

Modelling Cholesterol Metabolism and Atherosclerosis

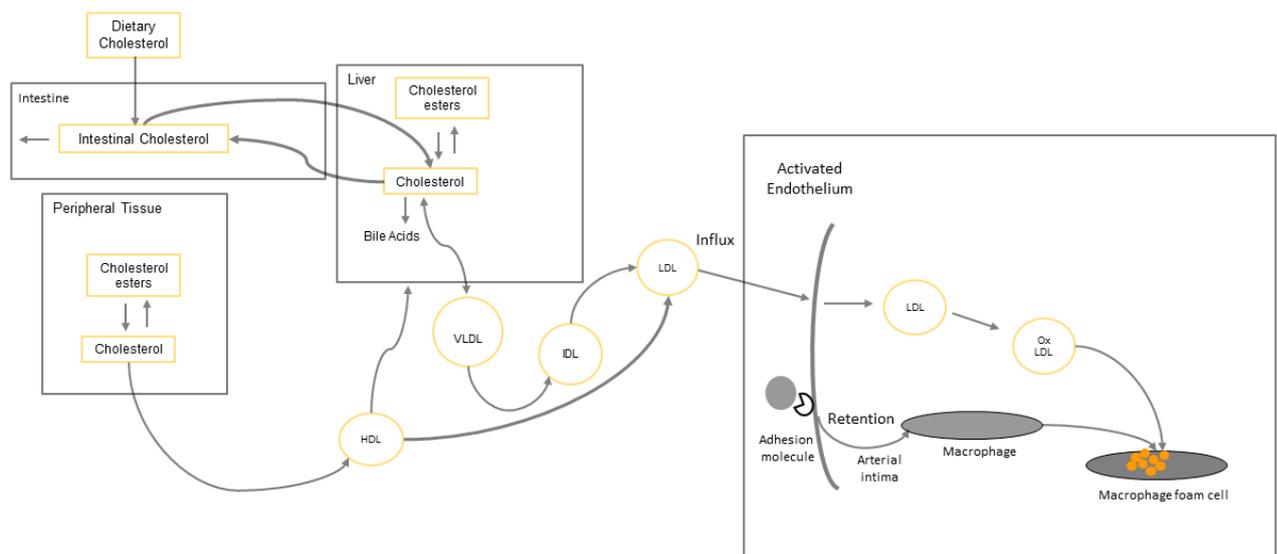
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Abstract

Atherosclerotic cardiovascular disease (ACVD) is the leading cause of morbidity and mortality amongst Western populations. Many risk factors have been identified for ACVD; however elevated low-density lipoprotein cholesterol (LDL-C) remains the gold standard. Cholesterol metabolism at the cellular and whole-body level is maintained by an array of interacting components. These regulatory mechanisms have complex behaviour. Likewise, the mechanisms which underpin atherogenesis are nontrivial and multifaceted. To help overcome the challenge of investigating these processes mathematical modelling, which is a core constituent of the systems biology paradigm has played a pivotal role in deciphering their dynamics. In so doing models have revealed new insights about the key drivers of ACVD. The aim of this review is four-fold; to provide an overview of cholesterol metabolism and atherosclerosis, to briefly introduce mathematical approaches used in this field, to critically discuss models of cholesterol metabolism and atherosclerosis, and to highlight areas where mathematical modelling could help to investigate in the future.

Graphical/Visual Abstract and Caption



[1. INTRODUCTION]

Cardiovascular disease (CVD) is the number one cause of death globally (Mc Namara et al., 2019). Over the last number of decades several risk factors have been identified for developing CVD, these include elevated low-density lipoprotein cholesterol (LDL-C) (FERENCE et al., 2017), physical inactivity (Lachman et al., 2018), genetic predisposition (Berberich & Hegele, 2019), obesity (Cercato & Fonseca, 2019), diabetes mellitus (Einarson et al., 2018), dysregulated fatty acid metabolism (Morgan et al., 2016), hypertension (Fuchs & Whelton, 2020), an unhealthy diet (Casas et al., 2018), tobacco smoking (Banks et al., 2019), and excessive alcohol consumption (Toma et al., 2017). Pathophysiologically, CVD is underpinned by atherosclerosis (Figure 1), a chronic inflammatory process defined by a cascade of events which commences with LDL permeating the arterial endothelium and entering the intima (Borén et al., 2020). Macrophages then engulf the LDL by phagocytosis becoming lipid laden foam cells, which collectively generate fatty streaks (Moore et al., 2018). Fatty streaks evolve into plaques, which can rupture resulting in a heart attack or stroke (Bentzon et al., 2014; Mortensen et al., 2020).

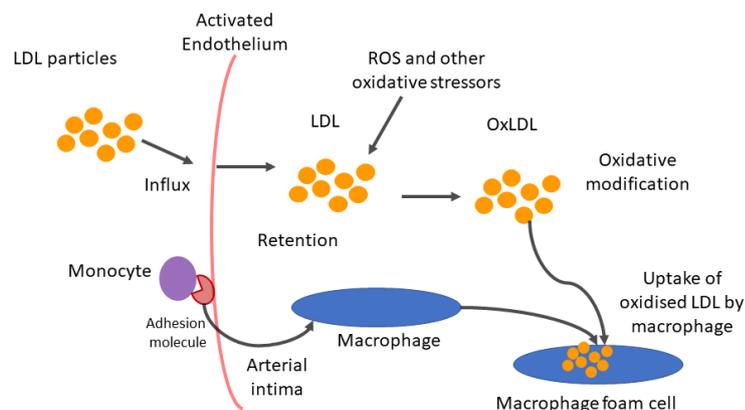


Figure 1. A strongly simplified graphical representation of the mechanisms which underpin atherosclerotic plaque formation. The process begins when LDL particles penetrate the endothelium and accumulate in the intima. There they undergo oxidative modification which makes them pro-inflammatory/immunogenic. Circulating monocytes then bind to adhesion molecules expressed by activated endothelial cells. These cells express scavenger receptors that facilitated the binding of lipoprotein particles. This converts them to foam cells. LDL, low density lipoprotein; OxLDL, Oxidised LDL; ROS, reactive oxygen species

Due to the key role of LDL in the aetiology of atherosclerosis, this has led to the establishment of the so called “cholesterol hypothesis” which postulates that

lowering LDL-C decreases atherosclerotic CVD (ACVD) risk (Jinnouchi et al., 2020). Based on this premise there is an ongoing medical imperative to identify therapeutic avenues which help to maintain optimal LDL-C levels, and lower ACVD risk. The pharmacological agents used to treat elevated LDL-C target key regulatory points in cholesterol metabolism (Zodda et al., 2018). These regulatory processes are inherently complex (Mc Auley & Mooney, 2018). Inter-individual variations in the behaviour of these mechanisms is well recognised and adds further to this challenge (Meshkini et al., 2017; Palmisano et al., 2018). Consequently, lipid lowering interventions are not universally effective, hence the continual drive to identify novel lipid lowering modalities (Davies et al., 2016).

When investigating cholesterol metabolism and its intersection with ACVD, it is imperative to recognise that this system becomes dysregulated with age (Mc Auley, 2018; Morgan et al., 2016a). A further layer of complexity is added due to the interplay between LDL and atherogenesis. During atherogenesis endothelium dysfunction facilitates LDL permeation, while the role of other mechanisms including neovascularization, vascular proliferation, apoptosis, matrix degradation, oxidative stress, and inflammation modulate this process (Yang et al., 2017). Arterial LDL uptake is also influenced by haemodynamic factors, including wall shear stress (WSS) (Cunningham & Gotlieb, 2005). The role of high-density lipoprotein cholesterol (HDL-C), ubiquitously regarded as atheroprotective, mainly due to its role in reverse cholesterol transport (RCT), also necessitates inclusion when investigating the pathophysiology of ACVD (Ouimet et al., 2019). Collectively, these factors present an intimidating picture of a multitude of variables interacting over time and space. As a result of this inherent difficulty mathematical models have been widely used to represent, examine, and analyse various aspects of cholesterol metabolism, and its intersection with atherosclerosis. In so doing they have yielded new insights into the factors which drive ACVD. In this review I will critically discuss these models and highlight areas of this field where mathematical modelling has the potential to elucidate in future.

