Elevated Nutrient Availability in Chondrocytes Leads to Changes in Glutamine Metabolism

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INTRODUCTION: The production of high-quality tissue engineered cartilage is key to developing improved biological-based approaches for treating articular cartilage injuries resulting from trauma or disease. While known for their anaerobic metabolism both *in vivo* and *in vitro*, chondrocytes exhibit improved biosynthesis under conditions that elicit mixed aerobic-anaerobic metabolism [1]. These conditions can be achieved through changes in nutrient availability, such as by elevated media culture volume [2]. In response to changes in nutrient availability, chondrocytes undergo a change in metabolic phenotype primarily driven by alterations in glucose metabolism [3]. However, glutamine also plays a key role in chondrocyte metabolism [4]. Thus, the aim of the present study was to characterize the role of glutamine in the metabolic transition caused by changes in nutrient availability.

METHODS: Isolated primary bovine articular chondrocytes were seeded on collagen II coated Millicell™ inserts in 3D high-density culture (2 x 10^6 cells / insert) and were cultivated under varying nutrient availabilities (8 to 32 mL of F12 media supplemented with 20% FBS) for 4 weeks under normoxic conditions with media exchanges every 48-72 hours. During the final media exchange cycle, constructs were fed with media supplemented with either 10 mM [U-^{13}C] glucose or 1 mM [U-^{13}C] glutamine. Conditioned media from the final exchange cycle was analyzed for glucose, lactate, glutamine, glutamate, and dissolved CO_2. Immediately after harvest, metabolites from the tissue constructs were extracted and the mass isotopomer distributions were obtained by mass spectrometry and HPLC [5], which were then paired with the external metabolite fluxes. Isotopomer Network Compartmental Analysis software (INCA 2.0) [6] was then utilized to assemble a tissue-specific network model and estimate the flux through the major metabolic pathways in chondrocyte central carbon metabolism. Confidence intervals for flux values were obtained through INCA by parameter continuation [6].

RESULTS: Both intermediate (8 mL) and elevated (16 mL) media volumes showed evidence of glutamine's participation in the TCA cycle via ^{13}C-glutamine tracing (Figure 1). Glutamine contributes carbons to key TCA cycle metabolites, including α-ketoglutarate, succinate, and fumarate in the lower TCA cycle. However, glutamine does not provide carbons to pyruvate or cis-aconitate, located before the glutamine entry point to the TCA cycle at α-ketoglutarate. ^{13}C-MFA revealed significant changes in metabolism as media availability was increased (Figure 2). Flux through glycolysis and the TCA cycle increased with increased culture media, whereas flux through lactate fermentation remained unchanged. Flux through glutamine metabolism into the TCA cycle also increased with increased culture volume, agreeing with the ^{13}C-glutamine results. The malate-aspartate shuttle appeared to be active, allowing glutamine carbons to enter glycolysis and lactate fermentation. Lastly, flux through the hexosamine biosynthetic pathway (HBS) was also observed to increase with increased culture volume.

DISCUSSION: We observe a shift from mostly anaerobic metabolism to a mixed aerobic-anaerobic metabolic phenotype as culture volume is increased. This change in metabolic phenotype is accompanied by an increase in glutamine metabolism and evidence of glutamine utilization in the TCA cycle. We have previously observed in these same culture conditions that elevated media volumes resulted in increased cartilaginous tissue biosynthesis (tissue weight, proteoglycans, collagen) [7]. The changes in metabolism agree with these results, as increased glutamine metabolism lends itself to proline production and ultimately collagen formation, and increased flux was observed in the HBS, the pathway responsible production of proteoglycans. The malate-aspartate shuttle (malate → pyruvate), although only thought to be active in chondrocytes [8], was confirmed through MFA modeling and provided a means of allowing glutamine carbons to enter metabolic pathways (glycolysis, lactate fermentation) typically only associated with glucose metabolism.

