RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Control of Cholesterol homeostasis by Entero-hepatic bile transport – Role of feedback mechanisms

Shekhar Mishra[#], Pramod R. Somvanshi[#], K. V. Venkatesh[*]

[*]*Department of Chemical Engineering, Indian Institute of Technology Bombay, Powai, Mumbai 400076, INDIA. Fax: +91-22-2572 6895; Tel: +91-22-2572 7223; E-mail: venks@iitb.ac.in*
[#] *SM and PRS contributed equally to this work.*

Abstract

Cholesterol homeostasis is achieved through a tight regulation between synthesis, dietary absorption, utilization for bile salts and excretion. These processes are regulated through three known feedbacks, namely auto-negative regulation on hepatic bile salt synthesis and positive regulation of intestinal bile salts on cholesterol absorption and cholesterol excretion. A model for the entero-hepatic cholesterol metabolism in conjunction with dietary inputs of cholesterol was used to obtain insights into the role of the feedbacks. The analysis demonstrated the significance of the negative feedback on maintaining physiological levels of cholesterol. Further, the positive feedbacks by the intestine bile salts on the cholesterol absorption /excretion processes play an important role in plasma cholesterol homeostasis. Under Familial Hypercholesterolemia (FH), perturbations in the hepato-intestinal reversible bile transport revealed that an increase in the transport of bile salts from intestine to liver decreased the hepatic cholesterol absorption rate. In such a condition, a 2-fold change in bile salt transport resulted in around 20% reduction in plasma cholesterol levels, thereby restoring it to normalcy. This suggests that the bile transport control strategy presents an alternative therapeutic method by effectively reducing the cholesterol absorption to attain cholesterol homeostasis.

Introduction

Liver plays a central role in the regulation of cholesterol levels in the body to maintain its homeostatic levels[1]. It houses the largest pool of free cholesterol, which is directed towards various sections of the body depending upon their needs. Intestine is responsible for the uptake of cholesterol from diet[2–5] and its excretion from the body. It has significant interactions with the liver that play an important role in the homeostasis[6]. High cholesterol levels in the blood can lead to deposition on the arterial walls which thicken as a result of the accumulation. This leads to a chronic inflammatory response in the walls causing atherosclerosis[7,8] and cardiovascular diseases[9–11]. The common causes for increase of cholesterol levels in the plasma are high intake of dietary cholesterol and/or impaired cholesterol synthesis, absorption and excretion.

Popular strategies for treatment of elevated cholesterol include the use of statins, bile acid sequestrants and cholesterol absorption inhibitors. Statins target the cholesterol biosynthesis pathway, which is responsible for most of the body's cholesterol needs[11–16]. Suppressing this pathway reduces the de novo cholesterol synthesis, thus decreasing plasma cholesterol. The purpose of cholesterol absorption in the intestine is to minimize loss of cholesterol that gets excreted with the biliary secretions; it also facilitates absorption of dietary cholesterol[2,4,17]. The inhibitors of this process, such as ezetimibe, inhibit the uptake of cholesterol from dietand are used to reduce cholesterol in the body. Abnormal cholesterol levels in the plasma may occur due to perturbations in the normal functioning of any of these processes. However, these processes are subject to drug therapies irrespective of the actual causes for elevated cholesterol. Despite the statin treatments, the events leading to atherosclerosis have not been reasonably controlled, providing us with room for more potential therapies to control total plasma cholesterol levels.

A prominent process of cholesterol utilization in the body is the synthesis of bile acids from cholesterol. Bile acid sequestrants promote the excretion of bile salts and boost the conversion of cholesterol into bile, thus reducing the blood cholesterol levels[18–21].In animal studies[22–24], it has been observed that despite a higher dietary intake of cholesterol, the plasma cholesterol levels did not increase substantially, owing to higher excretion of bile salts. The cholesterol homeostasis is strongly interconnected with bile metabolism and the mechanisms by which these two pathways regulate each other are still unclear. Therefore, developing a mathematical model of the biological network comprising of the interactions (feedbacks) between cholesterol and bile metabolism in entero-hepatic compartment and the analysis of the network is essential to

decipher the control mechanisms prevailing in the structure. For this purpose, we resorted to the integration of two different models in the literature[25,26] and analyzed the role of the feedbacks and the bile transport in cholesterol homeostasis.

