A Model of Cholesterol Metabolism and Transport

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Abstract

The need to combat heart disease, the number one cause of death in the United States, has called for greater understanding of cholesterol metabolism. Low and high density lipoprotein (LDL and HDL respectively) have been implicated in the incidence of heart disease and has driven recent research and mathematical models. In this report, we expand on current models of cholesterol metabolism focusing primarily on lipoprotein subfractions and turn to the intracellular workings of hepatocytes and peripheral cells to develop a nine-dimensional, non-linear model. The *de novo* cholesterol synthesis and bile acid synthesis, two targets of cholesterol lowering drugs which have been shown to prevent heart disease, are included in the model. Sensitivity analysis is performed on the model to determine that both *de novo* synthesis parameters and bile acid parameters are key parameters that affect lipoprotein concentrations. The model is also used to simulate cholesterol metabolism disorders as a verification of the model and to ensure that the behavior of the model correlates with clinical observations.

Acknowledgments

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(http://openwetware.org/wiki/User:Johnsy/Lipoprotein_Modelling)

Contents

1 Introduction **6**

2 Biology and Biochemistry of Cholesterol **8**
2.1 Cholesterol 8
2.2 Cholesterol Homeostasis 10
2.3 Bile Acid Biosynthesis 12
2.4 Lipoproteins and Cholesterol Internalization 13
2.4.1 LDL and LDL Receptors 18
2.4.2 HDL and Reverse Cholesterol Transport 19

3 The Current Model of Lipoprotein Metabolism **21**
3.1 Introduction 21
3.2 VLDL Modelling 21
3.3 IDL Modelling 23
3.4 LDL Modelling 23
3.5 LDL Receptor Modelling 24
3.6 Intracellular Cholesterol (IC) Modelling 25
3.7 HDL Modelling 26
3.8 Summary 26

4 Modelling *De Novo* Cholesterol Synthesis **27**
4.1 Introduction 27
4.2 The Model 28
4.3 Assumptions 28
4.4 Parameters 29
4.5 Justification of the Genetic Component 29
4.6 Quasi Steady State Approximation 31

5 Modelling Bile Acid Biosynthesis **32**
5.1 Introduction 32
5.2 The Model 32
5.3 Assumptions in Cholesterol Conversion 33
5.4 Enterophepatic Circulation Assumptions 34
5.5 Parameters 35
5.6 Justification of the Genetic Component 35
5.7 Quasi Steady State Approximation 37

6 A Model of the Hepatocyte **39**
6.1 Introduction . . . 39
6.2 The Model . . . 40
6.3 Parameters . . . 41
6.3.1 Lipoprotein Parameters . . . 41
6.3.2 *De Novo* Synthesis Parameters . . . 42
6.3.3 Bile Acid Synthesis Parameters . . . 42

7 A Model of a Peripheral Cell **44**
7.1 Introduction . . . 44
7.2 The Model . . . 44
7.3 Parameters . . . 45
7.3.1 Lipoprotein Parameters . . . 45
7.3.2 *De Novo* Synthesis Parameters . . . 46

8 A Model of the Blood Plasma **47**
8.1 Introduction . . . 47
8.2 The Model . . . 47
8.3 Parameters . . . 49

9 Statins and Bile Acid Binding Resins **50**
9.1 Statins . . . 50
9.1.1 Regulation of HMG-CoA Reductase . . . 50
9.1.2 Statin Models . . . 51
9.1.3 Changes to the Model . . . 52
9.2 Bile Acid Binding Resins . . . 53
9.2.1 The Model . . . 53

10 Model Analysis **54**
10.1 Time Analysis of the Model . . . 54
10.2 The Model at Equilibrium . . . 54
10.3 Sensitivity Analysis of the Model . . . 58
10.3.1 *De Novo* Synthesis Parameters . . . 58
10.3.2 Bile Acid Biosynthesis Parameters . . . 61
10.3.3 Bile Acid Recycling Parameters . . . 62
10.3.4 Other Parameters . . . 64
10.4 Statin Effects . . . 67
10.4.1 Statins in the Low Cholesterol Regime . . . 67
10.4.2 Statins in the High Cholesterol Regime . . . 69
10.5 Bile Acid Binding Resin Effects . . . 70
10.5.1 Resins in the Low Cholesterol Regime . . . 70
10.5.2 Resins in the High Cholesterol Regime . . . 70
10.6 Resins and Statins . . . 73
10.7 Modelling Daily Cholesterol Intake . . . 74
10.8 Disorders of Cholesterol Metabolism . . . 75
10.8.1 Tangier Disease . . . 75
10.8.2 Familial Hypercholesterolemia . . . 76
10.9 Cholesterol Degradation Effects . . . 77

11 Conclusion and Future Work **80**
11.1 Conclusion 80
11.2 Recommendations for Further Research 81

Chapter 1

Introduction and Motivation

Mathematical models have helped shape our current understanding of the way most of our world works, especially in the physical and macromolecular world where classical physics and Newtonian mechanics has dominated for several centuries. However, there is a strong drive to seek mathematical models to explain biological phenomena. Well established engineering fields such as electrical engineering or mechanical engineering have relied on mathematical models, but due to the dynamic nature of biological systems, modeling them is more difficult.

The health care industry has grown over the past decade, and the US alone spends roughly 16% of its GDP, equivalent to over $2 trillion per annum and is expected to grow at 6% (US Health & Human Services Data). With an increasing population, advancements in health care, and under changing health care consumer demands, the medical industry can no long afford to wait for serendipitous events to occur such as the discovery of the small pox vaccine or penicillin. By creating and building on simple mathematical models to reflect the physiology of the human body, we hope to reduce the research and development costs and times in the future and more precisely understand drug interactions through the use of technology. Although the gold standard of mathematical biology and systems biology is to create a complete model of all the pathways in the human body and be able to input molecular structures of drugs to test, we are far from achieving this. In the meantime, we can create models to simulate certain pathways and hopefully gain a more complete understanding of the effects of perturbations in the system.

Lipoprotein metabolism is of significance and should be studied because of its strong link to atherosclerosis and coronary heart disease (CHD), now the number one cause of death in the United States. Previous compartmental models of lipoprotein metabolism have been formulated, but they are fit to clinical data and do not fully reflect the biology and biochemistry behind the processes (Pont, 1998; Cobelli, 1987). Newer models of lipoprotein metabolism have focused on the macromolecular physiology of lipoproteins, mainly the production, delivery, and degradation of these particles and how intracellular cholesterol levels are affected by this pathway (August, 2007). However, lipoproteins are not the only source of cholesterol and the importance of this pathway varies with diet

and plasma cholesterol levels (Meddings, 1986). The other important pathway which contributes to intracellular cholesterol levels is the *de novo* pathway where cholesterol is synthesized from the precursor acetyl-CoA through a series of enzyme-dependent steps known as the HMG-CoA reductase pathway (Voet, 2004).

Because of its link to increasing coronary heart disease incidence levels, the lipoprotein levels, namely low-density lipoproteins (LDL) and high density lipoproteins (HDL) have been studied extensively. Drugs used to reduce levels of "bad" LDL cholesterol and raise levels of "good" HDL cholesterol have surfaced in the past few decades and remain as the major weapon in the arsenal against CHD. HMG-CoA reductase inhibitors (statins), drugs which have been clinically shown to decrease the LDL plasma cholesterol concentrations significantly, affect the *de novo* cholesterol synthesis pathway by inhibiting the rate-determining enzyme, HMG-CoA reductase. The biochemistry and structure of the enzyme is further discussed in the Biology and Biochemical Background section.

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, degradation, and transport of cholesterol and to understand the current models of lipoprotein metabolism which have recently been published. Then, we aim to expand the model to more accurately fit the biochemistry involved, especially incorporating the *de novo* biosynthesis of cholesterol and its degradation to bile acids in the liver.

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 and if the findings correlate well with previous models. 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 blood? We hope to be able to answer these questions through analysis of our model.

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.

Chapter 2

Biology and Biochemistry of Cholesterol

2.1 Cholesterol

Cholesterol is essential to all cells and living organisms. It is mainly incorporated into the plasma membranes of cells and regulates membrane fluidity conferring a higher degree of rigidity. Furthermore, it is the precursor of all steroid hormones and bile acids produced.

Cholesterol (Figure 2.1) is a 27 carbon compound derived from acetate. In all cells, this *de novo* synthesis pathway can be used as a method of producing cholesterol from naturally found precursor molecules. Acetyl-CoA, the two carbon precursor to all sterol molecules, is first converted to hydroxymethylglutaryl-CoA (HMG-CoA) by the action of two enzymes, thiolate and HMG-CoA synthase. HMG-CoA is then converted to mevalonate, another major intermediate in the cholesterol biosynthesis pathway, by the action of HMG-CoA reductase, the rate limiting step of cholesterol synthesis. Through a series of other intermediates, mevalonate (a 6 carbon compound) is converted to cholesterol, ubiquinone, dolichol, geranyl-geranylated proteins, and farnesylated proteins. (Voet, 2004)

Figure 2.2 outlines the steps in the synthesis of cholesterol from acetyl-CoA as well as listing all of the major enzymes involved. It is a truly amazing pathway that a simple two carbon molecule can be converted to a 27 carbon molecule

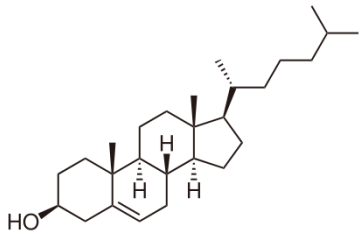

Figure 2.1: Chemical Structure of Cholesterol

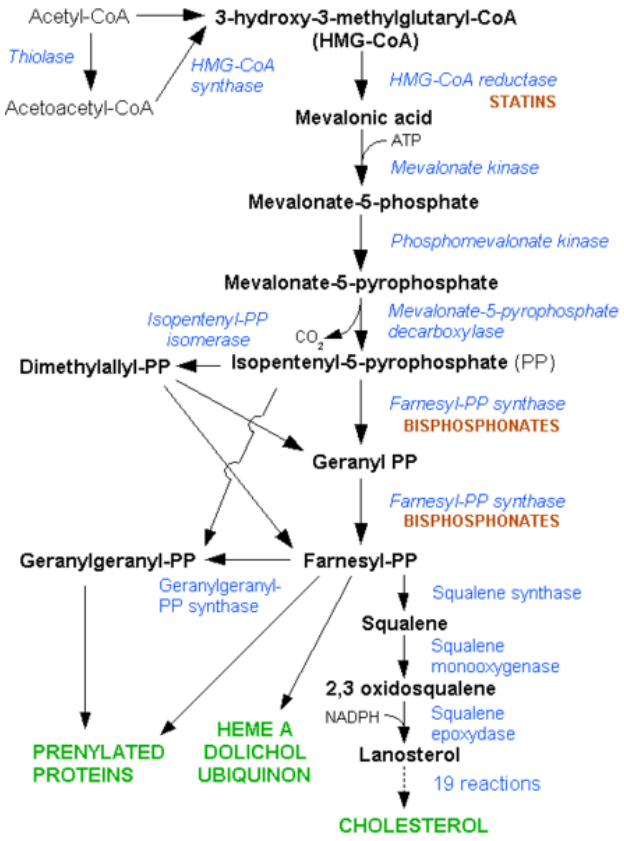

Figure 2.2: HMG-CoA Reductase Pathway

with a complex 4-ring structure in 30 enzymatic steps.

2.2 Cholesterol Homeostasis

The maintenance and regulation of cholesterol is important to the body as a means of preventing excess cholesterol from building up. Atherosclerosis and gall stone formation are just a couple of examples of what could happen when cholesterol is not effectively regulated and the need for continuous medication later in life results if homeostasis is not met.

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 (White, 1984).

HMG-CoA reductase is the enzyme governing the rate determining step of cholesterol synthesis, the conversion of HMG-CoA to mevalonate (see Figure 2.2). 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 remains attached when cholesterol levels are high (see figure 2.4). A decrease in cholesterol will cause the membrane protein to be cleaved and SREBP to enter the nucleus. Once inside the nucleus, SREBP binds to sterol regulatory elements (SRE) to initiate transcription of LDL receptors and HMG-CoA reductase enzyme in an attempt to increase the intracellular cholesterol levels. (Brown, 1997; Bischoff, 1992; Istvan, 2000; Rodwell, 2000; Smythe, 1998)

SREBP regulation is thus important in lipoprotein metabolism since it regulates the transcription of genes necessary for the synthesis of fatty acids and cholesterol. 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 without regulation. (Brown, 1997) The incorporation of SREBP into our model represents the genetic component of the control within our metabolic pathway. Few, if any, models combining genetic regulation with metabolic processes has ever been achieved due to the complexity involved as well as lack of concrete parameters.

SREBP is a membrane bound protein possessing two cleavage sites (see figure 2.3 below). Cleavage at site 1, the required sterol regulated 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 to facilitate transcription of cholesterol synthesizing genes. (Brown, 1997)

SREBP itself is regulated through the action of SCAP (SREBP cleavage

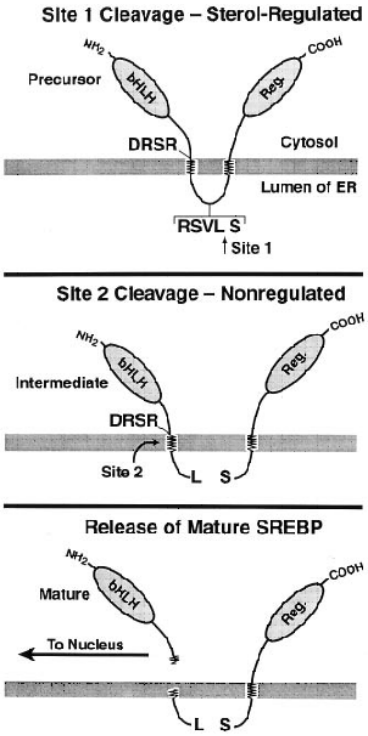

Figure 2.3: SREBP Cleavage Mechanism (Reproduced From Brown, 1997)

activating protein) which has a sterol-sensing domain and Insig-1 (see figure 2.4). In the presence of sterols, Insig-1 is kept bound to SCAP which is then unable to activate cleavage of the SREBP protein. In the absence of sterols, Insig-1 is no longer bound to SCAP and it can then activate cleavage of SREBP. This releases nuclear SREBP which migrates to the nucleus to activate gene expression, resulting in an increase in enzyme levels for cholesterol biosynthesis. (Brown, 1997; Lodish, 2003)

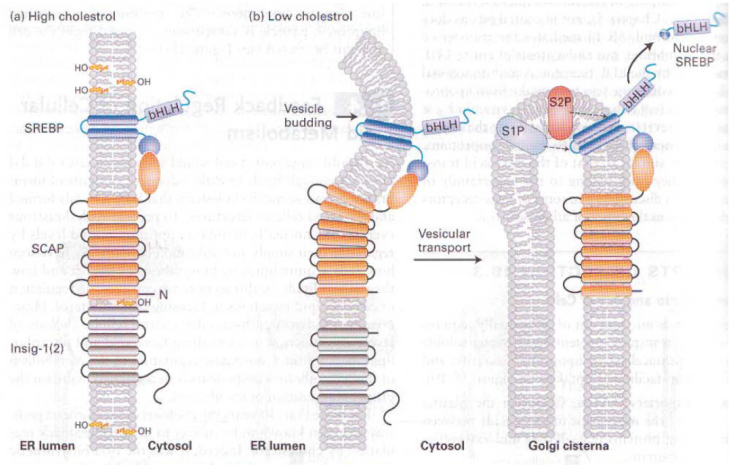

Figure 2.4: Insig-1, SCAP, and SREBP work together to regulate transcription of cholesterol synthesizing genes (Reproduced from Lodish, 2003)

2.3 Bile Acid Biosynthesis

The esterification and removal of free cholesterol is also a critical method by which cholesterol levels in the cell are regulated. The main method of this removal is the conversion of cholesterol to bile acids (also known as bile salts) in the liver (figure 2.5). The bile acids are secreted from the hepatocytes and stored in the gall bladder before being excreted 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 one of the only exits of cholesterol from the body, making it an important mechanism to maintain cholesterol homeostasis. The other major exit of cholesterol from the body is the removal of dead skin cells from the epidermis.

The production of bile acid is initiated by the cholesterol 7α hydroxylase enzyme (CYP7A1, or C7H as used in this report), the first and rate limiting step of the metabolic pathway (see figure 2.6 for the pathway).

Once the bile acids are produced, they are excreted from the cell via an ATP Binding Cassette (ABC) transporter ABCB11, also known as the bile salt export pump (BSEP) (Trauner, 2003). The bile acids are then stored in the gall bladder and are released during digestion. The release of the stored bile acids is regulated by the hormone cholecystokinin (CCK). The bile acids are then reabsorbed in the ileum by a sodium ion dependent bile salt transporter (ISBT).

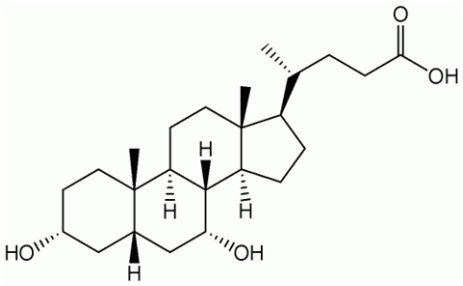

Figure 2.5: Structure of chenodeoxycholic acid, the most common bile acid found in the human body

Once reabsorbed, they travel through the hepatic portal vein and are returned to the liver where hepatocytes lined with bile salt transporters take up the bile acids to be recycled. Figure 2.7 summarizes the enterohepatic circulation as described above.

It is estimated that the human bile salt pool circulates 6-10 times per day with a daily excretion of about 20-40 g of bile salts. Approximately 0.5 g of bile salts are lost through fecal excretion each day. (Trauner, 2003)

The bile acids themselves function as negative inhibitors and also have an indirect effect on the production of cholesterol. The bile acids bind to nuclear hormone receptors which, similar to SREBP, regulate the gene expression of enzymes required for the bile acid synthetic pathway. (Trauner, 2003) If bile acid pools are low, for example if they are being depleted quickly and not recycled, then this activates the production of more enzymes facilitating the conversion of cholesterol to bile acids. The feedback to cholesterol occurs at this step. Once cholesterol levels are depleted in the body due to its conversion of bile acids, then this triggers SREBP to activate the *de novo* synthesis pathway and allow the production of receptors to take in LDL and IDL particles from the plasma. This will in turn push up the cholesterol pools in the hepatocytes such that equilibrium is once again reached.

2.4 Lipoproteins and Cholesterol Internalization

Within the cell, cholesterol is also kept at homeostasis by the action of LDL receptors found in almost all the cells in the body. They serve as flag for lipoprotein internalization and are also regulated through a genetic component via SREBP. These function as communicators with the blood plasma and in the liver, aid in globally regulating the plasma cholesterol pool. In peripheral cells, LDL receptors serve as an easy method by which the cell can gain access to the plasma cholesterol pool so that *de novo* synthesis does not need to occur. A cell would rather internalize cholesterol if available rather than expending energy to produce it. Studies have shown that lipoprotein internalization is accompanied by a decrease in *de novo* synthesis in all cells (Brown, 1977).

Lipoproteins (see figure 2.8) themselves are the means of transferring cholesterol throughout the body from where it is internalized from the diet to where it is needed. There are five main classes of lipoproteins distinguished by their size, density, contents, and surface proteins: chylomicrons, very low density lipopro-

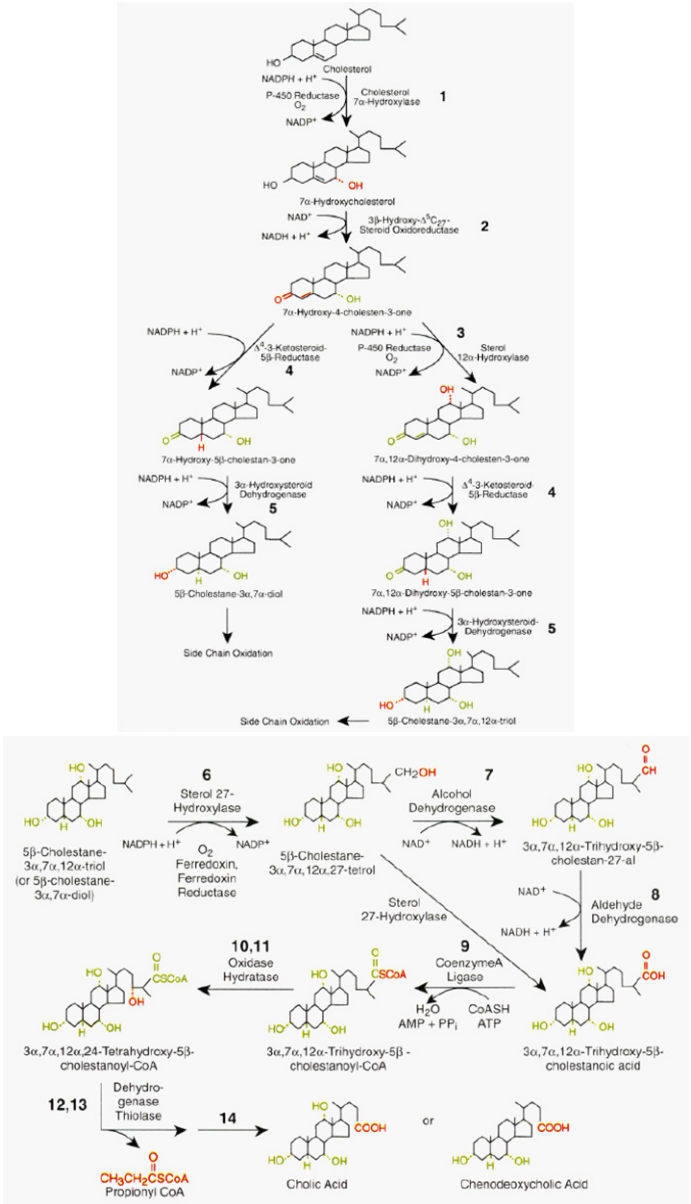

Figure 2.6: Bile Acid Biosynthesis Pathway

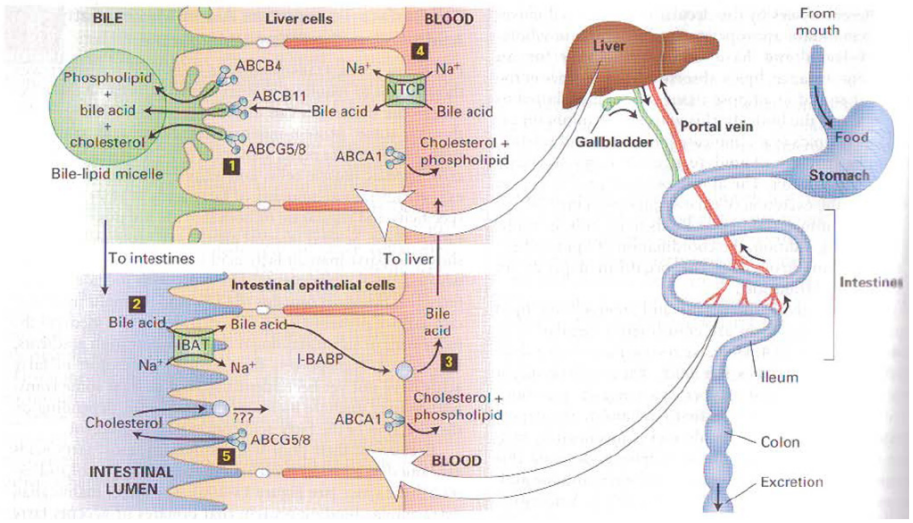

Figure 2.7: Major transport proteins in the liver and intestine taking part in the enterohepatic circulation of biliary lipids (Reproduced from Lodish, 2003)

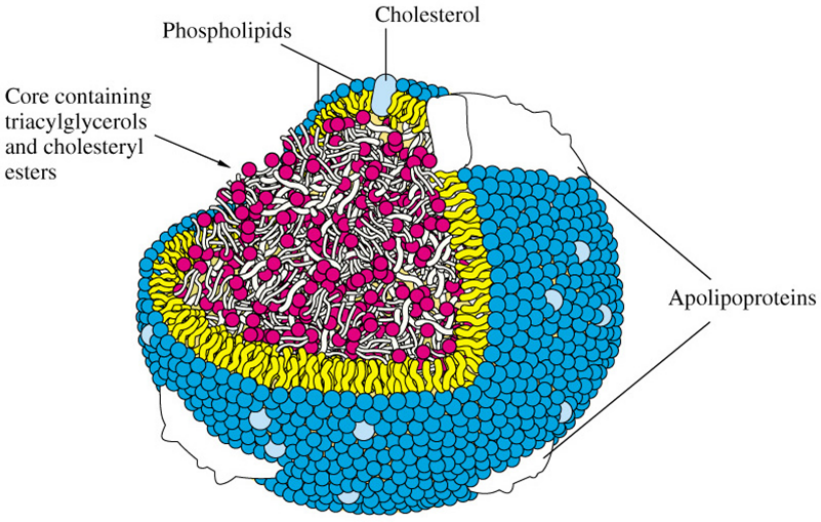

Figure 2.8: The Typical Structure of Lipoprotein

teins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins (LDL), and high density lipoproteins (HDL) (see Table 2.2 for a comparison between the different lipoproteins).

