STUDY OF THE DYNAMIC EFFECT OF CHOLESTEROL LOWERING DRUGS USING A MATHEMATICAL MODEL

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Abstract

It is widely accepted that cholesterol plays an important role in the infiltration and entrapment of low density lipoprotein (LDL) in the blood vessel walls (atherogenesis). The transport of cholesterol and other lipids through the circulatory system is facilitated by their packaging into lipoprotein carriers such as LDL. In fact, LDL is the major carrier of cholesterol in human plasma, and therefore it plays a crucial role in atherosclerosis. Small and dense lipoproteins (sdLDL) are held to be particularly atherogenic.
Fibrates can be used to correct the lipoprotein metabolism disorder associated with sdLDL. Fibrates act by lowering plasma triacylglycerol and very low density lipoprotein levels (VLDL), therefore correcting the underlying metabolic disturbance that causes hypercholesterolemia.
A deeper understanding of this system is necessary in order to design more effective drugs. In this work we present a mathematical model for lipoprotein metabolism. This model was used to study the effect of Niacin, a fibrate drug, on the levels of VLDL and sdLDLs. Simulations results show that high levels of triacylglycerol are essential for VLDL accumulation and sdLDL production. Addition of Niacin reduces triacylglycerol levels. As a result of this the production of VLDL decreases, which prevents the formation of sdLDL.

Keywords

Dynamic model, Hypertriglycerolemia, Cholesterol.

INTRODUCTION

Cholesterol is an amphipatic lipid which is essential for the structure cellular membranes and lipoproteic structures from blood serum. It is also a precursor for many steroids such as sexual hormones, bilic acid, corticosteroids and vitamin D (Yeagle 2005). Cholesterol is transported through plasma associated to lipoprotein molecules. The two main types are low density lipoproteins (LDL) and high density lipoproteins. (HDL) (Toth 2005).

However, even though cholesterol is crucial for many processes in the organism, many health problems have been associated to it and to LDL, such as hypercholesterolemia, hypertriglycerolemia, coronary heart disease and coronary artery disease, among others. A traditional measure of overall coronary health is the ratio of HDL to LDL and the levels of LDL or "bad cholesterol" (Walldius and Jungner 2005). Nowadays a series of drugs are used to treat lipid metabolism alterations, such as statins and fibrates. Fibrates act by lowering triglycerides levels in blood plasma and promote production of HDL apoAI or "good cholesterol". Niacin is a water soluble vitamin, a fibrate, and is used to treat abnormalities in plasma lipids metabolism and atherosclerotic cardiovascular diseases (Kamanna and Kashyap 2000). Niacin is an effective lipid regulating agent that reduces total cholesterol, and has been documented to reduce coronary events and contribute to regression of coronary atherosclerosis.

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In the past few years several modeling efforts have arisen, oriented to improving our understanding of cholesterol metabolism by capturing and representing different parts of it. Among these models we find a description of the gene network regulating cholesterol synthesis in the cell (Ratushny A.V., Ignatieva E.V. et al. 2000; Ratushny A.V., Likhoshvaia V.A. et al. 2003; Ratushny and Likhoshvai 2005), LDL size distribution (Das Gupta, Santos et al. 2005), and VLDL and LDL competition for particle binding and uptake (Panovska, Pickersgill et al. 2006). The model proposed in the present study shows a different approach, by considering the interactions between lipoproteins, lipids, and representing the effect of cholesterol lowering drugs.

In this work we developed a mathematical model for overall blood triglycerides and cholesterol content associated to lipoproteins and to describe the effect that Niacin would have in the distribution of these lipid molecules in lipoproteins. We compare three scenarios: normal lipid distribution, lipid distribution for hypertriglycerolemia and lipid distribution for hypertriglycerolemia after Niacin administration. This is a first attempt for describing the lipoprotein metabolism, and the effect of cholesterol lowering drugs in the system.

Background

In the human body the cholesterol and triacylglycerol are transported in the plasma by a family of macromolecules called lipoproteins, such as very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL) (Panovska, Pickersgill et al. 2006). Structurally VLDL and LDL are characterized by having apolipoprotein apoB-100 and HDL by apolipoprotein apoA-I (Lamarche, Rashid et al. 1999; Packard, Demant T. et al. 2000). These apolipoproteins promote the union of the lipoproteins to receptors and act as cofactors for enzymatic reactions (Andersson 2003).

