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M.J. Tindall, J.A.D. Wattis, B.J. O'Malley, L. Pickersgill, K.G. Jackson. A continuum receptor model of hepatic lipoprotein metabolism. *Journal of Theoretical Biology*, 2009, 257 (3), pp.371. 10.1016/j.jtbi.2008.11.016 . hal-00554536

HAL Id: hal-00554536

<https://hal.science/hal-00554536>

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Author's Accepted Manuscript

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PII: S0022-5193(08)00605-X
DOI: doi:10.1016/j.jtbi.2008.11.016
Reference: YJTBI5370

To appear in: *Journal of Theoretical Biology*

Received date: 4 April 2008
Revised date: 17 November 2008
Accepted date: 17 November 2008

Cite this article as: M.J. Tindall, J.A.D. Wattis, B.J. O'Malley, L. Pickersgill and K.G. Jackson, A continuum receptor model of hepatic lipoprotein metabolism, *Journal of Theoretical Biology* (2008), doi:[10.1016/j.jtbi.2008.11.016](https://doi.org/10.1016/j.jtbi.2008.11.016)

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Article Submitted to Journal of theoretical Biology

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A Continuum Receptor Model of Hepatic Lipoprotein Metabolism

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Abstract

A mathematical model describing the uptake of low density lipoprotein (LDL) and very low density lipoprotein (VLDL) particles by a single hepatocyte cell is formulated and solved. The model includes a description of the dynamic change in receptor density on the surface of the cell due to the binding and dissociation of the lipoprotein particles, the subsequent internalisation of bound particles, receptors and unbound receptors, the recycling of receptors to the cell surface, cholesterol dependent *de novo* receptor formation by the cell and the effect that particle uptake has on the cell's overall cholesterol content. The effect that blocking access to LDL receptors by VLDL, or internalisation of VLDL particles containing different amounts of apolipoprotein E (we will refer to these particles as VLDL-2 and VLDL-3) has on LDL uptake is explored. By comparison with experimental data we find that measures of cell cholesterol content are important in differentiating between the mechanisms by which VLDL is thought to inhibit LDL uptake. We extend our work to show that in the presence of both types of VLDL particle (VLDL-2 and VLDL-3), measuring relative LDL uptake does not allow differentiation between the results of blocking and internalisation of each VLDL particle to be made. Instead by considering the intracellular cholesterol content it is found that internalisation of VLDL-2 and VLDL-3 leads to the highest intracellular cholesterol concentration. A sensitivity analysis of the model reveals that binding, unbinding and internalisation rates, the fraction of receptors recycled and the rate at which the cholesterol dependent free receptors are created by the cell have important implications for the overall uptake dynamics of either VLDL or LDL particles and subsequent intracellular cholesterol concentration.

Keywords: Low density lipoprotein (LDL); Very low density lipoprotein (VLDL); Triglyceride rich lipoprotein (TRL); apolipoprotein E (ApoE); cholesterol; endocytosis.

1. Introduction

Elevated levels of plasma low density lipoprotein cholesterol (LDL-C) are a well established risk factor for coronary heart disease (CHD). The plasma LDL-C level is in part controlled by the rate at which low density lipoprotein (LDL) particles are removed from the circulation. The majority of LDL particles are removed from the bloodstream by the liver, in a process known as receptor mediated endocytosis. The dynamics of receptor-mediated endocytosis of LDL were described in a series of classical experiments by Goldstein and Brown; the first step in the endocytotic process involves binding of an LDL particle to specific hepatic LDL receptors (LDLR), which reside in specialised regions of the liver cell surface, known as clathrin-coated pits (Goldstein and Brown, 1974; Sato and Takano, 1995; Huff, 2003).

The LDL particle-receptor interaction is mediated by apolipoprotein B 100 (apoB 100) present on the surface of LDL particles. Upon binding to the LDLR, the lipoprotein particles and receptors are internalised into the cell, forming intracellular vesicles known as endosomes. Upon fusion of endosomes with lysosomes the LDL particles are degraded, releasing their constituent parts (cholesterol, fatty acids and amino acids). The LDLRs are either recycled to the cell surface, prior to lysosomal fusion, or are degraded. The expression of the LDLR at the cell surface is tightly coupled to the intracellular cholesterol concentration, i.e. LDLR expression is downregulated in response to an increase in cholesterol concentration, and upregulated in response to a decrease in cholesterol concentration. This negative feedback mechanism renders hepatocytes able to maintain cholesterol homeostasis independent of external LDL concentrations.

More recently *in vitro* experiments by Jackson et al. (2006) have demonstrated that the rate of LDL uptake is influenced by the presence of other plasma lipoproteins, namely the very low density lipoproteins (VLDL). VLDL particles isolated from individuals following consumption of meals of different fatty acid composition, compete with varying effectiveness with LDL for hepatic uptake. A comparable reduction in LDL uptake is

observed in the presence of VLDL isolated following polyunsaturated (PUFA), or monounsaturated (MUFA) fat-rich meals, but the effect is more pronounced with VLDL isolated following a saturated (SAFA) fat-rich meal. This difference is attributed to the variation in apolipoprotein E (apoE) content between VLDL isolated following a PUFA or MUFA-rich meal which carry an average of two molecules of apoE per particle (and hence we refer to them as VLDL-2), and VLDL particles isolated following a SAFA-rich meal which carry an average of three apoE molecules per particle (and hence are referred to as VLDL-3).

Mathematical modelling of lipoprotein metabolism to date has focused on modelling various aspects of particle uptake. Goldstein et al. (1984) modelled the distribution of free LDLR in coated pits on the surface of human fibroblasts. The receptors were assumed to move by diffusion and the work considered the mean time taken for receptors randomly introduced on the cell surface to reach a pit. Results showed that the rate of receptor diffusion was important in affecting free receptor density when pit recycling was introduced. Solana-Arellano et al. (1998) undertook similar work, but their model differed by considering the effect that radial advection of free LDLR has upon receptor distribution within the pits.

Harwood and Pellarin (1997) modelled the binding and internalisation of LDL particles and the subsequent recycling of receptors to the cell surface. Using experimental data they obtained good estimates on the change in free, bound and internalised LDLR and on the rates of LDL binding and unbinding from the cell surface, bound particle-receptor complex internalisation and subsequent breakdown of the complex and recycling of the receptors. The work of Knoblauch et al. (2000) and August et al. (2007) has modelled the interactions between various lipoprotein particles (LDL, VLDL, intermediate density lipoprotein (IDL), high density lipoprotein (HDL)) and their endocytosis. Knoblauch et al. (2000) used their model to show that decreasing LDLR causes an exponential increase in LDL levels, whilst other particle levels remain relatively constant. August et al. (2007) used their model of VLDL, IDL, LDL and the LDLR to calculate the various steady-

states of the metabolic system and how perturbations from these affect the intracellular cholesterol concentration. Their model exhibits low and high intracellular cholesterol levels (bistability) which the system can oscillate between when oscillations in VLDL production are introduced.

Given the importance of hepatic LDL uptake by endocytosis in governing plasma LDL-C levels, understanding the mechanisms by which LDL uptake is compromised is of importance to human health. In this paper we formulate a model to study the impact of the interplay between extracellular VLDL and LDL concentrations on cellular cholesterol homeostasis. An improved understanding of this area of biology is considered important in identifying mechanisms by which diet might influence plasma LDL-C levels, and hence, in the longer term, CHD risk. The work presented here focuses on the effect that VLDL particles, of different apoE content (i.e. VLDL-2 and VLDL-3) have on hepatic LDL uptake, and the amount of lipoprotein derived cholesterol entering the cultured hepatocytes in culture.

Our model includes a detailed description of LDL and VLDL particle binding, internalisation of receptor-lipoprotein complexes and the subsequent intracellular release of cholesterol. The model also includes a description of receptor recycling following internalisation, internalisation of free receptors (i.e. in the absence of lipoprotein binding), and de novo receptor production (a rate which is dependent upon the intracellular cholesterol concentration). We explore the influence that each of these processes has on the rate at which LDL particles are taken up by hepatocytes.

We use the model to address two hypotheses regarding the mechanism by which VLDL particles enriched in different amounts of apoE reduce the uptake of LDL by HepG2 cells.

- The first hypothesis states that VLDL particles reduce the uptake of LDL by HepG2 cells by blocking access to the LDLR. This can occur by either VLDL particles entering the pit, binding to LDLR, but not being internalised or VLDL particles being present in the pit, but not binding to LDLR.

- The second hypothesis states that VLDL particles enter the pits, bind to LDLR via apoE and are internalised by the cell.

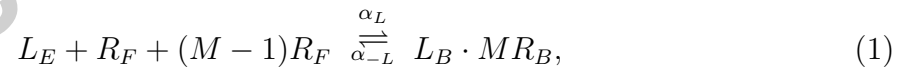
In addition we consider the influence of particle size and apoE content of VLDL on the hepatic uptake of LDL.

VLDL and LDL contain a comparable amount of cholesterol per particle, but the former are larger particles, and hence cover more LDLR than do LDL. Consequently, binding of one VLDL particle prevents several LDL particles from accessing LDLR. It is these issues and hypotheses which will be addressed in the work which follows.

2. Model Formulation

We formulate a model to consider the effects that combinations of LDL, VLDL-2 and VLDL-3 binding and internalisation have on the relative uptake of LDL by a hepatocyte and the cholesterol content of the cell. All of the processes we will consider are summarised in Figure 1.

The internalisation of LDL and VLDL particles along with the respective receptors involved on the surface of the cell is governed by the following reactions. In the case of LDL binding to the cell surface, the reaction sequence is as follows.



where one free LDL particle (L_E) binds to one free receptor (R_F) and in doing so occludes $(M - 1)$ other free receptors at a rate α_L . Receptor occlusion prevents them from participating in further binding events with any other lipoprotein particles. In doing so a bound receptor complex ($L_B \cdot MR_B$) is formed composed of a bound LDL particle (L_B) and M ‘bound’ receptors (R_B) (one actually bound and the remaining $(M - 1)$ occluded). The bound particles can unbind from the surface at a rate α_{-L} .