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 (Feher, 1997).

- **Phospholipid monolayer (PL)** - serves as a barrier between the hydrophobic interior containing triglycerides and cholesterol esters and the hydrophilic exterior defined by the extracellular fluid or blood plasma.
- **Apoproteins** - proteins which span the phospholipid monolayer and act as signaling molecules to identify the contents and type of lipoprotein. Each type of lipoprotein has a different apoprotein coat distinguishing them from each other. Apoproteins also serve to interact with the outside aqueous environment as receptor ligands.
- **Free cholesterol (FC)** embedded on the phospholipid monolayer whose polar alcohol groups on the unesterified cholesterol project into the aqueous environment.
- **Triglycerides (TG)** - one method by which fatty acids and lipids are transported, usually contained in the core of the lipoprotein. Different lipoprotein classes contain different proportions of triglycerides and cholesterol esters.
- **Cholesterol Esters (CE)** - esterified cholesterol attached to fatty acids (e.g. cholesterol linoleate) utilizing the hydroxyl group on cholesterol to form the ester bond. Cholesterol is transported mainly as cholesterol esters.

Lipoprotein	Cholesterol concentration g/l
[HDL-C]	0.5
[LDL-C]	1.25
[IDL-C]	0.2
[VLDL-C]	0.1

Table 2.1: Average Plasma Lipoprotein Cholesterol concentration for normolipidemic individuals (August, 2007)

In their transport of cholesterol around the body, the lipoproteins undergo four different types of modifications (Lodish, 2003) as seen in figure 2.9 below. In figure 2.9a, (1) the liver secretes VLDL particles which are hydrolyzed (2) by lipoprotein lipase to form IDL particles (3). The IDL particles are then converted to LDL particles (4), and are either taken up through endocytosis by LDL receptors in the liver (5) or in extrahepatic cells (6).

The transfer of dietary lipids (as seen in figure 2.9b) occurs through the use of chylomicrons. The dietary lipids are absorbed by the intestinal cells (1) which secrete chylomicrons (2). The chylomicrons undergo remodeling by lipoprotein lipase (3) forming smaller, cholesterol enriched chylomicron remnants (4). These are then taken up by the liver through receptor-mediated endocytosis.

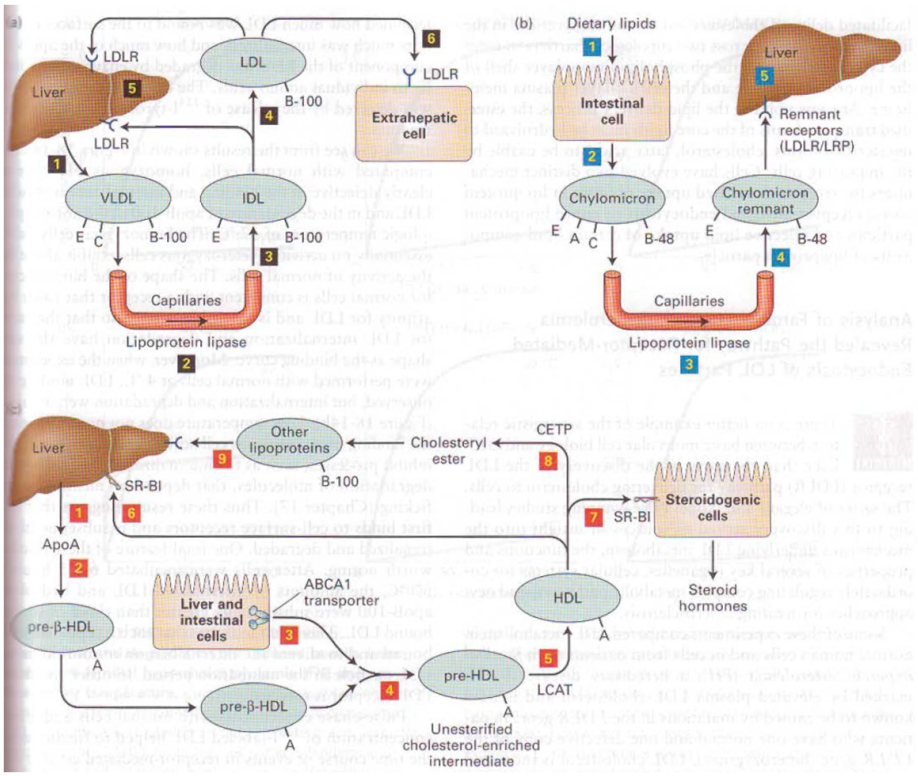

Figure 2.9: Lipoprotein remodeling and interconversions in the circulatory system (Reproduced from Lodish, 2003)

The reverse cholesterol transport utilizing HDL is shown in figure 2.9c. The liver first secretes pre-HDL particles (1-2) which are loaded via the ABCA1 transporter (3-4) with cholesterol from mature pre-HDL. Lecithin:cholesterol acyl transferase (LCAT) esterifies the cholesterol after its incorporation into HDL forming mature HDL (5). The cholesterol within HDL is then transferred back to the liver or steroidogenic cells (6-7) through the SR-B1 transporter. The cholesterol can also be transferred to other lipoproteins (mainly VLDL and LDL) through cholesterol ester transfer protein (CETP) (8). These other lipoproteins can then be taken up by the liver through receptor mediated and non-mediated endocytosis (9).

2.4.1 LDL and LDL Receptors

LDL particles contain the highest percentage of cholesterol (Table 2.2) and possessing a high plasma concentration of them has been implicated in the genesis of atherosclerosis. LDL particles are normally cleared through receptor mediated endocytosis in the liver (Figure 2.10). Once they are internalized, they are fused to lysosomes to be broken down and the cholesterol contained within them is transferred to the intracellular cholesterol pool by the function of the enzyme ACAT (Brown, 1979).

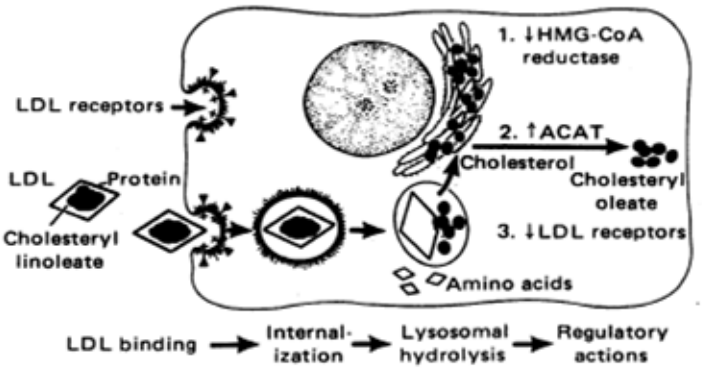

Figure 2.10: Internalization and degradation of LDL to release the contained cholesterol (reproduced from Brown, 1979)

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. (see figure 2.11) Although coated pits account for only 2% of the cell surface area, they contain approximately 50-80% of the LDL receptors (Brown, 1979). 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 an increase cholesterol concentration in the cell. Although research has shown that LDL particle internalization can be

-	**Chyl**	**VLDL**	**IDL**	**LDL**	**HDL**
Density g/ml	<0.95	0.95-1.006	1.006-1.019	1.019-1.063	1.063-1.21
Diameter nm	80-100	30-80	25-30	20-25	8-13
TG content %	90-95	50-65	35-40	4-6	7
CE %	2-4	8-14	20-35	34-35	10-20
FC %	1	4-7	7-11	6-15	5
PL %	2-6	12-16	16-24	22-26	25
Protein %	1-2	5-10	12-16	22-26	45
Major proteins % of total protein	A-1(31) C(32) E(10) B-48(5-8)	C(40-50) B-100(30-40) E(10-15)	B-100(60-80) C(10-20) E(10-15)	B-100(>95) C(<1) E(<1)	A-I(65) A-II(10-23) C(5-15) E(1-3)

Table 2.2: Lipoprotein structure

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 (Meddings, 1986). From patients with familial hypercholesterolemia with a phenotype showing the absence of LDL receptors, there was no detectable internalization of LDL particles suggesting that this phenomenon on receptor-independent internalization does not occur in humans (Brown, 1979). For the purposes of the model and to be consistent with previous models developed, we continue to assume that non-receptor mediated endocytosis of LDL particles does occur in humans.

2.4.2 HDL and Reverse Cholesterol Transport

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 (Sviridov, 2002). The protein cholesterol ester transfer protein (CETP) is responsible for this redistribution of cholesterol esters and triglycerides from the HDL particle to other lipoptein subfractions (Rye, 1999).

Apart from the HDL holoparticle being internalized in the liver, cholesterol can also be transferred out of the HDL particle to the hepatocyte by the action of SR-B1 proteins lining the hepatocyte. Effectively, they allow transfer of cholesterol from the HDL particle to the plasma membrane of the hepatocyte without destroying the HDL particle. The particle can then continue around the blood stream to collect more cholesterol or the HDL remnants can be taken up by the liver as a holoparticle (via receptor mediated endocytosis) (Lewis, 2005).

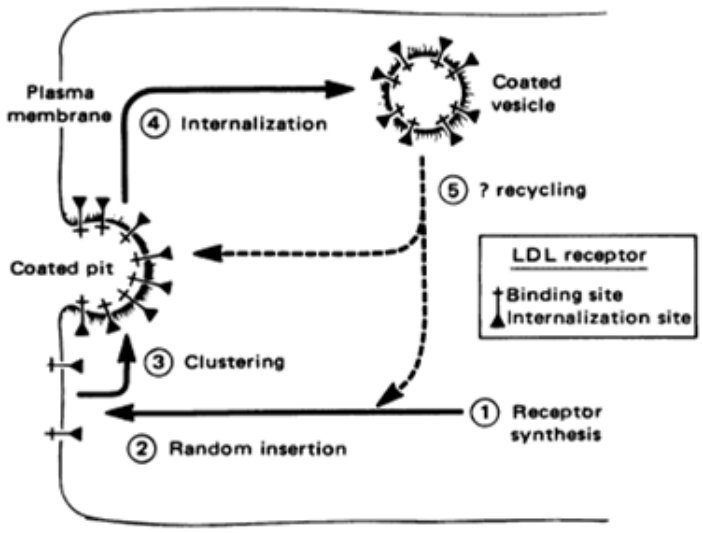

Figure 2.11: Receptor clustering, internalization and recycling (reproduced from Brown, 1979)

Chapter 3

The Current Model of Lipoprotein Metabolism

3.1 Introduction

Two models have been developed with regards to lipoprotein metabolism. The first is a general model (August, 2007) which attempted to address the problems with compartmental models developed in the past, namely to incorporate the biology behind lipoprotein metabolism phenomena and to see if the clinical data gathered from previous studies fit the model. The second model developed by Kathryn Cooper (Cooper, 2006) attempts to incorporate reverse cholesterol transport into the model as well as to satisfy mass conversation by considering the cholesterol content of the lipoproteins instead of a concentration of lipoprotein particles. The second model is described here as we will base our new model on this. A third model was also recently developed but focuses highly on the LDL intake and cholesterol receptor numbers instead of a general overview of lipoproteins (Panovska, 2006). The incorporation of clathrin coated pits and its better accuracy at describing the biochemistry involved in LDL internalization make this third model a possible extension to the model developed in this paper.

The system of equations of Kathryn's model is shown below derived from figure 3.1. A few key assumptions are made in this model that are carried forward to in the development of the model presented in this paper. We will go through each equation in turn describing the different assumptions made and the biochemistry behind each term.

3.2 VLDL Modelling

$$\begin{aligned}\frac{d[VLDL-C]}{dt} =& u_v - k_v[VLDL-C] \\ &+ c_v k_{cetp}[HDL-C][VLDL-C]([HDL-C]-a_v[VLDL-C])\end{aligned} \tag{3.1}$$

For the VLDL equation, it is assumed that the hepatocytes excrete VLDL particles at a constant rate leading to the first source term. Although within

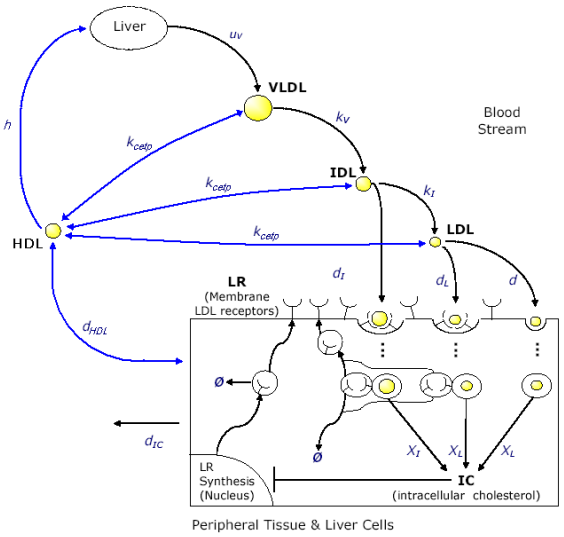

Figure 3.1: A Simplified View of Lipoprotein Metabolism (Reproduced from Kathryn Cooper)

hepatocytes the actual production of VLDL is strongly dependent on the intracellular cholesterol concentration, Kathryn's model only looks at peripheral cells and does not make any assumptions regarding hepatocytes. It is for this reason that the VLDL production is assumed constant and is not dependent on the intracellular cholesterol of the peripheral cell. VLDL is effectively lost in its remodelling into IDL by the action of lipoprotein lipase and is dependent upon the concentration of VLDL particles. The other source term represents the transfer of cholesterol from HDL to VLDL particles through CETP. One crucial assumption that is made is in the development of this HDL transfer term. In order for cholesterol transfer to occur, both particles must collide, represented by the multiplication of the VLDL-C and HDL-C terms. Furthermore, the transfer of cholesterol is dependent upon the difference in the cholesterol concentrations between the two particles. The factor a_v is a coefficient representing the weighting of the cholesterol gradient which determines the rate of cholesterol transfer. The inclusion of the c_v term in the equation represents the difference in the rate of transfer between different lipoprotein particle types. Each of the lipoprotein governing equations below have a similar term. Although this does not significantly affect the model, it is included for completeness in biochemical modelling.

Parameters used in the VLDL Model:

- u_v – Constant production of VLDL representing the input of cholesterol into the model from either the diet or *de ovo* synthesis. This parameter was swept between 0.0 and 3.0 $g(lh)^{-1}$ (August, 2007), which represents the dietary intake of cholesterol
- k_v – Fractional turnover rate of VLDL representing the conversion of VLDL to IDL - 0.3 h^{-1} (Packard, 2000)

- k_{cetp} – Rate of action of cholesterol ester transfer protein shuttling cholesterol from HDL to VLDL - 0.1 $gl^{-1}h^{-1}$ (Cooper, 2006)
- a_v – Attenuation coefficient for the cholesterol gradient - 2.5 (no units) (derived from the typical equilibrium concentrations of the cholesterol within HDL and VLDL)
- c_v – the rate of cholesterol transport from HDL to VLDL (the value of this parameter has no effect on the final model and is just included for completeness)

3.3 IDL Modelling

$$\begin{aligned}\frac{d[IDL-C]}{dt} =& k_v[VLDL-C] - k_i[IDL-C] \\ &+ c_i k_{cetp}[HDL-C][IDL-C]([HDL-C] - a_i[IDL-C]) \\ &- d_i[IDL-C]\phi_{LR}\end{aligned} \tag{3.2}$$

For the IDL equation, the main source term is the conversion of VLDL to IDL with another term describing the transfer of cholesterol from HDL to IDL with the help of CETP. The IDL particles are lost either by remodelling into LDL particles or by receptor-mediated endocytosis into cells. This receptor-mediated endocytosis occurs in both hepatic cells as well as extrahepatic cells. It is assumed the IDL is internalized in a similar fashion in both hepatocytes and peripheral cells.

Parameters used in the IDL Model (not introduced before):

- k_i – Fractional turnover rate of IDL representing the conversion of IDL to LDL - 0.05 h^{-1} (Packard, 2000)
- a_i – Attenuation coefficient for the cholesterol gradient - 5 (no units) (derived from the typical equilibrium concentrations of the cholesterol within HDL and IDL)
- d_i – receptor mediated endocytosis rate of internalization of IDL - 2 h^{-1} (August, 2007)
- c_i – the rate of cholesterol transport from HDL to IDL - (the value of this parameter has no effect on the final model and is just included for completeness)

3.4 LDL Modelling

$$\begin{aligned}\frac{d[LDL-C]}{dt} =& k_i[IDL-C] - d_l[LDL-C]\phi_{LR} - d[LDL-C] \\ &+ c_l k_{cetp}[HDL-C][LDL-C]([HDL-C] - a_l[LDL-C])\end{aligned} \tag{3.3}$$

For the LDL equation, the main source terms are the conversion of IDL to LDL and, like before, the transfer of cholesterol from HDL to LDL. LDL particles are also internalized into cells by receptor-mediated endocytosis. Furthermore, research has elucidated that LDL particles can also be internalized through non-receptor pathways. Although there is no concrete evidence that this occurs in humans, studies have shown that this phenomenon occurs in other animals and researchers have assumed that it does occur in humans (Dietschy, 1993; Meddings, 1986). It should be noted that the non-receptor internalization accounts for a very small fraction of LDL particles internalized into the cells with receptor-mediated endocytosis being the main driving force. This is evident in the differences between the rate coefficients. It is assumed that all the LDL is internalized in a similar fashion in both hepatocytes and peripheral cells.

Parameters used in the LDL Model (not introduced before):

- d_l – receptor mediated endocytosis rate of internalization of LDL - 0.01 h^{-1} (August, 2007)
- d – internalization of LDL particles not regulated by receptor mediated endocytosis - 0.0075 h^{-1} (Dietschy, 1993)
- a_l – Attenuation coefficient for the cholesterol gradient - 0.4 (derived from the typical equilibrium concentrations of the cholesterol within HDL and LDL)
- c_l – the rate of cholesterol transport from HDL to LDL (the value of this parameter has no effect on the final model and is just included for completeness)

3.5 LDL Receptor Modelling

$$\begin{aligned}\frac{d[\phi_{LR}]}{dt} = &- b(d_i\frac{[IDL-C]}{\chi_I} + d_l\frac{[LDL-C]}{\chi_L})\phi_{LR} \\ &+ c\frac{1-\phi_{LR}}{[IC]}\end{aligned} \tag{3.4}$$

For the receptor equation, the concentration of cholesterol must again be converted to a concentration of particles since we assume that it is the particles that are binding to the receptors while the internal cholesterol concentration within each lipoprotein has no bearing on the rate of internalization. The receptors are recycled back to the plasma membrane if cholesterol internalization is not sufficient for the needs of the cell and the actual number of receptors is genetically regulated through the action of SREBP (as discussed in the Biology and Biochemistry section).

Furthermore, the assumption is also taken that we are neglecting the effect of clathrin coated pits in our model and assuming that there is an equal chance of a lipoprotein attaching to a receptor at any given time. However, it has been shown experimentally that cells which do not express clathrin and are not able to form the coated pits are not able to internalize lipoproteins (Brown, 1979).

A derivation of the genetic regulatory component of the receptor recycling can be found in a separate paper (August, 2007).