For years researchers have studied the relevance of the lipoproteins in cardiovascular diseases, to determine the key atherogenics factors that allow the development of a state hypertriglyceridemia (HTG) (Krieger 1998; Toth 2005). It has been found that the higher the amount of HDL, the lower the risk for atherosclerotic disease. This relates to the fact that HDL takes part in the removal of excess cholesterol in the plasma, by taking it to the liver for its elimination (Lamarche, Rashid et al. 1999; Lewis and Rader 2005). On the other hand, small dense LDLs are an atherogenic factor since they promote the deposit of excess cholesterol in blood vessel walls. The enzymes that play a key role in lipoprotein metabolism are cholesteryl ester transfer protein (CETP), hepatic lipase (HL), and lipoprotein lipase (LPL) (Barter, Brewer et al. 2003; Santamarina-Fojo, Gonzalez-Navarro et al. 2004). In normal conditions, the liver produces VLDL which is lipolyzed by lipoprotein lipase (LPL). VLDL gives rise to a population of LDL particles. CETP removes cholesteryl ester (CE) and replaces it with triacylglycerol (TG) as the protein shuttles between VLDL, LDL and HDL particles. HL hydrolyzes triacylglycerol in triacylglycerol-enriched LDLs, and HDLs. This results in small dense particles of LDL and HDL.

For the development of hypertriglyceridemia (HTG) a threshold plasma triacylglycerol (TG) concentration has been determined, after which triacylglycerol-rich VLDL accumulates due to either overproduction in the liver or a low lipoprotein lipase activity or an excess of inhibitor of lipoprotein lipase (Packard, Demant T. et al. 2000; Packard 2003). This triggers the generation of small dense LDL, because an increase of triacylglycerol-rich VLDL's and increases the risk of coronary heart disease.

Correction of the dyslipidemia associated with small dense LDL is possible using drugs like Niacin. Niacin is an effective agent that reduces total plasma cholesterol, apoliprotein (apo) B, triglyceride (TG), VLDL, and LDL and increases HDL levels (Kamanna and Kashyap 2000; Tavintharan and Kashyap 2001). Niacin is believed to act by inhibiting fatty acid and TG synthesis, increasing intracellular apoB degradation and increasing apoA-I synthesis. This would reduce the production rate of VLDL by the liver, reduce LDL formation and enhance cholesterols' reverse transport which is HDL apoA-I dependent (Kamanna and Kashyap 2000).

Table 1: List of reactions considered.

Chemical Reactions	*Description*
$VLDL_{ce} \xrightarrow{k_1} LDL_{ce}$	$VLDL_{ce}$ hydrolysis
$VLDL_{tg} \xrightarrow{k_2} LDL_{tg}$	$VLDL_{tg}$ hydrolysis
$VLDL_{tg} \xrightarrow{k_3} CETPTG$	$VLDL_{tg}$ loses TG at rate k_3
$CETPCE \xrightarrow{k_4} VLDL_{ce}$	$CETPCE$ transfers CE at rate k_4
$HDL_{ce} \xrightarrow{k_5} CETPCE$	HDL_{ce} loses CE at rate k_5
$CETPTG \xrightarrow{k_6} HDL_{tg}$	$CETPTG$ transfers TG at rate k_6
$HDL_{tg} \xrightarrow{k_7} HDL_{tg-1}$	HDL_{tg} hydrolyzes at rate k_7
$LDL_{ce} \xrightarrow{k_8} CETPCE$	LDL_{ce} loses CE at rate k_8
$CETPTG \xrightarrow{k_9} LDL_{tg}$	$CETPTG$ transfers TG at rate k_9
$CETPCE \xrightarrow{k_{10}} LDL_{ce}$	$CETPCE$ transfers CE at rate k_{10}
$LDL_{tg} \xrightarrow{k_{11}} LDL_{tg-1}$	LDL_{tg} hydrolyzes at rate k_{11}

