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BEng Individual Project – Modeling Lipoprotein Metabolism and the Action of Statins

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Interim Report

1 Project Specification

Existing models of lipoprotein metabolism have been crucial to giving a further understanding of familial hypercholesterolemia and atherosclerosis. However, these models are relatively simple and make several assumptions that do not accurately reflect the biochemistry inside the cell. They currently focus on the extracellular concentrations of lipoprotein particles (LDL, HDL, VLDL, and IDL) as well as the intracellular concentrations of cholesterol which are derived from the endocytosis of lipoproteins.

The aims of this project are first to gain a good understanding of the biology and biochemistry involved in the production and transport of cholesterol and to understand the current models of lipoprotein metabolism which have recently been published. Then, we aim to extend the model to more accurately fit the biochemistry involved, especially incorporating the action of HMG-CoA reductase. This is the rate-limiting enzyme in the biosynthesis of cholesterol and the target of all HMG-CoA reductase inhibitors, better known as statins.

Once a more complete model is developed, we seek to understand the implications of the model, namely what effects are seen when parameters are perturbed. Some questions might be answered through a theoretical analysis of a model, but might not necessarily be derived from clinical studies. What other metabolites are affected with the incorporation of statins? What is the long term effect of statin use on the cell and important tissues? Are there other possible targets to control the level of cholesterol in the plasma?

Analysis of our model through stability and bifurcation analysis will hopefully give us insight into which parameters have the greatest effect on both intracellular cholesterol levels and plasma cholesterol levels. With these results in mind, pharmaceutical companies, nutritionists, and medical practitioners will be able to target specific intermediates in cholesterol transport and production depending on which parameters affect cholesterol levels the greatest.

2 Review of Background Literature

Cholesterol, a 27 carbon molecule, is produced from the precursor acetyl-CoA by the cells through a multistep process partially known as the mevalonate pathway. Free cholesterol is an integral part of the plasma membrane, conferring stability. Cholesterol is the also the basis of steroid hormones such as estrogens, critical to maintaining homeostasis within the human body.

The cholesterol homeostasis in the cell is achieved through three different methods: regulation of HMG-CoA reductase, regulation of LDL receptor synthesis, and regulation of the esterification and removal of free cholesterol. HMG-CoA reductase is enzyme governing

the rate determining step of cholesterol synthesis, the conversion of HMG-CoA to mevalonate. Regulation of HMG-CoA reductase itself is via transcription factors known as sterol regulatory element binding proteins as well as phosphorylation of the enzyme at serine residue 872. The sterol regulatory element binding protein (SREBP) responds to intracellular cholesterol concentrations and is also responsible for the regulation of LDL receptor synthesis, the second method of cholesterol control within the cell. SREBP is ordinarily found attached to the surface of the endoplasmic reticulum or nuclear membrane and is attached when cholesterol levels are high. A decrease in cholesterol will cause the membrane protein to the cleaved and SREBP to enter the nucleus. Once inside the nucleus, SREBP binds to sterol regulatory elements (SRE) and initiate transcription of LDL receptors and HMG-CoA reductase enzyme in an attempt to increase the intracellular cholesterol levels.

The esterification and removal of free cholesterol is also a critical method by which cholesterol levels in the cell are regulated. Cholesterol is converted to bile acids in the liver and stored in the gall bladder before being secreted into the small intestine. Bile acids help to emulsify fats, creating micelles and preparing them for absorption. Although up to 97% of the bile acid is reabsorbed by the small intestine, the 3% not reabsorbed represents the only exit of cholesterol from the body, making it an important mechanism to maintain cholesterol homeostasis.

There are five main classes of lipoproteins distinguished by their size, density, contents, and surface proteins: chylomicrons, very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins (LDL), and high density lipoproteins (HDL). Every lipoprotein contains five distinct elements: a phospholipid monolayer, apoproteins, free cholesterol located on the membrane, triglycerides, and cholesterol esters found in the center of the particle. Chylomicrons are responsible for delivering the fatty acids from the intestine to the liver, but are not regulated by the liver itself. VLDL particles are produced by the liver and are primarily responsible for transporting synthesized fat and cholesterol to the peripheral tissues. VLDL particles are degraded by the action of lipoprotein lipase (LPL) to IDL particles, of which approximately half are cleared from the plasma by receptor mediated endocytosis in the liver and the other half converted to LDL particles. The conversion of IDL to LDL also occurs via the enzyme LPL. LDL particles contain the highest percentage of cholesterol and a possessing a high concentration of them has been implicated in the genesis of atherosclerosis. LDL particles are normally cleared through receptor mediated endocytosis in the liver.

