University of Reading

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 cholesterols are associated with coronary heart disease.

Using the current model and a detailed biological diagram we construct a simpli ed 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 di erential 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 nd 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 nd that certain parameters have signi cant a ect 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 a ect that meals have on lipoprotein and cholesterol levels. Finally we suggest possible future work and further development of the model.

Acknowledgements

Firstly I would like to thank the ESPRC for their generous grant, without which it would not have been possible for me to study this course. I would also like to thank my supervisor Dr. Marcus Tindall for his guidance over the duration of this dissertation and Dr. Nick Biggs for his help and input.

I would like to thank all of my fellow MSc students for making the computer room an enjoyable and relaxing place to study. Many thanks also go to my boyfriend for all of the patience and support he has provided throughout the course of this dissertation.

Declaration

I con rm 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 uid around the liver may by 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 ow 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 di ers 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 a ect the uptake of LDL.

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

1.2 Lipoprotein Metabolism

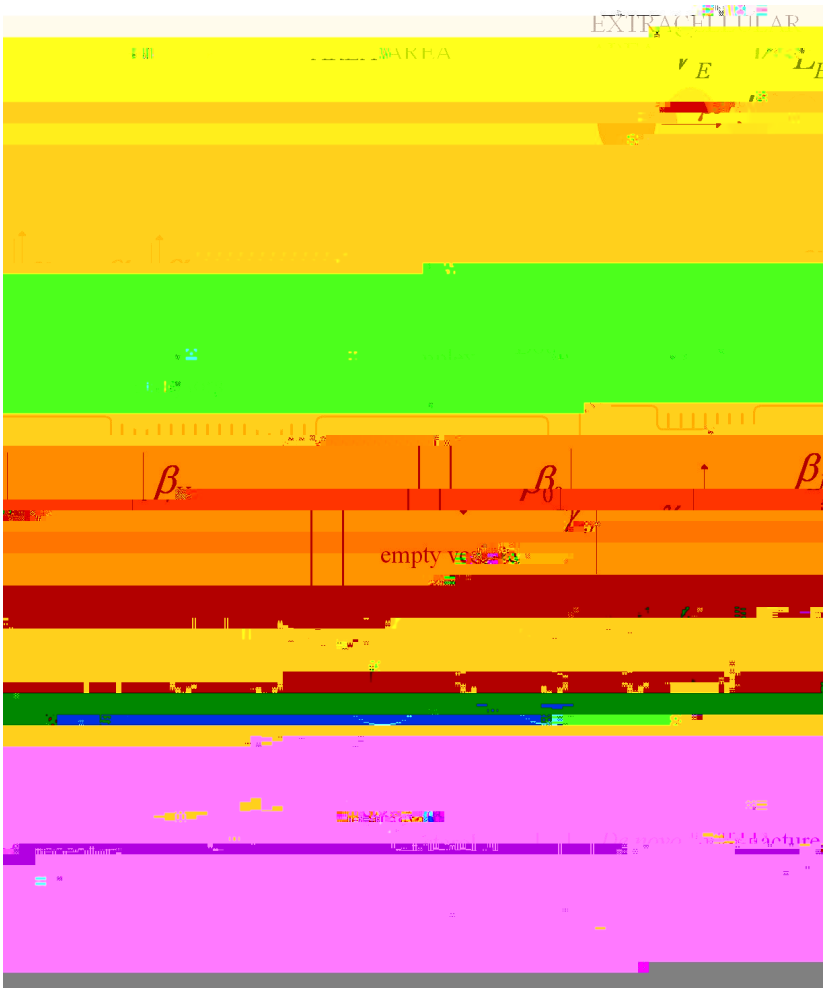

Figure 1.2: An illustrative summary of the processes involved in the uptake of LDL and VLDL particles by a hepatocyte cell, it also includes the breakdown of extracellular VLDL to extracellular LDL. The yellow circles represent LDL particles and the larger orange circles represent VLDL particles. See 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 uid and deliver cholesterol straight to the tissue. There is much more LDL in the blood plasma than in the extracellular uid 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 mediation 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 in uenced 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 is 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

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 a ect various parameters have on the intracellular cholesterol levels.

In chapter 3 we perform some asymptotic analysis on the non-linear system of ODE's,

Chapter 2

The Model

From a simpli cation of the main biological processes of lipoprotein metabolism some key reaction equations can be identi ed. From these reaction equations we form a system of ordinary di erential 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 rst process we observe is the extracellular VLDL particles breaking down into LDL particles within the circulation:

$$V_E \; ! \; L_E: \tag{2.1}$$

Here V_E and L_E refer to the extracellular VLDL and LDL particles and 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:

$$L_E + R_F + (M \quad 1)R_F \;) \; {}^{L*}_{L} \; L_B{:}MR_B: \tag{2.2}$$

One free LDLR, R_F, binds to one free LDL particle at rate

$$_{B}{}^{iMR}$$

The bound lipoprotein receptor complex is then internalised:

$$L_B{:}MR_B + \tilde{M}R_F \xrightarrow{\mu_L} L_I + (M + \tilde{M})R_I{:} \tag{2.3}$$

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 μ_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 μ_0:

$$PR_F \xrightarrow{\mu_0} PR_I{:} \tag{2.4}$$

The internalised LDL particles release cholesterol into the cell:

$$L_I \xrightarrow{\mu_L} R_L^{chol}C{:} \tag{2.5}$$

The internalised LDL particle, L_I, releases cholesterol at a rate μ_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, $V_B{:}NR_B$:

$$V_E + NR_F \underset{\nu}{\overset{\nu^*}{\rightleftharpoons}} V_B{:}NR_B{:} \tag{2.6}$$

One receptor is bound and $N - 1$ are blocked, this happens at a rate ν^*. The bound VLDL-receptor complex unbinds from the surface at rate ν.

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:

$$V_B{:}NR_B + \tilde{N}R_F \xrightarrow{\mu_V} (N + \tilde{N})R_I + V_I; \tag{2.7}$$

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

The internalised VLDL particles release cholesterol into the cell:

$l_B = [L_B]$, $v_B = [V_B]$, $l_l = [L_l]$ and $v_l = [V_l]$. 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, $_B$, is given by:

$$_B = M l_B + N v_B: \tag{2.18}$$

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

$$l_E(0) = 0; \quad l_B$$

$$W\frac{d\hat{I}_E}{d\hat{t}} = (\quad \hat{}_F\hat{I}_E + \quad {}_L\hat{I}_B + \quad \hat{v}_E); \tag{2.21}$$

$$\frac{d\hat{I}_B}{d\hat{t}} = \hat{}_F\hat{I}_E \qquad {}_L\hat{I}_B \qquad {}_L\hat{I}_B; \tag{2.22}$$

$$\frac{d\hat{I}_I}{d\hat{t}} = \quad {}_L\hat{I}_B \quad !\,{}_L\hat{I}_I; \tag{2.23}$$

$$W\frac{d\hat{v}_E}{d\hat{t}} = (\quad {}_V\,\hat{}_F\hat{v}_E + \quad {}_V\hat{v}_B \qquad \hat{v}_E + U_V); \tag{2.24}$$

$$\frac{d\hat{v}_B}{d\hat{t}} = \quad {}_V\,\hat{}_F\hat{v}_E \qquad {}_V\hat{v}_B \qquad {}_V\hat{v}_B; \tag{2.25}$$

$$\frac{d\hat{v}_I}{d\hat{t}} = \quad {}_V\hat{v}_B \quad !\,{}_V\hat{v}_I; \tag{2.26}$$

$$\frac{d\,\hat{}_F}{d\hat{t}} = \quad {}_{rr}\,\hat{}_I \quad {}_0\hat{}_F^P \quad m\hat{I}_E\,\hat{}_F \quad m\ {}_L\hat{I}_B + \frac{m\ {}_L\hat{I}_B\,\hat{}_F}{1 \quad \hat{}_F}$$

$$r \quad n\ {}_V\,\hat{}_F\hat{v}_E \quad n\ {}_V\hat{v}_B + \frac{n\ {}_V\hat{v}_B\,\hat{}_F}{1 \quad \hat{}_F} \quad = ; \tag{2.27}$$

$$\frac{d\,\hat{}_I}{d\hat{t}} = \quad \frac{s}{K + \hat{C}} + \quad {}_0 f\ \hat{}_F^P + f \quad 1 + \frac{\hat{}_F}{1 \quad \hat{}_F}$$

$$(m\ {}_L\hat{I}_B + n\ {}_V r\hat{v}_B) \qquad {}_{rr}\,\hat{}_I \quad = ; \tag{2.28}$$

$$\frac{d\hat{C}}{d\hat{t}} = \quad (!\,{}_L R_L^{chol}\hat{I}_I + !\,{}_V r R_V^{chol}\hat{v}_I) \qquad (\hat{C} \quad 1): \tag{2.29}$$

^d

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

$$!_L = \frac{L}{L_0}; \quad !_V = \frac{V}{L_0}; \qquad = \frac{}{L_0}: \tag{2.32}$$

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

$$_s = \frac{s}{C_e \ L_0^2}; \quad K = \frac{K}{C_e}; \qquad _{rr} = \frac{r}{L_0}: \tag{2.33}$$

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

$$r = \frac{v_0}{l_0}; \qquad = \frac{_0}{l_0}; \qquad = \frac{1}{R_L^{chol}}: \tag{2.34}$$

VLDL breakdown to LDL and constant rate of VLDL release:

$$= \frac{}{L_0}; \qquad U_V = \frac{U_V}{L_0}: \tag{2.35}$$

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

$$\begin{aligned} \hat{l}_E(0) &= 0; \quad \hat{l}_B(0) = 0; \quad \hat{l}_l(0) = 0; \\ \hat{v}_E(0) &= 1; \quad \hat{v}_B(0) = 0; \quad \hat{v}_l(0) = 0; \\ \hat{}_F(0) &= 1; \quad \hat{}_l(0) = 0; \quad \hat{C}(0) = 0{:}7C_e{:} \end{aligned} \tag{2.36}$$

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 di cult. 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 $= 1hr^{-1}$ 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 $g=ml$ and the peak values recorded after a fatty meal are 20 25 $g=ml$. LDL values show considerably less variation and are approximately 10 $g=ml$. Taking the weight of a small VLDL particle to be 6 $10^6 Da$ [5] and the weight of an LDL particle to be 2 $10^6 Da$ we calculate the initial values in terms of $particles=ml$ $medium$.

