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# Nutrient metabolism of the nucleus pulposus: A literature review

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ABSTRACT

Cells take in, consume, and synthesize nutrients for numerous physiological functions. This includes not only energy production but also macromolecule biosynthesis, which will further influence cellular signaling, redox homeostasis, and cell fate commitment. Therefore, alteration in cellular nutrient metabolism is associated with pathological conditions.

Intervertebral discs, particularly the nucleus pulposus (NP), are avascular and exhibit unique metabolic preferences. Clinical and preclinical studies have indicated a correlation between intervertebral degeneration (IDD) and systemic metabolic diseases such as diabetes, obesity, and dyslipidemia. However, a lack of understanding of the nutrient metabolism of NP cells is masking the underlying mechanism. Indeed, although previous studies indicated that glucose metabolism is essential for NP cells, the downstream metabolic pathways remain unknown, and the potential role of other nutrients, like amino acids and lipids, is understudied.

In this literature review, we summarize the current understanding of nutrient metabolism in NP cells and discuss other potential metabolic pathways by referring to a human NP transcriptomic dataset deposited to the Gene Expression Omnibus, which can provide us hints for future studies of nutrient metabolism in NP cells and novel therapies for IDD.

## Introduction

The intervertebral disc (IVD) serves as the major joint of the spine, contributing to its mobility and load-dispersion ability. The evolution from quadrupedal walking to standing gait, and the lifestyle changes in the modern era have increased the burden on the IVD in the human body. As a result, intervertebral disc degeneration (IDD)-related diseases have become a major social and medical concern [1,2]. However, there is currently no effective treatment for disc degeneration, and even surgical intervention is still limited to indirect approaches such as spinal fusion and decompression [3].

Research has been focusing on anti-oxidative, anti-apoptosis, and anti-inflammatory approaches for treating IDD [4–11], but the therapeutic effects are limited in the prevention of progression since these cellular changes are mostly observed in established IDD. Elucidation of cellular alterations that cause these stresses might provide a prophylactic approach.

Nutrient metabolism, i.e., how cells utilize/metabolize nutrients, has gained much attention as a therapeutic target in cancers [12–16], brain diseases [17,18], osteoarthritis [19–21], and immune diseases [22]. Now it is well-known that nutrient metabolism is not only for energy production, but also involves multifaceted physiological processes, such as protein/nucleotide synthesis, which will subsequently influence cellular signaling, redox homeostasis, cell proliferation/differentiation, and cell fate decision. Collectively, nutrient metabolism is the foundation of cellular functions, thus metabolic fluctuations can be the early signs of cellular dysfunctions, and interventions of nutrient metabolism can be a prophylactic approach.

Previous studies provide a solid correlation between metabolic alteration and IDD. Degenerated discs have lower pH, which partially resulted from increased glucose consumption and lactate production [23]. Mitochondrial dysfunction, increased mitochondrial ROS production, and diminished anti-oxidant capacity have also been pointed out in the degenerative NP [24,25]. Altered fatty acid metabolism is also suggested in IDD models [26]. Moreover, given that general metabolic diseases such as diabetes and obesity have been implicated as major risks for the IVD, altered metabolism can be not only the effect but also the cause of IDD [27–29]. Therefore, understanding the metabolic pathways in the NP can provide us both potential biomarkers and treatment targets for IDD.

Nutrient metabolism in the NP is also important for disc regeneration or repair. When the nutrient supply is significantly diminished due to degenerative changes of the endplates (EP) or the annulus fibrosus (AF), either decreasing the metabolic demands or increasing the metabolic ef-

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ficiency in NP cells may prevent cell apoptosis and further progression of IDD. During the healing process of disc herniation/disc injury, on the other hand, NP cells have to acquire a more active metabolic state. Nonetheless, whether NP cells possess such metabolic rewiring capabilities is unknown.

To date, moreover, the molecular interaction and the cause-effect relationship between metabolic pathways and NP functions are poorly understood. The importance of glucose metabolism in the NP has been demonstrated in previous studies [30], but how glucose is specifically used in the NP is still unclear. Besides, whether and how the other essential nutrients (amino acids and fats) are utilized in the NP remains mostly unknown.

In this review, we will summarize the current understanding regarding glucose, amino acid, and lipid metabolism in NP cells to visualize the whole picture of the metabolic pathways in the NP and find clues to tackle IDD from a metabolic perspective.

## A whole picture of the nutrient metabolism in the NP

### *The NP has unique nutrient accessibility*

The IVD consists of three major parts: the upper and lower vertebral bodies (VB), the surrounding AF, and the NP in the center. The NP is a highly hydrated, gelatinous core containing collagen fibers (predominantly collagen type II), and NP cells are chondrocyte-like cells with a low density of around 5000 cells/mm$^3$ in human lumbar discs [31]. Nonetheless, the NP is not dormant. In the murine lumbar NP, although the cell proliferation after puberty has slowed down (Fig. 1A: 3-week-old, Fig. 1B: 2-month-old), NP cells are constantly producing cartilaginous matrix proteins such as aggrecan (mRNA expression of *Acan* as shown in Fig. 1C,D). This biosynthetic process requires both energy and substrates metabolized from nutrients. However, of note, the NP is an avascular tissue, which indicates that they do not have direct access to systemically circulating nutrients [32–34], raising a question about the source of their nutrients.

