes. We found that *Igf2* null chondrocytes exhibit increased glycolysis and oxidative phosphorylation, leading to increased reactive oxygen species (ROS) levels and ATP production [34]. We then used an advanced two photon fluorescence imaging technique that captures the endogenous fluorescence emitted by FAD and NADH with high resolution [85]. Since FAD and NADH represent the oxidized and reduced cellular states, and FADH₂ and NAD⁺ are not fluorescent, the optical redox ratio of FAD/(FAD+NADH) fluorescence levels reflects the redox state in cells. When there is an increase in glycolysis with respect to oxidative phosphorylation, there is less conversion of NADH to NAD⁺, resulting in a decreased optical redox ratio. Thus, this ratio has been used to assess the balance of glycolysis and oxidative phosphorylation [85–88]. Interestingly, two photon imaging analysis suggested that the loss of *Igf2* causes a shift from oxidative phosphorylation to glycolysis, which can be restored by

exogenous IGF2 treatment [34••]. Considering that *Igf2* null cartilage showed premature hypertrophy, our work is consistent with the prior study demonstrating a glycolytic shift in hypertrophic chondrocytes [34••, 49••].

Little is known about the potential role of other signaling pathways on the regulation of glycolysis and oxidative phosphorylation in growth plate chondrocytes. Recent analysis using the Seahorse metabolic analyzer showed BMP2 could enhance oxygen consumption in articular chondrocytes [89]. Furthermore, glucose depletion also enhanced oxygen consumption, consistent with the “Crabtree” effect [90]. While Wnt signaling has not been shown to regulate these processes in growth plate chondrocytes, Wnt3a is known to induce a number of glycolytic proteins and stimulate glycolysis in osteoblasts [91, 92]. The importance of glucose metabolism in controlling the pace of cartilage development has been demonstrated in *Igf2* knockout mice, in which a glycolysis inhibitor, 3-bromopyruvate (3-BrPA), was able to rescue premature chondrocyte hypertrophy and shortened bone phenotype in *Igf2* null bones [34••]. When taken in the context of results obtained in articular chondrocytes showing that AMPK and ATP-citrate lyase are essential for regulating matrix production, our study clearly demonstrates the importance of tight control of glucose metabolism to ensure normal cartilage development [34••, 93, 94].

Pentose Phosphate Shunt

The pentose phosphate pathway (PPP) is another pathway for glucose utilization. In this pathway, G6P is converted to ribose 5-phosphate while generating reduced nicotinamide adenine dinucleotide phosphate (NADPH) (Fig. 1B) [95]. In addition, multiple sugars that are shared with glycolysis (such as glyceraldehyde 3-phosphate and fructose 6-phosphate) are generated. No ATP is consumed or produced in this process. The PPP is especially important because ribose 5-phosphate is the backbone of nucleic acids, which are required for cell proliferation and replacement. Additionally, NADPH serves as a major electron donor to balance the redox ratio and reduce oxidative stress [50, 95]. In growth plate chondrocytes, it was found that about 20% of oxygen is actually consumed by the PPP [96•, 97]. Interestingly, a higher level of glucose 6-phosphate dehydrogenase (G6PD), the first enzyme that acts on G6P in this pathway, was found in hypertrophic chondrocytes, concomitant with a lower level of G6P, but higher NADPH in these cells (Table 1) [96•]. This suggests that usage of the PPP is regulated during cartilage development. Since proliferating chondrocytes require a high level of ribose backbone for nucleic acid production, the increased PPP usage in hypertrophic chondrocytes implies that it may serve another purpose, perhaps as an energy source to compensate for the lowered oxidative phosphorylation level. Insulin and calcium supplementation were found to induce ¹⁴C incorporation into the PPP and to decrease the activity of glycolysis. This demonstrates that they regulate the balance of the PPP and glycolysis, which may directly influence chondrocyte proliferation and hypertrophy during cartilage template expansion [97]. In the case of articular chondrocytes, nitric oxide was found to attenuate the PPP and promote oxidative injury [98]. Whether the balance of the PPP versus glycolysis and oxidative phosphorylation is shifted by oxidative stress or other regulatory factors in developing cartilage remains to be determined.

Glycogen

Glucose can be stored as glycogen, a multi-branched polymer that ensures availability of glucose on-demand [99, 100]. Glycogen formation begins with conversion of G6P to glucose 1-phosphate (G1P) by phosphoglucomutase. A series of enzymes, including the glycosyltransferase glycogenin, glycogen synthase, and glycogen branching enzyme, subsequently facilitate the production of a chain of 8–12 glucose residues [99]. A key regulatory step in glycogen synthesis is the control of glycogen synthase by glycogen synthase kinase 3 (GSK3). GSK has two isoforms, GSK3 α and GSK3 β , that can be inactivated by a reversible phosphorylation reaction [101]. A decrease in GSK3 phosphorylation therefore leads to an increase in GSK3 activity, which subsequently decreases glycogen synthase activity and glycogen synthesis. When additional glucose is needed, glycogen is broken down in a process called glycogenolysis, facilitated by glycogen phosphorylase (to regenerate G6P for glycolysis and the PPP) and by glucose 6-phosphatase (G6Pase) to generate free glucose [100, 102]. The level of glycogen is thus balanced by the control of glycogen synthesis and glycogen breakdown (Fig. 1B).