Parameters used in the LDL Receptor Model (not introduced before):

- b – internalization rate of lipoproteins through LDL receptors (includes both IDL and LDL) - 0.1 (August, 2007)
- χ_I – Proportional cholesterol contained in IDL - 0.35 (representing the fraction of cholesterol esters and free cholesterol contained within the lipoprotein) (Adiels, 2002, August, 2007)
- χ_L – Proportional cholesterol contained in LDL - 0.45 (Adiels, 2002, August, 2007)
- c – receptor recycling rate - 0.05 $g(lh)^{-1}$ (August, 2007, Goldstein, 1977)

3.6 Intracellular Cholesterol (IC) Modelling

$$\begin{aligned}\frac{d[IC]}{dt} =&(d_i[IDL-C]+d_l[LDL-C])\phi_{LR}\\ &+d[LDL-C]\\ &-d_{HDL}[HDL-C]([IC]-p[HDL-C])\\ &-d_{IC}[IC]\end{aligned} \tag{3.5}$$

For the intracellular cholesterol equation, Kathryn assumes the model for a peripheral cell and not for the hepatocyte, however both cells internalize IDL and LDL in the same manner. The only difference is that there is a net loss of cholesterol to HDL from peripheral cells in contrast to a net gain from cholesterol from HDL in hepatocytes (as a result of HDL remodelling and endocytosis). Hence, the sink term for the transfer of cholesterol from the cell to HDL particles via ABCA1 is present in this model. The other degradation term for cholesterol is a general degradation term representing the utilization of cholesterol in the plasma membrane or for other cellular activities (such as production of steroid hormones in endocrine glands or production of bile salts). It should be noted that bile salts, although produced in very small quantities in peripheral cells, are mainly produced in the liver as one of the only means by which cholesterol is excreted from the body.

Since the use of cholesterol is different in both hepatocytes and peripheral cells, this equation will be different for our model. Furthermore, the *de novo* synthesis of cholesterol is not expressed here in Kathryn's model and it is assumed that all of the cholesterol is derived from lipoproteins. During feeding, this is probably the case, especially for high lipid western diets; however, *de novo* cholesterol synthesis does represent a major control step in the metabolic pathway.

Parameters used in the Intracellular Cholesterol Model (not presented before):

- d_{HDL} – transfer rate of cholesterol from peripheral cells to HDL - 0.1 h^{-1} (Johnson, 1988)

- p – Attenuation coefficient for cholesterol transfer to HDL from peripheral cells - 0.5 (derived from the typical equilibrium concentrations of the cholesterol within HDL and the intracellular cholesterol concentration)
- d_{IC} – degradation rate of cholesterol to bile acids and other cholesterol derivatives - 0.5 h^{-1} (an estimate)

3.7 HDL Modelling

$$\begin{aligned}\frac{d[HDL-C]}{dt} =& d_{HDL}[HDL-C]([IC]-p[HDL-C]) \\ &+ c_v k_{cetp}[HDL-C][VLDL-C]([HDL-C]-a_v[VLDL-C]) \\ &+ c_i k_{cetp}[HDL-C][IDL-C]([HDL-C]-a_i[IDL-C]) \\ &+ c_l k_{cetp}[HDL-C][LDL-C]([HDL-C]-a_l[LDL-C]) \\ &- h[HDL-C] \end{aligned} \tag{3.6}$$

For the HDL equation, the source term is only the transfer of cholesterol from peripheral cells via ABCA1 with the sink terms being the transfer of cholesterol from HDL to other lipoproteins as discussed above. Furthermore, one additional internalization term is added representing the transfer and endocytosis of HDL particles within the liver cells. There are two main methods by which the cholesterol from HDL particles is incorporated within hepatocytes. First, the cholesterol can be leeched out by SR-B1 receptors which only allow cholesterol transfer from inside the HDL particle directly into the membrane. The plasma membrane then forms vesicles which take the cholesterol into the cell to be used, stored, or excreted. HDL particles can also be taken up by endocytosis similar to IDL and LDL by hepatic endocytotic receptors. Both internalization methods are captured under one rate term in this model and is carried forward to the model that is developed in this paper. It should also be noted that since this model is only concerned with peripheral cells, the removal of HDL particles represents an output of cholesterol from this model.

Parameters in the HDL model (not introduced before):

- h – internalization rate of HDL by the liver - 0.024 h^{-1} (Chetiveaux, 2004)

3.8 Summary

Looking at the broader model as a whole, it can be seen that the introduction of VLDL particles represents the only cholesterol input into the dynamical system while the general cholesterol degradation term and the HDL removal term are the only outputs to the system. For this model, the *de novo* cholesterol synthesis and the action of statins is subsumed under the VLDL input. The biochemistry involved in these processes is more complex and is explored and included in the model developed in this paper.

Chapter 4

Modelling *De Novo* Cholesterol Synthesis

4.1 Introduction

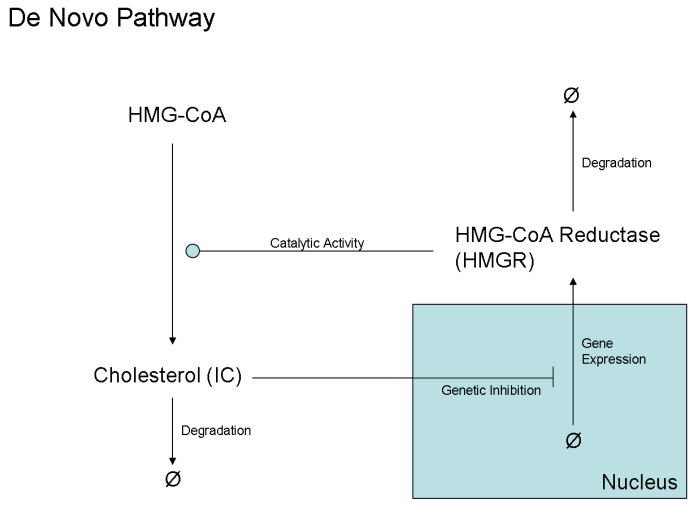

Figure 4.1: *De novo* synthesis of cholesterol pathway

We first start off with a diagram of our pathway showing all of the components that we wish to model in the de novo cholesterol biosynthesis (see Figure 4.1).

Recall that HMG-CoA reductase is the rate limiting step in the *de novo* synthesis of cholesterol and in our model, we assume that this rate is much slower than the other steps in the HMG-CoA reductease pathway. Hence, in order to simplify our model, we have ignored the other steps in the pathway. A further extension to this project would be to take each enzyme in turn and include it into the model to see if the rate limiting step actually dominates and if our assumption that the HMGR catalyzing step of the conversion of HMG-CoA to mevalonte dominates the entire pathway is a reasonable assumption.

Intracellular cholesterol (IC) itself regulates the gene expression of the HMGR enzyme via SREBP. In this model, we have considered that cholesterol acts as an inhibitor of transcription and have reduced our model by taking assumptions discussed later on. As a protein, HMGR enzyme is also degraded by the cell, attenuating the level of intracellular cholesterol if deemed to be enough.

We first outline a model that does not include the effect of HMG-CoA reductase inhibitors (statins), but will include this effect in a later chapter.

4.2 The Model

The system of equations used to model the de novo synthesis is shown below.

The equation for the production of HMG-CoA reductase taking into account the genetic inhibition from the intercellular cholesterol pools.

$$\frac{d[HMGR]}{dt} = \frac{k_1}{b_1 + [IC]} - d_1[HMGR] \tag{4.1}$$

The equation for the production of cholesterol is shown below (not considering the effect of statins).

$$\frac{d[IC]}{dt} = \frac{k_2[HMGR][HMG-CoA]}{k_{m1} + [HMG-CoA]} - d_{ic}[IC] \tag{4.2}$$

4.3 Assumptions

An increase in cholesterol will decrease the activity of the enzyme by reducing the gene expression via the action of SREBP (not shown). This is a gross simplification of the actual mechanism for controlling gene expression of sterol regulatory enzymes but has been taken to reduce the complexity of the model and to obtain a general understanding of the dynamics involved.

We have discarded the rest of the mevalonate pathway and have focused on the rate limiting step of cholesterol synthesis, the HMGR catalyzed step from HMG-CoA to mevalonate. The subsequent steps to form cholesterol from mevalonate are relatively fast and so are the steps from the cholesterol precursor acetyl-CoA to HMG-CoA. We have thus eliminated them from the model to simplify not only the number of parameters, but the species involved. In effect, all the rate constants that are involved in the complete pathway can be incorporated into k_2.

We have also assumed that there is sufficient HMG-CoA concentration for cholesterol production to take place and that the presence of HMGR will be the limiting factor in this enzymatic step. Furthermore, although the Michaelis-Menten model assumes that the enzyme concentration is kept constant, we are allowing the enzyme concentration to change over time. Strictly speaking, the Michaelis-Menten model should be modified, but we take the quasi-steady state approximation assuming that the enzyme will eventually be at equilibrium. This will further allow us to reduce the dimensionality of the model. To test whether or not our assumption regarding HMG-CoA is correct, we will subject this to sensitivity analysis in the Model Analysis chapter.

For now, we have just put in a degradation term, but this is only temporary until we are able to complete the entire model. The degradation term represents

the conversion of cholesterol to steroid hormones and bile acids (in the liver) and other sources of loss such as from sloughing off of the skin cells or the intestinal epithelium.

4.4 Parameters

- k_1 - transcription rate (see below for value)
- b_1 - attenuation factor for the regulation of HMGR by cholesterol (see below for value)
- d_1 - degradation rate of HMGR (see below for value)
- k_2 - rate constant for conversion of HMG-CoA to cholesterol mediated by HMGR (equivalent to k_{cat} value) - 22.6 $sec^{-1} = 79200\, h^{-1}$ (Theivagt,2006)
- k_{m1} - Michaelis-Menten constant for HMGR (using NADPH as substrate)- 20 μM = 0.02 gl^{-1} (Assuming an average HMG-CoA molecule has a molecular weight of 900 gl^{-1}) (Theivagt, 2006)
- d_{IC} - degradation rate of cholesterol to cholesterol derivatives (value irrelevant for developed model)

4.5 Justification of the Genetic Component

The level of HMGR enzyme is controlled by the SREBP transcription factor, but we have just assumed that cholesterol has a direct inhibitory effect on the transcription of HMGR genes and other cholesterol synthesizing genes. Figure 4.2 shows diagrammatically the steps required for the expression of HMGR enzyme.

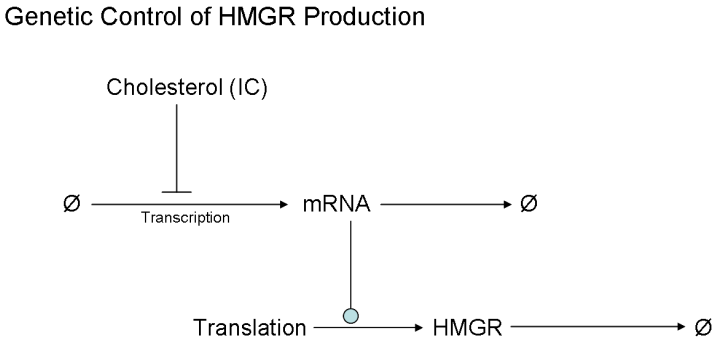

Figure 4.2: Transcription and Translation Pathway for HMGR

The equations used to model this genetic control are shown below.

$$\frac{d[mRNA]}{dt} = \frac{g_1 g_m^n}{g_m^n + [IC]^n} - w_1[mRNA] \tag{4.3}$$

$$\frac{d[HMGR]}{dt} = g_2[mRNA] - w_2[HMGR] \tag{4.4}$$

The parameters of the this model are defined below with relevant values found in literature:

- g_1 – transcription rate - 1 min^{-1} (Barrio, 2006)
- g_2 – translation rate - 1 min^{-1} (Barrio, 2006)
- g_m – DNA Dissociation Rate - 10-100 (no units) (Barrio, 2006)
- n – Hill cooperativity - assumed to be 1 for this case
- w_1 – mRNA degradation rate - 0.029 min^{-1} (Barrio, 2006)
- w_2 – HMGR degradation rate (assumed to be an ordinary protein degradation rate) - 0.031 min^{-1} (Barrio, 2006)

mRNA is produced by the transcription of the HMGR gene and is regulated with an inverse relationship to the concentration of intracellular cholesterol within the cell. The lower the cholesterol level, the higher the mRNA expression. The mRNA also degrades over time to prevent gene expression from continuing even if cholesterol levels are sufficiently high.

It is assumed that there are enough ribosomes and that the mRNA concentrations are the limiting factor in translation. Hence, the actual protein levels of HMRG are dependent upon the amount of mRNA in the cell and are degraded, like any other protein. Although different proteins degrade slightly differently depending upon tagging, we assume that HMGR is an ordinary protein and degrades on the order of average proteins.

We can take the assumption that the levels of mRNA remain constant in the cell to reduce the number of variables (quasi-steady state approximation). Although this may not be an accurate assumption to make, we assume that the mRNA production step is not rate limiting and it is the translation step that will determine the final concentration of enzyme in the cell.

$$\frac{d[mRNA]}{dt} = 0 \tag{4.5}$$

We can come up with the fixed point for the steady state concentration of mRNA and substitute it into our equation for HMGR.

$$[mRNA]^* = \frac{g_1 g_m^n}{w_1(g_m^n + [IC]^n)} \tag{4.6}$$

Now substituting into our equation for HMGR:

$$\frac{d[HMGR]}{dt} = \frac{g_1 g_2 g_m^n}{w_1(g_m^n + [IC]^n)} - w_2[HMGR] \tag{4.7}$$

If we now look at equation 4.1 from above, we can combine our constants together to simplify the number of parameters we have in our model. Below shows the simplified model with reduced parameters and their values calculated from the above parameters.

The Model:

$$\frac{d[HMGR]}{dt} = \frac{k_1}{b_1 + [IC]} - d_1[HMGR] \tag{4.8}$$

$$k_1 = \frac{g_1 g_2 g_m^n}{w_1} = \frac{1\ min^{-1} \times 1\ min^{-1} \times 55}{0.029\ min^{-1}} = 1896\ min^{-1} \tag{4.9}$$

$$b_1 = g_m = 55 \text{ (no units)} \tag{4.10}$$

$$d_1 = w_1 = 0.031\ min^{-1} = 1.86h^{-1} \tag{4.11}$$

Although it seems we have come up with feasible parameters for our model, it can be seen that the units for the value of k_1 are not feasible in light of equation 4.8. Because of the lack of detailed kinetic parameters regarding transcription and translation values, it will be necessary to perform a sensitivity analysis on these parameters, especially k_1 and b_1. This will be discussed further in the Model Analysis chapter.

4.6 Quasi Steady State Approximation

To further simplify the model and to reduce the number of variables that we must include in our model, we have decided to assume that the enzyme levels reach their equilibrium level quickly. From previous studies, the gene expression takes about 40 minutes to occur within the cell (Barrio, 2006), but we assume that this is relatively fast to the overall time scale of changes over a day to a few days in our model.

With this approximation, we can eliminate one of the variables as follows. First we assume that the rate of enzyme production is constant.

$$\frac{d[HMGR]}{dt} = 0 \tag{4.12}$$

We can then solve for the steady state concentration of HMGR.

$$[HMGR]^* = \frac{k_1}{d_1(b_1 + [IC])} \tag{4.13}$$

Now substituting this steady state concentration into our equation governing the intracellular cholesterol (IC) concentration, we obtain effectively a one dimensional system for the *de novo* synthesis of cholesterol.

$$\frac{d[IC]}{dt} = \frac{k_1}{d_1(b_1 + [IC])} \frac{k_2[HMG - CoA]}{k_{m1} + [HMG - CoA]} - d_{ic}[IC] \tag{4.14}$$

Equation (4.14) can now be used to represent the *de novo* synthesis of cholesterol in our full model. We can see that without cholesterol feeding and the internalization of lipoproteins, the intracellular cholesterol concentration is dependent upon itself such that a decrease in cholesterol will lead to an increase in the production rate (source term), whereas an increase in cholesterol will lead to a decrease in the production rate. The cholesterol levels are kept at equilibrium by the degradation to bile acids and other cholesterol derivatives in the cell.

Chapter 5

Modelling Bile Acid Biosynthesis

5.1 Introduction

In hepatocytes, cholesterol is converted to bile acids to be excreted into the intestine via the action of the enzyme cholesterol 7α hydroxylase (CYP7A1, C7H for short). The bile acid is excreted and stored in the gall bladder and is pooled until hormonal changes within the body signal the gall bladder to contract and excrete the bile acid into the gut during feeding. Bile acids are key to increasing the solubility of ingested lipids such that they can be absorbed in the intestine and later metabolized in the body. Approximately 95% of the bile acids are returned to the circulation through the ileum (termed enterohepatic circulation). The returned bile acids then inhibit the gene expression of C7H through a complex unknown pathway, but modelled simply as a direct inhibitor as seen in figure 5.1 (Myant, 1977). The "returned bile acids" are returned to the bile acid pool in the hepatocytes with a certain rate constant.

5.2 The Model

The equation governing the enzymatic degradation of cholesterol by the enzyme Cholesterol 7α Hydroxylase (C7H) is modelled using a simple Michaelis-Menten type equation as shown below. This equation will be combined with that from the *de novo* synthesis pathway to complete our hepatocyte model.

$$\frac{d[IC]}{dt} = -\frac{k_3[C7H][IC]}{k_{m2} + [IC]} \tag{5.1}$$

The equation for the synthesis of bile acids follows from the degradation of cholesterol assuming that in this model cholesterol is only degraded to bile acid.

$$\frac{d[BA]}{dt} = \frac{k_3[C7H][IC]}{k_{m2} + [IC]} - d_3\eta[BA] - r_1(1-\eta)[BA] + k_5[RBA] \tag{5.2}$$

Individual terms are described below:

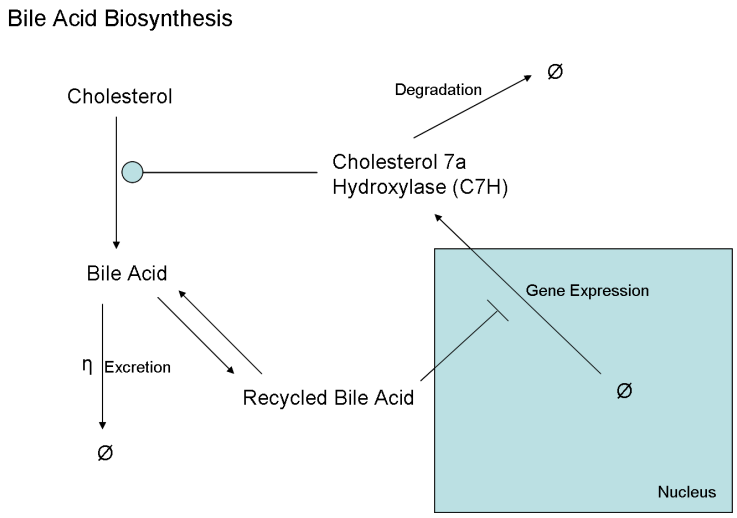

Figure 5.1: Bile Acid Synthesis Pathway

- $d_3\eta[BA]$ – This term describes the excretion of bile acids of which a fraction η is not returned to the liver (η is usually about 5% of the excreted bile acids).
- $r_1(1-\eta)[BA]$ – This term describes the excretion of bile acids which are recycled to the blood stream and are converted to "Returned Bile Acids" (RBA). This was done for convenience since the gene expression of C7H is dependent on the returned bile acid pool and not the bile acid pool already present in the hepatocyte (Myant, 1977).
- $k_5[RBA]$ – This term describes the conversion of Returned Bile Acids to the Bile Acid pool.

The equation governing the production of the enzyme C7H is described below. This is a genetically controlled production and will be justified and described further below. The justification follows similar lines to the genetic justification for the production of HMGR in the *de novo* synthesis pathway.

$$\frac{d[C7H]}{dt} = \frac{k_4}{b_2 + [RBA]} - d_4[C7H] \tag{5.3}$$

And finally, the equation relating to the returned bile acids is shown below.

$$\frac{d[RBA]}{dt} = r_1(1-\eta)[BA] - k_5[RBA] \tag{5.4}$$

5.3 Assumptions in Cholesterol Conversion

In the conversion of cholesterol to bile acid, there are several enzymes involved, but the rate limiting step is the conversion of cholesterol to 7α-hydroxy cholesterol by the action of a P450 cytochrome reductase (Russel, 1992). We assume that all the other steps in this conversion are fast and the rates are negligible

compared to that of the rate limiting step. Hence, we can assume that the overall rate of production of bile acids from cholesterol is defined by the kinetics of this step.

Furthermore, we also assume that the concentration of C7H is not constant and will vary over time defined by the equation above governing production of C7H. Although the derivation of the Michaelis-Menten equation assumed that the concentration of enzyme was kept constant, we assume that the steady state value of the enzyme will be reached before any significant changes in the level of enzyme. This is supported by the fact that there is a delay of approximately 40 minutes between regulation of gene expression and the presence or reduction of protein in the cell which is relatively fast compared to the hundreds of hours we are running our model for.

We currently assume that the only degradation by cholesterol is through bile acids, but we will couple the cholesterol governing equation with the same from the *de novo* synthesis pathway and from the lipoprotein pathway. By separating each component, we can compartmentalize and have modularity of the model.

Unlike the conversion of HMG-CoA to cholesterol, we cannot assume that the enzyme will be rate limiting as we are unsure of the cholesterol concentrations within the hepatocyte. It might turn out that the cholesterol concentration will be limiting the activity of the enzyme. Although studies that have probed the kinetic parameters of C7H have assumed cholesterol levels that are much higher than enzyme levels (Jelinek, 1990), in our model, there is a dynamical equilibrium set up between the amount of cholesterol in the cell and the amount converted to bile acids.

5.4 Enterophepatic Circulation Assumptions

A complex pathway is required for the return of bile acids from the ileum to the liver through chylomicrons and portal vein circulation. Unfortunately, the exact mechanism for this return is unknown (Redinger, 2003) and we just assume a linear relationship between the rate of return and the amount of bile acid present in the intercellular bile acid pool. Furthermore, we also assume a linear relationship in the rate of conversion from the recycled bile acid pool to the intercellular bile acid pool, since the literature review has not given us any significant insight into this metabolic pathway in hepatocytes.

We have also assumed that only the returned bile acid pool regulates the gene expression of C7H (Myant, 1977). In fact, there is a complex negative feedback through multiple nuclear hormone receptors from the returned bile acid which regulates the production of transcription factors that then regulate the transcription of the genes required for bile acid synthesis (Russell, 2003). As usual for modeling, we are neglecting these complications and simplifying our model as much as possible yet attempting to maintain the key biochemical elements involved.