The EP in the VB and the AF play important roles in supplying nutrients to the NP [35–37]. Capillaries arising from vertebral arteries end at the osteochondral junction of the EP, and those arising from surrounding soft tissues end in the outer layer of the AF (Fig. 2). Notably, the capillaries in the EP contribute to the majority of blood supply to the IVD [35]. The capillaries penetrate the subchondral EP and form loop-like buds adjacent to the cartilaginous EP. The cargo (nutrients) then move into the NP, mainly through diffusion. Urban's group and others have extensively studied and reviewed this process [35,38–41].

### *Glucose is the major nutrient in the NP but other nutrients might also be utilized*

Due to the inefficient nutrient trafficking machinery, small molecules such as glucose are considered the major fuel for the NP. Another environmental factor, oxygen, also primes the NP to a glucose-preferred metabolic state. Oxygen, albeit its tiny molecular size, still needs to enter the NP by diffusion. Consequently, the oxygen concentration falls steeply to less than 1% in the center of the human NP [42]. NP cells, therefore, adapt themselves to anaerobic glycolysis which does not need oxygen during ATP production [43].

However, glycolysis is not the only metabolic pathway in the NP. Of note, there are abundant functional mitochondria in NP cells, especially in the developing NP [25], and a recent study has indicated that maintaining the quality of mitochondria during aging is essential to keep the NP healthy [25,44]. Furthermore, metabolic assays showed that cultured rat NP cells have a substantial level of oxygen consumption [45] and isotypic carbon tracing studies also confirmed an active TCA cycle in NP cells [46].

Further evidence for mitochondrial respiration in NP cells is that reactive oxygen species (ROS), a byproduct mainly produced in the mi-

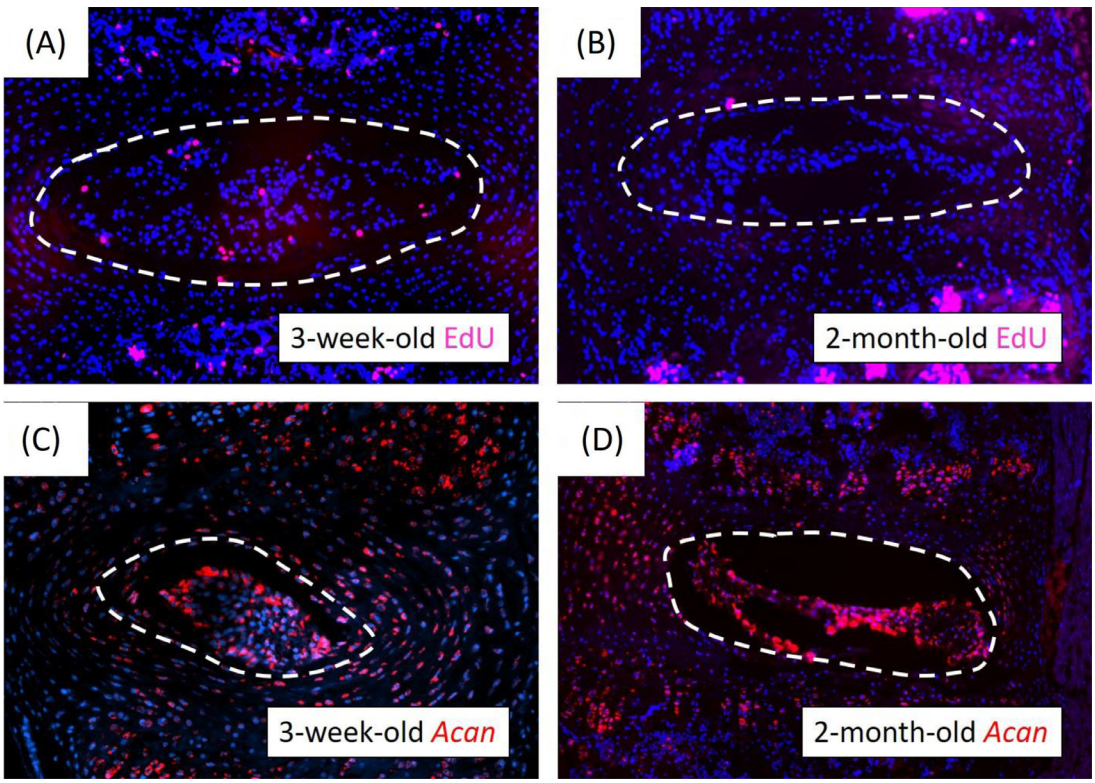


**Fig. 1. The activity of the NP.** (A, B) EdU incorporation in the 3-week-old and 2-month-old murine lumbar IVD. EdU (10 $\mu$g/g of mice) was injected 1 hour before harvest. (C, D) RNA in situ hybridization (RNAscope®) for *Aggrecan* in the 3-week-old and 2-month-old murine lumbar IVD. Dashed lines indicate the margin of the NP.


**Fig. 2. Vessels surrounding the murine lumbar IVD.** Dextran (500kMW: 200μl of 5mg/ml) was intravenously injected 5 minutes before harvest. The dashed line indicates the margin of the NP. Arrowheads indicate the capillaries ending at the osteochondral junction in the endplates and the outer layer of the AF.

tochondria by the electron transport chain (ETC), play an important role in the degeneration of the IVD (first described in [47], reviewed in [4,48,49]). This was a ground-breaking finding because it had been thought that NP cells abolish their ETC functionality. Given that mitochondria are functional in NP cells, other nutrient sources such as amino acids and lipids might also be metabolized.