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]

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]
	Ratio of initial LDL concentration to initial cholesterol.	2:94 10^{-4}	Tindall et al. (2009) [1]
$_L = \ _V$	Relative rate of LDL/VLDL internalisation.	1.28	Tindall et al. (2009) [1]
$_V$	Relative rate of VLDL binding to free receptors.	14	Tindall et al. (2009) [1]
$_L$	Rate of LDL unbinding from receptors.	0.279	Tindall et al. (2009) [1]
$_V$	Rate of VLDL unbinding from receptors.	0.140	Tindall et al. (2009) [1]
$_0$	Rate of internalisation of free receptors.	0	Tindall et al. (2009) [1]
$!_L = !_V$	Rate of digestion of internalised LDL/VLDL to cholesterol.	1.56	Tindall et al. (2009) [1]
	Ratio of receptor to LDL concentrations (rescaled).	2.74	Tindall et al. (2009) [1]

2.3 Solution

A sti system is a special system of ODE's. De nition of sti ness 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, $_i$, 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 sti 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 sti 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 sti systems. An alternative is the trapezium method which is unconditionally stable, however it produces oscillations and has a low level of accuracy.

In a sti system the required step-size to maintain accuracy can vary with each time step. There is a play o 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 e ciency due to the fact that it can

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 $y' = f(t; y)$ have the form:

$$\sum_{j=0}^{k} {}_j y_{n+j} = h \sum_{j=0}^{k} {}_j f_{n+j} \tag{2.37}$$

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

$$\sum_{j=0}^{k} {}_j y_{n+j} = h \, {}_0 f_n: \tag{2.38}$$

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 ine cient 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 sti 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 e cient in comparison to explicit methods which use extremely small time steps throughout.

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 di erent breakdown rates of VLDL to LDL particles and we experiment with di erent initial concentrations of extracellular LDL.

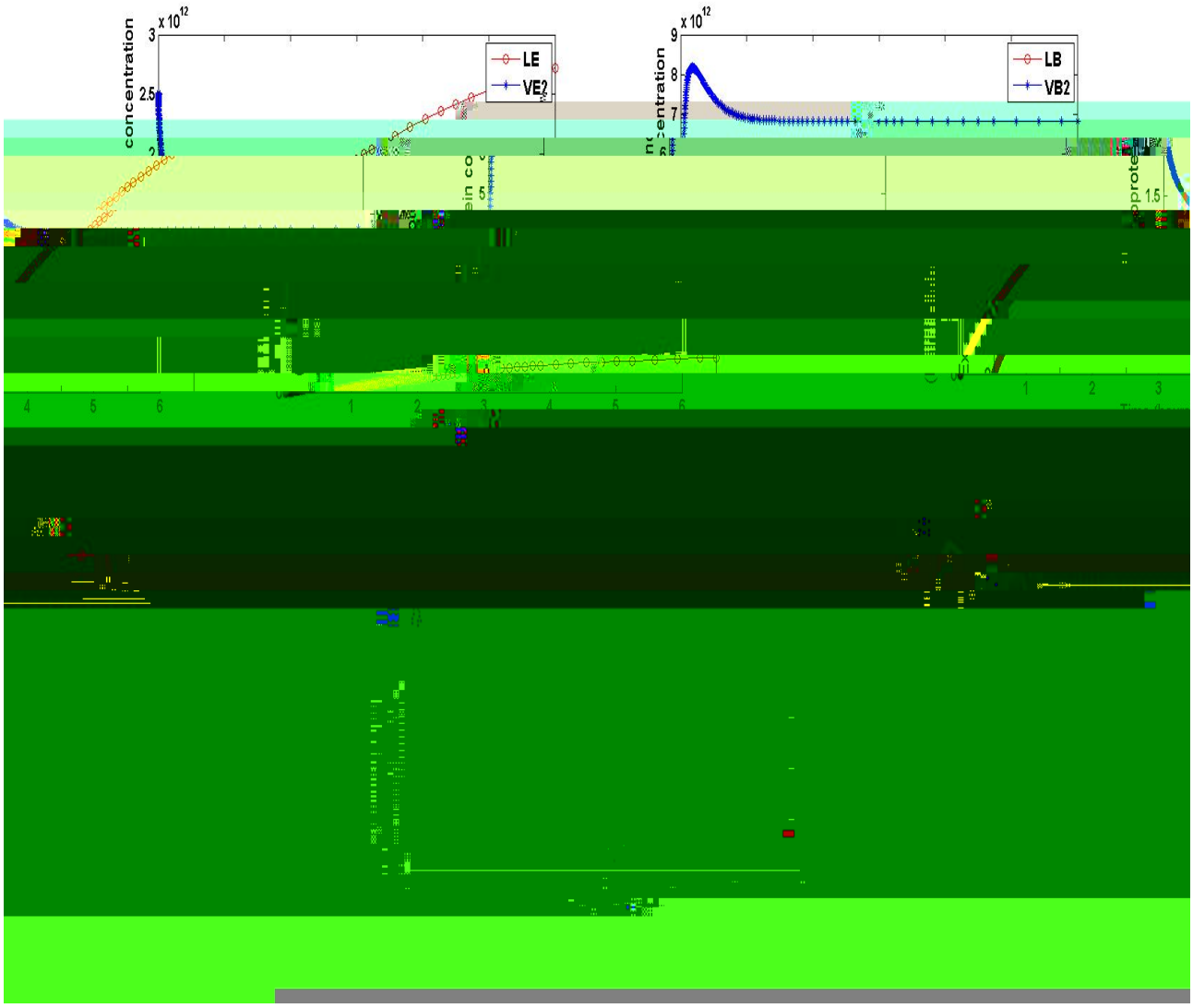

Figure 2.1: The change in extracellular, bound and internalised VLDL and LDL concentrations over a period of 6h. Parameter values are those stated in Table 2.2 with r=1, = 0:8 and extracellular LDL initially $\hat{I}_E$ = 0.

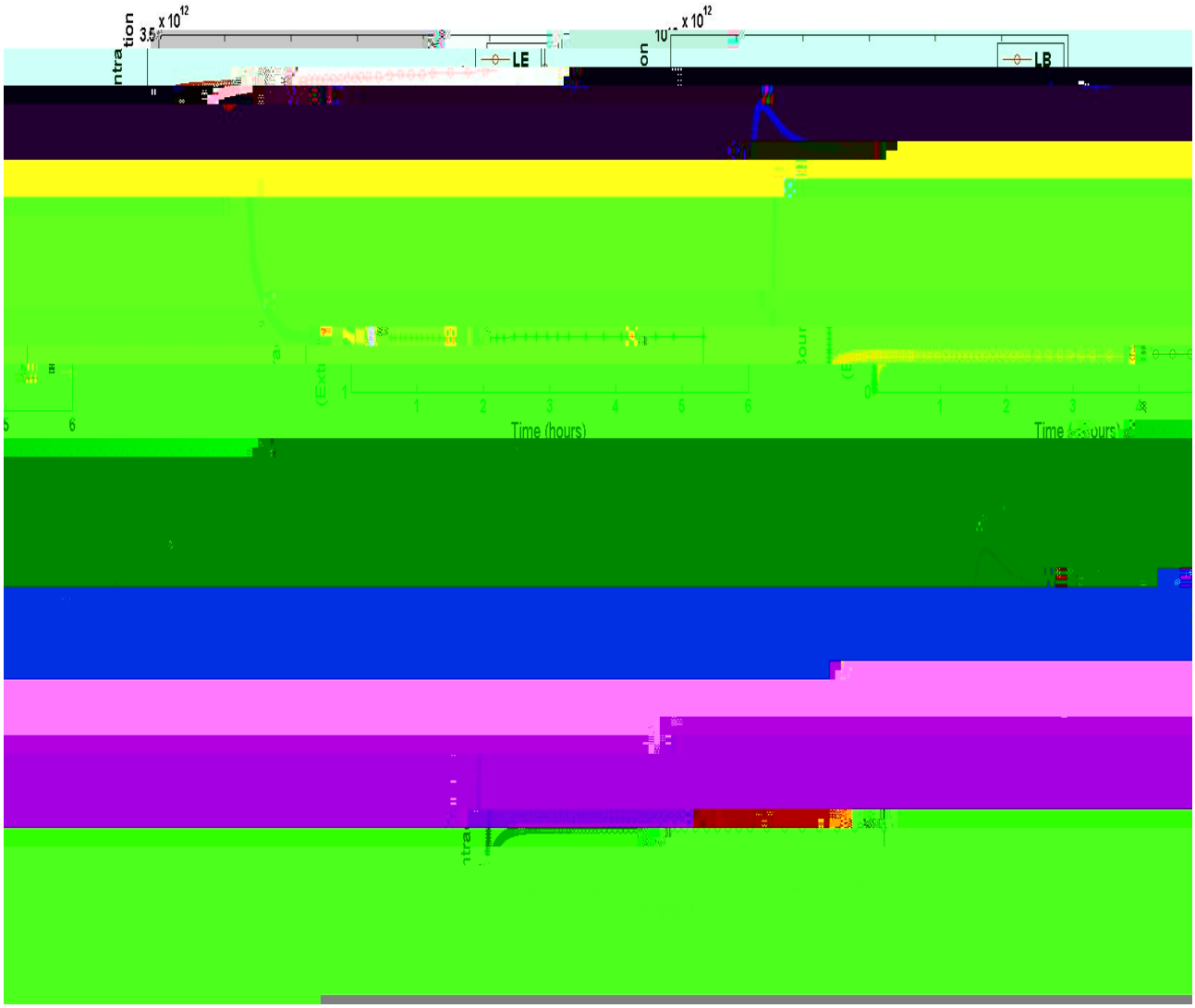

Figure 2.4: The change in extracellular, bound and internalised VLDL and LDL concentrations over a period of 6h. Parameter values are those stated in Table 2.2 with r=1, = 0:8. We now change the initial amount of extracellular LDL, $\hat{I}_E$ = 1 to simulate the values we would expect to be present biologically.

