Nonlinear dynamics of early atherosclerotic plaque formation may determine the efficacy of High Density Lipoproteins (HDL) in plaque regression

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Abstract

We use a computational model to explore the effect of foam cell accumulation on plaque regression following an increase in high density lipoprotein (HDL) influx into the plaque. Atherosclerotic plaque formation is the outcome of cellular and cytokine responses to low density lipoproteins (LDL) that penetrate the artery wall following an injury to the endothelium and become modified. We modelled the cells and cytokines that are most important in plaque formation using partial differential equations. The model included monocytes and macrophages, foam cells, macrophage chemoattractants, endothelium-stimulating cytokines, modified low density lipoproteins (mod LDL) and functional HDL. We included interactions both at the endothelium surface and inside the artery wall. The model predicted that when HDL influx is increased into a well-established plaque with large numbers of foam cells, the plaque may not regress but may continue to grow at a slower rate. If HDL influx is increased when a plaque is recently established and has fewer foam cells, then the plaque does regress. If modLDL influx into the plaque was lowered at the same time that HDL influx increased or the capacity of the HDL to remove cholesterol from foam cells was increased, then the plaque was more likely to regress. The predictions of the model were in qualitative agreement with experimental studies in mice and rabbits. The results suggest that the intrinsic dynamics of reverse cholesterol transport by HDL are important in determining the success of HDL raising in promoting plaque regression.

Introduction

There is a well-known correlation, at a population level, between high levels of high density lipoprotein cholesterol (HDL-C) in the blood plasma and a reduced risk of cardiovascular disease in humans [1–3].

Recent large clinical trials of drugs that are known to raise blood HDL-C have been disappointing [4–7]. The trials have either failed to show that increasing HDL-C reduces heart attacks or strokes in patients who already have LDL cholesterol (LDL-C) lowered by other therapy or they have had to be discontinued as the trial drug had significant adverse side effects or were found to have neutral effects.

This has led researchers to question the HDL hypothesis which predicts that HDL-raising will suppress cardiovascular disease and atherosclerotic plaque development via stimulation of reverse cholesterol transport (RCT). This is based on epidemiological data showing an association between higher plasma HDL-C levels and reduced

cardiovascular events. Clearly one potential explanation is that raising the amount of cholesterol carried by HDL particles may not raise the total number of HDL particles and so may not result in increased action by functional HDL [8, 9].

When the number of functional HDL particles is increased, there is experimental evidence from animal models that suggests that raising HDL may lead to plaque regression in recently established plaques [10–12] but not in older plaques [13–16]; it will only reduce the rate of progression in these older plaques. As plaques grow, they may develop lipid cores, calcification and collagen caps which early plaques do not have. These do not prevent LDL-C reduction leading to regression in late stage plaque [13, 15] which suggests that structures typical of late stage plaques do not prevent lipid from entering or exiting plaques. It is possible, therefore, that the failure of increased functional HDL to promote plaque regression in late plaque is due to something other than plaque structure.

Plaque formation and growth are complicated and include many interactions between cells, lipids, cytokines and other factors [17]. Many of these interactions are nonlinear; that is, the reaction rate is not proportional to the concentration or density of the inputs. For example, the uptake rate of modLDL by macrophages will saturate as the concentration of modLDL increases, because the rate that each macrophage can internalize modLDL is inherently limited by the number of receptors in the cell membrane or even by the area of the cell membrane itself. Therefore as modLDL availability increases, the rate that modLDL is removed by macrophages will not necessarily remain proportional to modLDL availability.

The consequences of nonlinear interactions are well known in biological systems such as ecology and neuroscience [18, 19]. The state of the system may undergo sudden dramatic switches in response to a small change in inputs or the effect of a change in inputs may depend on the current state of the system—for example, a change may produce an effect early in the development of the system, but not later. Mathematical and computational modeling shows that these effects are not random but qualitatively predictable.