The bile acids themselves return to from the ileum through the hepatic portal vein through the action of ileal sodium ion dependent bile salt trasnporter (ISBT) which facilitate the movement of bile acids across the cell and into the portal vein. The bile acids are then taken up by a transporter on the surface of the hepatocyte and then secreted into the bile duct again for another round of recycling. (Russell, 1999)

5.5 Parameters

- k_3 – rate constant for the conversion of cholesterol to bile acids via C7H (equivalent to k_cat value) - 3.59 min^{-1} (average value from P450 Type I and Type II) = 215 h^{-1} (Chiang, 1990) (estimated to be 10, to perform sensitivity analysis in a later section)
- km_2 – Michaelis-Menten constant for C7H - 41.5 μM (average value from P450 Type I and Type II) = 0.02 gl^{-1} (Assuming an average cholesterol molecule has a molar mass of 390 $gmol^{-1}$)(Chiang, 1990)
- d_3 – degradation (excretion) rate of bile acids which are not returned (rate at which ABCB11 transporter transfers bile acids from the hepatocyte into the bile) - 1 h^{-1} (An estimate is made here with sensitivity analysis performed in a later section)
- r_1 – "degradation" rate of bile acids to recycled bile acids - 0.1 h^{-1} (An estimate is made here with sensitivity analysis performed in a later section)
- k_5 – rate constant for the conversion of returned bile acid to bile acid (assume to be equal to r_1 as the conversion of recycled bile acids on the order of the recycling rate as the recycled acids is immediately directed to the bile acid pool)
- η – percentage of bile acids not recycled back to the liver - 5% (Redinger, 2003)
- k_4 – transcription rate (see section below for value)
- b_2 – attenuation factor for the regulation of C7H by RBA (see section below for value)
- d_4 - degradation rate of C7H (see section below for value)

5.6 Justification of the Genetic Component

The justification of the genetic component which regulates the expression of C7H is similar to that used with the justification of the HMGR enzyme. Here, we assume that it is the recycled bile acids which inhibit transcription of the mRNA and not the entire bile acid pool within the cell (see figure 5.2).

We can come up with similar equations to those before, and in fact, we assume that the same parameters are used since we take the assumption that the kinetics of transcription are the same for all genes and that all proteins can be treated similarly (ie have the same translation rate and the same degradation rate).

$$\frac{d[mRNA]}{dt} = \frac{g_1 g_m^n}{g_m^n + [IC]^n} - w_1[mRNA] \tag{5.5}$$

And for the translation of mRNA to C7H protein:

$$\frac{d[C7H]}{dt} = g_2[mRNA] - w_2[C7H] \tag{5.6}$$

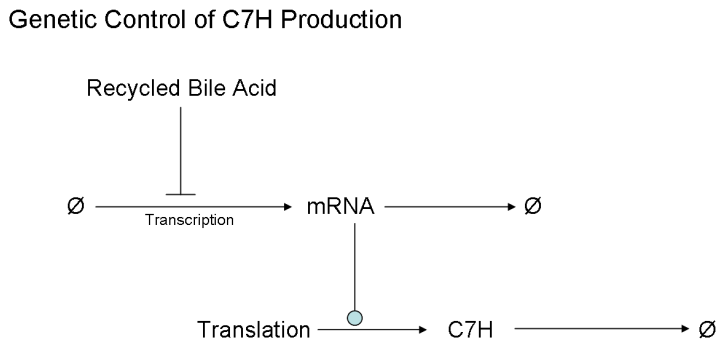

Figure 5.2: Transcription and Translation Pathway for C7H

The parameters for this model are the same as for the *de novo* synthesis model as we are assuming that all mRNA is regulated and degraded in the same way in the cells and that the proteins are also translated and degraded in a similar way.

- g_1 – transcription rate - 1 min^{-1} (Barrio, 2006)
- g_2 – translation rate - 1 min^{-1} (Barrio, 2006)
- g_m – DNA Dissociation Rate - 10-100 (no units) (Barrio, 2006)
- n – Hill cooperativity - assumed to be 1 for this case
- w_1 – mRNA degradation rate - 0.029 min^{-1} (Barrio, 2006)
- w_2 – HMGR degradation rate (assumed to be an ordinary protein degradation rate) - 0.031 min^{-1} (Barrio, 2006)

As from before, we can take the steady state approximation such that

$$\frac{d[mRNA]}{dt} = 0 \tag{5.7}$$

We can come up with the fixed point for the steady state concentration of mRNA and substitute it into our equation for C7H.

$$[mRNA]^* = \frac{g_1 g_m^n}{w_1(g_m^n + [RBA]^n)} \tag{5.8}$$

Now substituting into our equation for C7H:

$$\frac{d[C7H]}{dt} = \frac{g_1 g_2 g_m^n}{w_1(g_m^n + [RBA]^n)} - w_2[C7H] \tag{5.9}$$

We again make the assumption that the Hill cooperativity coefficient is equal to 1. Although the actual genetic regulation depends upon nuclear hormones receptors, we assume that one molecule of recycled bile acids will be sufficient to affect the gene expression of C7H.

We can again combine our constants together similar to what was done in the *de novo* synthesis modelling section. Below shows the simplified model with reduced parameters and their values calculated from the above parameters.

$$\frac{d[C7H]}{dt} = \frac{k_4}{b_2 + [RBA]} - d_4[C7H] \tag{5.10}$$

$$k_4 = \frac{g_1 g_2 g_m^n}{w_1} = \frac{1\ min^{-1} \times 1\ min^{-1} \times 55}{0.029\ min^{-1}} = 1896\ min^{-1} \tag{5.11}$$

$$b_2 = g_m = 55 \text{ (no units)} \tag{5.12}$$

$$d_4 = w_1 = 0.031\ min^{-1} = 1.86h^{-1} \tag{5.13}$$

Similar to the parameters calculated for the *de novo* synthesis modelling in the previous chapter, it will be necessary to do a sensitivity analysis on the transcription and translation values as the units don't match into our model. This will be performed in the Model Analysis chapter later on.

5.7 Quasi Steady State Approximation

Similar to what was performed with the *de novo* synthesis of cholesterol, we can again take the quasi-steady state approximation for several of the equations above to reduce our dimensionality. First, since the recycled bile acids are being converted to bile acids, we can also assume that the concentration of recycled bile acids will be at steady state such that:

$$\frac{d[RBA]}{dt} = 0 \tag{5.14}$$

Solving for the steady state concentration of returned bile acids, we obtain:

$$[RBA]^* = \frac{r_1}{k_5}(1-\eta)[BA] \tag{5.15}$$

We then assume that the concentration of the enzyme will be at steady state such that:

$$\frac{d[C7H]}{dt} = 0 \tag{5.16}$$

Solving for the steady state concentration of C7H, we obtain:

$$[C7H]^* = \frac{k_4}{d_4(b_2 + [RBA])} \tag{5.17}$$

Substituting our expression for the steady state concentration of the recycled bile acids:

$$[C7H]^* = \frac{k_4}{d_4(b_2 + \frac{r_1}{k_5}(1-\eta)[BA])} \tag{5.18}$$

Because we have assumed that the concentration of recycled bile acids is constant, our equation for the rate of change of bile acids is also simplified.

$$\frac{d[BA]}{dt} = \frac{k_3[C7H][IC]}{k_{m2} + [IC]} - d_3\eta[BA] \tag{5.19}$$

And our final equations governing both cholesterol levels and bile acid levels are shown below.

$$\frac{d[BA]}{dt} = \frac{k_4}{d_4(b_2 + \frac{r_1}{k_5}(1-\eta)[BA])} \frac{k_3[IC]}{k_{m2} + [IC]} - d_3\eta[BA] \tag{5.20}$$

$$\frac{d[IC]}{dt} = -\frac{k_4}{d_4(b_2 + \frac{r_1}{k_5}(1-\eta)[BA])} \frac{k_3[IC]}{k_{m2} + [IC]} \tag{5.21}$$

Now we can use equations (5.20) and (5.21) in our final model. The effect of bile acid binding resins will be discussed in a later section. Binding resins in effect inhibit the recycling of bile acids back to the liver, attenuating the η factor in our model.

Chapter 6

A Model of the Hepatocyte

6.1 Introduction

In the next three chapters, we formulate the final model incorporating the *de novo* synthesis and bile acid synthesis equations that we have derived in the past two chapters. We will also present the parameters relevant to our new model again and the values that we use for the final model analysis. The behavior of hepatic cell and peripheral cells are very different and we highlight them in this chapter and the next when we develop a model for the peripheral cells.

The hepatocyte is unique amongst all other cells in terms of cholesterol metabolism since it is the only cells in the body that are responsible for secreting cholesterol from the body via bile acids. Although other cells in the body are capable of performing the bile acid synthesis pathway, bile acid synthesis is predominantly in the liver by the action of the enzyme cholesterol 7α hydroxylase (Russel, 2003).

Furthermore, hepatocytes are also the major suppliers of VLDL and HDL nascent particles. VLDL acts as a method for redistributing cholesterol around the body through its conversion to IDL and further to LDL while HDL is responsible for reverse cholesterol transport, or moving excess cholesterol from peripheral cells back to the liver. In our model, we neglect the cholesterol output from the hepatocyte as the nascent particles do not contain significant amounts of cholesterol. Instead, we focus on the uptake of cholesterol from HDL molecules from either an endocytotic pathway where the entire lipoprotein is ingested or via selective removal of cholesterol without uptake of the whole particle. (Lewis, 2005) Figure 6.1 shows the main players in the cholesterol flux through the hepatocyte.

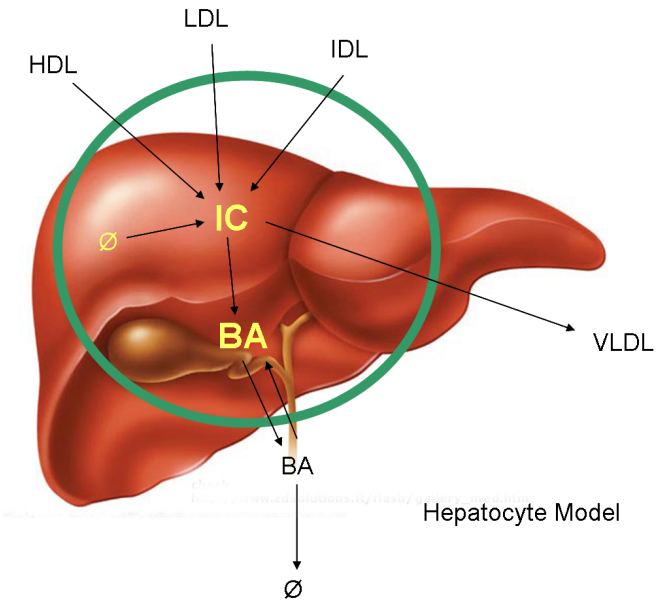

Figure 6.1: Simplified Model of the Hepatocyte

6.2 The Model

$$\begin{aligned}\frac{d[IC-H]}{dt} =& \frac{k_1}{d_1(b_1+[IC-H])}\frac{k_2[HMG-CoA]}{k_{m1}+[HMG-CoA]} \\ &- \frac{k_4}{d_4(b_2+\frac{r_1}{k_5}(1-\eta)[BA])}\frac{k_3[IC-H]}{k_{m2}+[IC-H]} \\ &+ (d_I[IDL-C]+d_L[LDL-C])\phi_{HLR} \\ &+ d_5[LDL-C] \\ &+ h[HDL-C] \\ &- u_v[IC-H] \\ &+ C_{chyl} \end{aligned} \tag{6.1}$$

As is seen in equation 6.1, there are seven different terms which relate specifically to the intracellular cholesterol in hepatocytes. The first term deals with the *de novo* synthesis of cholesterol as discussed in the *de novo* synthesis modelling chapter. The second term deals with the bile acid synthesis in the hepatocyte. We are assuming that this term is only specific to hepatocytes, even though bile acids are produced in the peripheral cells, but only a very small amount compared to the bile acid production in the liver. The third term is from the IDL and LDL receptor-mediated endocytosis. This term is further discussed below with the equation dealing with the LDL receptors on the liver. The $d_5[LDL-C]$ term deals with the non-receptor mediated endocytosis of the cell. This occurs in both hepatocytes and peripheral cells and will appear in the equation dealing the peripheral cholesterol. The $h[HDL-C]$ term is specific to hepatocytes and

represents the intake of cholesterol from LDL either by engulfing the holoparticle or by selective uptake of the cholesterol by SR-B1 as described in an earlier section. The $u_v[IC - H]$ term was added in because it was found in the literature that the production of VLDL particles was strongly dependent upon the level of intracellular cholesterol. Before, this term was a constant level, but for the purposes of our model and for mass conservation, we assume here that VLDL particles are excreted proportionally to the level of cholesterol in the cell. The final term (C_{chyl}) in the equation is our dietary input into the cell. Instead of lumping it into the u_v term as done in previous models, we have separated this term to better express the biochemistry involved. Strictly speaking, chylomicrons are excreted from the intestinal cells and are taken up by receptor mediated processes in the liver (see figure 2.9b), but in an attempt to simplify the model, we have neglected the actions of chylomicrons and used this term instead.

To model dietary intake of cholesterol, the level of C_{chyl} can be forced to oscillate with a fixed frequency and the lipoprotein response can be seen (August, 2007).

$$\frac{d[BA]}{dt} = \frac{k_4}{d_4(b_2 + \frac{r_1}{k_5}(1-\eta)[BA])} \frac{k_3[IC-H]}{k_{m2} + [IC-H]} - d_3\eta[BA] \tag{6.2}$$

In equation 6.2, the terms used to model the bile acid synthesis and recycling of the cell are shown. The equation is derived in the bile acid synthesis modelling section of the paper and is further described there along with the assumptions taken to reach the final equation.

For LDL Receptors

$$\frac{d[\phi_{HLR}]}{dt} = -b(d_i\frac{[IDL-C]}{\chi_I} + d_l\frac{[LDL-C]}{\chi_L})\phi_{HLR} + c\frac{1-\phi_{HLR}}{b_3 + [IC-H]} \tag{6.3}$$

In equation 6.3, the LDL receptor internalization and recycling are described. The first term describes the attachment of lipoproteins to the receptor such that those receptors are then no longer able to bind to another lipoprotein. This term is described in more detail in the lipoprotein modelling section.

6.3 Parameters

6.3.1 Lipoprotein Parameters

- d_i – receptor mediated endocytosis rate of internalization of IDL - 2 h^{-1} (August, 2007)
- d_l – receptor mediated endocytosis rate of internalization of LDL - 0.01 h^{-1} (August, 2007)
- χ_I – Proportional cholesterol contained in IDL - 0.35 (representing the fraction of cholesterol esters and free cholesterol contained within the lipoprotein) (Adiels, 2002, August, 2007)
- χ_L – Proportional cholesterol contained in LDL - 0.45 (Adiels, 2002, August, 2007)

- d_5 – internalization of LDL particles not regulated by receptor mediated endocytosis - 0.0075 h^{-1} (Dietschy, 1993)
- h – internalization rate of HDL by the liver - 0.024 h^{-1} (Chetiveaux, 2004)
- u_v – rate of production of VLDL particles - 1.5 h^{-1} (an estimate from previous studies (August, 2007 and Cooper, 2006))
- C_{chyl} – rate of intake of chylomicrons, one parameter that we wish to perturb in our system
- b – internalization rate of lipoproteins through LDL receptors (includes both IDL and LDL) - 0.1 (August, 2007)
- c – receptor recycling rate - 0.05 $g(lh)^{-1}$ (August, 2007, Goldstein, 1977)
- b_3 – attenuation factor for the regulation of LDL receptors by cholesterol - 0.01 (An estimate subject to sensitivity analysis)

6.3.2 *De Novo* Synthesis Parameters

We will estimate many of the parameters which do not fit the model that were calculated in the previous sections.

- k_1 - transcription rate - 0.1 $(g/l)^2h^{-1}$ (An estimate, subjected to sensitivity analysis)
- b_1 - attenuation factor for the regulation of HMGR by cholesterol - 0.01 g/l (An estimate, subjected to sensitivity analysis)
- d_1 - degradation rate of HMGR - 0.1 h^{-1} (An estimate, subjected to sensitivity analysis)
- k_2 - rate constant for conversion of HMG-CoA to cholesterol mediated by HMGR (equivalent to k_{cat} value) - 22.6 $sec^{-1} = 79200\, h^{-1}$ (Theivagt,2006) (In our model analysis, we estimate this to be 0.1 h^{-1} and perform sensitivity analysis since the value obtained from the literature is unreasonable)
- k_{m1} - Michaelis-Menten constant for HMGR (using NADPH as substrate)- 20 μM = 0.02 gl^{-1} (Assuming an average HMG-CoA molecule has a molecular weight of 900 gl^{-1}) (Theivagt, 2006)

6.3.3 Bile Acid Synthesis Parameters

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- k_3 – rate constant for the conversion of cholesterol to bile acids via C7H (equivalent to k_cat value) - 3.59 min^{-1} (average value from P450 Type I and Type II) = 215 h^{-1} (Chiang, 1990) (In our model analysis, we estimate this to be 10 h^{-1} and perform sensitivity analysis in a later section)
- km_2 – Michaelis-Menten constant for C7H - 41.5 μM (average value from P450 Type I and Type II) = 0.02 gl^{-1} (Assuming an average cholesterol molecule has a molar mass of 390 $gmol^{-1}$)(Chiang, 1990)

- d_3 – degradation (excretion) rate of bile acids which are not returned (rate at which ABCB11 transporter transfers bile acids from the hepatocyte into the bile) - 1 h^{-1} (An estimate is made here with sensitivity analysis performed in a later section)
- r_1 – "degradation" rate of bile acids to recycled bile acids - 0.01 h^{-1} (An estimate is made here with sensitivity analysis performed in a later section)
- k_5 – rate constant for the conversion of returned bile acid to bile acid (assume to be equal to r_1 as the conversion of recycled bile acids on the order of the recycling rate as the recycled acids is immediately directed to the bile acid pool)
- η – percentage of bile acids not recycled back to the liver - 5% (Redinger, 2003)
- k_4 – transcription rate - 0.1 $(g/l)^2 h^{-1}$ (An estimate as values from the literature are unrealistic to this model, to be subject to sensitivity analysis)
- b_2 – attenuation factor for the regulation of C7H by RBA - 0.01 g/l (An estimate as values from the literature are unrealistic to this model, to be subjected to sensitivity analysis)
- d_4 – degradation rate of C7H - 0.01 h^{-1} (An estimate as values from the literature are slightly unrealistic when put into the model, to be subjected to sensitivity analysis)

Chapter 7

A Model of a Peripheral Cell

7.1 Introduction

Peripheral cells are generally involved in autonomous cholesterol regulation and are usually only concerned about control within the cell itself. Both LDL and IDL particles are taken up by receptor mediated endocytosis and the cholesterol output is via HDL. *De novo* synthesis also occurs, but is usually a very small contributor to overall cholesterol concentrations since the cholesterol requirement is usually filled by the intake of lipoproteins. The cholesterol concentration is generally kept constant within the cell regardless of the concentration of lipoproteins in the blood plasma.

A general model of the peripheral cell is shown in figure 7.1.

7.2 The Model

For this compartment, we have two main equations governing cholesterol metabolism within these cells: Intracellular cholesterol and LDL receptors

$$\begin{aligned}\frac{d[IC-P]}{dt} =& \frac{k_1}{d_1(b_1+[IC-P])}\frac{k_2[HMG-CoA]}{k_{m1}+[HMG-CoA]} \\ &+ (d_I[IDL-C]+d_L[LDL-C])\phi_{PLR} \\ &+ d_5[LDL-C] \\ &- d_{HDL}[HDL-C]([IC-P]-p[HDL-C]) \end{aligned} \tag{7.1}$$

The equation 7.1 is similar to equation 6.1 except that we are now excluding the bile acid production term as we are neglecting it in peripheral cells. Furthermore, we have the transfer of cholesterol from the peripheral cell to the HDL particles via ABCA1 receptors. The receptor and non-receptor mediated endocytosis is conserved for both hepatocytes and peripheral cells.

$$\frac{d[\phi_{PLR}]}{dt} = -b(d_i\frac{[IDL-C]}{\chi_I} + d_l\frac{[LDL-C]}{\chi_L})\phi_{PLR} + c\frac{1-\phi_{PLR}}{b_3+[IC-P]} \tag{7.2}$$

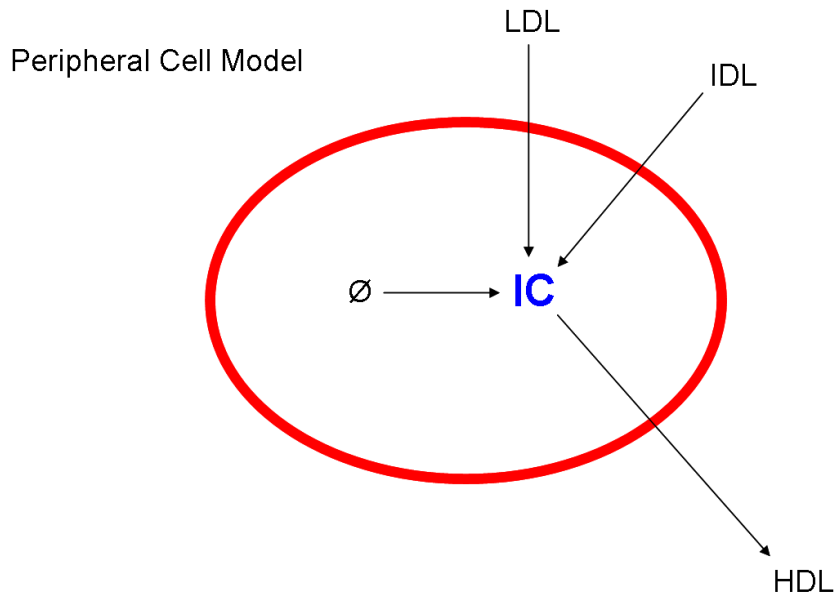

Figure 7.1: A simplified model of the cholesterol flows in a peripheral cell

Although the action of these receptors is exactly the same as that in the hepatocyte, we must separate the terms as the intracellular cholesterol levels are different for both populations as they are controlled separately. Hence the need for a ϕ_{PLR} for the peripheral cells and a ϕ_{HLR} for the hepatocytes.