The bound LDL-receptor complex is digested by the hepatocyte at a rate β_L according

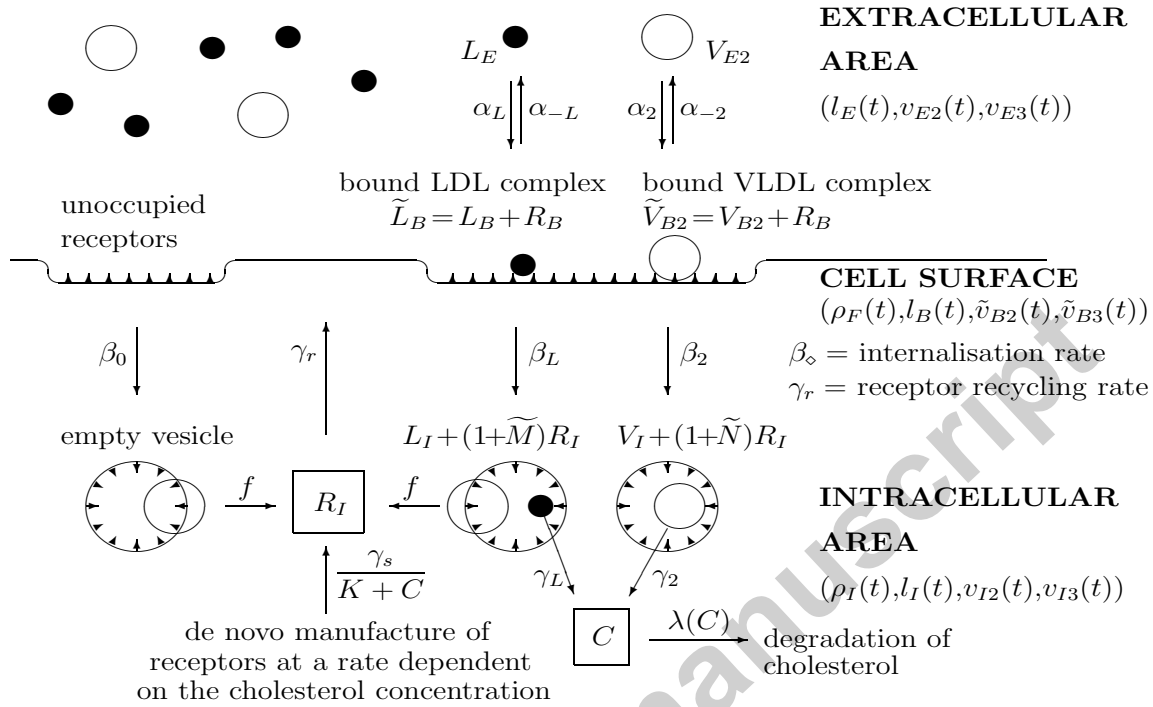
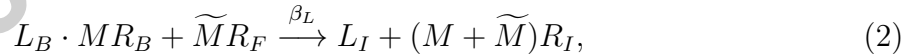


Figure 1: An illustrative summary of the processes considered in the uptake of LDL and VLDL particles by a single hepatocyte cell. Here filled circles in the extracellular region represent LDL particles whilst the larger unfilled circles represent VLDL particles. See the text for a detailed explanation of each process.

to the following reaction



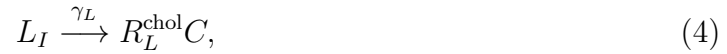
where R_I represents receptors inside the cell and L_I is an internalised LDL particle. Here \tilde{M} represents those free (unbound or occluded) receptors which are enclosed with the digested pit, but not attached or occluded by any bound lipoprotein particle. The quantity \tilde{M} will depend on the number of free receptors in a manner to be proposed in equation (25).

In addition, empty pits of P receptors are internalised at a different rate β_0 if all receptors

are unoccupied, a process which we denote

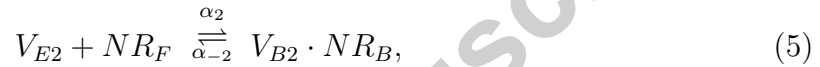


The cholesterol within internalised LDL particles is released into the cell. We write



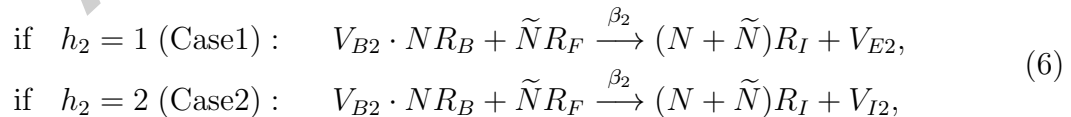
for some rate constant γ_L , R_L^{chol} being the number of cholesterol molecules contained in an LDL particle and C the intracellular cholesterol concentration.

The binding and internalisation of VLDL-2 and VLDL-3 particles follow similar reactions to those of LDL, namely

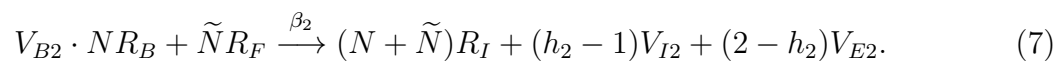


where V_{E2} is the concentration of extracellular VLDL-2 particles which bind to one free receptor and occlude $(N - 1)$ of them at a rate α_2 , which results in the bound complex $V_{B2} \cdot NR_B$. VLDL-2 particles can unbind from the cell surface at a rate α_{-2} .

The bound VLDL-2 complex ($V_{B2} \cdot NR_B$) is internalised at a rate β_2 , via one of two potential mechanisms. In the first case the bound VLDL-2 particle is not internalised, it remains bound to the surface of the cell, but the receptors associated with it are internalised. In the second case the VLDL-2 particle is taken into the cell along with the receptors, the internalised VLDL-2 particle is denoted V_{I2} , and in each case we assume that a number \tilde{N} of empty receptors are internalised as part of the pit. Using the parameter h_2 to denote the difference between these two cases we formulate them as follows



which can be combined into one equation as



The released cholesterol is given by



Following a similar formulation as VLDL-2, in the case of VLDL-3 we have

$$V_{E3} + QR_F \xrightleftharpoons[\alpha_3]{\alpha_3} V_{B3} \cdot QR_B, \quad (9)$$

where V_{E3} is an extracellular VLDL-3 particle which binds to one free receptor and occludes $(Q - 1)$ of them. The bound complex is internalised at a rate β_3 together with \tilde{Q} unbound and accessible receptors which happen to be in the same pit, yielding internalised receptors and VLDL-3 such that

$$\begin{aligned} \text{if } h_3 = 1 : & \quad V_{B3} \cdot QR_B + \tilde{Q}R_F \xrightarrow{\beta_3} (Q + \tilde{Q})R_I + V_{E3}, \\ \text{if } h_3 = 2 : & \quad V_{B3} \cdot QR_B + \tilde{Q}R_F \xrightarrow{\beta_3} (Q + \tilde{Q})R_I + V_{I3}, \end{aligned} \quad (10)$$

or

$$V_{B3} \cdot QR_B + \tilde{Q}R_F \xrightarrow{\beta_3} (Q + \tilde{Q})R_I + (h_3 - 1)V_{I3} + (2 - h_3)V_{E3}. \quad (11)$$

Here h_3 denotes the difference between the two cases (detailed above) for VLDL-3 particles. When both LDL and VLDL are present a number of different binding scenarios are possible with respect to receptor availability. For simplicity, and as a first approximation, we have assumed that receptors are either vacant or occupied. If they are vacant then they can be occupied by a bound LDL or VLDL particle. As demonstrated later, this approximation leads to a good agreement between model and experiments. More complex scenarios could be considered, for example, vacant receptors near a bound VLDL particle could be accessible to an LDL particle but not to a VLDL particle. Modelling this scenario would be vastly more complex and the ensuing analysis less straightforward.

Cholesterol is released according to



Finally, a fraction f of internalised receptors are returned to the surface of the cell. To model this, we postulate the existence of a store of receptors inside the cell, R_I . On breaking down the internalised vesicle, a fraction f of receptors are sent to the store; we assume that the remainder of receptors are degraded and lost from the system.

To replenish this loss, receptors are manufactured *de novo* at a rate inversely proportional of the cell's cholesterol content, which we model by $\gamma_S/(K + C)$, where γ_S is a production rate constant and K determines the strength of the nonlinearity (small K giving a strongly C -dependent production rate, and larger K giving an almost constant rate). From the store receptors are transported back to the surface of the cell, at some rate γ_r .

The inclusion of the parameters h_2 and h_3 allow us to assess the effect of internalisation of each type of VLDL particle ($h_2 = h_3 = 2$) or if $h_2 = h_3 = 1$, the effect of binding and blocking with no internalisation of VLDL-2 or VLDL-3. We may also assess the effect of one particle type being internalised and the other not, e.g. ($h_2 = 1, h_3 = 2$) is equivalent to VLDL-2 particles binding and blocking and VLDL-3 particles binding and being internalised.

2.1. Mathematical formulation

Applying the law of mass action to reactions (1)–(12) yields the following ordinary differential equations (ODEs). LDL binding is governed by

$$W \frac{dl_E}{dt} = -\alpha_L \rho_F l_E + \alpha_{-L} l_B, \quad (13)$$

$$\frac{dl_B}{dt} = \alpha_L \rho_F l_E - \alpha_{-L} l_B - \beta_L l_B, \quad (14)$$

$$\frac{dl_I}{dt} = \beta_L l_B - \gamma_L l_I, \quad (15)$$

where $l_E = [L_E]$, $\rho_F = [R_F]$, $l_B = [L_B \cdot MR_F]$ and $l_I = [L_I]$. Here W is the volume ratio of the culture medium containing l_e to the volume of hepatic cells, where the former is much larger than the latter which leads to $W \gg 1$. In the case of Jackson et al. (2006) $W \sim 1.5 \times 10^3$.

We utilise similar variables to represent the respective concentrations of free, bound and

internalised VLDL particles. With respect to VLDL-2

$$W \frac{dv_{E2}}{dt} = -\alpha_2 \rho_F v_{E2} + \alpha_{-2} v_{B2} + \beta_2 (2 - h_2) v_{B2}, \quad (16)$$

$$\frac{dv_{B2}}{dt} = \alpha_2 \rho_F v_{E2} - \alpha_{-2} v_{B2} - \beta_2 v_{B2}, \quad (17)$$

$$\frac{dv_{I2}}{dt} = (h_2 - 1)(\beta_2 v_{B2} - \gamma_2 v_{I2}), \quad (18)$$

and for VLDL-3

$$W \frac{dv_{E3}}{dt} = -\alpha_3 \rho_F v_{E3} + \alpha_{-3} v_{B3} + \beta_3 (2 - h_3) v_{B3}, \quad (19)$$

$$\frac{dv_{B3}}{dt} = \alpha_3 \rho_F v_{E3} - \alpha_{-3} v_{B3} - \beta_3 v_{B3}, \quad (20)$$

$$\frac{dv_{I3}}{dt} = (h_3 - 1)(\beta_3 v_{B3} - \gamma_3 v_{I3}), \quad (21)$$

where $v_{B2} = [V_{B2} \cdot NR_F]$ and $v_{B3} = [V_{B3} \cdot QR_F]$, respectively.

The change in free receptor density is governed by

$$\begin{aligned} \frac{d\rho_F}{dt} = & -M\alpha_L l_E \rho_F - \alpha_2 N v_{E2} \rho_F - \alpha_3 Q v_{E3} \rho_F + M\alpha_{-L} l_B + \alpha_{-2} N v_{B2} + \alpha_{-3} Q v_{B3} \\ & - P\beta_0 \rho_F^P - \beta_L \widetilde{M} l_B - \beta_2 \widetilde{N} v_{B2} - \beta_3 \widetilde{Q} v_{B3} + \gamma_r \rho_I, \end{aligned} \quad (22)$$

where the term involving γ_r represents the transport of free receptors onto the cell surface from the cell's internal receptor store R_I .

The quantities \widetilde{M} , \widetilde{N} and \widetilde{Q} are dependent on the average occupancy of the surface receptors. If there is an abundance of LDL and VLDL, then average occupancy will be high, and ρ_F will be low and few free receptors will be internalised in each pit, leading to small values of \widetilde{M} , \widetilde{N} and \widetilde{Q} . When ρ_F is large, the number of free receptors internalised with each pit will be much larger, as ρ_F increases, the number of free receptors should grow faster than ρ_F . If we assume that the total number of receptors is approximately constant over shorter timescales, such as that of pit internalisation, then the number of receptors on the surface of the cell is

$$\rho_B + \rho_F = M l_B + N v_{B2} + Q v_{B3} + \rho_F \approx \rho_K. \quad (23)$$

Upon internalisation of one pit, we assume a fraction of all receptors are internalised, this is given by

$$\rho_B + \rho_F = Ml_B + Nv_{B2} + Qv_{B3} + \widetilde{M}(\rho_F)l_B + \widetilde{N}(\rho_F)v_{B2} + \widetilde{Q}(\rho_F)v_{B3}. \quad (24)$$

We assume that the contribution of free receptors internalised with each particle is divided equitably between all bound particles, that is, $\widetilde{M}(\rho_F) = MT(\rho_F)$, $\widetilde{N}(\rho_F) = NT(\rho_F)$, and $\widetilde{Q}(\rho_F) = QT(\rho_F)$, where $T(\rho_F)$ is the total density of free receptors. Taking the difference of the previous two equations, and rearranging, we find

$$T(\rho_F) = \frac{\rho_F}{Ml_B + Nv_{B2} + Qv_{B3}} = \frac{\rho_F}{\rho_K - \rho_F} \quad (25)$$

Since the number of internalised receptors is small, a suitable value for ρ_K is ρ_0 .

