

A Computational Model of Mitochondrial Beta-Oxidation Highlighting the Implications on Uremia Disease in Human

Abhishek Sengupta, Sarika Saxena

Abstract- Enzyme deficiencies can segment the metabolic reactions in the mitochondrion and may spark to augmentation of specific substrates causing severe clinical manifestations. During the state of starvation mitochondrial oxidation of long-chain fatty acids procures a vital source of energy for the heart as well as for skeletal muscle. A computational kinetic network of reactions, compounds and parameters was constructed to correlate the mitochondrial fatty acid beta-oxidation to disease conditions. Carnitine deficiency limits the availability of the long chain acyl-CoAs inside the mitochondrial matrix. Majorly, carnitine is necessary for fatty acid transport to sites of beta-oxidation in the mitochondria. An increased ratio of long-chain acyl-carnitine (LCAC) to free carnitine, was observed when carnitine level was declined. This verifies that uremic patients have altered carnitine metabolism. Subjecting the constructed model of the biochemical reactions involved in fatty acid catabolism to further simulations at varying concentrations will provide predictive models to identify the disease targets.

Index Terms—Mitochondrial Beta-Oxidation, Carnitine Deficiency, Computational Model, Uremia.

I. INTRODUCTION

During the periods of fasting or starvation, fatty acids are the vital source of energy as they play a key role in catabolic stress. Deficiency in the process leads to reduced energy production, which is vital for highly functional and diligent organs like heart and skeletal muscles. The fatty acids, degenerated in beta-oxidation enter the cell via fatty acid protein transporters present on the surface of the cell. Coenzyme A group is added by fatty acid carnitine shuttle to the fatty acid inside the cell. The enzyme, Carnitine Palmitoyltransferase 1(CPT1) later converts the long chain acyl-CoA to long chain acyl-carnitine. The fatty acid moiety is transferred by carnitine translocase (CAT) across the inner mitochondrial membrane. CPT2 then converts the long chain acyl-carnitine back to long chain acyl-CoA [1]. The long chain acyl-CoA is now efficient enough to enter the fatty acid oxidation pathway, resulting in the production of one acetyl-CoA from each cycle of beta-oxidation. The NADH and FADH₂ produced by both beta-oxidation and the Tricarboxylic Acid (TCA) cycle from acetyl-CoA are used by the electron transport chain to produce ATP.

Tremendous knowledge and experiments has been harvested on these cellular pathways and the metabolites which signal for incorporating this information into a dynamic system. A kinetic model depicting metabolism of saturated fatty acids with special focus on the role of Carnitine is presented in this paper. Carnitine plays a pivotal role in transport of long chain acyl-CoAs to mitochondria. Loss of carnitine through dialytic membranes occurs in maintenance of hemodialysis, resulting in potential carnitine depletion and relative increments of esterified carnitine forms [2]. Certain data suggest that oxidation of long chain fatty acids (LCFA) may be impaired in uremia, and such a derangement could, partly, contribute to the myocardiopathy of uremia [3]. It was observed that patients had increased ratios of long-chain acyl-carnitine (LCAC) to free carnitine in their erythrocytes [4]. A comprehensive representation of all of the biosynthetic reactions of fatty acid transport via carnitine shuttle as well as its oxidation inside mitochondria is computationally designed and presented [5]. And also computer simulations of the system are performed which are subjected to validation by using the experimental data from various published literature and databases. Finally, the results are interpreted in a biological and clinical context followed by the discussion on limitations and further scope [6].

II. METHODOLOGY

The main aim was to construct a dynamic environment to correlate the mitochondrial fatty acid beta-oxidation to disease conditions. Extensive literature investigation revealed interesting facts and a large number of research progress in the area. The problem was selected based upon its relevance in the concerned field. During starving fatty acids serve as the main source of energy. The acetyl-CoA produced as a result of beta-oxidation further leads to the generation of ATP. Moreover, the acetyl-CoA forms ketone bodies which is fuel for brain. Disorders in the enzymes of this pathway are observed in a group of inherited metabolic conditions called Fatty Acid Oxidation Disorders (FAOD). This results in accumulation of fatty acids and decreased rate of metabolism. This work is an endeavor to build up a kinetic model to study the behavior of enzymes governing fatty acid transport and beta-oxidation spiral. A deterministic model was constructed since it is less time consuming than stochastic simulation of model. The initial step was to get familiar with the Cell Designer software, which is used for modeling and simulation complex biochemical systems. A model of beta-oxidation was created by taking reference from several books and journals. The concentrations of various substrates were collected by rigorous data search and information had to be

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scrutinized to see if it fit the model or not. Kinetic laws were generated by the plug-in SBML squeezer. SBML2LATEX was used to generate the PDF of the model in human readable format. It converts SBML files to pdf format. The final step was of performing simulation using COPASI, SOSlib and CellDesigner simulation platforms. Plots were generated by changing the concentration.