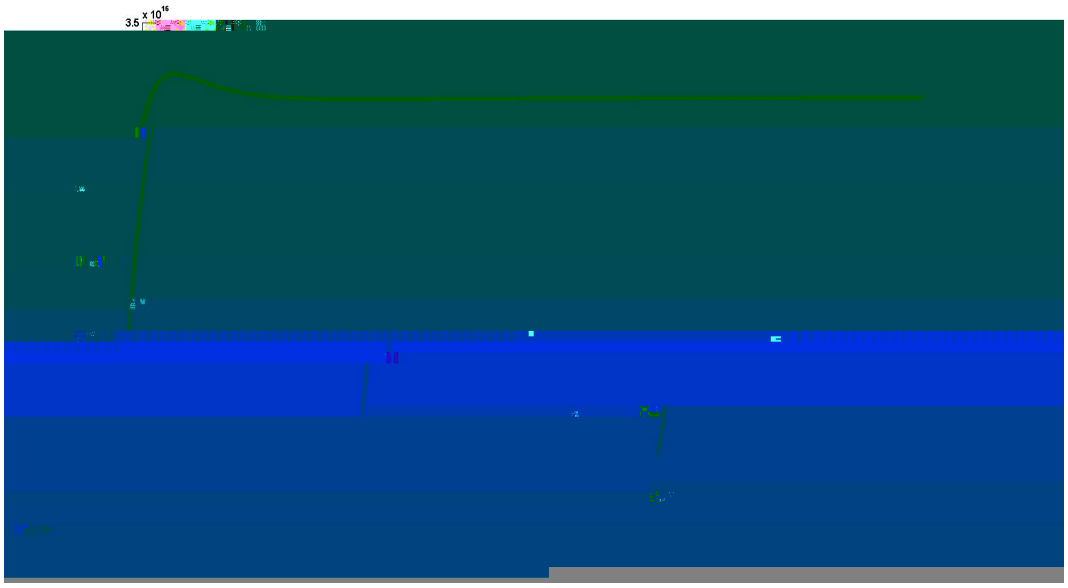

Figure 2.5: The change in intracellular cholesterol over a period of 6h. Parameter values are those stated in Table 2.2 with r=1, = 0:8. We now change the initial amount of extracellular LDL, $\hat{I}_E$ = 1 to simulate the values we would expect to be present biologically.

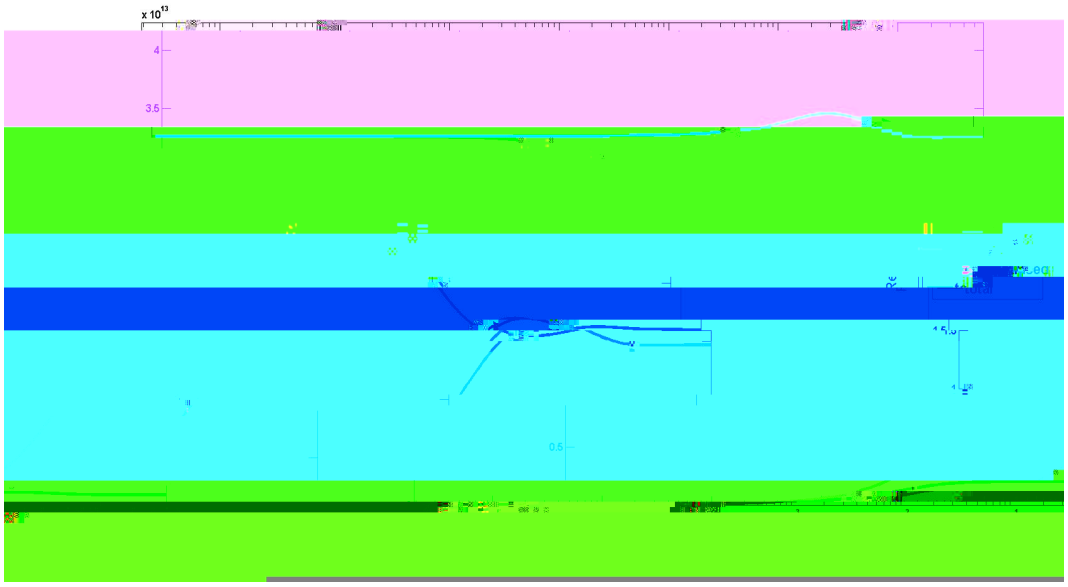

Figure 2.6: The change in time of free, bound, internalised and total receptor concentrations. Parameter values are those stated in Table 2.2 with r=1, = 0:8. We now change the initial amount of extracellular LDL, $\hat{I}_E$ = 1 to simulate the values we would expect to be present biologically.

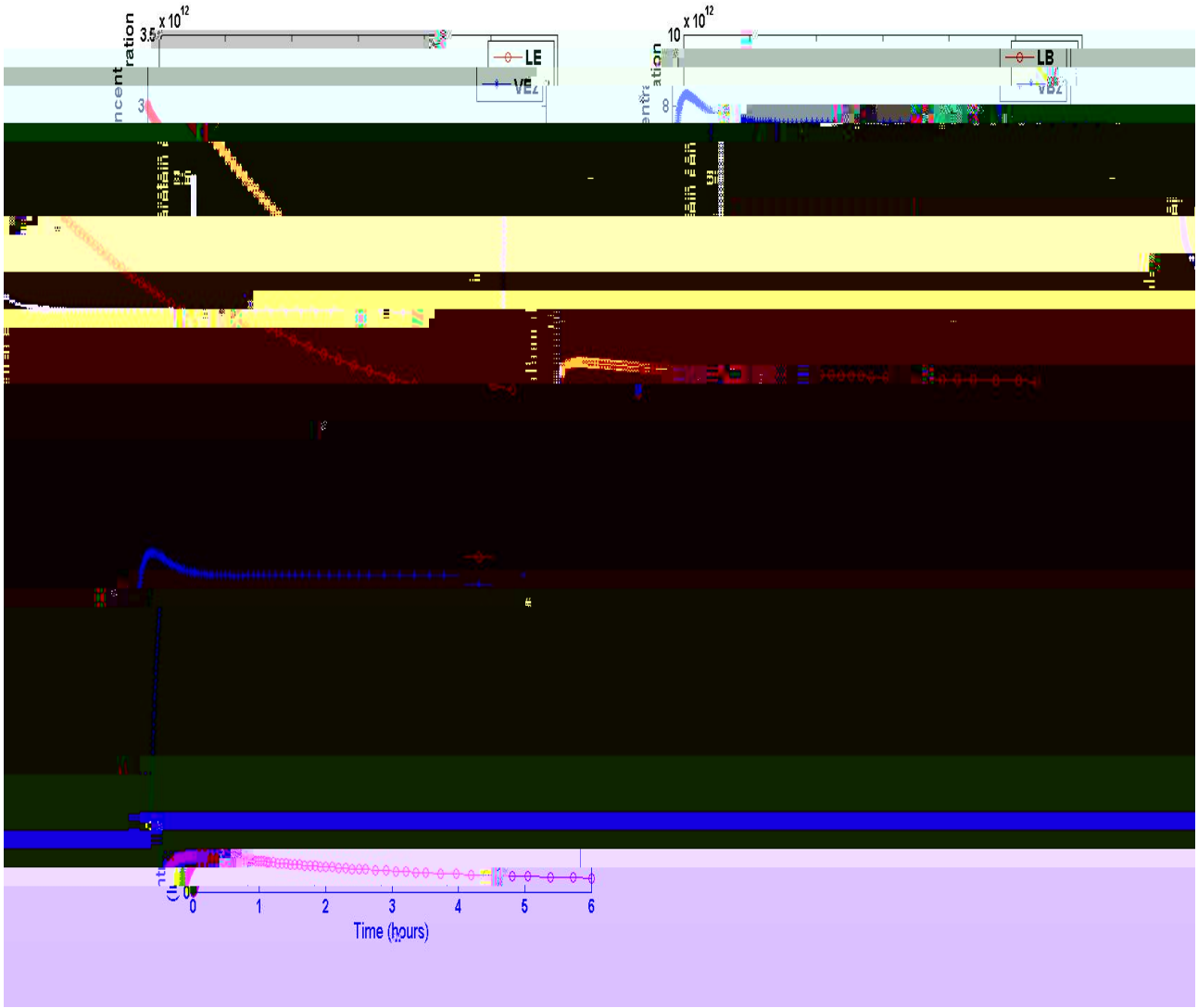

Figure 2.7: The change in extracellular, bound and internalised VLDL and LDL concentrations over a period of 6h. Parameter values are those stated in Table 2.2 with r=1, = 0:13. We continue with the initial amount of extracellular LDL, $\hat{I}_E = 1$.

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, = 0:13. We continue with the initial amount of extracellular LDL, $\hat{I}_E$ = 1.

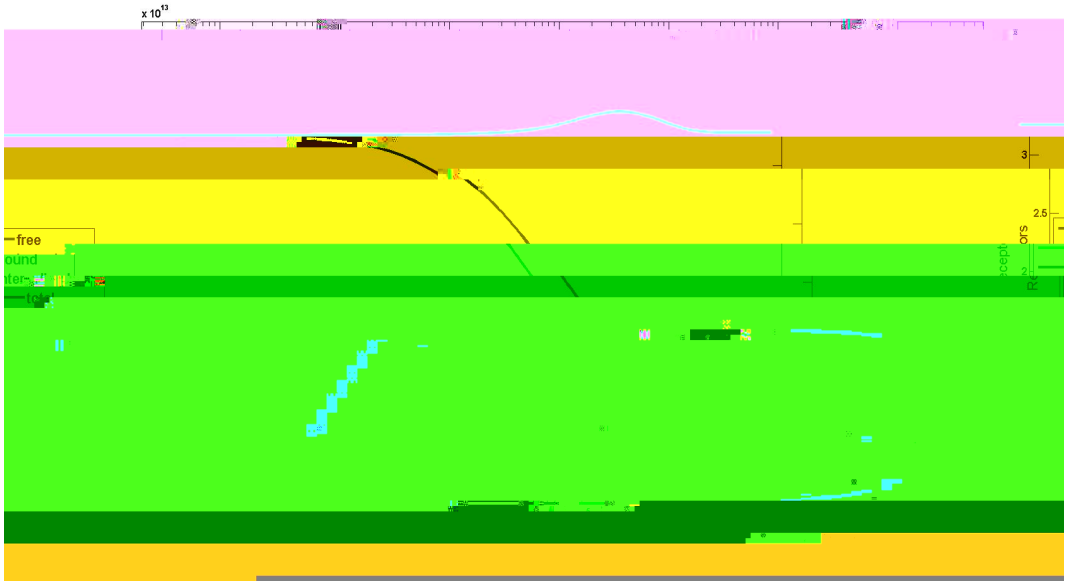

Figure 2.9: The change in time of free, bound, internalised and total receptor concentrations. Parameter values are those stated in Table 2.2 with r=1, = 0:13. We continue with the initial amount of extracellular LDL, $\hat{I}_E$ = 1.

