

University of Reading
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**Mathematical Modelling of the regulation
and uptake of dietary fats**

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Abstract

In this dissertation we extend a recent *in vitro* model of lipoprotein uptake by hepatocyte (liver) cells to the *in vivo* context. Lipoproteins are transporters of fat and cholesterol around the body; high levels of some cholesterol are associated with coronary heart disease.

Using the current model and a detailed biological diagram we construct a simplified model of the processes involved in lipoprotein metabolism. From this model we extract the main reaction equations. We then use the Law of Mass Action to form a system of non-linear ordinary differential equations (ODE's) from these reaction equations. The system of non-linear ODE's is non-dimensionalised and solved numerically.

We perform some Steady State analysis on the system of equations and find that only one steady state exists and we assess its stability numerically. Sensitivity analysis is performed on the non-dimensionalised system of ODE's and we find that certain parameters have significant affect on the model in comparison with others. We also perform some asymptotic analysis on the system of ODE's.

We then extend the model to try and capture the affect that meals have on lipoprotein and cholesterol levels. Finally we suggest possible future work and further development of the model.

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Declaration

I confirm that this is my own work, and the use of all material from other sources has been properly and fully acknowledged.

Signed..... Date.....

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Chapter 1

Introduction

The work presented in this dissertation relates to the mathematical modelling of lipoprotein uptake by hepatocyte cells. Lipoproteins are transporters of fat and cholesterol around the body. Cholesterol is made and used by the body for protecting nerves, making cell tissues and producing hormones. As well as our body producing it, it is also present in much of the food we eat.

When we eat a fatty meal the liver responds by releasing higher than normal amounts of very low density lipoproteins (VLDL). These VLDL are suspended in the blood plasma and travel in circulation. VLDL present in the extracellular fluid around the liver may be metabolised by the hepatocyte cells. The majority of VLDL is not in the liver and while in circulation VLDL break down into low density lipoproteins (LDL).

LDL carries cholesterol to the cells, it also carries cholesterol into the artery walls. It can be retained on the artery wall before being absorbed and as result attracts macrophages; macrophages are cells that rid the body of other worn-out cells and other debris. These macrophages engulf the LDL particles and form plaque which builds up on the artery walls.

The growth of these plaques slowly blocks blood flow in the arteries. A worst case scenario is when a piece of plaque ruptures and the subsequent blood clot may cause a heart attack or stroke. The successful modelling of lipoprotein metabolism could reveal possible key processes that could be targeted when developing new drugs for the treatment of Coronary Heart Disease (CHD).

1.1 Lipoproteins

A lipoprotein is a biochemical assembly that contains both proteins and lipids. The major lipoproteins classes are chylomicrons, VLDL, LDL, intermediate-density lipoproteins (IDL) and high-density lipoproteins (HDL). We are interested in lipoproteins which enable fats to be carried in the blood stream.

Lipoproteins are formed from Triglycerols (TAG), Cholesterol Esters (CE) and apolipoproteins. TAG have many uses, one of the most important of which is as a source of energy for the body. Apolipoproteins are proteins that bind to fats (lipids). They play an important role in the breakdown of lipoproteins and in their uptake by the hepatocyte cells.

We can see a large reduction in LDL after a fatty meal, even more so after a saturated fatty meal. We specify one type of VLDL particle although elsewhere people have noted that the number of apolipoprotein E (apoE) molecules on each VLDL particle differs depending on the diet of a particular person [1]. VLDL particles isolated following a poly-unsaturated or mono-unsaturated fat meal are VLDL-2 (i.e. on average they have 2 apoE molecules per particle). Following a meal high in saturated fat our isolated VLDL particles carry an average of 3 apoE molecules each, VLDL-3. Thus we can infer that the apoE content of the VLDL particles affect the uptake of LDL.

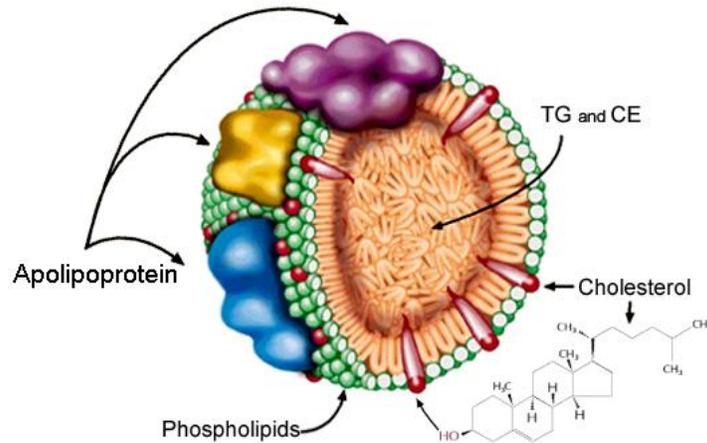


Figure 1.1: Lipoprotein Particle: Image adapted from www.peprotech.com

1.2 Lipoprotein Metabolism

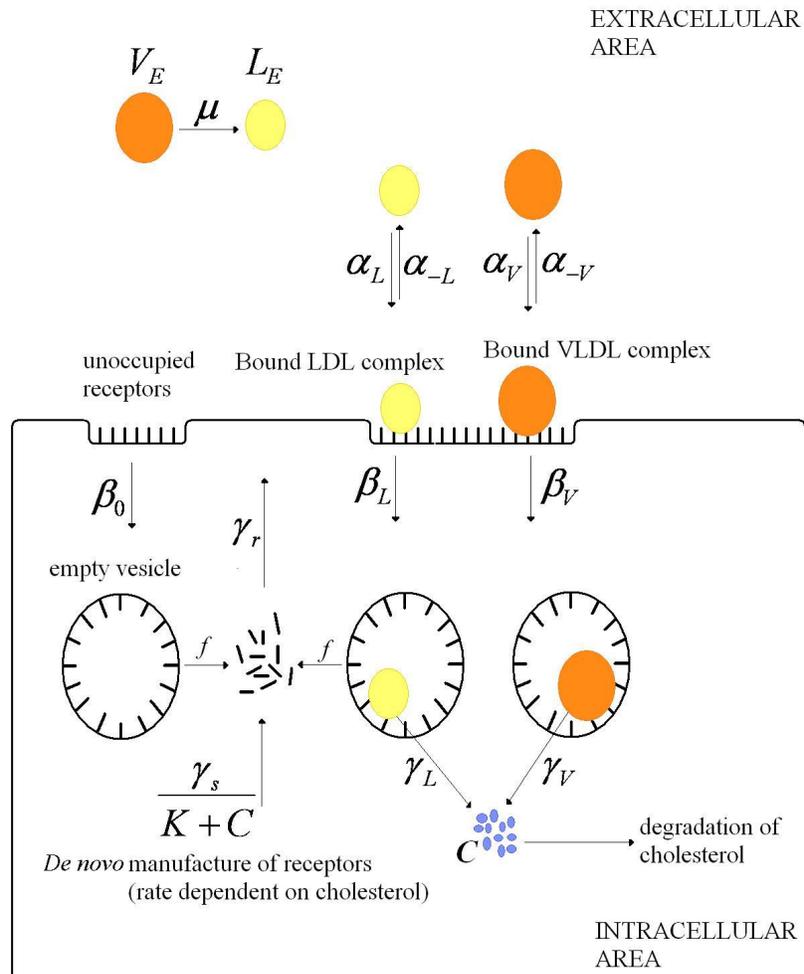


Figure 1.2: An illustrative summary of the processes involved in the uptake of LDL and VLDL particles by a hepatocyte cell, it also includes the breakdown of extracellular VLDL to extracellular LDL. The yellow circles represent LDL particles and the larger orange circles represent VLDL particles. See text for more detailed information on each of the processes.

After a meal containing fats the liver secretes the triglyceride-rich lipoprotein VLDL. Once secreted, VLDL particles travel round the body suspended in blood plasma. In the circulation they acquire apolipoproteins and there is progressive removal of triglycerides from their core by lipoprotein lipase. After enzymes in the plasma remove large chunks of triglycerides the particles become LDL, which are smaller denser particles with a similar amount of cholesterol compared to VLDL.

LDL particles are small enough to move through tissue fluid and deliver cholesterol straight to the tissue. There is much more LDL in the blood plasma than in the extracellular fluid surrounding tissue; approximately 90% of all LDL is found in the blood plasma.

Figure 1.2 depicts the processes involved in the metabolism of LDL particles in the liver. LDL in the liver can be absorbed and digested by hepatocyte cells. On the surface of each hepatocyte cell there are pits full of LDL receptors, (LDLR). The LDL particle binds to the LDLR, this interaction is mediated by the apolipoprotein B100, which is present on the surface of the LDL particle.

When the LDL particle has bound with the LDLR the pit collapses around the bound complex and forms an endosome which is internalised by the cell. The endosome fuses with lysosomes, present within the hepatic cell. The resulting vesicle breaks up and releases cholesterol and LDL protein (amino acids). LDLR are either degraded or recycled to the surface.

Empty pits of LDLR collapse and form empty vesicles which are absorbed by the cell. Some internalised LDLR are recycled to the cell surface, whilst others are broken down inside the cell. The *de novo* rate of receptor recycling is dependent on the cholesterol concentration within the cell and thus the amount of LDLR at the surface of the cell is proportional to the intracellular cholesterol concentration.

Most VLDL breaks down into intermediate density lipoprotein (IDL) which then breaks down into LDL while in circulation, however it can also be absorbed by the hepatocyte cells in the same way as LDL. LDL uptake is influenced by the presence of VLDL since VLDL competes for uptake on the surface of the hepatocyte cell. VLDL is absorbed in the same way as LDL but is larger and thus blocks more LDLR.

1.3 Mathematical Modelling of Lipoprotein Metabolism

Mathematical models of lipoprotein metabolism vary in focus. Some models focus on modelling the *in vitro* case, looking at very specific cellular level dynamics: the binding and internalisation of specific lipoproteins. Other models try to look at the whole system and develop *in vivo* models, these usually involve the breakdown of larger lipoproteins into smaller ones.

August et al. [2] present a model that combines the cascading breakdown of VLDL to IDL and then to LDL with cellular level dynamics: binding, internalisation and digestion of these lipoproteins. In this model the rate at which the liver excretes VLDL is assumed constant. There are some limitations to this model, the first being it assumes that IDL and LDL can both bind with receptors and be internalised by the hepatocyte cell but VLDL particles cannot. The model does not differentiate between free, bound or internalised receptors, so the system of differential equations is relatively simple. The model is sensitive to different types of lipoprotein, it recognises VLDL, LDL, IDL and HDL.

Analysis of the model reveals three steady states, two of which are asymptotically stable. Sensitivity analysis is also performed with the conclusion that LDL concentration is sensitive to changes in the most parameter values whereas intracellular cholesterol is not. This conclusion has a flaw, in the model the LDL is very loosely controlled whereas the intracellular cholesterol levels are very tightly controlled. The model explores the bi stability between a high and a low cholesterol state. They conclude that the main control parameters are the constant injection of VLDL from the liver and the rate at which intracellular cholesterol degrades.

Wattis. J. O'Malley et al. [3] present a model that focuses on the cellular level dynamics. It assumes VLDL and LDL particles are internalised at the same rate. It recognises two different types of VLDL particle; VLDL-2 and VLDL-3 particles, as defined in section 2.2 Where VLDL-2 refers to a VLDL particle that has two molecules of apoE on the surface and VLDL-3 has three molecules of apoE. Like most *in vitro* models it does not model the breakdown of VLDL to LDL and simply assumes an initial value for the number of these lipoprotein particles. It is presented as an initial value problem but does not have a constant injection of VLDL. A constant injection of VLDL is accepted by biologists as a more realistic view of how VLDL enters circulation from the liver.

They conclude that a key process is the binding of VLDL particles to LDLR via apoE

molecules, which inhibits the uptake of LDL particles. The competition is attributed to the apoE content of the VLDL particles. As a result of this they observe a higher proportion of bound VLDL particles to bound LDL particles, compared to the proportion of free VLDL particles compared to free LDL particles in circulation.

Tindall et al. [1] present a purely *in vitro* model with the aim at observing the competition on the surface of the hepatocyte cells between VLDL and LDL particles. The model includes a description of the change in receptor density on the surface of the cell as well as a detailed explanation of particle binding and digestion by the cell. It also includes detail on receptor recycling and intracellular cholesterol release. The model differentiates between two types of VLDL, VLDL-2 and VLDL-3. However it does not include details of the other lipoprotein particles, IDL and HDL, or the cascade between the different types. The model considers a single hepatocyte cell saturated by extracellular fluid, in which lipoproteins are suspended. It considers the extracellular space; the volume of extracellular fluid in proportion to the volume of the hepatocyte cell, $W = 1500$, this is highly unrealistic biologically.

We present a model that combines the detailed cellular level dynamics from the Tindall et al. [1] model with the *in vivo* case of August et al., [2]. The model includes detailed cellular level dynamics: particle binding, internalisation of the receptor lipoprotein complex (endosomes) and the resultant release of cholesterol within the cell. It also includes the breakdown of VLDL particles into LDL particles while the lipoproteins are in circulation. To simulate the liver constantly injecting VLDL into the circulation we include a constant rate of extracellular VLDL. We assume we can roughly model the *in vivo* case by treating the liver as one large cell and using the extracellular space to represent the proportion of blood plasma in the liver compared to the amount in the rest of the circulation. We begin with a high initial concentration of extracellular VLDL to simulate the peak values we would see after a fatty meal.

1.4 Outline

In the following chapter we discuss the development of the model. We begin by identifying key biological processes present in lipoprotein metabolism and use them to form reaction equations. These are used to form a system of non-linear ODE's which are non-dimensionalised and solved numerically. Different initial conditions are discussed and the

results illustrated. We solve the system in equilibrium and numerically illustrate the stability of the steady states found. We perform some sensitivity analysis on the model to see the affect various parameters have on the intracellular cholesterol levels.

In chapter 3 we perform some asymptotic analysis on the non-linear system of ODE's, we present and discuss these results.

We extend the model further in chapter 4 to capture the affect of consuming three fatty meals within 24 hours. We finish by drawing some conclusions and outlining future work.

Chapter 2

The Model

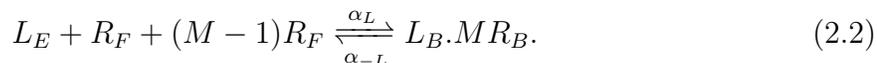
From a simplification of the main biological processes of lipoprotein metabolism some key reaction equations can be identified. From these reaction equations we form a system of ordinary differential equations which we non-dimensionalised and then solve numerically. Numerical analysis is performed on the steady states of this system. Sensitivity analysis of the model is carried out and the results discussed.

The first process we observe is the extracellular VLDL particles breaking down into LDL particles within the circulation:



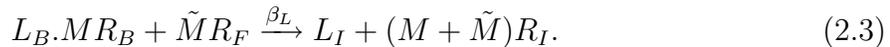
Here V_E and L_E refer to the extracellular VLDL and LDL particles and μ is the rate of breakdown. The extracellular VLDL, V_E is released by the liver at a constant rate U_V .

We now move on to looking at processes directly relating to the metabolism of lipoprotein particles within the liver. The extracellular LDL particles bind to the LDLR on the surface of the hepatocyte cell:



One free LDLR, R_F , binds to one free LDL particle at rate α_L . This binding process blocks $M - 1$ other free receptors in the pit, in total M LDLR are either blocked or bound. The bound complex, $L_B.MR_B$, is composed of both the bound and blocked receptors, where α_{-L} is the rate the bound complexes unbind from the surface.

The bound lipoprotein receptor complex is then internalised:



The pit collapses on itself to form an endosome which contains \tilde{M} free receptors in addition to the M receptors that are part of the bound complex. The endosome is internalised by the hepatocyte cell, where L_I and R_I are the internalised LDL particle and receptors respectively and β_L is the rate at which the hepatocyte cell internalises the endosome.

Simultaneous to these processes occurring, empty pits containing P free receptors on the cell surface are also being internalised at a rate β_0 :



The internalised LDL particles release cholesterol into the cell:



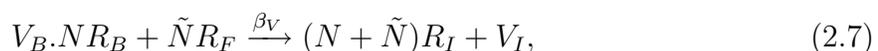
The internalised LDL particle, L_I , releases cholesterol at a rate γ_L . C is the intracellular cholesterol concentration and R_L^{chol} the average number of cholesterol molecules per LDL particle.

The binding and internalisation of the VLDL particles is similar to that of LDL particles. First the extracellular VLDL particle, V_E , binds to the LDLR to form a bound receptor lipoprotein complex, $V_B.NR_B$:



One receptor is bound and $N - 1$ are blocked, this happens at a rate α_V . The bound VLDL-receptor complex unbinds from the surface at rate α_{-V} .

The bound complex is internalised and we assume the VLDL particle is ingested in a similar way to LDL particles, i.e. it does not sit in the pit blocking the receptors but is digested by the cell:



\tilde{N} free receptors are enclosed by the pit when it forms an endosome which is then internalised at rate β_V . Subsequently $N + \tilde{N}$ internalised receptors are released and a fraction, f , of these will be recycled to the cell surface. One VLDL particle, V_I , is also released within the cell.

The internalised VLDL particles release cholesterol into the cell:



The cholesterol is released at rate γ_V , where R_V^{chol} is the number of cholesterol molecules per VLDL particle. Details on all the parameter rates is provided in section 2.2.