[2. THE REGULATION OF CHOLESTEROL METABOLISM]

Whole body cholesterol metabolism is regulated by an array of mechanisms. These processes respond to perturbations in cholesterol intake, absorption, synthesis, and excretion (Luo et al., 2020; Mooney & Mc Auley, 2016). In humans the dietary intake of cholesterol is generally low (~300mg in males and females), consequently the majority of intestinal cholesterol is endogenously derived (Mok et al., 1979; Williams Sr et al., 2015). Several mechanisms oversee the absorption process. Cholesterol esterase, hydrolyses the ester bond of cholesteryl esters, liberating free cholesterol (FC) and fatty acids (Chang et al., 2009). The polytopic transmembrane protein Niemann-Pick C1-Like 1 (NPC1L1) controls cholesterol absorption into the enterocyte (Altmann et al., 2004). Following this ATP-binding cassette (ABC)

transporters G5 and G8 (ABCG5/G8) regulate the cholesterol efflux from the enterocyte to the lumen (Yu et al., 2014). Acetyl CoA acetyltransferase 2 (ACAT2) catalyses the re-esterification of FC with apolipoprotein B-48 (apoB-48), phospholipids and triglycerides (TGs) to create a chylomicron (CM) (Nguyen et al., 2012). The TG core of circulating CMs are hydrolysed by lipoprotein lipase (LPL) (He et al., 2018). The TG-depleted CM remnants (CR), which are enriched in cholesteryl esters are removed from the circulation by hepatic remnant receptors (Cooper, 1997). The liver is the hub of cholesterol metabolism, and it is here that cholesterol is packaged together with TGs into very low-density lipoproteins (VLDLs) (Feingold & Grunfeld, 2015). Like CM, VLDLs are hydrolysed at the endothelium by LPL to generate intermediate density lipoproteins (IDLs) (Chait et al., 2020). In contrast to CR however, IDLs have two opposing metabolic fates; IDLs can be removed by the liver, or they are catabolised further to LDLs (Ooi et al., 2012). The LDL receptor (LDLr) and LDLr-related protein 1 (LRP1) play a critical role in removing LDL from the circulation (Goldstein & Brown, 2009; Lillis et al., 2005). This process is intimately connected to intracellular sterol levels. A rise in intracellular cholesterol triggers, insulin-induced genes (Insigs) proteins (Lee et al., 2020). Insig-1 and Insig-2 bind to sterol regulatory element-binding protein cleavage-activating protein (SCAP) in the endoplasmic reticulum (ER), limiting the migration of the SCAP/sterol regulatory element-binding protein (SREBP) complex to the Golgi (Brown et al., 2018). A decline in sterol concentration precipitates the migration of SREBP-2 to the Golgi; where it is split by subtilisin kexin isozyme/Site-1 protease (SKI-1/S1P), and the intramembranous metalloprotease Site-2 protease (S2P); this liberates the NH-terminal domain of SREBP-2 from the membrane (Elagoz et al., 2002). Two N-terminal fragments dimerize, then interact with importin- β , and then migrate to the nucleus, to trigger SREBP-2-regulated gene promoters (S. J. Lee et al., 2003). Regulation also occurs during LDLr synthesis. Nuclear SREBP-2 augments the transcription of proprotein convertase subtilisin/kexin type 9 (PCSK9) (Jeong et al., 2008; Sobati et al., 2020). PCSK9 elevates the degradation of LDLr, which subsequently limits plasma LDL removal. Elevated intracellular cholesterol inhibits the release of SREBP-2 from the ER (Radhakrishnan et al., 2008). This reduces PCSK9 transcription which in turn elevates LDLr (Lagace, 2014). Collectively these mechanisms help to regulate plasma LDL-C levels. Therefore, it is unsurprising that a recent study found that elevated levels of PCSK9 in male patients have been associated with peripheral artery disease (Kheirkhah et al., 2020). Moreover, PCSK9 is recognised as a target which can significantly lower LDL-C by up to 60% (Sabatine et al., 2015). There is little doubt PCSK9 will continue to be a therapeutic target in the future.

RCT is also crucial to maintaining whole body cholesterol metabolism. Essentially this process involves the removal of excess peripheral cholesterol (Marques et al., 2018). The initial stage of RCT involves the generation of nascent HDL. Nascent

HDL are derived from cellular lipids and extracellular lipid-free or lipid-poor apolipoprotein AI (apoAI), in a process mainly regulated by ATP-binding cassette transporter 1 (ABCA1) activity (Wang & Smith, 2014). Lecithin-cholesterol acyltransferase (LCAT) esterifies nascent HDL cholesterol as they progress to mature HDL particles. HDLs migrate to the liver via direct hepatic uptake by scavenger ester class B type I (SR-BI), or indirectly via the action of cholesteryl ester transfer protein (CETP), which redistributes cholesterol to apoB-by the liver (Zannis et al., 2006). Cholesterol can then be converted to bile acids for excretion. As a result RCT is widely accepted as an antiatherogenic process (Ouimet et al., 2019). Consequently, RCT has also been the subject of therapeutic investigations which have sought to identify novel therapeutic strategies for optimising cholesterol metabolism.

[3. AN OVERVIEW OF ATHEROSCLEROSIS]

ASCVD is defined by atherosclerosis. Atherosclerosis is a chronic inflammatory process which is underpinned by a series of biological processes which result in the formation of fatty streaks and eventually a plaque. The process begins when apoB-containing lipoproteins such as LDL, permeate the arterial endothelium and penetrate the intima (Abdolmaleki et al., 2019; Sniderman et al., 2019). Circulating monocytes then bind to adhesion molecules expressed by activated endothelial cells (Pamukcu et al., 2010). These cells express scavenger receptors that facilitate the attachment of lipoproteins (Ding et al., 2018). Monocyte derived macrophages engulf the lipoprotein by phagocytosis, becoming lipid laden foam cells (Moroni et al., 2019). Macrophages are central to creating a microenvironment which is defined by inflammation as they generate reactive oxygen species (ROS) and secrete a range of proinflammatory molecules including chemokines and cytokines (Jacinto et al., 2018). In addition to inflammation and oxidative stress, other factors including vascular proliferation, neovascularization, apoptosis, and matrix degradation transform these laden macrophage cells ("fatty streaks") into complex rupture-susceptible plaques (Costopoulos et al., 2017). As alluded to at the beginning of this section central to this process is LDL. Over many decades a wealth of evidence has associated this apo-B containing lipoprotein within the aetiology of atherosclerosis (Silverman et al., 2016; Vega & Grundy, 2019). Thus, the LDL particle has become firmly embedded within the atherogenesis paradigm. Consequently there is an unrelenting biomedical drive to identify pharmacological modalities to lower LDL-C (Klein-Szanto & Bassi, 2019).

In addition to elevated LDL-C, genetics, diet, physical inactivity, and smoking are established risk factors for atherosclerosis (Ng et al., 2020). However, in recent years many other risk factors have been identified for ACVD (Libby, 2021). For

example, it has been revealed that hormonal changes could influence the aetiology ACVD (Mc Auley & Mooney, 2014). Moreover, the gut microbiome is increasingly being recognised as a modulator of atherosclerosis (Jonsson & Bäckhed, 2017). It has been found that perturbations to the gut microbiota interfere with lipid metabolism (Schoeler & Caesar, 2019). Moreover, alterations to the gut microbiome are thought to drive key inflammatory pathways. Inflammation is central to the pathogenesis of atherosclerosis (Karl et al., 2018).

Emerging evidence also suggests that epigenetics influences atherosclerosis progression. For example, the plasticity of macrophages appear to be partly controlled by epigenetic enzymes (Chen et al., 2020). Interestingly, the promoters of many genes associated with age related disease undergo hypermethylation with age (Morgan et al., 2018; Tabaei & Tabaei, 2019). Epigenetic change is often brought about because of the environment. In fact, air pollution aggravates pro-inflammatory pathways (Bove & Ghiselli, 2018). These nascent revelations underscore the complexity of atherosclerosis. This review will unveil how mathematical modelling can help to represent and investigate this complexity.

[4. MODELLING APPROACHES]

At the core of all computational systems biology models is mathematics (Mc Auley et al., 2009; Mc Auley et al., 2015; Salcedo-Sora & Mc Auley, 2016). Many theoretical approaches can be used to model biological systems (Mc Auley et al., 2017; Mooney et al., 2016). The technique that is used is largely dependent on the nature of the biological system (Mc Auley et al., 2013). However, the most widely used technique employed to model cholesterol metabolism has been ordinary differential equations (ODEs) (Mc Auley & Mooney, 2015b; Mc Auley, 2019a). These equations are referred to as ordinary because they depend on one independent variable only (time). They are suitable when it is necessary to model dependent variables with respect to time. For example, this could be a variable such as the concentration of LDL-C. Several computational tools are routinely employed to solve ODEs, and it is not necessary for biologists to be overly familiar with mathematics (Mc Auley & Mooney, 2015a; Mc Auley, 2019b).