7.3 Parameters

7.3.1 Lipoprotein Parameters

- d_i – receptor mediated endocytosis rate of internalization of IDL - 2 h^{-1} (August, 2007)
- d_l – receptor mediated endocytosis rate of internalization of LDL - 0.01 h^{-1} (August, 2007)
- χ_I – Proportional cholesterol contained in IDL - 0.35 (representing the fraction of cholesterol esters and free cholesterol contained within the lipoprotein) (Adiels, 2002, August, 2007)
- χ_L – Proportional cholesterol contained in LDL - 0.45 (Adiels, 2002, August, 2007)
- d_5 – internalization of LDL particles not regulated by receptor mediated endocytosis - 0.0075 h^{-1} (Dietschy, 1993)
- b – internalization rate of lipoproteins through LDL receptors (includes both IDL and LDL) - 0.1 (August, 2007)
- c – receptor recycling rate - 0.05 $g(lh)^{-1}$ (August, 2007, Goldstein, 1977)

- b_3 – attenuation factor for the regulation of LDL receptors by cholesterol - 0.01 (An estimate subject to sensitivity analysis)
- d_{HDL} – transfer rate of cholesterol from peripheral cells to HDL - 0.1 $l(gh)^{-1}$ (Johnson, 1988)
- p – Attenuation coefficient for cholesterol transfer to HDL from peripheral cells - 0.5 (derived from the typical equilibrium concentrations of the cholesterol within HDL and the intracellular cholesterol concentration)

7.3.2 *De Novo* Synthesis Parameters

- k_1 - transcription rate - 0.1 $(g/l)^2 h^{-1}$ (An estimate, subjected to sensitivity analysis)
- b_1 - attenuation factor for the regulation of HMGR by cholesterol - 0.01 g/l (An estimate, subjected to sensitivity analysis)
- d_1 - degradation rate of HMGR - 0.1 h^{-1} (An estimate, subjected to sensitivity analysis)
- k_2 - rate constant for conversion of HMG-CoA to cholesterol mediated by HMGR (equivalent to k_{cat} value) - 22.6 $sec^{-1} = 79200\, h^{-1}$ (Theivagt,2006) (In our model analysis, we estimate this to be 0.1 h^{-1} and perform sensitivity analysis since the value obtained from the literature is unreasonable)
- k_{m1} - Michaelis-Menten constant for HMGR (using NADPH as substrate)- 20 μM = 0.02 gl^{-1} (Assuming an average HMG-CoA molecule has a molecular weight of 900 gl^{-1}) (Theivagt, 2006)

Chapter 8

A Model of the Blood Plasma

8.1 Introduction

Probably the most well studied portion is the conversion of VLDL to IDL and the further conversion to LDL. These lipoproteins are present in the plasma and serve to shuttle cholesterol from the liver to the extrahepatic cells as well as to take excess cholesterol from these extrahepatic cell back to the liver (reverse cholesterol transport). As mentioned previously, these lipoproteins are the mediators between our two compartments we are considering and is the most clinically relevant aspect of the project. Clinicians refer to both LDL and HDL levels as markers for cardiovascular disease and prescribe medications to correct these levels. Although it is probably the intracellular cholesterol levels that are actually responsible for the atherosclerosis phenomenon, the difficulty to assess intracellular cholesterol levels in patients has prevented it's use. Hence, clinicians still rely on statistics that have shown a correlation between lipoprotein levels and the prevalence of coronary heart disease (CHD).

The figure 8.1 summarizes the major players when modelling the transport of cholesterol in blood plasma.

Here, we are concerned with all of the lipoproteins and the rate of change over time starting with VLDL, IDL, LDL, and HDL. The equations governing these are shown in the next section.

8.2 The Model

$$\begin{aligned}\frac{d[VLDL-C]}{dt} =& u_v[IC-H] - k_v[VLDL-C] \\ &+ k_{cetp}[HDL-C][VLDL-C]([HDL-C] - a_v[VLDL-C])\end{aligned} \tag{8.1}$$

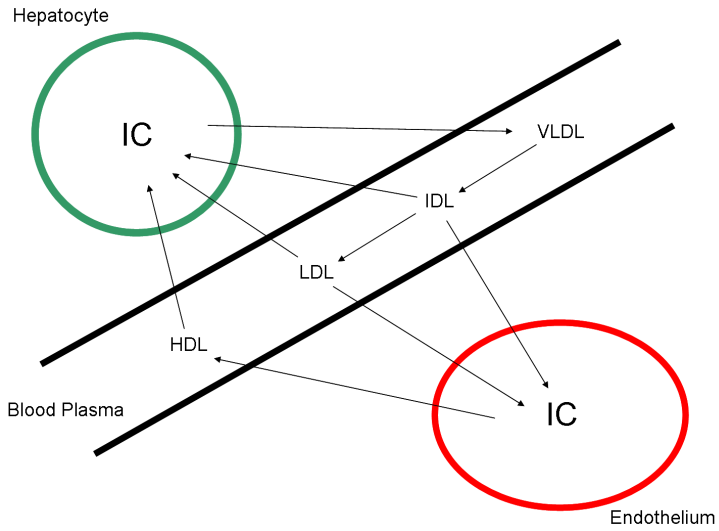

Figure 8.1: The plasma liipoprotein model

$$\begin{aligned}\frac{d[IDL-C]}{dt} =& k_v[VLDL-C] - k_i[IDL-C] \\ &+ k_{cetp}[HDL-C][IDL-C]([HDL-C] - a_i[IDL-C]) \\ &- d_i[IDL-C]\phi_{HLR} - d_i[IDL-C]\phi_{PLR} \end{aligned} \tag{8.2}$$

$$\begin{aligned}\frac{d[LDL-C]}{dt} =& k_i[IDL-C] - d_5[LDL-C] \\ &- d_l[LDL-C]\phi_{HLR} - d_l[LDL-C]\phi_{PLR} \\ &+ k_{cetp}[HDL-C][LDL-C]([HDL-C] - a_l[LDL-C]) \end{aligned} \tag{8.3}$$

$$\begin{aligned}\frac{d[HDL-C]}{dt} =& d_{HDL}[HDL-C]([IC-P] - p[HDL-C]) \\ &+ k_{cetp}[HDL-C][VLDL-C]([HDL-C] - a_v[VLDL-C]) \\ &+ k_{cetp}[HDL-C][IDL-C]([HDL-C] - a_i[IDL-C]) \\ &+ k_{cetp}[HDL-C][LDL-C]([HDL-C] - a_l[LDL-C]) \\ &- h[HDL-C] \end{aligned} \tag{8.4}$$

All of the equations (8.1 to 8.4) are derived from the model developed by Kathryn Cooper as explained further in the lipoprotein modelling section with slight modifications. We have neglected the c_v, c_i, and c_l terms in the equations and have assumed that the rate of cholesterol transfer will be similar for all the lipoproteins. Also, the production of VLDL is only dependent upon the intracellular cholesterol levels in hepatocytes.

8.3 Parameters

- u_v – Rate of production of VLDL particles - 1 h^{-1} (An estimate since we have now changed the meaning of this parameter from the previous models. Sensitivity analysis is performed on this parameter in a later section)
- k_v – Fractional turnover rate of VLDL representing the conversion of VLDL to IDL - 0.3 h^{-1} (Packard, 2000)
- k_{cetp} – Rate of action of cholesterol ester transfer protein shuttling cholesterol from HDL to VLDL - 0.1 $gl^{-1}h^{-1}$ (Cooper, 2006)
- a_v – Attenuation coefficient for the cholesterol gradient - 2.5 (no units) (derived from the typical equilibrium concentrations of the cholesterol within HDL and VLDL)
- k_i – Fractional turnover rate of IDL representing the conversion of IDL to LDL - 0.05 h^{-1} (Packard, 2000)
- a_i – Attenuation coefficient for the cholesterol gradient - 5 (no units) (derived from the typical equilibrium concentrations of the cholesterol within HDL and IDL)
- d_i – receptor mediated endocytosis rate of internalization of IDL - 2 h^{-1} (August, 2007)
- d_l – receptor mediated endocytosis rate of internalization of LDL - 0.01 h^{-1} (August, 2007)
- d_5 – internalization of LDL particles not regulated by receptor mediated endocytosis - 0.0075 h^{-1} (Dietschy, 1993)
- a_l – Attenuation coefficient for the cholesterol gradient - 0.4 (derived from the typical equilibrium concentrations of the cholesterol within HDL and LDL)
- p – Attenuation coefficient for cholesterol transfer to HDL from peripheral cells - 0.5 (derived from the typical equilibrium concentrations of the cholesterol within HDL and the intracellular cholesterol concentration)
- d_{HDL} – transfer rate of cholesterol from peripheral cells to HDL - 0.1 h^{-1} (Johnson, 1988)
- h – internalization rate of HDL by the liver - 0.024 h^{-1} (Chetiveaux, 2004)

Chapter 9

Statins and Bile Acid Binding Resins

9.1 Statins

Statins have become one of the largest selling drugs around the world as there is an increase in the incidence of cardiovascular diseases (CVD). Since LDL and HDL cholesterol have been implicated in the formation of atherosclerotic plaques which may result in myocardial infarction (heart attack) or stroke if an embolism occurs, most drugs that help to reduce the incidence of CVD have focused on lowering the "bad" (LDL) cholesterol levels while increasing the "good" (HDL) cholesterol levels.

9.1.1 Regulation of HMG-CoA Reductase

Regulation is achieved by three main mechanisms

- Transcription and Translation of HMGR - the transcription and translation level of HMGR increases when concentrations of mevalonate pathway intermediates and products are low
- Degradation of HMGR - when these intermediates and products of the mevalonate pathway are high, HMGR degradation is increased leading to a lower concentration of enzyme within the cell
- Phosphorylation - action of the enzyme is aided by phosphorylation at serine residue 872 (S872). Phosphorylation at this site is controlled by AMP-activated protein kinases as well as HMG-CoA phosphorylation

The active site of HMGR contains two binding pockets for HMG-CoA and NADP+, the two substrates necessary to produce mevalonate from HMG-CoA. Phosphorylation occurs at S872 and the phosphorylation by AMP-activated protein kinase and HMG-CoA phosphorylase is thought to activate the enzyme (Istvan, 2000)

Statins act as a reversible allosteric inhibitor of the enzyme HMG-CoA reductase (discussed in the Biology and Biochemistry section) by mimicking the

transition state (Istvan, 2001). It does so by binding to the active site where HMG-CoA binds to prevent the the attachment of HMG-CoA, the actual substrate of the enzyme. Not only do they prevent the substrate from binding, they also confer a structural change to the HMGR enzyme rendering it non-functional (Stancu, 2001). Figure 9.1 shows the chemical structure of statins which closely mimic the ring structure of HMG-CoA.

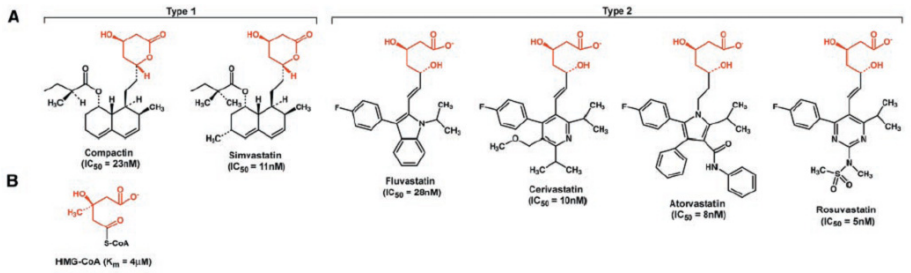

Figure 9.1: Chemical structure of statins as compared to HMG-CoA. Note the similarities in structure highlighted in red. (Reproduced from Istvan, 2001)

9.1.2 Statin Models

Kinetic models have been tested on HMGR to determine the mechanism of action for statins (Louis-Flamberg, 1990). It was determined that the kinetic model fit with an allosteric inhibitor which can be modelled with equation 9.1. ($[S]$ is the concentration of the substrate: HMG-CoA, $[I]$ is the concentration of inhibitor: statins)

$$v_i = \frac{V_{max}[S]}{K_m + [S] + \frac{K_m}{K_i}[I]} \tag{9.1}$$

Since we are not assuming that the enzyme levels are constant, we can decompose the V_{max} term as seen in equation 9.2, where $[E]$ is the concentration of HMGR enzyme

$$v_i = \frac{V_{max}[S]}{K_m + [S] + \frac{K_m}{K_i}[I]} = \frac{k_{cat}[E][S]}{K_m + [S] + \frac{K_m}{K_i}[I]} \tag{9.2}$$

Kinetic parameters were obtained by fitting data to different curves and minimizing the error between the curve and the data points and are shown below.

- k_{cat} – maximum rate of conversion of HMG-CoA to mevalonate - 22 sec^{-1} (Theivagt, 2006)
- K_m – Michaelis-Menten rate constant for HMGR - 20 μM (Theivagt, 2006)
- K_i – dissociation constant for statins - 0.2 - 3.6 nM (Louis-Flamberg, 1990) (In the model analysis, we assume that the $K_i = 0.01$ and subject this to sensitivity analysis.)

We must now convert these parameters into values that are compatible with our equation. Because most results are expressed in grams, liters, and hours, we convert the above parameters into our wanted units.

For k_{cat}:

$$k_{cat} = 22sec^{-1} = 79200h^{-1} \tag{9.3}$$

For K_m, we take from the literature that the HMG-CoA molecular weight is about 900 g/mol which allows the conversion to g/l.

$$K_m = 20 \times 10^{-6}M \times 900g/mol = 0.02g/l \tag{9.4}$$

For K_i, the relevant molecular weight is the average statin which is approximately 400 g/mol, allowing the conversion to g/l.

$$K_i = 2 \times 10^{-9}M \times 400g/mol = 8 \times 10^{-7}g/l \tag{9.5}$$

9.1.3 Changes to the Model

In order to take the effect of statins into account, we only have to slightly modify the hepatic intracellular cholesterol equation ($[IC-H]$) as statins target the *de novo* synthesis of cholesterol within hepatocytes (Stancu, 2001). We assume that there is no effect on the *de novo* synthesis of cholesterol in peripheral cells. The modified version of equation 6.1 is shown below (equation 9.6)

$$\begin{aligned}\frac{d[IC-H]}{dt} =& \frac{k_1}{d_1(b_1+[IC-H])}\frac{k_2[HMG-CoA]}{k_{m1}+[HMG-CoA]+\frac{k_{m1}}{k_i}[Statins]} \\ &- \frac{k_4}{d_4(b_2+\frac{r_1}{k_5}(1-\eta)[BA])}\frac{k_3[IC-H]}{k_{m2}+[IC-H]} \\ &+ (d_I[IDL-C]+d_L[LDL-C])\phi_{HLR} \\ &+ d_5[LDL-C] \\ &+ h[HDL-C] \\ &- u_v[IC-H] \\ &+ C_{chyl}\end{aligned} \tag{9.6}$$

In equation 9.6, the k_{m1} value is equivalent to the K_m value seen in equation 9.2). Also, the k_2 value is equilvalent to the k_{cat} in equation 9.2. From the equation, we realize that the parameter values that we obtained might not be completely feasible, especially for k_2 and for k_i. Although they were given in the literature, it will be good to perform sensitivity analysis on these values to ensure that an estimate will not cause significant changes to the model. It is also good to note that the effect of k_1, k_2, and d_1 will affect the model in the same way so we can lump these parameters together when performing our sensitivity analysis (Described in the Model Analysis chapter under *De Novo* Synthesis Parameters section).

9.2 Bile Acid Binding Resins

Apart from statins, the other major drug used to treat hypercholesterolemia is bile acid resins. Studies have shown a significant decrease in LDL and total cholesterol levels with cholestryamine (a bile acid binding resin) treatment as compared to patients treated with diet alone (Levy, 1984). Bile acid resins work by preventing the uptake of bile acids in the ileum and allowing them to be excreted by the feces. As it prevents bile acids from returning, the effect of resins will also only affect the hepatic cholesterol and bile acid production (Einarsson, 1991).

9.2.1 The Model

Since no current models exist for the action of bile acid binding resins and no kinetic parameters have been found in the literature, we must develop our own model to describe this phenomenon. The key assumption in developing this model is that the presence of resins will have a linear effect on preventing the recycling of bile acids. The greater the resins, the greater the excretion of bile acid through the feces. From the Biology and Biochemistry chapter, we realize that this will directly affect the value of η, the fraction of bile acids which is returned to the liver. We can attenuate this by adding another constant β to describe the action of resins as seen in the modified equations below (equations 9.7 and 9.8) describing the hepatic intracellular cholesterol and bile acids.

$$\begin{aligned}\frac{d[IC-H]}{dt} =& \frac{k_1}{d_1(b_1+[IC-H])}\frac{k_2[HMG-CoA]}{k_{m1}+[HMG-CoA]+\frac{k_{m1}}{k_i}[Statins]} \\ &- \frac{k_4}{d_4(b_2+\frac{r_1}{k_5}(1-\beta\eta)[BA])}\frac{k_3[IC-H]}{k_{m2}+[IC-H]} \\ &+ (d_I[IDL-C]+d_L[LDL-C])\phi_{HLR} \\ &+ d_5[LDL-C] \\ &+ h[HDL-C] \\ &- u_v[IC-H] \\ &+ C_{chyl} \end{aligned} \tag{9.7}$$

$$\frac{d[BA]}{dt} = \frac{k_4}{d_4(b_2+\frac{r_1}{k_5}(1-\beta\eta)[BA])}\frac{k_3[IC-H]}{k_{m2}+[IC-H]} - d_3\beta\eta[BA] \tag{9.8}$$

It is also important to see that the value of β will always be greater than 1 as resins will never cause a greater proportion of bile acids to be reabsorbed in the ileum.

Chapter 10

Analysis of the Model

In order to obtain more information about the model and to see if it is feasible in light of clinical studies and laboratory research done to extract cholesterol values in cells, we must analyze the model to see if solutions are feasible and that the model is robust to parameter changes. Parameter changes could be induced by dietary intake of cholesterol (affecting the C_{chyl} term). We will first take a look at the model without the effect of statins and bile acid binding resins.

10.1 Time Analysis of the Model

To get a general overview of what happens to the levels of lipoproteins and cholesterol in the cells over time, we first numerically integrate our system with respect to time. Since we have a 9-dimensional system, it is difficult to predict the behavior over time. As is shown in figure 10.1 below, the system does reach a steady state value over time. It appears that in all models (this and previous models), the cholesterol concentrations reach steady state.

We would like to verify that the model reaches steady state since we would like to plot the steady state value over time when we change out input parameters (C_{chyl}). Once we are sure that an equilibrium solution exists for a given input range (by using the ode45 function in Matlab), then we can plot the equilibrium solution versus our parameter easily using the fsolve function.

We also checked the sensitivity to initial conditions regarding the model at the given parameter values and conclude that the model is not dependent on initial conditions and that it will eventually reach a steady state value for the values of chylomicron input that we are interested in.

10.2 The Model at Equilibrium

Since we know that our system will reach steady state after long periods of time, we can explore what happens when we perturb our first control parameter, the dietary intake of cholesterol as described by C_{chyl}. Here, we have neglected the effects of statins and bile acid binding resins and have left the analysis of these drugs to a later section. The daily intake of cholesterol is about 500 mg per day for an average individual (assume 70 kg). When converting this to an intake

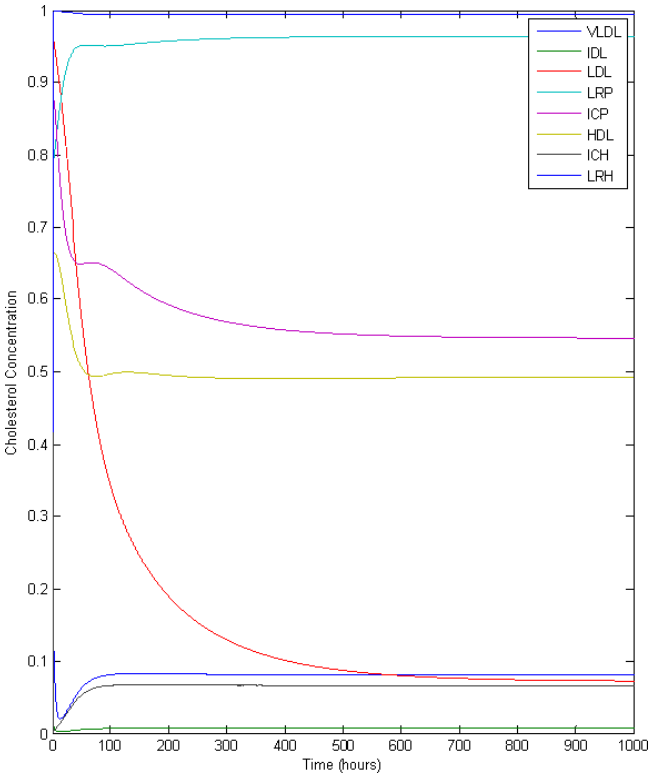

Figure 10.1: Development of the model over time (excluding the bile acid synthesis)

rate, this equates to approximately $3 \times 10^{-4} g(lh)^{-1}$, which is much less than our maximum daily intake of 2 $g(lh)^{-1}$.

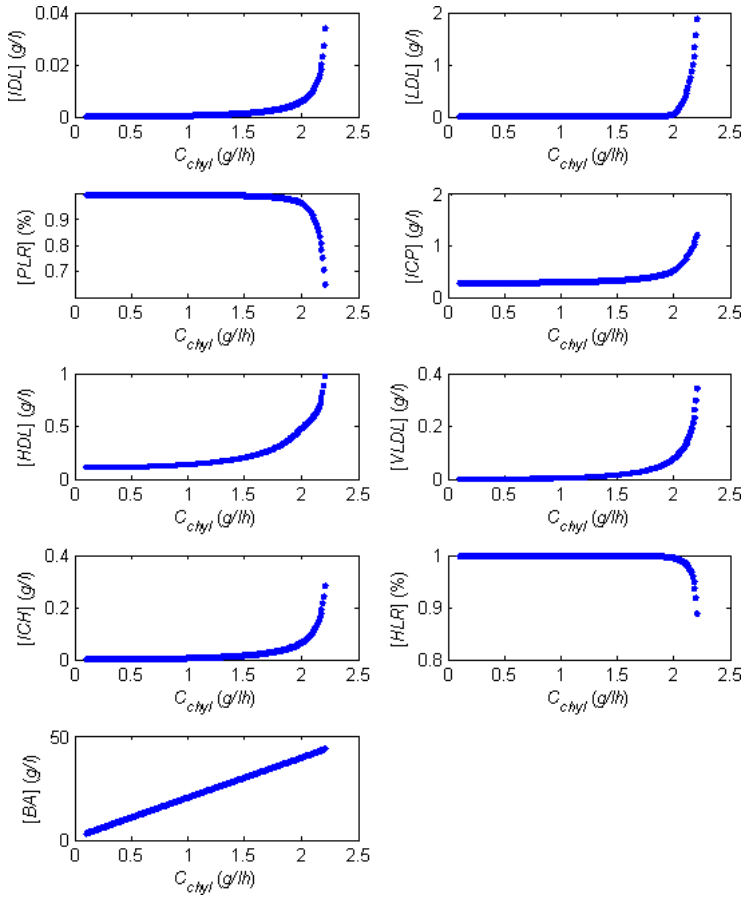

Figure 10.2: Plot of equilibrium solutions versus chylomicron input (C_{chyl})

As in previous models, we again see the emergence of a low cholesterol regime and a high cholesterol regime which is clearly demarcated when the lipoprotein receptor percentage suddenly drops around 2.2 $g(lh)^{-1}$ of chylomicron input. Below this level, all the cholesterol concentrations remain relatively low and constant. Above the threshold chylomicron input, the cholesterol concentrations begin to rise and the receptor percentage drops significantly. Although in previous models a definite high cholesterol regime was seen, in this model, the high cholesterol regime corresponds to the concentrations of cholesterol blowing up to infinity.