Glucose, lipids, and amino acids are the three major nutrients and an intact energy metabolism network is critical for normal physiological functions of cells (Fig. 3). Glucose is a hydrophilic solute with a small molecular weight, which is thus the most readily available nutrient in the avascular matrix [50]. Amino acids are not solely the building blocks for proteins but are also essential for other cellular activities such as supplying the TCA cycle with carbons to produce ATP and providing substrates for antioxidants (e.g. glutathione). Lipids have the highest capability of storing and yielding energy, and they are components required for the synthesis of cell membranes, hormones, and signaling molecules. We will briefly summarize the current understanding of the metabolism of these three nutrients in the following sections.

## Glucose

### *Anaerobic glycolysis and mitochondrial respiration in the NP*

After uptake via glucose transporters (GLUTs) into the cell, glucose will first undergo glycolysis to produce pyruvate. In anaerobic glycolysis, pyruvate will be fermented into lactate by lactate dehydrogenase (LDH). In the oxidative pathway, on the other hand, pyruvate will be converted to acetyl-CoA by pyruvate dehydrogenase (PDH) and then enter the TCA cycle [51].

Parallel to glycolysis, glycolytic intermediates such as glucose-6-phosphate (G6P) are used in the Pentose Phosphate Pathway (PPP) to produce nicotinamide adenine dinucleotide phosphate (NADPH) and ribose-5-phosphate (R5P) which are essential for cellular redox homeostasis (the glutathione redox cycle) and nucleotide synthesis, respectively. Another glycolytic intermediate, 3-phosphoglyceric acid (3PG), is used in the serine biosynthesis pathway which is important for several biosynthesis processes in addition to proteins, e.g. purine for the nucleotides and the sphingolipids for the cell membrane [51].

The hypoxic NP primarily uses glucose since it can be metabolized to produce ATP without using oxygen [52]. NP cells cultured in a medium without glucose will lose viability within 24 hours [53]. Studies have shown that NP cells, especially in the center of the NP, convert most of the glucose into lactate and produce little $CO_2$, suggesting that glucose primarily fluxes into anaerobic glycolysis rather than entering the TCA cycle in mitochondria (See Fig. 3) [37,50].

The final product of anaerobic glycolysis is lactic acid, whose accumulation results in an acidic environment. The pH value of the healthy mammalian NP is reported to be a pH 6.9-7.1 [23]. Studies have shown that a low pH especially below pH 6.8 will cause a steep decrease in cell viability and the rate of matrix protein synthesis [23].

Reassuringly, the NP has its own defensive system to prevent a low pH. NP cells have been shown to resist this increasing acidification by pumping out protons via $Na^+/H^+$ exchangers and $H^+$-ATPases [54], MCT4 (lactate and $H^+$ efflux, [46]), or recycling extracellular $HCO_3^-$ produced by carbonic anhydrases [55].

Lactate is not just a waste but also serves as fuel in the IVD. It has recently been reported that AF cells recycle the lactate secreted from NP cells via MCT1, a lactate importer contrary to MCT4 a lactate exporter in the NP. AF cells then convert lactate to pyruvate which then goes into the TCA cycle for ATP production [56].

It is worth mentioning that glucose can also be used for oxidative metabolism in the mitochondria in NP cells, albeit to a lesser extent. In an in vivo canine model, Urban's group found that there is substantial oxygen consumption in NP cells, and the usage of oxygen increases by mechanical stimulus [57]. With an explant culture system, they demonstrated that inhibiting oxidative phosphorylation in the mitochondria (the electron transport chain) impaired matrix production by NP cells [43]. They further showed that the rate of anaerobic glycolysis is dependent on the oxygen level, as NP cells decrease glucose uptake and lactate production at higher oxygen levels and vice versa [43,50]. Therefore, the balance between anaerobic glycolysis and mitochondrial respiration may vary depending on the location in the NP (the outer region of the NP might use more oxidative metabolism due to higher $O_2$ levels), physiological conditions (development, degeneration, etc.), and functional status (resting, standing, actively mobilizing, etc.).

### *Key players that regulate glycolysis in the NP*

To understand how the NP uses glucose and what kinds of cell functions are associated with glucose metabolism, in vivo genetic animal models, which manipulate NP glucose metabolism, are desired. We first reanalyzed single cell RNA sequencing (scRNAseq) data from human NPs deposited into the GEO to examine the expression of genes related to glucose metabolism as well as other metabolic pathways in NP cells ([58], GSE160756, GSM4878539_umi_hNP_2).

Under unsupervised clustering, two clusters were identified (Fig. 4A). Cells in Cluster 0 express high levels of well-known markers for chondrocyte-like NP cells (*ACAN, MIA, SOX9, COL2A1, PAX1, A2M* [59,60]), whereas cells in cluster 1 express monocyte/macrophage markers *(HLA-DRA, ITGAM, CD68*) [61–63], as shown in Fig. 4B.

Multiple key molecules in the glycolysis, *HIF1A* ("HIF-1$\alpha$" in Fig. 3), *SLC2A1* (GLUT1), *PGK1 (*phosphoglycerate kinase that catalyzes the conversion from 1,3-biphosphoglycerate to 3-phosphoglycerate and produces ATP), *PDHA1* (pyruvate dehydrogenase, converts pyruvate to acetyl-CoA. "PDH" in Fig. 3), and *LDHA* (lactate dehydrogenase, converts pyruvate to lactate. "LDH" in Fig. 3) are expressed in human NP cells (Fig. 4C). Corresponding pathways for these enzymes can be referred to Fig. 3.