III. RESULTS

A detailed comprehensive study of fatty acid transport and beta-oxidation in human has resulted in the creation of a model using a structured diagram editor, CellDesigner. The model provides a clear picture of the pathway depicting reactions and the enzymes involved in an organized manner. The model depicted in the Figure 1 which shows an orthogonal layout outsmarts the pre-existing models of beta-oxidation in its simplicity and clarity. Further, dynamic simulations were performed to check the validity of the model. Four compartments for depicting mitochondrial outer membrane, inter mitochondrial space, mitochondrial inner membrane and mitochondrial matrix were structured and default represented the cytoplasm. A dynamic comprehensive model environment was created by connecting each reaction with another. Substrates were denoted by “simple molecules”, while the proteins were denoted by “generic proteins”, ions were denoted by “ion”. “State transition” arrow represented conversion, “transport” arrow denoted transport, and “association” arrow denoted association and “dissociation” arrow denoted dissociation. Reversibility of the reaction could be set by checking the reversible option to true or false. Enzymes were connected using the “catalysis” arrow or the “inhibition” arrow as per its activity.

A plot of dynamic simulation of the constructed model was performed showing comparative time course simulation in CellDesigner and COPASI referred to Figure 2(a), (b) in the text. The graphs are plotted to prove that simulation results are reliable. Though the same results are obtained using both the software, COPASI is more algorithm intensive whereas Graphical User Interface of CellDesigner is user-friendly. Plotting is better in COPASI as intuitive scaling if done by the simulator. A plot showing concentration rates as a function of time was plotted using COPASI. From the graph as shown in Figure 3(a) it could easily be inferred that all reaction tends to obtain a constant rate of reaction as the slope becomes zero. A concentration versus time plot of NADH and NAD⁺ was plotted simultaneously as shown in Figure 3(b) where the green line denotes NAD⁺ and blue line denotes concentration change of NADH with respect to time in seconds. A plot of change in concentrations of C22 trans-enoyl-CoA and C22 L3Hydroxy acyl-CoA as shown in Figure 3(c) was plotted over period of time. C22 trans-enoyl-CoA is denoted in blue and C22 L3Hydroxy acyl-CoA is denoted in green. This denotes the second step of beta-oxidation after conversion of C22 acetyl-CoA to C22 trans-enoyl-CoA. The concentration of substrate has a fast decrease initially but later equilibrium is attained. Similarly the product concentration increases with time

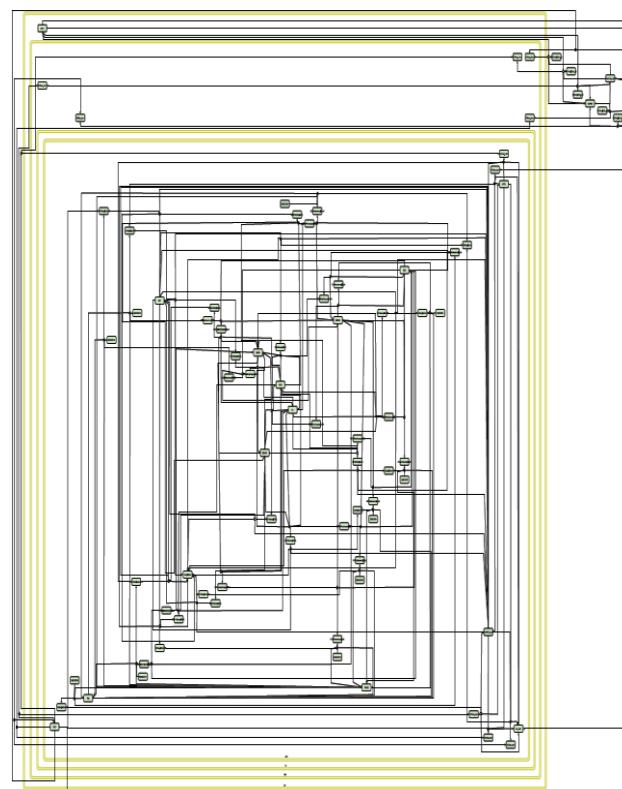


Figure 1: Orthogonal layout of a detailed comprehensive study of fatty acid transport and beta-oxidation in human