Here, we highlight on the major interactions in the entero-hepatic cholesterol metabolism. The intestinal cholesterol is absorbed in the liver where it is converted into the hepatic bile salts (HBS). Bile salts are known to act as carriers of cholesterol to facilitate micelle formation. A part of hepatic cholesterol pool is also transported to blood to maintain plasma cholesterol levels. In the liver, an auto-negative feedback of bile salts regulates itsown synthesis. The bile salts from the liver are transported into the intestinal bile salt (IBS) pool. The intestinal cholesterol (IC) pool has two inlets – biliary cholesterol release from liver and dietary cholesterol and two outlets – cholesterol excretion and cholesterol absorption back into the liver. The intestinal bile salts are required forfacilitatingthe excretionas well as absorption of intestinal cholesterol thereby positively regulating thetwo processes. Thus, three main feedbacks may be identified in the entero-hepatic cholesterol metabolism.

Cholesterol homeostasis mainly depends on the three primary feedbacks prevalent in the entero-hepatic cholesterol metabolism. The focus of the current study is to explore the mechanisms that play a role in cholesterol and bile homeostasis and suggest an alternative therapeutic strategy to control cholesterol levels.Towards this, we develop and utilize a mathematical model to obtain insights into the role of these feedbacks on cholesterol homeostasis. The importance of the feedback mechanism in relation to varying dietary cholesterols is analyzed. The feedback analysis reveals that increased bile excretion has a dual role in reducing cholesterol levels -a lower steady state levelof intestinal bile salts reduces the strength of the positive feedback on cholesterol absorption into the liver, thereby reducing the overall plasma cholesterol levels; secondly, increased bile excretion boosts bile synthesis thus increasing cholesterol utilization. The analysis indicated that the auto-negative feedback on the synthesis of the hepatic bile salts and the positive feedback on the hepatic cholesterol absorption play an important role in regulating cholesterol levels. Therefore, the entero-hepatic reversible bile transport is a possible site for perturbation/control to restore the plasma cholesterol levels under hypercholesterolemia.

Material and Method-Model Development and Analysis

The whole body model for cholesterol metabolism developed by McAuley et al. (2012)[26], which includes intestine, liver, peripheral and blood compartments was adopted. From literature[27–31], we know that other factors like dietary nutrients and components, along with lifestyle effects also play a significant role in determining cholesterol levels in the body. The effects of dietary components on cholesterol metabolism were taken from the system dynamics model written by Demirezen&Barlas (2009)[25]. Figure 1 showsthe interactions in the entero-hepatic compartments in cholesterol metabolism. The rate equations applicable for the process of synthesis, absorption and excretion of cholesterol and bile salts are listed in the supplementary information.

