A Stoichiometric Matrix Model of the Biochemical Network of Central Energy Metabolism in Human Cells for Understanding Chondrocyte Mechanotransduction

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Introduction: Osteoarthritis (OA) involves the metabolic dysfunction of chondrocytes found in articular cartilage and is the most common joint disorder worldwide [1-3]. Chondrocytes are principally responsible for the synthesis and maintenance of articular cartilage including the structural proteins comprising the extracellular and pericellular matrices, chiefly Types II and VI collagen and aggrecan [4-6]. We hypothesize that physiological compression of chondrocytes will result in direction of central energy metabolism to production of collagen and associated proteins as a mechanism of cellular mechanotransduction. The objectives of this study were to (1) develop a stoichiometric network model of human central energy metabolism and (2) use this model to predict fluxes in order to maximize production of precursors to cartilage matrix proteins.

Methods: MATHEMATICAL MODEL Construction - Central energy metabolism was modeled using a stoichiometric matrix, with each row representing a specific metabolite and each column a separate reaction. Matrix values corresponded to the stoichiometric relationship of the specific metabolite in the specified reaction resulting in a matrix representing 52 metabolites in 37 physiological reactions. For example, when glucose is converted into glucose-6-phosphate via hydrolysis of a single ATP molecule, the stoichiometric values for glucose and ATP would be -1, and the entries for glucose-6-phosphate, ADP, and H+ would be +1. Stoichiometric relationships were determined from the extensive published literature on human central metabolism [7].

GENERALIZED HYPOTHESIS TESTING - The stoichiometric matrix was used to predict metabolite flux using linear programming in MATLAB. Multiple objective functions were defined based on the amino acid sequence of the protein of interest (e.g. type II collagen). The resulting minimizations predicted the flux vectors through the biochemical reactions of central metabolism that would theoretically result in the maximal production of the specific protein. Objective functions were defined using a 38th reaction based on the primary structure of the protein of interest as published in the NCBI database [8,9], and each non-essential amino acid was classified according to its central metabolism precursor. The relative relationships between these precursors then determined the stoichiometric relationship of the objective function.

NETWORK ANALYSIS AND PREDICTION OF SPECIFIC MODES - The network was analyzed using Elementary Flux Mode Analysis by CellNetAnalyzer, a third party module written for use in the MATLAB environment.

Results: The stoichiometric matrix was constructed to include glycolysis, the pentose phosphate shunt, the tricarboxylic acid cycle, oxidative phosphorylation, and two anaplerotic reactions used by the cell to replenish carbon skeletons removed from the TCA cycle. We found 12 elementary flux modes for this network. Linear programming of the model elucidated unique fluxes that predict maximal synthesis relative to glucose input of Types II and VI collagen as well as the precursor peptide for aggrecan, a
structural protein in cartilage (Figures 1-2). Specificity of predicted fluxes for proteins of interest was inferred for specific reactions via comparison of the fluxes for the proteins of interest with negative control fluxes (Figure 2).

**Discussion:** The creation of a central metabolism model for human chondrocytes and the prediction of unique flux vectors specific maximizing production of a target protein (e.g. Type II Collagen) are major advances which will enable metabolic engineering of human cells. This study provides a framework for comparison with experimental data to better elucidate how cells alter their energy metabolism to respond to external stimuli.

We found distinct flux vectors for matrix proteins (type II collagen, type VI collagen, and aggrecan) compared with negative control proteins (Figure 2). This analysis indicates that the predicted fluxes unique when compared to the negative controls of cyclooxygenase 2 (COX-2) and albumin and that there are differences between the predictions for the various matrix proteins. These results suggest specific biochemical reactions for engineering matrix production by chondrocytes.

Development of this model enables testing of our hypothesis by compressing chondrocytes in physiological conditions and using metabolomics to find an experimental flux vector to compare with these hypothetical flux vectors. This experimental flux vector can be expressed as a linear combination of the elementary flux modes for better comparison between experimental flux vector and the theoretical flux vectors. Further studies will focus on this comparison to better understand how osteoarthritic chondrocytes respond at a metabolic level to mechanical loading as well as expanding our network model to better represent the cellular metabolism as a whole.

**Significance:** The significance of this work is that comparison of experimental flux data of osteoarthritic chondrocytes with predicted flux vectors determined herein can lead to a better understanding of the sources of metabolic dysfunction within the diseased chondrocytes. It is still unknown whether OA is a single disease, or if it is the phenotypic outcome of multiple underlying diseases [10]. A system-wide understanding of the source or sources of dysfunction will lead to targets for novel therapeutics that may treat the underlying causes of osteoarthritis, rather than treating the disease’s symptoms.