SIGNIFICANCE: Utilizing nutrient-based cellular regulation for biomass and signaling offers the potential for use in a wide range of tissue engineering applications. Glutamine's role in chondrocyte metabolism and function is pivotal, and investigating these connections may also yield insights into osteoarthritis, which has recently been shown to includes a metabolic phenotype [9].

REFERENCES: [1] Khan *et al.* (2009) *Biotechnol Prog* 25:508; [2] Tarantino *et al.* (2021) *Biotechnol Bioeng* 118:4119; [3] Tarantino *et al.* (2023) *In Review;* [4] Stegen *et al.* (2020) *Dev Cell* 53:530; [5] Tchigvintsev *et al.* (2013) *Chem Biol* 12:1386; [6] Rahim *et al.* (2022) *Metab Eng* 69:275; [7] Tarantino *et al.* (2023) *TERMIS-AM*; [8] Hollander *et al.* (2019) *Curr Osteoporo Rep* 17:59; [9] Courties *et al.* (2019) *Curr Opin Rheumatol* 29;214.

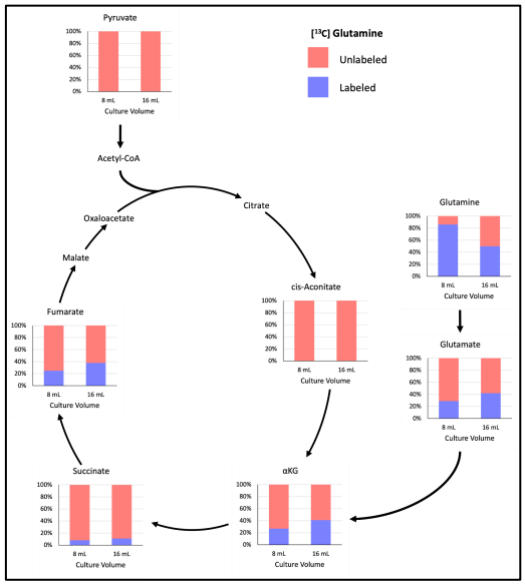

Figure 1: Contribution of glutamine to TCA Cycle metabolism. Fraction of [^{13}C] labelled TCA cycle intermediates in tissue-engineered chondrocytes incubated with [U-^{13}C] glutamine.

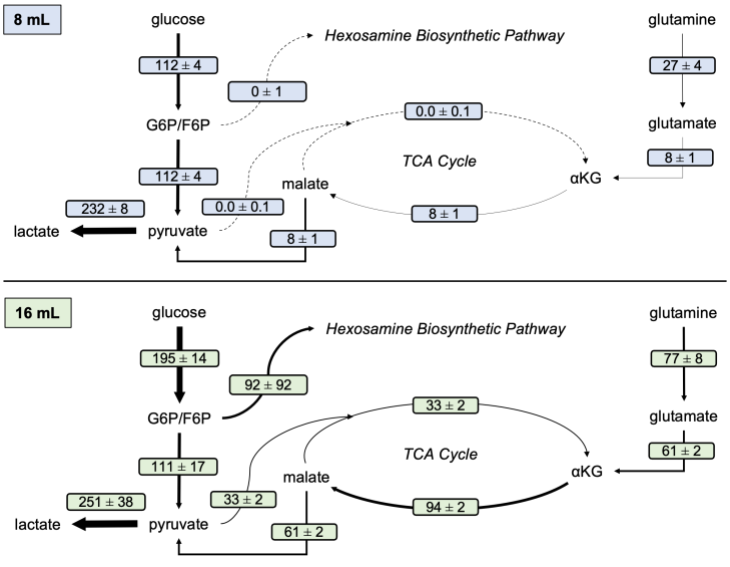

Figure 2: Flux maps of chondrocyte metabolism at elevated culture volumes. Fluxes [nmol/h] are presented as net flux ± confidence interval. Arrow thickness is proportional to the net flux, with dashed lines representing zero net flux.