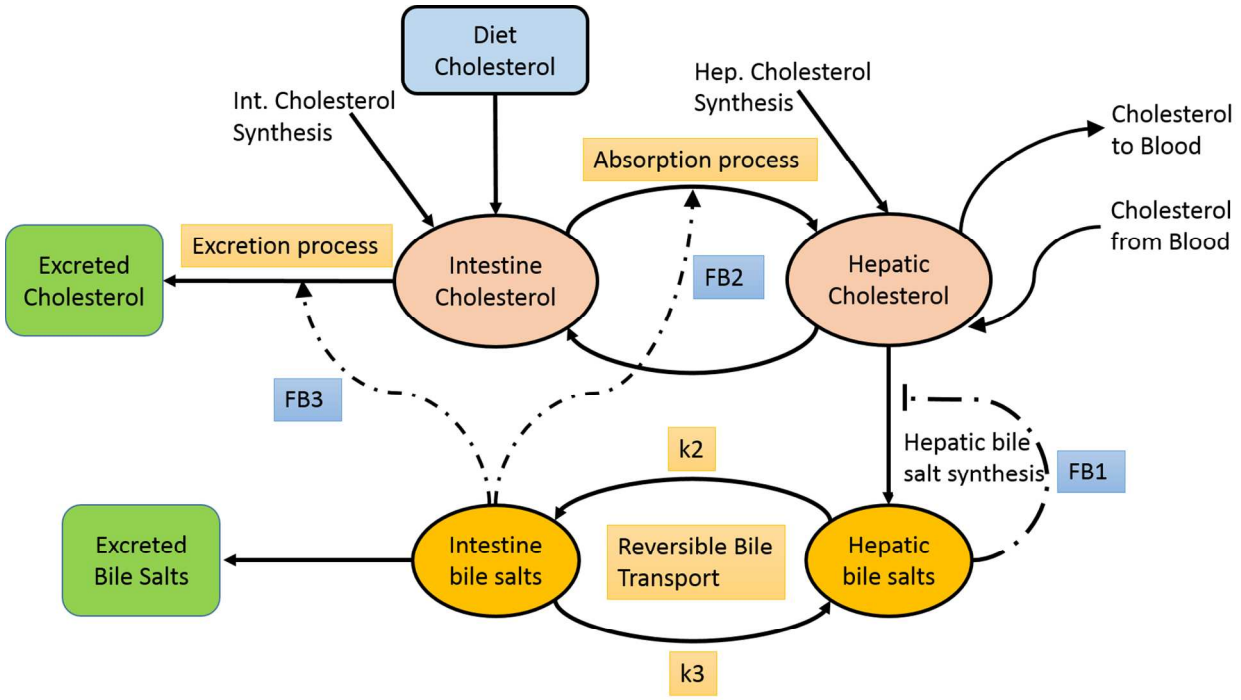

Figure 1 - Module depicts the interactions in entero-hepatic cholesterol and bile metabolism. The dietary cholesterol is absorbed into the intestine; from there it is further transported to liver. The part of hepatic cholesterol is used for bile synthesis and for maintaining the plasma cholesterol level. FB1 represents the auto-negative feedback on hepatic bile salts. The hepatic bile salts are reversibly transported to intestine. The intestinal bile salts help in hepatic cholesterol absorption and intestinal cholesterol excretion thereby resulting into a positive feedback represented by FB2 and FB3, respectively.

The two models, based on different modeling frameworks, were integrated and analyzed in the present study. One of the models by Demirezen&Barlas (2009)[25] used the principle of systems dynamics, wherein the concept of stock and flow variables is used to emulate a rough idea of the time dynamics of the model. The system dynamics approach allows us to break down extremely

complex non-linear systems into systems with stocks, rate flows and internal feedback loops thus allowing for a preliminary analysis of the dynamics under limited mechanistic details. The authors used empirical data to express the rate kinetics of various reaction pathways. The systems dynamic model accounts for changes in the cholesterol trends due to various external attributes like effect of dietary components on cholesterol biosynthesis, effect of body weight, effect of exercise etc. One of the limitations of this method is that it ignores the actual mechanisms occurring inside the system as most rate expressions (flows) are based on empirical data with a black box assumption. Therefore, the impact of mechanistic changes in any variable upon the system's phenotypic response will not be reflected in the results. The other model we resorted to was the mechanistic model by McAuley et al. (2012)[26]. This model implements the law of mass action and Michaelis-Menten formulation to express rate kinetics of every step involved in the cholesterol metabolism. It accounts for the actual mechanisms in a greater detail, but ignores the effect of external factors. These two models form the basis of the integrated model that we have presented (See supplementary material).

Our effort has been to merge the two models, thus being able to imitate not only the mechanistic processes with greater detail, but also the effects of the above mentioned external factors. To incorporate the effects of external factors into the current model, we translated the empirical data into convenient Hill expressions to fit the data points and included these effects into the model by McAuley et al. (2012)[26]. The model was simulated in MATLAB (www.mathworks.in) using ODE15s solver. The algorithm used for obtaining the effect of feedback mechanisms and parameters is shown in Figure 2.

