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METABOLIC CONSIDERATION OF EPIPHYSEAL GROWTH: SURVIVAL RESPONSES IN A TAXING ENVIRONMENT

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Abstract

The goal of this review is to examine some of the metabolic features of the maturing chondrocyte within the epiphyseal growth plate. The energy status of the tissue is examined in light of the energy needs of the tissue and the availability of oxygen. The role of HIF, PHD's and other proteins concerned with transduction of the oxemic response is considered and related to chondrocyte survival in a complex extracellular matrix.

Keywords

chondrocyte; epiphyseal growth plate; metabolism; oxygen; HIF; PHD; mTOR; Akt; AMPK

Introduction to Energy Metabolism

The defining characteristic of life is the ability to extract energy from organic and even inorganic compounds. A range of strategies have been evolved that permit organisms to utilize the free energy contained within organic nutrients or reduced inorganic compounds. These strategies enhance organism survival in taxing environments: extremes of temperature, pH and ionic strength, and nutrient and oxygen (O₂) availability. In almost all cases, the blueprint is to trap the available energy by completely or partially oxidizing nutrients, commonly lipids and carbohydrates. Carbohydrates, such as glucose, can be partially oxidized by a highly regulated process, glycolysis, releasing a small proportion of the energy as ATP:

The glycolytic pathway has a very long evolutionary history. All human tissues can generate energy by this route. Of interest to the field of skeletal biology is the observation that in the growth plate, this ancient conserved system is used by chondrocytes to directly generate ATP (by substrate level phosphorylation) and to reduce NAD. The reduced form of NAD (NADH) serves to maintain the activity of the glycolytic pathway, and if the molecule gains access to the mitochondrion, it can be used to drive energy formation through the efficient cytochrome-dependent oxidative phosphorylation system (Fig 1).

In the presence of O₂, intermediates of the glycolytic pathway as well as lipids and amino acids can undergo oxidative metabolism. In the mitochondrion, these molecules are completely oxidized by the enzymes of the tricarboxylic (citric) acid (TCA) cycle and generate a large

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amount of useful energy (15 fold greater than glycolysis). In this latter case, the energy can be released as heat (a strategy employed by bears and hibernating animals to keep warm), or as energy-rich nucleotides (most commonly ATP, as well as other nucleoside phosphates):

Measures of Energy Status

A considerable number of chemical and thermodynamic measurements have been performed to evaluate the energetic status of cells and tissues. The most obvious approach is to determine the concentration of selected energy rich compounds such as NADH (the redox couple NAD/NADH); ATP (or more specifically the ratio of ATP/ADP and AMP); the generation of lactate (or the pyruvate/lactate ratio); and creatine phosphate (creatine phosphate/creatine). Another very useful determinant is the rate of O₂ consumption and the ratio of Pi/O₂ ratio. The actual concentration of O₂ in aqueous media is dependent on the partial pressure of O₂ in air (Henry's Law). As the O₂ content of air is about 21%, this is equivalent to 150 Torr (air pressure at 1 atmosphere = 760 Torr); in aqueous solution the concentration is 132mmol/L. In the lungs, the O₂ partial pressure is 100 Torr while in venous blood the value is closer to 15 Torr. Not surprisingly, in the mitochondrion (the major O₂ consuming organelle), the O₂ tension can be reduced to 0 Torr. The take home message here is that the O₂ tension value, a critical regulator of the energy state, is site and tissue specific.

In non-proliferating cells, the greater part of the free energy is used for maintenance of the activity of membrane pumps to preserve the ionic (osmotic) milieu of the cell, and of course to mediate biosynthetic reactions such as those required for generation of the extracellular matrix of bone and cartilage. In proliferating chondrocytes, much of the free energy yield is diverted to drive reactions required for cell division and the biosynthesis of cellular constituents.