Figures 2.1- 2.9 are the numerical solution to the system of non-linear di erential equations (2.21)-(2.29). They show the change in VLDL and LDL concentration as well

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

We continue with the value = 0:8 but change the non-dimensionalised initial conditions and solve the system of equations with $\hat{I}_E = 1$, 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 gure 2.5 is less pronounced than that of gure 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 gure 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 a ected 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 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:

$$0 = \hat{\lambda}_F \hat{l}_E + {}_L\hat{l}_B + \hat{v}_E; \tag{2.39}$$

$$0 = \hat{\lambda}_F \hat{l}_E - {}_L\hat{l}_B - {}_L\hat{l}_B; \tag{2.40}$$

$$0 = {}_L\hat{l}_B - !_L \hat{l}_I; \tag{2.41}$$

$$0 = {}_V\hat{\lambda}_F \hat{v}_E + {}_V\hat{v}_B - \hat{v}_E + U_V; \tag{2.42}$$

$$0 = {}_V\hat{\lambda}_F \hat{v}_E - {}_V\hat{v}_B - {}_V\hat{v}_B; \tag{2.43}$$

$$0 = {}_V\hat{v}_B - !_V \hat{v}_I; \tag{2.44}$$

$$0 = {}_{rr}\hat{\lambda}_l - {}_0\hat{\lambda}_F^P - m\hat{l}_E \hat{\lambda}_F$$

$$\hat{I}_B = \frac{\hat{v}_E}{L}; \tag{2.48}$$

$$\hat{I}_I = \frac{\hat{v}_E}{!_L}; \tag{2.49}$$

$$\hat{v}_B = \frac{U_V \quad \hat{v}_E}{V}; \tag{2.50}$$

$$\hat{v}_I =$$

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

$$\hat{}_I = \frac{1}{rr} \quad \frac{s}{K + \hat{C}} + f \quad 1 + \frac{\hat{}_F}{1 \quad \hat{}_F} \qquad (m \ _L \hat{I}_B + rn \ _V \hat{v}_B) \ : \tag{2.57}$$

To find a solution we make an approximation of the steady states of $\hat{I}_E$ and $\hat{v}_E$ using their initial values. All other variables can be expressed in terms of $\hat{I}_E$ 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 $\hat{I}_E = 1$ 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 $= 0.8$ 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	$6 \quad 10^{-5}$	0.344
L_I	Internalised LDL particles.	$5 \quad 10^{-5}$	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
$_F$	Free Receptors.	0.913	0.433
$_I$	Internalised Receptors.	0.970	0.144
C	Intracellular Cholesterol Concentration.	3.25	3.27

Note for the $\hat{v}_E = 1$ and:

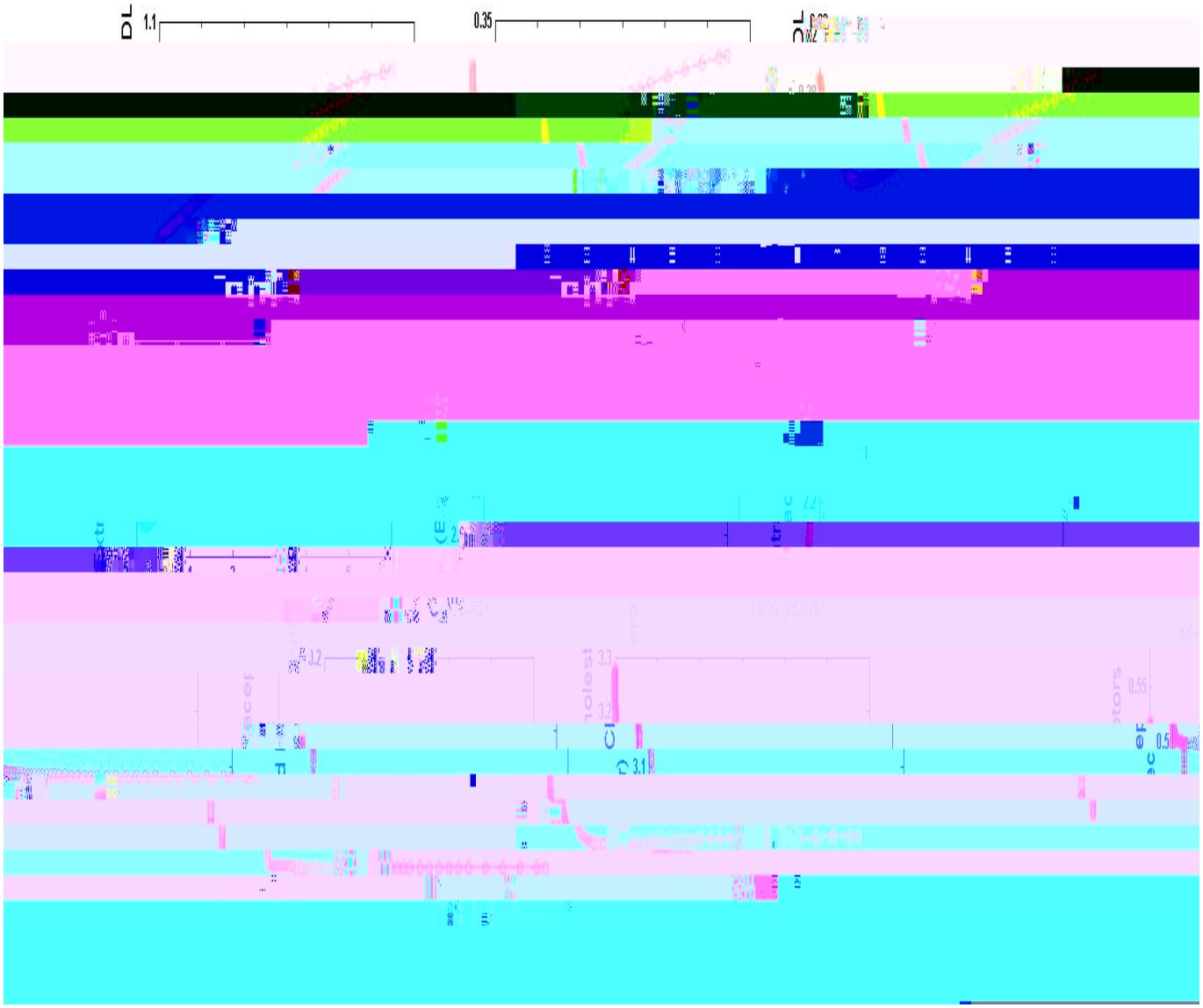

Figure 2.10: The development of steady states for all non-dimensional variables. Parameter values are those stated in Table 2.2 with r=1 and = 0:8. Initial conditions are from the Table 2.3 (Non-Trivial).

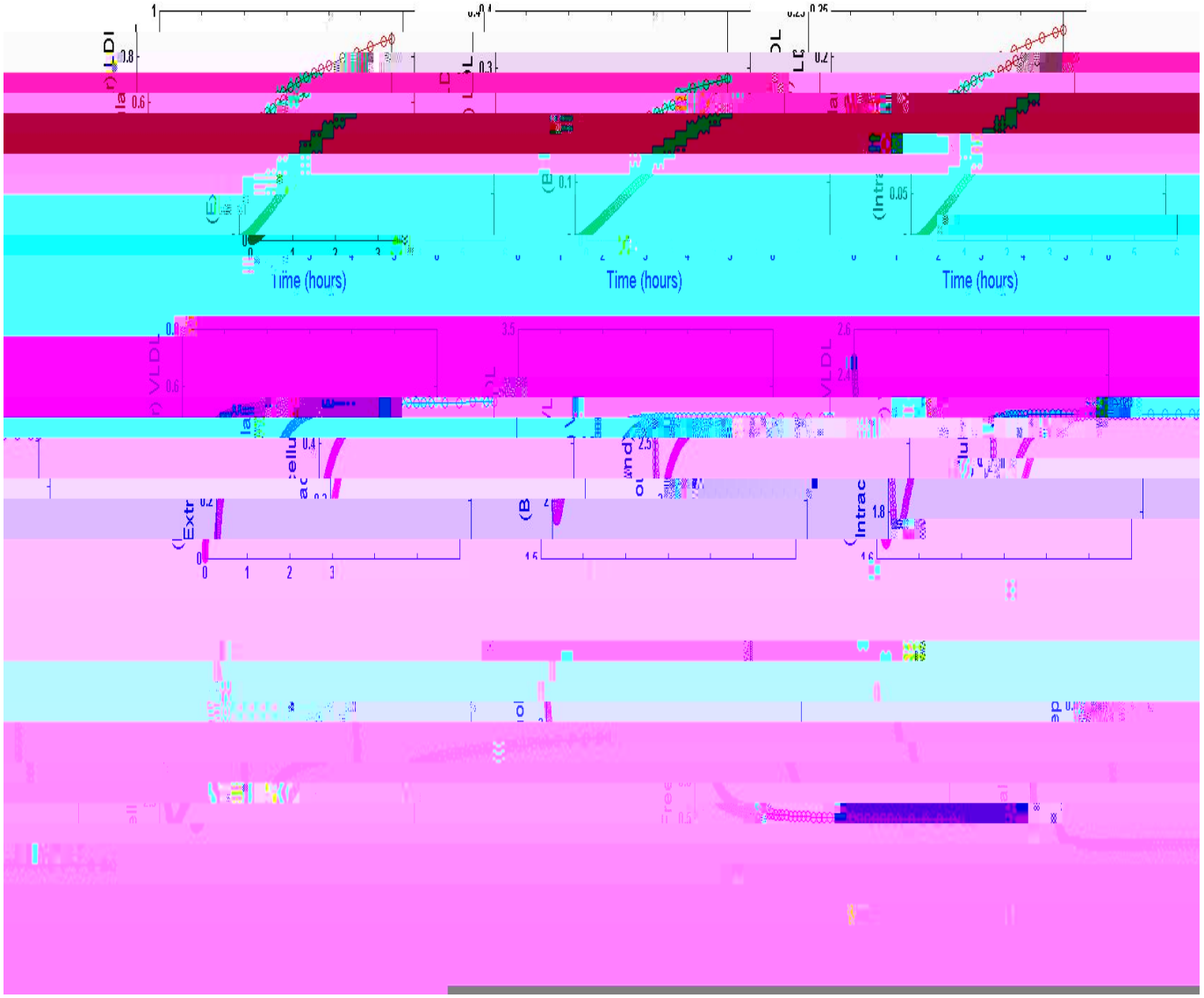

Figure 2.11: The development of steady states for all non-dimensional variables. Parameter values are those stated in Table 2.2 with r=1 and = 0:8. Initial conditions are from the Table 2.3 (Trivial).