We propose a computational model for early plaque growth which includes the action of HDL and predicts that plaques will not regress if functional HDL influx into the plaque is increased too late in plaque development. This failure of HDL to promote plaque regression is due purely to the inherent dynamics of plaque growth and of HDL action. Increasing functional HDL influx too late in plaque development only attenuates plaque growth and does not enable plaque regression.

Previous mathematical and computational models for plaque development that include the action of HDL have focused on the relative importance of different functions of HDL [20] or predicting risk as a function of the balance between HDL-C and LDL-C [21]. Here we focus specifically on the impact of the timing of changes in the rate of HDL influx on plaque development and regression.

Results

Figure 1 shows schematically how the model works. We assume that the injured endothelial cells allow LDL into the intima, the layer of the artery wall nearest to the bloodstream. This LDL rapidly becomes modLDL and sets up an immune reaction in the endothelial cells on the boundary. In response to modLDL, endothelial cells produce monocyte chemoattractants such as MCP-1, which draw monocytes into the intima. Endothelium-stimulating (ES) cytokines such as TNF-α, produced in the intima, stimulate the endothelial cells to produce adhesion molecules which act on monocytes in the blood stream and further increase the rate of monocyte recruitment. Inside the intima the monocytes rapidly differentiate into macrophages which move towards

modLDL, and consume it. Stimulated by modLDL consumption, macrophages produce more monocyte chemoattractant and also ES cytokines. As macrophages consume modLDL, they become foam cells. The model applies to plaque development before the formation of the lipid core or collagen cap.

Figure 1. Schematic diagram of the model Diagram of processes in the endothelium and intima in early plaque formation with a flow-chart representation of the model interactions on the endothelium (top) and in the intima (bottom) between modLDL, monocytes/macrophages, chemoattactants, ES cytokines, foam cells and HDL. A plaque is initiated when the endothelium is injured and allows LDL to enter. The LDL particles become oxidised or modified in other ways. These modLDL particles provoke an immune reaction that causes monocytes to enter the blood vessel wall from the blood stream and differentiate into macrophages which consume modLDL. The macrophages become filled with cholesterol and take on a foamy appearance under the microscope. If the cholesterol is not removed from the cells, these foam cells accumulate in the intima. HDL particles transport cholesterol out of foam cells and cause the plaque either to regress or grow more slowly. HDL also acts to reduce the modification of LDL and the excitation of the endothelium which reduces the rate that monocytes enter the plaque.

Functional HDL modulates these processes in the model by reducing the oxidation of LDL so that there is less modLDL entering the intima; by reducing the number of adhesion molecules on the damaged endothelium, which in turn reduces the recruitment of monocytes; and by facilitating RCT from foam cells which then revert to macrophages. There is provision in the model for only some of the macrophages to revert to the original inflammatory type (M1). When macrophages revert to the anti-inflammatory (M2) type, they no longer play any part in the model.

The solutions of the model produce spatial profiles across the intima of modLDL, macrophages and foam cells. These profiles change over time as the plaque develops. The model plaques start with no modLDL, cytokines or macrophages present in the intima. At first, each profile has a maximum at, or close to, the endothelium. As the plaque develops, both cells and cytokines become evenly spatially distributed across the intima. The density of macrophages in the intima in these model plaques always settles to a fixed equilibrium, which does not change after an initial period of growth, but the density of foam cells may either continue to grow or settle to a fixed equilibrium where the plaque is neither growing nor shrinking (Figure 2).

Figure 2. Macrophage and foam cell density over time. (a) Macrophage density in the intima and (b) foam cell density when the plaque grows unboundedly. Plots (c) and (d) show macrophage and foam cell density respectively where foam cell numbers settle to a fixed equilibrium. Note: all variables are scaled with respect to intima width and indicative time scale.