Energy Metabolism in Cartilage

The force required for longitudinal growth of the axial skeleton is generated by cells contained within the epiphyseal cartilage. During periods of active growth, within a 24–48 hour period the chondrocytes progress from a proliferative state to terminal differentiation and subsequently death [1,2]. Outside these discontinuous periods of growth, the transit time of chondrocytes in the epiphysis is much slower resulting in a decreased rate of entry and deletion from the cartilage. Investigators have used many of the measurements discussed earlier to probe the metabolic state of cells in the rapidly growing tissues. Results of these studies have shown that the chondrocytes are glycolytic i.e. they generate 2 moles of ATP per mole of glucose. Since the energy yield is so low, the question is frequently raised: how can chondrocytes generate sufficient energy to meet their own housekeeping functions, while at the same time synthesize the proteins of the extracellular matrix and even initiate energy demanding reactions required for activation of their own death (apoptosis)? The answer is relatively simple. Compared with high efficiency mitochondrial oxidative phosphorylation, glycolytic activity is rapid and a small number of ATP molecules are generated at a very fast rate [3]. In the mammalian cell, in the trade-off between rate and yield, rate turns out to be the most critical value. Indeed, during the period of active growth, it is likely that the very high rate of generation (and delivery) of ATP from glycolysis, to energy consuming systems of the cell, is optimally adapted for the needs of the chondrocyte. The fact that these cells utilize glycolysis in place of more efficient oxidative phosphorylation may be an evolutionary adaptation for a limited fuel source. Within the dense cartilage matrix, and surrounded by rapidly developing bone and bone marrow, cells that can rapidly utilize and process nutrients have a biological advantage.

Oxygen Metabolism in the Growth Plate

It is important to consider the factors that “force” chondrocytes at specific developmental stages to generate energy through the glycolytic pathway. It should be acknowledged that a genetic program initially directs cells to generate energy through a specific metabolic pathway and synthesize its own local environment. These two activities are synchronous and provide maximal adaptation to a changing milieu. One defining micro-environmental factor is the rate of O₂ delivery by the vascular elements. While the vascular supply to the plate varies enormously between species, the plate is supplied with blood derived from the epiphyseal and metaphyseal vessels. These vessels are linked by interconnecting vessels that traverse the plate. For small molecules such as O₂, the rate of delivery appears to be similar, irrespective of the vascular route [4]. In the perichondrium, a ring vessel close to the hypertrophic zone anastomoses with both the metaphyseal and epiphyseal arteries [5]. Farnum and co-workers [4] have speculated that the ring vessel and its associated plexus may serve to regulate in a paracrine fashion the vasculature of the growth plate and even modify the PTHrP- Ihh negative feedback loop.

Since cartilage is almost avascular, and, compared with most other tissues, the rate of O₂ delivery is low, it is worthwhile asking the following questions. First, what is the O₂ tension in the growth plate and, how does the oxemic state regulate nutrient utilization? A second question concerns the influence of a specific metabolic state: how does the metabolism influence critical activities of the maturing chondrocyte?

There is now hard data on the O₂ tension in the growth plate. Earlier attempts to measure the pO₂ in the epiphysis were invasive and required that electrodes were inserted into the tissue. While there were limitations in the use of these devices (inability to define the topography of the region evaluated and the high rate of O₂ consumption of the electrode itself) the data indicated that the tissue O₂ tension was low [6]. More recently, our group and other workers used a derivative of metrinidazole, which is fragmented by nitroreductases, to provide a non-invasive read out of the O₂ tension in situ. These studies showed that the tension in cartilage was low and probably about 2–5% O₂ [7]. In an excellent study by Schipani et al [8] [9] it was confirmed that the tension in the plate was low. These workers revealed that there was an O₂ gradient in the growth plate with lowest levels of O₂ in chondrocytes of the core hypertrophic zone. The low pO₂ of this zone probably reflects the oxidative activity of surrounding cells as well as the anatomical location. Within the depth of the plate, the O₂ tension of these maturing growth plate cells would be expected to be lower than the more superficial proliferative chondrocytes or the deeper hypertrophic cells. The second factor influencing core cells oxidative activity is the metabolic activity of the surrounding chondrocytes. Although their O₂ requirements are low, these cells would act as a sink and utilize available O₂ for oxidative functions. In this way they would further deplete the tissue of available O₂. Noteworthy, it is likely that the low pO₂ serves as a break on other oxidative activities of the maturing chondrocyte. From this perspective, because of limitations in oxidative metabolism, chondrocytes in this region would be expected to be susceptible to genetic and environmental insults.