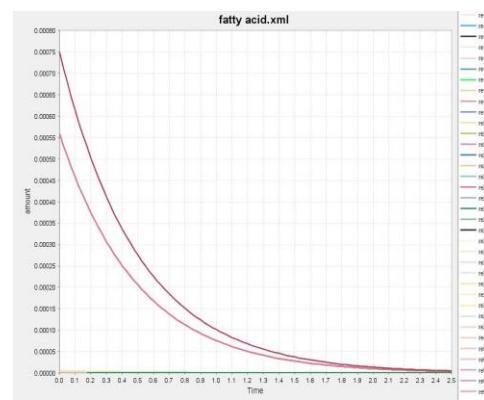


Fig 2(a)
Reaction Fluxes

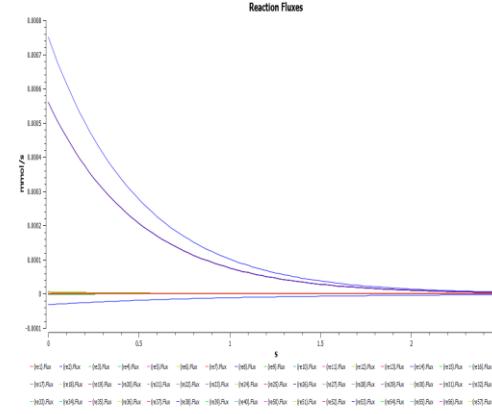


Fig 2(b)

Figure 2: A plot of dynamic simulation using (a) CellDesigner, (b) COPASI

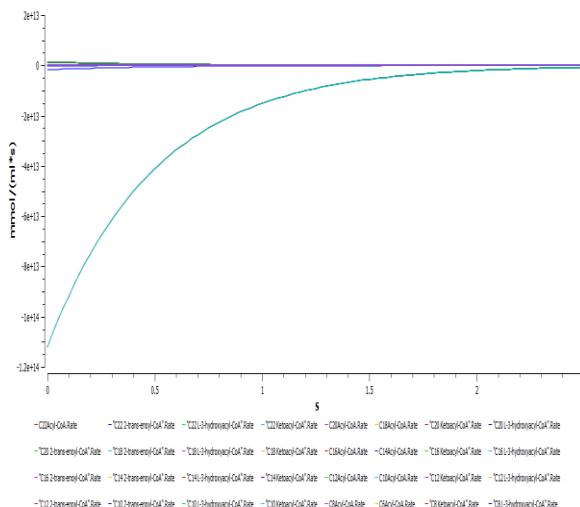


Fig 3(a)

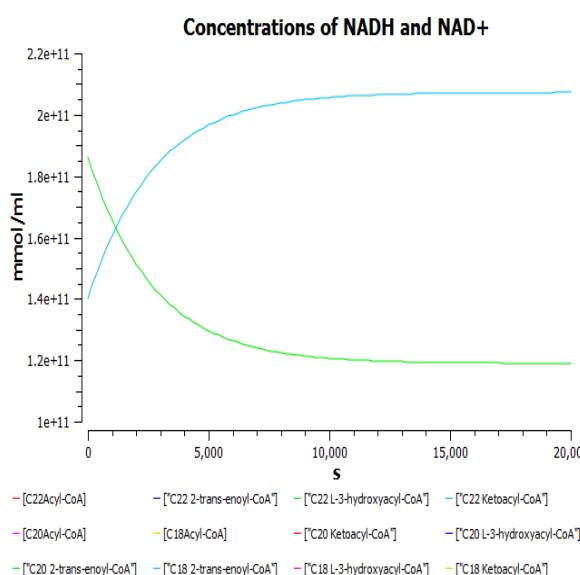


Fig 3(b)

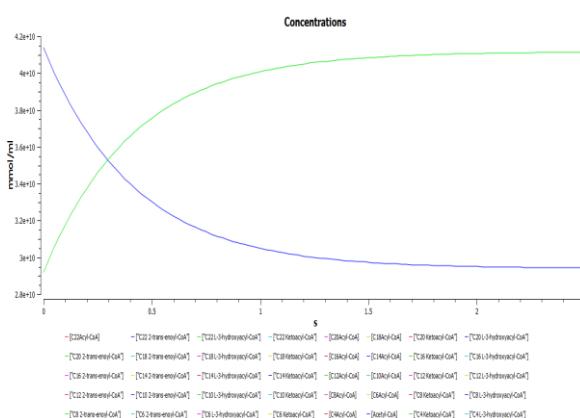


Fig 3(c)