The cell is also able to produce a constant rate of free receptors, that is, at a background ‘de novo’ production. In addition, when cholesterol levels are low the cell also responds by producing more receptors which are sent to the cell surface. When the second process dominates the former, we assume that the first process can be accommodated by adjusting the parameters in the model for the second.

The concentration of receptors inside the cell’s store is denoted by ρ_I and is governed by

$$\frac{d\rho_I}{dt} = Pf\beta_0\rho_F^P + f\beta_L(M + \widetilde{M})l_B + f\beta_2(N + \widetilde{N})v_{B2} + f\beta_3(Q + \widetilde{Q})v_{B3} - \gamma_r\rho_I + \frac{\gamma_S}{K+C}. \quad (26)$$

This represents a combination of those receptors in the cell’s internal store and those still in internalised vesicles where they are part of a complex with l_I . Once released from the complex, we assume that only a fraction, f , of these go to the store. New receptors manufactured by the cell are also assumed to be deposited in the receptor store, via two mechanisms: passive production at some slow rate, and by an active process which depends on the cell’s internal cholesterol level (the γ_S term). Receptors are assumed to leave the store at a constant rate γ_r . Ideally this rate is dependent upon the number of receptors in the store, which is kept low by having rapid transport from the store to the surface. The rate at which receptors are added to the store is more complex, since this

comes from two sources: the imperfect recycling of internalised receptors and the de novo construction of receptors at a rate which depends on the cell's internal cholesterol level. Assuming γ_r to be a constant is thus a good first approximation to a series of complex processes which are subject to significant modulations in time.

The rate of internal receptor formation is not dependent upon the number of free receptors present - these receptors are simply internalised passively as the clathrin pit containing the LDL receptors undergoes endocytosis and the LDL receptors and their bound lipoproteins are internalised by the cell.

Intracellular cholesterol concentrations are governed by

$$\frac{dC}{dt} = (\gamma_L R_L^{chol} l_I + \gamma_2 R_{V2}^{chol} v_{I2} + \gamma_3 R_{V3}^{chol} v_{I3}) - \lambda(C - C_e), \quad (27)$$

where R_L^{chol} , R_{V2}^{chol} and R_{V3}^{chol} represent the quantity of cholesterol per LDL, VLDL-2 and VLDL-3 particle respectively, λ describes either the rate of cholesterol synthesis or utilisation, depending on whether intracellular cholesterol levels are below or above C_e , the maximum intracellular cholesterol concentration of an hepatocyte.

We further note that the concentration of bound receptors on the surface of the cell is defined by

$$\rho_B = M l_B + N v_{B2} + Q v_{B3}. \quad (28)$$

The initial conditions for the above equations are defined by

$$\begin{aligned} l_E(0) = l_0, \quad l_B(0) = 0, \quad l_I(0) = 0, \quad v_{E2}(0) = v_{02}, \quad v_{B2}(0) = 0, \\ v_{I2}(0) = 0, \quad v_{E3}(0) = v_{03}, \quad v_{B3}(0) = 0, \quad v_{I3}(0) = 0, \\ \rho_F(0) = \rho_0, \quad \rho_I(0) = 0 \quad \text{and} \quad C(0) = 0.7C_e. \end{aligned} \quad (29)$$

We note with the condition on $C(0)$ that we have assumed the cell has an initial intracellular cholesterol concentration of 70% of its maximal value, denoted C_e . This assumption reproduces the initial conditions of Jackson et al. (2006) where the HepG2 cells were incubated in lipid poor medium to upregulate LDLR levels.

2.2. Non-dimensionalisation

The governing equations are non-dimensionalised according to the following re-scalings

$$\begin{aligned}
 t &= \frac{\hat{t}}{\alpha_L \rho_0}, & l_E &= l_0 \hat{l}_E, & l_B &= l_0 \hat{l}_B, & l_I &= l_0 \hat{l}_I, & v_{E2} &= v_{02} \hat{v}_{E2}, \\
 v_{B2} &= v_{02} \hat{v}_{B2}, & v_{I2} &= v_{02} \hat{v}_{I2}, & v_{E3} &= v_{02} \hat{v}_{E3}, & v_{B3} &= v_{02} \hat{v}_{B3}, \\
 v_{I3} &= v_{02} \hat{v}_{I3}, & \rho_F &= \rho_0 \hat{\rho}_F, & \rho_I &= \rho_0 \hat{\rho}_I, & \text{and } C &= C_e \hat{c}.
 \end{aligned}$$

Applying this non-dimensionalisation to equations (13)-(27) yields the following set of equations. We note that we have replaced M , N and Q by m , n and q .

$$W \frac{d\hat{l}_E}{d\hat{t}} = -\hat{l}_E \hat{\rho}_F + \psi_L \hat{l}_B, \quad (30)$$

$$\frac{d\hat{l}_B}{d\hat{t}} = \hat{\rho}_F \hat{l}_E - \psi_L \hat{l}_B - \chi_L \hat{l}_B, \quad (31)$$

$$\frac{d\hat{l}_I}{d\hat{t}} = \chi_L \hat{l}_B - \omega_L \hat{l}_I, \quad (32)$$

$$W \frac{d\hat{v}_{E2}}{d\hat{t}} = -\phi_2 \hat{\rho}_F \hat{v}_{E2} + \psi_2 \hat{v}_{B2} + (2 - h_2) \chi_2 \hat{v}_{B2}, \quad (33)$$

$$\frac{d\hat{v}_{B2}}{d\hat{t}} = \phi_2 \hat{\rho}_F \hat{v}_{E2} - \psi_2 \hat{v}_{B2} - \chi_2 \hat{v}_{B2}, \quad (34)$$

$$\frac{d\hat{v}_{I2}}{d\hat{t}} = (h_2 - 1) (\chi_2 \hat{v}_{B2} - \omega_2 \hat{v}_{I2}), \quad (35)$$

$$W \frac{d\hat{v}_{E3}}{d\hat{t}} = -\phi_3 \hat{\rho}_F \hat{v}_{E3} + \psi_3 \hat{v}_{B3} + (2 - h_3) \chi_3 \hat{v}_{B3}, \quad (36)$$

$$\frac{d\hat{v}_{B3}}{d\hat{t}} = \phi_3 \hat{\rho}_F \hat{v}_{E3} - \psi_3 \hat{v}_{B3} - \chi_3 \hat{v}_{B3}, \quad (37)$$

$$\frac{d\hat{v}_{I3}}{d\hat{t}} = (h_3 - 1) (\chi_3 \hat{v}_{B3} - \omega_3 \hat{v}_{I3}), \quad (38)$$

$$\begin{aligned} \sigma \frac{d\hat{\rho}_F}{dt} = & \gamma_{rr}\sigma\hat{\rho}_I - \chi_0\hat{\rho}_F^P - \left(m\hat{l}_E\hat{\rho}_F - m\psi_L\hat{l}_B + \frac{m\chi_L\hat{l}_B\hat{\rho}_F}{1-\hat{\rho}_F} \right) \\ & - r \left(n\phi_2\hat{\rho}_F\hat{v}_{E2} - n\psi_2\hat{v}_{B2} + \frac{n\chi_2\hat{v}_{B2}\hat{\rho}_F}{1-\hat{\rho}_F} \right) \\ & - r \left(q\phi_3\hat{\rho}_F\hat{v}_{E3} - q\psi_3\hat{v}_{B3} + \frac{q\chi_3\hat{v}_{B3}\hat{\rho}_F}{1-\hat{\rho}_F} \right), \end{aligned} \quad (39)$$

$$\begin{aligned} \sigma \frac{d\hat{\rho}_I}{dt} = & \frac{\gamma_s^*\sigma}{\bar{K} + \hat{c}} + \chi_0 f \hat{\rho}_F^P + f \left(1 + \frac{\hat{\rho}_F}{1-\hat{\rho}_F} \right) \left(m\chi_L\hat{l}_B + n\chi_2 r \hat{v}_{B2} + q\chi_3 r \hat{v}_{B3} \right) \\ & - \sigma \gamma_{rr} \hat{\rho}_I, \end{aligned} \quad (40)$$

$$\frac{d\hat{c}}{dt} = \Upsilon \left(\omega_L R_L^{\text{chol}} \hat{l}_I + \omega_2 r R_{V2}^{\text{chol}} \hat{v}_{I2} + \omega_3 r R_{V3}^{\text{chol}} \hat{v}_{I3} \right) - \lambda^* (\hat{c} - 1), \quad (41)$$

where the non-dimensional binding and unbinding rates are

$$\phi_2 = \frac{\alpha_2}{\alpha_L}, \quad \phi_3 = \frac{\alpha_3}{\alpha_L}, \quad \psi_L = \frac{\alpha_{-L}}{\alpha_L \rho_0}, \quad \psi_2 = \frac{\alpha_{-2}}{\alpha_L \rho_0}, \quad \psi_3 = \frac{\alpha_{-3}}{\alpha_L \rho_0}, \quad (42)$$

and the internalisation rates are

$$\chi_0 = \frac{\beta_0 P \rho_0^{P-1}}{\alpha_L l_0}, \quad \chi_L = \frac{\beta_L}{\alpha_L \rho_0}, \quad \chi_2 = \frac{\beta_2}{\alpha_L \rho_0}, \quad \chi_3 = \frac{\beta_3}{\alpha_L \rho_0}. \quad (43)$$

The parameters

$$\omega_L = \frac{\gamma_L}{\alpha_L \rho_0}, \quad \omega_2 = \frac{\gamma_2}{\alpha_L \rho_0}, \quad \omega_3 = \frac{\gamma_3}{\alpha_L \rho_0}, \quad \lambda^* = \frac{\lambda}{\alpha_L \rho_0}, \quad (44)$$

describe the rate of LDL, VLDL-2, VLDL-3 and cholesterol breakdown respectively and

$$\gamma_s^* = \frac{\gamma_s}{C_e \alpha_L \rho_0^2}, \quad \bar{K} = \frac{K}{C_e}, \quad \gamma_{rr} = \frac{\gamma_r}{\alpha_L \rho_0} \quad (45)$$

are related to receptor production and recycling, and the remainder give the relative sizes of LDL/VLDL particles, pits and relative concentrations

$$r = \frac{v_{02}}{l_0}, \quad \sigma = \frac{\rho_0}{l_0}, \quad \Upsilon = \frac{1}{R_L^{\text{chol}}}. \quad (46)$$

Finally, the initial conditions for the non-dimensional system of equations are

$$\begin{aligned} \hat{l}_E(0) = 1, \quad \hat{l}_B(0) = 0, \quad \hat{l}_I(0) = 0, \quad \hat{v}_{E2}(0) \leq 1, \quad \hat{v}_{B2}(0) = 0, \\ \hat{v}_{I2}(0) = 0, \quad \hat{v}_{E3}(0) = v_0, \quad \hat{v}_{B3}(0) = 0, \quad \hat{v}_{I3}(0) = 0, \\ \hat{\rho}_F(0) = 1, \quad \hat{\rho}_I(0) = 0 \quad \text{and} \quad \hat{C}(0) = 0.7 \end{aligned} \quad (47)$$

Since v_{02} can take a range of values (between 2.5 and 20 $\mu\text{g}/\text{ml}$ - see Table 1), we use the upper value to non-dimensionalise the system. The initial data for $\hat{v}_{E2}(0)$ will thus be in the range $0 \leq \hat{v}_{E2}(0) \leq 1$. For notational convenience in the work which follows the hats will be dropped.

2.3. Parameter values

Our model formulation requires knowledge of the initial number of free receptors for a hepatocyte, the average number of receptors covered by LDL and VLDL containing two and three molecules of apoE and their respective rates of binding, internalisation and rate of return of receptors to the cell surface as well as the internal receptor recycling and production dynamics of the cell.