Despite their wide-spread application ODEs have several drawbacks. For instance, their continuous and deterministic framework makes them inappropriate for representing random behaviour at the molecular level. Probabilistic models can be used to overcome this. For example, stochastic differential equations can be used to represent discrete random collisions at the molecular level in biological systems (Wilkinson, 2006). ODEs also fall short when it is necessary to represent biological systems with more than one independent variable. In this situation it is appropriate to use partial differential equations (PDEs). PDEs differ from ODEs as they are

multivariable functions with partial derivatives, and they are routinely employed in this field when modelling arterial structure and blood flow.

Boolean models have also been used in this field (Schwab et al., 2020). A Boolean model is made of network of discrete variables. They are ideal for representing gene networks because they can easily represent the qualitative nature of gene expression data that is commonplace in molecular biology. This helps to overcome the need for quantitative data which is a requirement of enzyme kinetic based ODE models (Barbuti et al., 2020). An example of their application in this field is that they have been employed to study gene regulatory network which underpins intracellular cholesterol homeostasis (Kervizic & Corcos, 2008). Crucially however the advantage of Boolean models is also their disadvantage. Their major limitation is that they are incapable of including biological mechanisms or enzyme kinetics.

Physiologically based kinetic (PBK) models have also been employed in this field. PBL models as their names suggests are concerned with the kinetics of physiological processes in the whole body. As a mathematical framework they facilitate the integration of data relating to absorption, distribution, metabolism and excretion of a chemical within the body (Paini et al., 2019). Due to their integrated nature, they are an ideal way to model whole-body cholesterol metabolism. Closely related to PBK models are systems pharmacology models. These are mechanistic models which focus specifically on drug interactions with biological processes (Bradshaw et al., 2019). Their goal is to understand how a biological system or disease responds to pharmacological modulation.

In recent years, the field of cholesterol and atherosclerosis modelling has benefitted from advances in systems biology. It is now straightforward to exchange and update mathematical models of biological systems. This is largely due to the introduction of the systems biology markup language (SBML), a community-orientated XML-based file format created specifically to facilitate model exchange (Hucka et al., 2003). SBML models can be archived in the online repository BioModels (Malik-Sheriff et al., 2019). This makes models of cholesterol metabolism/atherosclerosis increasingly easier to adapt and modify as our knowledge of a biological system progresses. It is also becoming increasingly straightforward to represent the topology of a model. This is due to the use of systems biology graphical notation (SBGN) (Rougly et al., 2019), a standardised graphical schema which is used to visually capture mathematical models of biological systems.

[5. LDLR MEDIATED ENDOCYTOSIS AND LIPOPROTEIN DYNAMICS]

Modelling in this field originated in the 1970s, with the theoretical conceptualisation of LDL transport and accumulation in the arterial wall (Bratzler et al., 1977). It was

assumed arterial LDL uptake obeyed first order reaction kinetics (Truskey, 1985; Truskey et al., 1984). Based on this premise a set of ODEs was developed to represent LDL binding, internalization, and degradation. A model which adopted this framework was created by Chun and colleagues, where ODEs represented the recycling of low density lipoproteins (LDLr) in human skin fibroblasts (Chun et al., 1985). Model output reinforced experimental findings that oxysterol/hydroxycholesterol down-regulates cell surface receptor production. It also suggested LDL breakdown and the intracellular build-up of cholesterol does not inhibit the downregulation of LDLr synthesis. Harwood and Pellarin also created a kinetic model of LDL dynamics. (Harwood & Pellarin, 1997). This model focused on LDLr mediated endocytosis. Using modelling together with labelled [14C] sucrose-LDL in Hep-G2 cells enabled the kinetics of LDLr-mediated endocytosis to be determined. The model suggested that LDL-LDLr interactions are defined by a receptor recycling process that is rapid and serves to maintain 80% of LDLr on the cell surface in a steady-state. Interestingly, mathematical modelling has also been used to reveal that avidity (binding 'tightness') is the major regulator of LDLr activity (Shankaran et al., 2007).

Mathematical modelling has also played a role in exploring the dynamic relationship between LDL, very low-density lipoprotein (VLDL) and LDLr mediated endocytosis. For instance, Wattis and colleagues investigated the response of LDLr to a single bolus of extracellular LDL, versus a continuous supply of LDL (Wattis et al., 2008). The model suggested competition between LDL and very low-density lipoproteins (VLDLs), for binding to cellular surface pits, impacts intracellular cholesterol concentration. Specifically, it revealed that under constant low-level delivery of lipoproteins to the cellular surface, more VLDL than LDL will occupy the pits. This suggests VLDLs are more effective competitors for receptor binding. Tindall and colleagues also included VLDLs in a system which represented hepatocyte LDL/VLDL uptake (Tindall et al., 2009).

Simulations suggested the binding, unbinding, and internalisation rates, together with the fraction of receptors recycled, are key to LDL/VLDL uptake. Similarly, Pearson and colleagues modelled the binding and uptake of LDL/VLDL in cultured hepatocytes (Pearson et al., 2009). Model findings matched *in vitro* data which described LDL uptake in cultured hepatocytes. Translated to an *in vivo* context this suggests competition between LDL and VLDL for cellular pits modulates intracellular cholesterol. Due to the crucial role PC subtilisin/kexin type 9 (PCSK9) plays in the degradation of LDLr it is unsurprising more recent models in this area have focused on this molecule (Lagace, 2014). To investigate PCSK9 Gadkar et al. (2014) created a model of virtual subjects, based on preclinical data (Gadkar et al., 2014). Model output revealed a combination of anti-PCSK9, and atorvastatin was more effective at lowering LDL-C than anti-PCSK9 on its own. Sokolov et al. (2019)(Sokolov et al., 2019) also created a model to investigate PCSK9. This ODE model was calibrated by estimating plasma kinetic parameters for key lipoproteins. The model was

validated with quantitative formation on PCSK9/LDL-C together with data on non-HDL-C, TC, TG, and apoB dynamics from clinical trials. The model suggested LDL-C decreases proportionally to PCSK9 reduction for both monoclonal antibodies and small interfering RNAs.

[6. INTRACELLULAR CHOLESTEROL METABOLISM]

The mechanisms which control the cholesterol biosynthesis pathway are well recognised as a therapeutic target for lowering LDL-C (Istvan & Deisenhofer, 2001). Due to its complexity, containing feedback loops and enzymatic regulators, modelling has offered an ideal way to study this system. Yuan and colleagues constructed one of the first models of intracellular kinetic model of cholesterol homeostasis (Yuan et al., 1991) (Figure 2). By formalising the conception outlined figure 2 it was demonstrated that for a slowly increasing extracellular LDL concentration the intracellular degradation of LDL initially rises, and then reaches a saturation point.

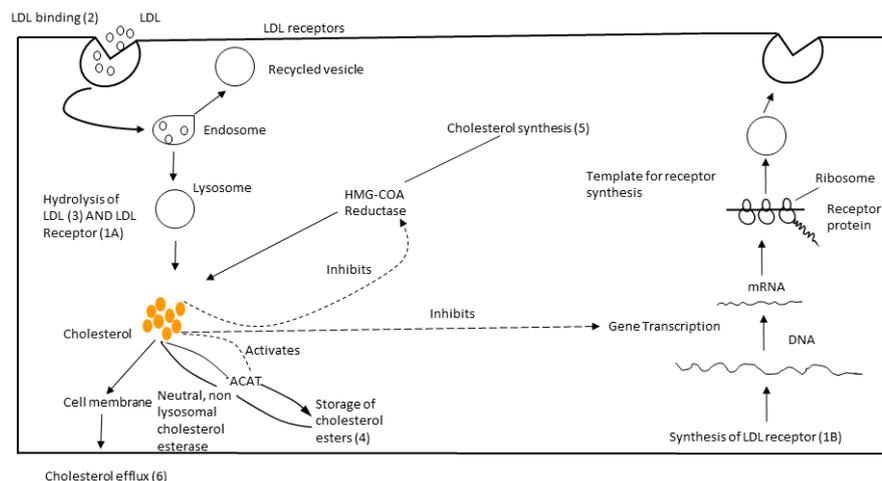


Figure 2. Graphical representation of an early model of intracellular cholesterol metabolism by Yuan et al. (1991). The diagram captures the steps involved in the LDL receptor-mediated pathway in cultured human fibroblasts. The model has 6 steps: (1A, B) hydrolysis and synthesis of LDLrs, (2) LDLr binding/internalization of LDL, (3) lysosomal hydrolysis of LDL, (4) storage of cholesteryl esters (CE), (5) regulation of cholesterol synthesis, and (6) the efflux of free cholesterol (FC). This

model examined the relationship between intracellular FC concentration and extracellular LDL levels.