There are but a few studies of the oxemic characteristics of isolated epiphyseal chondrocytes, although a report by Haselgrove et al [9] indicated that the uptake was low and dependent in a non-linear fashion on the O₂ tension. This group noted that above 20 M, the consumption rate remained constant, about 4 nmoles/mg protein/min; the half maximal velocities were about 5.8 M for pre-hypertrophic cells and 0.8M for hypertrophic chondrocytes. Parallel studies with articular chondrocytes indicated that the O₂ consumption rate is an order of magnitude lower than growth plate cells [10]. Note, for both articular chondrocytes and epiphyseal cells, these values are well below the O₂ tension of inspired air.

Both the invasive as well as the non-invasive studies of the growth plate as well as studies of isolated chondrocytes lent strong support to the notion that cells in the growth plate were hypoxic^{*} and resided in a low O₂ environment. It is likely that an O₂ concentration gradient exists between the vascular elements and the cartilage, and between the cells and the O₂ consuming (mitochondrial) systems. Thus, gradients exist between cartilage and the surrounding tissues and within the cells themselves. However, the architecture of the gradients has not been clearly delineated. It is possible that lipid bilayers in membranes vesicles and lipoproteins may help to direct gradient formation[#]. Whatever the pathway, it is likely that the gradient is most marked in the core central region of the cartilage. Cells located at the calcification front (the chondro-osseous junction), which is closest to the metaphyseal blood vessels, would be at a higher oxemic state than the post-mitotic core chondrocytes.

Since chondrocytes are located in an avascular tissue, with possibly a paucity of glucose and other nutrients the question is raised, what are the energetic consequences of residing in such a microenvironment? There are a series of rules that govern these interactions. Of these, the most relevant is called the *Crabtree Effect*. In many tissues including cartilage, high glucose levels inhibit respiration (O₂ utilization). If there is a low level of O₂ delivery, cells decrease the rate of O₂ utilization (“conformity principle”). However, as the pO₂ falls, ATP generation through oxidative phosphorylation is decreased and functionally there is electron transport arrest. However, ATP continues to be generated by glycolysis (this is a modification of the well known *Pasteur Effect*). These adaptational responses are exhibited by most, but not all tissues: for example, brain cells do not accommodate to this change, and die if there is a sustained decrease in O₂ [11].

A second factor influencing energy metabolism is the nutrient concentration. Since almost all earlier studies have focused on glucose, we only discuss this nutrient, even though it is likely that lipids and amino acids are probably metabolized by chondrocytes. The Crabtree effect points to high levels of glucose inhibiting O₂ utilization. However, in reality, the low vascularity of cartilage and its high glycolytic rate are likely to work together to lower the actual amount of glucose available for energy metabolism^{*}.

In a study of articular chondrocytes Otte [12] published that there was severe depletion of glucose in articular cartilage. Likewise Cryer et al [13] reported that in the deep zone of articular cartilage the glucose concentration was 1 mM or less. In a recent study of articular chondrocytes Heywood et al [10] refined these observations by noting that O₂ consumption was regulated by the glucose concentration up to a concentration of 2.7 mM. Before ending this discussion, it is recognized that many of the studies mentioned above were performed in articular cartilage and the results are extrapolated to epiphyseal cartilage. It is argued here, that unlike articular cartilage, during growth, the endochondral cartilage is a highly cellular and functionally active tissue. From this perspective, the gradients in both O₂ and glucose are likely to be more extreme than those reported in articular cartilage.

^{*}Note the term hypoxia is often used to describe the oxemic state of growth plate cells. This term implies that the O₂ needs of the cell are not being met by the rate of O₂ delivery from the blood supply. Whether this is the case is debatable as the glycolytic chondrocytes require very little O₂.