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:

$$\hat{}_F = \frac{m\hat{l}_B(\ _L + \ _L) + rn\hat{v}_B(\ _V + \ _V) + \frac{s}{K+\hat{C}}}{m\hat{l}_E + rn\ _V\hat{v}_E}: \tag{2.58}$$

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	$6 \quad 10^{\ 5}$	0.344
L_I	Internalised LDL particles.	$5 \quad 10^{\ 5}$	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
$\ _F$	Free Receptors.	2770	0.467
$\ _I$	Internalised Receptors.	0.003	0.153
C	Intracellular Cholesterol Concentration.	3.25	3.27

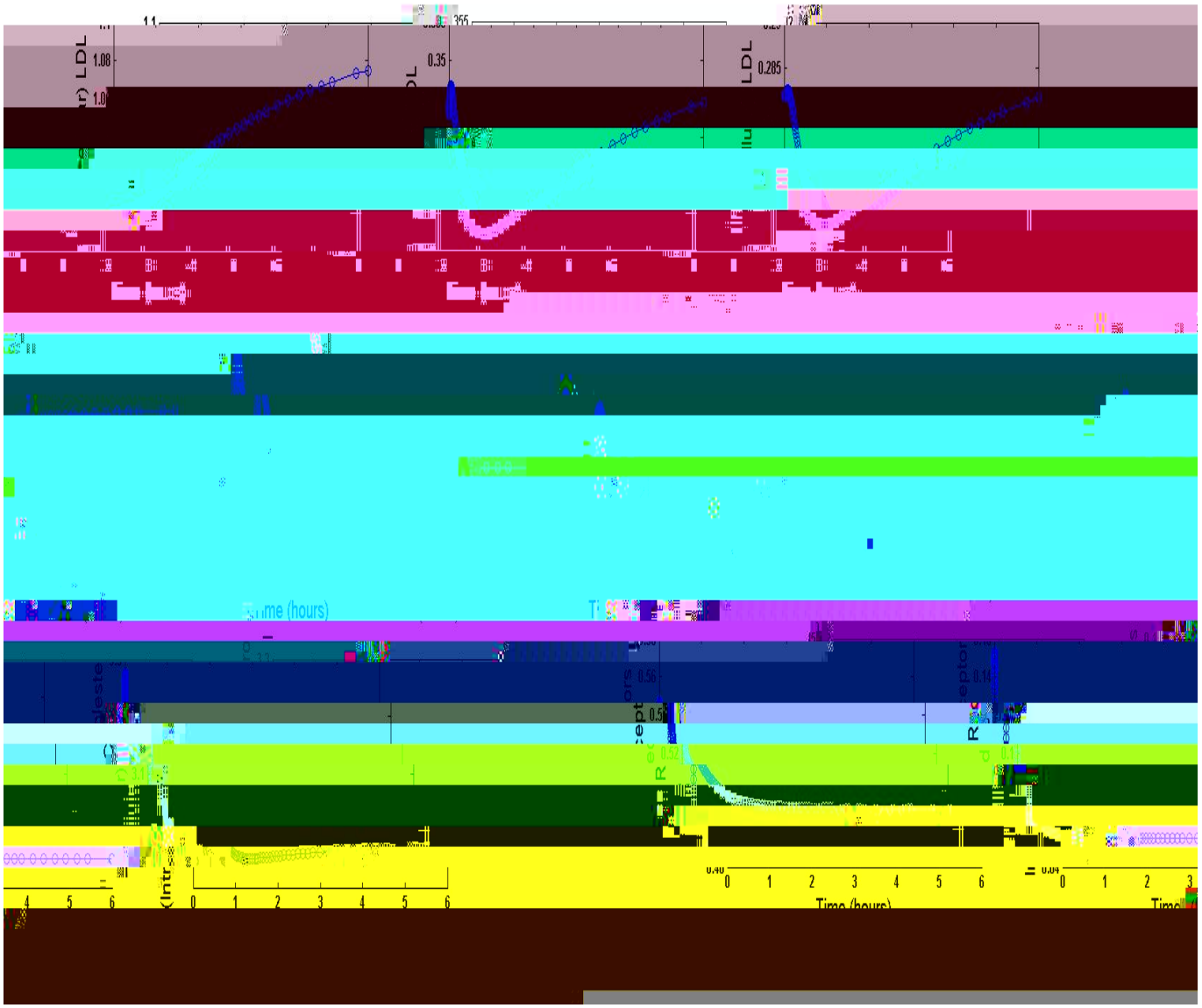

Figure 2.12: The development of steady states for all non-dimensional variables assuming $f = 1$. Parameter values are those stated in Table 2.2 with $r=1$ and $= 0.8$. Initial conditions are from the Table 2.4 (Non-Trivial).

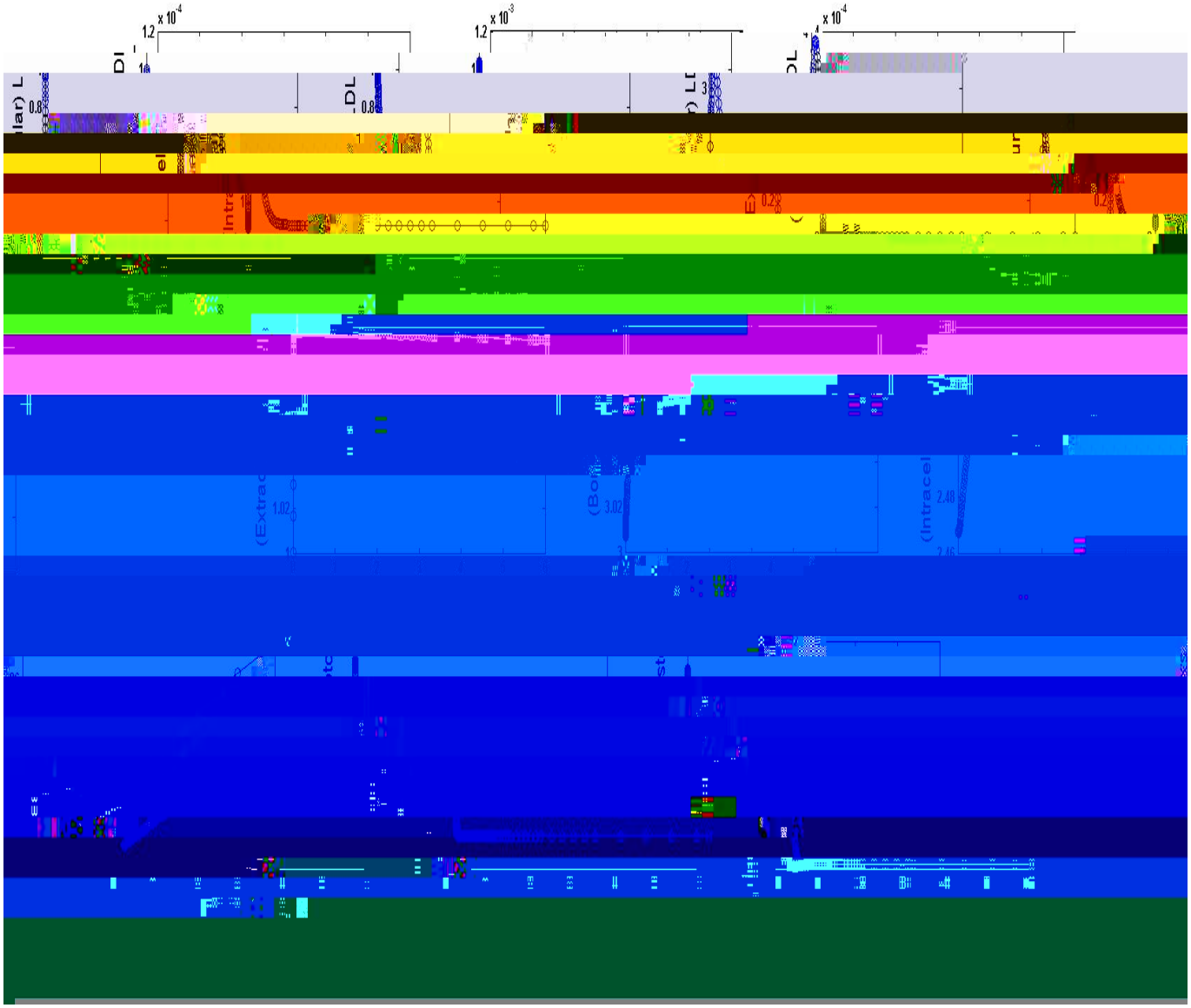

Figure 2.13: The development of steady states for all non-dimensional variables assuming $f = 1$. Parameter values are those stated in Table 2.2 with $r=1$ and $= 0.8$. Initial conditions are from the Table 2.4 (Trivial).