Applying the law of mass action [6] to the reaction equations (2.1)-(2.8) leads to a system of non-linear ODE's:

$$W \frac{dl_E}{dt} = -\alpha_L \rho_F l_E + \alpha_{-L} l_B + \mu v_E, \quad (2.9)$$

$$\frac{dl_B}{dt} = \alpha_L \rho_F l_E - \alpha_{-L} l_B - \beta_L l_B, \quad (2.10)$$

$$\frac{dl_I}{dt} = \beta_L l_B - \gamma_L l_I, \quad (2.11)$$

$$W \frac{dv_E}{dt} = -\alpha_V \rho_F v_E + \alpha_{-V} v_B - \mu v_E + U_V, \quad (2.12)$$

$$\frac{dv_B}{dt} = \alpha_V \rho_F v_E - \alpha_{-V} v_B - \beta_V v_B, \quad (2.13)$$

$$\frac{dv_I}{dt} = \beta_V v_B - \gamma_V v_I, \quad (2.14)$$

$$\begin{aligned} \frac{d\rho_F}{dt} &= -M\alpha_L l_E \rho_F - N\alpha_V v_E \rho_F + M\alpha_{-L} l_B + N\alpha_{-V} v_B \\ &\quad - P\beta_0 \rho_F^P - \beta_L \tilde{M} l_B - \beta_V \tilde{N} v_B, \end{aligned} \quad (2.15)$$

$$\begin{aligned} \frac{d\rho_I}{dt} &= Pf\beta_0 \rho_F^P + f\beta_L(M + \tilde{M})l_B + f\beta_V(N + \tilde{N})v_B \\ &\quad - \gamma_r \rho_I + \frac{\gamma_s}{K + C}, \end{aligned} \quad (2.16)$$

$$\frac{dc}{dt} = \gamma_L R_L^{chol} l_I + \gamma_V R_V^{chol} v_I - \lambda(C - C_e). \quad (2.17)$$

We use similar variables to represent the concentrations of free, bound and internalised LDL and VLDL particles and receptors: $l_E = [L_E]$, $v_E = [V_E]$, $\rho_F = [R_F]$, $\rho_I = [R_I]$,

$l_B = [L_B]$, $v_B = [V_B]$, $l_I = [L_I]$ and $v_I = [V_I]$. Previous models of the *in vitro* case took W as the ratio of volume of the cell culture medium to the volume of the hepatic cell. We model W as the ratio of the volume of blood plasma in the circulation to the volume of blood plasma in the liver.

We also note that the concentration of bound receptors on the surface, ρ_B , is given by:

$$\rho_B = Ml_B + Nv_B. \quad (2.18)$$

Initially we assume all extracellular LDL occurs as a result of the breakdown of VLDL and thus the initial conditions are defined:

$$\begin{aligned} l_E(0) &= 0, & l_B(0) &= 0, & l_I(0) &= 0, \\ v_E(0) &= v_0, & v_B(0) &= 0, & v_I(0) &= 0, \\ \rho_F(0) &= \rho_0, & \rho_I(0) &= 0, & C(0) &= 0.7C_e. \end{aligned} \quad (2.19)$$

Note that we assume the cell initially has an intracellular cholesterol concentration of 70% of its maximal value C_e .

2.1 Non-Dimensionalisation

The system of ODE's (2.9)-(2.17) is non-dimensionalised using the following re-scalings:

$$\begin{aligned} t &= \frac{\hat{t}}{\alpha_L \rho_0}, & l_E &= l_0 \hat{l}_E, & l_B &= l_0 \hat{l}_B, \\ l_I &= l_0 \hat{l}_I, & v_E &= v_0 \hat{v}_E, & v_B &= v_0 \hat{v}_B, \\ v_I &= v_0 \hat{v}_I, & \rho_F &= \rho_0 \hat{\rho}_F, & \rho_I &= \rho_0 \hat{\rho}_I \\ C(0) &= C_e \hat{C}. \end{aligned} \quad (2.20)$$

This gives the following system of equations:

$$W \frac{d\hat{l}_E}{d\hat{t}} = (-\hat{\rho}_F \hat{l}_E + \psi_L \hat{l}_B + \zeta \hat{v}_E), \quad (2.21)$$

$$\frac{d\hat{l}_B}{d\hat{t}} = \hat{\rho}_F \hat{l}_E - \psi_L \hat{l}_B - \chi_L \hat{l}_B, \quad (2.22)$$

$$\frac{d\hat{l}_I}{d\hat{t}} = \chi_L \hat{l}_B - \omega_L \hat{l}_I, \quad (2.23)$$

$$W \frac{d\hat{v}_E}{d\hat{t}} = (-\phi_V \hat{\rho}_F \hat{v}_E + \psi_V \hat{v}_B - \zeta \hat{v}_E + \bar{U}_V), \quad (2.24)$$

$$\frac{d\hat{v}_B}{d\hat{t}} = \phi_V \hat{\rho}_F \hat{v}_E - \psi_V \hat{v}_B - \chi_V \hat{v}_B, \quad (2.25)$$

$$\frac{d\hat{v}_I}{d\hat{t}} = \chi_V \hat{v}_B - \omega_V \hat{v}_I, \quad (2.26)$$

$$\begin{aligned} \frac{d\hat{\rho}_F}{d\hat{t}} = & \left(\gamma_{rr} \sigma \hat{\rho}_I - \chi_0 \hat{\rho}_F^P - \left(m \hat{l}_E \hat{\rho}_F - m \psi_L \hat{l}_B + \frac{m \chi_L \hat{l}_B \hat{\rho}_F}{1 - \hat{\rho}_F} \right) \right. \\ & \left. - r \left(n \phi_V \hat{\rho}_F \hat{v}_E - n \psi_V \hat{v}_B + \frac{n \chi_V \hat{v}_B \hat{\rho}_F}{1 - \hat{\rho}_F} \right) \right) / \sigma, \end{aligned} \quad (2.27)$$

$$\begin{aligned} \frac{d\hat{\rho}_I}{d\hat{t}} = & \left(\frac{\gamma_s^* \sigma}{\bar{K} + \hat{C}} + \chi_0 f \hat{\rho}_F^P + f \left(1 + \frac{\hat{\rho}_F}{1 - \hat{\rho}_F} \right) \right. \\ & \left. \times (m \chi_L \hat{l}_B + n \chi_V r \hat{v}_B) - \sigma \gamma_{rr} \hat{\rho}_I \right) / \sigma, \end{aligned} \quad (2.28)$$

$$\frac{d\hat{C}}{d\hat{t}} = \Upsilon (\omega_L R_L^{chol} \hat{l}_I + \omega_V r R_V^{chol} \hat{v}_I) - \lambda^* (\hat{C} - 1). \quad (2.29)$$

This leads to the following non-dimensional parameters. Firstly we have the binding and unbinding rates of the lipoproteins with the LDLR:

$$\phi_V = \frac{\alpha_V}{\alpha_L}, \quad \psi_L = \frac{\alpha_{-L}}{\alpha_L \rho_0}, \quad \psi_V = \frac{\alpha_{-V}}{\alpha_L \rho_0} \quad (2.30)$$

The internalisation rates of the endosomes become:

$$\chi_0 = \frac{\beta_0 P \rho_0^{P-1}}{\alpha_L l_0}, \quad \chi_L = \frac{\beta_L}{\alpha_L \rho_0}, \quad \chi_V = \frac{\beta_V}{\alpha_L \rho_0}. \quad (2.31)$$

The rates of internalised LDL, VLDL and cholesterol breakdown are:

$$\omega_L = \frac{\gamma_L}{\alpha_L \rho_0}, \quad \omega_V = \frac{\gamma_V}{\alpha_L \rho_0}, \quad \lambda^* = \frac{\lambda}{\alpha_L \rho_0}. \quad (2.32)$$

De novo receptor production, receptor dependent cholesterol regulation and receptor recycling:

$$\gamma_s^* = \frac{\gamma_s}{C_e \alpha_L \rho_0^2}, \quad \bar{K} = \frac{K}{C_e}, \quad \gamma_{rr} = \frac{\gamma_r}{\alpha_L \rho_0}. \quad (2.33)$$

Relative size of LDL-VLDL particles, pits and relative concentrations:

$$r = \frac{v_0}{l_0}, \quad \sigma = \frac{\rho_0}{l_0}, \quad \Upsilon = \frac{1}{R_L^{chol}}. \quad (2.34)$$

VLDL breakdown to LDL and constant rate of VLDL release:

$$\zeta = \frac{\mu}{\alpha_L \rho_0}, \quad \bar{U}_V = \frac{U_V}{\alpha_L \rho_0}. \quad (2.35)$$

Finally the initial conditions for the non-dimensionalised system are:

$$\begin{aligned} \hat{l}_E(0) &= 0, & \hat{l}_B(0) &= 0, & \hat{l}_I(0) &= 0, \\ \hat{v}_E(0) &= 1, & \hat{v}_B(0) &= 0, & \hat{v}_I(0) &= 0, \\ \hat{\rho}_F(0) &= 1, & \hat{\rho}_I(0) &= 0, & \hat{C}(0) &= 0.7C_e. \end{aligned} \quad (2.36)$$

2.2 Parameterisation

A literature search provided the relevant information on most of the model parameters, see Table 2.2. At any one time approximately 10 – 15% of a person’s blood is in their liver [8]. We take that as a proportion 10 : 1 of volume of blood in the body to volume of blood in the liver, so we take $W = 10$.

Finding a current value for the breakdown of VLDL to LDL is difficult. In previous literature it is noted that the breakdown of VLDL to IDL is $0.3h^{-1}$ and IDL to LDL is $0.1h^{-1}$ [2]. However these values gave spurious results when input to the model. Investigation

into the source of these values found that they were taken using a tracer experiment on 20 healthy subjects with varying lipoprotein levels [4]. All other model parameters were gained *in vitro* which explains why they do not work with our other parameters. After talks with Dr Kim Jackson, a member of the Food and Nutrition Sciences department, we decided to take $\mu = 1hr^{-1}$ as a reasonable estimate. We assume it takes VLDL particles on average 1 hour to breakdown into LDL.

The initial values of VLDL were taken to be the peak values measured after a fatty meal. The value of extracellular VLDL at a fasting state is $15\mu g/ml$ and the peak values recorded after a fatty meal are $20 - 25\mu g/ml$. LDL values show considerably less variation and are approximately $10\mu g/ml$. Taking the weight of a small VLDL particle to be $6 \times 10^6 Da$ [5] and the weight of an LDL particle to be $2 \times 10^6 Da$ we calculate the initial values in terms of *particles/ml medium*.

We take the fasting level of VLDL to be our base line value; the value we expect VLDL to tend towards. We took the value of constant injection of VLDL as $0.3g(lh)^{-1}$ [2] and used the information about particle weight to convert this to a value in *particle/ml/s*.

All dimensional values, including those calculated and those sourced from the literature can be found in table 2.2.

Parameter	Description	Value	Reference
P	Number of pits per cell.	180	Tindall et al. (2009) [1]
M	Number of receptors covered by a bound LDL particle.	1	Tindall et al. (2009) [1]
N	Number of receptors covered by a bound VLDL particle.	2	Tindall et al. (2009) [1]
α_L	Rate of LDL binding to free receptors.	6.66×10^{-17} ml/molecules s	Tindall et al. (2009) [1]
α_V	Rate of VLDL binding to free receptors.	$14.0 \alpha_L$	Tindall et al. (2009) [1]
β_L	Rate of LDL internalisation.	$2.7 \times 10^{-3} s^{-1}$	Tindall et al. (2009) [1]
β_V	Rate of VLDL internalisation.	β_L	Tindall et al. (2009) [1]
β_0	Rate of unbound receptors internalisation.	0	Tindall et al. (2009) [1]
α_{-L}	Rate of LDL unbinding from receptors.	$5.9 \times 10^{-4} s^{-1}$	Tindall et al. (2009) [1]
α_{-V}	Rate of VLDL unbinding from receptors.	$0.5 \alpha_{-L}$	Tindall et al. (2009) [1]
γ_L	Rate of conversion of internalised LDL to cholesterol.	$\propto 1/300$ s	Tindall et al. (2009) [1]
γ_V	Rate of receptor recycling from bound VLDL.	γ_L	Tindall et al. (2009) [1]
γ_r	Rate of receptor recycling.	$0.001 s^{-1}$	Tindall et al. (2009) [1]
f	Fraction of receptors recycled.	0.9	Tindall et al. (2009) [1]
K	Constant for receptor production.	$2C_e$	Tindall et al. (2009) [1]
γ_s	Rate of free receptor production by cell.	1.8×10^{30} molecules/ml s	Tindall et al. (2009) [1]
C	Rate of breakdown of cholesterol.	$3.3 \times 10^4 s^{-1}$	Tindall et al. (2009) [1]
R_L^{chol}	Average cholesterol content per LDL particle.	3400	Tindall et al. (2009) [1]
R_V^{chol}	Average cholesterol content per VLDL particle.	3100	Tindall et al. (2009) [1]
ρ_0	Initial concentration of free receptors.	2.5×10^4 /cell	Tindall et al. (2009) [1]
C_e	Maximum cholesterol content of a hepatocyte.	2.65×10^{19} molecules/ml	Tindall et al. (2009) [1]
ρ_0	Initial concentration of free receptors.	3.26×10^{13} receptors/ml	Tindall et al. (2009) [1]
l_0	Initial concentration of LDL particles (mass/vol).	10 μ g/ml	Tindall et al. (2009) [1]
l_0	Initial concentration of LDL particles (no./vol).	3.011×10^{13} particles/ml	Gustafson et al. (1965) [5]
v_0	Typical concentration of VLDL particles (mass/vol).	15-25 μ g/ml	Gustafson et al. (1965) [5]
v_0	Initial concentration of VLDL particles (no./vol).	2.509×10^{12} particles/ml medium	Gustafson et al. (1965) [5]
W	Volume ratio of blood plasma in circulation to blood plasma in the liver.	10	Assumed
μ	Rate of VLDL-LDL breakdown.	$2.78 \times 10^{-4} s^{-1}$, $1.74 \times 10^{-3} s^{-1}$	Assumed*
U_V	Constant rate of VLDL released.	8.364×10^{-3} particles/ml/s	August et al. (2007) [2]

Table 2.1: Dimensional Parameter Values. * μ is assumed to be the value discussed in section 2.2, in addition to this a faster rate was used in the solutions to follow.

Parameter	Description	Value	Reference
m	Relative size of LDL to pit.	1	Tindall et al. (2009) [1]
n	Relative size of VLDL to LDL.	2	Tindall et al. (2009) [1]
p	Relative size of pit to LDL.	180	Tindall et al. (2009) [1]
r	Ratio of VLDL concentration to LDL concentration.	0.25, 1, 2	Tindall et al. (2009) [1]
Υ	Ratio of initial LDL concentration to initial cholesterol.	2.94×10^{-4}	Tindall et al. (2009) [1]
$\chi_L = \chi_V$	Relative rate of LDL/VLDL internalisation.	1.28	Tindall et al. (2009) [1]
ϕ_V	Relative rate of VLDL binding to free receptors.	14	Tindall et al. (2009) [1]
ψ_L	Rate of LDL unbinding from receptors.	0.279	Tindall et al. (2009) [1]
ψ_V	Rate of VLDL unbinding from receptors.	0.140	Tindall et al. (2009) [1]
χ_0	Rate of internalisation of free receptors.	0	Tindall et al. (2009) [1]
$\omega_L = \omega_V$	Rate of digestion of internalised LDL/VLDL to cholesterol.	1.56	Tindall et al. (2009) [1]
σ	Ratio of receptor to LDL concentrations (rescaled).	2.74	Tindall et al. (2009) [1]
γ_{rr}	Rate of receptor recycling.	30	Tindall et al. (2009) [1]*
f	Fraction of receptors recycled.	0.9	Tindall et al. (2009) [1]
γ_s^*	Rate of <i>de novo</i> receptor production.	0.54	Tindall et al. (2009) [1]*
K	Constant regulating cholesterol dependence of receptor production.	2.0	Tindall et al. (2009) [1]
λ^*	Rate of breakdown of cholesterol.	1.56	Tindall et al. (2009) [1]
W	Volume ratio of cell culture medium to cell volume.	100	Assumed
ζ	Relative rate of VLDL-LDL breakdown.	0.13, 0.8	Assumed
\dot{U}_V	Constant rate of VLDL released.	3.85	August et al. (2007) [2]

Table 2.2: Non-Dimensional Parameter Values. *These non-dimensionalised values cannot be obtained from the transformations (2.33). This due to the fact that we have not accounted for the number of *receptors/molecule*. Unfortunately this information was unavailable in the literature but these non-dimensional values have been verified by Dr Marcus Tindall

2.3 Solution

A stiff system is a special system of ODE's. Definition of stiffness varies. It is generally taken to mean a system where some terms can lead to rapid variation in the solution which can lead to instability when using most numerical schemes. The eigenvalues of the Jacobian matrix, λ_i , completely characterize the stability of the system. In our system the large variation in orders of magnitude of the non-dimensionalised parameters, see Table 2.2, causes extreme variation of eigenvalues in the Jacobian, creating a stiff system.

For this complicated system of equations we seek a numerical solution. The most general and commonly used numerical methods are Euler's method, ADAMS method and fourth order Runge-Kutta among others. Adams-Bashforth and Euler's methods are explicit methods which are unsuitable for solving stiff systems due to the extremely small time steps that need to be taken in order to keep the error bounded. Adams-Moulton and fourth order Runge-Kutta methods are implicit schemes which allows larger time steps to be taken, however their stability regions are inappropriate for solving stiff systems. An alternative is the trapezium method which is unconditionally stable, however it produces oscillations and has a low level of accuracy.

In a stiff system the required step-size to maintain accuracy can vary with each time step. There is a play off between taking larger time steps with a complicated implicit solution at each time step against taking incredibly small time steps with a simple explicit solution. In comparison with these other numerical methods Gear's method has a much higher level of precision and it maintains calculation efficiency due to the fact that it can change its step size without manual interference. It also uses less calculation time in each step when solving implicit equations compared with other numerical methods. Gear's method is a backward differentiation formula (BDF) using Milne's Device.

Gear's Method

We solve the stiff system of ODE's (2.21)-(2.29) using the stiff ODE solver ode15s in MATLAB. ode15s is a variable order solver based on the numerical differentiation formulas (NDFs) which allows the use of Gear's method.

Gear's method uses a form of the Backward Differentiation formula (BDF). At each step Gear's uses Milne's device to choose which backward differentiation formula to use. Milne's device uses two multistep methods of the same order, one explicit and the second implicit to estimate the local error of the implicit method. The aim of using Milne's method is to get the two step method to maximum possible order [C W Gear] [7].

BDFs are a set of implicit methods for the numerical integration of ODE's. They are implicit linear multistep methods that approximately calculate the derivative of a function using the solution at a previous time step.