Physiologically, in the low cholesterol regime, the receptor production is enough to regulate the cholseterol within the cells. In the high cholesterol regime when cholesterol levels are too high, it was speculated in previous models that the increase in cholesterol concentrations overall correlated with the non-receptor mediated endocytosis having a greater effect with increasing concentration of cholesterol. In this model, it shows that the system cannot cope with such high cholesterol intakes (although the maximum intake of 2 $g(lh)^{-1}$

is physiologically unfeasible). By looking at the model more carefully, we can somewhat gauge the reason for this high cholesterol regime. Looking at the bile acid equation (equation 5.20), we see that if the cholesterol concentration goes too high (greater than k_{m2}), then the source term of bile acid synthesis (the enzymatic production term) becomes constant. Furthermore, the production of more bile acids will also tend to reduce the production of C7H enzyme. The combined effect of these two limit the conversion of cholesterol to bile acids at very high cholesterol levels and the cholesterol concentrations will tend to infinity as they are pooled within the body and cannot be excreted (using our assumption that bile acids are the only exit of cholesterol from the body).

One other interesting thing to note about the model is that intracellular cholesterol concentration is actually lower overall in the hepatocytes versus the peripheral cells. Although we could not find any studies which compared the cholesterol content in both cells, we speculate that the reason for this observation is that hepatocytes have more outflows of cholesterol and can regulate the cholesterol content within the cell more effectively than in peripheral cells. The presence of a large outflow of bile acids that are responsive to the intracellular cholesterol concentrations allows the cell to quickly expend excess cholesterol from the body. In peripheral cells, only HDL particles remove excess cholesterol making it more difficult to exercise higher control of its intracellular cholesterol levels.

One of the assumptions that we made which could potentially alter the behavior of the system is that non-receptor mediated endocytosis occurs for LDL particles. Our initial hypothesis is that the high cholesterol regime is characterized by the lack of receptors in the cell because it has received enough cholesterol and that lipoproteins are driven into the cell via diffusion. If we remove this term, we actually see no difference in the behavior of the system (results not shown), so we speculate that there is another mechanism at work and the two cholesterol regimes are inherent to the model regardless of whether non-receptor mediated endocytosis occurs or not.

If we look back at figure 10.2, we can also see that the bile acid level continues to increase almost linearly as we increase the cholesterol intake. It would even seem that at high values of C_{chyl}, the bile acid level becomes physiologically unfeasible. We must be careful when interpreting this because in our model, we have neglected to take into account the bile acid pooling in the gall bladder. An increase in bile acid wouldn't be unexpected since the bile acids will eventually be excreted and stored in the gall bladder. The higher the cholesterol intake, the faster the bile acid storage occurs. To fully complete this model, it would be necessary to include an equation describing the pooling of bile acids within the gall bladder with their periodic release corresponding to feeding times.

It is also interesting to note that the bile acid response to cholesterol intake is different than the other variables such as lipoproteins and intracellular cholesterol which show a distinct flat curve following by a sharp increase. Although it is unclear why this occurs in our highly non-linear model, doing a more thorough equilibrium analysis on the equations may yield a linear response to variations in C_{chyl}.

10.3 Sensitivity Analysis of the Model

10.3.1 *De Novo* Synthesis Parameters

As mentioned before, it is quite difficult to determine accurate values of the parameters for the *de novo* cholesterol synthesis because the values provided in literature do not link up exactly to our model. Hence, we must test the sensitivity to the equilibrium solutions when we change the value of these parameters by a few orders of magnitude. If we look again at the equations for intracellular cholesterol in the hepatocyte and the peripheral cells (equations 6.1 and 7.1), we can see that three parameters can be lumped together as changing them will have similar consequences to the model: k_1, k_2, and d_1. Changing one of these values by an order of magnitude will in effect change the entire group of parameters by an order of magnitude. Although strictly speaking we should be changing the parameters to remain within physiological conditions, there is a lack of concrete data on the kinetics of various enzymes and we have taken several approximations in our model. It thus makes sense to consider all three parameters at once and change them all at the same time.

The results of the sensitivity analysis (figure 10.3) show that our model is quite sensitive to changes in the value of k_1. We have simulated a 100x fold increase in the value of k_1 (black to red in figure 10.3), and we see general increases in the lipoprotein concentrations, although they are less than 2 orders of magnitude larger. One other significant change that is seen when altering k_1 is that the lipoprotein receptor percentage of the peripheral cells in the low cholesterol regime is not at its maximum capacity. Instead, only about 80% of the reeptors are displayed when the value of k_1 is increased 100 fold. In the hepatocytes, the receptor percentage remains unchanged.

Changing all three variables at the same time translates to changing the translation rate of HMGR, catalytic conversion rate of HMGR, and the degradation rate of HMGR at the same time. We can say that our analysis probes what happens when we affect how HMGR enzyme acts on the entire system and how important it is to cholesterol homeostasis overall. The change in receptor percentage in peripheral cells is probably due to the lack of control mechanisms that are found in hepatocytes. If the HMGR production rate is increased significantly (as simulated by a 100 fold change in k_1), it seems that this *de novo* production of cholesterol can account for up to 20% of intracellular cholesterol with lipoprotein internalization responsible for the remaining 80%. In hepatocytes, the bile acid synthesis neutralizes any addition to the hepatic intracellular cholesterol pools and receptor percentage remains at 100% in the low cholesterol regime.

One other parameter we wish to perform sensitivity analysis on is b_1, the attenuation coefficient for the regulation of LDL receptors by cholesterol. Going back to the the justification of the genetic component, the value of b_1 corresponds to the DNA binding coefficient, K_m. This value is the cholesterol concentration value at which the transcription rate is half maximum. Since transcription events don't occur continuously, but are only on the order of a few molecules of mRNA being produced, it is difficult to model this with a continuous function, calling for sensitivity analysis on the value that we have obtained from the literature.

The result of the sensitivity analysis (figure 10.4) shows that changes of

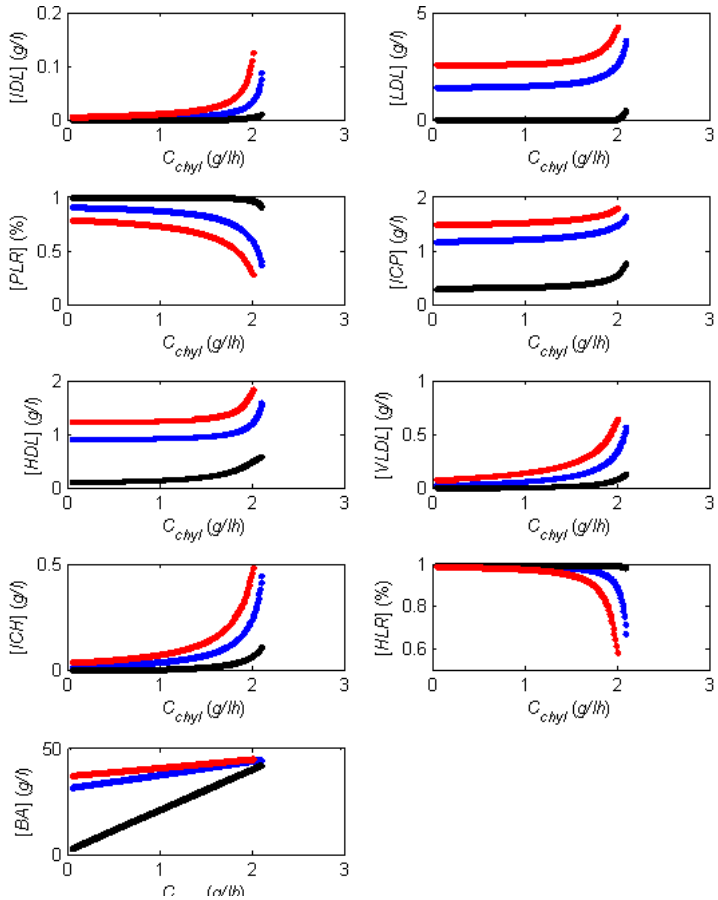

Figure 10.3: Plot of equilibrium solutions versus chylomicron input (C_{chyl}) with a 100 fold increase in the *de novo* synthesis chacteristic parameter ($k_1 \times k_2/d_1$). Red ($k_1 = 1$), Black (original) ($k_1 = 0.01$), Blue ($k_1 = 0.5$)

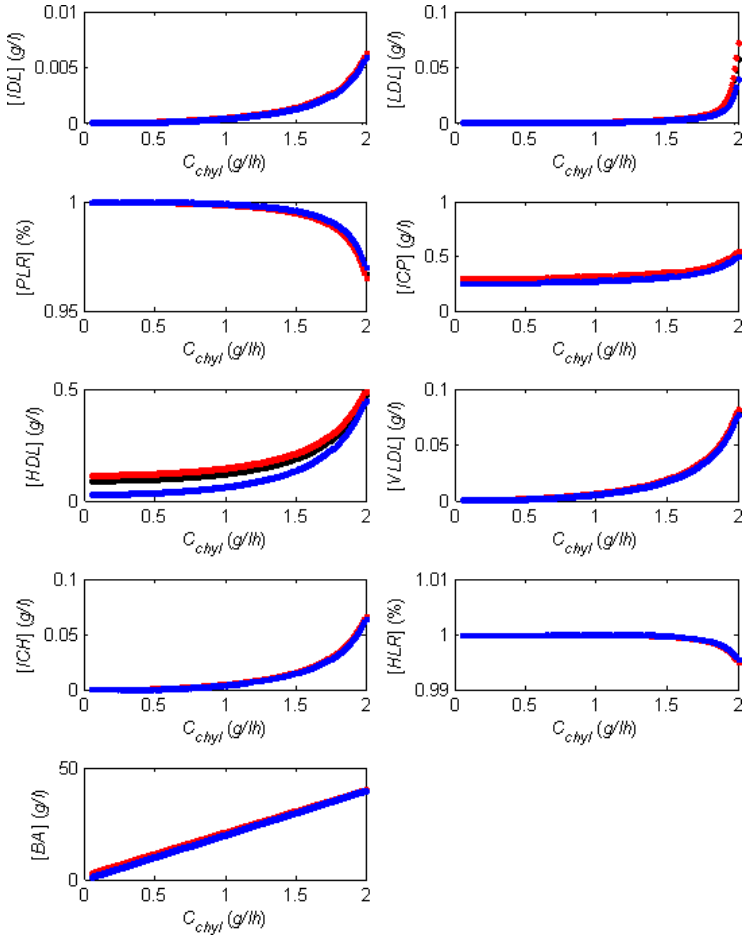

Figure 10.4: Plot of equilibrium solutions versus chylomicron input (C_{chyl}) with a 100 fold increase in the parameter b_1. Red $b_1 = 0.01$ (original), Black $b_1 = 0.1$, Blue $b_1 = 1$

three orders of magnitude below and above our estimated value do not have a significant effect on the equilibrium values as we change the dietary cholesterol input (C_{chyl}). Having done our analysis, it is safe to say that the effect of b_1 is negligible and that we can take any estimated value of b_1 without worrying too much about affecting the equilibrium value of our model. The reason for the insensitivity of b_1 is probably because the concentration of cholesterol is much higher than the value of b_1. Because we are adding the cholesterol concentration to this small value, the effect is negligible to the overall model.

What is interesting about changing the value of b_1 is that it has an effect on the HDL cholesterol pool. A increase in the value of b_1 will lead to a decrease in the value of HDL while all other lipoprotein pools remain relatively constant. Although this is only seen at lower chylomicron inputs and the actual difference is almost negligible, it goes to show that there are some unintended effects when extending the model.

10.3.2 Bile Acid Biosynthesis Parameters

Since we faced a similar problem with coming up with the correct values for the bile acid biosynthesis parameters, a sensitivity analysis should be done to ascertain whether or not the model is robust to these parameters. Similar to the *de novo* synthesis parameters, we will once again consider the three parameters affect the bile acid synthesis term in a similar way: k_3, k_4, and d_4 from equation 6.1. Again, changing only the value of k_3 by an order of magnitude will effectively change the entire set of parameters at the same time.

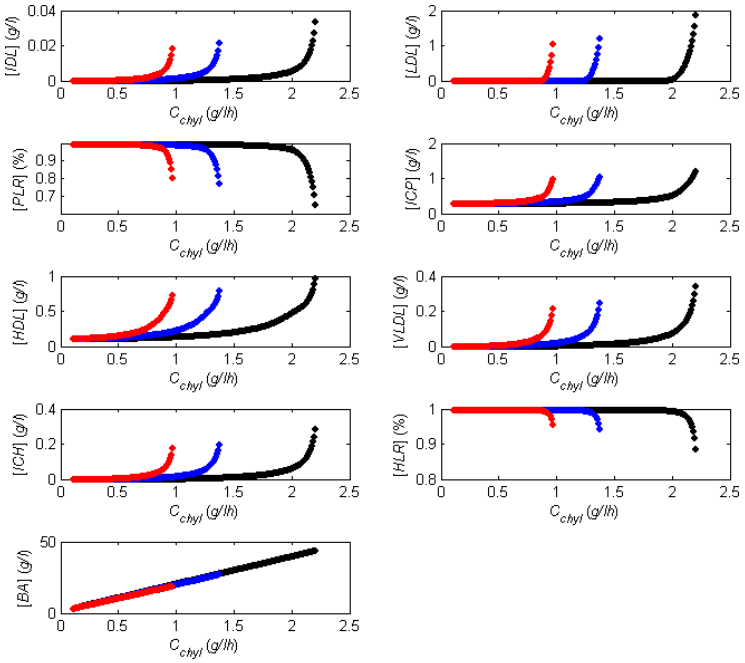

Figure 10.5: Plot of equilibrium solutions versus chylomicron input (C_{chyl}) with a 5 fold increase in the bile acid synthesis chacteristic parameter ($k_3 \times k_4\ d_4$). Black (original) $k3 = 10$, Blue $k3 = 4$, Red $k3 = 2$.

Biologically speaking, we are again considering all of the parameters related to cholesterol 7α hydroxylase, so by doing sensitivity analysis, we are seeing the effects of changing C7H enzyme on the entire system as we saw with the sensitivity analysis of HMGR enzyme. What our analysis shows is a significant change in the transition point between the low and high cholesterol regimes (see figure 10.5). The lipoprotein cholesterol concentrations remain of similar value compared to the original parameter values. Although it doesn't appear that there is a linear relationship between an increase in the value of k_3 and the corresponding increase in the transition point, the change is significant for only a 10 fold increase in k_3.

We have chosen our value of k_3 to be used in the model based upon what might be considered a clinically relevant cholesterol intake. Although the daily intake of cholesterol is much lower than 2 $g(lh)^{-1}$, we decided to make this the cutoff point as even if one is physically "stuffed" with cholesterol (ie C_{chyl} value above physiologically relevant values), the cholesterol in the body does not tend to infinity.

Clinically, this might be significant because by finding a way to alter the characteristics of C7H medically, we can increase the normal cholesterol input concentration limit such that one always remains in the low cholesterol regime. By remaining in this low cholesterol regime, the patient not only has decreased LDL concentrations, but also a lower risk of CHD.

10.3.3 Bile Acid Recycling Parameters

Another parameter we have not been able to find concrete data for is the recycling terms r_1 and k_5, the rate of recycling of bile acids and the rate of conversion of recycled bile acids back to the cellular bile acid pool respectively. The process of returning bile acids from the intestine to the liver occurs over several steps and transporters while our model assumes that there is one parameter that describes the entire transport mechanism. Because we have made an estimate as to the value of this parameter, it is necessary to do sensitivity analysis to see if changes in this parameter will affect the dynamics of the model. As with the previous sensitivity analysis done, we can lump both parameters together as their conglomerate value has one effect on the entire system.

Biologically, altering the ratio of r_1 and k_5 changes the weighting on how C7H affects the cholesterol to bile acid conversion. A higher ratio would mean stronger inhibition of C7H and the decrease in conversion of cholesterol to bile acids. A lower ratio implies a weaker inhibition, and hence a slower conversion of cholesterol to bile acids. Our sensitivity analysis shows that with an increase in r_1 value, there is a decrease in the transition concentration between the low and high cholesterol regimes (see figure 10.6). This is opposite to what was seen with the bile acid synthesis parameters where an increase in the parameter values lead to an increase in the transition point.

This phenomenon can also be clinically relevant as any way of reducing this ratio could also bring about the same changes as with the bile acid synthesis parameters, enabling one to stay within the low cholesterol regime even with an increase in cholesterol intake.

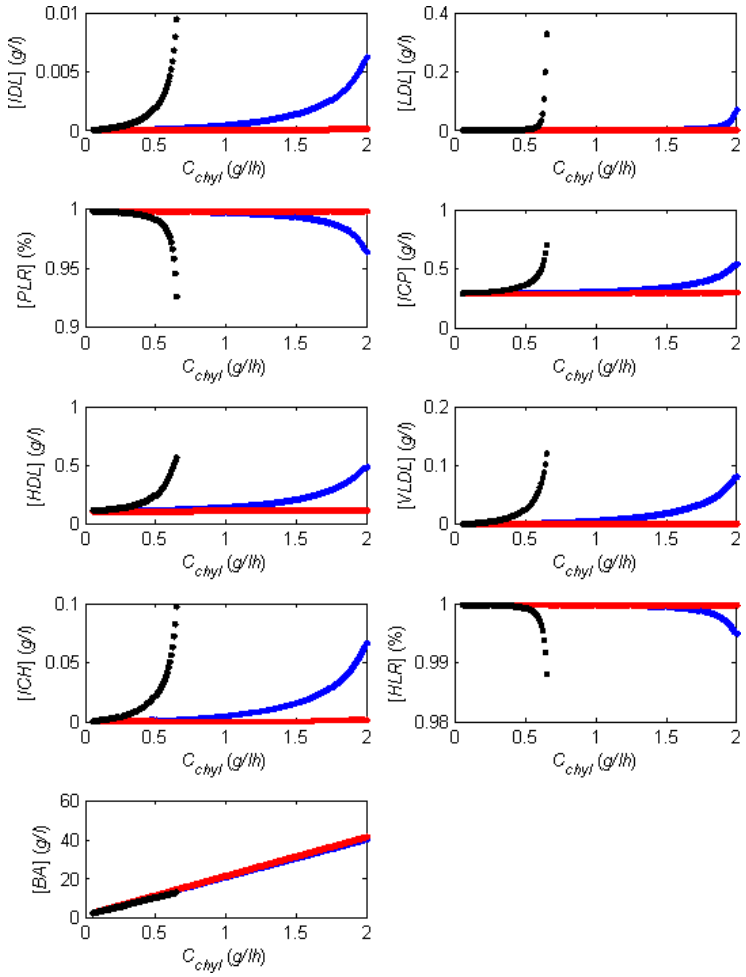

Figure 10.6: Plot of equilibrium solutions versus chylomicron input (C_{chyl}) with a 100 fold increase in the bile acid recycling parameter r_1. Red $r_1 = 1$, Blue (original) $r_1 = 0.1$, Black $r_1 = 0.01$

10.3.4 Other Parameters

The first other parameter we wish to do sensitivity analysis on is b_2, or the attenuation coefficient of C7H in response to bile acid concentration. We expect that the sensitivity will be similar to b_1 for the *de novo* synthesis term as they are both derived from considering the transcription inhibition and utilize similar parameters.

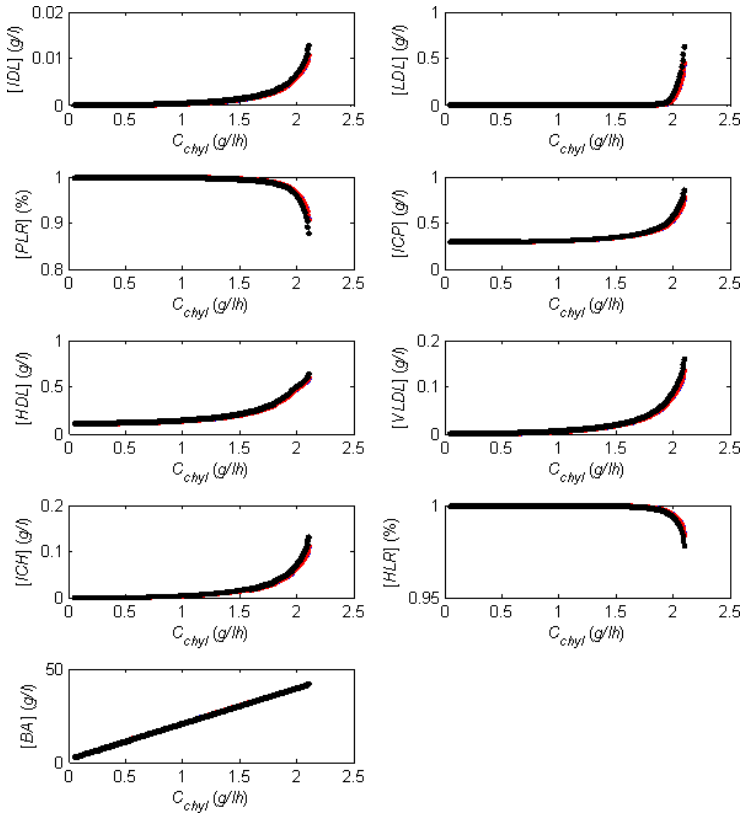

Figure 10.7: Plot of equilibrium solutions versus chylomicron input (C_{chyl}) with a 100 fold increase in the parameter b_2. Blue (original) $b_2 = 0.01$, Red = $b_2 = 0.1$, Black = $b_2 = 1$

Our analysis shows that the sensitivity of the system to changes in b_2 is similar to that of b_1 and that the model is relatively robust to changes in this parameter (see figure 10.7). Even though our value of b_2 is an estimate, we are not too concerned with the nominal value as the effect of this is trivial. Similar to the *de novo* synthesis, we speculate that the reason for the insensitivity to changes in this parameter is that the concentration of bile acids is kept at a higher level than the value of b_2 (which is derived from the DNA binding constant). Compared to the sensitivity analysis of b_1, we do not see the change in HDL concentration anymore perhaps because it is not affecting the peripheral cells. When we were concerned with b_1 (the attenuation coefficient for HMGR), the only exit for cholesterol in the peripheral cells is to transfer it to HDL. Hence, with an allowed increase in production of cholesterol in the peripheral cell, then

there must be an increase in transfer to HDL to maintain the cholesterol at homeostasis.