Figure 3(a): A plot showing concentration rates as a function of time which infers that all the reactions tend to obtain a constant rate of reaction as the slope becomes zero.
Figure 3(b): A concentration versus time plot of NADH and NAD⁺ depicted together. Green line denotes NAD⁺ and blue line denotes concentration change of NADH with respect to time.

time. **Figure 3 (c):** A plot of change in concentrations of C22 trans-enoyl-CoA and C22 L3Hydroxy acyl-CoA over period of time in seconds. C22 trans-enoyl-CoA is denoted in blue and C22 L3Hydroxy acyl-CoA is denoted in green. This is the second step of beta-oxidation after conversion of C22 acetyl-CoA to C22 trans-enoyl-CoA. The concentration of substrate decreases initially very fast but later equilibrium is attained. Similarly the product concentration increases with time.

IV. DISCUSSION

Comparative time course simulation plots generated for the model encompass same plots on performing dynamic simulation of the model using the two separate software programs. This justifies that the simulation results which are plotted graphs are reliable. COPASI program enables the user to choose from a wide range of algorithms, whereas Graphical User Interface of CellDesigner is better. Concentration rates as a function of time were studied. From the graph, plotted in COPASI, it could easily be inferred that all reactions tend to obtain a constant rate of reaction as the slope becomes zero. Time versus concentration plot of NADH reveals that its concentration increases with time, which is in accordance with the published literature. A plot of concentration as a function of time using COPASI output assistant for NAD⁺. In agreement with the concentration increase of NADH, the concentration of NAD⁺ decreases with time (as shown in plot of NADH with time). Concentrations versus time plot of NADH and NAD⁺ were obtained in a single graph to study their correlation. Green line denotes NAD⁺ and blue line denotes concentration change of NADH with respect to time. The second step of beta-oxidation was studied, after the conversion of C22 acetyl-CoA to C22 trans-enoyl-CoA. A plot of change in concentrations of C22 trans-enoyl-CoA (blue) and C22 L3Hydroxy acyl-CoA (green) over period of time was generated. As a result of dynamic simulation it could be seen that, concentration of substrate decreases initially very fast but later equilibrium is attained and it is also notable that the product concentration increases with time. Scatter plots were generated as well, to gain insights into beta-oxidation of C22 Acyl CoA, the reactions were in accordance with classical pathway. A scatter plot of C22 Hydroxy Acyl-CoA (x-axis) versus C22 Trans-enoyl-CoA (y-axis) shows that these two have negative correlation. This is logically validated by the fact that with metabolic cycle progression concentration of C22 Trans-enoyl-CoA decreases whereas that of Hydroxy-Acyl-CoA increases [7]. Related 2D bar charts were created to study the concentrations of long chain acyl-CoAs, long chain carnitines, free acyl-CoAs, and free carnitine in diseased condition of uremia as shown in Figure 4(a-e) [8]. The results depict the increased ratios of long-chain acylcarnitine (LCAC) to free carnitine which is in accordance with the experimental observations [9].

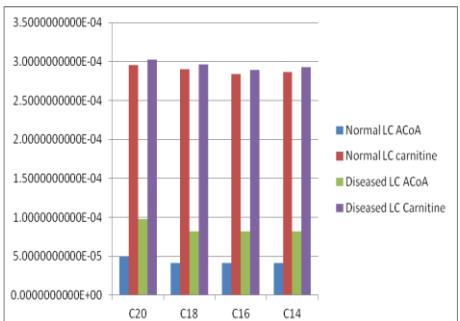


Fig 4(a)

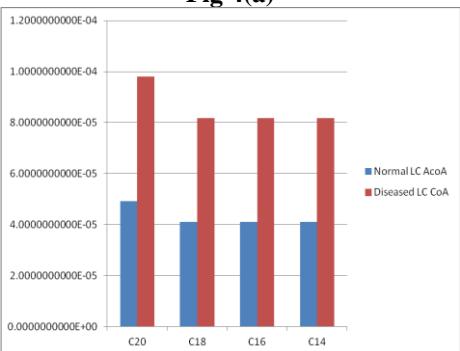


Fig 4(b)