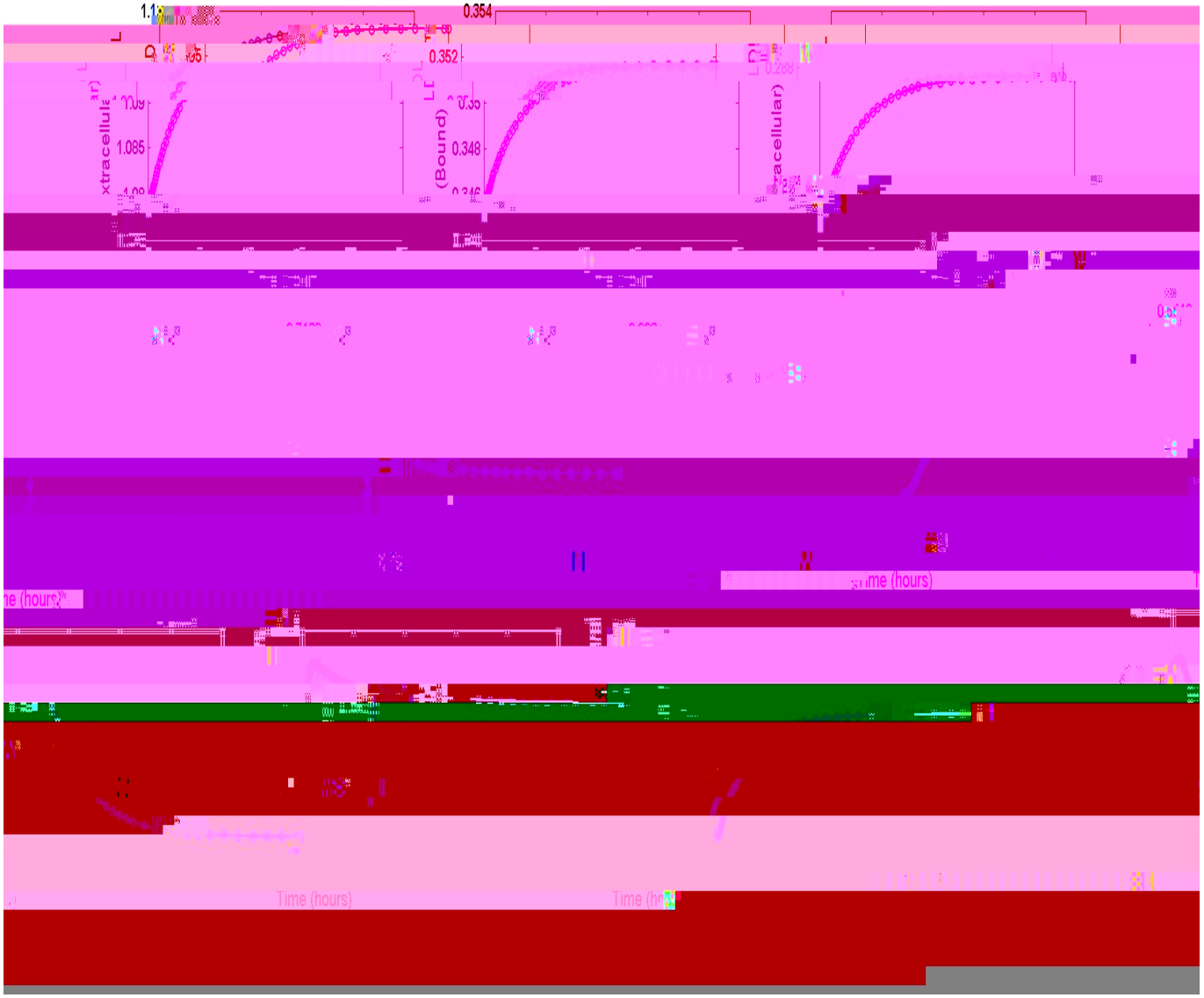

Figure 2.14: The development of steady states for all non-dimensional variables. Parameter values are those stated in Table 2.2 with r=1 and = 0:8. Initial conditions are from the Table 2.4.

Due to numerical inaccuracies we can see that we have no 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.

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 a ect on a speci c model output. In our model we are interested in how parameter variation within the model a ects the intracellular cholesterol content of the cell. Global sensitivity analysis looks at the relationships parameters have to each other and the e ect 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 a ect 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 a ect while with others the change is more subtle so we have separated the parameters to re ect this.

Figure 2.15: Sensitivity Analysis: Parameters with large e ect

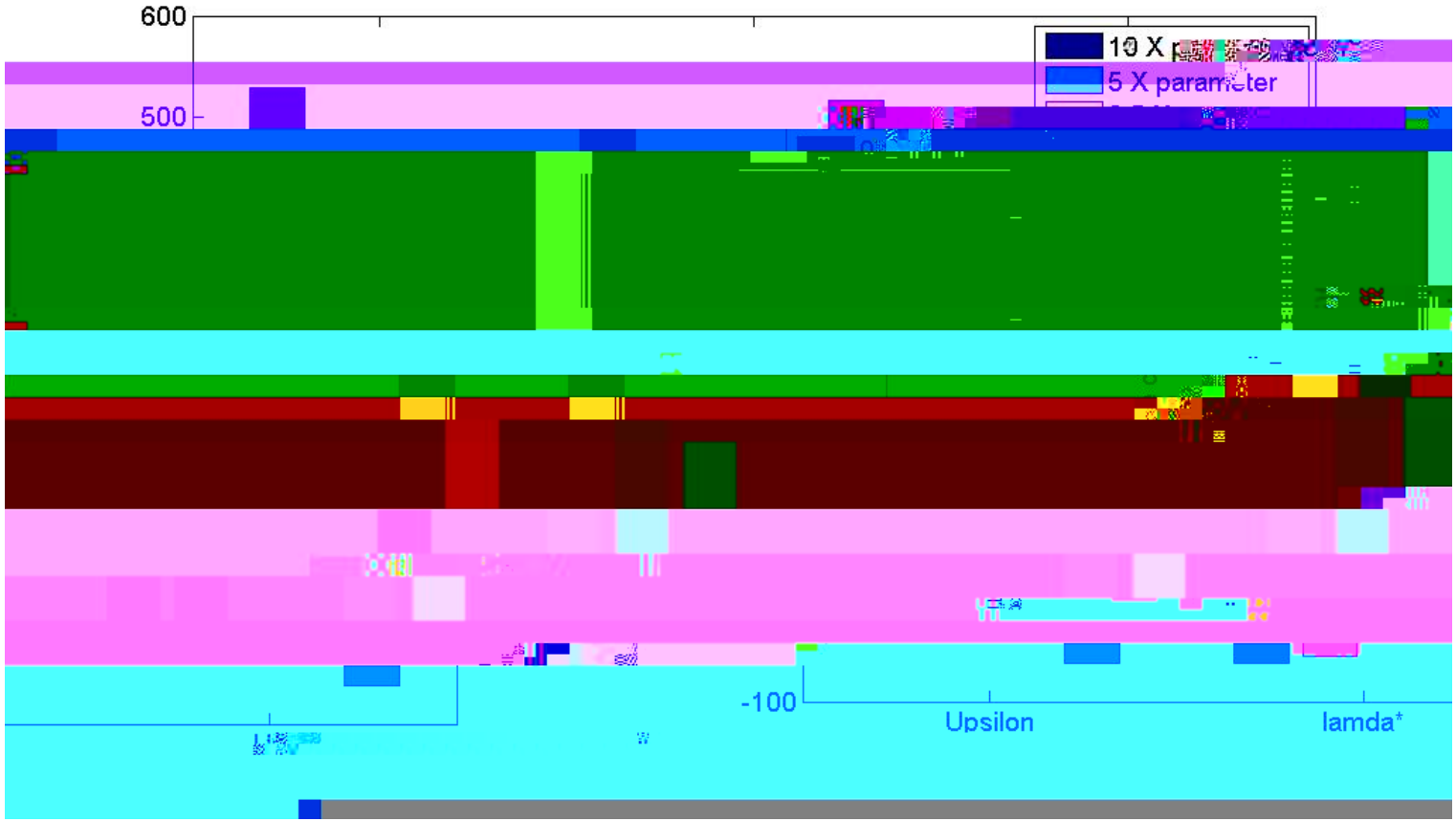

Figure 2.16: Sensitivity Analysis: Parameters with some e ect

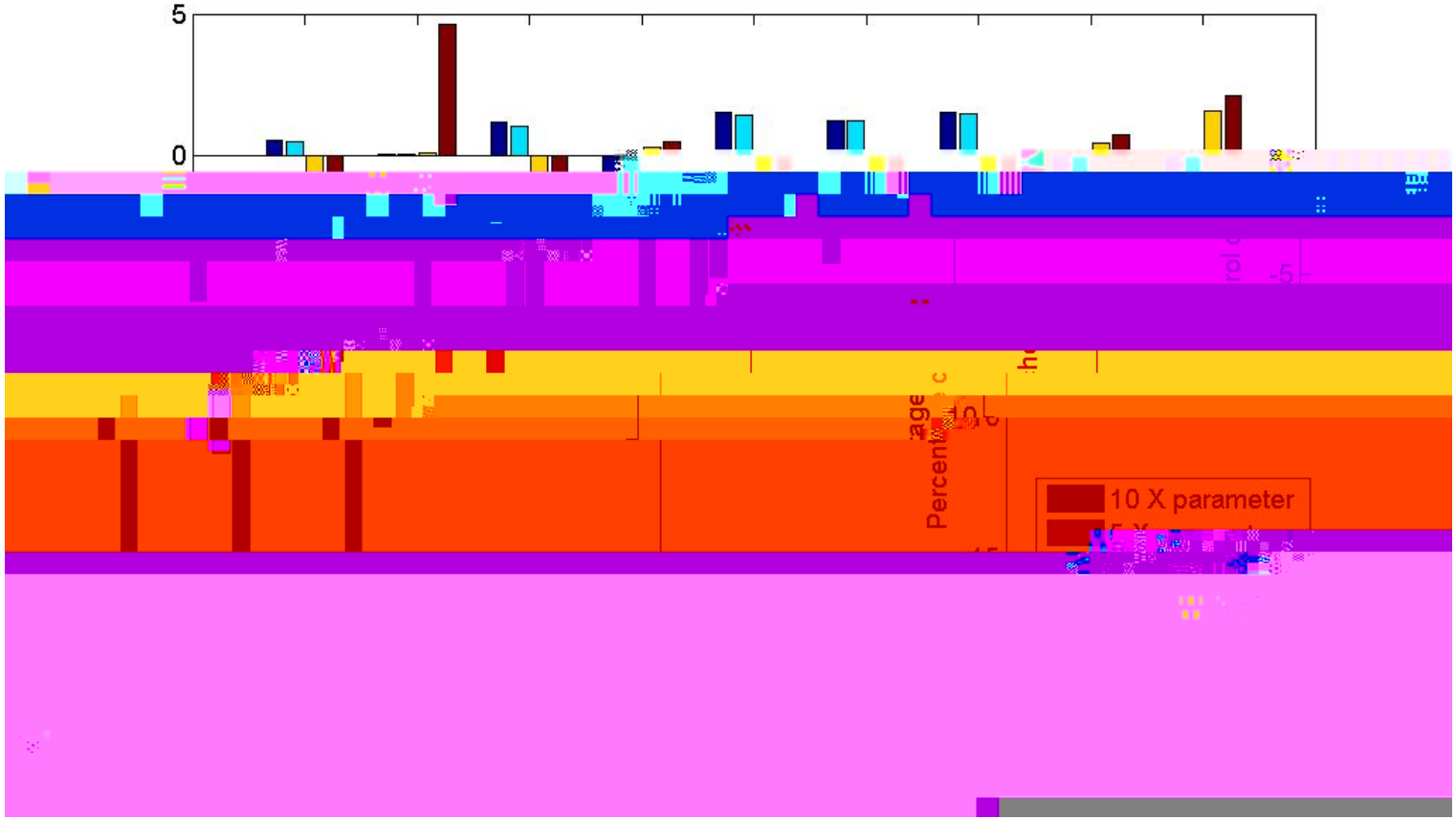

Figure 2.15 shows us that the parameters that have the most a ect on cholesterol are , and U_V. We expect to reduce cholesterol if it is increased since it is the rate of breakdown of cholesterol. Increasing U_V also logically increases cholesterol since if the concentration of extracellular lipoproteins 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 $_{rr}$, see gure 2.17, has such a negligible a ect. 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 signi cantly lower. As expected if we decrease the rate of *de novo* receptor production $_s$, see gure 2.16, the cholesterol is signi cantly 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 a ect the intracellular cholesterol concentration signi cantly. Figures 2.16 and 2.17 show what we would expect, increasing the unbinding rates $_L$; $_V$ reduces the intracellular cholesterol concentration. Increasing the relative binding rate of VLDL particles $_V$ increased intracellular cholesterol concentration.