The general multistep methods which solve $\mathbf{y}' = \mathbf{f}(t, \mathbf{y})$ have the form:

$$\sum_{j=0}^k \alpha_j \mathbf{y}_{n+j} = h \sum_{j=0}^k \beta_j \mathbf{f}_{n+j} \quad (2.37)$$

where \mathbf{f}_i denotes $\mathbf{f}(t_i, \mathbf{y}_i)$, h is the step size of the method and α and β relate to the particular linear multi-step method being used. All methods from the BDF family have $\beta_i = 0$ for all $i > 0$. We can therefore simplify (2.37):

$$\sum_{j=0}^k \alpha_j \mathbf{y}_{n+j} = h \beta_0 \mathbf{f}_n. \quad (2.38)$$

These BDFs are implicit methods and require the solution of non-linear equations at each step. These nonlinear equations are typically solved using the Newton-Raphson Method.

In general Gear's method is considered inefficient in comparison to other numerical methods due to the continual re-calculation of time-step size, however it is exactly this that makes it appropriate for solving stiff systems. The re-calculation allows it to vary its time-step according to the stability of the scheme whilst maintaining accuracy. The re-calculation is costly computationally but overall is efficient in comparison to explicit methods which use extremely small time steps throughout.

MATLAB also provides two other ODE solvers that are suitable for stiff systems, ode23s and ode23tb. Both of these solvers offer lower accuracy than ode15s.

In the following section we use ode15s to provide numerical solutions for (2.21)-(2.29) in various scenarios.

2.4 Simulations and Results

Figures 2.1-2.9 are simulations of various scenarios using a peak initial concentration of extracellular VLDL. We test different breakdown rates of VLDL to LDL particles and we experiment with different initial concentrations of extracellular LDL.

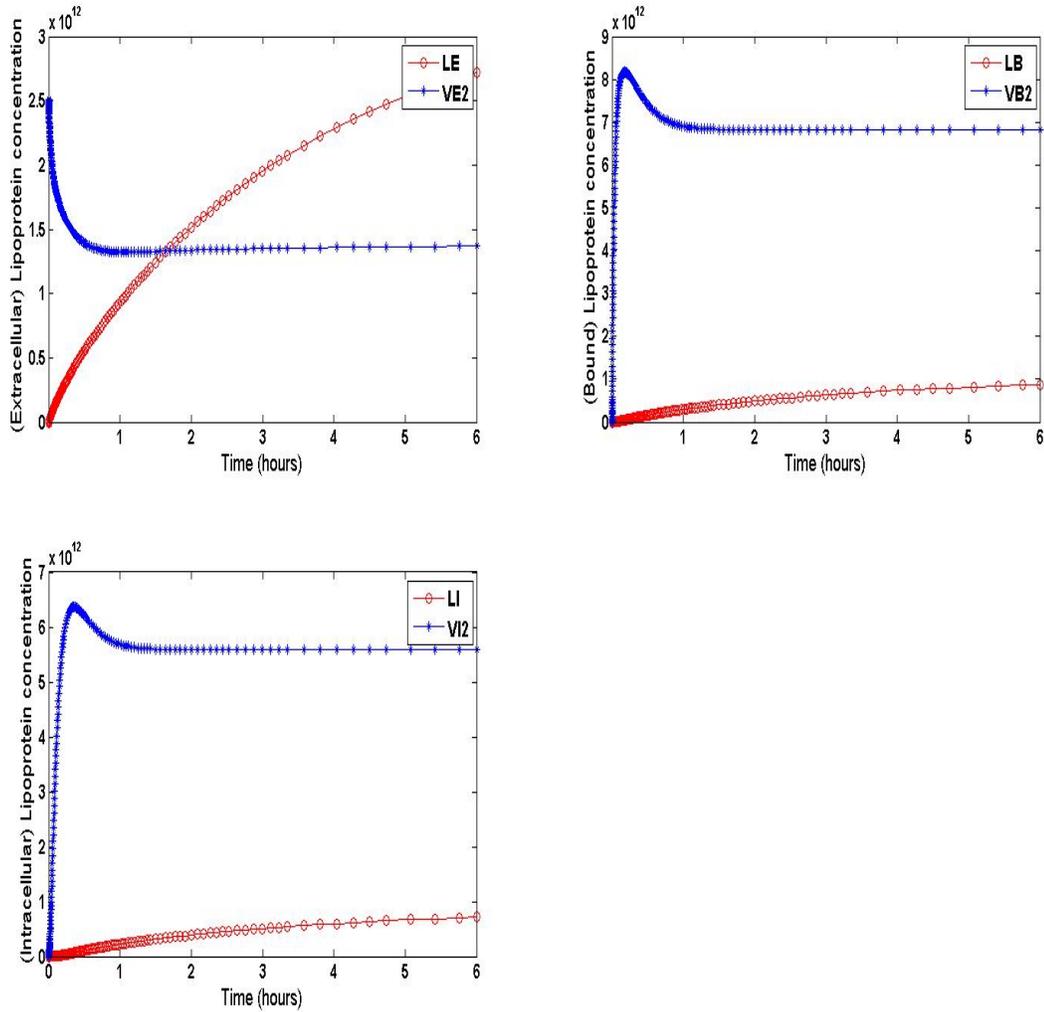


Figure 2.1: The change in extracellular, bound and internalised VLDL and LDL concentrations over a period of 6h. Parameter values are those stated in Table 2.2 with $r=1$, $\zeta = 0.8$ and extracellular LDL initially $\hat{l}_E = 0$.

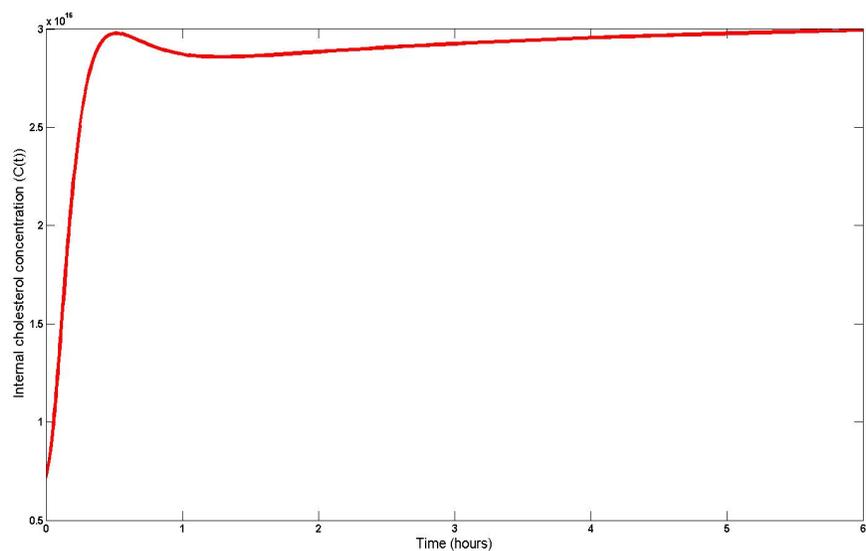


Figure 2.2: The change in intracellular cholesterol over a period of 6h. Parameter values are those stated in Table 2.2 with $r=1$, $\zeta = 0.8$ and extracellular LDL initially $\hat{l}_E = 0$.

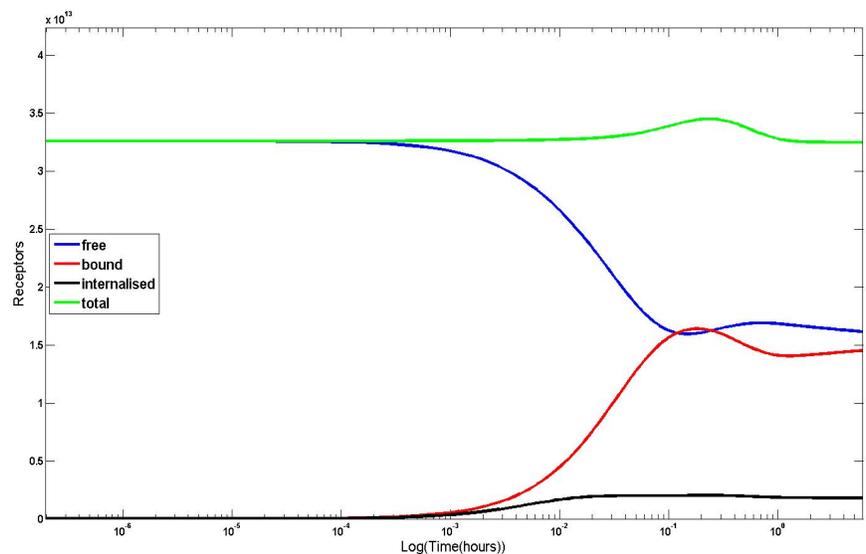


Figure 2.3: The change in time of free, bound, internalised and total receptor concentrations. Parameter values are those stated in Table 2.2 with $r=1$, $\zeta = 0.8$ and extracellular LDL initially $\hat{l}_E = 0$.

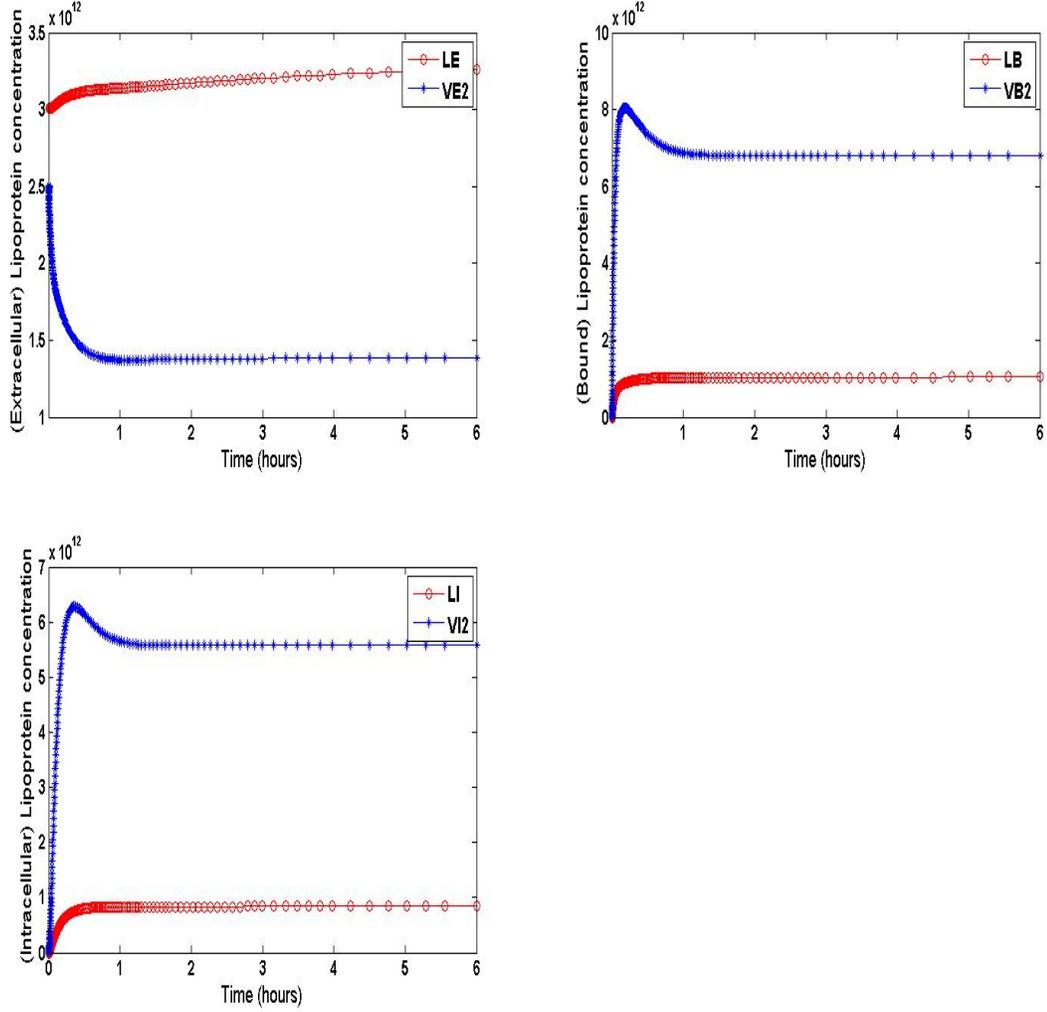


Figure 2.4: The change in extracellular, bound and internalised VLDL and LDL concentrations over a period of 6h. Parameter values are those stated in Table 2.2 with $r=1$, $\zeta = 0.8$. We now change the initial amount of extracellular LDL, $\hat{l}_E = 1$ to simulate the values we would expect to be present biologically.

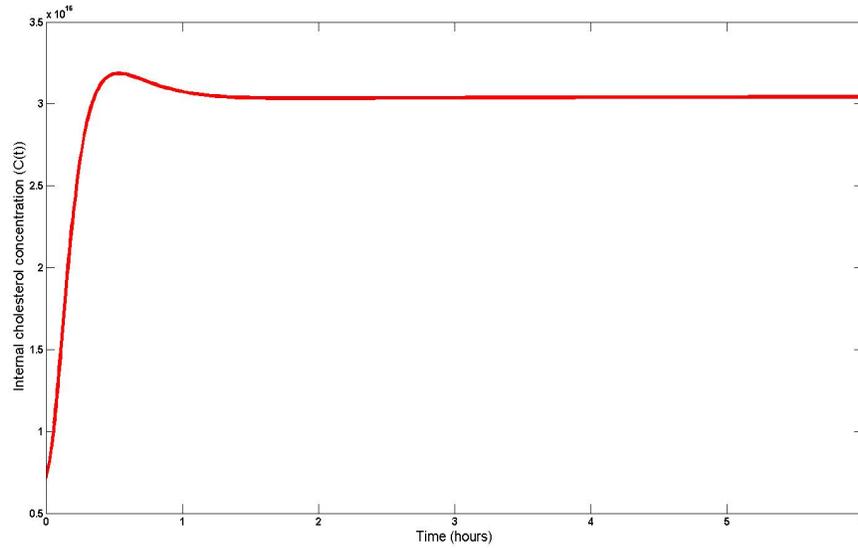


Figure 2.5: The change in intracellular cholesterol over a period of 6h. Parameter values are those stated in Table 2.2 with $r=1$, $\zeta = 0.8$. We now change the initial amount of extracellular LDL, $\hat{l}_E = 1$ to simulate the values we would expect to be present biologically.

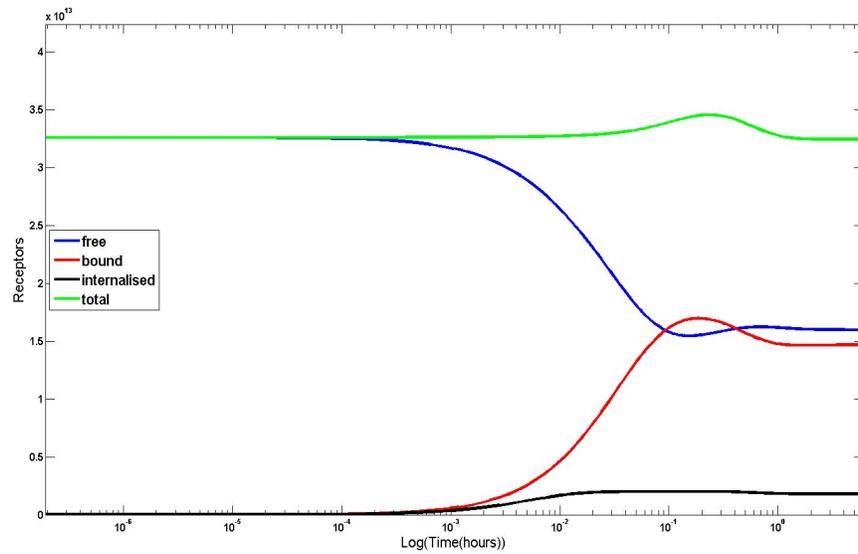


Figure 2.6: The change in time of free, bound, internalised and total receptor concentrations. Parameter values are those stated in Table 2.2 with $r=1$, $\zeta = 0.8$. We now change the initial amount of extracellular LDL, $\hat{l}_E = 1$ to simulate the values we would expect to be present biologically.