We would also like to see the effect of changing the value of d_3, or the rate at which the bile acids are excreted from the body (equivalent to being degraded). We expect that the model will exhibit sensivitity to this parameter because it alters the equilibrium value of the bile acids. This bile acid excretion term will not be able to handle any dietary cholesterol intake that is too high, and so by increasing the value of d_3, we are effectively increasing the threshold. That is exactly seen in figure 10.8.

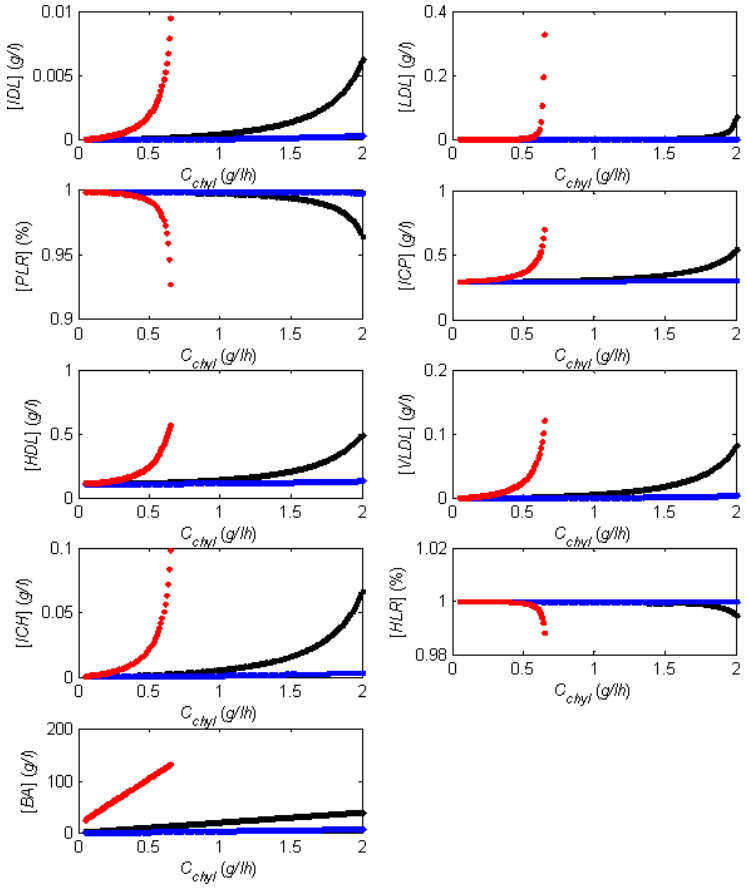

Figure 10.8: Plot of equilibrium solutions versus chylomicron input (C_{chyl}) with a 50 fold difference in the parameter d_3. ($d_3 = 0.1$ (Red line), $d_3 = 1$ (Black line), $d_3 = 5$ (Blue line))

Hence, it is important to note that by changing the levels of bile acids at equilibrium and the net flows through the system, we can drastically change the characteristics of the system, making all of the bile acid biosynthesis parameters clinically significant. Bile acid binding resins achieve a lowering of the cholesterol levels by increasing the net flux of cholesterol through the system as discussed later on.

The only other parameter that we wish to perform sensitivity analysis on is

the production of VLDL particles from hepatocytes (equation 6.1). We can see that this is the other exit of cholesterol from hepatocytes and may be important to maintaining the homeostasis of cholesterol within hepatocytes. In previous models (August, 2007 and Cooper, 2006), this parameter was swept between 0 and 3 $g(lh)^{-1}$ since they only considered the cholesterol pool of peripheral cells and not of hepatocytes. The VLDL production uniquely occurs in hepatocytes and is highly dependent upon the cholseterol levels within the cell. Although we estimate the value of u_v to be around 1 h^{-1}, we have performed a sensitivity analysis to see what happens to changes in the value of u_v.

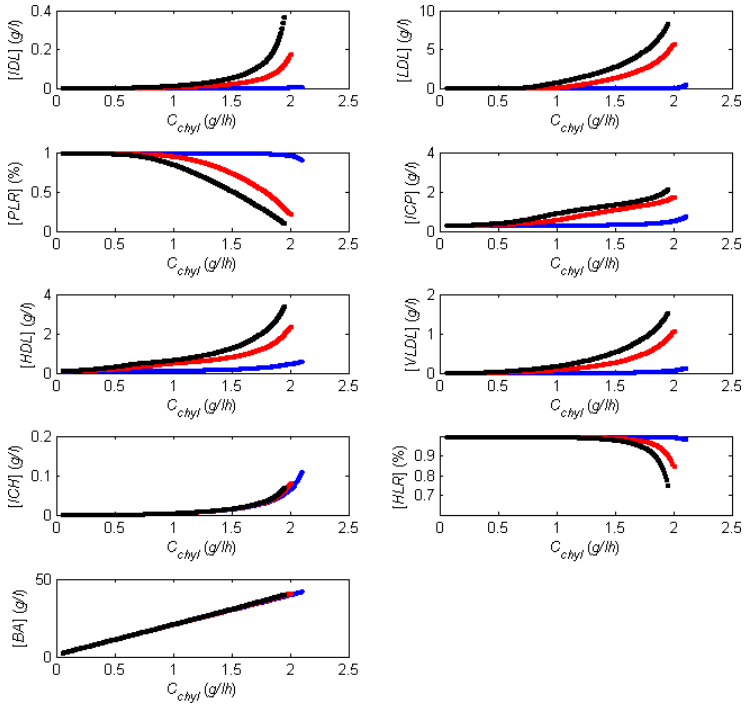

Figure 10.9: Plot of equilibrium solutions versus chylomicron input (C_{chyl}) with a change in the parameter u_v from 3.5 to 10. Blue (original) $u_v = 3.5$, Red $u_v = 5$, Black $u_v = 10$

Changing u_v biologically refers to altering the rate of production of VLDL given the level of cholesterol within the hepatocyte. From our sensitivity analysis, we can conclude the model is surprisingly sensitive to the value of u_v keeping all other parameters constant (see figure 10.9). Since u_v is another outlet for cholesterol from the hepatocytes and we have seen that the bile acid pathway eventually saturates at high levels of cholesterol intake, we speculated and confirmed that changes in u_v will alter the equilibrium value significantly. However, even though the control is occurring in the hepatocyte, we can also see in figure 10.9 that increasing the value of u_v has a greater effect on the peripheral cells than on the hepatocytes, an unexpected result.

By increasing the rate of production of VLDL, we also increase the equilibrium value of all the other lipoproteins. Since the intake of cholesterol into the peripheral cells is dependent upon the concentration of lipoproteins, it will

respond by allowing more endocytosis of LDL and IDL particles. Increase this intake results in an increase in peripheral cell IC concentration which decreases the receptor recycling and production leading to a decline in the receptor values.

In contrast to the peripheral cells, the hepatocytes do not exhibit such sensitivity to increases in u_v and only at very high cholesterol intake ($C_{chyl} > 1.5$) do we begin to see any significant differences in receptor fraction. Hepatocytes have the bile acid synthesis pathway to regulate the intracellular cholesterol levels more highly and can thus take higher values of cholesterol intake while still being able to maintain homeostasis, explaining why changing values of u_v have a greater effect on the peripheral cells than on hepatocytes.

Since we are varying quite a number of variables, one aspect of further work is to see how combinations of perturbations affect the model. Here we have assumed that our initial estimates for values were the correct values and have performed sensitivity analysis assuming that any perturbation will be changing from the physiological parameters. However, in the human body, almost all pathways are linked meaning that if one parameter changes, then it is almost certain that one other parameter will also change (either directly or inversely).

To obtain a clearer picture of the effects of these parameters, a Monte Carlo simulation should be done to test changes in all the parameters at once. Further research should also go into studying the complex interactions more specifically between *de novo* cholesterol synthesis, bile acid biosynthesis, and the internalization of cholesterol via lipoproteins. Only with a full understanding can be better appreciate the complexity of the system and more accurately model changes in parameters.

10.4 Statin Effects

One of the major section of this project was to come up with a model to take into account drug interactions with the cholesterol metabolism pathway. We have first gone into detail regarding the *de novo* cholesterol synthesis and how statins affect the rate limiting step in the pathway catalyzed by HMGR. Now we test the effect of statins in this section in the two different cholesterol regimes that we observed when plotting the equilibrium solutions to our system.

10.4.1 Statins in the Low Cholesterol Regime

Generally statins would not be prescribed if a patient does not have an elevated cholesterol level, but does it make a difference if the drugs are prescribed for a slight elevation of cholesterol levels (as determined by LDL levels)? In the low cholesterol regime, we assume that we are dealing with cholesterol dietary inputs (C_{chyl}) between 0 and 1 $g(lh)^{-1}$. We now sweep the statin parameters to see the effect it has on the other variables.

From our analysis in figure 10.10, we can see that there is an approximately 11% decrease in the LDL cholesterol levels if we increase the statin dose to 0.5 g/l. Evidence has shown that statins only reduce the LDL cholesterol concentration by about 30-60% in patients when clinical studies were done (Schachter, 2005). This may also reflect exercise and dietary limitations which have the effect of lowering the LDL cholesterol significantly as seen before (C_{chyl} decrease affects equilibrium LDL concentration).

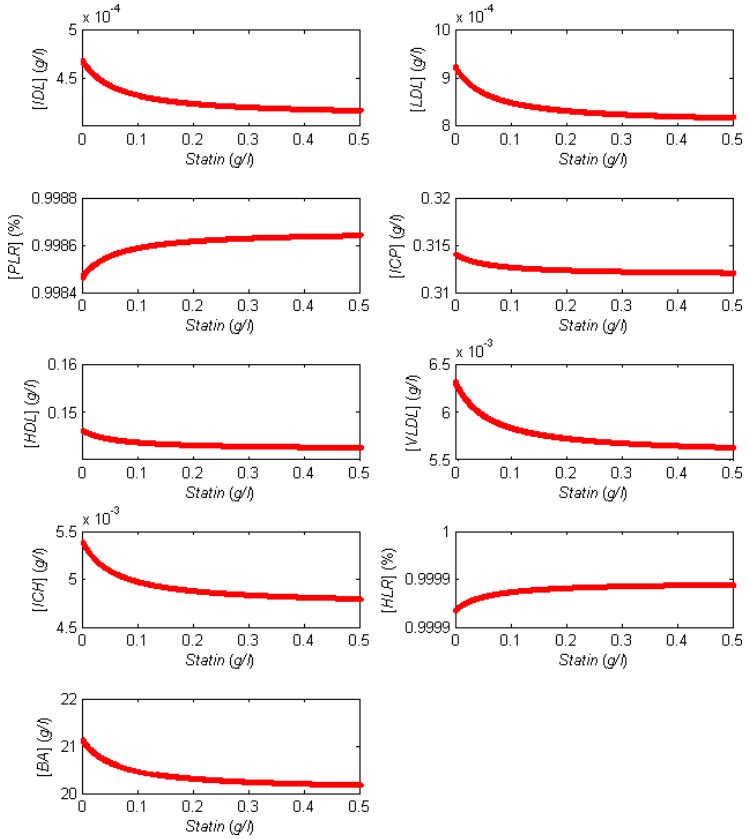

Figure 10.10: Plot of equilibrium solutions versus statin input with a low dietary input ($C_{chyl} = 1$)

One thing that is missing from our model is the increase in HDL of about 10% as reported in clinical studies (Schachter, 2005). It is not understood how statins affect the HDL cholesterol, but we were hoping that it might appear in the model. This just goes to show that there is a deficiency in our model that we are not taking into consideration. Until further studies have been shown regarding the precise effect of statins on HDL concentrations, we still have yet to correct this deficiency in our model.

10.4.2 Statins in the High Cholesterol Regime

In the high cholesterol regime, we can see a slightly greater decrease in the LDL cholesterol levels (of about 15%) when statins are included. In the high cholesterol regime, we have set the dietary intake to be 2 $g(lh)^{-1}$ (C_{chyl} level). In figure 10.11, we can see the effect of statins in this regime.

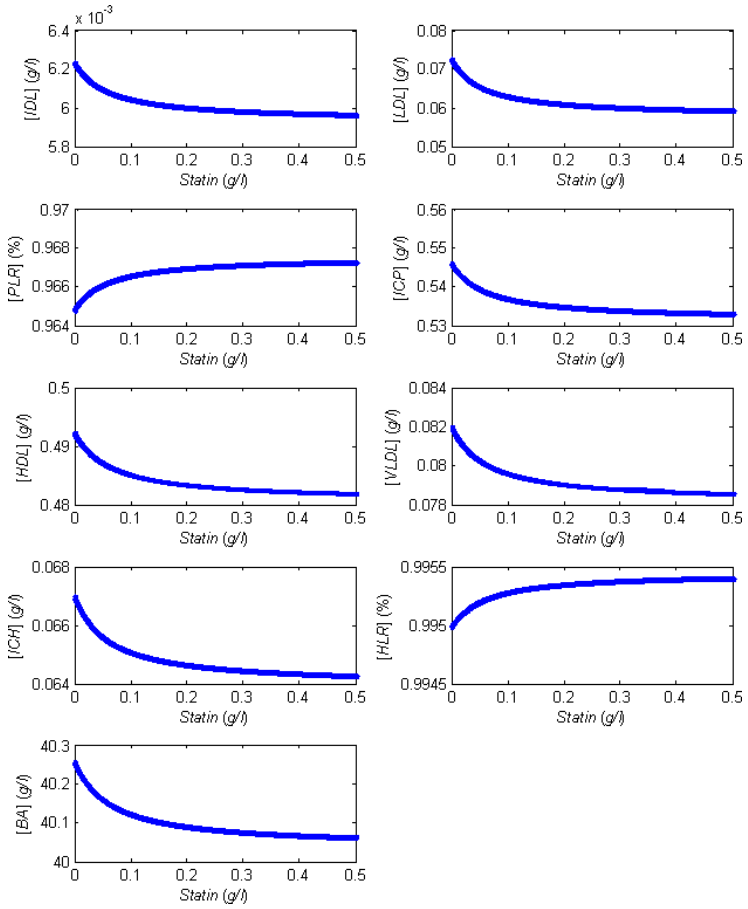

Figure 10.11: Plot of equilibrium solutions versus statin input with a high dietary input ($C_{chyl} = 2$)

Although we expect the effect of statins to be quite high, we observe that statins only affect cholesterol levels slightly and our model does not completely correlate with the clinical data. One reason for this is that in patients prescribed

statins, there is usually a medical condition causing abnormally high levels of cholesterol, for example familial hypercholesterolemia or Tanger's disease. A high dietary intake of cholesterol supplemented with statins would probably not be the best way to lower the cholesterol level, although we do see a slight decrease in cholesterol levels. It has already been seen that decreasing the dietary cholesterol intake has a strong effect on lowering the overall cholesterol concentrations, suggesting that dietary intake of cholesterol is an important factor in preventing atherosclerosis. Only when diet restrictions are insufficient to lower cholesterol is drug interventions necessary.

10.5 Bile Acid Binding Resin Effects

As discussed in a previous section, bile acids serve to increase the amount of cholesterol that is excreted from the body (increasing the value of η in terms of our model). In this section, we investigate the use of bile acid resins in both the low cholesterol regime ($C_{chyl} = 1$) and a higher dietary intake ($C_{chyl} = 2$). Studies have shown that the cholesterol concentrations can decrease by as much as 25% with the use of bile acid binding resins alone.

Physiologically, there is a complex regulatory effect when using resins that should be taken into account and investigated. Using resins will deplete the returned bile acid pool, which causes an increase in the production of C7H enzyme, resulting in an increased conversion of cholesterol to bile acid. Under normal circumstances, the body maintains the homeostasis by increasing the amount of HGMR enzyme to once again bring up the cholesterol concentration to equilibrium, having no net effect on the overall cholesterol levels.

It is for this reason that bile acid binding resins are usually coupled with statins to prevent the cell from producing more cholesterol via the *de novo* pathway and to utilize the cholesterol gained from lipoprotein endocytosis.

10.5.1 Resins in the Low Cholesterol Regime

In the low cholesterol regime, we see quite a substantial effect on the cholesterol concentrations (see figure 10.12). With an increase in the value of η from the normal 5% to 20%, there is already a decrease in LDL concentrations by 70%. Biologically, this might be due to the increase in flux through the cholesterol metabolic pathway, decreasing the bile acid threshold and allowing more cholesterol to flow through. The net effect (as seen), is that the lipoprotein concentrations are decreased.

10.5.2 Resins in the High Cholesterol Regime

When we increase the dietary intake of cholesterol, we again see a similar effect to that found when $C_{chyl} = 1$. There is again a decrease in LDL concentrations of about 70% with the explaination being the same as above. Again what is interesting to note about both the equilibrium solutions in the low and high cholesterol regimes is that we do not see an increase in HDL concentrations as would be seen clinically. Perhaps this can also be explained by this model being for a normal patient with an abnormally high intake of cholesterol.

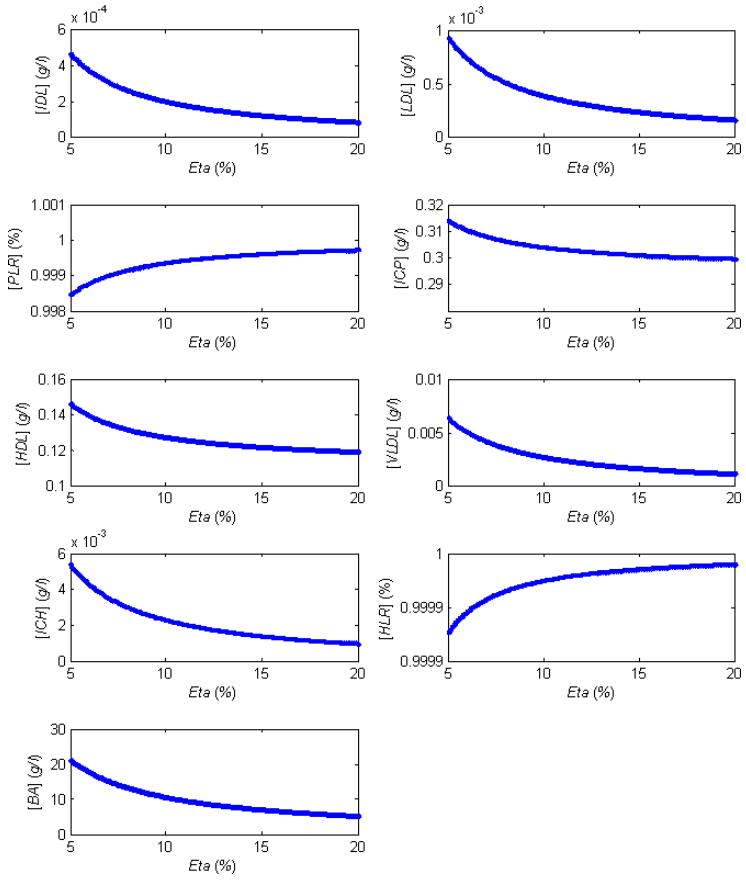

Figure 10.12: Plot of equilibrium solutions versus resin input with a low dietary input ($C_{chyl} = 1$)

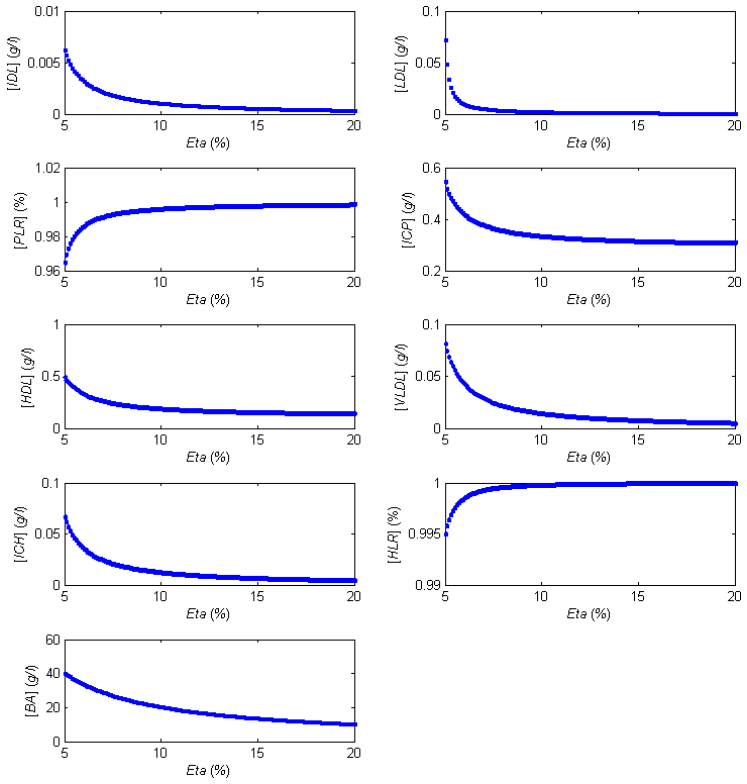

Figure 10.13: Plot of equilibrium solutions versus resin input with a high dietary input ($C_{chyl} = 2$)

10.6 Resins and Statins

As mentioned before, the effect of resins can be countered by the production of more cholesterol via the *de novo* synthesis pathway due to the robust nature of the cell in maintaining cholesterol concentrations. Hence, for severe hypercholesterolemia, a combination of both drugs are given such that the intracellular cholesterol would be dominated by cholesterol derived from the internalization of lipoproteins. This results in the LDL concentrations decreasing and hopefully also decreasing the risk of atherosclerosis and CHD.