Figure 3(a) shows a bifurcation diagram, computed from the model, which gives qualitative information about foam cell accumulation at different rates of HDL influx. The vertical black arrows represent model plaques (labeled A to E) that are developing with a fixed rate of HDL influx. The direction of the arrows indicates whether the plaque is growing or shrinking. The horizontal dashed lines represent instantaneous changes in HDL influx. The solid curve represents the fixed equilibrium and is known mathematically, as an attractor. Plaques grow or shrink so as to get closer to the equilibrium. The dashed curve is known as the repellor. Plaques grow or shrink so that they move away from this curve. The dashed and solid curves meet at a point known as a bifurcation point or tipping point. We have labeled the rate of HDL influx at the bifurcation point Σ_h. When the HDL influx is below Σ_h, the density of foam cells in the

model plaque will always increase as no equilibrium state exists. Figure 3(b) shows the accumulation of foam cells with time in each of the model plaques represented in Figure 3(a).

Figure 3. Model predictions from bifurcation diagram (a) Bifurcation diagram showing the density of foam cells at equilibrium as a function of the rate of influx of functional HDL. The solid blue curve represents the attracting equilibrium and the dashed red curve the repellor. The black arrows and lines represent various plaques labeled A–E whose size changes with time; the dashed horizontal lines represent rapid changes in HDL influx rate; the vertical arrows represent changes with time due to intrinsic dynamics in the model tissue. (b) Plaques A–E plotted as a function of scaled time. As shown in the bifurcation diagram plaques B, C and E tend to the fixed equilibrium which has low foam cell density, but the density of foam cells in plaques A and D continue to grow. The scales on the axes are a qualitative indication only as there is limited information about the exact values of the input parameters in the model.

Plaque A represents a plaque with low HDL influx. Over time, the density of foam cells in Plaque A continues to grow, as shown in Figure 3(b). Plaque B on the other hand represents a plaque with a high rate of functional HDL influx. The density of foam cells in this plaque grows with time, but approaches the fixed equilibrium represented by the solid curve in Figure 3(a).

The effect of changing the rate of functional HDL influx in the model depends on the timing and magnitude of the change. Plaque C grows as the same rate as Plaque A initially, but after Plaque C has grown for a short time, the rate of influx of functional HDL into the plaque is instantaneously increased to the same HDL influx rate as Plaque B. When this happens, Plaque C loses foam cells via RCT and regresses to the fixed equilibrium foam cell density. Plaques D and E also grow in the same way as Plaques A and C initially, but the rate of HDL influx is increased later than for Plaque C. For Plaque D the HDL influx rate is increased to the same value as Plaque C but the plaque continues to grow, albeit at a slower rate than for Plaque A. The difference between plaques D and C is the timing of the increase in HDL influx; in Plaque C the plaque was smaller when HDL influx increased and the new influx rate moves the plaque to between the dashed and solid curves in Figure 3(a). The plaque develops so that is gets closer to the fixed equilibrium, represented by the solid curve and further from the repellor which is represented by the dashed curve. Plaque D has grown sufficiently that, when HDL influx is increased, the plaque is above the dashed curve and so foam cells continue to accumulate as the plaque moves away from the repellor. If the rate of influx of HDL is increased at the same time as in Plaque D but by a larger amount, then the plaque will fall in the region between the solid and dashed curves and will regress (Plaque E).

In general, when the rate of HDL influx into the plaque is less than Σ_h (the HDL influx rate at the bifurcation point), there is no equilibrium and so the density of foam cells in the plaque continues to grow. If the rate of HDL influx is greater than Σ_h then there is an equilibrium or attractor where the density of foam cells in the plaque does not change. If the HDL influx remains constant and high enough, then a newly initiated plaque will approach this equilibrium and remain small. If HDL influx later drops below Σ_h the plaque will start to grow again. A subsequent increase in HDL influx may or may not lead to plaque regression and a consequent return to the small plaque with a fixed density of foam cells; this will depend on the timing and magnitude of the change in HDL influx.

The placement of the bifurcation point depends on various physiological parameters in the model (Figure 4). As the cholesterol efflux capacity of HDL increases, the bifurcation point moves left and so plaques can settle to a fixed equilibrium at lower rates of HDL influx. As the rate of LDL influx and consequent generation of modLDL

increases, the bifurcation point moves right, so that the HDL influx rate that is required for a plaque to be at equilibrium increases. As the proportion of foam cells that revert to M2 type macrophages after cholesterol efflux increases, the repellor curve becomes steeper and so an increase in HDL influx is more likely to promote regression. Other parameters that affect the shape and placement of the curve in the bifurcation diagram, include the response of the endothelium to modLDL and the density of monocytes available in the blood stream (Figure 5).