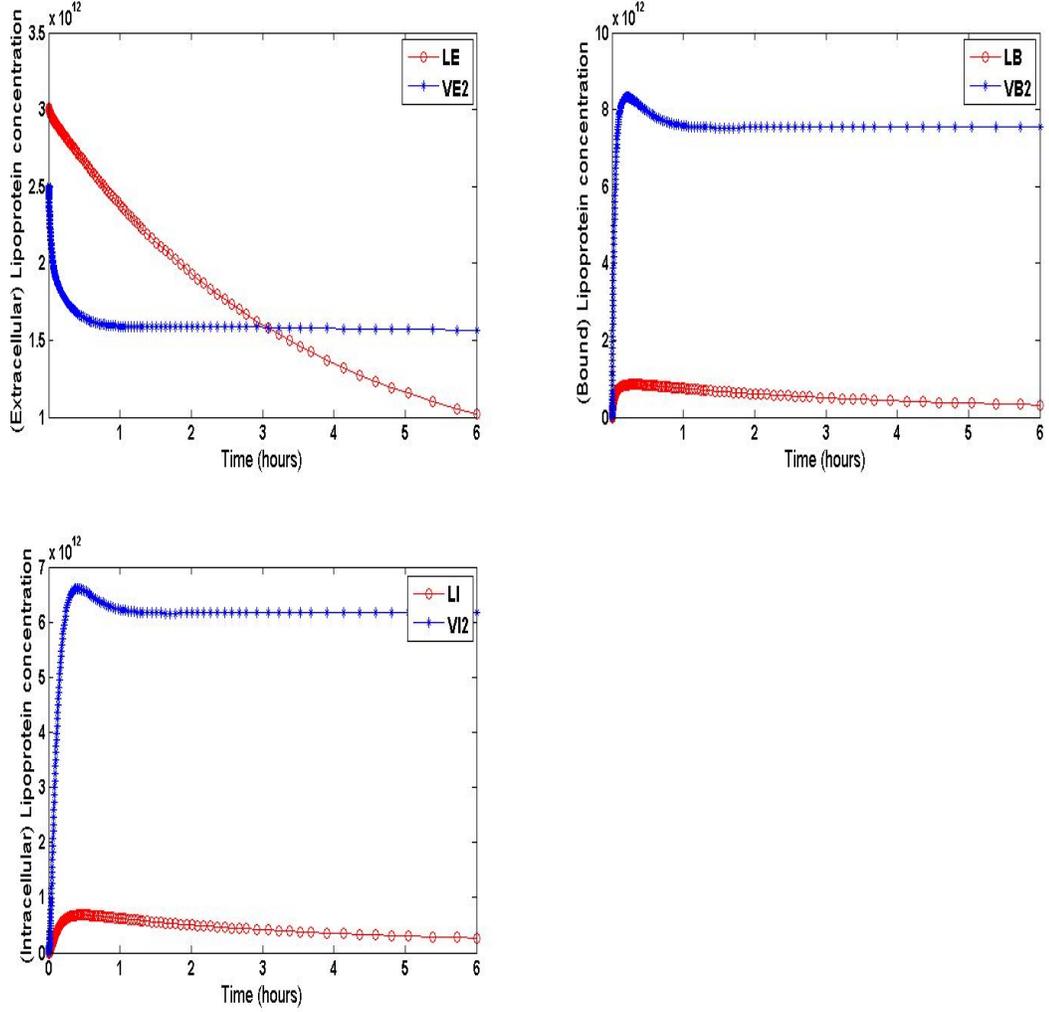


Figure 2.7: The change in extracellular, bound and internalised VLDL and LDL concentrations over a period of 6h. Parameter values are those stated in Table 2.2 with $r=1$, $\zeta = 0.13$. We continue with the initial amount of extracellular LDL, $\hat{l}_E = 1$.

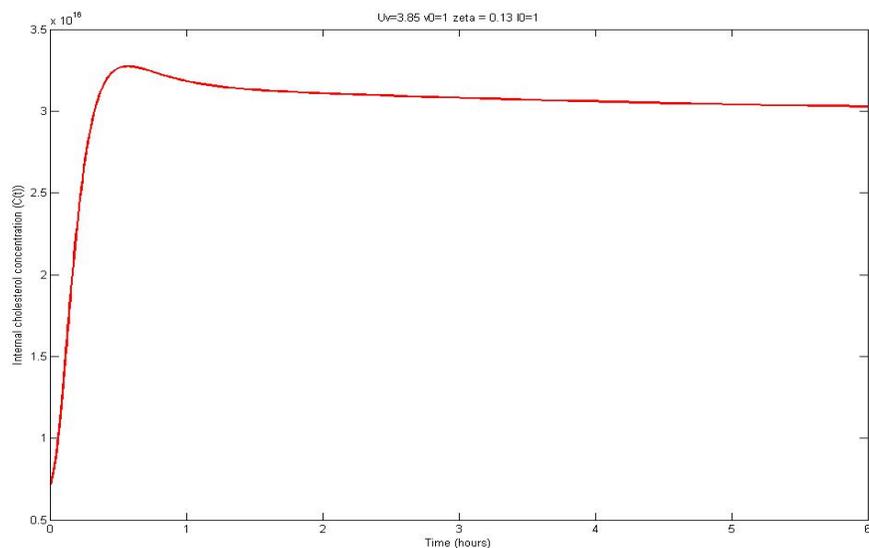


Figure 2.8: The change in intracellular cholesterol over a period of 6h. Parameter values are those stated in Table 2.2 with $r=1$, $\zeta = 0.13$. We continue with the initial amount of extracellular LDL, $\hat{l}_E = 1$.

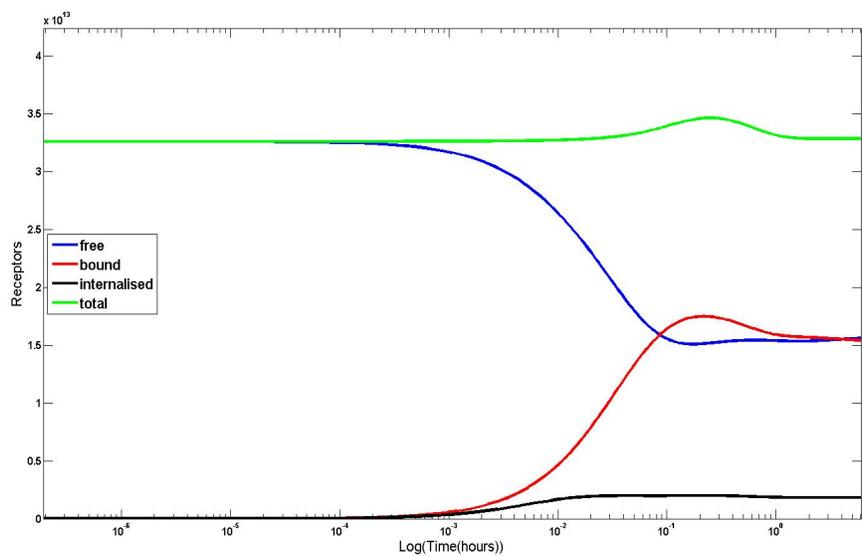


Figure 2.9: The change in time of free, bound, internalised and total receptor concentrations. Parameter values are those stated in Table 2.2 with $r=1$, $\zeta = 0.13$. We continue with the initial amount of extracellular LDL, $\hat{l}_E = 1$.

Figures 2.1- 2.9 are the numerical solution to the system of non-linear differential equations (2.21)-(2.29). They show the change in VLDL and LDL concentration as well as the change in intracellular cholesterol levels and the concentrations of free, bound, internalised and total receptors. The time span of 6 hours was used to represent a realistic time between meals that LDL and VLDL would have to normalise.

In Figures 2.1, 2.2 and 2.3 we make the breakdown rate of VLDL to LDL, ζ , higher than the values discussed with Dr Kim Jackson and found in the literature [2]. It is through experimentation that we found this value provided the behaviour we would expect of the model. As discussed in chapter 2 we initially suppose that there is no LDL present in the system. This is not a biologically realistic situation as LDL levels usually remain fairly constant with mild fluctuations.

Figure 2.1 shows that the extracellular VLDL very quickly depletes to its base line amount. Within an hour it has depleted to below 1.5×10^{12} particles/ml medium which corresponds to the base line level of $15\mu g/ml$ discussed in section 2.2. We also see the extracellular concentration of LDL quickly increasing towards the value we would expect 3.01×10^{12} particles/ml medium. We begin with no bound or internalised VLDL so the graphs show an initial spike as the high concentration of extracellular VLDL is bound and internalised. After an hour these concentrations become constant due to the fact that extracellular VLDL has become constant. The bound and internalised LDL increases with more of a constant state, this is due to the lack of extracellular LDL in the system at the beginning. It is interesting to note there appears to be little or no competition between extracellular VLDL and LDL particles for uptake by the receptors. As the extracellular LDL increases we might expect to see the concentration of bound VLDL decrease as more LDL is bound to the free receptors but it remains at a steady state.

The intracellular cholesterol concentration, figure 2.2, shows a quick increase as the lipoprotein particles are bound and internalised. It reaches it's peak value and then has a sudden decrease before tending back towards it's maximum. This behaviour is explained when we observe figure 2.3. The *de novo* receptor recycling links the number of free receptors on the cell surface to the intracellular cholesterol concentration. The intracellular cholesterol concentration reaching it's peak value causes the cell to stop producing as many receptors, reducing the number available on the surface for lipoprotein particles to bind to, which produces the dip in the cholesterol concentration within the cell. We

also see that the total number of receptors increases briefly when we have this spike in cholesterol but then settles back to a steady state.

We continue with the value $\zeta = 0.8$ but change the non-dimensionalised initial conditions and solve the system of equations with $\hat{l}_E = 1$, which represents a typical level of extracellular LDL *in vivo*. Figure 2.4 shows the behaviour we would expect. The extracellular LDL concentration remains relatively constant, it shows a slight increase but this can be attributed to numerical accuracy errors. Again we see the spike in bound and internal VLDL concentrations which correspond to the large injection of extracellular VLDL. The bound and internalised LDL concentrations quickly tend to steady states.

The peak of the intracellular cholesterol concentration in figure 2.5 is less pronounced than that of figure 2.2, this expected as we do not have the sudden increase in extracellular LDL. The intracellular cholesterol concentration quickly reaches an equilibrium state. In figure 2.6 the receptors have reached a steady state by the end of the time period.

Figures 2.7-2.9 were included to illustrate the problem with assuming a slower breakdown rate of VLDL to LDL particles. Figure 2.7 shows the extracellular concentration of LDL decreasing to 0 within 6 hours. As a result of this the bound and internalised LDL also tend towards 0. The extracellular, bound and internalised VLDL concentrations are not affected except that we see all the steady states they tend towards are higher due to the fact that less particles are broken down into LDL.

Figures 2.1- 2.9 show that modelled LDL levels are more sensitive to changes in the breakdown rate ζ than they are to the initial conditions.

2.5 Steady State Analysis

The steady state of the system occurs when all process are in equilibrium. To find the steady state, or steady states, we set the system of equations (2.21)-(2.29) to zero which gives a new set of equations to solve:

$$0 = -\hat{\rho}_F \hat{l}_E + \psi_L \hat{l}_B + \zeta \hat{v}_E, \quad (2.39)$$

$$0 = \hat{\rho}_F \hat{l}_E - \psi_L \hat{l}_B - \chi_L \hat{l}_B, \quad (2.40)$$

$$0 = \chi_L \hat{l}_B - \omega_L \hat{l}_I, \quad (2.41)$$

$$0 = -\phi_V \hat{\rho}_F \hat{v}_E + \psi_V \hat{v}_B - \zeta \hat{v}_E + \bar{U}_V, \quad (2.42)$$

$$0 = \phi_V \hat{\rho}_F \hat{v}_E - \psi_V \hat{v}_B - \chi_V \hat{v}_B, \quad (2.43)$$

$$0 = \chi_V \hat{v}_B - \omega_V \hat{v}_I, \quad (2.44)$$

$$0 = \gamma_{rr} \sigma \hat{\rho}_I - \chi_0 \hat{\rho}_F^P - \left(m \hat{l}_E \hat{\rho}_F - m \psi_L \hat{l}_B + \frac{m \chi_L \hat{l}_B \hat{\rho}_F}{1 - \hat{\rho}_F} \right) - r \left(n \phi_V \hat{\rho}_F \hat{v}_E - n \psi_V \hat{v}_B + \frac{n \chi_V \hat{v}_B \hat{\rho}_F}{1 - \hat{\rho}_F} \right), \quad (2.45)$$

$$0 = \frac{\gamma_s^* \sigma}{\bar{K} + \hat{C}} + \chi_0 f \hat{\rho}_F^P + f \left(1 + \frac{\hat{\rho}_F}{1 - \hat{\rho}_F} \right) \times (m \chi_L \hat{l}_B + n \chi_V r \hat{v}_B) - \sigma \gamma_{rr} \hat{\rho}_I, \quad (2.46)$$

$$0 = \Upsilon (\omega_L R_L^{chol} \hat{l}_I + \omega_V r R_V^{chol} \hat{v}_I) - \lambda^* (\hat{C} - 1) \quad (2.47)$$

We sum (2.39) and (2.40) and combine this result with (2.41) to find that:

$$\zeta \hat{v}_E = \chi_L \hat{l}_B = \omega_L \hat{l}_I.$$

From (2.42), (2.43) and (2.44) we have the relationships:

$$\bar{U}_V - \zeta \hat{v}_E = \chi_V \hat{v}_B = \omega_V \hat{v}_I.$$

Using these equations we substitute into (2.45)-(2.47) and can re-write many of our parameters in terms of the extracellular VLDL concentration.

$$\hat{l}_B = \frac{\zeta \hat{v}_E}{\chi_L}, \quad (2.48)$$

$$\hat{l}_I = \frac{\zeta \hat{v}_E}{\omega_L}, \quad (2.49)$$

$$\hat{v}_B = \frac{\bar{U}_V - \zeta \hat{v}_E}{\chi_V}, \quad (2.50)$$

$$\hat{v}_I = \frac{\bar{U}_V - \zeta \hat{v}_E}{\omega_V}, \quad (2.51)$$

$$\hat{C} = \frac{\Upsilon}{\lambda^*} ((R_L^{chol} - r R_V^{chol}) \zeta \hat{v}_E + r R_V^{chol} \bar{U}_V) + 1, \quad (2.52)$$

We can write an expression for $\hat{\rho}_F$ in terms of the variables in (2.48) to (2.52)

$$\begin{aligned} 0 &= \chi_0 \hat{\rho}_F^P (f - 1) + \frac{\hat{\rho}_F}{1 - \hat{\rho}_F} (f - 1) (m \chi_L \hat{l}_B + n r \chi_V \hat{v}_B) \\ &\quad - \hat{\rho}_F (m \hat{l}_E + r n \phi_V \hat{v}_E) + m \hat{l}_B (\psi_L + f \chi_L) + r n \hat{v}_B (\psi_V + f \chi_V) \\ &\quad + \frac{\gamma_s^* \sigma}{\bar{K} + \hat{C}} \end{aligned} \quad (2.53)$$

Unfortunately this is not a simple system to solve. We can initially simplify it by noticing that $\chi_0 = 0$ which makes it easy to re-arrange (2.53) into a quadratic of the form :

$$A \hat{\rho}_F^2 + B \hat{\rho}_F + C = 0, \quad (2.54)$$

where

$$\begin{aligned} A &= m \hat{l}_E + r n \phi_V \hat{v}_E \\ B &= - \left(m \hat{l}_E + r n \phi_V \hat{v}_E + m \hat{l}_B (\psi_L + \chi_L) + r n \hat{v}_B (\psi_V + \chi_V) + \frac{\gamma_s^* \sigma}{\bar{K} + \hat{C}} \right) \\ C &= m \hat{l}_B (\psi_L + f \chi_L) + r n \hat{v}_B (\psi_V + f \chi_V) + \frac{\gamma_s^* \sigma}{\bar{K} + \hat{C}} = 0. \end{aligned} \quad (2.55)$$

The solution is given by:

$$\hat{\rho}_F = \frac{-B \pm \sqrt{B^2 - 4AC}}{2A}. \quad (2.56)$$

We take the biologically realistic solution and subsequently we can formulate an equation for $\hat{\rho}_I$.

$$\hat{\rho}_I = \frac{1}{\gamma_{rr}\sigma} \left(\frac{\gamma_s^*\sigma}{\bar{K} + \hat{C}} + f \left(1 + \frac{\hat{\rho}_F}{1 - \hat{\rho}_F} \right) \times (m\chi_L\hat{l}_B + rn\chi_V\hat{v}_B) \right). \quad (2.57)$$

To find a solution we make an approximation of the steady states of \hat{l}_E and \hat{v}_E using their initial values. All other variables can be expressed in terms of \hat{l}_E and \hat{v}_E when in steady state. We try two solutions, the first is the trivial solution that extracellular VLDL and LDL concentrations = 0. This is a highly unrealistic biologically but it is interesting in terms of the mathematical system. The next solution we try is $\hat{l}_E = 1$ and $\hat{v}_E = 0.55$, these are the non-dimensionalised values and represent the base line values we expect for the extracellular lipoprotein particles. When we used $\hat{v}_E = 1$ this represented a peak value in the extracellular VLDL but from figure 2.4 we can see this clearly is not it's steady state as the behaviour shows it tends towards the VLDL levels present at a fasting state. From this point onwards we are using the non-dimensional value $\zeta = 0.8$ for the breakdown of VLDL to LDL particles.

Table 2.3: Steady State Solutions.

Variable	Description	Trivial	Non-Trivial
L_E	Extracellular LDL particles	0.0001	1
L_B	Bound LDL particles	6×10^{-5}	0.344
L_I	Internalised LDL particles.	5×10^{-5}	0.282
V_E	Extracellular VLDL particles.	0.0001	0.55
V_B	Bound VLDL particles.	3.01	2.66
V_I	Internalised VLDL particles.	2.47	2.19
ρ_F	Free Receptors.	0.913	0.433
ρ_I	Internalised Receptors.	0.970	0.144
C	Intracellular Cholesterol Concentration.	3.25	3.27

Note for the trivial steady states we actually take $\hat{v}_E = \hat{l}_E = 0.0001$ to avoid a division by zero.