One of the most intriguing early models of cholesterol metabolism is that of Shefer et al. (1995) (Shefer et al., 1995). This model tested a treatment for hypercholesteremia by using a snake venom derived immobilized enzyme within a modelled bioreactor. Venom altered LDL, expediting the hepatic removal of this lipoprotein. In 2007 August and colleagues broadened the scope in the field by creating a model of lipoprotein catabolism which also examined intracellular cholesterol homeostasis (August et al., 2007). ODEs captured the processes associated with the transit of lipoproteins from the liver to the plasma. By using stability analysis, the model revealed that intracellular cholesterol levels are tightly regulated. Conversely, the model demonstrated that plasma concentrations of cholesterol can fluctuate significantly when key regulatory parameters are altered. Specifically, intermediate density lipoprotein-cholesterol (IDL-C) and LDL-C. This cardinal finding emphasised that plasma cholesterol unlike intracellular cholesterol is not subject to rigid homeostatic regulation. This resonates with experimental observations which have identified significantly inter-individual variability in plasma LDL-C (Abbott et al., 1983).

More recent models have examined lipoprotein dynamics in greater breath. For example, Sips and colleagues created a murine kinetic model of lipoprotein metabolism (Sips et al., 2014). The model was used for the longitudinal analysis of C57Bl/6J mice following a pharmaceutical intervention with a liver X receptor (LXR) agonist. More recently, Boolean networks have been used to represent cholesterol biosynthesis. Kervizic and Corcos created a Boolean network model of cholesterol biosynthesis (Kervizic & Corcos, 2008). The model was able to simulate the action of statins. In 2013 Mazein and colleagues created largely qualitative representations of cholesterol biosynthesis (Mazein et al., 2013) The pathway was represented in SBGN. This system was subsequently modelled more quantitatively (Watterson et al., 2013). Informed by data from primary bone marrow derived macrophage cells infected with murine cytomegalovirus or treated with IFN γ ; the model revealed that the down regulation of enzymes central to cholesterol biosynthesis induced a graduated flux reduction. The graduated reduction was mediated by the coordinate regulation of multiple enzymes. It was suggested, the coordinate regulation of cholesterol biosynthesis demonstrates a long-term evolutionary advantage over single enzyme regulation, and that pharmaceutical therapies could target this system with greater specificity based on this finding. Blattmann and colleagues also developed a model of intracellular cholesterol regulation to investigate different pharmacological agents (Blattmann et al., 2017) Based on experimental evidence that cells and tissues have a heterogeneous response to pharmacological agents; the aim was to capture the variability in intracellular drug response after a variety of perturbations in different cell lines. Models were built using mass spectrometry data

and simulations revealed cell-line-specific drug-response phenotypes. A systems pharmacology approach was also adopted by Benson et al. (2017). Informed by representations of the cholesterol biosynthesis pathway from the KEGG database (Kanehisa & Goto, 2000), SBGN diagrams were generated (Benson et al., 2017).

Building the model revealed a significant challenge to modelling work of this nature. It was found that online kinetic databases contain many inconsistencies in key enzymatical parameters. In 2018 Pool and colleagues created a model of the mevalonate pathway (Pool, Currie, et al., 2018). The model revealed that 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGCR), farnesyl-PP and squalene synthase together with negative feedback between cholesterol and sterol regulatory element-binding protein 2 (SREBP-2), are central to the regulation of the mevalonate cascade.

Interestingly, Pool et al. (2018) combined the Bhattacharya et al 2014 and Tindall et al. (2009) models of cholesterol biosynthesis and lipoprotein metabolism to create a more integrated representation of cholesterol metabolism (Pool, Sweby, et al., 2018). The merged system found that a small perturbation to the VLDL to LDL conversion rate significantly impacted extracellular LDL levels. It was posited this could be a therapeutic avenue for reducing LDL-C. Toroghi and colleagues also modelled the cholesterol biosynthesis pathway encased within a multi-scale whole-body model of LDL-C regulation (Toroghi et al., 2019). The model explored combination therapy involving anti-PCSK9 and atorvastatin drugs. Simulations suggested this approach is necessary to reduce LDL-C levels in individuals with high HMGCR activity. Most recently, Morgan and Mc Auley developed an intracellular representation of hepatic cholesterol homeostasis and its intersection with aging (Morgan & Mc Auley, 2020). This model found that a ROS induced rise in HMGCR had a limited impact on cholesterol homeostasis. In contrast an age-related decline in hepatic Acetyl-CoA acetyltransferase 2 activity raised FC levels. Important regulatory features of the Mc Auley et al.(2012) model were also used by Bourgin and colleagues in an approach which used three methodologies to study cholesterol metabolism (Bourgin et al., 2020). These were bacterial/Bile salt hydrolase assays, isotope tracer studies using mice and mathematical modelling. The novelty of the work centred on the influence of gut bacteria on cholesterol plasma cholesterol levels. Bayesian analysis was used to determine unknown parameters, and the sensitivity of the model was explored with local and global parameter scans. The model predicted that bile salt degradation is a key driver of a reduction in plasma cholesterol.

[7. WHOLE BODY CHOLESTEROL METABOLISM]

Considerable efforts have been made in the last decade to represent cholesterol metabolism more holistically (Morgan et al., 2017). For instance, Van de Pas and

colleagues created a 21 reaction PBK model of cholesterol metabolism (van de Pas et al., 2012). The model predicted that an increase in dietary cholesterol leads to increased total cholesterol (TC), increased non-HDL-C, and marginally lower HDL-C level. Van de Pas et al and colleagues extended this model to observe plasma cholesterol concentrations as a function of pravastatin dose (van de Pas et al., 2014) A population of 7,609 virtual individuals was generated using Monte Carlo simulation, and the response to a 40 mg/d pravastatin dose was simulated for each subject. The model predicted that hepatic and peripheral cholesterol synthesis were key factors in determining a non-HDL-C reduction by statins. Other models have also represented whole-body cholesterol metabolism. For example, Eussen and colleagues created a model which captured the interlocking features of cholesterol metabolism (Eussen et al., 2011). Compartments represented the liver, extrahepatic tissue, and blood. The model demonstrated that 2g/day of plant sterols reduced LDL-C levels by ~8%-9%, in conjunction with statins. In addition to not being encoded in SBML a further model drawback is that it overlooks some key regulators of cholesterol metabolism. Addressing this issue Mc Auley and colleagues created the first whole body model of cholesterol metabolism using ODEs (Mc Auley et al., 2012). The model was represented using systems biology graphical notation (SBGN) (Rouhny et al., 2019), a standardised graphical schema which is used to visually capture mathematical models of biological systems. The model was also encoded in SBML. In addition to being encoded in SBGN/SBML the most pertinent feature of the model was that it acted as a framework to examine the age-onset dysregulation of cholesterol metabolism. The model suggested that a 30% increase in cholesterol absorption between the ages of 20 and 60-years results in 34 mg/dl increase in LDL-C. However, the key model finding was that a 50% drop in hepatic LDLr by 65 years was the main driver of a significant rise in LDL-C. The adaptability of this model was emphasised by Mishra and colleagues when it was used to identify a flip response in plasma cholesterol levels due to altering the sensitivity of cholesterol absorption and intestinal bile salt levels (Mishra et al., 2014). Moreover, the utility of this model was further emphasised by Paalvast and colleagues (Paalvast et al., 2015) who found that after one year of simvastatin therapy LDL-C reduced by 33% in model simulations. This was similar to *in vivo* findings which have shown that this regime reduces LDL-C levels by ~30-40% (Corti et al., 2001; Schachter, 2005). Hrydziuszko et al. (2014) also created a whole-body model of this system by distilling cholesterol metabolism into liver and blood compartments, represented by two ODEs (Hrydziuszko et al., 2014). The model demonstrated how variations in cholesterol synthesis impacts plasma cholesterol levels.

[8. REVERSE CHOLESTEROL TRANSPORT]

Modulating RCT offers the possibility of lowering CVD risk (Kontush, 2020; Ouimet et al., 2019) and models have been utilised to explore this process in greater depth. For instance, Hübner and colleagues used modelling to focus on a key aspect of RCT.