Whilst excessive LDL particles have been shown to cause atherosclerosis to occur, HDL particles help curb these effects by a process called reverse cholesterol transport. Nascent HDL particles are also secreted by the liver, but maturation occurs only after the proper apoproteins have been transferred from the processing of VLDL and IDL particles by the enzyme LPL. HDL particles also pick up excess cholesterol from cells and transport them back to the liver where they are internalized by receptor mediated endocytosis.

LDL receptors are crucial to maintaining cholesterol homeostasis as discussed earlier. The receptors themselves are produced by normal protein synthesis pathways and are finished in the Golgi apparatus before being randomly inserted into the plasma membrane. For internalization of LDL particles to occur when bound to LDL receptors, the presence of a clathrin coated pit is necessary. Although coated pits account for only 2% of the cell surface area, they contain approximately 50-80% of the LDL receptors. Once internalized, the new vesicle is transported to a lysosome where the lipoprotein is degraded, the cholesterol released into the cell, and the LDL receptors are either degraded or recycled back to the

surface of the cell. The recycling process occurs naturally approximately every 10 minutes and is thought to be downregulated with the increase cholesterol concentration in the cell. Although research has shown that LDL particle internalization can be receptor-independent in rats and hamsters, there have been no clinical or in vitro studies on human cells to prove the existence of this pathway. From patients with familial hypercholesterolemia with a phenotype showing the absence of LDL receptors, there was no detectible internalization of LDL particles suggesting that this phenomenon on receptor-independent internalization does not occur in humans.

HDL is also important as a transporter of cholesterol and has been implicated in a reduction of atherosclerosis and plasma cholesterol concentration. HDL's main pathway is known as reverse cholesterol transport which occurs in five steps. First, cholesterol is transferred from the cells to nascent HDL molecules which are secreted by the liver. This is an important rate determining step mediated by ATP binding cassette transporter A-1 (ABCA1). Next, the free cholesterol obtained from the cells is esterified by lecithin cholesterol acyl transferase (LCAT). The cholesterol esters are transferred to other lipoproteins such as LDL particles which in turn transfer their triglycerides to HDL particles. The nascent HDL molecule is then remodeled into mature HDL and in the final stage, the particles are taken up by receptor-mediated endocytosis through to be via scavenger receptor (SRB1).

SREBP regulation is also important in lipoprotein metabolism since it regulates the transcription of genes necessary for the synthesis of fatty acids and cholesterol, two major building blocks of membranes. In depth biochemical understanding of how this transcription factor operates could allow us to incorporate SREBP into our model thus clarifying a parameter which was previously ignored or lumped into assumptions. The importance of SREBP is highlighted by experiments where SREBP was constitutively produced without the ability to bind to the membrane. The pseudo-mature SREBP was able to enter the nucleus and initiate transcription of cholesterol synthesis genes resulting in high levels of intracellular cholesterol.

SREBP is a membrane bound protein possessing two cleavage sites. Cleavage at site 1, the required initial cleavage, separates two membrane bound segments of premature SREBP. Cleavage at site 2 is the activating cleavage, splicing mature SREBP from the membrane bound protein allowing it to enter the nucleus.

SREBP itself is regulated through the action of SCAP (SREBP cleavage activating protein) which has a sterol-sensing domain. In the absence of sterols, SCAP activates cleavage of the SREBP membrane bound protein resulting in an increase in enzyme levels for cholesterol biosynthesis.

For a more in depth details into the biology and biochemistry behind lipoprotein metabolism, please visit the project website at:

http://www.openwetware.org/wiki/User:Johnsy/Lipoprotein_Modelling

3 Implementation Plan

Implementation of this project relies on understanding the current models of lipoprotein metabolism and the biochemistry involved in creating the model. A solid understanding of the biochemistry is also fundamental to extending the current models to more accurate reflect what is going on within the cells.

The first models developed have been compartmental models where only surface bound LDL particles and their internalized products were considered. They assumed an initial concentration of surface bound lipoproteins and considered a rate of internalization proportional to the number of bound lipoproteins. From there, more complex models were devised to incorporate the production of VLDL, its conversion to IDL and to LDL, the internalization of LDL, and the change in intracellular cholesterol concentration. This model is fully developed and analyzed in a paper published in March 2007 by August, et al.

The paper mentioned derived a five-dimensional model to simulate the changes in lipoprotein concentration in the blood and intracellular cholesterol concentrations. The equations for the original model are reprinted below.

$$\frac{d[VLDL]}{dt} = -k_V[VLDL] + u_V \quad (1)$$

$$\frac{d[IDL]}{dt} = k_V[VLDL] - k_I[IDL] - d_I[IDL]\phi_{LR} \quad (2)$$

$$\frac{d[LDL]}{dt} = k_I[IDL] - d_L[IDL]\phi_{LR} - d[LDL] \quad (3)$$

$$\frac{d\phi_{LR}}{dt} = -b(d_I[IDL] + d_L[LDL])\phi_{LR} + c\frac{1-\phi_{LR}}{[IC]} \quad (4)$$

$$\frac{d[IC]}{dt} = -(\chi_I d_I[IDL] + \chi_L d_L[LDL])\phi_{LR} + \chi_L d[LDL] - d_{IC}[IC] \quad (5)$$

The stability analysis of this and the subsequent models are an area of future work for the second term.