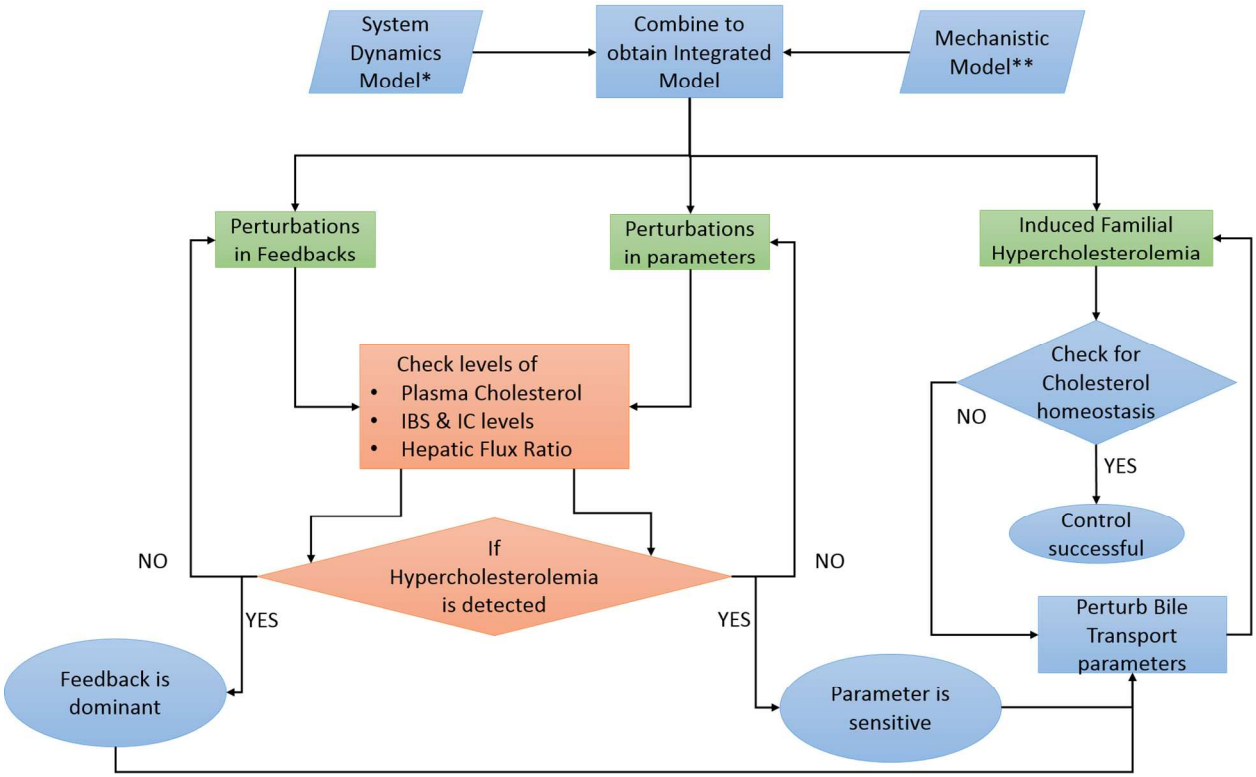

Figure 2 –Flowchart representation of the algorithm followed in this paper to investigate the effects of feedback and parametric perturbations in the mathematical model. The flowchart also shows the implementation of the Bile Transport Strategy, which uses the results of the feedback and parametric perturbations. * represents the Systems Dynamics model by Demirezen&Barlas (2009); ** represents the mechanistic model by McAuley et al. (2012).

The model incorporates theabsorption of dietary cholesterol into intestinal cholesterol pool which is further transported to hepatic cholesterol. Hepatic cholesterol is converted into hepatic bile salts. Further, the model explicitly accounts for the following mechanisms. A negative feedback of bile salts is maintained on its own synthesis. The bile salt from the liver is transported into the intestine bile salt pool and a reverse transport is also operational that helps to equilibrate the entero-hepatic bile salt balance. The intestinal bile salt pool regulates the outlets of intestinal cholesterol – excretion and absorption into liver.Part of hepatic cholesterol is secreted into the intestinal cholesterol pool through biliary cholesterol release[17,18,32,33].These interactions and transport processes collectively regulate cholesterol homeostasis. The model thus contains three feedbacks namely, 1)an auto-negative feedback on hepatic bile synthesis-FB1, 2) a positive feedback of intestinal bile salts on hepatic cholesterol absorption-FB2 and 3) a positive feedback of intestinal bile salts on intestinal cholesterol excretion-FB3.

The developed model was used to obtain steady state input-output relationships for plasma cholesterol, intestinal cholesterol and intestinal bile salt levels in relation to varying levels of cholesterol in diet. The model was also used to obtain the effect of the three feedbacks present in the network. To monitor the effect of perturbations on the net cholesterol accumulation in the liver, the ratio of intestinal cholesterol absorption to the sum of hepatic bile synthesis biliary cholesterol release was recorded. Since this represents the ratio of cholesterol input to cholesterol output from the liver, changes in this ratio represent the effective plasma cholesterol levels. At every value of dietary cholesterol, a thousand simulations were run in which parameters of the module were randomly selected to be varied in a range of 10% on either side of their original values. The variation in the cholesterol values were recorded for all the simulations.The mean and standard deviation of total plasma cholesterol, intestinal cholesterol, intestinal bile salts, cholesterol flux to blood and input-output hepatic flux ratio were calculated. The extent of spread around the mean of these values at each dietary level was used as an indicatorof the robustness due to perturbation of each feedback.