We have been able to obtain a large number of parameter values from published experimental literature as listed in Table 1. Details on how these values have been obtained from current literature sources are given in Appendix A. Where possible we have utilised values from experiments using HepG2 cells. The non-dimensional parameters are listed in Table 2.

3. Model solutions

3.1. Solution method

The system of governing equations (30) – (41), with the respective initial conditions (equation (47)), and parameter values detailed in Table 2 constitute a stiff system of ODEs. The stiff ODE solver ode15s (Gear's method) in Matlab was used to solve the respective equations.

Parameter	Description	Value
P	Number of pits per cell.	180
	Number of receptors per cell.	35,000
	Number of receptors per pit (only 70% in pits).	180
	Radius of an LDL particle.	10 nm
	Radius of a VLDL particle	15–40 nm
M	Typical radius of a pit.	100 nm
	Number of receptors covered by a bound LDL.	1
N	Number of receptors covered by a bound VLDL-2.	2
Q	Number of receptors covered by a bound VLDL-3.	3.6
$\bar{M}, \bar{N}, \bar{Q}$	Maximum number of additional free receptors internalised.	M, N, Q .
α_L	Rate of LDL binding to free receptors.	6.66×10^{-17} ml/molecules/s
α_2, α_3	Rate of VLDL-2, VLDL-3 binding to free receptors.	$14.0\alpha_L, 24\alpha_L$
β_L	Rate of LDL internalisation.	2.7×10^{-3} /s
β_2, β_3	Rate of VLDL-2 and VLDL-3 internalisation.	β_L
β_0	Rate of unbound receptor internalisation.	0
α_{-L}	Rate of LDL unbinding from receptors.	5.9×10^{-4} /s
α_{-2}, α_{-3}	Rate of VLDL-2, VLDL-3 unbinding from receptors.	$0.5\alpha_{-L}, 0.33\alpha_{-L}$
γ_L	Rate of conversion of internalised LDL to cholesterol.	$\sim 1/300$ /s
γ_2, γ_3	Rate of receptor recycling from bound VLDL.	γ_L
γ_r	Rate of receptor recycling.	0.01/s
f	Fraction of receptors recycled.	0.9
K	Constant for receptor production.	$2C_e$
γ_s	Rate of free receptor production by cell.	1.8×10^{30} molecules/ml/s
λ	Rate of breakdown of cholesterol.	3.3×10^{-3} /s
R_L^{chol}	Average cholesterol content per LDL particle.	3400
R_{V-2}^{chol}	Average cholesterol content per VLDL-2 particle.	3100
R_{V-3}^{chol}	Average cholesterol content per VLDL-3 particle.	3900
ρ_0	Initial concentration of free receptors.	2.5×10^4 /cell
C_e	Maximum cholesterol content of a hepatocyte.	2.65×10^{19} molecules/ml
ρ_0	Initial concentration of free receptors.	2.17×10^{10} receptors/ml
l_0	Initial concentration of LDL particles (mass/vol).	10 μ g/ml
l_0	Initial concentration of LDL particles (no./vol).	1.17×10^{13} particles/ml
v_{02}/v_{03}	Typical concentration of VLDL particles (mass/vol).	2.5, 10 and 20 μ g/ml.
v_{02}/v_{03}	Initial concentration of VLDL particles (no./vol).	$2.95 \times 10^{12}, 1.17 \times 10^{13}$ and 2.35×10^{13} particles/ml medium.
W	Volume ratio of cell culture medium to cell volume.	1.50×10^3
h_2, h_3	Hypothesis: if $h = 2$, VLDL is internalised if $h = 1$, VLDL blocks and is not internalised.	1,2

Table 1: Dimensional parameter values. Details on the sources used to obtain these values are given in Appendix A.

Parameter	Description	Value
m	Relative size of LDL to pit.	1
n	Relative size of VLDL-2 to LDL.	2
q	Relative size of VLDL-3 to LDL.	3.6
p	Relative size of pit to LDL.	180
r	Ratio of VLDL concentration to LDL concentration.	0.25, 1, 2
Υ	Ratio of initial LDL concentration to initial cholesterol.	2.94×10^{-4}
$\chi_L = \chi_2 = \chi_3$	Relative rate of LDL/VLDL internalisation.	1.28
ϕ_2	Relative rate of VLDL-2 binding to free receptors.	14
ϕ_3	Relative rate of VLDL-3 binding to free receptors.	24
ψ_L	Rate of LDL unbinding from receptors.	0.279
ψ_2	Rate of VLDL-2 unbinding from receptors.	0.140
ψ_3	Rate of VLDL-3 unbinding from receptors.	0.093
χ_0	Rate of internalisation of free receptors.	0
$\omega_L = \omega_2 = \omega_3$	Rate of digestion of internalised LDL/VLDL to cholesterol.	1.56
σ	Ratio of receptor- to LDL-concentrations (rescaled).	2.74
γ_{rr}	Rate of receptor recycling.	30
f	Fraction of receptors recycled.	0.9
γ_s^*	Rate of de novo receptor production.	0.54
\bar{K}	Constant regulating cholesterol-dependence of receptor production.	2.0
λ^*	Rate of cholesterol breakdown.	1.56
W	Volume ratio of cell culture medium to cell volume.	1.50×10^3
$h_{2,3}$	Hypothesis (allowing VLDL blockage or internalisation).	1,2

Table 2: Non-dimensional parameter values.

3.2. Results

Before proceeding to a full analysis of our model we compare model solutions to those already in the literature for the case when only LDL is present. Harwood and Pellarin (1997) formulated a model similar to ours which considered LDL uptake, but did not include the effects of cholesterol dependent free receptor formation by the cell. Instead they assumed the cellular receptor concentration remained constant. Likewise the process of the internalised LDL-receptor complex breakdown differs slightly from that presented here; we assume a certain number of receptors are degraded ($f \neq 1$). In comparing the change in free, bound and internalised receptors in the presence of LDL only as shown in Figure 2, we have found our model results agree well with those reported in Harwood and Pellarin (1997). Both models show an exponential decrease in the number of free receptors, as the concentration of extracellular LDL is increased, and a subsequent similar increase in the number of bound receptors. At concentrations of LDL greater than $10\mu\text{g/ml}$, 75%

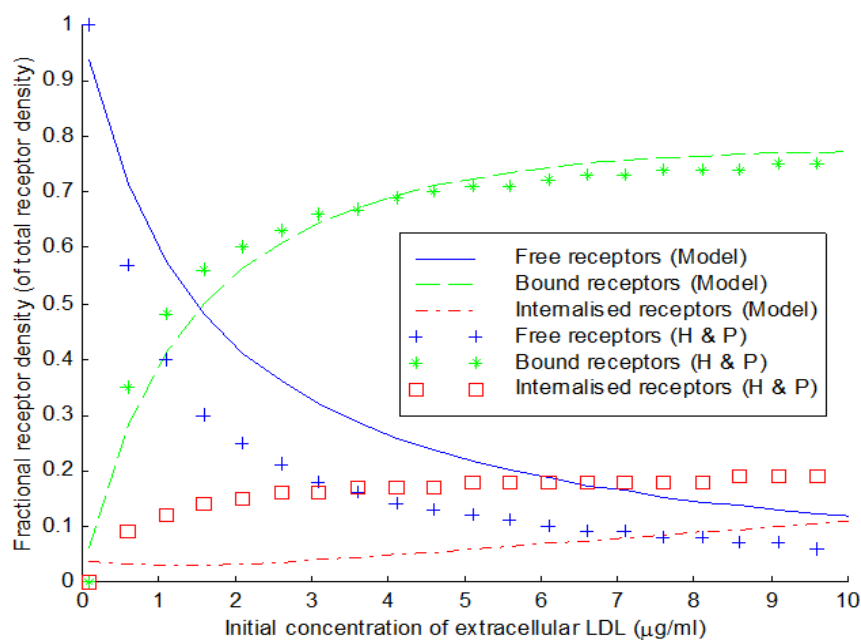


Figure 2: Comparison of our model (LDL only) with that of Harwood and Pellarin (1997). The subtle difference between the two models with respect to internalised and free receptors is a result of differing receptor recycling kinetics (see text for further details). We have utilised the rates for our model as shown in Table 2.

of the receptors are bound and approximately 10% are free. The number of internalised receptors is uniformly small, lying between 10% and 20% for the whole range of LDL concentrations. We now proceed to investigate our model behaviour in the presence of both LDL and each VLDL particle type.

Our model has a total of twelve variable outputs which can be used to test the effect that parameter variations have on the model output. An example of the dynamic change in LDL, VLDL, receptor and cholesterol concentrations is shown in Figures 3 and 4, respectively. Note the difference in scale between the extracellular, bound and internalised concentrations for each particle type. It is noted that the initial change in concentration of extracellular LDL, VLDL particles and free receptors is quite rapid (Figure 3). This is a consequence of rapid binding of particles to a receptor surface initially devoid of any

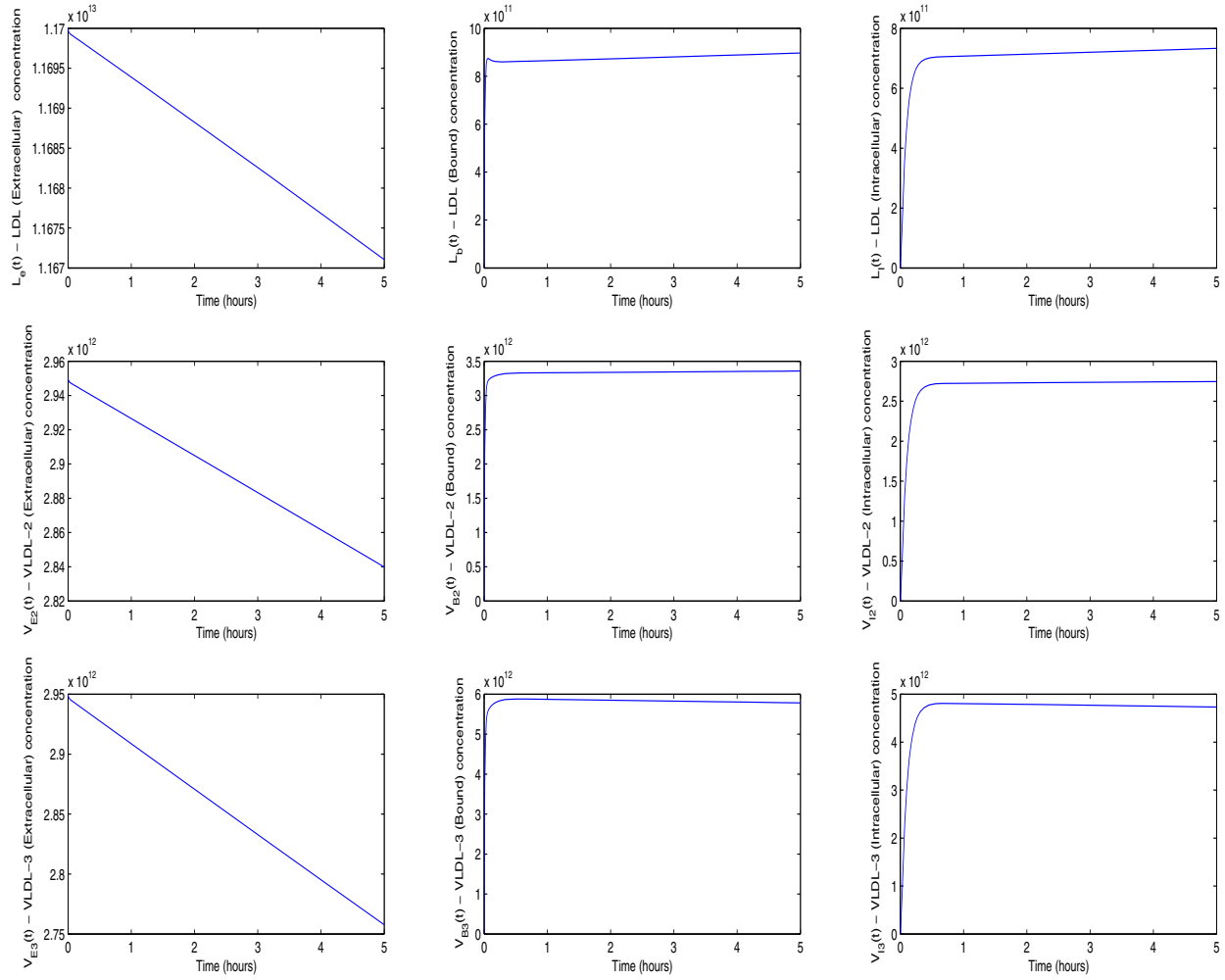


Figure 3: The change in extracellular, bound and internalised LDL and VLDL concentrations over a period of five hours. Parameter values are those stated in Table 2 with both VLDL-2 and VLDL-3 particles present and being internalised by the cell ($h_1 = 2 = h_2$) and $r = 1$. Units of concentration are particles/ml.