Initially it seems surprising that the rate of breakdown 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 gure 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 uid 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 uctuation 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 di cult and involve a fair amount of ad-hoc analysis.

We begin by identifying relatively small and large parameters. We take $= \frac{1}{W} = 0{:}1$, unfortunately our non-dimensionalised rate of VLDL breakdown 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 and to account for the fact that is of the same order we also express it as a multiple of .

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

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

$$\frac{d(R_{F0} + \ R_{F1} + O(\ ^2))}{dT} = \ _{rr} \ (R_{I0} + \ R_{I1} + O(\ ^2))$$
$$(m(L_{E0} + \ L_{E1} + O(\ ^2))(R_{F0} + \ R_{F1} + O(\ ^2))$$
$$m \ _L(L_{B0} + \ L_{B1} + O(\ ^2))$$
$$+ \ \frac{m \ _L(L_{B0} + \ L_{B1} + O(\ ^2))(R_{F0} + \ R_{F1} + O(\ ^2))}{1 \quad (R_{F0} + \ R_{F1} + O(\ ^2))})$$
$$r(n \ _V(R_{F0} + \ R_{F1} + O(\ ^2))(V_{E0} + \ V_{E1} + O(\ ^2))$$
$$n \ _V(V_{B0} + \ V_{B1} + O(\ ^2))$$
$$+ \ \frac{n \ _V(V_{B0} + \ V_{B1} + O(\ ^2))(R_{F0} + \ R_{F1} + O(\ ^2))}{1 \quad (R_{F0} + \ R_{F1} + O(\ ^2))}); \tag{3.9}$$

$$\frac{d(C_0 + \ C_1 + O(\ ^2))}{dT} = (\ !_L R_L^{chol}(L_{I0} + \ L_{I1} + O(\ ^2)) + \ !_V r R_V^{chol}(V_{I0} + \ V_{I1} + O(\ ^2))$$
$$((C_0 + \ C_1 + O(\ ^2)) \quad 1) = 0: \tag{3.10}$$

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

$$\frac{dL_{E0}}{}$$

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 $\hat{\Gamma}_E \quad L_{E0}$, $\hat{\Gamma}_B \quad L_{B0}$ 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, = 0:8. Using initial values 2.36 but with extracellular LDL, $\hat{\Gamma}_E$ Tf 10.599 1.79582L2.va-

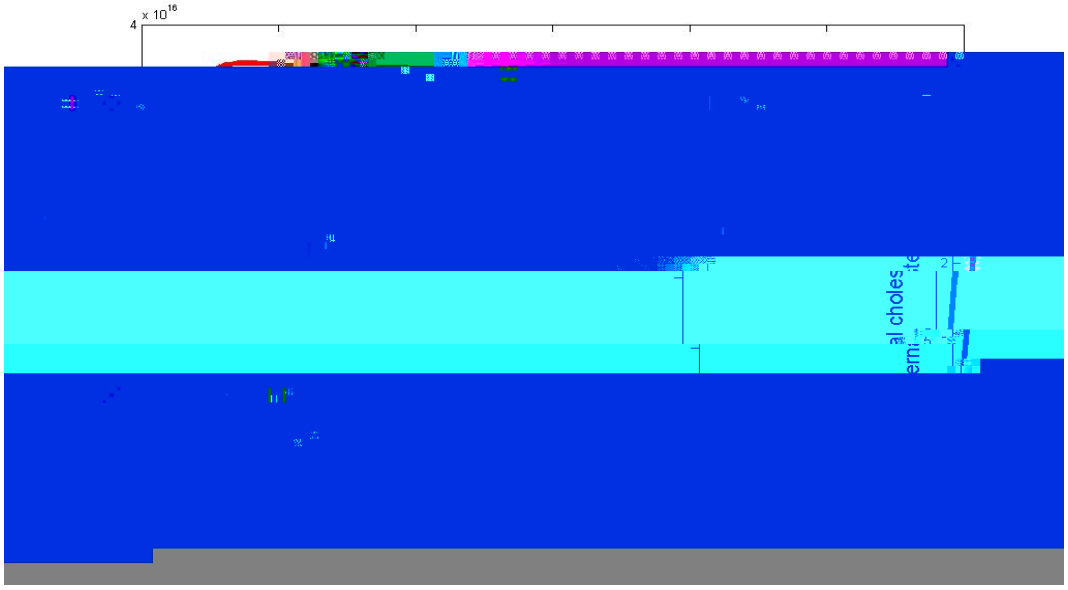

Figure 3.2: The change in intracellular cholesterol over a period of 6h using the reduced system 3.11-3.20. Parameter values are those stated in Table 2.2 with r=1, = 0:8. Using initial values 2.36 but with extracellular LDL, $\hat{I}_E$ = 1.

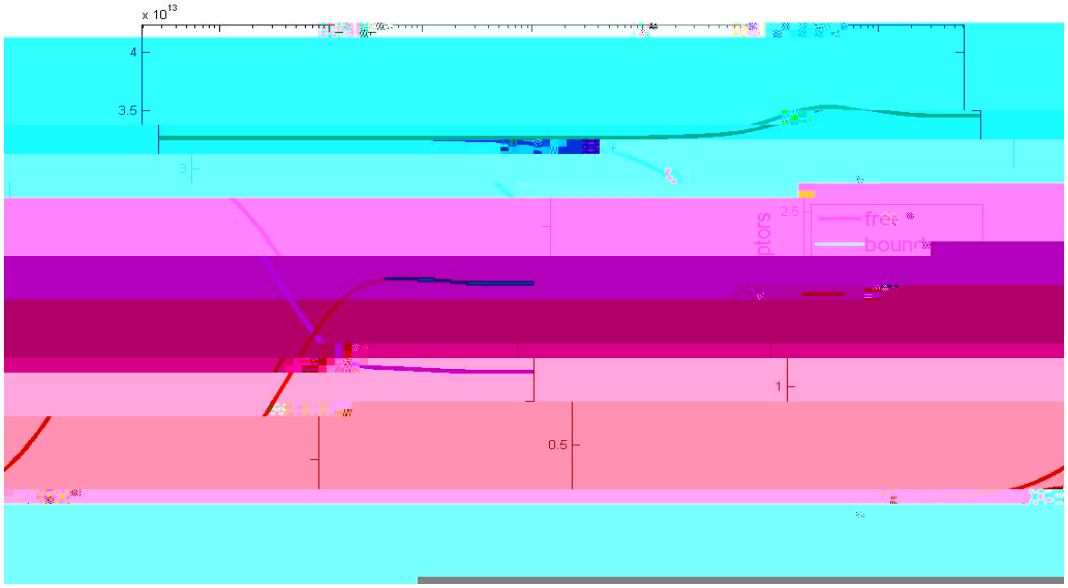

Figure 3.3: The change in time of free, bound, internalised and total receptor concentrations using the reduced system 3.11-3.20. Parameter values are those stated in Table 2.2 with r=1, = 0:8. Using initial values 2.36 but with extracellular LDL, $\hat{I}_E$ = 1.

Comparing the graphs of lipoprotein concentrations gures 3.1 and 2.4, the reduced

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 signi cant 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