The model simulated impaired ABCA1 activity (Hübner et al., 2008). The model was able to successfully replicate familial hypoalphalipoproteinemia data that found that a reduction in ABCA1 activity significantly reduces cholesterol levels in all HDL fractions. Gadhar and colleagues also focused on RCT. This model investigated several HDL targets to determine the most effective way to improve RCT (Gadkar et al., 2016). The model suggested the induction of ApoA-I synthesis is an effective way to lower CVD risk because of its ability to increase RCT over an extended time-period. Jansen et al. (2016) and colleagues also investigated RCT. Model reactions were informed by blood samples taken from 15 healthy individuals. The model predicted a selective CE efflux of 8.4 $\mu\text{M}/\text{h}$ out of LDL, an efflux which according to the model is necessary for the loss of CE from LDL during their retention, and hence for the decrease in LDL size (Jansen et al., 2016).

Cholesteryl ester transfer protein (CETP) is key to RCT. It facilitates the transfer of cholesterol esters (CE) from HDL, in exchange for triglycerides (TGs) from VLDL and LDL. Low CETP increases HDL formation (Morton & Liu, 2020), thus CETP inhibition is a key pharmacological target (Tall & Rader, 2018). To address this Potter and colleagues (2009) developed a model where CETP binds with CE and TG creating “stores” of CE and TG, that exchange with lipoproteins (Potter et al., 2009). The model suggested the net transfer activity of CETP is acutely impacted by the relative preference for homo-exchange (a TG enters, and a TG leaves the lipoprotein) versus heteroexchange (a TG enters the lipoprotein).

In 2016 the Mc Auley et al. (2012) model was updated to focus on RCT and aging. (Morgan et al., 2016b) The model explored the potential role of CETP polymorphisms in healthspan. By investigating the effects of aging in conjunction with three different CETP genotypes. Aging together with an atheroprotective CETP genotype, conferring low CETP activity, resulted in a 0.6% increase in LDL-C. In comparison, aging with a genotype commensurate with a high CETP activity, resulted in a 1.6% increase in LDL-C. This finding emphasized the potential role CETP genotypes have in contributing to a healthy aging phenotype. The model also found that lipoprotein processing was extremely sensitive to global parameter perturbations, a finding which resonated with August et al. (2007). Jansen and colleagues also focused on CETP by adapting the Potter et al (2009) model to estimate CETP TG flux (Jansen et al., 2019). It was assumed if CETP mediates an exchange between two lipoproteins or between a CETP loaded with CE or TG, neither CE nor TG is preferred. The probability of CETP associating with a lipoprotein was estimated to be proportional to its surface area. In contrast to previous models, it was assumed the lipoprotein core only consists of TG and CE. Using this framework, the model was able to consolidate experimental data associated with HDL and LDL dynamics. Lu and colleagues used mathematical modelling to explore RCT in even greater depth. In this model. HDL dynamics were explicitly represented (Lu et al., 2014). This model included the geometric structure of HDL, linked to how many ApoA-I molecules are on its surface. The model

focussed on CETP inhibition and ABCA1 up-regulation. Simulations found that while CETP inhibition did not result in an increased RCT rate, ABCA1 up-regulation increased both HDL-C and RCT rate.

[9. MODELLING THE PATHOPHYSIOLOGY OF ATHEROSCLEROSIS]

Multiple processes are at work during atherogenesis. These pathogenic interactions are ongoing throughout life (Pollock et al., 2019). Consequently, models of atherosclerosis need to have a broad outlook. An early model which explored the pathophysiology of atherosclerosis is that of Cobbold et al. (2002). This model incorporated the build-up of cholesterol within sub-endothelial cells to generate a fatty streak, rationalizing that cholesterol deposition is due to LDL-C oxidation (Cobbold et al., 2002). According to the model an *in silico* dietary intervention with ascorbate did not significantly abrogate lipid peroxidation. Interestingly, the model predicted that increasing the intima influx rate of HDL, decreased oxidant levels, thus implying an anti-atherosclerotic role for HDL in addition to its role in RCT. Crucially however, this model failed to include the detailed role of the immune system in atherogenesis. To address this Ibragimov and colleagues incorporated inflammation into an atherosclerotic model (Ibragimov et al., 2005). The model captured the localization of immune cells, cholesterol accumulation, debris, and the formation of a cap of smooth muscle cells (SMC). Simulations revealed that disease is initiated when the immune response cells are unable to adjust to increasing debris in the presence of a discrete amount of oxidised LDL (oxLDL). A more integrated model of LDL oxidation was created by Kim and colleagues (Kim et al., 2011). The model revealed that LDL and oxLDL mass accumulate focally in the internal carotid artery and external carotid artery plaques. Similarly, Gessaghi et al. (2011) (Gessaghi et al., 2011) created a model of LDL accumulation and intimal thickening. Plaque growth was modelled as a function of oxLDL in the intima. Pappalardo et al. also focused on the oxidation of LDL (Pappalardo et al., 2008). By using an agent-based model (ABM), they included chitotriosidase and captured the dynamics of atheroma formation in subjects with high LDL. An ABM is a rule-based model for representing complex multi-component biological systems (Soheilypour & Mofrad, 2018). The model implied that initial LDL levels are a key determinant of foam generation, a prediction echoing experimental observations (Ameli et al., 1996).

Further depth was added to modelling in this area by Little and Gola who created a model of atherosclerotic lesion formation (Little et al., 2009). The model predicted that the equilibrium level of monocyte Chemoattractant Protein-1 (MCP-1) increases directly with LDL (specifically oxLDL), a finding which has been observed experimentally (Shi et al., 2005). Sun and colleagues investigated the impact of low WSS on endothelial integrity/atherogenesis (Sun et al., 2009). The model showed

that LDL accumulation occurred in low WSS regions, while Van Schalkwijk et al. (2009) examined how lipoprotein size distribution determines CVD risk. The model captured production, remodelling, and lipoprotein uptake, and the assumption was made that each process depends on lipoprotein size (van Schalkwijk et al., 2009). A key feature of this model was its ability to successfully simulate familial hypercholesterolemia.

In contrast to previous work Ougrinovskaia and colleagues introduced a time scale of months/years to model atherosclerosis (Ougrinovskaia et al., 2010). The model excluded microscopic processes, such as LDL modification in the intima. Core elements of the inflammatory response were represented and attention was played to the interactions between modified LDL and activated macrophages. The model suggested that macrophage proliferation and constant signalling to endothelial cells, rather than an increase in the influx of LDL, underpin atherogenesis. This model was repurposed by Cohen and colleagues to investigate the athero-protectiveness of HDL. By adding three extra ODEs the authors were able to examine the effects of HDL on lesion formation (Cohen et al., 2014).

Gomez-Cabrero and colleagues utilised optimization search algorithms, machine-learning, classification techniques and cluster-based analysis to identify key features of atherosclerosis (Gomez-Cabrero et al., 2011). Crucially, the model could be interpreted based on several underlying behaviours despite having a large parameter set. A more integrated model of atherosclerosis was created by Hao and Friedman (2014). This model was able to account for 1) atherogenesis; 2) HDL and LDL oxidation; 3) oxLDL transformation to foam cells; 4) the velocity of macrophages, T-cell and SMCs into the intima (Hao & Friedman, 2014). Model simulations revealed that combined LDL and HDL levels govern whether a plaque will expand or decline. This work was subsequently extended to investigate how certain interventions could abrogate plaque growth, these included separate treatments with anti-miR33, TGF- β and miR-145 (Friedman & Hao, 2015).

More recent models which have focused on the pathophysiology of atherosclerosis have become increasingly more detailed. For instance, Pichardo-Almaraz and colleagues created a model that consists of two parts: LDL oxidation, and the immune response (the PDPK model by Kim et al); and a “mechanical” model which describes plaque growth, and the effect of blood flow on plaque progression (Pichardo-Almaraz et al., 2015). The model was calibrated to replicate plaque growth for a given population treated with simvastatin. Similar topologies have been adopted by others (Deyranlou et al., 2015; Iasiello et al., 2016; S. Kim & Giddens, 2015). For instance, Chalmers and colleagues modelled atherogenesis by capturing the dynamics of time and space using partial differential equations (PDEs) (Chalmers et al., 2015). The model revealed the complex interplay between LDL and the immune system in atherosclerosis progression. The model suggested it is unrealistic to

expect a linear response to therapies, such as raising HDL/lowering LDL or reducing inflammation. Other models have used PDEs to model this phenomenon (Yang et al., 2016). In particular, Chalmers and colleagues created a model using six PDEs to examine the impact of foam cell accumulation on plaque regression as a result of HDL influx (Chalmers et al., 2017). The model included monocytes, macrophages, foam cells, macrophage chemoattractants, endothelium-stimulating cytokines, LDL and HDL. Interactions were modelled at the endothelium surface and inside the artery wall. The model suggested that the intrinsic dynamics of RCT by HDL are important in determining plaque regression. In contrast other approaches have modelled atherosclerosis by introducing stochasticity. For instance, Simonetto and colleagues used a stochastic model to investigate atherosclerosis (Simonetto et al., 2017). Although there was limited focus on the role cholesterol plays atherosclerosis.