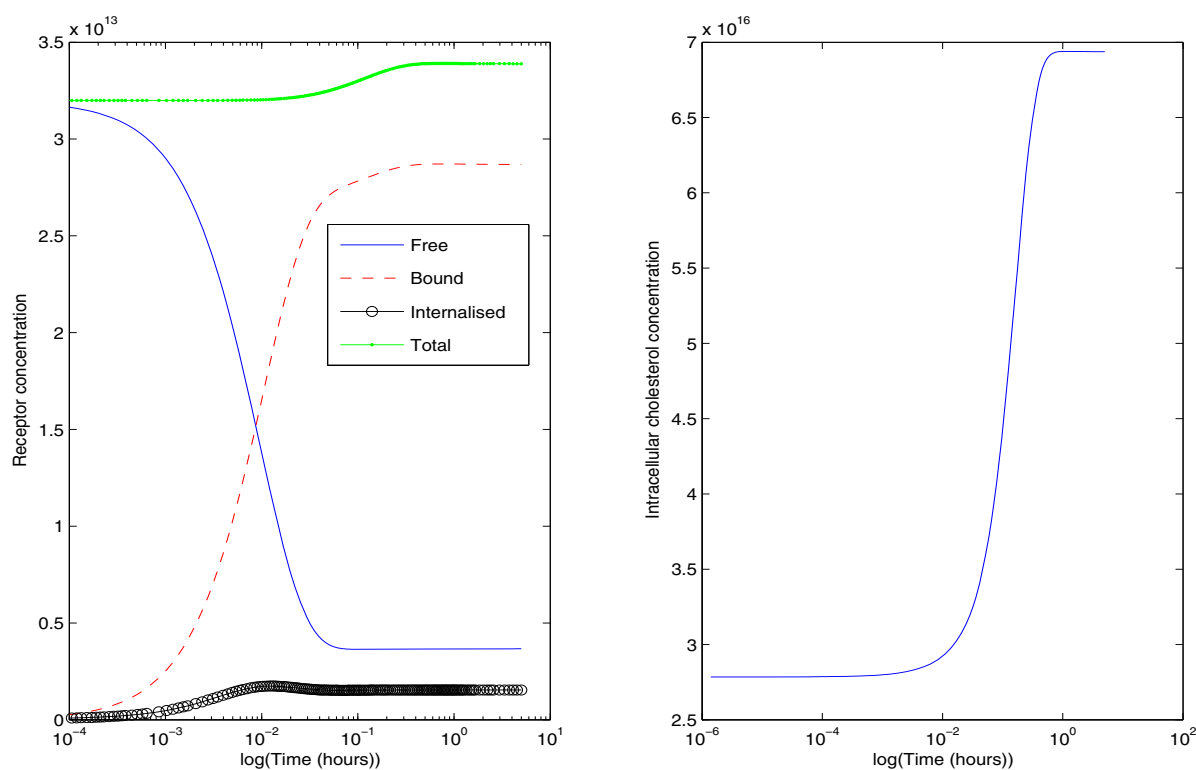


Figure 4: The change in time of (a) free, bound, internalised and total receptor concentrations and (b) intracellular cholesterol concentration. Parameter values are those stated in Table 2 with both VLDL-2 and VLDL-3 particles present and being internalised by the cell ($h_1 = 2 = h_2$). Here receptors are measured in particles/ml and the cholesterol concentration in molecules/ml.

bound particles. Competition between particles for free receptors after this initial binding leads to the observed slow decrease in extracellular particle concentrations.

However, assessing the effect of each phenomena included in our model over such a wide range of output variables is infeasible. For simplicity, as well as to be able to compare our results with the experimental data of Jackson et al. (2006), we consider two measures: the relative LDL uptake; and the relative cholesterol uptake by the cell.

We define the *absolute* LDL uptake by choosing a time, T , at which we measure the

concentration $l_E(t)$, and define

$$\text{LDLU}_{abs}(v_{02}, v_{03}) = [l_E(T) - l_E(0)]_{v_{E2}(0)=v_{02}, v_{E3}(0)=v_{03}}, \quad (48)$$

where the subscripts denote the initial data imposed on v_{E2} and v_{E3} . The *relative* LDL-uptake is defined as the difference between two simulations of the system: one with some arbitrary initial concentrations of VLDL-2 or VLDL-3, and the other with no VLDL-2 or VLDL-3 present at $t = 0$. Hence

$$\text{LDLU}_{rel} = \text{LDLU}_{abs}(v_{02}, v_{03}) - \text{LDLU}_{abs}(0, 0), \quad (49)$$

where the initial concentration of extracellular LDL, $l_E(0)$, is the same in each experiment.

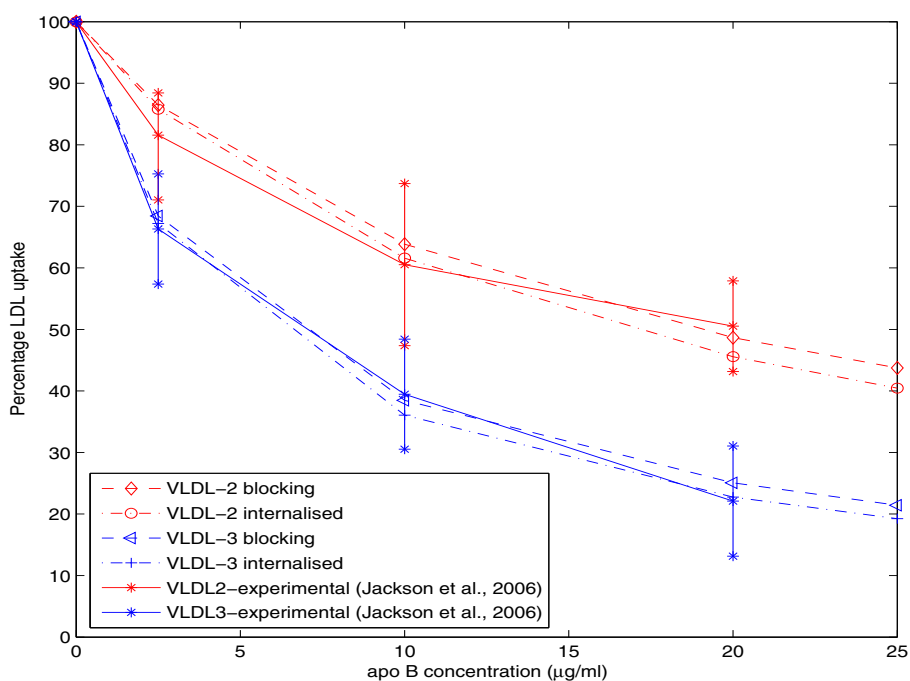
The relative cholesterol level of the cell is defined in a similar way as

$$C_{rel}(T) = C(T)|_{v_{E2}=v_{E3}=v_0}^{h=1,2} - C(T)|_{v_{E2}=v_{E3}=0}. \quad (50)$$

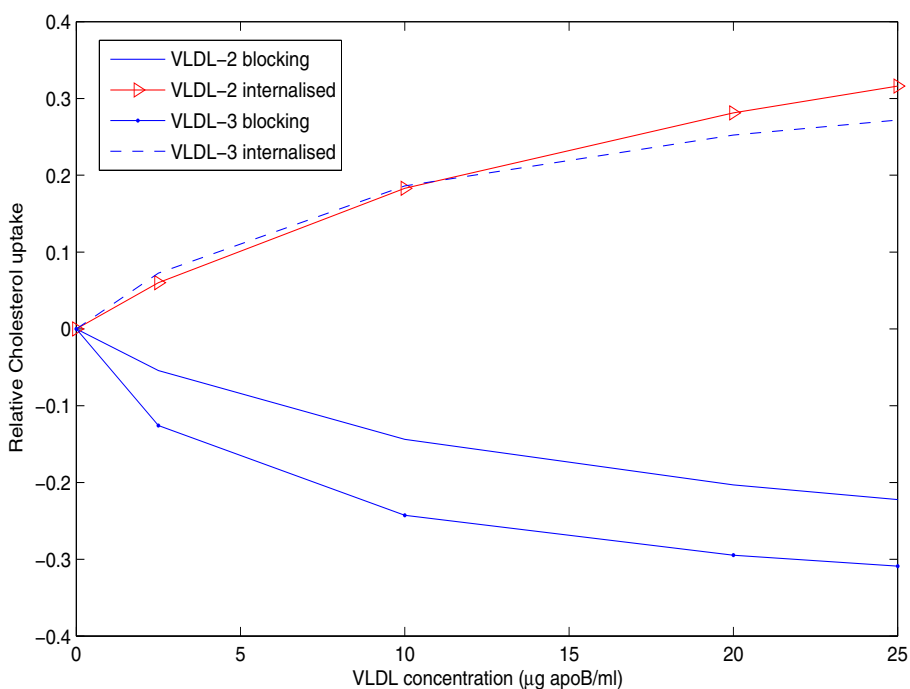
Each of these measures will be used to consider the effect that variations in respective parameters have on the model behaviour in the following sections. We note that $\text{LDLU}_{rel} < 0$ corresponds to less LDL being taken up in the presence of either particle of VLDL type.

Figure 5(a) shows the difference in relative LDL uptake as defined by equation (49) when either VLDL-2 or VLDL-3 are present. The LDL uptake (both experimental and from our model) is expressed as a percentage of LDL uptake when no VLDL is present. This allows us to quantitatively compare our results with those of Jackson et al. (2006), without the need for adjustment of the results due to experimental factors not included in our model, for instance the effect of temperature. The result of Figure 5(a) shows our model results are in good quantitative agreement with experimental data.

Figure 5(a) also demonstrates that it is difficult to discern between LDL uptake in respect of VLDL blocking or internalisation, when either VLDL-2 or VLDL-3 are present. At most a difference in uptake is observed for *either* VLDL-2 or VLDL-3 due to different binding and unbinding rates and to a lesser degree, the size of each particle. However, a comparison of the intracellular cholesterol concentration as shown in Figure 5(b) demonstrates the distinct difference that VLDL blocking and binding have. In each case, internalisation leads



(a)



(b)

Figure 5: (a) Model predictions of the percentage change in relative LDL uptake in the presence of either VLDL-2 or VLDL-3 compared with experimental data from Jackson et al. (2006). Here relative LDL uptake is defined by equation (49) and is expressed as a percentage of that when only LDL is present. (b) The relative difference in cholesterol uptake is defined by equation (50). Both LDL and cholesterol uptake are compared at a time of five hours and parameter values are as detailed in Table 2.

to more cholesterol being taken up by the cell than blocking; not an unexpected result. The cholesterol uptake for VLDL-2 and VLDL-3 is similar due to the small difference in the cholesterol content of each particle. As the number of initial VLDL particles increases so does the cholesterol uptake difference for the internalised particles, albeit marginally. The binding rates of VLDL-2 and VLDL-3, and to some degree size of each particle, has a greater effect on differing between the cholesterol uptake when either VLDL-2 or VLDL-3 blocks LDL uptake.