[#]Chemical measurements have shown that O₂ is more soluble in hydrocarbons than in aqueous tissues fluid.

^{*}Whether these simple relationships explains the low level of O₂ utilization by chondrocytes is unlikely especially as the K_m of mitochondria for O₂ is very low (10⁻⁶–10⁻⁸M) i.e. even if the O₂ tension is very low it would be in excess of what is needed to promote oxidative phosphorylation.

Oxidative Metabolism of Articular Cartilage

To digress for a moment, articular cartilage resembles the growth plate in that there is a paucity of blood vessels, and gradients in oxygen supply have been reported [14]. Moreover, commonly enlarged (hypertrophic) cells are present in the avascular deep zone.

In osteoarthritis, the number of hypertrophic chondrocytes is elevated and there is evidence of increased apoptosis. Moreover, the induction of hypertrophy, a process that is functionally appropriate for the growth plate, where the increase in cell volume is a major cause of tissue growth, leads to development of an extracellular matrix in articular cartilage that is chemically and mechanically flawed.

Two metabolic factors may serve to disturb the normal function of articular chondrocytes. First, there is increased activity of the subchondral bone and fibrillation of the superficial and mid-zones of the articular cartilage. It would not be unreasonable to assume that cells buried in the cartilage receive nutrients and oxygen from the blood capillaries and vascular channels of the subchondral bone. From this perspective, any disruption in the bone vascular supply would impact on the viability and health of the cells of cartilage and possibly exacerbate development of disease. Thus, a chronic disruption of the metabolic state of subchondral osteoblasts could have a profound impact on the biosynthetic activities of the overlying chondrocytes.

The second type of change is linked to changes in metabolism and alterations in the osmotic pressure in the tissue due to loss of aggrecan. Early in the development of the osteoarthritic lesion, there is a marked change in aggrecan distribution. Since aggrecan provides cartilage with its osmotic properties, this physical change would influence the mechanical properties of the tissue. Moreover, the anaerobic generation of lactate maintains the tissue osmotic pressure. If there is complete oxidation of glucose to CO₂ and H₂O the tissue osmotic pressure would be disturbed and cell function would be degraded [15]. Hence, changes in chondrocyte metabolism, or possibly alterations in the chondrocyte osmotic pressure, would be expected to adversely influence cell function. In the growth plate, the low O₂ tension serves to enhance glycolysis and therefore the turgor pressure within the developing epiphysis is maintained.

Regulation of the Oxemic Response by PHD and HIF Proteins

If there are systemic problems with the conformity principle, the question remains how is the oxemic response sensed by the cell? Other questions that may also be asked include: how is the oxemic signal transduced; how does a change in signal modify cell behavior? These questions can now be answered thanks to a number of new discoveries by a small group of talented investigators. There is strong support for the hypothesis that chondrocytes contain one or more O₂ sensor proteins, PHDs (prolyl hydroxylases)*. Like their enzymatic relatives, the collagen prolyl hydroxylases, these enzymes are Fe²⁺ and oxoglutarate dependent dioxygenases. When activated, they enhance the hydroxylation of specific prolyl and asparagyl residues of the transcription protein, Hypoxia Inducible Factor (HIF) (Fig 2). It is this family of proteins that respond to the oxemic status and through their interaction with HIF regulate glycolysis as well as a number of related cellular activities [16–18]. Indeed, in an earlier study we showed the presence of PHD isoenzymes in isolated chondrocytes and the growth plate itself [19]. Noteworthy, normoxic conditions promoted the recognition of the proline-hydroxylated form of HIF by the von Hippel Lindau tumor suppressor protein (pVHL), a novel E3 ubiquitin ligase. Once bound, the complex is targeted for polyubiquitination and degradation by the proteasome [20]. Conversely, under hypoxic conditions, oxygen-sensitive

* A second group of sensor proteins (Factor Inhibiting HIF) have been reported by Semenza. There are currently no reports of their presence in cartilage.

prolyl-hydroxylase activity is reduced, pVHL-mediated ubiquitination is suppressed and HIF-1 α translocates into the nucleus. Within the nucleus it binds to HIF-1 β . This complex binds to specific hypoxic responsive elements (HRE), thereby initiating the transcription of specific target genes. Mice lacking pVHL in cartilage are viable, but grow more slowly than the wild type and develop a severe form of dwarfism. Chondrocyte proliferation within the VHL null growth plates is reduced and atypical large cells are present in the resting zone [21].