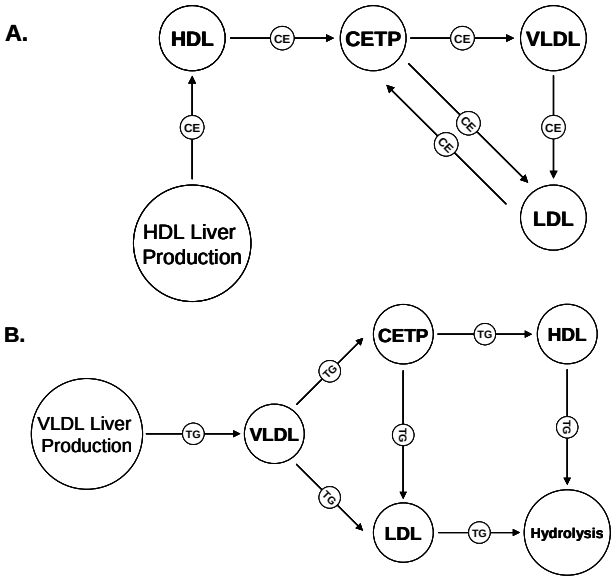

Figure 1: Representation for the transfer of triglycerides and cholesterol among phospholipids proteins. An arrow indicates that transfer between those nodes is possible, in the arrows direction. A.: Graph for the distribution of cholesteryl ester (CE) in cholesterol metabolism. Nodes represent CE associated to the species considered. B.: Graph for the distribution of triacylglycerol (TG) in cholesterol metabolism. Nodes represent TG associated to the species considered.

MATHEMATICAL REPRESENTATION OF THE SYSTEM

We developed a mathematical model for the system in Figure 1, which represents the transfer of triglycerides and cholesterol among phospholipids proteins, in order to study the effect of cholesterol lowering drugs. The reactions considered are listed in Table 1.

For the models'construction eight species were considered: HDL_{CE}, HDL_{TG}, LDL_{CE}, LDL_{TG}, $VLDL_{CE}$, $VLDL_{TG}$, $CETP_{CE}$ and $CETP_{TG}$. Every species contains an average concentration of CE or TG respectively.

To determine the distribution of CE and TG in cholesterol metabolism a series of biological relationships were established from literature (Guerin, Dolphin et al. 1994; Andersson 2003; Barter, Brewer et al. 2003; Packard 2003; Santamarina-Fojo, Gonzalez-Navarro et al. 2004; Das Gupta, Santos et al. 2005; Lewis and Rader 2005).

The dynamic of protein interactions and mass transfers were conceptually represented as graphs, shown in Figure 1.

These graphs show the feasible transferences of lipids between lipoproteins.

Figure 1-A shows the representation of cholesteryl ester (CE) metabolism.HDL_{CE} is synthesized directly in the liver (Lewis and Rader 2005). Cholesteryl ester transfer protein (CETP) catalyzes CE transfer between lipoproteins, and Lipoprotein lipase (LPL) catalyzes the conversion of $VLDL_{CE}$ into LDL_{CE}. Figure 1-B shows the representation of triacylglycerol (TG) metabolism. $VLDL_{TG}$ is produced directly in the liver. CETP is involved in TG transfer between lipoproteins. LDL_{TG} is the catabolic product of $VLDL_{TG}$ by the action of LPL. Additionally, HDL_{TG} and LDL_{TG} are hydrolyzed by the action of hepatic lipase (HL) (Barter, Brewer et al. 2003; Santamarina-Fojo, Gonzalez-Navarro et al. 2004; Lewis and Rader 2005). This reduces the levels of HDL_{TG}, and LDL_{TG} in the system.

The set of equations derived for the system illustrated in Figure 1 is the following:

$$\frac{dVLDL_{CE}}{dt} = CETP_{CE} \cdot k_4 - VLDL_{CE} \cdot k_1 \quad (1)$$

$$\frac{dVLDL_{TG}}{dt} = VLDL_{TG\_prod} - VLDL_{TG} \cdot k_2 - VLDL_{TG} \cdot k_3 \quad (2)$$

$$\frac{dLDL_{CE}}{dt} = VLDL_{CE} \cdot k_1 + CETP_{CE} \cdot k_{10} - LDL_{CE} \cdot k_8 \quad (3)$$

$$\frac{dLDL_{TG}}{dt} = VLDL_{TG} \cdot k_2 + CETP_{TG} \cdot k_9 - LDL_{TG} \cdot k_{11} \quad (4)$$

$$\frac{dHDL_{CE}}{dt} = HDL_{CE\_Prod} - HDL_{CE} \cdot k_5 \quad (5)$$

$$\frac{dHDL_{TG}}{dt} = CETP_{TG} \cdot k_6 - HDL_{TG} \cdot k_7 \quad (6)$$

$$\frac{dCETP_{CE}}{dt} = HDL_{CE} \cdot k_5 + LDL_{CE} \cdot k_8 - CETP_{CE} \cdot k_4 - CETP_{CE} \cdot k_{10} \quad (7)$$

$$\frac{dCETP_{TG}}{dt} = VLDL_{TG} \cdot k_3 - CETP_{TG} \cdot k_6 - CETP_{TG} \cdot k_9 \quad (8)$$