Figure 4. Equilibrium foam cell density for changing physiological parameters Foam cell density at equilibrium (the attractor) and the repellor are plotted against HDL influx showing the effect of (a) increasing the cholesterol efflux capacity (CEC) of HDL particles; (b) increasing the influx of modLDL into the plaque and (c) increasing the proportion of macrophages that revert to M2 type after RCT. The attracting equilibria are indicated by the solid curves; the repellors by dashed curves. In (a) as cholesterol efflux capacity increases, the bifurcation point moves left so that the equilibrium exists for lower rates of HDL influx. In (b) as modLDL influx increases the bifurcation point moves right so equilibrium only exists for higher rates of HDL influx. In (c) as the proportion of foam cells revert to M2 rather than M1 macrophages increases, the slope of the repellor curve increases so that it is more likely that a plaque will fall below the repellor curve if HDL influx is increased and consequently will regress. The blue curve in each plot is the same curve as in Figure 3(a).

Figure 5. The effect of different HDL actions on equilibrium foam cell density Plots showing where equilibrium plaques with a fixed density of foam cells exist as a function of σ_h the rate of influx of functional HDL and (a) σ_m which governs the rate of influx of monocytes and may be thought of as the availability of monocytes in the blood stream; (b) $\frac{\gamma_\ell}{\alpha_\ell}$ which governs the rate that LDL is modified and hence the rate that modLDL enters the model plaque; and (c) $\frac{\gamma_m}{\alpha_m}$ which governs the excitability of the endothelium in response to modLDL and its propensity to express adhesion molecules and thereby recruit macrophages to enter the lesion. In each plot, the curve is the locus of the bifurcation point.

Reverse cholesterol transport by HDL in the model is key to determining whether plaques continually grow or settle to an equilibrium where foam cells do not accumulate. Removing RCT from the model removes the attracting equilibrium so that plaques with fixed foam cell density cannot form and the only possibility is that plaques continue to grow. The effect of reducing CEC is shown in Figure 4(a). As CEC decreases, the bifurcation point moves right. When the HDL has no capacity to remove cholesterol from foam cell—that is, there is no RCT—then the bifurcation point and the fixed equilibrium disappear entirely. Similar information is presented in a different way in Figure 6. The anti-inflammatory effect of HDL on the endothelium and its anti-oxidative action alone is insufficient for plaque regression or insufficient even for equilibrium in this model.

1 Discussion

The results of this model suggest that the history of plaque growth is as important as the functional HDL influx rate that the plaque experiences. If a plaque is well-established with a substantial foam cell density, then the model predicts that raising HDL by a moderate amount may not lead to regression but only to a slower rate

Figure 6. The existence of equilibrium plaque as cholesterol efflux capacity and HDL influx This plot shows where equilibrium plaques with fixed density of foam cells exist as a function of σ_h the rate of influx of functional HDL and ν_N which specifies the cholesterol efflux capacity of HDL and governs the rate of reverse cholesterol transport from foam cells. The solid curve is the locus of the bifurcation point; that is, of Σ_h as ν_N changes. This curve separates the region where an equilibrium plaque exists from the region where there is no equilibrium. As the cholesterol efflux capacity of the functional HDL particles decreases, the bifurcation point occurs at increasingly higher values of σ_h than when cholesterol efflux capacity is high. If cholesterol transport rates are very low then very high rates of HDL influx are required for a plaque to exist in equilibrium. On the locus of the bifurcation point, as $\nu_N \to 0$ then $\sigma_h \to \infty$ which suggests that there it is impossible to have an equilibrium plaque when there is no reverse cholesterol transport.

of growth. Conversely, raising HDL influx very early in plaque growth or by a large amount will lead to regression.