There are major difference between the two groups of prolyl hydroxylases - the PHDs and the collagen prolyl hydroxylases. Firstly, they are specific for their substrate protein (HIF versus collagen). Secondly, the K_m for O₂ is very different for the two groups of enzymes. While the K_m for the collagen enzyme for O₂ is 40 M, the K_m 's of the PHDs for O₂ is between 230–250 M, a value which is in the physiological range [16]. Based on the knowledge that the PHD enzyme system is sensitive to the O₂ status of the cell and hydroxylates prolyl residues on HIF, the question can be raised: does HIF activity regulate chondrocyte metabolism, and if so, how does the protein transduce the oxemic response? Studies by Semenza and others showed that HIF transcriptionally controls the activity of more than 200 genes, many of which are required for regulation of the glycolytic pathway [22,23]. In this way, a fall in the pO₂ would activate HIF and enhance anaerobic glycolysis; moreover as we will discuss later, HIF serves to inhibit mitochondrial activity, thereby decreasing the O₂ needs of the cell.

In an early study of the growth plate it was shown that HIF was highly expressed in hypertrophic chondrocytes. Studying mutants with conditionally inactivated HIF-1 α gene, Schipani et al [8] reported that mice exhibited a hypocellular hypertrophic zone, and the presence of apoptotic cells in both the proliferative and hypertrophic zones. In addition, there was an irregular and disorganized metaphysis.. While the mechanism for this effect has not been scrutinized, it is likely that deletion of this transcription factor prevents chondrocytes from adapting to the oxemic state of the tissue. Thus, many of the glycolytic genes remain inactivated and the cell is unable to promote glucose metabolism through the glycolytic pathway. Moreover, as HIF regulates the activity of genes linked to tissue oxygenation (VEGF) it is likely that it also influences vascularization of the underlying bone. As a result, there is disturbed function of cells at the chondro-osseous junction and a loss of normal tissue architecture. Accordingly, HIF activity plays a major role in chondrocyte metabolism and long bone growth

In a series of recent studies, it was shown that while HIF promotes glycolysis, it also regulates energy generation at the mitochondrial level. HIF inhibits the activity of pyruvate dehydrogenase the enzyme which converts pyruvate into acetyl CoA, a critical substrate for enzymes of the TCA cycle [24]. Since the activity of this enzyme is carefully controlled by stimulatory (Ca²⁺, insulin etc) and inhibitory (phosphorylation) reactions, it is regarded as a rate-limiting enzyme. When stabilized, HIF promotes activation of a kinase (pyruvate dehydrogenase kinase) that phosphorylates and inhibits pyruvate dehydrogenase. As the pyruvate concentration rises, it is converted into lactate by lactate dehydrogenase, another HIF regulated gene (see Fig 1). Lactate serves to re-generate NAD, thereby promoting glycolysis in the hypoxic state. Thus, the low O₂ supply to cartilage is sensed by one or more PHD's leading to stabilization of HIF. HIF then upregulates enzymes involved with glycolysis and down regulates O₂ consuming reactions at the mitochondrial level. Based on these exciting findings, PHD-HIF system must be viewed as a key regulator of chondrocyte metabolism.

Whether the PHD-HIF system can account for all of the metabolic events that govern chondrocyte maturation in the growth plate is unlikely. For example, constitutive activation of HIF-1 results in suppression of cell growth. If this same effect is exhibited by maturing chondrocytes, then the needs for hypertrophy could not be met. Thus, during hypertrophy when there is a substantial increase in the cell size, chondrocytes would not be able to maintain a

high level of membrane biogenesis. This latter activity depends on the synthesis of acetyl-CoA and an activated pyruvate dehydrogenase complex. HIF-dependent down regulation of mitochondrial activity and a decrease in metabolite flux into the mitochondrion would compromise these activities.