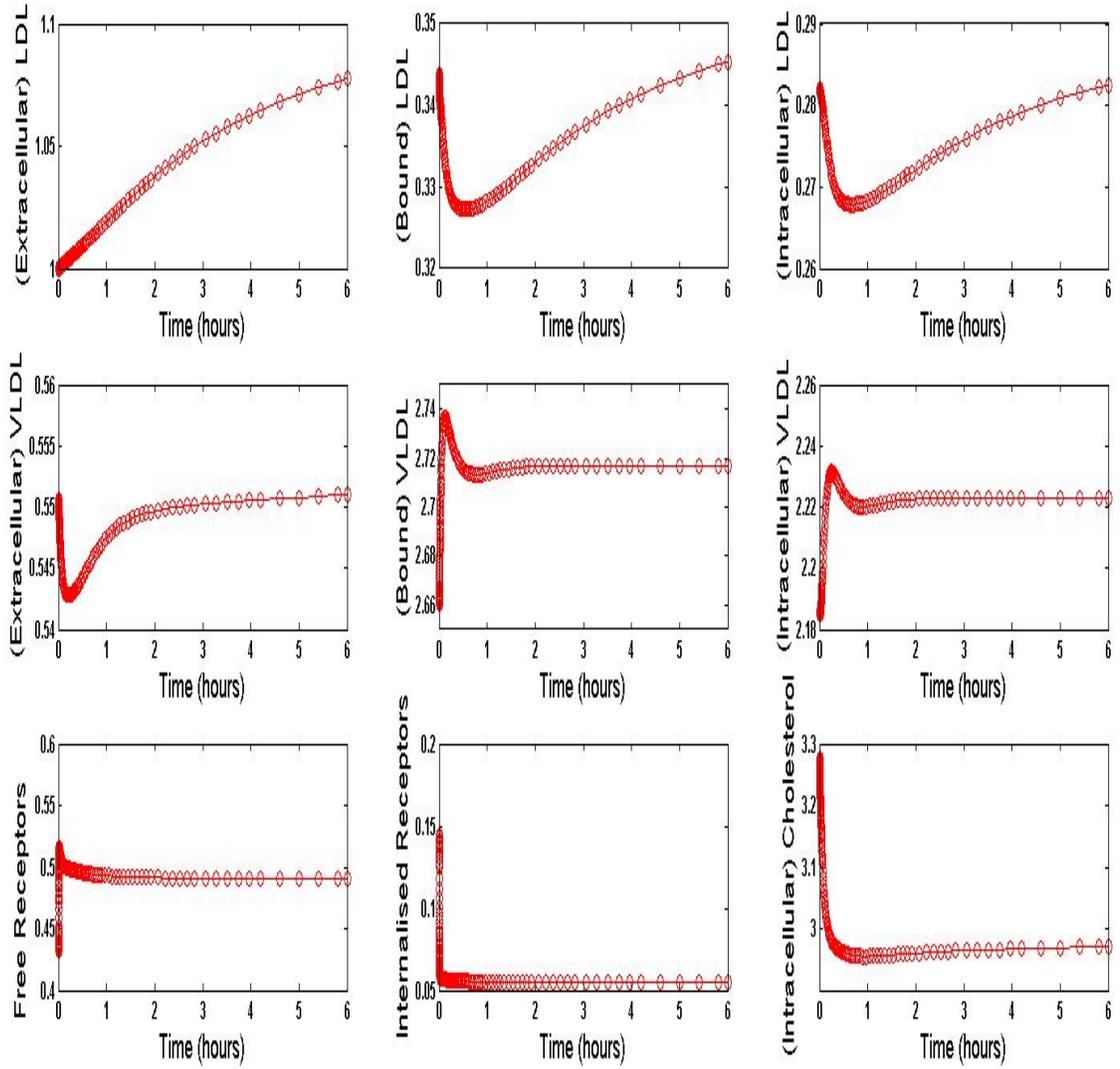


Figure 2.10: The development of steady states for all non-dimensional variables. Parameter values are those stated in Table 2.2 with $r=1$ and $\zeta = 0.8$. Initial conditions are from the Table 2.3 (Non-Trivial).

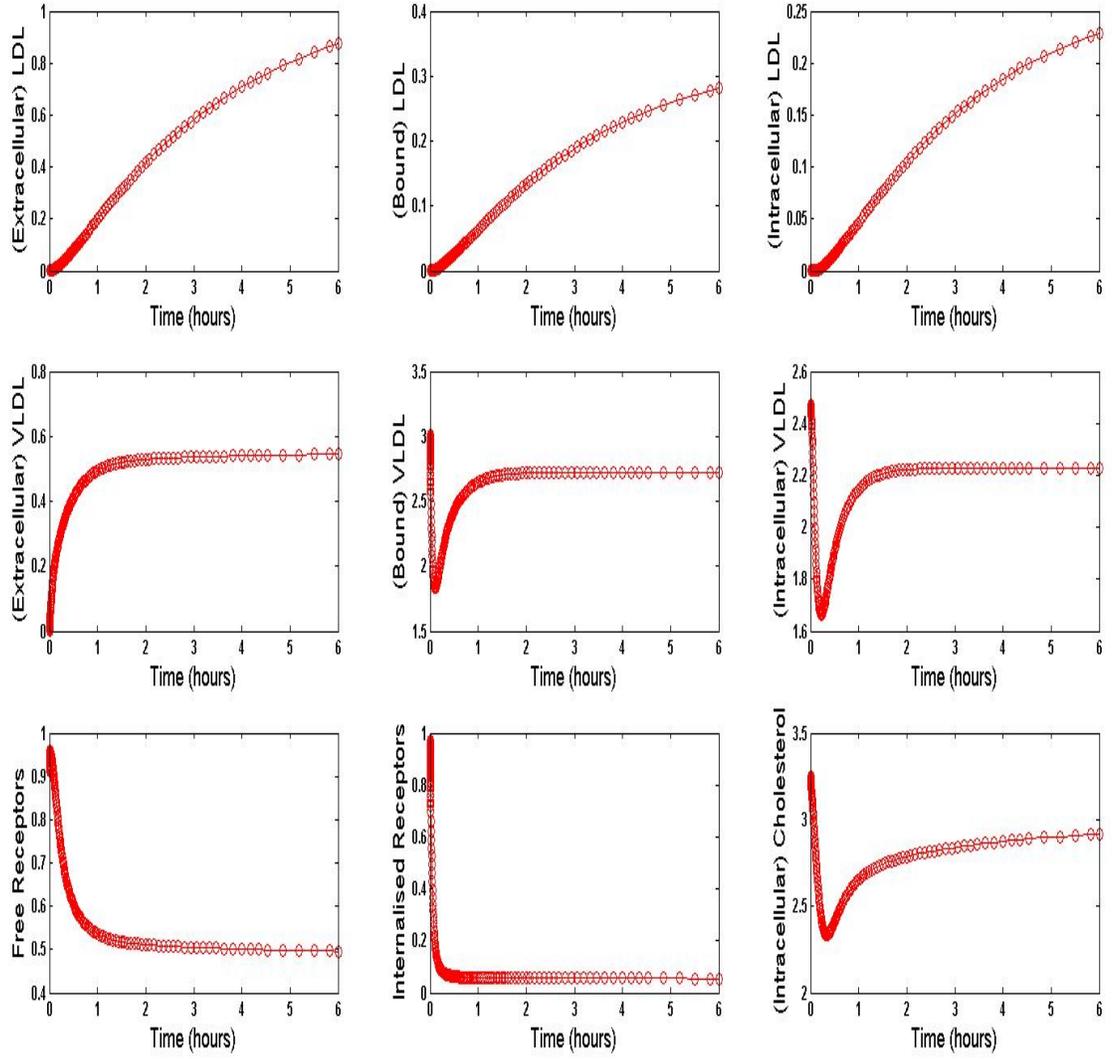


Figure 2.11: The development of steady states for all non-dimensional variables. Parameter values are those stated in Table 2.2 with $r=1$ and $\zeta = 0.8$. Initial conditions are from the Table 2.3 (Trivial).

If we assume that all receptors are recycled, i.e. $f = 1$, we can produce some much simpler equations for the steady state analysis. The relationships (2.48) to (2.52) remain the same. But (2.57) can be simplified:

$$\hat{\rho}_F = \frac{m\hat{l}_B(\psi_L + \chi_L) + rn\hat{v}_B(\psi_V + \chi_V) + \frac{\gamma_s^*\sigma}{K+C}}{m\hat{l}_E + rn\phi_V\hat{v}_E}. \quad (2.58)$$

Table 2.4: Reduced Steady State Solutions.

Variable	Description	Trivial	Non-Trivial
L_E	Extracellular LDL particles	0.0001	1
L_B	Bound LDL particles	6×10^{-5}	0.344
L_I	Internalised LDL particles.	5×10^{-5}	0.282
V_E	Extracellular VLDL particles.	0.0001	0.55
V_B	Bound VLDL particles.	3.01	2.66
V_I	Internalised VLDL particles.	2.47	2.19
ρ_F	Free Receptors.	2770	0.467
ρ_I	Internalised Receptors.	0.003	0.153
C	Intracellular Cholesterol Concentration.	3.25	3.27

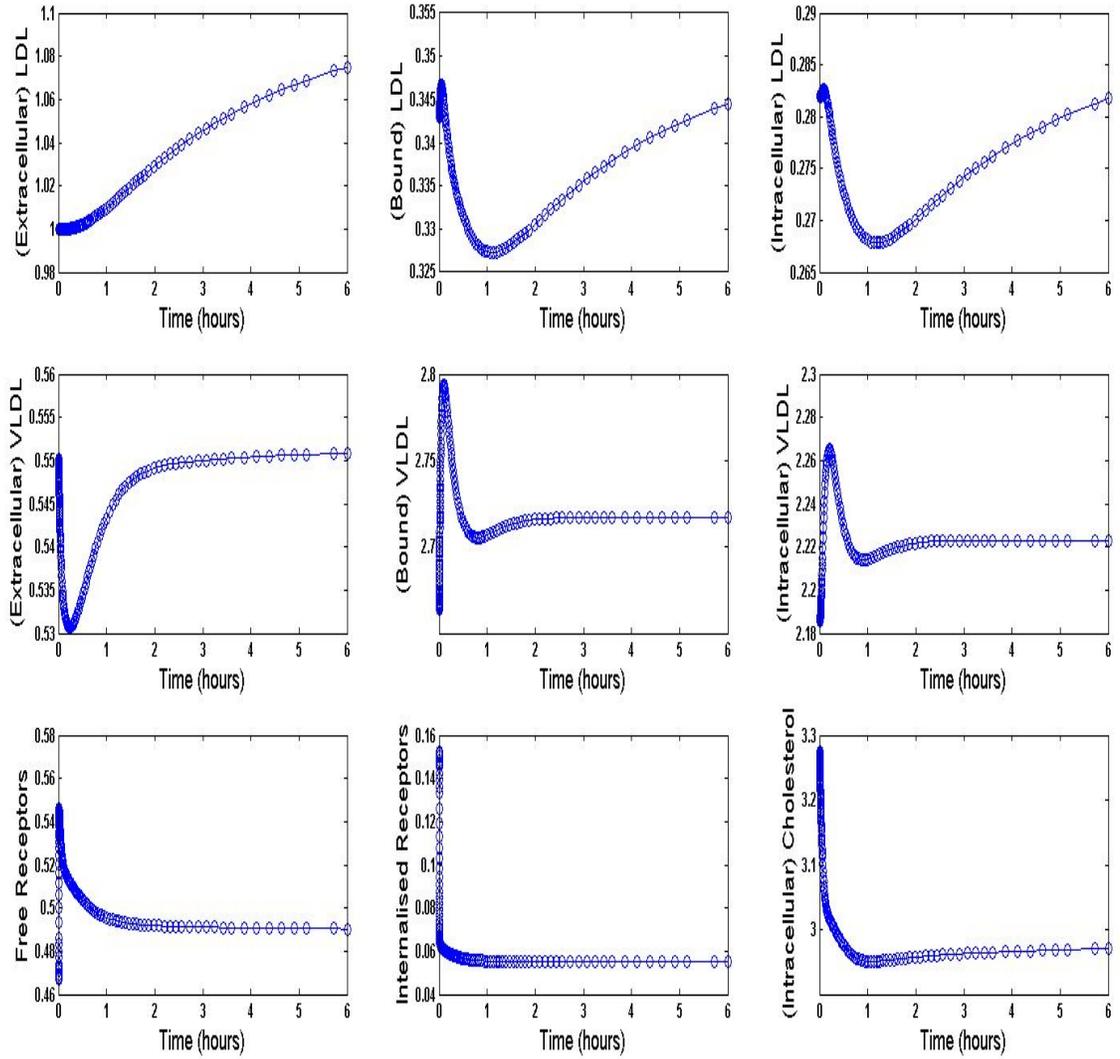


Figure 2.12: The development of steady states for all non-dimensional variables assuming $f = 1$. Parameter values are those stated in Table 2.2 with $r=1$ and $\zeta = 0.8$. Initial conditions are from the Table 2.4 (Non-Trivial).

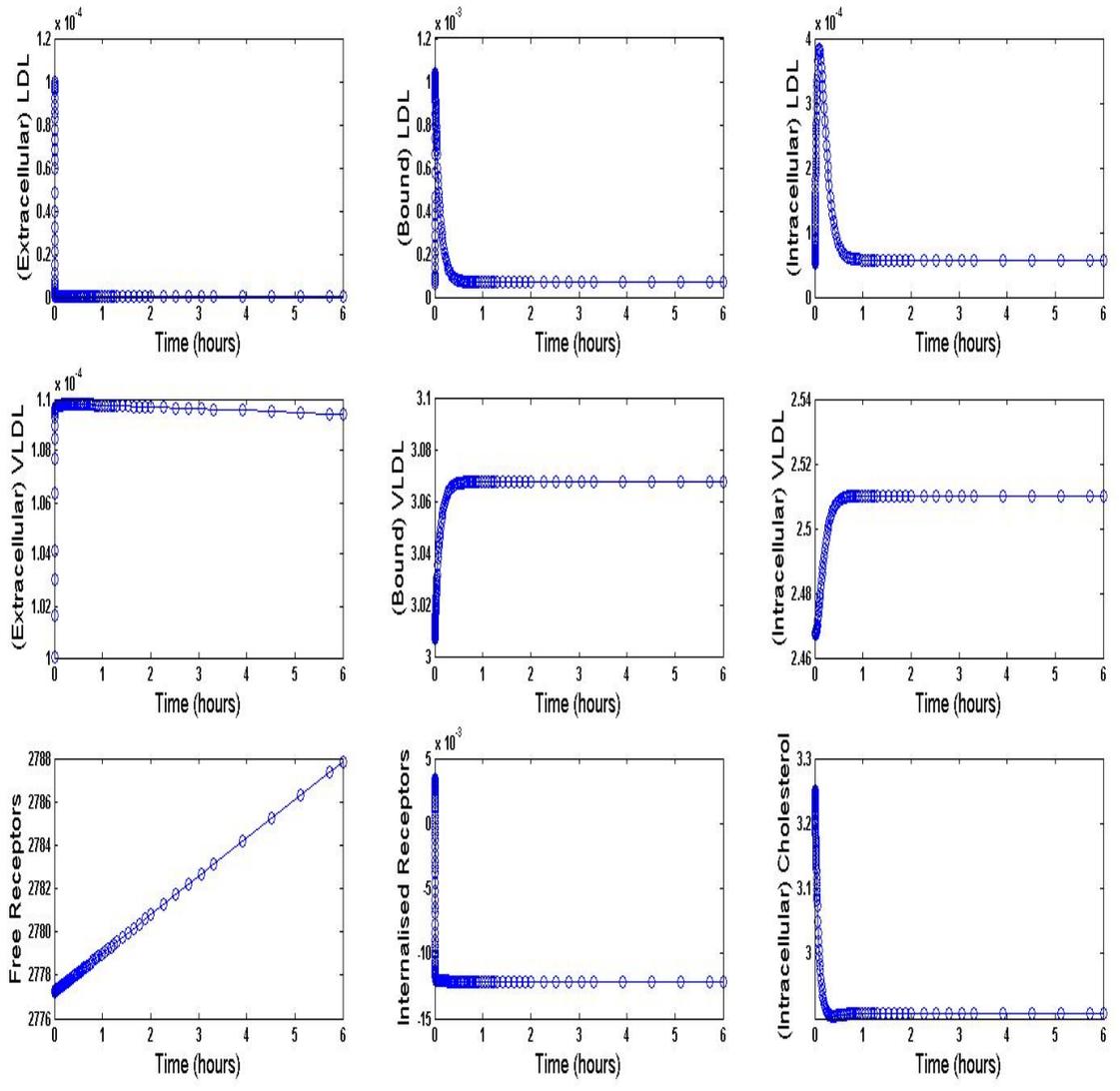


Figure 2.13: The development of steady states for all non-dimensional variables assuming $f = 1$. Parameter values are those stated in Table 2.2 with $r=1$ and $\zeta = 0.8$. Initial conditions are from the Table 2.4 (Trivial).

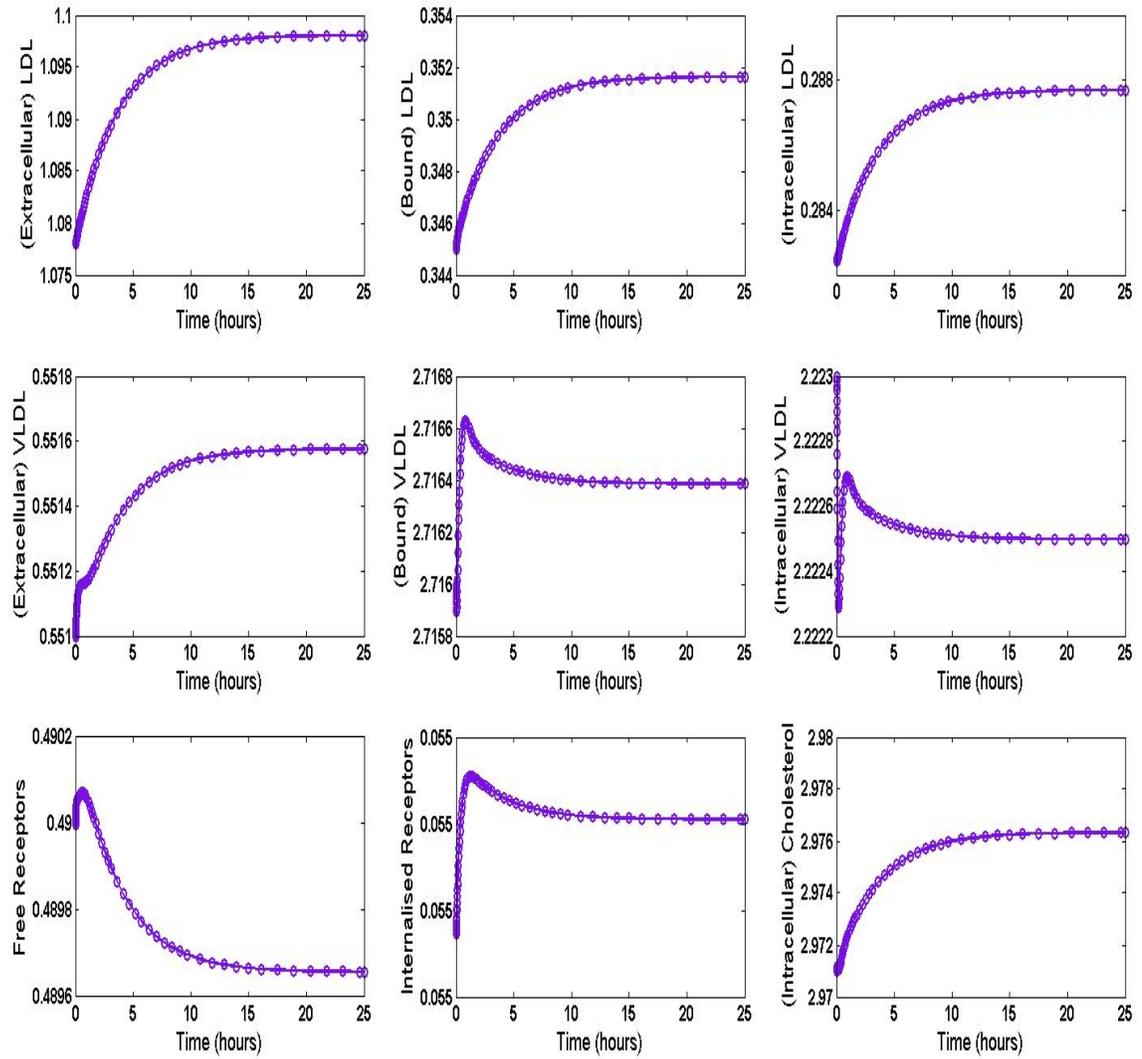


Figure 2.14: The development of steady states for all non-dimensional variables. Parameter values are those stated in Table 2.2 with $r=1$ and $\zeta = 0.8$. Initial conditions are from the Table 2.4.