Glycogen presence in the growth plate has been established for over a century [102–105]. Electron microscopy and histological analyses indicated that glycogen particles are present in both the proliferating and hypertrophic zones, with greater aggregation in the hypertrophic zone [56•, 103–106]. GSK3 β was also found to be mainly in the prehypertrophic and hypertrophic zones, but GSK3 α was uniformly expressed in all zones of the growth plate (Table 1) [107]. Mice lacking *Igf1* exhibited less phosphorylated GSK3 β and less glycogen in the growth plate; furthermore, *Igf1R* null mice showed diminished glycogen stores, suggesting that IGF1 controls GSK3 β activity and glycogen levels in growth plate chondrocytes [56•, 108]. Our laboratory found that glycogen levels are also lower in *Igf2* null chondrocytes, particularly in the prehypertrophic zone [34••]. This coincided with diminished expression of the prehypertrophic marker IHH, as if *Igf2* null chondrocytes had prematurely entered the hypertrophic phase from the proliferating phase [34••]. Considering *Igf2* null chondrocytes exhibited overactive glucose uptake, glycolysis, and oxidative phosphorylation, it appears that the lack of IGF2 sacrifices glycogen synthesis in favor of using other glucose metabolism pathways. Therefore, IGF2 plays a pivotal role in balancing the various pathways of glucose utilization [34••].

Enzymes involved in glycogen breakdown are also expressed in chondrocytes [102]. Glycogen phosphorylase and G6Pase are both strongly expressed in the proliferating zone and upper hypertrophic zone, suggesting active glycogen metabolism at these stages [102].

The roles of glycogen synthesis and utilization in the growth plate are still unclear. Inhibition of phosphorylated GSK3 β increased the length of the proliferating zone and the bone as a whole, and decreased that of the hypertrophic zone [107]. However, cartilage-specific knockout of GSK3 β in vivo resulted in compensatory upregulation of GSK3 α and no skeletal phenotype [107]. Since GSK3 β is a key component of other signaling pathways, most notably the Wnt pathway, it cannot be concluded that GSK3 β affects cartilage growth solely through regulating glycogen synthesis [109]. Additional genetic manipulation of

components specific to glycogen metabolism is needed to reach a more definitive conclusion.

Conclusion

Mutations and dysregulation of genes controlling growth plate development are known to cause multiple skeletal growth abnormalities. For example, IGF1 deficiency is prevalent in children with short stature [110, 111]. *IGF2* mutation and insufficiency lead to growth restriction consistent with Silver-Russell Syndrome, whereas IGF2 overexpression results in Beckwith-Wiedemann Syndrome and gigantism [60, 112–115]. *FGFR3* mutations cause achondroplasia, a major form of dwarfism [116]. Aside from genetic skeletal diseases, growth abnormality is also common in children with Type I diabetes and in chronic inflammatory diseases like juvenile idiopathic arthritis (JIA) and cystic fibrosis [117–119]. Insulin, IGFs, and FGFs, as well as pro-inflammatory cytokines such as IL1, IL6, and TNF α , are all known to regulate glucose metabolism and growth plate cartilage development [50–52, 58, 59, 120–125]. Although glucose metabolism has not been assessed in many growth abnormalities, the incidence of hypoglycemia in Beckwith-Wiedemann syndrome was found to reach approximately 50% [126]. It is entirely possible that dysregulation of glucose metabolism is not only casually associated with the abnormalities of cartilage growth, but is also the driving force of these changes.

The investigation of glucose metabolism was initiated decades ago, with elegant and rich biochemical characterization of multiple aspects, including glucose uptake, glycolysis, oxidative phosphorylation, the pentose phosphate pathway, and glycogen synthesis and glycogenolysis. In the current era, metabolism is increasingly recognized as a central control for countless biological processes, but how glucose metabolic pathways control cartilage development is still largely unknown, and their regulation in chondrocytes remains poorly understood. This new dimension of study may lead to a unifying mechanism explaining the actions of the elaborate web of signaling molecules, and provide insights into designing treatment options for a variety of growth abnormalities.

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Table 1

Transporters and enzymes involved in glucose metabolism in developing cartilage

Process	Protein	Function	Protein expression		
			Resting	Proliferating	Hypertrophic
Glucose transport	GLUT1	Glucose transport ($K_M \sim 3$ mM)			++ (upper)
	GLUT2	Glucose transport ($K_M \sim 17$ mM)			++
	GLUT3	Glucose transport ($K_M \sim 1.5$ mM)			++
	GLUT4	Glucose transport ($K_M \sim 6.6$ mM) Insulin-responsive			++
Glycolysis	LDH	Pyruvate \rightarrow lactate NAD ⁺ \rightarrow NADH	+	++	+++
	GAPDH	Glyceraldehyde 3-phosphate \rightarrow 1,3-bisphosphoglycerate NAD ⁺ \rightarrow NADH	+	++	+++
Citric acid cycle	SDH	Succinate \rightarrow fumarate	+	++	++
Fatty acid oxidation	HOAD	Involved in converting fatty acids to acetyl-CoA NAD ⁺ \rightarrow NADH	+	++	+++
Pentose phosphate pathway	G6PD	G6P \rightarrow 6-phosphogluconolactone NADP ⁺ \rightarrow NADPH	+	++	+++
Glycogen	GS	Glucose \rightarrow glycogen		+	+
	GSK	Inactivates glycogen synthase	+	+	+
	Glycogen phosphorylase	Glycogen \rightarrow glucose	+	+	+
			(GSK α)	(GSK α)	(GSK α and β)
					+
					(upper)