Other Sensors of Metabolic Stress

Since glucose is the major energetic fuel of the body, its utilization is very carefully conserved. Indeed, a glance at a standard text on biochemistry provides insight into the multitude of hormones, and autocrine and paracrine factors that regulate glucose transport into the cell. In this context, we have shown that HIF is a key intracellular regulator of glucose utilization by the maturing chondrocyte. Not surprisingly, a number of other mechanisms have evolved to regulate glucose utilization. For example, in some cells, the activity of the PI3K/Akt signaling pathway is dependent on glucose metabolism, and when stimulated there is a marked increase in the uptake of glucose [25]. If the stimulation is maintained, there is an accumulation of the end products of aerobic glycolysis, pyruvate, and NADH. While pyruvate is required to maintain the mitochondrial membrane potential, some can also be utilized for lipid (membrane) biogenesis and the maintenance of non-essential amino acid pool. Hence, activation of this signaling pathway provides a mechanism to permit chondrocyte maturation within a glycolytic environment.

In addition to promoting glucose transport and glycolysis, Akt activates another environmental sensor, mTOR. The mammalian target of rapamycin (mTOR) is a serine-threonine protein kinase that integrates inputs from multiple pathways: in addition, it serves a circumscribed role with respect to actin organization and membrane trafficking. From a metabolic viewpoint, it acts as a sensor of the nutrient (mainly leucine and other amino acids) and the energy and oxemic status of the cell. Thus, hypoxia and energy (ATP) depletion results in mTOR inhibition [26]. Although the mechanism of mTOR inhibition by hypoxia has not been clearly delineated, recent studies have shown that mTOR inhibition by hypoxia is mediated by a second hypoxia-inducible gene, *REDD-1*. In addition to hypoxic induction, *REDD-1* can be induced by either ATP depletion or direct activation of the AMP-activated protein kinase (AMPK). These studies have shown that *REDD-1* is a critical transducer of the cellular response to energy depletion and hypoxia through the mTOR pathway (Fig 3) [27–29].

The mechanism by which energy depletion inhibits mTOR involves activation of AMP-activated protein kinase (AMPK) [30]. This protein is a highly conserved energy sensor that is present in all eukaryotic cells. Since it is sensitive to the AMP level in the cell, its activity is dependent on the activity of a second enzyme, adenylate kinase which responds to the ADP concentration, resynthesizing ATP and generating AMP:

Changes in AMPK activities are tissue specific and dramatic. In some tissues there is a marked effect on glycolysis and glucose utilization, in others, there are changes in fatty acid synthesis, fatty acid oxidation and lipolysis. There is also some evidence that AMPK can effect protein synthesis via activation of elongation factor EF-2.

In summary, it is now becoming clear that aside from HIF-PHD system, there exists a number of biochemical sensor proteins which monitor microenvironmental signals. These metabolic sensors are geared to evaluating and responding to three main sets of stimuli: the energy status of the cell, nutrient availability and oxemic status. It is also becoming very clear that there is considerable crosstalk between each of these sensors and one or more major signal transduction pathways in the cell. Together, these proteins provide close scrutiny of intracellular activities

ensuring that metabolic behavior is carefully coordinated with protein synthetic activity, proliferative behavior and cell fate.