These results suggest that it is only possible to discern between the different hypotheses proposed by Jackson et al. (2006), and the effects of VLDL blocking and internalisation, when the intracellular cholesterol concentration of the cell is considered. Measuring LDL uptake alone does not allow the effect of VLDL on LDL internalisation to be quantified. When intracellular cholesterol concentration is considered, the hypothesis that VLDL blocking leads to a reduction in cholesterol content of the cell appears to be correct (hypothesis one). This result needs to be considered in the timeframe of the experiment and ultimately *in vivo*; if more time is allowed for internalisation then more LDL will be taken up. LDLR blocking by VLDL merely slows the uptake rate. Our results also show that if hypothesis two is correct then a further increase in intracellular cholesterol, due to the uptake of both LDL and VLDL, should be observed. Without further details on intracellular cholesterol levels in the cell it is difficult to therefore determine which of these hypotheses is fully correct. We note that binding and internalisation of VLDL is so rapid compared to that of LDL that any subsequent reduction in cholesterol uptake due to the presence of VLDL, is not observed. If any blocking by VLDL of LDL occurs when VLDL is internalised, it is small if at all relevant.

3.3. LDL and cholesterol uptake in the presence of VLDL-2 and VLDL-3

The above results lead us to ask how LDL and cholesterol uptake are affected when both VLDL particle types are present in the medium with LDL. Given the possibility that either VLDL-2 or VLDL-3 may block cell receptors or be internalised we have four cases

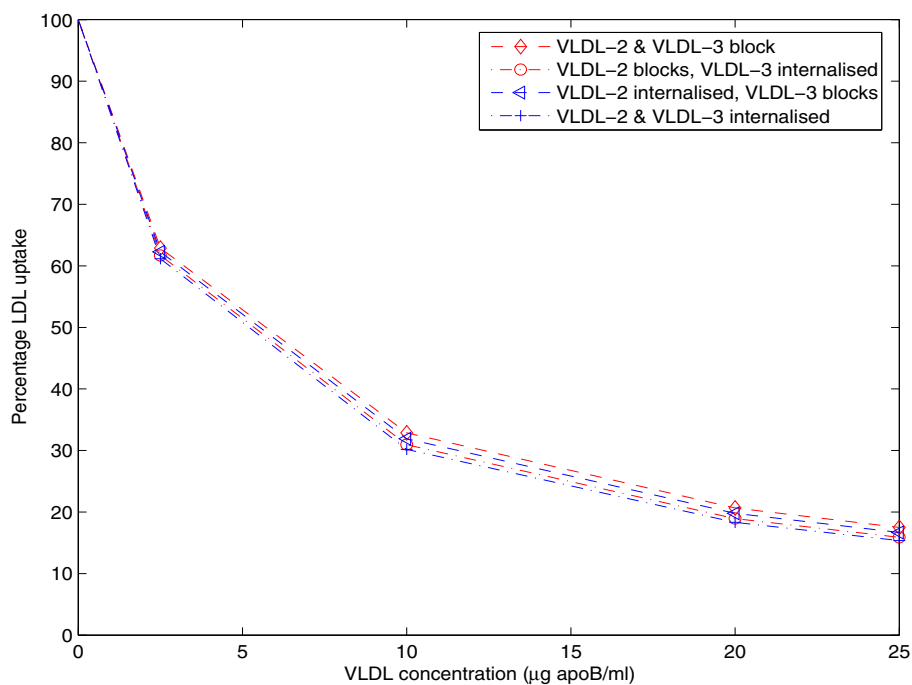
to consider: (i) VLDL-2 and VLDL-3 both block receptors; (ii) VLDL-2 blocks and VLDL-3 is internalised; (iii) VLDL-2 is internalised and VLDL-3 blocks; and (iv) VLDL-2 and VLDL-3 are both internalised. Our model predicts that there is no appreciable difference in LDL uptake for each of the four cases as shown in Figure 6(a). A marginal difference in relative cholesterol uptake by the cell is, however, predicted as shown in Figure 6(b). Again this difference in cholesterol uptake is a result of the variation in cholesterol content between each VLDL particle type.

3.4. Sensitivity analysis

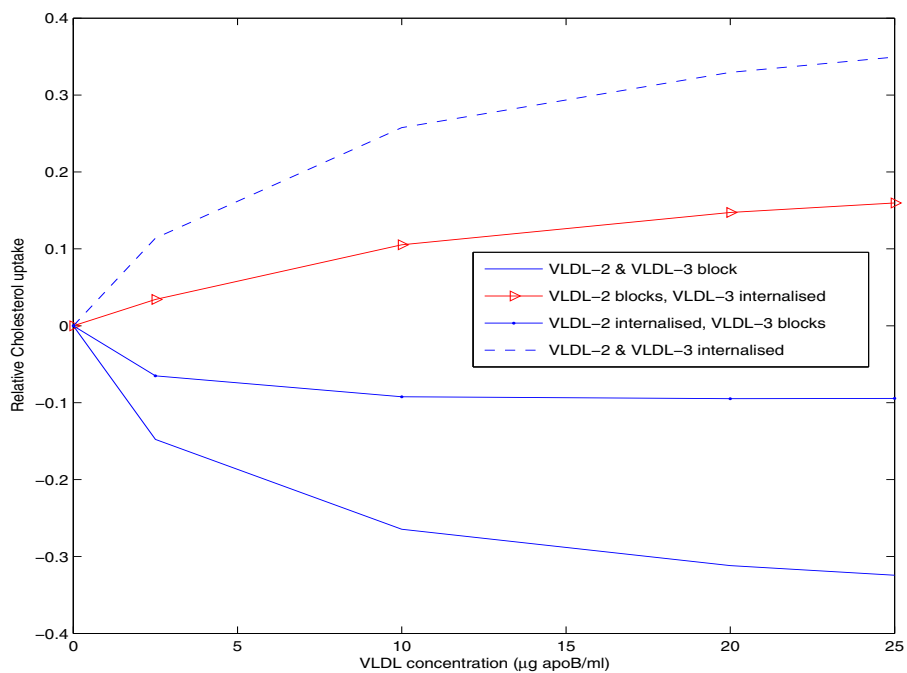
Sensitivity analysis allows us to understand the effect that variations in parameter values have on the model outcomes and how robust the reported outcomes are. In the following sections we explore the effect that variations in parameters affecting key mechanisms incorporated into our model have on the relative LDL and cholesterol uptake by hepatocytes. In each case the sensitivity analysis involves varying the respective parameter of interest whilst keeping all other parameters (as detailed in Table 2) fixed. We consider only those cases where either VLDL-2 or VLDL-3 are present in the medium with LDL, not all three species as considered in Section 3.3. Variations in parameter values are explored over a number of order of magnitudes as indicated.

3.4.1. Initial VLDL concentration (*apoB* concentration)

Increasing the initial concentration of VLDL particles leads to a saturation effect whereby at approximately $400\mu\text{g}$ of apoB/ml no further LDL or cholesterol is taken up by the cell. This result is shown in Figures 7(a) and (b). Increasing the number of VLDL particles decreases the number of receptors available to free LDL particles to bind to the cell surface; a consequence of the difference in the size of LDL and VLDL particles and the subsequent number of receptors they occlude. Subsequently at a critical initial VLDL concentration, no further reduction in LDL or cholesterol uptake is observed with increasing VLDL concentration.

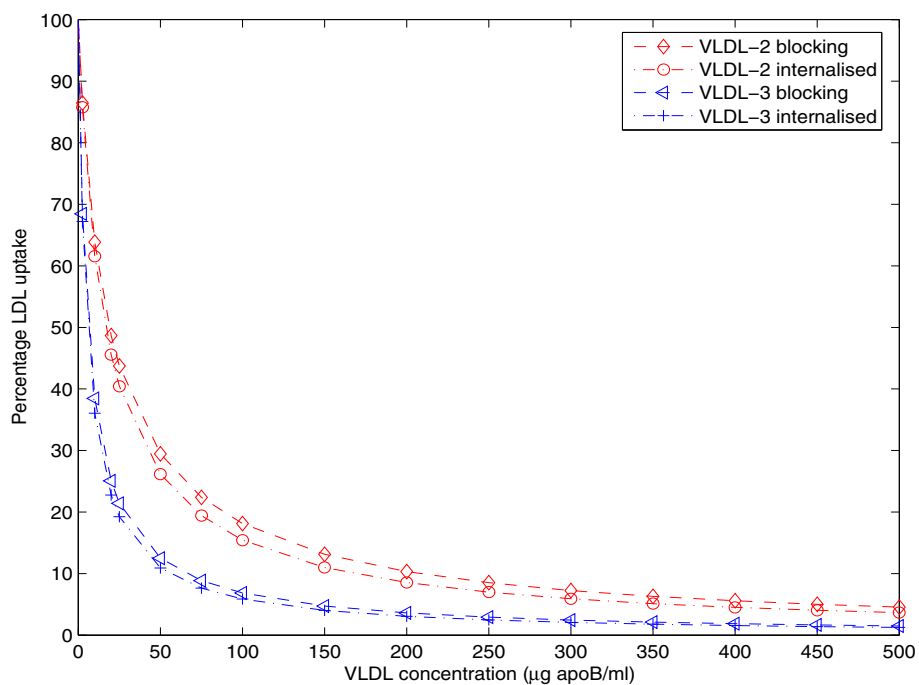


(a)

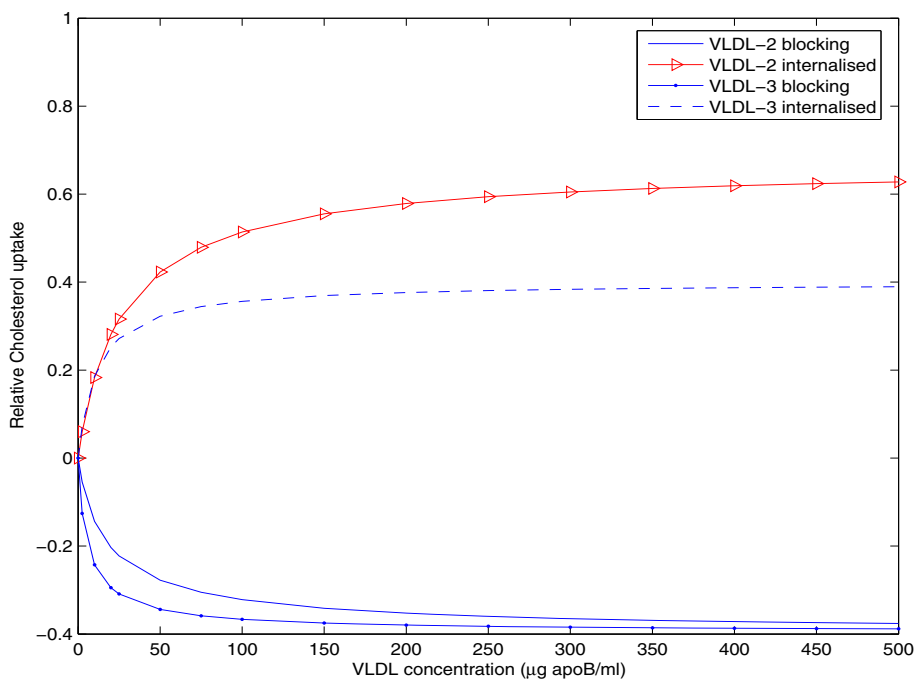


(b)

Figure 6: Model predictions of the: (a) percentage change in relative LDL uptake; and (b) relative cholesterol uptake in the presence of both VLDL-2 and VLDL-3. Here uptake is measured after a time of five hours and parameter values are as detailed in Table 2.