The model results agree with experimental studies on rabbits and on ApoE-/- and Ldlr-/- mice. A study on rabbits that were fed an atherogenic diet for eight weeks and then injected weekly with HDL particles showed that the extent of fatty streaks in the aorta declined in the treatment group compared to both the baseline and the control groups after 30 days [10]. Another study using Ldlr-/- mice injected with a human ApoA-I adenovirus after five weeks on an atherogenic diet before sacrifice four weeks later, showed a reduction in aortic lesion size compared to both control and baseline groups [11]. Another, similar study on Ldlr-/- mice with a short initiation phase did not produce regression but did demonstrate a greatly reduced progression rate so that 24 weeks after injection the treatment group had lesions that were 50% smaller than the control group [12].

Studies with Ldlr-/- or ApoE-/- mice with a period of plaque initiation of 6 months or longer followed either by human ApoA-I gene transfer [13, 14] or by transplant of the aorta into a wild-type mouse [15] did not produce regression even several months after treatment but did show that plaques in the treatment group did not progress as rapidly as plaques in control animals. In another study [16] ApoE-/- mice were given ApoA-I via infusion or gene transfer after either 8 or 34 weeks on atherogenic diets. Plaques in mice that were given infusions or gene transfer after 8 weeks grew more slowly than in untreated mice. There was no difference in plaque size between untreated mice and mice that were treated at 34 weeks and so, presumably, had larger plaques.

The results of our model are consistent with the results of these studies which, together, suggest that an increase in functional HDL availability early in the life of a plaque is more likely to produce regression than a later increase in HDL influx. This hypothesis has obvious implications for HDL-raising therapies.

Reducing the influx of modLDL into the plaque moves the bifurcation point to the left so that the equilibrium with low foam cell density will exist for lower rates of influx of HDL (Figure 4(b)). This allows us to compare the predictions of the model to the results of a study by Feig et al. [22]. In this study ApoE-/- mice were fed an atherogenic diet for 16 weeks and their aortas were each transplanted into genetically different mice so that the transplanted aortas were in an environment with raised HDL compared to the donor mouse, lowered LDL or both. The recipient animals were on a chow diet and sacrificed after one week. Table 1, together with Figure 7, summarizes the results of this study and their interpretation in our model. In each case, the experimental results can be explained qualitatively using our model and each of the explanations are consistent with one another.

The existence of the bifurcation point at the critical value of HDL influx Σ_h suggests

Table 1. Model results compared with experiments This table shows the results of different treatments by Feig et al [22] and the corresponding prediction of the model.

Recipient mouse	Change in blood HDL-C and LDL-C.	Observed change in lesion area from baseline size.	Figure with bifurcation diagram.	Explanation from model.
ApoE$^{-/-}$	No change: HDL-C remains low; LDL-C remains high	Increasing trend	Figure 7(a)	No change in plaque behaviour. Plaque continues to grow.
hAI/ApoE$^{-/-}$	HDL-C rises to normal levels; LDL-C remains high	Significant decrease ($P < 0.05$)	Figure 7(b)	Change in functional HDL levels puts plaque to the right of the bifurcation point and below repellor so the model plaque regresses.
ApoAI$^{-/-}$	HDL-C remains low; LDL-C falls to low levels	Decreasing trend	Figure 7(c)	Decrease in LDL moves the curve left; the plaque can now regress to the equilibrium.
Wild Type	HDL-C rises to normal levels LDL-C falls to normal levels	Significant decrease ($P < 0.05$)	Figure 7(d)	Decrease in LDL moves curve left. Increase in HDL moves the plaque right. Plaque tends to an equilibrium that is smaller than for the ApoAI$^{-/-}$ recipient

Figure 7. Diagrammatic representation of model predictions described in Table 1 Sketch of predicted changes in plaque size after transplant into recipient mice: (a) ApoE-/-; (b) hAI/ApoE-/-; (c)ApoA-I-/-; (d) Wildtype. The curves in each plot represent the attractor and repellors. The solid curves represent the attractor or fixed equilibrium solution and the dashed curves represent the repellors. Where there are two sets of curves, the heavier set of curves corresponds to the plaque after transplant and the lighter curve corresponds to the plaque before transplant. The solid arrow represents plaque growth before transplant and the dashed arrow represents post-transplant changes.

why low HDL-C is consistently correlated with increased risk of CVD, but high HDL-C is not necessarily correlated with low CVD risk [9]. If this switch exists in vivo between continual plaque growth with no equilibrium plaque size and an accessible equilibrium, then further increases in HDL-C provide very little benefit once HDL-C is sufficiently high for there to be enough functional HDL for a plaque to settle at equilibrium.