Due to numerical inaccuracies we can see that we have not quite met the steady states. However all the graphs show that the model quickly corrects this and very quickly tends towards the steady states of the system. This suggests a high level of stability in the model. Figure 2.10 shows that the model clearly does not tend towards our calculated value for the internalised receptor concentration. The other values do not vary by large orders of magnitude. There is insufficient time to see whether the extracellular LDL concentration will become constant. The extracellular, bound and internalised concentrations of VLDL fluctuate slightly but do return to a steady state which supports the behaviour seen in figures 2.1-2.9 where the stability of the VLDL concentrations was unaffected by the changes in the different LDL concentrations.

Figure 2.11 shows the trivial 'zero' steady state is clearly unstable. The extracellular, bound and internalised LDL concentrations all immediately increase as does the extracellular VLDL concentration. Because this steady state is not something we would expect to see biologically it is unsurprising that it is unstable. Encouragingly it is tending towards a state we would expect to see, with all the variables tending towards the same values as in figure 2.10.

The reduced solution, where we assume all internalised receptors are recycled, $f = 1$, shows similar results to the full steady state solution. Figure 2.12 shows extremely similar results to figure 2.10 including the large drop in the internalised receptor concentration. This supports the argument that although our steady states were calculated correctly the steady state of the solutions differ due to numerical error and inaccuracies.

Figure 2.13 shows the reduced solution for the trivial case which due to a division by a number close to 0, results in some unrealistic results. Clearly the assumption that all receptors are recycled can not be used for all initial conditions. Although the solutions of this steady state are highly unrealistic they do demonstrate that the model shows stability with hugely varying initial conditions.

Figures 2.12 and 2.10 both show the system tending towards the same steady state so taking the solution from these we test to see if this numerical steady state is stable. Figure 2.14 does show some extremely small oscillations, note: scale on the graphs. In general all parameters are tending towards a steady state. The value of the internal receptor concentration is lower than our steady state solution indicates but it shows numerical stability.

2.6 Sensitivity Analysis

Sensitivity analysis is a way of determining how the output of a model can be apportioned to the varying sources of input. Local sensitivity analysis works by varying an individual parameter within the system, keeping all others constant measuring the affect on a specific model output. In our model we are interested in how parameter variation within the model affects the intracellular cholesterol content of the cell. Global sensitivity analysis looks at the relationships parameters have to each other and the effect they have together on the model output.

Sensitivity analysis is a useful tool in determining the robustness of the model in relation to the parameters. It is important for us to understand how accurate our model is when considering further development. Global sensitivity analysis is especially useful when considering parametric uncertainties within the model. For biological models, information about parameter values is becoming clearer very quickly so it is important to have an accurate model in place.

We perform local sensitivity analysis on all the variable parameters within the system to see which has the most affect on the intracellular cholesterol levels. This information is important in understanding which processes to target when developing drugs to lower cholesterol levels.

Parameters are varied by factors of 5 and we measure percentage cholesterol change. Some parameters have a large affect while with others the change is more subtle so we have separated the parameters to reflect this.

Some criticisms of local sensitivity analysis are that it does not preserve the relationships currently existing between parameters. For example we know that at steady state we expect the number of extracellular VLDL breaking down in LDL to be equal to the amount LDL binding, but varying the binding and breakdown rates individually will change the affect this relationship has on the model output.

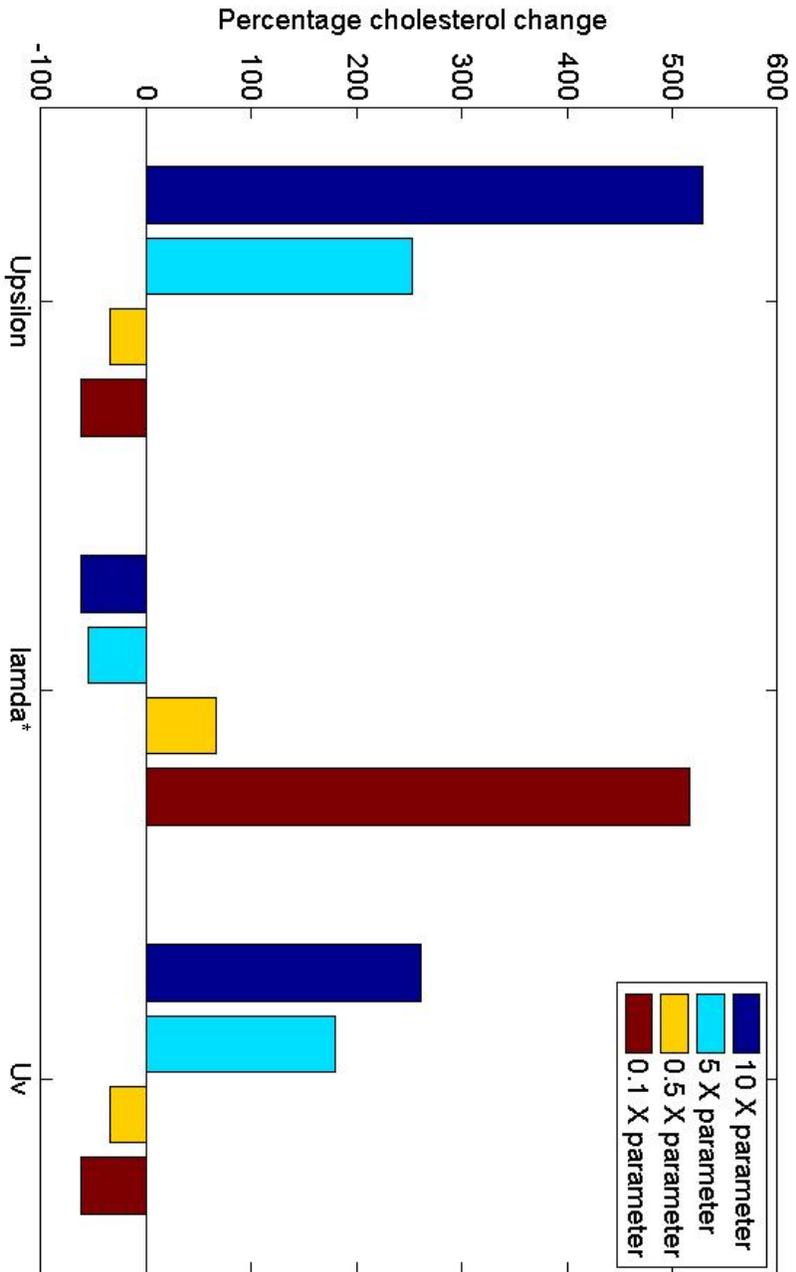
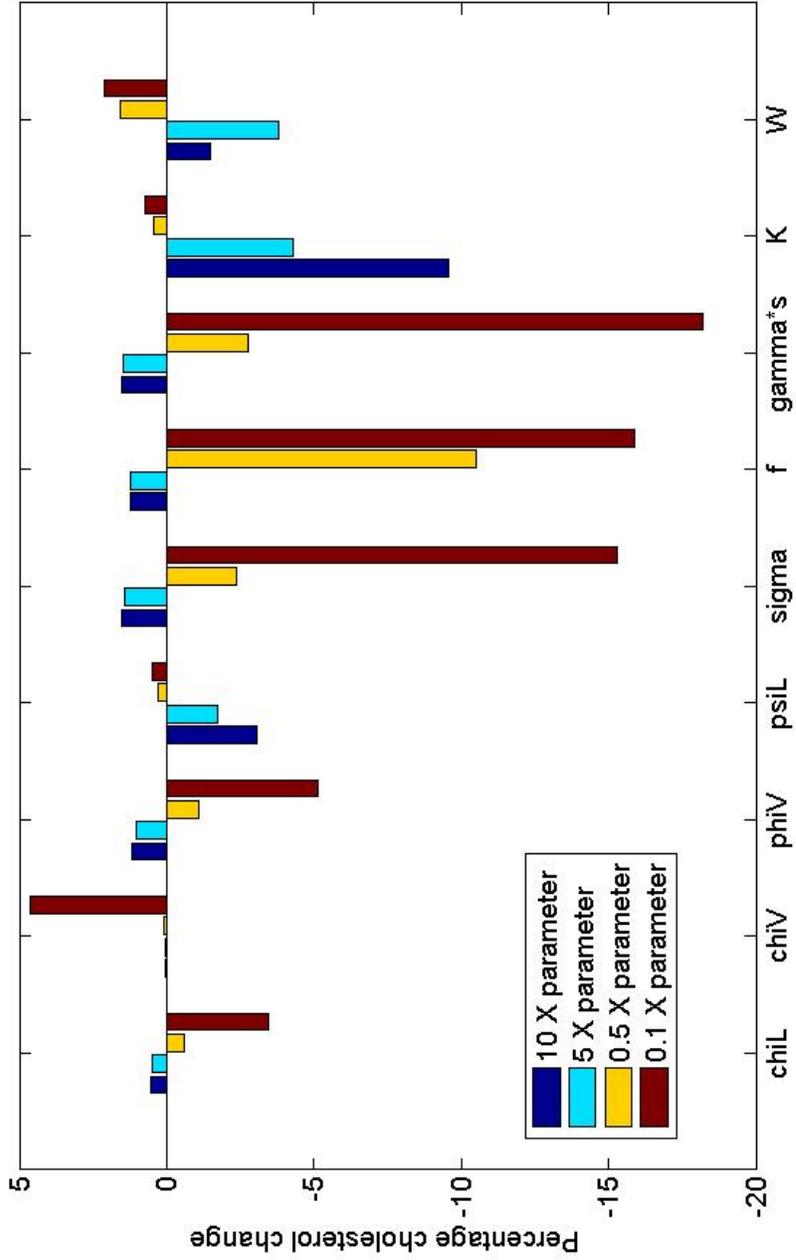


Figure 2.15: Sensitivity Analysis: Parameters with large effect

Figure 2.16: Sensitivity Analysis: Parameters with some effect



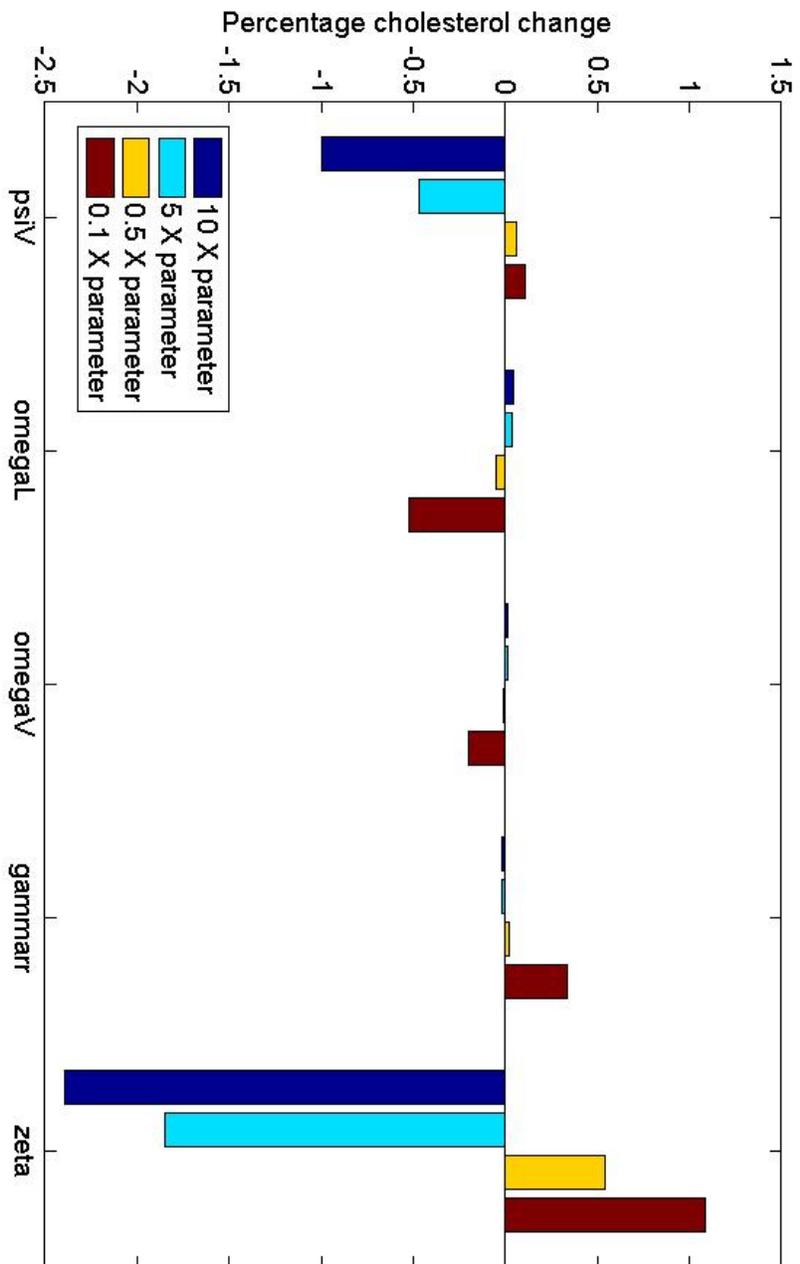


Figure 2.17: Sensitivity Analysis: Parameters with negligible effect

Figure 2.15 shows us that the parameters that have the most affect on cholesterol are Υ , λ^* and \bar{U}_V . We expect λ^* to reduce cholesterol if it is increased since it is the rate of breakdown of cholesterol. Increasing \bar{U}_V also logically increases cholesterol since if the concentration of extracellular lipoproteins is higher this will increase the concentration of bound lipoproteins, which in turn increases the amount internalised so more cholesterol will be released within the cell.

It is very interesting that the rate of receptor recycling γ_{rr} , see figure 2.17, has such a negligible affect. We would expect that if the rate of receptors recycled is low, there will be less LDLR on the surface and so less lipoproteins internalised and thus the cholesterol to be significantly lower. As expected if we decrease the rate of *de novo* receptor production γ_s^* , see figure 2.16, the cholesterol is significantly reduced. The intracellular cholesterol concentration is also reduced if we decrease the fraction of receptors recycled, f . This receptor production is directly proportional to the intracellular cholesterol levels.

The binding and unbinding of lipoproteins on the surface of the cell do not affect the intracellular cholesterol concentration significantly. Figures 2.16 and 2.17 show what we would expect, increasing the unbinding rates ψ_L, ψ_V reduces the intracellular cholesterol concentration. Increasing the relative binding rate of VLDL particles ϕ_V increased intracellular cholesterol concentration.

Initially it seems surprising that the rate of breakdown ζ being increased reduces intracellular cholesterol concentration. This increase will produce a much higher concentration of LDL particles and a lower concentration of VLDL particles. Whilst LDL particles do have a higher average cholesterol content per particle, VLDL particles bind to the LDLR 14 times quicker. The VLDL particles are larger and bind more quickly than LDL particles but are internalised at the same rate so they block the LDL particles out of the pits.

2.7 Discussion

We make a large assumption that we can model the liver as one large cell and thus extend *in vitro* parameter values to the *in vivo* case. This is not accurate which can be seen in figure 2.4. We expect the extracellular VLDL levels to decrease slowly over the 6 hour period but they reduce to their base line levels within an hour.

The model does not locate VLDL particles and thus assumes all particles have an equal chance of binding to the liver. In reality only a small fraction of the VLDL particles would be in the extracellular fluid around the hepatocyte cells and most of the VLDL particles would degrade to IDL or LDL particles whilst in circulation.

The model does not account for the fraction of LDL that is absorbed by the tissues and is not metabolised by the liver. A truly *in vivo* model would account for all of the pathways a lipoprotein particle could take.

Overall, the model does display the behaviour we expect, tending towards a steady state but it is very limited in its ability to capture the change in VLDL concentrations. The model does not attempt to model the varying output of VLDL from the liver.

The issue of whether VLDL particles block the pits on the surface of the hepatocyte cell or whether they are internalised has not been addressed. We assumed all extracellular VLDL particles are internalised. In reality we would not expect the initial values of bound and internalised VLDL and LDL particles to be 0, nor would we expect the internalised receptor concentration to be 0.

When we start with the expected value of LDL we see that the spike in VLDL causes a larger fluctuation in the receptors. At one point the number of bound receptors is greater than the number of free, this is the result of the increase in overall number of particles attaching to the hepatocyte cell.

The sensitivity analysis is useful because scientists are interested in cholesterol levels. However it is high levels of the extracellular LDL concentration that cause plaque in the arteries, it would be interesting to perform some sensitivity analysis using this as the output measure.

Chapter 3

Asymptotic Analysis

We apply some asymptotic analysis to the system of non-linear ODE's (2.21)-(2.29) to simplify the system. In some cases obtaining asymptotic estimates is straightforward, but there are many situations where this can be quite difficult and involve a fair amount of ad-hoc analysis.