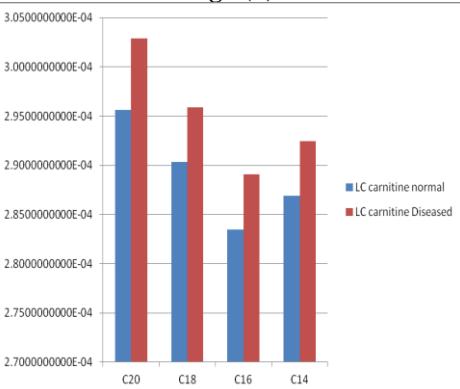


Fig 4(c)

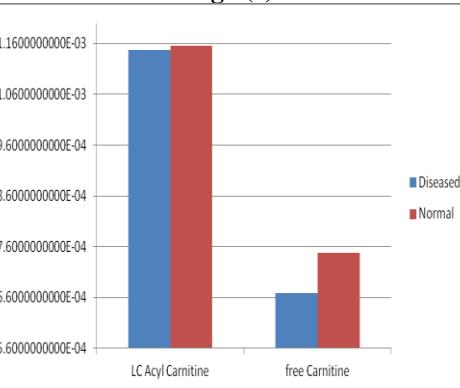


Fig 4(d)

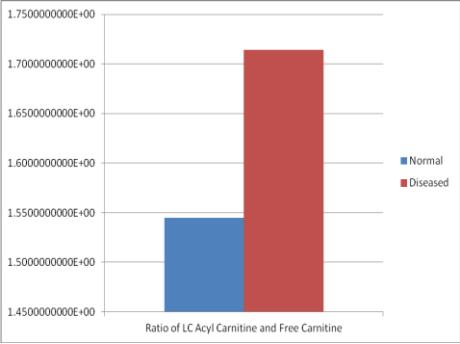


Fig 4(e)

Figure 4(a): A 2D Bar chart depicting relative concentrations of Long Chain carnitine and Long Chain acyl-CoA in normal and diseased conditions. **Figure 4(b):** A 2D Bar chart depicting relative concentrations of Long Chain acyl-CoAs in normal and diseased conditions. **Figure 4(c):** A 2D Bar chart depicting relative concentrations of Long Chain carnitine in normal and diseased conditions. **Figure 4(d):** A 2D Bar chart depicting relative concentrations of Long Chain acyl-carnitine and free carnitine in normal and diseased conditions. The results are in accordance with the disease condition of Uremia. **Figure 4(e):** A 2D Bar chart depicting ratio of relative concentrations of Long Chain acyl-carnitine and free carnitine in normal and diseased conditions. The results are in accordance with the disease condition of Uremia.

V. CONCLUSIONS

A systems biology approach to create biochemical networks using modeling tools is being frequently applied today to gain physiological insights into the dynamics of metabolic pathways. Beta-oxidation model generated computationally in this work is a highly simplified version of the classical pathway. Based on kinetic laws, the model could be used to test hypothesis and validate them for experiments which would prove to be more cost effective and time saving. This comprehensive kinetic model was generated beginning from the scratch. The references for detailed model were taken from literature and research papers and since while making the model its comprehensibility was kept in mind hence it is advanced and easier to understand. The results generated by the model were validated logically and by literature resources. The model could be used to provide predictive information on substrate concentration and enzymatic activity in any diseased condition. Further modifications and refinements in the model may provide possible clues for the disorders associated with the beta-oxidation pathway and the carnitine transport. This model can be used as sub model with other anabolic and catabolic processes of energy production pathways to create a more accurate and clear picture of the complex network of mitochondrial energy production in human. A system wide analysis of metabolic network can be done to find the causes and potential targets of drugs for various diseases. The limitations of the model are that it does not include odd chained fatty acid, unsaturated fatty acid oxidation. Further, CPT1 enzyme shows its role to control the rate limiting step of beta-oxidation of fatty acids, i.e., transport of the long chain acyl carnitines in the matrix of mitochondria. Malonyl CoA is an inhibitor of CPT1 and is dependent on ATP and AMP levels. If energy content is low, to replenish the need of ATP, an energy restoration cascade takes place which converts acetyl CoA into malonyl CoA, which inhibits the CPT1 enzyme, the pathway can easily be merged with this model and tested for significant outcomes and hypothesis.

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