The model also highlights the key role of the cholesterol efflux capacity (CEC) of HDL. If CEC is high then plaques will reach an equilibrium for lower values of HDL influx than if CEC is low. When CEC is low, a plaque is more likely to continue to grow. This result agrees with recent population-level data on the relation between CEC and heart attack and stroke [23].

This computational model has many nonlinear terms that model different facets of cell and cytokine action in plaque growth and further work is required to determine which of these dominate in plaque formation and regression. Accurate values for most of the parameters in the model cannot yet be obtained from the experimental literature and consequently many of these are estimates or values that are obtained from other

contexts or from general considerations of how cells and cytokines behave. Nevertheless, this analysis strongly suggests that the effects of nonlinear interactions may be significant in the dynamics of plaque formation and regression. Several laboratory research groups [24–26] have recently begun to characterize macrophage movement and plaque growth in terms of dynamics. It is important to understand, in such a complicated and multifactorial process, that nonlinear interactions occur and may lead to discontinuous switches and changes due to bifurcations. These bifurcations will not occur only for changing levels of functional HDL influx but may also occur for other physiological variables, such as the availability of monocytes in the blood stream or the influx of LDL particles into the plaque [27].

As yet, there is only indirect evidence that these types of nonlinear switches occur in plaques in vivo, but if they do, there is likely to be implications for the management of plaque growth and for strategies to promote plaque regression. This study, for example, suggests that, if the modeling is valid, then the influx rate of functional HDL into the arterial wall must either be increased sufficiently early in the life of the plaque or be sufficiently substantial so that the attracting equilibrium is accessible under the newly increased rate of HDL influx. This raises questions about the dynamics of HDL action and RCT in plaques in addition to the plethora of questions that already surround drug interventions to increase HDL levels in patients at high risk of CVD.

In conclusion this study suggests, via a computational model that there may be a bifurcation point in plaque growth. (The bifurcation point is also called the tipping point in other scientific contexts.) Plaques with HDL influx below this point will continue to grow but plaques with HDL influx above this point may settle to an equilibrium where they do not grow. The model also suggests that both the timing and magnitude of any increase in HDL influx into plaque will determine whether or not a plaque regresses or continues to grow. Whether or not a plaque remains small or regresses to equilibrium depends also on the influx of LDL into the plaque and the cholesterol efflux capacity of the functional HDL. These results suggest that therapy that raises functional HDL may be most effective in preventing the growth of small plaques rather than promoting the regression of large plaques.

A theory of the dynamics of early plaque growth and regression has the capacity, not only to generate new hypotheses, but also to provide explanations of anomalous observations and a framework to synthesize research results. Developing this theory and devising biologically valid computational and mathematical models to encapsulate theoretical ideas will require care and persistence and is unlikely to be simple or straightforward. This analysis is one early step in the journey.

Materials and Methods

The model consists of six partial differential equations with associated boundary conditions. We solved the equations in one spatial dimension where the endothelial boundary, between the artery wall and the bloodstream was at $x = 0$. The other boundary is at the interface between the intima and the media which is the next outward layer of the artery wall. The spatial dimension was scaled by the width of the intima, so that the medial boundary was at $x = 1$.

The six dependent variables were ℓ, h, p and q, the concentrations of modLDL, HDL, monocyte chemoattractant and ES cytokine respectively and m and N, respectively the density of macrophages and foam cells in the tissue. We assumed that the timescales of LDL modification and the differentiation of monocytes into macrophages were much faster than the timescales of other events in the model, so that LDL essentially enters the intima in modified form and as monocytes enter the intima, they instantaneously become macrophages that can consume modLDL.