An important assumption was made regarding the production of LDL from VLDL. In normal conditions VLDL are TG-rich lipoproteins (TRL) and low lipoprotein lipase (LpL) is the enzyme responsible for the hydrolysis of TRL into smaller remnant lipoproteins. The model considers average quantities of CE or TG in lipoproteins, and CETP. Then, the system is split into two parts: one involved in cholesteryl ester transfer (CE) and one involved in

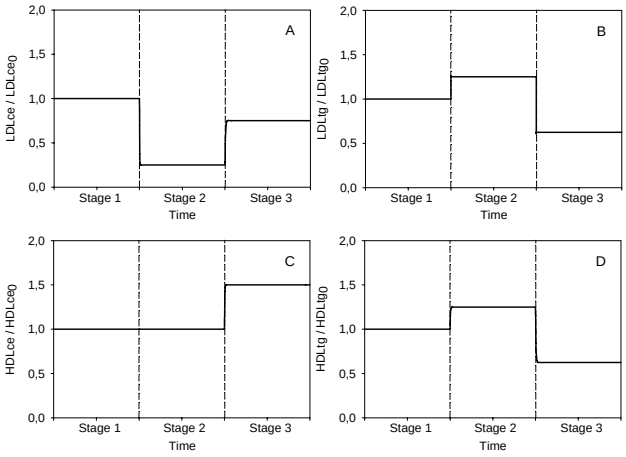

Figure 2: Simulation results for lipid distribution in normal conditions (Stage 1), hypertriglycerolemia (Stage 2), and niacin treatment (Stage 3). A: LDL_{CE} content; B: LDL_{TG} content; C: HDL_{CE} content; C: HDL_{TG} content.

triacylglycerol transfer. These two species, were defined in order to represent separately the concentration of the different lipoproteins. The initial average of CE, and TG in the species was established according to the composition of plasma lipoproteins found in literature (Andersson 2003). The following values were used for TG content in VLDL, LDL, and HDL: 50%, 9% and 8%, while for CE content were used 22%, 45%, and 30%, respectively. Consequently we used average experimental concentrations obtained from literature as our initial concentrations for simulation purposes (Guerin, Dolphin et al. 1994).

Initial distribution of total CETP was considered as 65% as a complex $CETP_{CE}$, and the rest as $CETP_{TG}$. based on experimental data from literature (Hannuksela, Marcel et al. 1992; Desrumaux, Athias et al. 1999). Also, we considered that CETP is always available for lipid transportation.

For simulation, we considered a scenario, represented as consecutive stages. Stage 1 corresponds to normal plasma triacylglycerol levels,

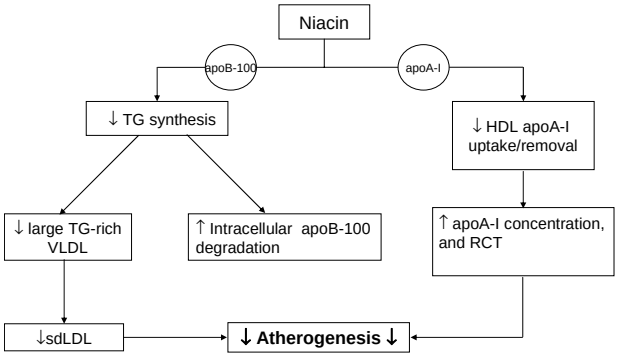

Figure 3: Effect of niacin in lipid metabolism.

and is associated to the set of parameters that represent the condition of a normolipidemic subject (Augustin, Freeze et al. 1978; Colvin, Moriguchi et al. 1998; Lassel, Guerin et al. 1998; Cohn, Patterson et al. 2004). Stage 2 represent the hypertrygliceromelia development. In this case, we introduced perturbations associated with the reactions involved in HTG to represent what has been reported to occur in this disease (Lamarche, Rashid et al. 1999; Andersson 2003; Barter, Brewer et al. 2003; Packard 2003; Santamarina-Fojo, Gonzalez-Navarro et al. 2004; Das Gupta, Santos et al. 2005).