HIF-1A is one of the master regulators of glucose metabolism [64,65]. Importantly, in rat and human degenerated discs, the expres-

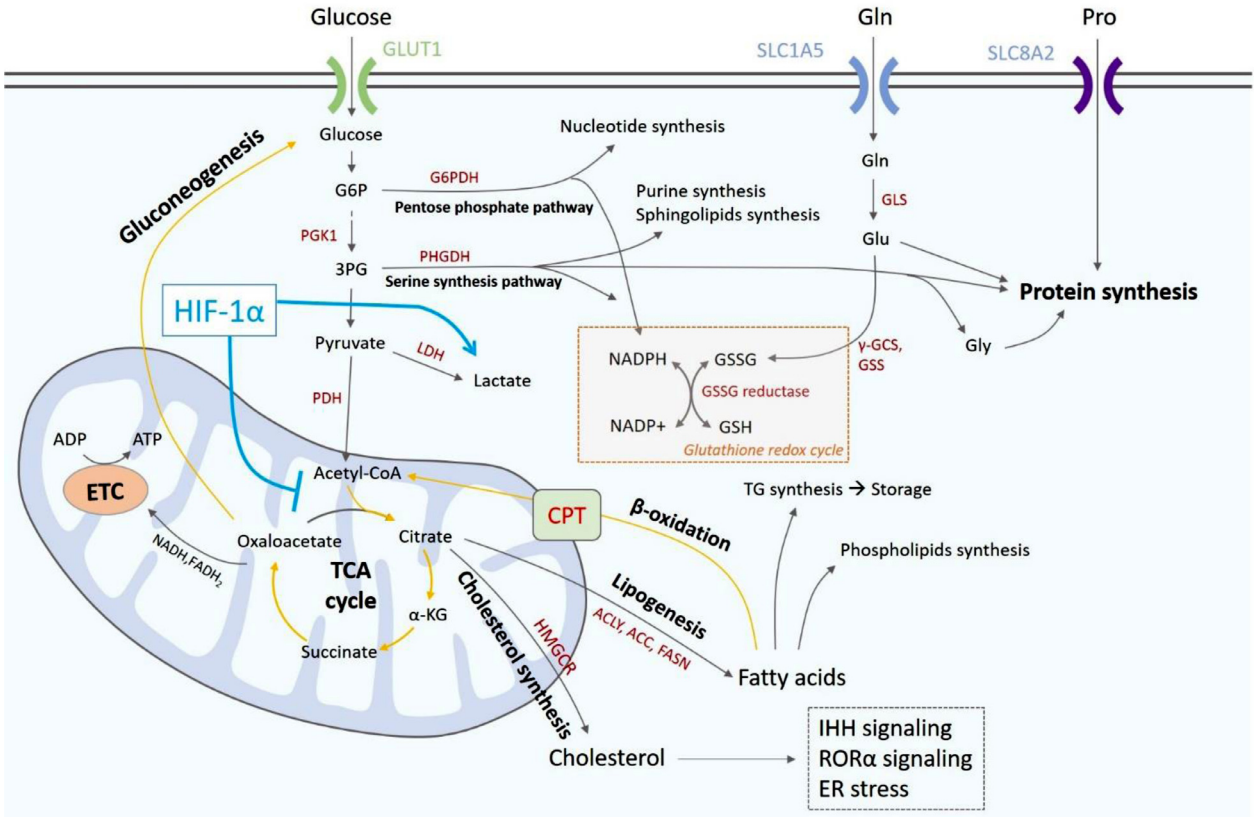


**Fig. 3. Schematic diagram of the metabolic pathways**. Glucose, amino acid, and lipid metabolism included in this review were depicted. The blue arrow indicates positive regulation, and the blue bar indicates inhibitory regulation. Yellow arrows indicate gluconeogenesis starting from fatty acids. Abbreviations: TG = triglyceride, CPT = carnitine palmitoyltransferase, ACLY = ATP citrate lyase, ACC = acetyl-CoA carboxylase, FASN = fatty acid synthase, HMGCR = 3-hydroxy-3-methylglutaryl-CoA reductase, G6P = glucose-6-phosphate, 3PG = 3-phosphoglycerate, PGK1 = phosphoglycerate kinase 1, PDH = pyruvate dehydrogenase, PHGDH = phosphoglycerate dehydrogenase, G6PDH = glucose-6-phosphate dehydrogenase, LDH = lactate dehydrogenase, Gly = glycine, Pro = proline, Gln = glutamine, Glu = glutamate, GLS = glutaminase, GSH = reduced glutathione, GSSG = oxidized glutathione, NADPH = nicotinamide adenine dinucleotide phosphate, TCA cycle= tricarboxylic acid cycle, $\alpha$-KG = $\alpha$-ketoglutarate, ADP = adenosine diphosphate, ATP = adenosine triphosphate, NADH = reduced nicotinamide adenine dinucleotide, FADH = reduced flavin adenine dinucleotide, $\gamma$GCS = gamma-glutamylcysteine synthetase, GSS = glutathione synthetase.

sion level of HIF1A decreases [67,68], suggesting its essential role in maintaining IVD health.

HIF-1A is consistently expressed as a functional transcriptional factor in rat, human, and sheep NP cells [66]. NP cells upregulate the expression of HIF-1a and HIF-2a responding to hypoxia [66,69,70], which then suppresses mitochondrial respiration while increasing glycolysis to minimize the oxygen consumption of cells to prevent severe hypoxia (reviewed in [71,72]).

Loss of *Hif1a* in murine notochordal cells (inducible Cre recombinase driven by *Foxa2*) results in massive cell death and fibrocartilaginous NP formation due to the lack of *Pgk1* expression and ATP production from glycolysis [73]. The roles of HIF1A in the IVD have been comprehensively reviewed [71,72].