(a)



(b)

Figure 7: The saturating effect of increased initial concentrations of VLDL particles on the relative uptake of: (a) LDL; and (b) cholesterol.

3.4.2. Particle dynamics (binding, dissociation and internalisation)

The rate of VLDL binding (ϕ_2, ϕ_3) is important in differentiating between LDL uptake by either VLDL-2 or VLDL-3. When the rate of VLDL-2 and VLDL-3 binding are equal there is little difference in the relative LDL uptake by the cell, although a difference in cholesterol uptake for the blocking and internalisation of each VLDL particle is observed. Setting the number of receptors VLDL-2 and VLDL-3 particles occlude to be equal ($N = Q$) reduces this difference even further. VLDL binding rates thus play an important role in discerning between LDL and cholesterol uptake in the presence of VLDL.

The dissociation rate of LDL (ψ_L) has no effect on LDL uptake even when it is increased 1000 fold ($\psi_L = 100$) in the presence of VLDL. Increased LDL dissociation does lead to an increase in cholesterol uptake when either VLDL-2 or VLDL-3 are internalised, but for $\psi_L > 10$ cholesterol uptake when either VLDL particle blocks is approximately zero. As we are comparing relative LDL uptake, i.e. LDL uptake with and without VLDL present, the increased dissociation of LDL means very few LDL particles, either in the presence or absence of VLDL, are internalised. Increasing the rate of VLDL dissociation up to a thousand-fold has the expected effect of increasing LDL uptake, but decreasing the amount of cholesterol taken up by the cell. The relationship between LDL and cholesterol uptake in the presence of VLDL, as shown in Figures 5(a) and (b), is maintained.

Our model assumes that the rate of internalisation (χ_L, χ_2, χ_3) of all particle types is equivalent. Increasing the rate of LDL internalisation or internalisation of both VLDL particles by up to three orders of magnitude does not affect LDL or cholesterol uptake. However, decreasing the rate of VLDL internalisation by a tenth leads to approximately a 30% reduction in cholesterol content of the cell in respect of VLDL internalisation (irrespective of VLDL-2 or VLDL-3). A further magnitude of order reduction leads to a negative relative cholesterol value for internalised particles when the initial VLDL concentration is high. The amount of cholesterol uptake with respect to blocking is unchanged. These results show that it is rapid binding of VLDL to the surface receptors that effects

the blocking and subsequent uptake of LDL. A reduction in VLDL internalisation merely reduces the time taken for the VLDL-particle complex to become internalised and thus the cholesterol content to increase. If we were to wait longer than the simulation time here of five hours then the cholesterol content of the cell would be greater.

3.4.3. Receptor dynamics (Recycling, production and unbound internalisation)

Increasing the rate of receptor recycling (γ_{rr}) slightly above $\gamma_{rr}=30$ has the effect of reducing the already small number of internalised receptors (see Figure 4) to zero. Reducing this rate by 1/10th leads to an increase in internalised receptors and a difference in the transient number of free and bound receptors. Neither increasing or decreasing γ_{rr} changes the relative LDL or cholesterol uptake. These results are a consequence of equations (39) and (40) - a reduction in receptor recycling leads to less receptors reaching the surface, thus the observed transient reduction in subsequently bound and free receptors (the binding rates of the particles remains the same). Increasing the rate simply recycles more receptors back to the surface, but given there are so few receptors initially internalised, increasing γ_{rr} does not greatly affect the overall receptor concentration on the cell surface. Thus we observe no appreciable differences in relative LDL or cholesterol uptake.

In contrast, reducing the fraction of internalised receptors which are returned to the surface of the cell by approximately 20% (from $f = 0.9$ to $f = 0.7$) leads to a 50% reduction in the number of free, bound and total concentration of receptors. This subsequently results in an average 10% reduction in LDL uptake and 70% reduction in intracellular cholesterol for internalised VLDL. A reduction in free receptors leads to increased competition between LDL and VLDL particles, which given the size of the latter, with less receptors available fewer LDL particles are internalised in the presence of VLDL. The fraction of receptors which are returned to the surface is thus clearly important in affecting the competition between LDL and VLDL for free LDLR.

The cholesterol dependent rate of receptor production or *de novo* production is dependent upon the magnitude of γ_s^* and \bar{K} . According to equation (40) an increase in γ_s^* and/or

decrease in \bar{K} should lead to an increase in the internalised receptor concentration. We have found that the concentration of free, bound and internalised receptors is very sensitive to changes in γ_s^* and \bar{K} . Increasing γ_s^* or \bar{K} leads to an increase in free and subsequently bound receptors. This in turn causes an observable increase in LDL uptake (approximately 10%) and subsequently intracellular cholesterol concentration when VLDL is internalised (no change in intracellular cholesterol levels when VLDL blocks is observed). These results show the system is susceptible to small changes in the rate at which the cell creates intracellular receptors. Any receptors placed on the cell surface quickly become bound due to the fast binding of the respective particles.

Increasing the rate of free receptor internalisation (χ_0) from zero to $\chi_0 = 1 \times 10^6$ had no effect on receptor concentration or relative LDL or cholesterol, both in terms of transient and steady-state behaviour. Finally, we note that increasing the rate of cholesterol breakdown (λ) does not affect the LDL uptake, but does reduce the relative cholesterol uptake.

4. Summary and Conclusions

A continuum mathematical model describing the uptake of LDL particles by a hepatocyte cell in the presence of VLDL-2 and VLDL-3 particles has been formulated and solved. The model includes descriptions of particle binding and dissociation, internalisation, receptor recycling, *de novo* cholesterol dependent receptor formation, internalisation of free receptors and cholesterol breakdown by the cell. Blocking of free receptors due to the type and size of particle binding is also included. The model has been parameterised with data from the experimental literature.

By comparing with experimental data from Jackson et al. (2006) our model has demonstrated that in order to differentiate between the effects of LDL uptake in the case of VLDL blocking LDLR or being internalised, measuring LDL uptake alone is not sufficient

in distinguishing between the different scenarios. Instead a measure of the intracellular cholesterol concentration is required.

When the intracellular cholesterol concentration is considered, we have shown that blocking of LDLR by VLDL leads to a reduction in the cholesterol content of the cell. When either VLDL particle is internalised the intracellular cholesterol concentration increases. In respect of the two hypotheses regarding the effects of VLDL receptor blocking or internalisation on relative LDL or intracellular cholesterol uptake (as detailed in the Introduction), we conclude that blocking of LDLR leads to a decrease in intracellular cholesterol. In the case where VLDL is internalised, the binding and internalisation is so rapid in comparison to LDL binding that the effect of VLDL blocking in this case is negligible. Given receptor recycling is the same order of magnitude as binding, any receptors lost due to VLDL binding and internalisation are quickly replaced, allowing further LDL and VLDL particles to bind and internalise. This subsequently leads to an increase in intracellular cholesterol levels. From these results we conclude that VLDL blocking without internalisation leads to a reduction in intracellular cholesterol levels, but internalisation of any VLDL particles leads to an increase in such levels.

We have used our model to hypothesise how LDL and cholesterol uptake are affected by the presence of *both* VLDL-2 and VLDL-3. Four scenarios were considered here: (i) VLDL-2 and VLDL-3 both blocking LDLR; (ii) VLDL-2 blocking receptors and VLDL-3 being internalised; (iii) VLDL-2 being internalised and VLDL-3 blocking receptors; and (iv) both VLDL particles being internalised. No discernible difference in LDL uptake is observed between each scenario, however, consideration of the intracellular cholesterol concentration shows that the greatest increase in cholesterol obtained when both VLDL particles are internalised. Subsequently less cholesterol is taken up by the cell when both VLDL particles block LDLR. These results are not dissimilar to that when either VLDL-2 or VLDL-3 are present.

Our model further predicts that increasing the initial concentration of VLDL particles (of either type) leads to a 'saturation' in LDL and cholesterol uptake. When the initial

VLDL concentration reaches approximately $400\mu\text{g apoB/ml}$, LDL and cholesterol uptake increases only marginally upon further increases in VLDL concentration; a result of VLDL particles ‘saturating’ or simply blocking LDLR sites on the cell surface thereby impeding any further increase in uptake of LDL or VLDL particles.

By conducting a sensitivity analysis, the effect of particle (binding, dissociation and internalisation) and receptor (recycling, production and unbound internalisation) dynamics on LDL uptake and intracellular cholesterol concentration has been measured. Our main findings are as follows.

- The relative rates of LDL and VLDL particle binding play important roles in affecting the competition between LDL and VLDL particles for LDLR and subsequently LDL and cholesterol uptake.
- Increased LDL dissociation in the presence of VLDL does not affect LDL uptake and leads to only a slight increase in intracellular cholesterol concentration when either VLDL-2 or VLDL-3 are internalised. LDL uptake is increased when VLDL dissociation is increased, but intracellular cholesterol concentration subsequently falls in the case when VLDL dissociation is high and VLDL is internalised.
- Decreasing the rate of VLDL internalisation leads to lower intracellular cholesterol levels when VLDL is internalised.
- Reducing the fraction of internalised receptors recycled back to the cell surface leads to appreciable reductions in LDL uptake and intracellular cholesterol concentrations.
- The concentration of free and bound receptors, and subsequently LDL uptake and intracellular cholesterol concentration, are sensitive to the rate of receptor *de novo* production. Doubling of this rate leads to an increase in free and bound receptors, which gives an average 10% increase in LDL uptake and 70% increase in intracellular cholesterol concentration as a consequence of reduced total receptor concentrations.

These results show that the relative magnitude of each mechanism, be it related to binding

or receptor dynamics, is important in affecting the overall uptake of particles by the cell. For instance, if particle binding and internalisation are rapid, but the quantity of receptors recycled is slow then competition for receptors on the cell surface will increase. This result is reflected in an initial rapid increase in intracellular cholesterol levels which only increase marginally with a greater incubation period.

Our results here require further experimental evidence before concrete conclusions can be drawn, however, they demonstrate the relative importance of binding and receptor dynamics and how each can have important consequences on intracellular cholesterol concentration. Such mechanisms need to be taken into account when attempting to alter cholesterol uptake by the cell and likewise devising treatments for dysregulated lipoprotein metabolism.

A. Parameter values

The work in this manuscript is concerned with describing lipoprotein particle uptake by a hepatocyte (HepG2) cell. Where possible we have sourced values relating to particle and receptor dynamics for HepG2 cells from the literature. In cases where information on such cells has been unavailable we have used those values from other cell lines (as indicated below), which we believe are most representative of the mechanism being considered. In what follows N_A represents Avogadro's number.

Typically a cell's surface contains between 20,000 and 50,000 receptors, of which only 50–80% are found in hemispherical indentations known as pits (data for human fibroblasts taken from Brown and Goldstein (1979)). The median of these two values is 35,000 receptors per cell, a figure similar to that obtained by Harwood and Pellarin (1997) (they quote a figure of 154 fm LDL receptors/mg cell protein, which upon a conversion factor of 300 mg cell protein/ml, and a cell volume of 1 pl, we obtain 30,000 LDL receptors per HepG2 cell). A typical pit radius is approximately 100 nm giving an area of $3.14 \times 10^{-14} \text{m}^2$. The average surface area of a cell is estimated to be $2.85 \times 10^{-10} \text{m}^2$, with around 2% of the

cell's surface area given over to pits. This implies that there are about 180 pits per cell, and each pit contains approximately 180 receptors.