Metabolism, Oxemia and the Future Studies in Skeletal Biology

Currently, many of the most critical studies of the growth plate have used molecular genetic approaches for cataloging genes or group of genes that are concerned with expression of the chondrocytic phenotype. In addition, use of knockout, gene silencing and gain of function techniques has permitted investigators to probe the importance of a specific gene or gene complexes in regulating tissue development and function. Although these studies have provided much needed critical new insights into the genetic control of the maturation process, it is now clear that this information needs to be related to the functional status of the tissue. For this reason, it is once again necessary to open those large textbooks of biochemistry and turn once again to those sections that deal with the complexities of intermediary metabolism, regulation and energy conservation. It is only by relating biochemical understandings at the metabolic level to the emerging information at the gene level will it be possible to fully comprehend the functional status of tissues and thereby fully delineate complexities of the life process. In his book, Robert Hazen noted:

This comparative simple chemical cycle [the citric acid cycle] is an engine that can bootstrap all of biochemistry including the key molecules of genetics (*Genesis: the Scientific Quest for Life's Origins*, 2005 The National Academy of Sciences Press, pp192)

One final comment concerns the import of the findings discussed above and the way cartilage biologists perform cell-based studies. In the past few years, there has been recognition that it is necessary to mimic in culture those conditions that exist *in vivo*. Hence, chondrocyte cell culture is frequently performed in bioreactors using cells embedded in scaffolds, and maturation is activated with agents such as retinoids or BMP's. Only infrequently is attention directed at the pO₂ of the gas phase and the O₂ content of the medium. If we are to continue to explore how chondrocytes regulate their own function and elaborate a mineralizable matrix, then attention needs to be given to defining critical microenvironmental factors that influence chondrocyte function. The two that have been discussed in this review are the nutrient (glucose) content of the medium and the oxemic status of the tissue. It is clear that high glucose medium will impact the mechanism of energy generation and serve as a poor mimic of the external nutrient environment. Likewise, maintaining cartilage cells in an environment that is 130 Torr or more, completely defies logic. Moreover, since the response to hypoxia is mediated by HIF, the oxemic state can modify the expression of 200–300 genes that are transcriptionally regulated by this protein. Accordingly, to perform studies of cartilage in conditions that are optimum for alcohol formation may be important in beer fermentation (a worthy activity in its own right), but they obfuscate and frustrate our understanding of the birth, life and death of not just chondrocytes, but all skeletal tissues.

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Figure 1.

Schematic of the glycolytic pathway. Chondrocytes of the epiphyseal growth plate exist in a low O_2 microenvironment. Under these conditions, the cells generate ATP by glycolysis. Thus, glucose is converted to pyruvate, and under anaerobic conditions, this molecule is reduced to lactate. During the latter step, NADH generated during glycolysis is oxidized to NAD, an important co-enzyme for maintenance of the glycolytic pathway. Some pyruvate can be converted to acetyl CoA, by the enzyme pyruvate dehydrogenase (PDH); this molecule serves as an intermediate in the tricarboxylic (citric) acid cycle in the mitochondrion. The low oxemic environment also suppresses the cycle and the flow of electrons into the respiratory chain. This is achieved by stimulating the production and activity of pyruvate dehydrogenase kinase (PDK), an enzyme that inhibits PDH.

Figure 2.

Mechanism of O₂ sensing by chondrocytes. The low oxemic microenvironment experienced by chondrocytes in the growth plate results in the stabilization of the α subunit of the transcription factor HIF-1. Subsequently, this subunit is transported to the nucleus where it dimerizes with the β subunit. The heterodimer binds the Hypoxia Response Elements (HRE) and stimulates transcription. In normoxia, the α subunit is hydroxylated at two proline residues by HIF prolyl hydroxylases (PHDs). Bound to VHL, the modified protein is tagged for ubiquitin-mediated proteosomal degradation.

Figure 3.

Regulation of chondrocyte function in a "taxing" environment. During maturation, cells of the growth plate respond to nutrients and growth factors by changing the activities of a number of key regulatory proteins. Growth factors stimulate Akt signaling. Once activated there is an increase in glucose uptake which serves to sustain energy generation by glycolysis, and promote cell proliferation and survival. Changes in the level of amino acids and nutrients promote the activity of mTOR and increase protein translation and cell growth. When ATP levels decrease, AMPK is activated and mTOR is suppressed. In this case, there is reduced energy-consuming reactions, such as those required for translation. In a similar manner, low energy and hypoxia activate REDD-1. When activated, this protein blocks mTOR and sustains cellular ATP levels.