Finally, Stage 3 corresponds to the action of niacin in the system, and its effect is incorporated in the model as illustrated in Figure 3. This stage represents how niacin administration can change the rates of VLDL production, and HDL levels in the system (Jin, Kamanna et al. 1997; Jin, Kamanna et al. 1999; Kamanna and Kashyap 2000).

RESULTS AND DISCUSSION

The model constructed represents the dynamic evolution of the lipid content of lipoproteins for three different stages: i) Stage 1 simulates normal plasma triacylglycerol levels; ii) Stage 2 corresponds to the hypertrygliceromelia development; and Stage 3 indicates the action of niacin in the system.

Figure 2 shows the dynamic distribution of CE, and TG in lipoproteins at the different stages. These graphs are a percentage distribution of TG, and CE content in the lipoprotein population after the simulation of the three stages.

For LDL the concentration of LDL_{CE}, and LDL_{TG} at normal conditions reaches a steady state which is set as the initial condition for the following stage. HTG was represented as an increase of the residence time of $VLDL_{TG}$ in the system. When the perturbations associated to HTG are introduced, LDL_{CE}, and LDL_{TG} decrease, and increase respectively (see Figure 2-A, 2-B). The reduction of LDL_{CE} can be interpreted as the result of triacylglycerol-rich VLDL accumulation, TG is transferred to LDL, which leads to a reduced CE content in LDL. While the new LDL distribution, which contains a higher percentage of LDL_{TG}, is associated to the VLDL accumulation, TG is transferred to LDL, which leads to an increased TG content in LDL and to an increased activity of HL on LDL. After the system reaches steady state, the action of niacin is applied (Jin, Kamanna et al. 1997; Jin, Kamanna et al. 1999). As a result of the action of niacin LDL_{TG} is further reduced, while LDL_{CE} increases (see Figure 2-A, 2-B). This corresponds to the modulating effect of niacin in LDL profile (Kamanna and Kashyap 2000).

Figure2-C and 2-D show the distribution of CE,

and TG in HDL respectively at the different stages. When the system is perturbed from normal steady state conditions (HTG), we observe no changes in HDL_{CE} while HDL_{TG} increases. Although the CE content is the same as in normal conditions, the distribution of HDL has a higher TG than CE content with respect to the initial distribution. These changes in TG content are consistent with reported HTG effects in lipid redistribution among lipoproteins: triacylglycerol-rich VLDL accumulates, leading to the transfer of TG to HDL (Lamarche, Rashid et al. 1999; Lewis and Rader 2005). After the system reached a new steady state, we evaluated the action of niacin. Perturbations (see Figure 3) were imposed for the effect of niacin according to literature: increase HDL_{CE} and reduction in $VLDL_{TG}$ synthesis rates. (Jin, Kamanna et al. 1997; Jin, Kamanna et al. 1999). As a result of the action of niacin, HDL_{CE} levels were higher than normal, and TG content in HDL was lower than in the normal scenario.

CONCLUSIONS

In this work we present a dynamic model which represents the effect of metabolic alterations such as HTG in CE and TG content in lipoproteins. The differences observed for lipoproteins' TG content are as expected biologically. As TG levels increase in HTG conditions there is a reduction in the relative content of CE in lipoproteins. When niacin is administered, TG content in lipoproteins is reduced

Although this modeling work is still in early stages, it allowed us to study the dynamic effect of cholesterol lowering drugs in lipid content distribution for lipoproteins.

GLOSSARY

Variable	*Description*
VLDL	Very low density lipoprotein.
LDL	Low density lipoprotein.
HDL	High density lipoprotein.
HDL_{CE}	Average quantity of CE in HDL.
HDL_{TG}	Average quantity of TG in HDL .
LDL_{CE}	Average quantity of CE in LDL.
LDL_{TG}	Average quantity of TG in LDL.
$VLDL_{CE}$	Average quantity of CE in VLDL.
$VLDL_{TG}$	Average quantity of TG in VLDL.
CETPCE	Amount of CETP with CE (CETP-CE).
CETPTG	Amount of CETP with TG (CETP-TG).
CE	Cholesteryl ester.
TG	Triacylglycerol.
CETP	Cholesteryl ester transfer protein.
LPL	Lipoprotein lipase.
HL	Hepatic Lipase

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