GLUTs are the membranous glucose transporter in mammalian cells. Among the multiple isoforms of GLUTs, GLUT1 (encoded by *SLC2A1*) is highly expressed in NP cells [26,74]. Deletion of *Glut1* in chondrocytes impaired proliferation and maturation, which resulted in a significantly delayed skeletal development, indicating that glucose uptake is essential for chondrocyte function [75]. Given that NP cells partially exhibit similar characteristics to chondrocytes, this conditional knockout model can also be applied to NP cells to further characterize the role of GLUTs in the NP.

PGK1, PDHA1, and LDHA are key enzymes in glycolysis [51]. Pgk1 deletion can lead to blockage of the entire glycolysis flux, whereas *Pdha1* or *Ldha* depletion can diminish glucose flux to the TCA cycle or lactate production, respectively. Genetic manipulations of these molecules in NP cells can provide us specific knowledge of how glucose is metabolized in vivo.

*Usage of glucose beyond glycolysis*

As aforementioned, glucose can be used for several anabolic processes (Fig. 3). PPP branches at G6P in the glycolysis pathway, producing R5P and NADPH which are essential for nucleotide synthesis and redox homeostasis, respectively. The rate-limiting enzyme in this pathway is G6PDH [76]. Serine synthesis pathway branching at 3PG, on the other hand, is also important for purine synthesis (nucleotides) and NADPH synthesis (anti-oxidant), which is catalyzed by PHGDH [77]. Moreover, the intermediate citrate in the TCA cycle can be exported from mitochondria to the cytosol by citrate/isocitrate carrier (SLC25A1), which will then be used for de novo lipogenesis. This process is catalyzed by ATP-citrate lyase (ACLY), acetyl-CoA carboxylase (ACACA), and fatty acid synthase (FASN) [78–80] (refer to Fig. 3). These key enzymes are all expressed in human NP cells (Fig. 4C), and genetic manipulation of these molecules can provide us a more comprehensive understanding of glucose metabolism in NP.

**Amino acids**

There are nine essential amino acids, which cannot be synthesized in the human body, and thirteen non-essential amino acids, forming a complex, intertwined network of metabolic pathways. Amino acids are