A more detailed model was created by Bhui and colleagues who developed a hybrid ABM-computational fluid dynamic model (Bhui & Hayenga, 2017). The ABM represented cellular components such as LDL, Tissue Necrosis Factor alpha (TNF- α), Interlukin-10 (IL-10) and Interlukin-1 beta (IL-1 β) while the CFD component represented model hemodynamics. Adopting this hybrid framework enabled plaque progression to be tracked. Guo and colleagues also focused on arterial pathophysiology by creating a model which encapsulated lipid deposition, inflammation and angiogenesis (Guo et al., 2019). The model revealed the crucial role the inflammatory microenvironment plays in the evolution of a plaque. Similarly Santra and colleagues developed a model which evaluated LDL within different arterial layers (Santra et al., 2020). The model suggested the rate of LDL accumulation is higher in the intima layer than the media layer for both hyperlipidemia and hypertension, implying the intima layer is pivotal to plaque formation. However, among the models which have centred on the detailed pathophysiology of atherosclerosis, the model by Parton et al. (2019) stands out because it is encoded in SBGN and SBML. (Parton et al., 2019). The model complements previous work which has encased atherosclerosis in these frameworks (Bekkar et al., 2018), and well-designed simulations pin-pointed a combination of pharmacological agents which effectively reversed atheroma development.

[10. Multi-Physics Models of Atherosclerosis]

To model the dynamics of blood flow and the anatomical features of the artery it has been necessary to use approaches from physics, and PDEs have been central to this (El Khatib et al., 2019; Mihai et al., 2012). An early model which utilised this approach is that of Tzeghai et al. (1986). This model represented LDL accumulation and degradation in the arterial lumen (Tzeghai et al., 1986). By representing leaky junction-cell turnover (Weinbaum et al., 1985); the model aimed to elucidate the

impact of convection on macromolecular transport across an artery wall. Results revealed that a change of arterial LDL particle equilibrium occurred because of a small number of leaky junctions. The model was subsequently modified to predict that elevated convection has a significant bearing on macromolecular transport (Weinbaum et al., 1988; Wen et al., 1988); a prediction suggested to account for findings which observed this phenomenon after the endothelium became damaged. Neuman and colleagues also investigated how hemodynamics and endothelium integrity influence arterial wall flux (Neumann et al., 1990). Model findings suggested that changes to endothelial integrity result in increased permeability, convection, and incorporation of cholesterol in the arterial wall. On a similar theme Stangeby and Ethier created a model of coupled blood-wall arterial LDL transport. Based on the idea that the behaviour of fluid and LDL particles at the blood wall boundary impinge on the resulting interface concentration (Stangeby & Ethier, 2002). Blood was viewed as a homogenous, isothermal fluid, and PDEs described luminal and transmural flow in the artery lumen. Simulations revealed increased permeation of LDL into the distal side of the artery stenosis; an intriguing finding which awaits experimental confirmation.

More recent models have focused on atheroma progression in coronary arteries (Cilla et al., 2014; Hidalgo et al., 2014; Jesionek & Kostur, 2015). Karimi and colleagues created a model which predicted that LDL oxidation rate is a driver of the concentration of LDL in the intima (Karimi et al., 2013). The same authors assembled a model which examined the role of leaky junctions in the transport of LDL from the lumen into the arterial wall (Karimi et al., 2013). The model predicted that an increase in leaky junctions' augments LDL concentration inside the arterial wall. Sun and colleagues examined the influence of pulsatile flow on arterial wall LDL transport (Sun et al., 2007). Blood flow was represented with PDEs, and arterial LDL convection modelled by Darcy's law, an equation which represents fluid flow in a porous medium. The model identified a significant difference between steady flow and pulsatile flow. This implied that a steady flow is inadequate for predicting transmural LDL transport. Other models have focused on the multilayer nature of the aortic wall. For instance, Dabagh and colleagues created a multi-layered model of the aortic wall exploring LDL transport under hypertension (Dabagh et al., 2009) PDEs represented the flow and convection associated with LDL transit. The model suggested LDL accumulation through subendothelial layers is the indirect effect of hypertension increasing endothelial permeability by impacting cell turnover. Lantz and Karlsson focused more on blood flow within the aorta, and how LDL surface concentration alters during a cardiac cycle (Lantz & Karlsson, 2012). Hemodynamics were informed by magnetic resonance imaging data, and the model suggested that perturbing blood flow significantly affected LDL surface concentration.

Synergies between hypertension and hypercholesterolaemia accelerate mortality from ACVD in model organisms (Ning et al., 2018). For instance, in the

hyperlipidaemic Watanabe rabbit hypertension causes a 3.5-fold increase in aortic lesions (Chobanian et al., 1989). To represent this crosstalk, Yang and Vafai created a model of LDL transport in the arterial wall and lumen (Yang & Vafai, 2006). The aim was to elucidate the parameters responsible for increased LDL uptake during hypertensive conditions. A weakness of this model is that it does not represent pulsation and how LDL transport is affected by arterial curves/bifurcations. Also, focusing on hypertension Ai and Vafai (2006) developed a model of LDL transport in a stenosed artery (Ai & Vafai, 2006). The model revealed that hypertension significantly increases the transmural filtration and concentration polarization at the lumen/endothelium interface. Dabagh and colleagues created a multi-layered model of the aortic wall exploring LDL transport under hypertension (Dabagh et al., 2009). PDEs represented the flow and convection associated with LDL transit. The model suggested LDL accumulation through subendothelial layers is the indirect effect of hypertension increasing endothelial permeability by impacting cell turnover.

It is important to recognise that atherosclerosis has a propensity to occur at bifurcations within medium sized arteries (Hurtubise et al., 2016). This anatomical feature has been represented within model topology. Kenjereš and de Loor created a multi-layered model of carotid artery bifurcation (Kenjereš & de Loor, 2014). A significant dependence between blood flow pattern, WSS distributions and LDL uptake in the artery wall was identified. It is important to underscore the clinical significance of WSS. Atherosclerotic lesions have been found to occur mainly in areas of low WSS and have been associated with increased plaque burden (Urschel et al., 2021). Other models have focused on wall strains as a modulator of atherosclerosis. For instance, Hansen and colleagues used a two-dimensional model to test the hypothesis that arterial LDL transport is governed by first principal wall strains, and that location and progression of atherosclerotic plaques are results of this relationship (Hansen et al., 2014). Blood flow was modelled using PDEs, and the arterial wall was represented by a strain energy function. A correlation was identified between highly strained areas of the arterial wall and atherosclerotic plaque locations.

More recent models have adopted a multi-physics approach to model atherogenesis. Tomaso and colleagues created a multiscale model of atherosclerosis (Di Tomaso et al., 2011). The model revealed that plaque location was dependent on WSS, while plaque, size, number, and growth was dependent on mean LDL. Thon and colleagues also used this methodology to capture the different time scales associated with atherogenesis (Thon et al., 2018). The model was calibrated with data from murine experiments. Model results suggested pulsatile blood flow was crucial to atherogenesis progression. In contrast the model suggested aorta

compliance has a negligible impact on atherogenesis. The work has two key limitations. Firstly, model conclusions were based on a single data set. Secondly, the model is coarse grained, oversimplifying several key processes. These issues were addressed to an extent when Thon and colleagues created a model consisting of three reduced ODE sub-models (Thon et al., 2018). Each sub-model was informed by *in vitro* experimental data. Model simulations suggested that it would be worthwhile if experimental work focused on the balance between FC and CE inside macrophages. It was postulated this difference could help predict long-term atherosclerotic plaque outcomes. Similarly, Roustaei and colleagues (2018) developed a five-layer Fluid Structure Interaction (FSI) model to investigate LDL permeation into the coronary arterial wall (Roustaei et al., 2018). It was found luminal distension prompted by FSI reduces WSS along the lumen/wall interface, particularly during hypertension. This resulted in lowered endothelial resistance against LDL permeation. Other models adopted a slightly different approach, for example, Watson and colleagues developed a multiphase model on a one-dimensional cartesian domain to investigate early fibrous cap formation in the atherosclerotic plaque (Watson et al., 2018). It was found that cap thickness is potentially sensitive to the balance between SMC apoptosis and SMC recruitment from the media. Similarly, Lasiello and colleagues created a multi-layer model of LDL accumulation (Lasiello et al., 2018). Most significantly however, Thon and colleagues This work focuses on the arterial penetration of HDL and recruitment of macrophages as contributors to atherosclerosis (Thon et al., 2019). An area which had been overlooked by modelling. The model differentiates between progression-prone and progression resistant plaques. Plaques were defined by, WSS exposure, and plasma levels of LDL-C and HDL-C. The model suggested advective transport of lipoproteins through the activated endothelium by transmural flow is critical to atherogenesis, while advective transport within the artery is not.