The size of pits varies dramatically. Lucius et al. (2003) show a distribution of diameters from 60nm to 500nm. The mean of the distributions illustrated in Figures 3A and 3B therein yields an average radius of 85–90 nm. The distribution is not Gaussian, and has a much slower decay at larger cluster sizes than at smaller. We take a typical pit radius to be 100 nm. Assuming a uniform distribution of receptors inside the pit, each receptor occupies an area of 350 nm².

LDL particles are spherical with radii of 10nm (Goldstein et al., 1979), thus have a cross-sectional area of 300 nm² and on average cover 0.86 receptors on binding; rounding up, we take $M = 1$, since we discount the possibility that 10 LDL particles could bind to just 9 receptors. VLDL particles are larger (Palmer et al., 2005), having radii in the range 15–40nm, although this distribution is also highly skewed. We assume VLDL-2 particles are toward the lower end of this (15nm) and so cover 700nm²; thus in addition to binding to a receptor, it will occlude others. We take a total figure of $N = 2$. VLDL-3 particles are larger (20nm) so occupy 1260nm², enough to cover $Q = 3.6$ receptors on average.

P : Number of free receptors per pit.

Simulation value: 180

M, N, Q : Number of free receptors occluded when an LDL, VLDL-2, VLDL-3 particle binds in a pit.

Simulation values: $M = 1, N = 2, Q = 3.6$

$\bar{M}, \bar{N}, \bar{Q}$: These functions denote the number of vacant receptors internalised with an occupied pit (see equation (25) and surrounding text for details). When a pit is taken into the cell, as well as the bound receptors, other, unoccupied, receptors are also internalised; the number of these depends on the occupancy of the pit. At high occupancies (low ρ_F) few will be internalised, but at lower occupancies (high ρ_F) many will be internalised.

Simulation values: $\widetilde{M}(\rho_F) = MT(\rho_F)$, $\widetilde{N} = NT(\rho_F)$, $\widetilde{Q} = QT(\rho_F)$, with $T(\rho_F) = \rho_F/(\rho_0 - \rho_F)$.

A.1. Parameters describing concentrations

In deriving the model, we have implicitly assumed that the concentration variables are measured in number of particles per unit volume. In order to be compatible with our model we need to convert all concentrations from mass per unit volume to numbers per unit volume.

ρ_0 : Taking a value of 92.4 ng of apoB per mg cell protein from Harwood and Pellarin (1997) we find 1.09×10^{11} receptors per mg cell protein, or $(1.09 \times 10^{11}/\bar{V}_c)N_A$ receptors per ml, estimates cell volume as 1 pl and protein content as 300 pg.

Simulation value: 3.26×10^{13} receptors per ml.

l_0 : In the experimental setup of Jackson et al. (2006) the initial concentration of LDL is 10 μg per ml of cell medium. We convert this value to the number of particles per ml by taking into account of the molecular weight of the apoB particles, i.e. we write 10 μg per ml of cell medium = $1 \times 10^{-5} g$ /molecular weight of apoB $\times 10^{-3} L$ = 19.5×10^{-9} mol per L of cell medium = $19.5 \times 10^{-9} \times N_A$ particles per L of cell medium = 1.17×10^{13} lipoprotein particles per ml of cell medium.

Simulation value: 1.17×10^{13} particles per ml.

v_{02} and v_{03} : In the experimental setup of Jackson et al. (2006) three different initial values of VLDL are used: 2.5 μg per ml of cell medium, 10 μg per ml of cell medium and 20 μg per ml of cell medium. Using the same transformations as for l_0 we obtain the three values for the initial VLDL concentration to be respectively 2.95×10^{12} , 1.17×10^{13} and 2.38×10^{13} lipoprotein particles per ml of cell medium.

Simulation values: $v_{02} = v_{03} = 2.95 \times 10^{12}$, 1.17×10^{13} , 2.38×10^{13} lipoprotein particles per ml of cell medium.

A.2. Kinetic rate parameters describing the transport of lipoprotein particles across the cell membrane

α_L : Rate of LDL binding to free receptors. In Harwood and Pellarin (1997) (equation (1) and from the top line of Table 2) our α_L is denoted k_1 and given the value $k_1 = 4 \times 10^4/\text{Ms}$. Since molarity, M, is the number of moles per litre, we have $\alpha_L = 0.666 \times 10^{-16}$ ml/s.

Simulation value: $\alpha_L = 6.66 \times 10^{-17}$ ml/s.

α_{-L} : Rate of LDL dissociation from receptors. We take the average of three very similar values found in the literature. Harwood & Pellarin give a figure of $5.9 \times 10^{-4}/\text{sec}$, quoted as k_2 at the top of the penultimate column in Table 2 of Harwood and Pellarin (1997). Further data which confirms that this is the correct order of magnitude is available from Dergunov et al. (2000) who quote two reaction constants for the reversible binding of LDL as well as reaction constants for VLDL-2 and VLDL-3 binding (though they use the terminology of low plasma triglyceride (TG_l) and intermediate or high plasma triglyceride (TG_i, TG_h)). Table 4 of Dergunov et al. (2000) quotes a reaction constant for LDL of $Ka = 1.04 \times 10^8 \text{ M}^{-1}$. Noting that $1 \text{ M} = 10^{-3} \text{ mol/ml}$ and $N = 6 \times 10^{23} \text{ mol}^{-1}$, we find $Ka = 1.7 \times 10^{-13} \text{ ml}$. Since $Ka = \alpha_L/\alpha_{-L}$, $\alpha_{-L} = \alpha_L/Ka = 3.9 \times 10^{-4} /\text{sec}$. Dergunov et al. (2000) also quote $Ka = 0.85 \times 10^8 \text{ M}^{-1}$ which is equivalent to $Ka = 1.4 \times 10^{-13} \text{ ml}$, and yields $\alpha_{-L} = 4.7 \times 10^{-4} /\text{sec}$. The three values 5.9, 4.7 and 3.9×10^{-4} per sec are all similar. Considering the equation $\dot{l}_b = \alpha_L \rho_F l_e - \alpha_{-L} l_b - \beta_L l_b$; at steady-state $\dot{l}_b = 0$, and the term which provides a net influx of l_b is balanced by two loss terms. Since $\beta_L > \alpha_{-L}$, most bound LDL protein particles are internalised; however, a proportion ($\alpha_{-L}/(\alpha_{-L} + \beta_L) \approx 18\%$) of binding events end with dissociation rather than internalisation.

Simulation value: $\alpha_{-L} = 5.9 \times 10^{-4}/\text{s}$

α_2, α_3 : Rate of VLDL-2 and VLDL-3 binding respectively. Whilst LDL particles have only one apoB molecule, VLDL-2 particles have two apoE and one apoB molecules,

and VLDL-3 particles have three apoE molecules and one apoB. Experimental results by Mahley and Jr. (2000) suggest that the affinity of apoE to bind to the receptors is significantly greater than that of apoB. Using these estimates as a guide, and in order to obtain a fit to the data of Jackson et al. (2006), we have obtained the following values for the two rates of VLDL binding.

Simulation values: $\alpha_2 = 14\alpha_L$, $\alpha_3 = 24\alpha_L$.

α_{-2} , α_{-3} : Rate of VLDL-2 and VLDL-3 dissociation from receptors. Even though VLDL particles cover more than one receptor, we still expect them to typically bind with only one LDL receptor, so we assume that VLDL dissociation will occur at a similar rate to LDL dissociation. However, the precise value of the VLDL dissociation rates is determined by fitting to the data (Jackson et al., 2006) on the reduction in LDL uptake caused by the presence of VLDL.

Simulation values: $\alpha_{-2} = 12\alpha_{-L}$, $\alpha_{-3} = 2\alpha_{-L}$.

β_L : The internalisation rate of LDL.

A value of $2.7 \times 10^{-3}/s$ is quoted by Harwood and Pellarin (1997) which agrees with that of Goldstein et al. (1979) and Brown and Goldstein (1979), who quote a pit lifetime of 5 minutes, or 300 seconds.

Simulation value: $2.7 \times 10^{-3}/s$.

$\beta_{2,3}$: Rate of VLDL-2 and VLDL-3 internalisation.

Since the internalisation rate has units of ‘per sec’ (not pits per sec or receptors per sec) the rate applies to all bound entities; that is, the rate is a property of the pit, not what is bound to receptors in the pit; hence we assume $\beta_2 = \beta_3 = \beta_L$.

Simulation values: $\beta = \beta_2 = \beta_3 = \beta_L = 2.7 \times 10^{-3}/s$

β_0 : Rate of internalisation of empty receptors due to the empty pits being internalised.

Since our model internalises large numbers of empty receptors when the external concentration of LDL and VLDL is low, this additional effect is expected to be small and thus will be neglected, this is equivalent to our assuming $\beta_0 \sim 0$.

Simulation value: $\beta_0 = 0$.

A.3. Kinetic rate parameters for the cell's internal processes

γ_L : Rate at which internalised LDL particles are degraded to release cholesterol into the cell. No data is currently available, but Brown and Goldstein (1979) quote a time of ten minutes for marked particle ingestion to measurement of related cholesterol concentration. This time includes a number of mechanisms included in our model and we thus assume a figure of five minutes to be more realistic for γ_L .

Simulation value: $\frac{1}{300}/s$.

$\gamma_{2,3}$: Rate at which internalised VLDL particles are degraded to release cholesterol into the cell. We assume that cholesterol from VLDL particles is released at the same rate, and associated receptors return to the surface at the same rate, so that $\gamma_2 = \gamma_3 = \gamma_L$.

Simulation value: $\gamma_2 = \gamma_3 = \gamma_L$.

γ_s, K : These parameters control the rate of free receptor production by the cell, which is regulated by the cell's internal free cholesterol concentration (C). By writing $\gamma_s = \gamma_{test}(K + 1)$, and varying γ_{test} , the steady-state number of receptors can be imposed, and then varying K determines the rate to which the steady-state is approached. Smaller values of K make the receptor-production rate more sensitive to the cell's cholesterol level, and so lead to more rapid convergence to steady-state. Large values of K give a more uniform receptor-production rate, which means it takes longer to replace non-recycled receptors, and longer to reach steady-state.

Simulation values: $K = 2C_e, \gamma_s = 1.8 \times 10^{30}/ml/s$.

γ_r : Rate of receptor recycling. The recycling rate of receptors from inside the cell back to the surface is fast. Whilst there must be some mechanism for returning receptors to the cell surface (our γ_r term), the parameter γ_r should be taken to be large, which has the consequence that ρ_I will be kept small. In our notation equation (1) of Harwood and Pellarin (1997), is $L_i + \rho_I \rightarrow L_i + \rho_F$, and the rate constant $k_5 = 0.653$ per min (Table 1) corresponds to $\gamma_r = 0.0108$ per second.

Simulation value: 0.0109/s

f : Fraction of internalised receptors returned to the cell surface. Results in Dunn et al. (1989) suggest that 70-100% of internalised receptors return to the cell surface following endocytosis. Therefore we take $f \in [0.7, 1]$.

Simulation value: 0.9

R_L^{chol} , R_{V-2}^{chol} , R_{V-3}^{chol} : Average cholesterol content per LDL (Panovska et al., 2005), VLDL-2 and VLDL-3 particle (Jackson et al., 2006).

Simulation values: $R_L^{chol} = 3400$, $R_{V-2}^{chol} = 3100$, $R_{V-3}^{chol} = 3900$.

$\lambda(C)$: Rate of esterification (breakdown) of cholesterol. We use this single term to cover a combination of processes: firstly the continual slow exchange between the two types of cholesterol stored in the cell (free and esterified, Havekes et al. (1987), the cycling conversion occurring over a 24 hour timespan); and secondly the loss of free cholesterol through the manufacture of bile which is excreted by the liver. The value is optimised by fitting to the endocytosis of LDL particles, see Pearson et al. (2008); Wattis et al. (2008) for details.

Simulation value: $\lambda = 3.3 \times 10^{-3}/s$.

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