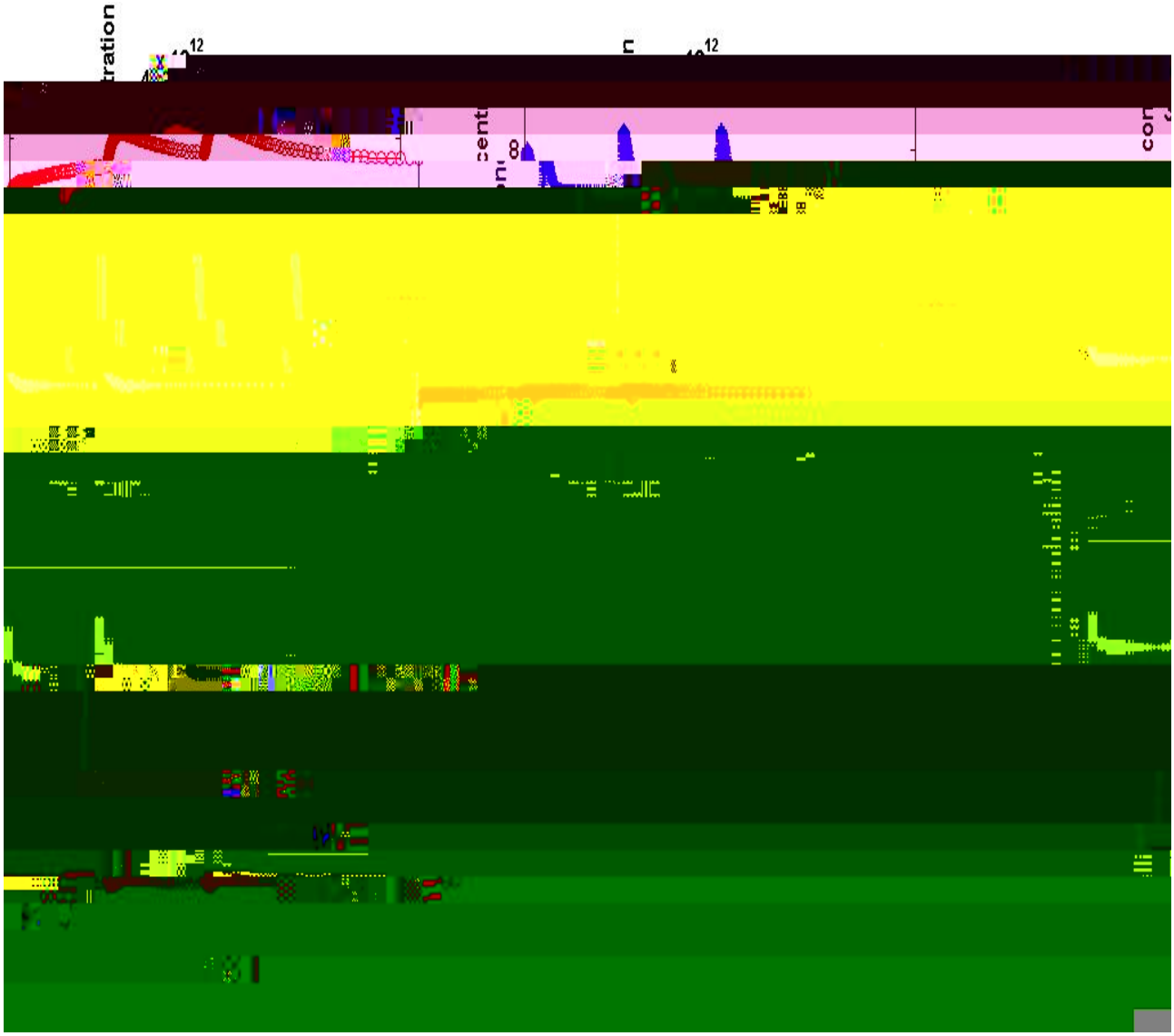

Figure 4.1: The change in extracellular, bound and internalised VLDL and LDL concentrations over a period of 24h with an injection of extracellular VLDL at 0h, 6h and 12h. Parameter values are those stated in Table 2.2 with r=1, = 0:8. Using initial values 2.36 but with extracellular LDL, $\hat{I}_E$ = 1.

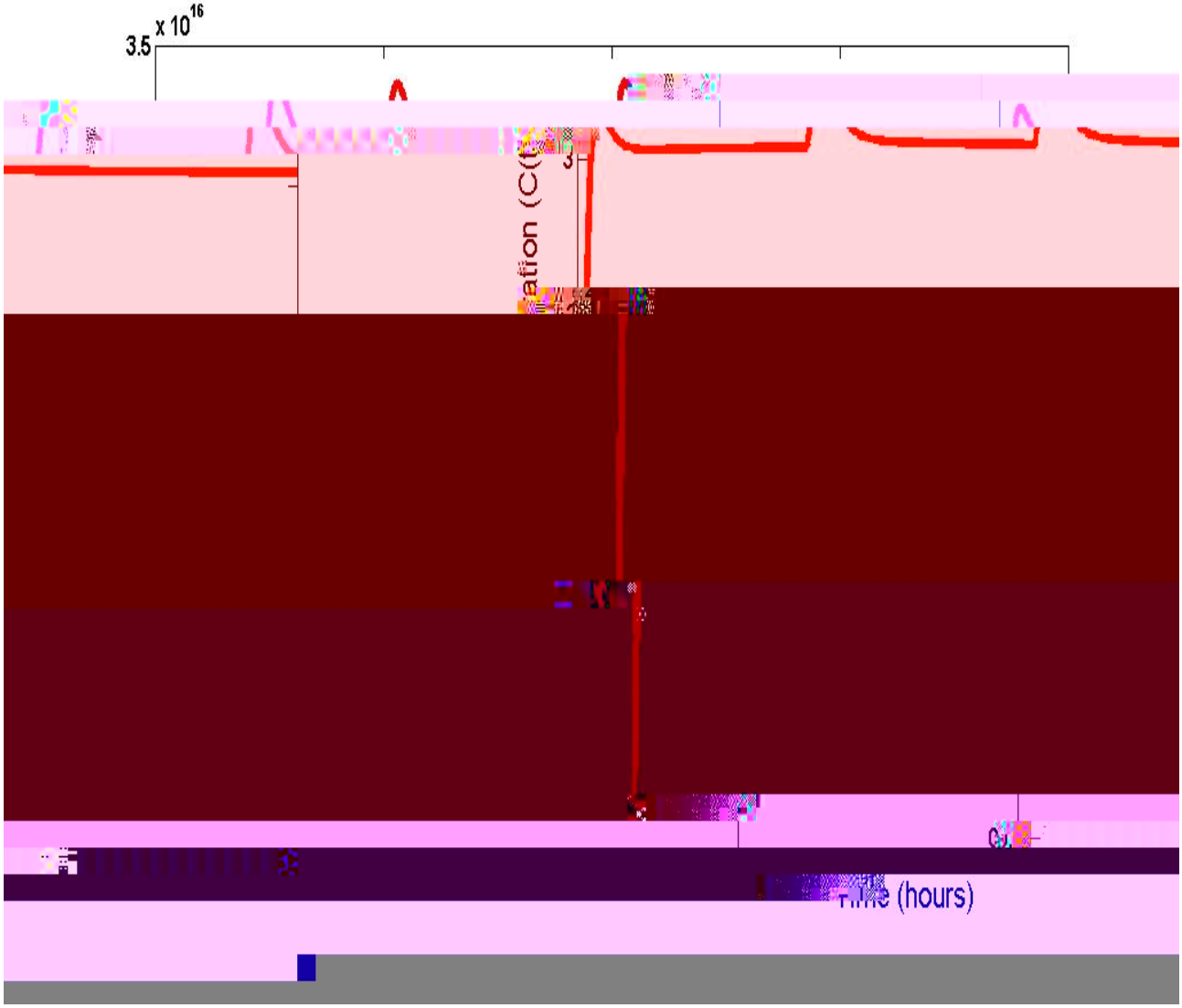

Figure 4.2: The change in intracellular cholesterol over a period over 24h with an injection of extracellular VLDL at 0h, 6h and 12h. Parameter values are those stated in Table 2.2 with r=1, = 0:8. Using initial values 2.36 but with extracellular LDL, $\hat{I}_E$ = 1.

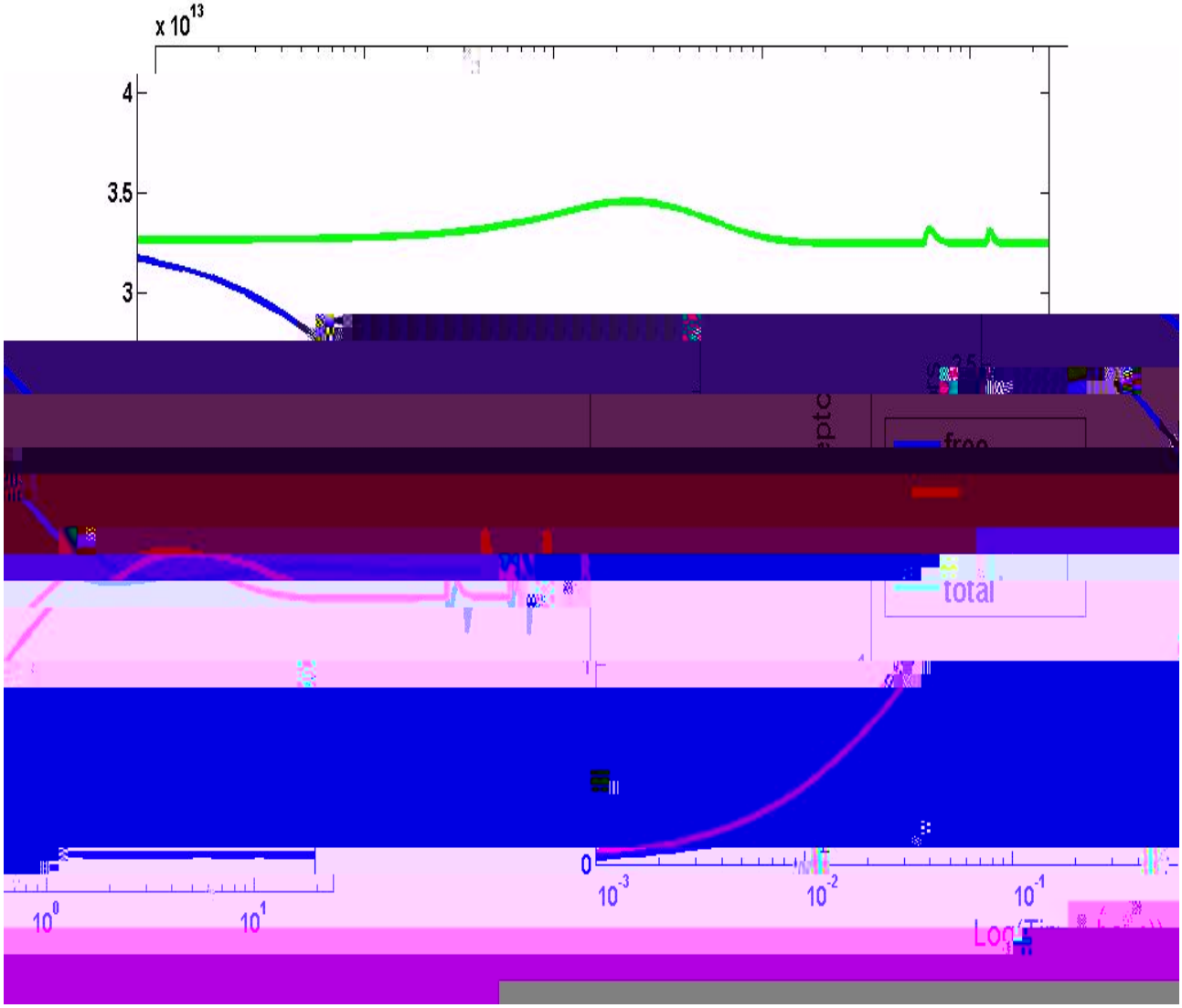

Figure 4.3: The change in time of free, bound, internalised and total receptor concentrations over 24h with an injection of extracellular VLDL at 0h,6h and 12h. Parameter values are those stated in Table 2.2 with r=1, = 0:8. Using initial values 2.36 but with extracellular LDL, $\hat{I}_E$ = 1.

4.2 Discussion

The actual behaviour of VLDL secretion is more uid. It only varies slightly around a

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 gure 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 brie y increase but quickly settles back towards the steady state.

There are other e ects the model is incapable of capturing, for example the second meal a ect. From the rst 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 a ect due to the constant release of extracellular VLDL

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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