11. FUTURE PERSPECTIVES

[11.1 Emerging Modulators of Cholesterol Homeostasis]

When considering the future of modelling in this field a worthwhile place to begin is cholesterol biosynthesis. Several areas of cholesterol biosynthesis require further elucidation, and mathematical modelling can help with this. For example, in addition to HMGCR other enzymes are key regulators of cholesterol biosynthesis (Sharpe & Brown, 2013). Squalene monooxygenase (SQLE) is suggested to be an additional rate-limiting enzyme in cholesterol synthesis downstream of HMGCR (Gill et al., 2011). Modelling is essential to elucidate how perturbations to the activity of SQLE impacts the flux of the cholesterol biosynthesis pathway. There is also a growing need to investigate the effects aging has on cholesterol biosynthesis. Specifically, it is possible aging impacts key enzymatic regulatory points in cholesterol biosynthesis. For instance, ROS are synonymous with the aging process(Liguori et

al., 2018). Intriguingly, rodent studies have suggested ROS increase HMGCR activity (Mc Auley & Mooney, 2017; Pallottini et al., 2005, 2007). Most recently, it was found that treating rat HepG2 cells with 500 μ M of ROS inducing H₂O₂ led to a significant increase in HMGCR (Seo et al., 2019). This change elicited a corresponding rise in hepatic FC. This could explain why hepatic FC has been observed to rise significantly with age in rats (Mulas et al., 2005). However, this relationship requires further investigation which modelling could help to explore. Other factors associated with aging modulate cholesterol biosynthesis. Both the mammalian target of rapamycin complex (mTORc) and sirtuins are implicated in the regulation of lipid metabolism (Johnson & Stolzing, 2019). These metabolic networks comprise a myriad of interacting processes, and it is recognised that a deep understanding of their dynamics may only be achieved by using computational simulation and analysis (Mc Auley et al., 2015).

Other biochemical processes which influence intracellular cholesterol homeostasis have been largely overlooked by models to date. For example, recent experimental findings have revealed that lysosomes are crucial to the regulation of cholesterol homeostasis (Meng et al., 2020). Intriguingly, it has been suggested lysosome status is key to a pro-longevity phenotype (Folick et al., 2015). Cholesterol arrives at lysosomes via receptor mediated endocytosis of lipoproteins. The transport of cholesterol to lysosomes involves Niemann-Pick C (NPC) 1 and NPC2 proteins (Wheeler & Sillence, 2020). At the lysosome cholesterol levels are detected by the lysosomal membrane, resulting in cellular regulation via mTORc (Castellano et al., 2017; Chu et al., 2015). Intriguingly, it has recently be suggested that lysosomal dysregulation could modulate ACVD risk in humans (Trivedi et al., 2020). It has also been found that lysosome status is key to a pro-longevity phenotype in *Caenorhabditis elegans* (Folick et al., 2015). For these reasons it is important future models include lysosomal regulation. The interplay between bile acid metabolism and cholesterol homeostasis also requires greater scrutiny. Bile acid composition is central to cholesterol homeostasis and alterations to it change cholesterol excretion (Chiang, 2017). The cytochrome P450 family 2 subfamily c polypeptide 70 has recently been identified as pivotal to cholesterol excretion in mice (de Boer et al., 2020). To date no model has incorporated this mechanism. It is also essential future models are reflective of emerging findings in relation to lipid rafts. Lipid rafts are cholesterol and sphingolipids rich microdomains of the plasma membrane. They are involved in transduction, specifically during inflammatory responses. It is thought the cholesterol content of lipid rafts have the potential to interact with inflammatory signalling pathways, impacting the pathophysiology of ACVD (Lemaire-Ewing et al., 2012). To gain a deeper understanding of this relationship future mathematical models will need to acknowledge these processes.

[11.2 Novel Insights into Atherosclerosis]

Understanding how cholesterol is regulated cellularly underscores whole-body cholesterol metabolism and the pathogenesis of atherosclerosis. This review has revealed a broad range of models which have represented cholesterol metabolism and atherosclerosis at varying degrees of scale. However, to date no model has successfully integrated cholesterol biosynthesis, whole body metabolism and the key elements underpinning atherosclerosis. It is imperative future integrated models take account of novel experimental findings which have deepened our understanding of cholesterol metabolism and atherosclerosis. Several recent developments have focused on the cellular and molecular mechanisms which underpin atherosclerosis. For instance, it has been recognised that LDL are physiochemically and metabolically heterogeneous. For example, the biochemical composition of small dense LDL (sdLDL) increases its inflammatory potential, and are more significantly associated with atherogenesis than larger LDL (Chapman et al., 2020). In coronary heart disease (CHD) patients, a greater concentration of sdLDL was observed, when compared to control males (Koba et al., 2008). In this investigation, men with untreated CHD had a mean sdLDL-C concentration of 37mg/dL, in comparison to male controls who had a mean of 28.9mg/dL; individuals with treated CHD had levels of 33.1mg/dL. Moreover, the severity of CHD was associated with sdLDL-C levels. Males with mild CHD, had concentrations of 28.9mg/dL when compared to 39.9mg/dL for individuals with acute CHD. Recently several genotypes have been associated with the different LDL-C subfractions (Dai et al., 2021). The relationship between sdLDL and ACVD could have several underlying mechanisms. For example, sdLDL have an increased residence time compared to larger LDL-C (Thongtang et al., 2017). This reason for this might be due to a reduced clearance rate by hepatic LDLr. However, large LDL have a reduced affinity for LDLr in comparison to intermediate sized LDL in normocholesterolaemia patients (Campos & Monsó, 1990). In addition, vitamin E is diminished in sdLDL-C, which increases their potential for oxidation (Khalil et al., 2017). Importantly, LDL-C size has been associated with response to cholesterol lowering treatment. As an example, sdLDL have been associated with a reduced fractional change in response to statins when compared to larger LDL particles (Shim et al., 2015).

Recently the modified LDL particle lipoprotein (a) [Lp(a)] has also been suggested to be a key driver of atherosclerosis (Rehberger Likozar et al., 2020). Lp(a) has an extra apolipoprotein (a) covalently bonded to apolipoprotein B-100 of LDL (Klingel et al., 2019). Although its role in atherosclerosis remains to be fully elucidated it is likely ROS induces the formation of pro-inflammatory and pro-atherogenic oxidation-specific epitopes (Leibundgut et al., 2013). The Pakistan Risk of Myocardial Infarction Study revealed) found that Lp(a) was elevated in patients with confirmed first-onset acute myocardial infarction when compared to controls (Saleheen et al., 2017). Interestingly, reducing plasma PCSK9; lowers the levels of highly atherogenic lipoprotein (a) [Lp(a)] particles by 30-35% (Stein, 2013; Stein & Raal, 2014).