We begin by identifying relatively small and large parameters. We take $\epsilon = \frac{1}{W} = 0.1$, unfortunately our non-dimensionalised rate of VLDL breakdown ζ is of the same order so we must be aware of this in the asymptotics we employ. We begin by expressing the variables in terms of ϵ and to account for the fact that ζ is of the same order we also express it as a multiple of ϵ .

$$\begin{aligned}\hat{l}_E &= L_{E0} + \epsilon L_{E1} + O(\epsilon^2) \\ \hat{l}_B &= L_{B0} + \epsilon L_{B1} + O(\epsilon^2) \\ \hat{l}_I &= L_{I0} + \epsilon L_{I1} + O(\epsilon^2) \\ \hat{v}_E &= V_{E0} + \epsilon V_{E1} + O(\epsilon^2) \\ \hat{v}_B &= V_{B0} + \epsilon V_{B1} + O(\epsilon^2) \\ \hat{v}_I &= V_{I0} + \epsilon V_{I1} + O(\epsilon^2) \\ \hat{\rho}_F &= R_{F0} + \epsilon R_{F1} + O(\epsilon^2) \\ \hat{\rho}_I &= R_{I0} + \epsilon R_{I1} + O(\epsilon^2) \\ \hat{C} &= C_0 + \epsilon C_1 + O(\epsilon^2) \\ \zeta &= H\epsilon\end{aligned}\tag{3.1}$$

Note $\hat{t} = T$ has been replaced to simplify the notation, all parameters and variables are

still non-dimensional. We now substitute (3.1) into equations (2.21) - (2.29), we have used the fact the $\chi_0 = 0$ to simplify the equations:

$$\begin{aligned} \frac{1}{\epsilon} \frac{d(L_{E0} + \epsilon L_{E1} + O(\epsilon^2))}{dT} &= -(R_{F0} + \epsilon R_{F1} + O(\epsilon^2))(L_{E0} + \epsilon L_{E1} + O(\epsilon^2)) \\ &+ \psi_L(L_{B0} + \epsilon L_{B1} + O(\epsilon^2)) \\ &+ H\epsilon(V_{E0} + \epsilon V_{E1} + O(\epsilon^2)), \end{aligned} \quad (3.2)$$

$$\begin{aligned} \frac{d(L_{B0} + \epsilon L_{B1} + O(\epsilon^2))}{dT} &= (R_{F0} + \epsilon R_{F1} + O(\epsilon^2))(L_{E0} + \epsilon L_{E1} + O(\epsilon^2)) \\ &- (\psi_L + \chi_V)(L_{B0} + \epsilon L_{B1} + O(\epsilon^2)), \end{aligned} \quad (3.3)$$

$$\begin{aligned} \frac{d(L_{I0} + \epsilon L_{I1} + O(\epsilon^2))}{dT} &= \chi_L(L_{B0} + \epsilon L_{B1} + O(\epsilon^2)) \\ &- \omega_L(L_{I0} + \epsilon L_{I1} + O(\epsilon^2)), \end{aligned} \quad (3.4)$$

$$\begin{aligned} \frac{1}{\epsilon} \frac{d(V_{E0} + \epsilon V_{E1} + O(\epsilon^2))}{dT} &= -\phi_V(R_{F0} + \epsilon R_{F1} + O(\epsilon^2))(V_{E0} + \epsilon V_{E1} + O(\epsilon^2)) \\ &+ \psi_V(V_{B0} + \epsilon V_{B1} + O(\epsilon^2)) - H\epsilon(V_{E0} + \epsilon V_{E1} + O(\epsilon^2)) \\ &+ \bar{U}_V, \end{aligned} \quad (3.5)$$

$$\begin{aligned} \frac{d(V_{B0} + \epsilon V_{B1} + O(\epsilon^2))}{dT} &= \phi_V(R_{F0} + \epsilon R_{F1} + O(\epsilon^2))(V_{E0} + \epsilon V_{E1} + O(\epsilon^2)) \\ &- (\psi_V + \chi_V)(V_{B0} + \epsilon V_{B1} + O(\epsilon^2)), \end{aligned} \quad (3.6)$$

$$\begin{aligned} \frac{d(V_{I0} + \epsilon V_{I1} + O(\epsilon^2))}{dT} &= \chi_V(V_{B0} + \epsilon V_{B1} + O(\epsilon^2)) \\ &- \omega_V(V_{I0} + \epsilon V_{I1} + O(\epsilon^2)), \end{aligned} \quad (3.7)$$

$$\begin{aligned} \sigma \frac{d(R_{I0} + \epsilon R_{I1} + O(\epsilon^2))}{dT} &= \frac{\gamma_s^* \sigma}{\bar{K} + (C_0 + \epsilon C_1 + O(\epsilon^2))} + f \frac{1}{1 - (R_{F0} + \epsilon R_{F1} + O(\epsilon^2))} \\ &\times (m\chi_L(L_{B0} + \epsilon L_{B1} + O(\epsilon^2)) + n\chi_V r(V_{B0} + \epsilon V_{B1} + O(\epsilon^2))) \\ &- \sigma \gamma_{rr}(R_{I0} + \epsilon R_{I1} + O(\epsilon^2)), \end{aligned} \quad (3.8)$$

$$\begin{aligned}
\sigma \frac{d(R_{F0} + \epsilon R_{F1} + O(\epsilon^2))}{dT} &= \gamma_{rr} \sigma (R_{I0} + \epsilon R_{I1} + O(\epsilon^2)) \\
&- (m(L_{E0} + \epsilon L_{E1} + O(\epsilon^2))(R_{F0} + \epsilon R_{F1} + O(\epsilon^2)) \\
&- m\psi_L(L_{B0} + \epsilon L_{B1} + O(\epsilon^2)) \\
&+ \frac{m\chi_L(L_{B0} + \epsilon L_{B1} + O(\epsilon^2))(R_{F0} + \epsilon R_{F1} + O(\epsilon^2))}{1 - (R_{F0} + \epsilon R_{F1} + O(\epsilon^2))}) \\
&- r(n\phi_V(R_{F0} + \epsilon R_{F1} + O(\epsilon^2))(V_{E0} + \epsilon V_{E1} + O(\epsilon^2)) \\
&- n\psi_V(V_{B0} + \epsilon V_{B1} + O(\epsilon^2)) \\
&+ \frac{n\chi_V(V_{B0} + \epsilon V_{B1} + O(\epsilon^2))(R_{F0} + \epsilon R_{F1} + O(\epsilon^2))}{1 - (R_{F0} + \epsilon R_{F1} + O(\epsilon^2))}), \tag{3.9}
\end{aligned}$$

$$\begin{aligned}
\frac{d(C_0 + \epsilon C_1 + O(\epsilon^2))}{dT} &= \Upsilon(\omega_L R_L^{chol}(L_{I0} + \epsilon L_{I1} + O(\epsilon^2)) + \omega_V r R_V^{chol}(V_{I0} + \epsilon V_{I1} + O(\epsilon^2)) \\
&- \lambda^*((C_0 + \epsilon C_1 + O(\epsilon^2)) - 1) = 0. \tag{3.10}
\end{aligned}$$

Reducing the non-linear system of equations (3.2)-(3.10) to $O(1)$ results in the following non-linear system of ODE's:

$$\frac{dL_{E0}}{T} = 0 \tag{3.11}$$

$$\frac{dL_{B0}}{T} = R_{F0}L_{E0} - (\psi_L + \chi_L)L_{B0} \tag{3.12}$$

$$\frac{dL_{I0}}{T} = \chi_L L_{B0} - \omega_L L_{I0} \tag{3.13}$$

$$\frac{dV_{E0}}{T} = 0 \tag{3.14}$$

$$\frac{dV_{B0}}{T} = \phi_V R_{F0} V_{E0} - (\psi_V + \chi_V) V_{B0} \tag{3.15}$$

$$\frac{dv_{I0}}{T} = \chi_V V_{B0} - \omega_V V_{I0} \tag{3.16}$$

$$\begin{aligned}
\frac{dR_{F0}}{T} &= (\gamma_{rr} \sigma R_{I0} - \left(m L_{E0} R_{F0} - m \psi_L L_{B0} + \frac{m \chi_L L_{B0} R_{F0}}{1 - R_{F0}} \right) \\
&- r \left(n \phi_V R_{F0} V_{E0} - n \psi_V V_{B0} + \frac{n \chi_V V_{B0} R_{F0}}{1 - R_{F0}} \right) / \sigma \tag{3.17}
\end{aligned}$$

$$\frac{dR_{I0}}{T} = \left(\frac{\gamma_s^* \sigma}{\bar{K} + C_0} + f \frac{1}{1 - R_{F0}} \right) \tag{3.18}$$

$$\times (m \chi_L L_{B0} + n r \chi_V V_{B0}) - \sigma \gamma_{rr} R_{I0} / \sigma \tag{3.19}$$

$$\frac{dC_0}{T} = \Upsilon(\omega_L R_L^{chol} L_{I0} + \omega_V r R_V^{chol} V_{I0}) - \lambda^*(C_0 - 1) \tag{3.20}$$

Solving to $O(1)$ gives L_{E0} and V_{E0} constant with time. We solve this simpler system numerically using ode15s as in chapter 2 and take $\hat{l}_E \approx L_{E0}$, $\hat{l}_B \approx L_{B0}$ etcetera.

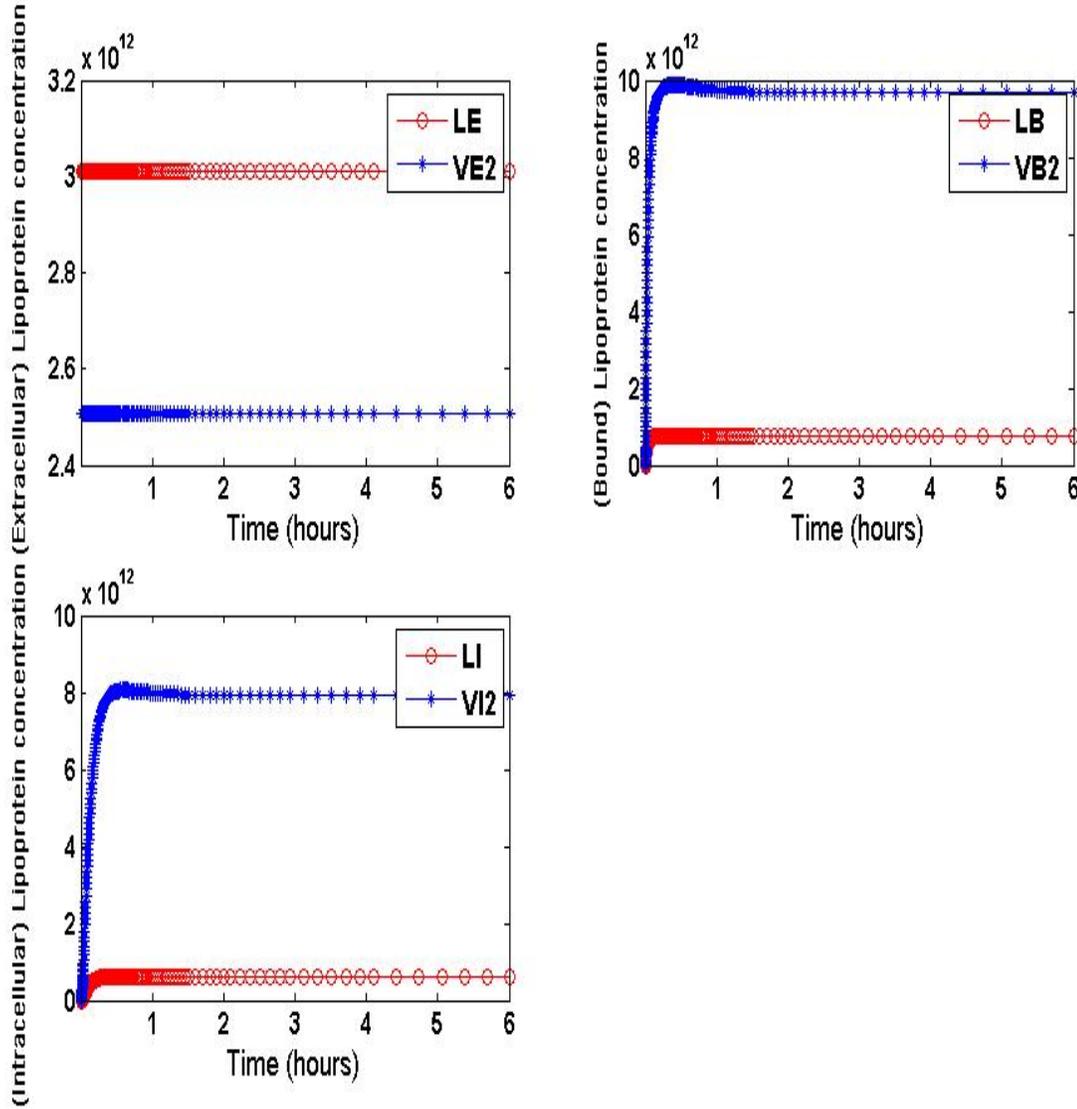


Figure 3.1: The change in extracellular, bound and internalised VLDL and LDL concentrations over a period of 6h using the reduced system 3.11-3.20. Parameter values are those stated in Table 2.2 with $r=1$, $\zeta = 0.8$. Using initial values 2.36 but with extracellular LDL, $\hat{l}_E = 1$.

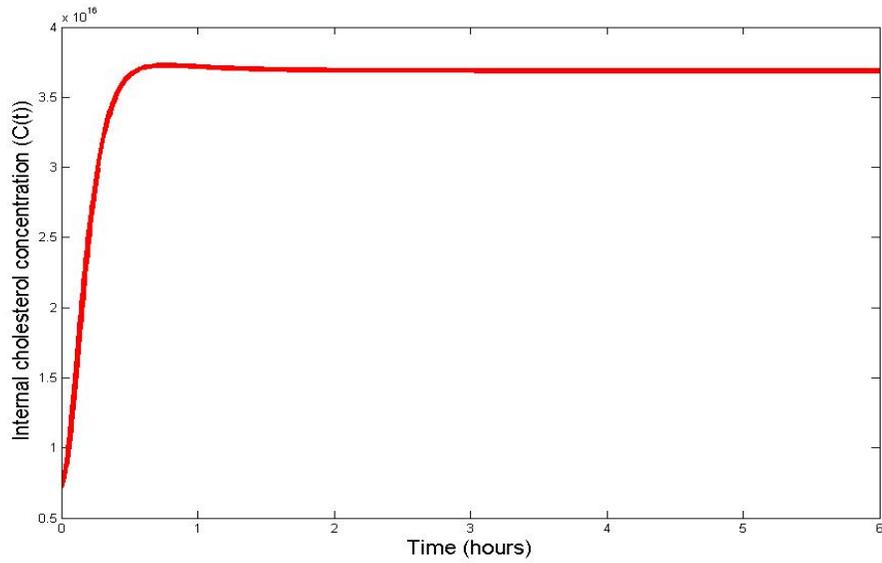


Figure 3.2: The change in intracellular cholesterol over a period of 6h using the reduced system 3.11-3.20. Parameter values are those stated in Table 2.2 with $r=1$, $\zeta = 0.8$. Using initial values 2.36 but with extracellular LDL, $\hat{l}_E = 1$.

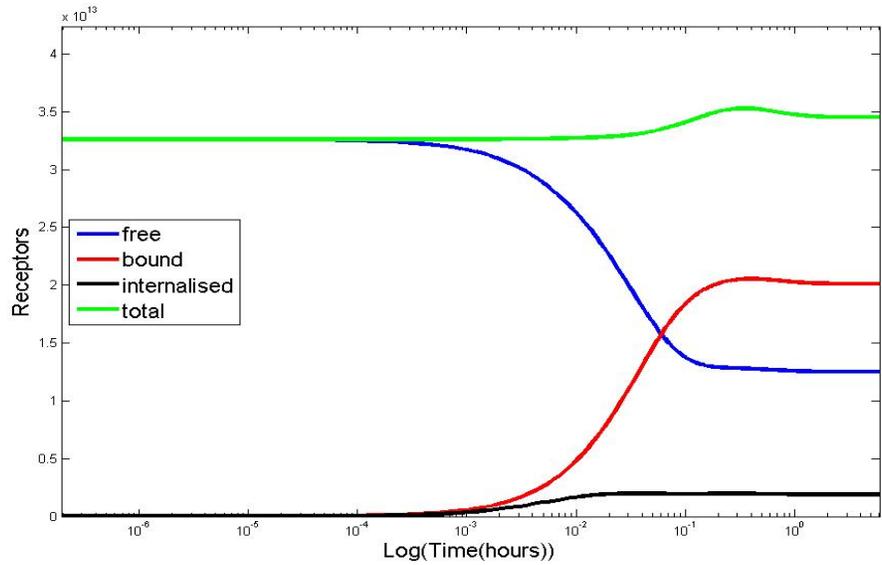


Figure 3.3: The change in time of free, bound, internalised and total receptor concentrations using the reduced system 3.11-3.20. Parameter values are those stated in Table 2.2 with $r=1$, $\zeta = 0.8$. Using initial values 2.36 but with extracellular LDL, $\hat{l}_E = 1$.

Comparing the graphs of lipoprotein concentrations figures 3.1 and 2.4, the reduced asymptotic system does not capture the sudden decrease in extracellular VLDL to its fasting levels since it is assumed to be constant. The values of the bound LDL and VLDL concentrations are higher than in the full model solution, this is due to the fact that the extracellular VLDL is constantly at its peak amount.

Figure 3.3 shows the total concentration of receptors is higher than in figure 2.6 and as the bound and free receptors tend towards a steady state their places have swapped. The asymptotic solution concludes there will be more bound receptors than free receptors whereas the full model solution from Chapter 2 finds the opposite; there will be more free receptors than bound receptors. This difference is due to the fast binding rate of extracellular VLDL particles. The peak values of extracellular VLDL concentration displayed in the asymptotic model mean that any free receptors will quickly be bound.