We assume that each macrophage consumes modLDL at a rate that is proportional to the concentration of modLDL when that concentration is low. When modLDL concentration is high, the rate of consumption tends to a fixed upper limit. In the model, these saturating kinetics for modLDL consumption by a single cell are represented by the function $\ell/(1+\ell)$ where ℓ has been scaled so that the constant in the denominator is 1.

The first term in each equation models the diffusion or random motion of the lipid particles, cytokines or cells in the tissue. The last term, except in the equation for foam cell density N, models linear loss due to chemical decay, cell apoptosis or other processes not explicitly included in the model.

For chemoattractant, concentration p and ES cytokine, concentration q, the second term on the right hand side models production by macrophages stimulated by their consumption of modLDL. This rate of production is, therefore, proportional to the rate that macrophages consume modLDL:

$$\frac{\partial p}{\partial t} = D_p \frac{\partial^2 p}{\partial x^2} + \mu_p \frac{\ell m}{1+\ell} - d_p p\,; \tag{1}$$

$$\frac{\partial q}{\partial t} = D_q \frac{\partial^2 q}{\partial x^2} + \mu_q \frac{\ell m}{1+\ell} - d_q q\,. \tag{2}$$

For modLDL the second term models the loss of modLDL due to consumption by macrophages and for HDL the second term models loss of functional HDL as it receives lipids via RCT from foam cells:

$$\frac{\partial \ell}{\partial t} = D_\ell \frac{\partial^2 \ell}{\partial x^2} - \mu_\ell \frac{\ell m}{1+\ell} - d_\ell \ell\,; \tag{3}$$

$$\frac{\partial h}{\partial t} = D_h \frac{\partial^2 h}{\partial x^2} - \nu_h \frac{hN}{\kappa + h} - d_h h\,. \tag{4}$$

Macrophages move directedly towards modLDL (second term) and convert to foam cells as they consume modLDL (third term). A proportion θ of foam cells revert to M1 (inflammatory) macrophages after RCT (fourth term):

$$\frac{\partial m}{\partial t} = D_m \frac{\partial^2 m}{\partial x^2} - \chi_m \frac{\partial}{\partial x}\left(m \frac{\partial \ell}{\partial x}\right) - \mu_m \frac{\ell m}{1+\ell} + \theta \nu_N \frac{hN}{\kappa + h} - d_m m\,. \tag{5}$$

Foam cells are generated as macrophages consume modLDL (second term) and revert to macrophages as HDL removes lipids (third term):

$$\frac{\partial N}{\partial t} = D_N \frac{\partial^2 N}{\partial x^2} + \mu_m \frac{\ell m}{1+\ell} - \nu_N \frac{hN}{\kappa + h}\,. \tag{6}$$

At the endothelial boundary $x = 0$ the boundary conditions encapsulate the activity of the endothelium. HDL flows into the domain, ES cytokines flow outwards at a rate proportional to the concentration of ES cytokines in the intima, foam cells do not cross this boundary:

$$J_h = \sigma_h\,; \tag{7}$$

$$J_q = -\sigma_q q\,; \tag{8}$$

$$J_N = 0\,. \tag{9}$$

where J_h, J_q and J_N is the inward flux of HDL, ES cytokines and foam cells respectively across the boundary. The flux of modLDL across $x = 0$, J_ℓ, is modulated by the presence of HDL which reduces LDL oxidation. The flux of monocyte

chemoattractant J_m is determined by the rate that it is produced by endothelial cells, a function of ES cytokine and modLDL concentrations:

$$J_\ell = \sigma_\ell \frac{1 + h/\alpha_\ell}{1 + h/\gamma_\ell}\,; \tag{10}$$

$$J_p = \sigma_{p_1} \frac{\ell}{\beta_p + \ell} + \sigma_{p_2} q\,. \tag{11}$$

The inward flux of macrophages is dependent on endothelial excitation by ES cytokines, by the action of monocyte chemoattractants and the anti-inflammatory effects of HDL.

$$J_m = \sigma_m \frac{1 + h/\alpha_m}{1 + h/\gamma_m}(1 + Aq)(p - P_0)H(p - P_0), \tag{12}$$

where $H(x)$ is the Heaviside function.