Moreover, it has been calculated that a 65.7mg/dL reduction in Lp(a) would result in a 45% lifetime risk reduction for CHD. A growing body of recent evidence also suggests that the innate immune cells known as dendritic cells (DC), have an important role to play in instigating atherosclerosis. DC have the capacity to take up oxLDL and have been identified within arterial lesions. Moreover, the adaptive immune cells known as T lymphocytes, are suggested to be “executors” of the DC immunity in atherogenesis (Sun et al., 2020). In addition, although ROS are established within the prevailing paradigm which accounts for atherosclerosis (Kattoor et al., 2017). Recently, new evidence has come to light about the relationship between ROS and atherosclerosis. ROS are involved in the formation of 7-Ketocholesterol (7KC) (Helmschrodt et al., 2013). 7KC is generated when cholesterol oxidation occurs on the C7 position of the steroid (Anderson et al., 2020). 7KC is the most abundant oxysterol present in OxLDL (Anderson et al., 2020). The pernicious impact of C7 is underscored by its ability to exacerbate ROS production. It is the most toxic oxysterol within an atherosclerotic plaque (Brown et al., 1997). Given this finding it is incumbent that future models include this mechanism. It is also important models describe in greater mechanistic detail the role of oxysterol sensors, such as LXRs. LXR is a ligand acceptor for many oxysterols, including potentially 7KC (Okabe et al., 2013). LXRs work in tandem with the sterol SREBP2. SREBP2 provokes the transcription of ATP-binding cassette transporter 1 by producing oxysterol ligands for LXRs (Wong et al., 2006). The importance of LXRs are underscored by the fact that the dysregulation of LXR activity modulates atherogenesis in mice (Wang & Tontonoz, 2018). Therefore, it is clear many questions remain to be addressed about 7KC and LXRs. Our view of how LDL enters the endothelium is undergoing a conceptual shift. The traditional view that this process occurs by passive filtration is being heavily challenged. It is being gradually superseded by a view which suggests LDL transcytosis across the endothelium requires interaction of scavenger receptor class B type 1 (SR-B1) with a cytoplasmic protein (Armstrong et al., 2015). This view is supported by the observation that SR-B1 expression is higher in atherosclerotic arteries (Huang et al., 2019). Future models will have to take account of this conceptual shift. Models will also need to pay careful attention to the potential role of the HDL particle in atherosclerosis. Many questions remain unanswered. For instance, what are the mechanisms whereby HDL modulates the atherogenicity of LDL? It has been suggested that the main role of HDL within plaque tissue is anti-inflammatory/anti-oxidative, i.e. apoA1 (the major apolipoprotein of HDL) helps to quench ROS levels, and inflammation which is key to the oxidation of LDL (Barrett et al., 2019). However, this question requires further examination and modelling could help investigate this.

[11.3 Mathematical Modelling Challenges]

A significant challenge in this field is the issue of scale. This review revealed a broad range of models. These models represent cholesterol metabolism/atherosclerosis at

varying degrees of granularity. If this field is to develop a holistic model of cholesterol metabolism and atherosclerosis model merging needs to be addressed. Progress in computational system biology could help with this. For example, several models discussed in this review have been encoded in SBML. This should in theory facilitate model merging. From the BioModels database it is possible to download SBML code for the following models, Gomez-Cabrero et al. (2011), Mc Auley et al. (2012), Watterson et al. (2013) Morgan et al. (2016), Parton et al. (2019). Figure 3 represents a merged SBGN diagram of the Mc Auley et al. (2012) and the Gomez-Cabrero et al. (2011) models. The diagram underlines that it is theoretically possible to merge models of whole-body model of cholesterol metabolism and atherogenesis. To facilitate the merging process, the most recent version of the computational systems biology software tool COPASI (Hoops et al., 2006) has a model merging feature. When combined it is possible models have overlapping features. Reduction methodologies can be used to alleviate this. These techniques eliminate overlapping components and remove aspects of the model which have a negligible impact on model output (Snowden et al., 2017). When considering the challenge of merging models, it is also important to recognise that many of the models discussed in this review have been developed using a variety of different theoretical frameworks. While most of the models were coded using ODEs, in many instances this is not the case. Some models have been coded using frameworks such as PDEs or ABM, and although not widely applied to this field yet, it is important to recognise that stochastic approaches could be needed to model aspects of atherosclerosis progression. In this instance the model would need to combine deterministic integrations of ODEs with a stochastic simulation algorithm. Several systems biology software tools such as COPASI, Snoopy (Herajy et al., 2017), Virtual Cell (Resasco et al., 2012) have hybrid capabilities.

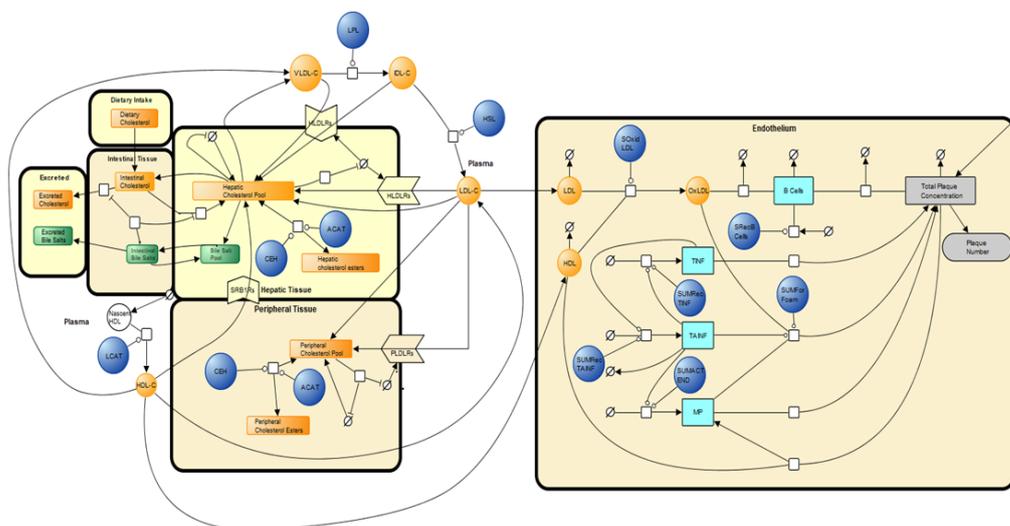


Figure 3. Proposed combined model of whole-body cholesterol metabolism and its intersection with the mechanisms which underpin atherosclerosis. The diagram was created by merging the Mc Auley et al. (2012) model of whole-body cholesterol metabolism with the Gomez-Cabrero et al. (2011) model of atherosclerosis. The intersection points of the two models are LDL and HDL.

A further challenge centres on model parameterisation. Despite significant efforts by the systems biology community to create repositories of kinetic parameters, model parameterisation remains an outstanding problem. This challenge was highlighted by Benson et al. (2017) (Benson et al., 2017) when building a model of cholesterol biosynthesis. Their efforts to assemble a mathematical model of the mevalonate arm of the pathway was stifled due to “gaps and inconsistencies” in the kinetic data. The authors reported instances of incomplete kinetic data in the primary literature. This undermined their ability to assemble model reactions. For example, V_{max} values were reported instead of K_{cat} . This is a problem which is inherent in the older literature, and it is difficult to envisage experiments being conducted in the future which will rectify this measurement challenge. Consequently, the computational systems biology community has focused recent efforts on techniques for parameter estimation. A variety of approaches have been utilised in this field (Penas et al., 2017). These have included genetic algorithms, particle swarm and simulated annealing optimization methods (Ismail et al., 2017). Due to the paucity/inconsistency of kinetic information associated with cholesterol metabolism it will be necessary to apply these techniques in this field.

[12 Conclusions]

There has been a long history of mathematical modelling applied to various aspects of cholesterol metabolism and ACVD. Due to the growing adoption of the systems biology paradigm, the use of models for understanding the biological processes which underscores these systems has increased significantly. Depending on the level of abstraction and the nature of the biological phenomenon being modelled a variety of modelling approaches have been employed. Most models have been built using ODEs. However, PDEs, ABM, and probabilistic models have also been utilised. Regardless of the framework the models have provided a wealth of information about cholesterol metabolism and the drivers of atherosclerosis. Recent years has witnessed the emergence of models which have captured cholesterol metabolism/atherosclerosis in a more integrated manner. However, a model which fully captures the detailed interactions between cholesterol metabolism and atherosclerosis remains elusive. Building such a model will be challenging. Crucially it will need to capture key recent findings in this area and will need to overcome long-

standing obstacles in this field such as the integration of biological mechanism at different levels of scale. Even if this issue is overcome, the problem of parametrising such a model is computationally demanding. Currently the biological data does not exist to facilitate the parametrisation of a model of this nature. However, given that modelling approaches are the most realistic way in which to gain a truly integrated appreciation of the dynamics of a biological system across time and space there is an imperative that future experiments are designed to dovetail with mathematical models. By adopting this cross-disciplinary approach this will help with both the design of improved experiments and the advent of more realistic models which can help to augment our understanding of cholesterol metabolism, and the processes which drive the pathogenesis of ACVD.

Research Resources

Acknowledgments

I would like to acknowledge Callum Davies for Creating Figure 3

Notes

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