The intracellular cholesterol level in the asymptotic solution, figure 3.2, behaves in a similar way to the full solution, figure 2.5. It does tend towards a higher value but we expect this due to the higher extracellular VLDL concentration inputting more cholesterol into the system.

Clearly taking these equations to $O(1)$ does not properly represent the system. We could take it to $O(\epsilon)$ and then solve for L_{E1} to C_1 and take $\hat{l}_E \approx L_{E0} + L_{E1}$ etcetera. This creates an even more complicated system of equations than the one we are already solving so it is not a logical step.

If we had been solving the *in vitro* case where $W = 1500$, representing the extracellular space, we would expect the results of the numerical solution to be very similar to the results of this asymptotic analysis.

Chapter 4

Meal Approach

Chapters 2 and 3 covered modelling approaches considering a sudden peak in extracellular VLDL concentration. Over a 24 hour period we would expect three significant peaks in VLDL in respect to the three main meals a person consumes. We now try to adapt the model to make it more realistic in terms of the change in concentrations of lipoproteins we would expect in the blood plasma.

The non-dimensionalised, non-linear system of ODE's (2.21)-(2.29) was solved the same way as in Chapter 2, over a 6 hour period. After the 6 hour period an injection of $\hat{v}_E = 0.45$ was added to the extracellular VLDL concentration to simulate the livers peak excretion after a fatty meal. This was repeated after another 6 hours. The model was run for another 12 hours after this to simulate the fasting period overnight when no food is consumed.

4.1 Simulation and Results

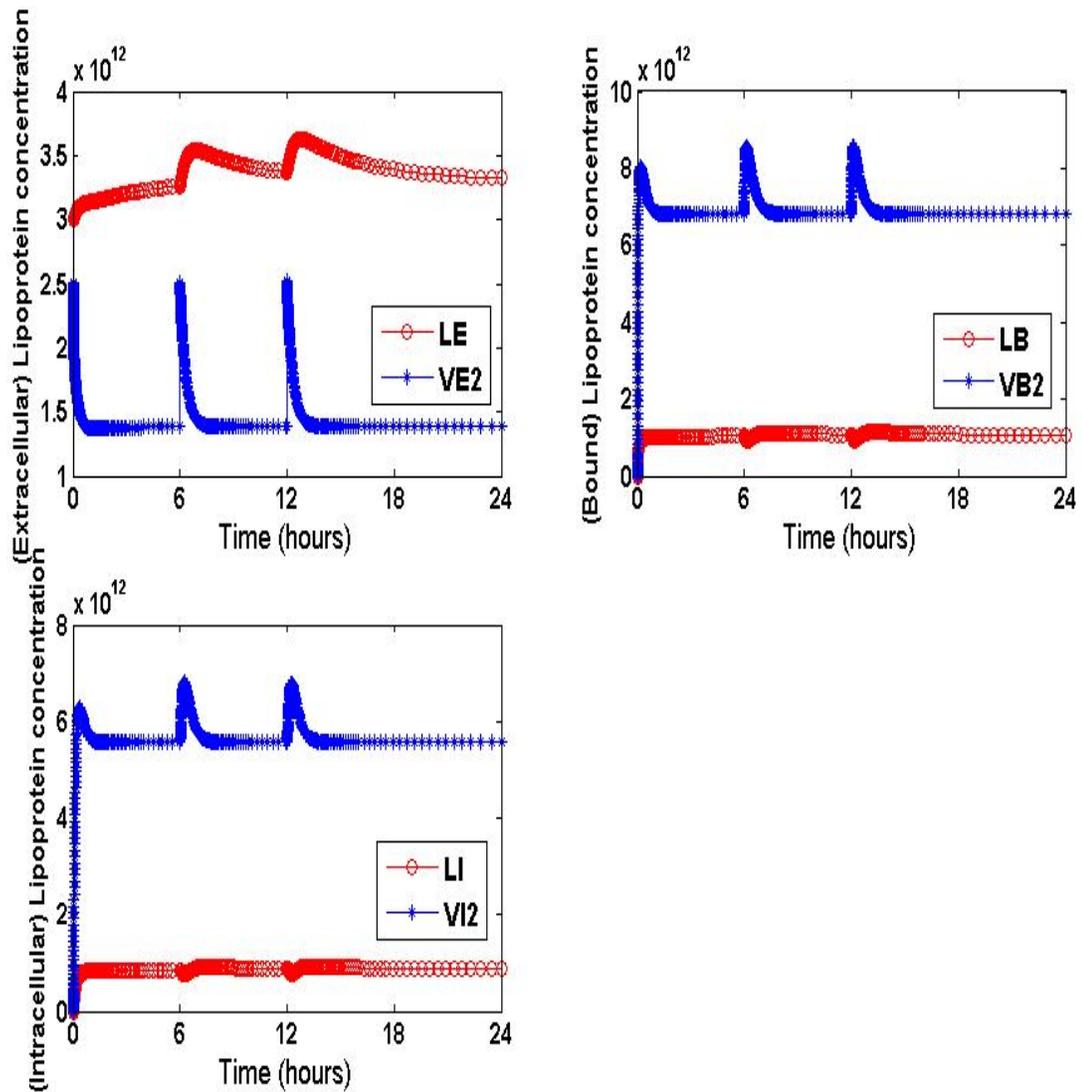


Figure 4.1: The change in extracellular, bound and internalised VLDL and LDL concentrations over a period of 24h with an injection of extracellular VLDL at 0h, 6h and 12h. Parameter values are those stated in Table 2.2 with $r=1$, $\zeta = 0.8$. Using initial values 2.36 but with extracellular LDL, $\hat{l}_E = 1$.

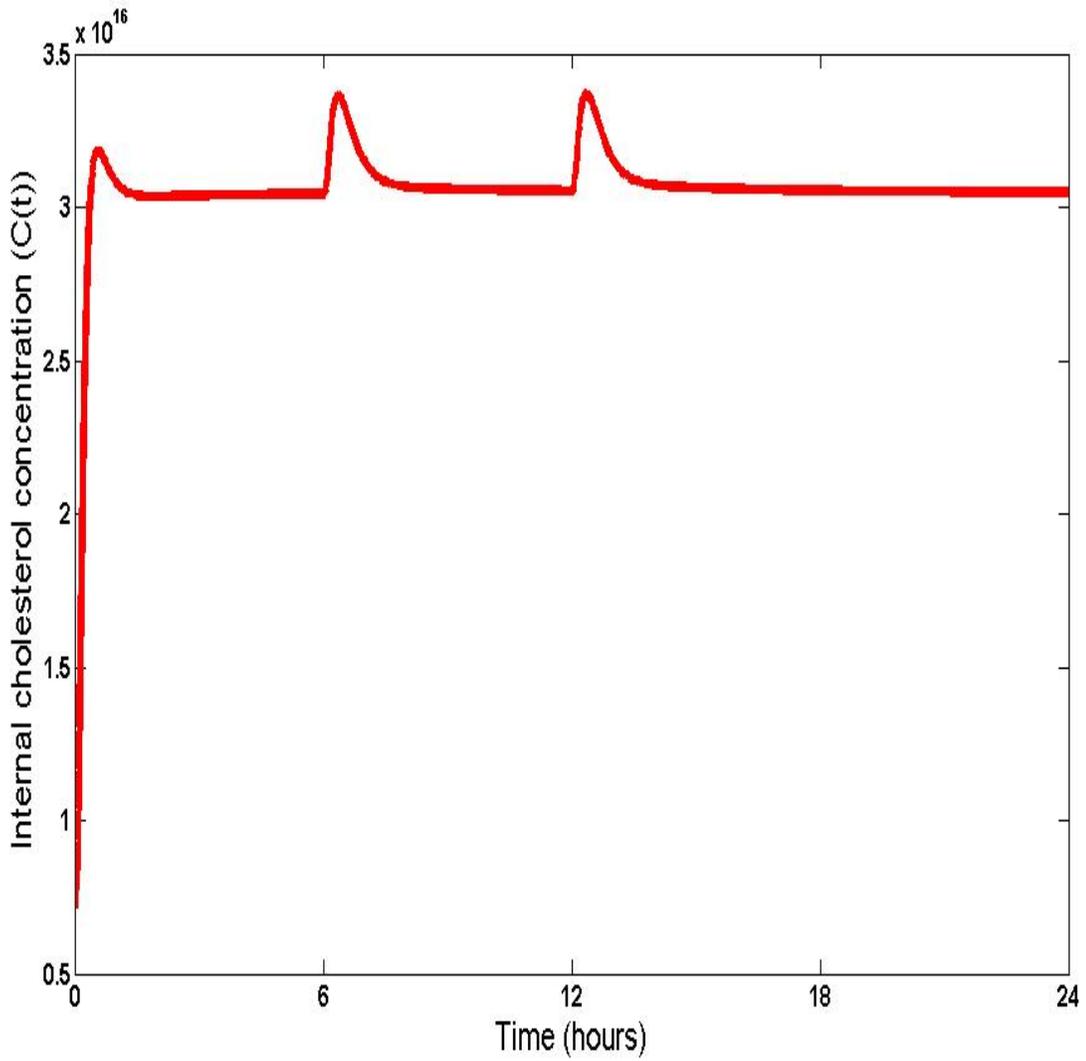


Figure 4.2: The change in intracellular cholesterol over a period over 24h with an injection of extracellular VLDL at 0h, 6h and 12h. Parameter values are those stated in Table 2.2 with $r=1$, $\zeta = 0.8$. Using initial values 2.36 but with extracellular LDL, $\hat{l}_E = 1$.

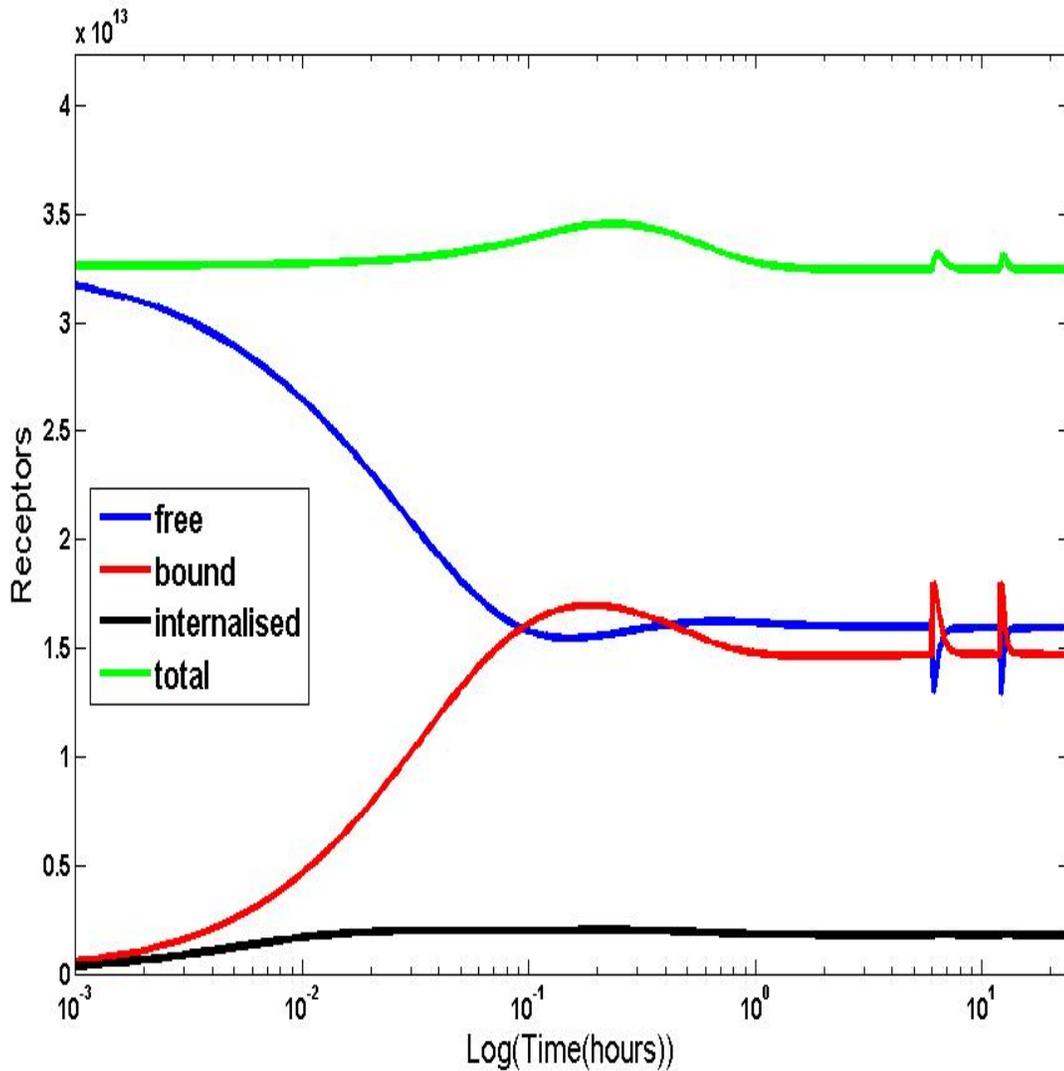


Figure 4.3: The change in time of free, bound, internalised and total receptor concentrations over 24h with an injection of extracellular VLDL at 0h,6h and 12h. Parameter values are those stated in Table 2.2 with $r=1$, $\zeta = 0.8$. Using initial values 2.36 but with extracellular LDL, $\hat{l}_E = 1$.

4.2 Discussion

The actual behaviour of VLDL secretion is more fluid. It only varies slightly around a base amount and the peak levels would happen a few hours after a fatty meal with a delay as they are released. We would also expect the level of LDL to remain constant. Figure 4.1 shows a very fast decrease in extracellular VLDL. We also see a slow increase in the extracellular LDL concentration which does not completely reduce over the 24 hour

period. We would expect the extracellular LDL to remain constant.

Figure 4.2 shows that the intracellular cholesterol levels peak shortly after the extracellular VLDL concentration peaks, but this settles back very quickly to a steady state level. The receptors, see figure 4.3, behave similarly. There is a sharp increase in bound receptors which correlates to the high concentration of VLDL and subsequently a decrease in the number of free receptors as they are being bound. The total number of receptors does briefly increase but quickly settles back towards the steady state.

There are other effects the model is incapable of capturing, for example the second meal affect. From the first meal after a fasting state, e.g. breakfast, the liver is slow to release extracellular VLDL particles, if a second meal is received in quick succession, e.g. lunch, it releases a higher amount of VLDL. This is because after a fasting state the liver tries to conserve energy and so releases the extracellular VLDL particles slowly. The model cannot capture this affect due to the constant release of extracellular VLDL particles.

Chapter 5

Conclusions and Further Work

5.1 Conclusions

In this dissertation we have formulated and studied a mathematical model for lipoprotein metabolism by combining a recent *in vitro* model with the *in vivo* case.

Assumptions were made about the suitability of modelling the liver as one large hepatocyte cell and changing the meaning of current model values, W . We also assumed that the apoE content of VLDL particles was unimportant and did not differentiate between VLDL-2 and VLDL-3 particles.

Only one biologically realistic steady state of this system exists and its values are dependent on the concentrations of extracellular VLDL and LDL. From numerical experimentation it appears stable. In general the model displays a high level of stability, which will be useful for future development.

There were significant inaccuracies in the numerical steady state solution preventing the system from reaching its true equilibrium.

Sensitivity analysis of the system has shown the most significant parameters affecting intracellular cholesterol levels are the rate the liver secretes extracellular VLDL particles and the ratio of LDL concentration to intracellular cholesterol. This intuitively makes sense, a higher number of VLDL particles in circulation will result in more particles binding, being internalised and subsequently releasing cholesterol within the cell.

We have observed the breakdown rate of extracellular VLDL to LDL particles has significant affect on the model results and the intracellular cholesterol concentration.

There were difficulties obtaining this parameter.

It is important that parameter values are gathered in a similar way. There was also insufficient experimental data for comparison; lipoprotein and cholesterol levels are usually measured over a course of weeks or months and they are measured in comparison to another biological parameter.

Asymptotic analysis was shown to be unsuitable for the model, unless modelling steady state solutions where the extracellular concentration of VLDL should not significantly vary.

The extended model in Chapter 4 was a step towards making this approach truly *in vivo*. Due to its inability to model the delay in extracellular VLDL release after a fatty meal or capture the second meal effect it still has room for many developments.

5.2 Further Work

The literature has shown that the apoE content of VLDL particles is very influential in their uptake. The parameter information for VLDL-2 and VLDL-3 particles is known [1] so including this difference in the model would be the first development. To improve the accuracy of the model, better information on some of the parameter values is needed.

Later this could develop into a more sensitive model including IDL and LDL particles. For this development it would be important to obtain the relevant parameter information.

In the liver the volume of extracellular fluid is twice that of blood. The rough approximation of modelling the liver as one large cell is flawed in this respect. This could be rectified by calculating the precise proportion of extracellular lipoprotein particles in the extracellular fluid of the liver. Then using the total volume of blood in circulation in proportion to this value for W .

A further problem with the approximation of W used in the model is that it assumes all extracellular lipoproteins have an equal chance of binding with the LDLR. However a lipoprotein in circulation not in contact with the liver has 0 chance of being metabolised by a hepatocyte cell. The location of the lipoprotein will determine if it will degrade from a VLDL to an LDL particle before it reaches a hepatocyte cell. If it has already degraded into an LDL particle it may move into the tissue or become retained on an artery wall as opposed to returning to the liver.

An alternative modelling approach would be to track individual lipoprotein particles. This could directly address the issues above. However a particle tracking approach is costly computationally and would mean completely reforming the model. The model currently uses concentrations of particles. The concentrations of extracellular particles could be split and the appropriate parameter assigned depending on the location. The chance of being at either location would change with each time step. This would still add a lot of complexity to the model which could lead to numerical instability.

To further develop this model and make it into a better representation of what we would expect, a time delay and second meal effect would need to be included. It would be relatively simple do both of these. The time delay could be captured by making the injection of VLDL into a time dependent function rather than the constant value we have used. We could incorporate the second meal effect into this by delaying the peak values in relation to the time the last peak occurred.

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