To add to the model already developed, we consider again very simple compartments. For example, if we were to incorporate the action of HMG-CoA reductase into the model, we can first model the change in intracellular cholesterol concentrations and analyze such a system behaves without lipoprotein internalization. HMG Co-A reductase is an enzyme and can be simply modeled using Michaelis-Menten kinetics with the equation below.

$$\frac{d[IC]}{dt} = V_{HCR}\frac{[HMG-CoA]}{K_{HCR}+[HMG-CoA]} \quad (6)$$

In the equation above, HMG-CoA is the substrate of the reductase enzyme, V_{HCR} is the maximum turnover rate of the enzyme, and K_{HCR} is the michaelis-menten constant for the enzyme (the concentration at which the enzyme is at half its maximum turnover rate). We also know that intracellular cholesterol levels affect the production of HMG-CoA reductase enzyme, and hence its activity. We can model this as an inverse relationship with the rate whereby an increase in intracellular cholesterol concentration will inhibit the activity of the enzyme. A decrease in intracellular cholesterol concentration will boost the biosynthesis of cholesterol in the cell.

$$\frac{d[IC]}{dt} = V_{HCR}\frac{[HMG-CoA]}{[IC](K_{HCR}+[HMG-CoA])} \quad (7)$$

One further addition to the equation would be to consider the action of HMG-CoA reductase inhibitors, or statins. Statins block enzymatic activity by competitive inhibition of the HMG-CoA and the NADP+ active site. It is thought that the cyclic ring characteristic of statins is able to bind reversibly to the nicotinamide binding pocket to block prevent the activity of the enzyme. Statins are thought to also be able to inhibit the HMG-CoA binding pocket as well.

The first statin, mevastatin, is a fungal metabolite which was discovered serendipitously in 1976. From then on, researcher have discovered and synthesized several more potent statins.

As we are considering a competitive inhibitor of the enzyme, we can model this as a change in the michaelis-menten factor K_{HCR} as shown in the equation below.

$$\frac{d[IC]}{dt} = V_{HCR}\frac{[HMG-CoA]}{[IC](\alpha K_{HCR}+[HMG-CoA])} \quad (8)$$

The value of the factor is always greater than 1, as that is the level at which the enzyme performs optimally. Further analysis of this model is presented in the preliminary results section.

Thus, by adding this michaelis-menten term into equation (5) of the original model, we can incorporate the *de novo* pathway, a crucial source of cholesterol neglected in the original model. With the same stability and bifurcation analysis as is done in the published paper, the project aims to get a greater understanding of disease processes that occur in the human body relating to fluctuating cholesterol levels. We hope also to get an understanding of the exact effect of statins in the metabolic system as well as which processes to target to effectively reduced LDL concentration, two open questions not answered in the original model.

A further model derived by K. Cooper in 2006 (unpublished results) takes into account the reverse cholesterol transport phenomenon and goes further to include the HDL flux in the plasma. One major difference in the approach taken in this revised model as compared to the model presented above in equations (1) to (5) is that actual cholesterol concentrations are considered and not the concentration of the lipoprotein particles themselves. Instead of LDL particle concentration, the model exploits the mean cholesterol content to derive an equation describing the cholesterol concentration inside all of the LDL particles. This may spring from the fact that cholesterol efflux and transport is main culprit in the formation of atherosclerotic plaques. Although concentrations of lipoproteins do have an effect, the complex relationship between the concentration of particles and emergence of atherosclerosis is unclear. Clinical studies have shown that high HDL concentration could be the result of several different blocks in the reverse transport pathway, not all of them leading to a decrease in cardiovascular disease risk.