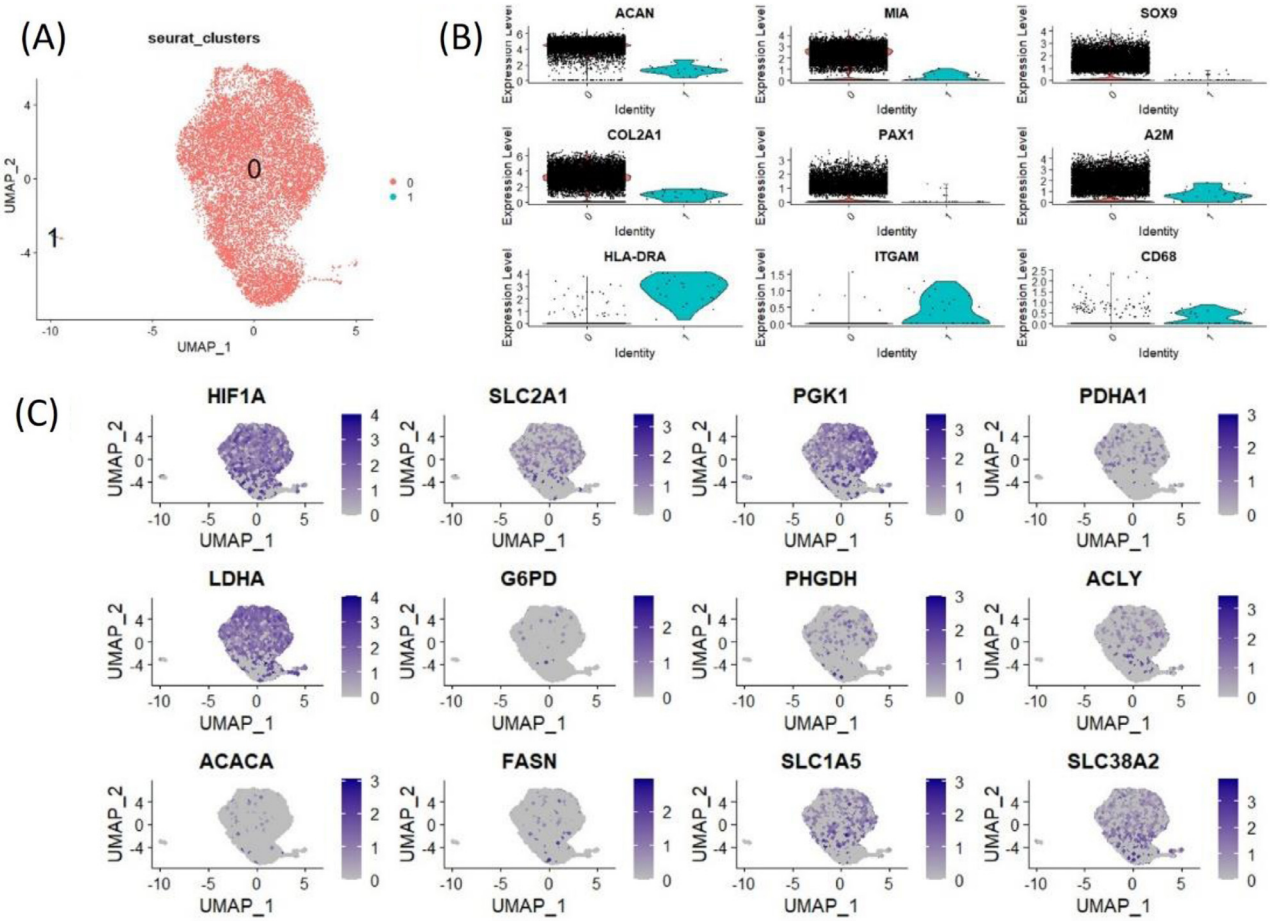


**Fig. 4. Gene expressions in human NP cells from deposited single cell RNA sequencing data (GSE160756, GSM4878539_umi_hNP3).** (A) The UMAP analysis was performed using the Seurat package in R Studio. Cells with more than 200 and less than 7000 features and less than 10% of mitochondrial genes were included. FindNeighbors (dimensions = 1:20), FindClusters (Resolution = 0.03), and RunUMAP (dimensions = 1:20) functions were used for clustering. (B) Violin plots of marker genes indicate that cluster 0 is NP cells (ACAN, MIA, SOX9, COL2A1, PAX1, A2M), and cluster 1 is monocytes/macrophages (HLA-DRA, ITGAM, CD68). (C) Feature plots of genes involved in glycolysis (HIF1A, SLC2A1, PGK1, PDHA1, LDHA), PPP (G6PD), serine synthesis (PHGDH), lipogenesis (ACLY, ACACA, FASN), and amino acid transporters (SLC1A5, SLC38A2).

used for protein synthesis. Additionally, their metabolites are involved in a wide range of cellular physiological processes including the synthesis of nucleotides via purine, TCA cycle anaplerosis (supplementation of intermediates in the TCA cycle), and redox homeostasis (synthesis of anti-oxidants). The importance of amino acid metabolism in skeletal cells, especially in chondrocytes, is increasingly acknowledged and well-reviewed by Devignes et al. [81]. Amino acids are even smaller molecules (~110 Da) than glucose (180 Da), suggesting that they may diffuse into the NP. Indeed, we confirmed the expression of two key amino acid transporters, SLC1A5 [82] and SLC38A2 [83] in human NP cells (Fig. 4C), suggesting that NP cells have the potential to uptake amino acids. However, the utilization of amino acids in the NP has not been demonstrated.

*Protein production*

The primary role of amino acids is to be the building blocks for proteins. Studies have suggested that inhibiting proline and alanine uptake in osteoblasts could directly affect osteogenic differentiation by affecting the production of osteoblast-associated proteins [83,84]. NP cells also produce a unique class of proteins that have different compositions of amino acids from other types of cells.

For instance, NP cells actively produce aggrecan, one of the most abundant molecules in the NP matrices (Fig. 1C-D). We calculated the amino acid compositions in the human aggrecan core protein sequence (UniPort, P16112 PGCA_HUMAN. Accessed November 2, 2022. https://www.uniprot.org/uniprotkb/P16112/entry), and found glutamate (Glu, 11%), serine (Ser, 12%), glycine (Gly, 12%), and proline (Pro, 9%) are especially enriched (Fig. 5).

These are all non-essential amino acids, which can be either imported from outside or bio-synthesized endogenously. Glu can be converted from Gln by glutaminase (GLS), and Gln is transported into the cell by SLC1A5 (Fig. 3). Ser can be imported into cells via several transporters including SLC5A1 [85], but it is also actively synthesized in the cell from G3P, a glycolysis intermediate (Fig. 3). Ser is also the precursor for Gly synthesis [85].

Several essential amino acids are also found in aggrecan such as threonine, valine, and isoleucine, etc. The high compositions of these amino acids in aggrecan suggest that their metabolism is essential for the production of aggrecan in the NP. Further studies focusing on the uptake, biosynthesis, and downstream metabolic pathways of amino acids enriched in proteins that are essential for NP development and homeostasis, including aggrecan, type II collagen, and SOX9, can provide us novel clues for the regulation of NP matrix production.

*Redox homeostasis*

Another essential role of amino acids is in the regulation of cellular redox homeostasis. Targeting the increasing reactive oxygen species (ROS) levels in IDD is a spotlighted research field [86]. Although the un-

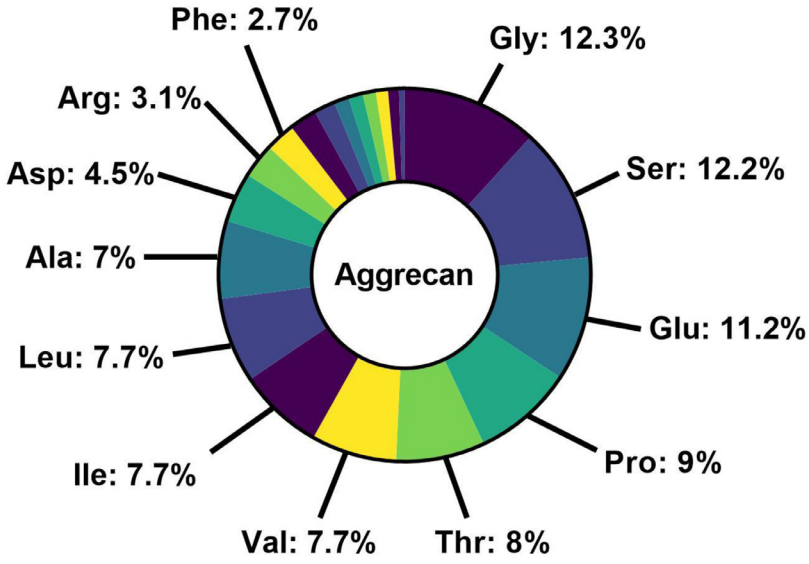


**Fig. 5. Amino acids compositions of human aggrecan core protein.** Gly = glycine, Ser = serine, Glu = glutamate, Pro = proline, Thr = threonine, Val = valine, Ile = Isoleucine, Leu = leucine, Ala = alanine, Asp = aspartate, Arg = arginine, Phe = phenylalanine.

derlying mechanism has not been fully elucidated, the increasing ROS is partially associated with the hypoxic environment of the IVD.