From the analysis of our model as seen in figure 10.14 (showing LDL levels only), it would seem that resins provide a much greater effect than statins. Changing the value of η by even 0.3% allows for a decrease in almost 70% of LDL levels. Combined, they do produce a even greater decrease in the LDL concentrations, confirming that our model correlates to drug treatment schemes.

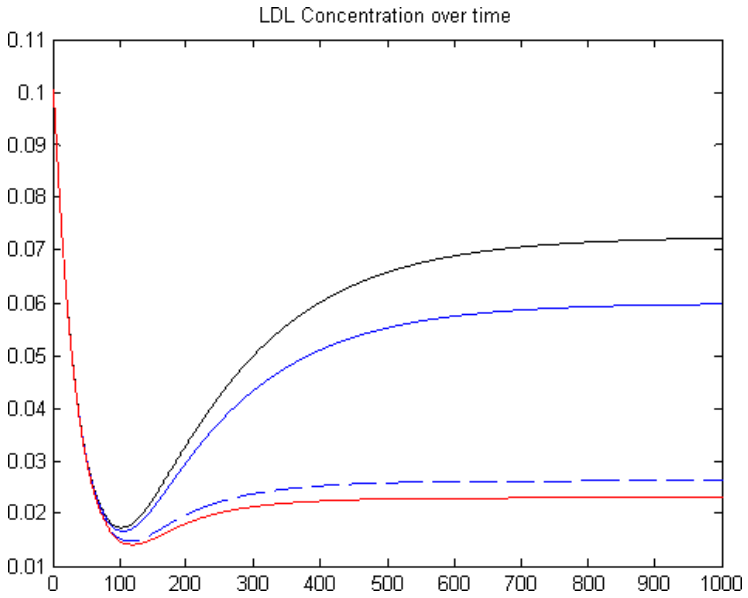

Figure 10.14: LDL concentration over time seeing the effect of statins (solid blue), resins (dashed blue), and a combination of both medications (red) with a high dietary cholesterol intake ($C_{chyl} = 2$). Statin treatment was set at 0.3 g/l and resin treatment was assumed to produce a net increase of excretion by 0.3% (for $\eta = 0.053$).

It is again important to note that this simulation would be on an individual who has normal cholesterol metabolism but has a high daily cholesterol intake. Although we see a decrease in cholesterol levels, we speculate that in a disease state where cholesterol levels are elevated not because of dietary intake but because of other disorders, we would see a greater decrease in the LDL cholesterol levels. This will be explored further in another section on disorders of cholesterol metabolism.

10.7 Modelling Daily Cholesterol Intake

Investigating an oscillatory input can give us insight into what happens in every day life where feeding is not a continuous stream, but at discrete intervals. Although an actual model of this would probably be to perturb the system at given time intervals corresponding to the meals during the day, we can simplify this to a sinusoidal input of cholesterol from the diet (changing C_{chyl}).

We initially set the period of the oscillations equal to one day to see the effects as was done in previous papers with simpler models (see August, 2007). What we hope to find out is that a similar behavior exists from previous models and hope to extract any new or interesting findings when expanding the low-dimensional model into a higher dimensional model taking into account more physiological detail.

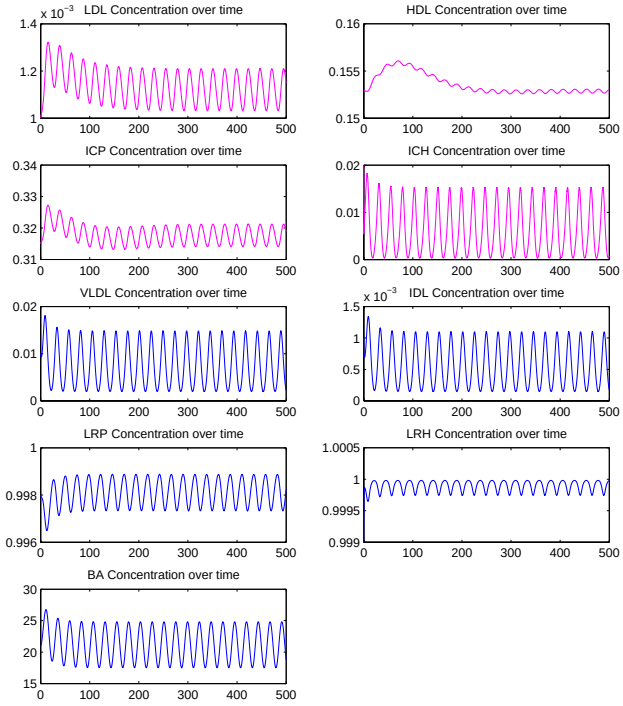

Figure 10.15: Reaction to forced oscillations with a period of a day

The results of the forced oscillation is seen in figure 10.15. Looking closely, we first note that the response from IDL is slightly greater than LDL. This is consistent with the findings in August's 2007 paper. Further points of interest are that the receptor fraction remains almost at 1 throughout, that HDL is less responsive to cyclic changes than the other lipoprotein levels, and that the changes to intracellular cholesterol levels in both the peripheral cells and the hepatocytes are of the same order.

Since we are still operating before the bifurcation point ($C_{chyl} = 2$), we do not expect to see large variations in the receptor fraction, although this result, coupled with the observation that intracellular cholesterol levels are also fairly constant suggests that all the cells within the body have a good mechanism for coping with changes in cholesterol intake. Human life is dependent on the maintenance of this cholesterol homeostasis and analysis of our model has hinted at how this regulation is achieved and how effective it is.

10.8 Disorders of Cholesterol Metabolism

10.8.1 Tangier Disease

Tangier disease is a autosomal recessive genetic disorder characterized by defective ABCA1 cholesterol transporter that transfers cholesterol from peripheral cells to HDL. People with this condition generally suffer from very low HDL levels, peripheral neuropathy, and frequently premature coronary artery disease (CAD) (Rust, 1999). Because of this impairment of cholesterol transfer, there is an overall decrease in plasma cholesterol (Assmann, 2001). Patients with Tangier disease usually present with large, yellow-orange tonsils.

In terms of our model, the ABCA1 function can be easily switched off by setting $d_{HDL} = 0$, resulting in the cessation of cholesterol transfer from the peripheral cell to the HDL particles. As expected (figure 10.16), we see a decrease in HDL concentration as well as LDL concentration confirming that the model relates well to what is observed *in vivo* (Assmann, 2001).

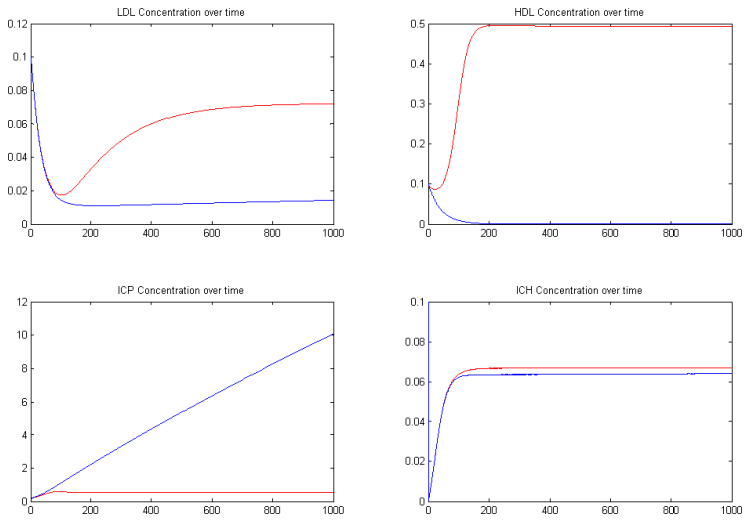

Figure 10.16: LDL, HDL, and intracellular cholesterol concentrations for the peripheral cell (ICP) and hepatocyte (ICH) over time comparing a normal individual (red) and with Tangier Disease (blue)

Another expected aspect of the model is that the intracellular cholesterol in peripheral cells tends to become very high. Since we assumed that the HDL transfer is the only exit of cholesterol for the cell, the concentration inside the cell hence will tend to infinity. On the other hand, the hepatocytes tend to be

fairly steady in maintaining the cholesterol levels with an actual decrease in the cholesterol input. This might be due to the lack of HDL molecules entering the hepatocyte.

There is currently no treatment for Tangier Disease due to its genetic nature, but physicians prevent the risk of CAD by lowering the morbidity of other risk factors such as smoking, dietary intake, and hypertension. (Assmann, 2001)

10.8.2 Familial Hypercholesterolemia

Familial Hypercholesterolemia (FH) is also a recessive genetic disorder occurring in about 1 in 500 people (Goldstein, 2001). The primary deficit in FH patients is the gene specifying the LDL receptors for plasma lipoproteins. This results in a decrease in the rate of removal of LDL with a corresponding increase in the LDL concentrations in the plasma. The LDL particles are deposited in scavenger cells and other cell types if the concentration becomes too high in the plasma.

In our model, we can simulate this by setting the recycling term (c) to zero such that there will be no receptors present on the cell surface anymore. There is an approximately an increase of 20 fold over normal LDL concentrations which correlates well with clinical investigations (up to 1000 mg/dl for patient with FH compared to 50-100 mg/dl for patients without FH). The result over time is shown in figure 10.17 with a dramatic increase in LDL concentrations as expected.

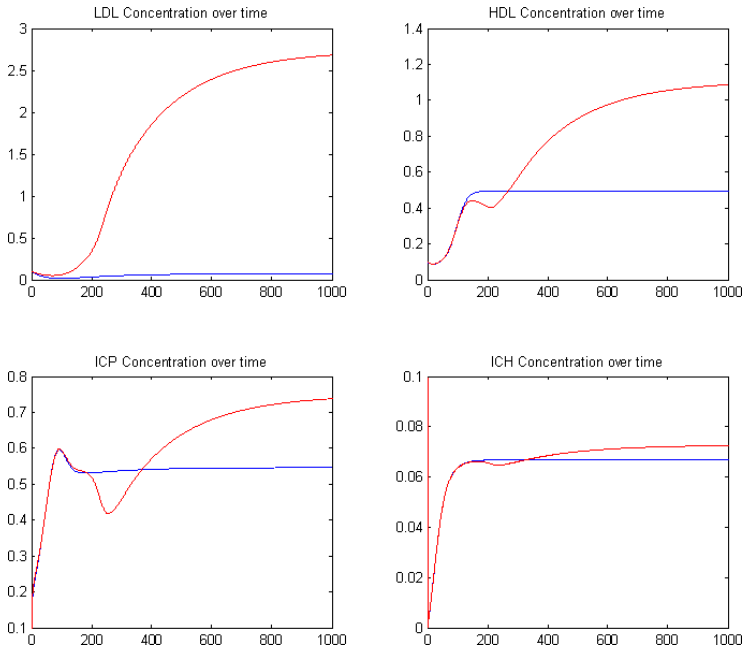

Figure 10.17: Model of Familial Hypercholesterolemia (FH) (red) over time compared to normal individuals (blue).

It is also important to note that there is a general increase in the intracellular cholesterol in both peripheral cells and hepatocytes, with the peripheral cell

having the greater change of the two.

Statins are usually prescribed to treat FH and we have simulated this in our model. Unfortunately, we do not see a significant decrease in the LDL, HDL, or intracellular cholesterol concentrations (see figure 10.18), but the intracellular cholesterol concentrations are more in line with normal values. When statin treatment is coupled to resins, we see a significant decrease in the LDL cholesterol by almost one-third for a very small increase in the value of η ($\eta = 0.053$)(data not shown). This suggests that for sever FH, a treatment coupling statins and resins is very effective in lowering cholesterol levels and could potentially lower the risk of atherosclerosis.

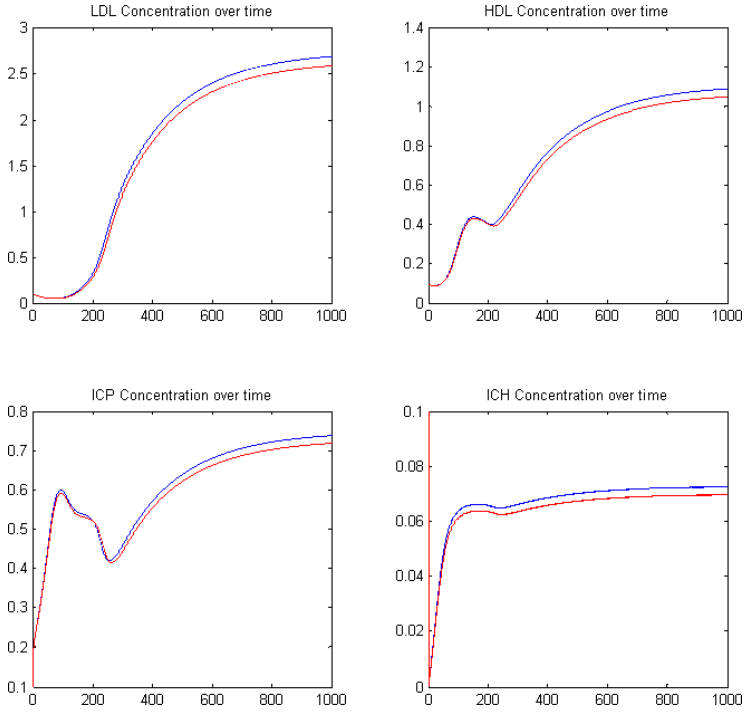

Figure 10.18: Model of FH without statin intervention (blue) and with statin treatment (statin = 0.3) (red).

10.9 Cholesterol Degradation Effects

In our model, we assumed that the only output of cholesterol is the bile acids and we saw that the bile acids would reach a saturation level if cholesterol concentration were high enough, preventing us from seeing what happens past a certain dietary intake. One way to prevent the cholesterol values from going to infinity would be to include another degradation term to the intracellular cholesterol equation. This might correspond to the production of steroid hormones, storage of cholesterol, use in cell signaling, or any of a multitude of uses for cholesterol. Metabolic pathways of cholesterol ending in hormonal production or protein modification used to anchor Hedgehog signaling proteins to cell

membranes (Peters, 2004).

We have modified the cholesterol term as shown in equation 10.1. We have included the effect of statins and bile acid binding resins into the equation for a complete model.

$$\begin{aligned}\frac{d[IC-H]}{dt} =& \frac{k_1}{d_1(b_1+[IC-H])}\frac{k_2[HMG-CoA]}{k_{m1}+[HMG-CoA]+\frac{k_{m1}}{k_i}[Statins]} \\ &- \frac{k_4}{d_4(b_2+\frac{r_1}{k_5}(1-\eta)[BA])}\frac{k_3[IC-H]}{k_{m2}+[IC-H]} \\ &+ (d_I[IDL-C]+d_L[LDL-C])\phi_{HLR} \\ &+ d_5[LDL-C] \\ &+ h[HDL-C] \\ &- u_v[IC-H] \\ &+ C_{chyl} \\ &- d_{ic}[IC-H] \end{aligned} \tag{10.1}$$

Since the d_{ic} parameter is a lump of several parameters, it will be difficult to find the exact value, so we have estimated this value to be 0.5 h^{-1}.

We can see in figure 10.19 that instead of not getting any solutions for the high cholesterol regime, we do now obtain values. The values in the low cholesterol regime were compared to those generated in figure 10.2 and similar values were obtained.

In our original model, we decided not to include the d_{ic} term because the bile acid production is the main excretion pathway for cholesterol. However, we do realize its importance to prevent solutions from blowing up in the overall model, but again cholesterol intake past 2 $g(lh)^{-1}$ is already highly unlikely and not physiologically possible and its clinical relevance becomes negligible. Also, cholesterol concentrations seen in the high cholesterol regimes in figure 10.19 are also physiologically unreasonable and unfeasible, suggesting that the d_{ic} term can be neglected when considering physiological ranges.

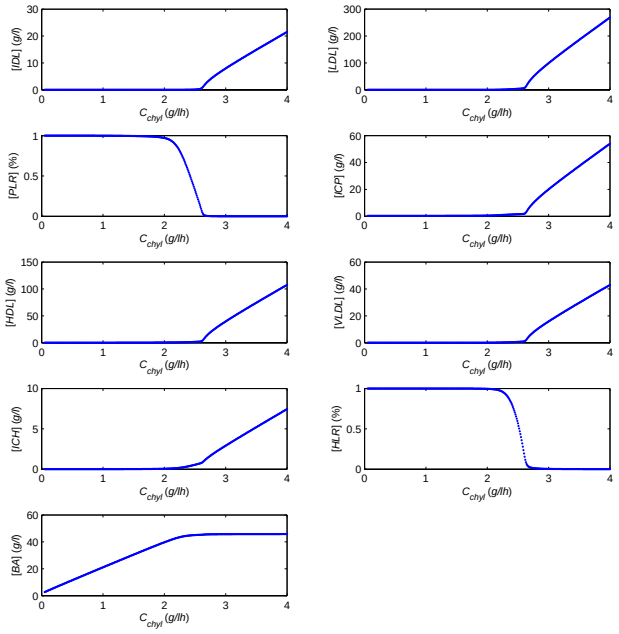

Figure 10.19: Plot of equilibrium solutions versus chylomicron input (C_{chyl}) with a general cholesterol degradation term (d_{ic}) added, now showing the high cholesterol regime without values going to infinity

Chapter 11

Conclusion and Future Work

11.1 Conclusion

In this report we sought to extend the current models of cholesterol metabolism to incorporate specifically the *de novo* cholesterol synthesis and bile acid synthesis pathways. The biology and biochemistry behind these two phenomenon was discussed in detail and a model was created based upon several assumptions regarding rate limiting steps and transport processes of cholesterol throughout the body. Having come up with equations for the *de novo* and bile acid synthesis, we worked toward having a three compartment model motivated by knowing that hepatocytes are key players in globally controlling cholesterol homeostasis while the peripheral cells focus on their own local needs.

The model was then tested and sensitivity analysis performed on the parameters which we were unsure, had to estimate, or values from the literature did not make physical sense when used in our model. The sensitivity analysis in this paper focused primarily on the parameters for the *de novo* and bile acid synthesis pathways and not for the lipoprotein metabolism pathway firmly established in previous studies (Cooper, 2006 and August, 2007). As a verification procedure, the model was compared to previous models by Elias August and Kathryn Cooper to determine the feasibility and validity of the values obtained. Crucially, the existence of a low cholesterol branch and a high cholesterol branch were still seen, but were not of the same form as seen in previous models. The high cholesterol branch corresponded to all cholesterol values increasing toward infinity over time and did not reach an equilibrium value. The low cholesterol branch remained present in our model.

Our sensitivity analysis found that changes to the bile acid production and excretion term (changing k_3, d_3, and r_1) dramatically affected the transition point between the low cholesterol regime and the high cholesterol regime (where cholesterol concentrations increase over time to infinity). When in the low cholesterol regime, it was seen that the model was robust to changes in most parameters.

The effect of medications such as statins and bile acid binding resins was also a driving factor for including the *de novo* synthesis and bile acid produc-

tion terms into the model. Including drug effects into our model did reduce cholesterol concentrations overall, especially LDL concentrations as CHD has been closely linked to high LDL levels. What was not seen was the increase in HDL with the inclusion of statins. Although research has determined the mechanism for action of statins on the HMGR enzyme, we are still unsure of any other interactions this drug might have on other metabolic pathways which could also affect HDL levels.

Daily intake of cholesterol was also simulated in our model to see the sensitivity of our variables with a forced oscillatory input. The overall stability of the system, even to large changes in cholesterol intake, suggested that our model was robust in terms of dealing with varying cholesterol concentrations and that perhaps the human body is also robust at coping with a high cholesterol intake.

Lastly, as a verification with real world scenarios, we modeled the effect of Tangier disease and familial hypercholesterolemia in our system to see if changes in such a model reflected what is seen clinically. Results show that our model adheres to clinical observations in both cases, suggesting that this exercise could be used to investigate possible mechanisms and novel treatments for these diseases and others related to cholesterol metabolism.

In our research, we have answered the question of the effects of statins on some of the players in the cholesterol metabolic pathway are (Davidson, 2001), but have opened up further questions such as what other effects to statins and bile acid binding resins have on both peripheral and hepatic cells. Although the model attempts to incorporate the knowledge of cholesterol metabolism as of today, there are several unknown interactions of statins that contribute to clinical observations which are not seen with this model. We can only hope that more research is done to complete the puzzle of cholesterol metabolism to effectively tackle the primary cause of death in the United States.

11.2 Recommendations for Further Research

Although we have extensively tested the model developed in this paper, we have neglected to rigorously perform sensitivity and stability analysis on our system as was done on previous models (August, 2007). Our sensitivity analysis of each parameter was based on the assumption that the parameter values that we estimated were correct. A more rigorous method for verifying that the model is robust to changes in all parameters would be to perform a Monte Carlo sensitivity analysis. This randomly changes all the variables in parallel to ensure that we are not biasing our analysis.

In terms of biochemistry, the literature review found a substantial lack of concrete values related to many of the parameters. The reason for this being that we have lumped in several steps into one parameter and there is no value for the conglomerate of steps, and that some parameter values are just difficult to obtain. Mathematical biologists and laboratory based biochemists should cooperate in coming up with pathway models and doing experiments to ascertain the parameter values. Unfortunately, biology itself is rather temperamental when it comes to experiments done at such small scale only compounding on the difficulty of finding such parameter values.

Perhaps to get around the problem of needing to come up with parameters which describe entire pathways (as we did for the *de novo* synthesis pathway),

further studies could improve the detail of the model and incorporate more or even all of the enzymatic and transport pathways involved in cholesterol metabolism. Many of these individual steps have been characterized in detail allowing for a more accurate model to be developed. On the other hand, small errors in individual steps could lead to disaster when modelling.

Another possible extension of this is in terms of drug development. We have shown that our model can be used to accurately describe what occurs in both Tangier disease and FH. One could use this model to perturb different aspects which might be impossible *in vivo*, for example the transcription rate of HMGR enzyme. Strong changes in LDL cholesterol could signify potential points where interventional drugs could limit the risk of CHD and atherosclerosis, paving a novel way for experimentation. Current methods of finding novel drugs mainly by trial and error are outdated and must be replaced by modern technology which is cheaper and more efficient.

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