Another key aspect of lipoprotein modeling that should be incorporated into the final model is the effect of binding bile acids. Since this is already a proven treatment to help lower cholesterol levels, it is important that we can take this into effect to model the action of both statins and binding resin effects simultaneously (both are usually given to lower high cholesterol levels). Bile acid bind resins would affect the cholesterol degradation term as it prevents recycling of the bile acids back to the liver. Thus, an increase in resins would lead to an increase in depletion of intracellular cholesterol (equation 5). To keep in line with accurate biochemistry, we should also include the recycling of these bile acids. First define the fraction of bile acids recovered as η and d_{BA} is the rate of conversion of cholesterol to bile acids. If we only consider this phenomenon, we can derive the equation below (without considering resins. Our source term is the fraction that is recycled while the sink term in the equation is the fraction that is not recycled.

$$\frac{d[IC]}{dt} = \eta d_{BA}[IC] - (1-\eta)d_{BA}[IC] \quad (9)$$

If we take into consideration resins, this decreases the percentage of cholesterol recycled by affecting the value of η (bounded $0 < \eta < 1$). We can add the effect of resins in by

multiplying it by another constant ξ, which will be bounded between 0 and 1 (1 having no effect and 0 having greatest effect on cholesterol concentration).

$$\frac{d[IC]}{dt} = \xi\eta d_{BA}[IC] - (1 - \xi\eta)d_{BA}[IC] \quad (10)$$

Further investigation into the implications of adding this equation to the total model (equation 4) is contained in the implementation section. Much work still has to be done in terms of combining all the individual models into a complete model and seeing if the complete model still accurate predicts clinically significant events relating to diseases such as atherosclerosis.

4 Evaluation Section

Evaluating the success of this project is determined by considering the feasibility of the model as well as the inclusion of as many biochemical aspects of lipoprotein metabolism as possible. Furthermore, it is important that the results obtained still correlate with concentration values of cholesterol and lipoproteins obtained through clinical studies.

A second stage of success which we hope to achieve is to develop a further understanding of how the entire lipoprotein metabolic system is integrated. A full and complete model will be able to tell us more information than what can be derived from clinical studies. For example, although we know that high levels of LDL are a risk factor for developing atherosclerosis, our analysis of the mathematical model might show that a perturbation in another parameter might have a much greater effect.

It is unrealistic to believe that we could create the perfect model since current medical research has not given us the values of many of the parameters contained in the model. Many values for previous models have had to be inferred or guessed leading to slight errors and perhaps a skewed understanding of the analysis that results. This is one of the major biological milestones necessary to overcome before being able to fully develop a complete mathematical model. Hopefully by advancing the mathematical modeling, we can spur an advancement in the measurement of biological values relevant to modeling.

5 Preliminary Results Section

Preliminary results include investigating the basic models and a qualitative understanding of a few key aspects of the derived model in the Implementation plan section. Matlab was used to solve for the time trajectories of the models using the 4th order Runge-Kutta algorithm for solving differential equations. We began by considering the biosynthesis of cholesterol via the *de novo* pathway and restricting ourselves to looking only within the cell. The equation derived below does not take into account the cholesterol contribution that lipoproteins make to the over intracellular cholesterol concentration.

$$\frac{d[IC]}{dt} = V_{HCR}\frac{[HMG-CoA]}{[IC](\alpha K_{HCR}+[HMG-CoA])} - k_d[IC] \quad (11)$$

This is a non-linear one dimensional differential equation whose stability is quite easy to determine. We assume that the concentration of HMG-CoA is constant in the cell and we denote this by a constant *H*. Solving for the fixed point of the system gives the equation below (13).

$$[IC]^* = \sqrt{\frac{V_{HCR}H}{k_d(\alpha K_{HCR}+H)}} \quad (12)$$

As we know from the discussion in the Implementation section, the value of α is always greater than 1 (1 being the value at which the enzyme has the highest turnover rate and as α increases, the enzyme becomes less efficient at catalyzing the reaction). This observation is in line with the effect that statins have. Now, we speculate that the concentration of statins is directly proportional to the value of α, and we can possibly find the value of α by looking at data gathered clinically.

We can also look at the effect that bile acid binding resins have on the concentration of intracellular cholesterol as we did with statins. Now, we are only considered with the uptake of recycled bile acids which go to maintaining the cholesterol concentrations in the cell as well as the intracellular production of cholesterol by the *de novo* pathway.

$$\frac{d[IC]}{dt} = V_{HCR}\frac{[HMG-CoA]}{[IC](\alpha K_{HCR}+[HMG-CoA])} + \xi\eta d_{BA}[IC] - (1-\xi\eta)d_{BA}[IC] \quad (13)$$

Looking again at equation 10, we can now obtain the fixed point of the non-linear system to be the following relation shown in equation 14.

$$[IC]^* = \sqrt{\frac{V_{HCR}H}{d_{BA}(\alpha K_{HCR}+H)(1-2\xi\eta)}} \quad (14)$$

Again, the effect of bile acid resins (ξ) has a decreasing effect on the concentration by reducing the amount of cholesterol that is recycled back into the hepatocytes (as determined by η). Unlike α, the value of ξ will decrease with increasing bile acid resins showing an inverse proportion. We can also look at experimental data to determine a calibration curve for the values of ξ.

6 Preliminary Bibliography and References

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