$O_2$ is the terminal receptor of electrons in the ETC. Insufficient $O_2$ causes a buildup of equivalents (NADH and $FADH_2$) in the mitochondria, which results in excessive electrons leaking to $O_2$. The excessive electrons partially reduce $O_2$ into superoxide ($O_2^-$) and then generate hydrogen peroxide ($H_2O_2$) [87].

Cells have their own anti-oxidant defensive system, in which, the glutathione (GSH) redox cycle plays the essential role (reviewed in [88,89], Fig. 3). In short, reduced GSH receives the excessive electrons from ROS and transforms into oxidized glutathione (GSSG). Then, GSSG is reduced back to GSH by glutathione reductase and passes the electron to $H^+$ which is provided by NADPH. The two essential members in this cycle, GSH and NADPH, are closely connected to amino acid metabolism.

GSH can be made from Gln, the most abundant amino acid in the blood. Gln is first converted into Glu by GLS, and then together with cysteine Glu is converted into GSH by gamma-glutamylcysteine synthetase and glutathione synthetase [90]. Impairment of this pathway by knocking down or knocking out GLS in periosteal cells or chondrocytes showed a decreased GSH/GSSG ratio and increased ROS [91,92].

The major source of NADPH, on the other hand, is PPP shunting from the intermediate G6P in glycolysis (Fig. 3). Another contributor to the cellular NADPH production is serine catabolism driven by PHGDH [93,94]. PHGDH depletion in chondrocytes lowered serine production, increased ROS, and caused cell death [77].

Collectively, a healthy amino acid metabolism is required for maintaining cellular redox homeostasis, which is associated with IDD. Elucidation of the disruption of this metabolic network in IDD may lead to novel anti-oxidative treatments.

## Lipids

Lipid metabolism is grabbing increasing attention in skeletal diseases [95,96], but few studies have focused on NP cells. Nonetheless, clinical studies have found a correlation between altered systemic lipid profiles and spinal diseases [97–100], but the underlying molecular mechanisms remain elusive. Some recent studies focused on the lipid metabolism in chondrocytes, which are relatives of NP cells. Here, we will seek hints for lipid metabolism in NPs referring to some of the insights in chondrocytes.

### *Cholesterol*

The majority of lipids that are stored in human bodies are in the forms of cholesterols, triglycerides, and phospholipids. Cholesterol can be either obtained from food or biosynthesized in cells. After uptake in the guts, cholesterol will be transported to peripheral tissues via circulation in the form of lipoproteins that contain apolipoproteins. The lipoproteins will then be taken up by peripheral cells by lipoprotein receptors such as low-density lipoprotein receptor (LDLR) and low-density lipoprotein receptor-related proteins (LRPs) [19,95].

Cholesterol can be also synthesized in most cells. The cholesterol biosynthesis pathway takes place in the cytosol and endoplasmic reticulum (ER) and consists of more than 20 steps. Simply, the rate-limiting step is the conversion from acetyl-CoA to hydroxy-3-methyglutaryl coenzyme A (HMG-CoA) by HMG-CoA reductase (HMGCR) (Fig. 3). HMG-CoA is then converted to mevalonate, then squalene, and finally cholesterol [101].

Systemically, cholesterol is the precursor for all steroid hormones, sex hormones, vitamin D, and bile acids [101]. At cellular levels, cholesterol is an essential component of the cell membranes. Moreover, it is essential for chondrocyte maturation (hypertrophy) in multiple cellular signaling pathways such as the Hedgehog and nuclear receptor retinoid-related orphan receptor-alpha (ROR$\alpha$) signaling pathway [102,103].

However, in the setting of osteoarthritis, in which hypertrophy of chondrocytes is the hallmark of degeneration, cholesterol seems to have a detrimental effect. In both murine and human osteoarthritic (OA) chondrocytes, there is an enhanced cholesterol uptake and processing pathway (oxidized low-density lipoprotein receptor 1-cholesterol hydroxylases-ROR$\alpha$ signaling). Consequently, OA chondrocytes produce more oxysterol (the end product of cholesterol excretion pathways) into circulation. Indeed, a high cholesterol diet exacerbated OA progression [104]. The inhibition of cholesterol synthesis, in contrast, is beneficial for chondrocyte function. Studies showed that inhibition of cholesterol synthesis by statin attenuated OA progression [105,106], or enhanced endochondral ossification [107]. Taken together, these findings suggest that finely controlled cholesterol metabolism and related signaling are highly associated with chondrocyte health.

Clinical studies identified that hypercholesterolemia is a risk factor for IDD [108,109]. Sasani et al. fed rabbits with a high cholesterol diet and identified fat accumulation in the surrounding tissues of the NP, which caused IDD by the disruption of blood supply to the IVD [100]. However, the proposed underlying mechanisms are focused on the cardiovascular dysfunctions surrounding the IVD caused by hypercholesterolemia. Thus, the involvement of cellular cholesterol metabolism in the NP is still unclear.