All variables had no flux boundary conditions on $x = 1$.

PDEs were solved using the software package FlexPDE and the bifurcation curves for the steady states of the set of equations were found numerically using AUTO [28]. The complete set of parameter values are given in Table 2.

It is possible to formulate models for plaque development which have a moving boundary that enables the increasing numbers of foam cells to distort the intima [33]. Here we are primarily concerned with HDL dynamics rather than intimal thickening and made the simplifying assumption that the domain was fixed; that is, that the influx of modLDL and macrophages did not significantly distort the intima.

Table 2. Parameter values used in the model. The values below have been rescaled in space and time in order to normalise the intima width. We take an intima width of 40 μm [29]. We use a time scale of $\sim 7.7 \times 10^6$ s (approximately 89 days) to rescale the equations. Further details on the rescaling can be found in [27].

Parameter	Value after rescaling	Reasoning/Description
D_ℓ	10^4	Diffusion of LDL paricles ~ 2 μm^2/s. [30]
μ_ℓ	10^5	Estimated consumption rate of LDL by macrophages.
d_ℓ	10^1	Estimated decay rate of LDL.
σ_ℓ	10^3	Estimated influx rate of LDL.
D_p	10^6	Diffusion of chemoattractant ~ 200 μm^2/s. [31]
μ_p	10^6	Estimated production rate of chemoattractants by macrophages consuming LDL.
d_p	10^3	Decay rate of chemoattractant $\sim 10^{-4}$/s. [32]
σ_{p_1}	10^5	Estimated production rate of chemoattractants by endothelial cells by LDL stimulation.
σ_{p_2}	10^4	Estimated production rate of chemoattractants by endothelial cells by ES cytokine stimulation.
β_p	10^0	Estimated saturation constant of LDL stimulation of endothelial cells in the production of chemoattractant.
D_m	10^2	$\ll D_\ell$. Estimated random movement of macrophages.
χ_m	10^3	Estimated chemotactic term due to scavenging of LDL.
μ_m	10^2	$\ll \mu_\ell$. Estimated coversion rate of macrophages to foam cells via LDL consumption.
d_m	10^0	Estimated decay rate of macrophages.
σ_m	10^{-3}	Estimated adhesion efficacy on inward migration of monocytes into the intima.
A	10^{-2}	Estimated efficacy of ES cytokines on the transmigration of monocytes into the intima.
P_0	10^{-1}	Estimated background chemoattractant levels.
D_q	10^6	Diffusion of chemoattractant ~ 200 μm^2/s. [31]
μ_q	10^6	Estimated production rate of ES cytokines by macrophages consuming LDL.
d_q	10^3	Decay rate of chemoattractant $\sim 10^{-4}$/s. [32]
σ_q	10^0	Estimated outflux of ES cytokines
D_N	10^{-2}	$\ll D_m$. Estimated random movement of macrophages.
ν_N	10^1	Estimated coversion rate of foam cells to macrophages via HDL reverse cholesterol transport.
D_h	10^4	Assumed same as D_ℓ.
ν_h	10^4	Estimated consumption rate of HDL through reverse cholesterol transport.
d_h	10^1	Estimated decay rate of HDL.
κ	10^0	Estimated saturated constant of HDL in reverse cholesterol transport.
σ_h	Varies	Estimated influx of HDL into the intima.
α_ℓ	10^1	With γ_ℓ, estimated efficacy of HDL on the oxidation of LDL.
γ_ℓ	10^{-1}	$\ll \alpha_\ell$.
α_m	10^1	With γ_ℓ, estimated efficacy of HDL on the oxidation of LDL.
γ_m	10^{-1}	$\ll \alpha_m$.

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Acknowledgments