Importantly, a very recent study detected elevated cholesterol levels in degenerative NP tissue both in humans and rats. Furthermore, in vitro investigations revealed that cholesterol burden upregulated the ex-

pression of sterol regulatory element-binding protein 1 (SREBP1), which caused endoplasmic reticulum stress (ER stress). Accordingly, knocking down SREBP1 attenuated cholesterol-induced cell apoptosis and restored the production of aggrecan and collagen type II [110]. This study investigated the cellular cholesterol metabolism in the NP for the first time and provided us a potential treatment target for IDD induced by altered systemic lipid profiles.

### *Triglycerides and fatty acids*

Triglycerides consist of three fatty acids with a glycerol backbone. When needed, fatty acids in the triglycerides are released via lipolysis mediated by adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and monoacylglycerol lipase (MGL) [111,112]. For catabolic purposes, these free fatty acids are transformed into acyl-carnitine and transported into the mitochondria for further degradation via the carnitine shuttle. Acyl-carnitine is then converted into acyl-CoA by Carnitine-Palmitoyl-Transferase II (CPT-2) and enters FAO (Fatty Acid $\beta$ Oxidation) [113]. In this reaction, the bond between $\alpha$- and $\beta$-carbon next to the first carbon with the CoA segment is repeatedly cut to finally produce Acetyl-CoA (two-carbon metabolites). Acetyl-CoA then fluxes into the TCA cycle.

Recent studies accumulated circumstantial evidence for FAO in NP cells. Agrawal et al. demonstrated active FAO in rat NP cells by adding radiolabeled fatty acid ([$^3$H] labeled palmitate/myristate) and subsequently detecting the metabolite ([$^3$H] $H_2O$) [114]. In the study by Tsingas et al., ablation of Sox9 in aggrecan-expressing cells caused drastic degeneration in the adult IVD, and interestingly, transcriptomic analyses revealed that fatty acid metabolism-related genes were downregulated [26]. More interestingly, scRNAseq data from monkeys identified a new marker for notochordal NP cells, Apolipoprotein E (ApoE, [115]), which is a binding protein to lipoproteins involved in lipid transportation and intracellular lipid metabolism in other cell types [116]. These findings imply that the NP cells have the potential to use fatty acids.

To further study lipid metabolism in NP cells, several fundamental questions must be answered. How do the NP cells access fatty acids? The first possibility is *de novo* lipogenesis. Lipids can be synthesized from citrate, the metabolite from the TCA cycle. Another possibility is cell-to-cell lipid trafficking. Fatty acids are considered too large to freely diffuse in the avascular matrices. However, earlier studies showed that even large molecules (20000 molecular weight Dextran) can slowly diffuse in the cartilaginous matrix [117,118]. NP cells might transport fatty acids in short distances via yet unknown mechanisms.

Another question is, why do NP cells, at least partially, use fatty acids? There are also two possible reasons, which are energy storage and phospholipids synthesis.

Firstly, in the center of the NP, even glucose concentration steeply decreases, which means NP cells have very limited access to exogenous nutrients [35]. Fatty acids can be converted to triglycerides and stored in the form of lipid droplets within the cell. When cells cannot access exogenous nutrients, the lipid droplets can be degraded to fatty acids and subsequently acetyl-CoA, which can directly serve as an endogenous energy source via the TCA cycle [119,120], or provide substrates for macromolecule neogenesis such as glucose and amino acids (Fig. 3, yellow arrows). However, whether this oxygen-demanding metabolic pathway can efficiently occur in the hypoxic NP needs further investigation.

Secondly, Fatty acids can be used for the synthesis of phospholipids, which are not only constituting cellular membranes but also essential for membrane protein regulation/recognition and cell membrane signaling [121–123].

Lipid metabolism in NP cells is mostly unknown. To elucidate the connection between systemic lipid metabolism and the progression of IDD, investigations of the molecular mechanisms of lipid utilization in NP cells are highly expected.

## Concluding remarks and future directions

We systemically reviewed the nutrient metabolism of NP cells including glucose, amino acids, and lipids (Fig. 3). NP cells primarily use glucose, but amino acids and lipids might also play an unignorable role in maintaining cellular homeostasis. To date, however, the nutrient metabolism of the NP is understudied, leaving numerous questions to be answered. What are the nutrients used in the NP? How are these nutrients accessed by NP cells? What are they respectively used for? How are the different nutrient metabolism pathways interconnected? What kind of metabolic alterations happen in IDD? And eventually, how to treat IDD by targeting metabolic pathways?

Earlier studies of the complex metabolic system in the NP are mostly limited to in vitro observations or in vivo phenotypic observations, largely due to the technical limitations to timely detect the metabolic change in vivo or to reproduce the metabolic conditions in vitro. In addition, difficulties of isolating and culturing NP cells, species-dependent characters, different mechanical stresses between humans and animals, and lack of established degenerative models are all standing in the way.

Notably, there is a considerable advance in recent methodologies for metabolic studies, such as metabolomics (tandem mass spectrometry and Mass Spectrometry Imaging) [124], NMR spectroscopy [125], and a recent fascinating computational model which can simulate the metabolic flux in a single cell resolution by using scRNAseq [126]. The application of these methods can help us investigate the metabolic status of cells more comprehensively.

Nutrient metabolism at the cellular level is closely related to all cellular activities and therefore linked to the underlying mechanisms of various cellular dysfunctions, including degenerative changes like IDD. Furthermore, the connection between local diseases and systemic conditions is becoming increasingly acknowledged, in which nutrient metabolism could play a pivotal role. Therefore, in conclusion, we expect that chronic diseases such as IDD, which correlate closely with systemic conditions, will increasingly be spotlighted in future metabolic research.

## Declarations of Competing Interest

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