Results

Steady-stateresponse for varying dietary cholesterol

The developed model was simulated to obtain the dynamic concentration profile of the Total Blood Cholesterol for different values of dietary cholesterol and for different network perturbations, as shown in Figure 3. Under normal physiological conditions, the profile shows an invariant dynamic response indicating homeostatic levelwith total plasma cholesterol at 5.38mM.The total plasma cholesterol is represented by the summation of Low Density Lipoprotein Cholesterol (LDLC), Very-Low Density Lipoprotein Cholesterol (VLDLC), Intermediate Density Lipoprotein Cholesterol (IDLC) and High Density Lipoprotein Cholesterol (HDLC). Thus, the parameter values used in the model yield physiologically relevant steady state values. Figure 3 (A) shows the behavior of total plasma cholesterol for different dietary cholesterol values. A 2-fold reduction in dietary cholesterol intake reduces total cholesterol to 5.25 mM but a 4-fold increase from normal diet cholesterol results in an increase of 20%. In Figure 3 (B), perturbing the negative feedback on bile synthesis causes total plasma cholesterol to rise by 15%, whereas perturbation of the intestinal bile salt feedback reduces the cholesterol value to 5.25 mM.

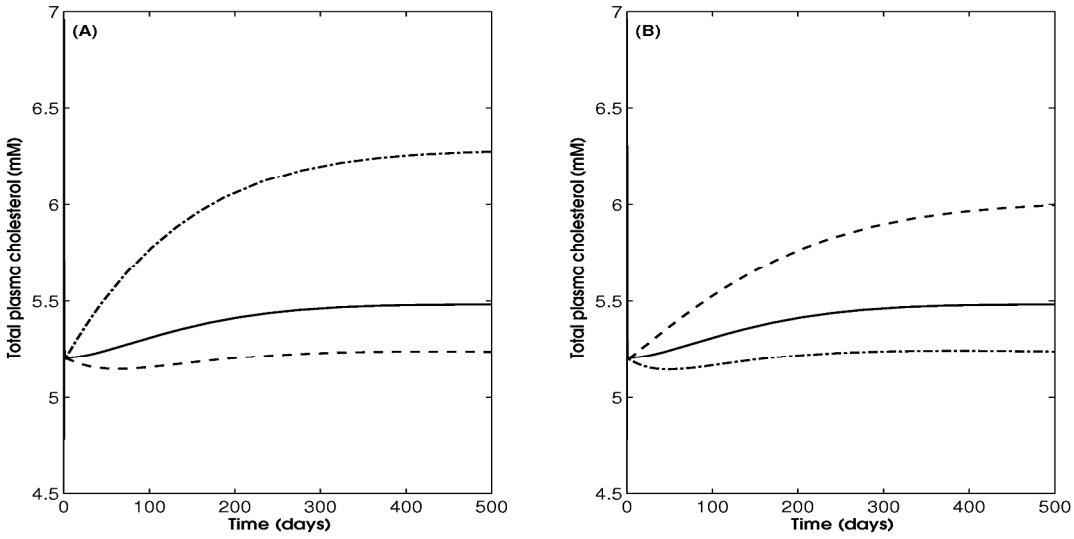

Figure 3 - Dynamic response of total plasma cholesterol in response to (A) Variation in dietary cholesterol values. Solid line represents normal dietary cholesterol, dashed line represents a 2-fold reduction in dietary cholesterol and dashed-dotted line represents a 4-fold increase in dietary cholesterol (B) Perturbations in different feedback regulations. Solid line represents normal phenotypic functioning, dashed line represents absence of FB1, and dashed-dotted line represents absence of FB2 (as in Fig.1).

Figure 3 also shows the dynamic response for this module lacking the negative feedback on the hepatic bile salt synthesis[34,35]. The above analysis suggests that the negative feedback on the hepatic bile salt synthesis is essential in regulating blood cholesterol levels. Removal of this negative regulation causes the dominance of the indirect positive feedback loop (see Fig.1), thereby leading to higher cholesterol levels.

Role of individual feedbacks on homeostasis

The analysis of the model indicated that the system attains a monostable steady state at different initial conditions. In order to analyze the system performance at different levels of dietary cholesterol the steady state values were obtained and plotted against different dietary cholesterol levels with and without feedback perturbations. The system performance was evaluated using four metrics namely,Total Plasma Cholesterol, Intestinal Cholesterol (IC) Levels, Intestinal Bile Salts (IBS), and the Ratio of input to output fluxes for the hepatic cholesterol pool. Figure 4 shows the effect of the dietary cholesterol on the four metrics for three different cases - normal physiological conditions, absence of negative feedback on the hepatic bile salts (HBS), and the absence of positive feedback on intestinal cholesterol absorption by intestinal bile salts.

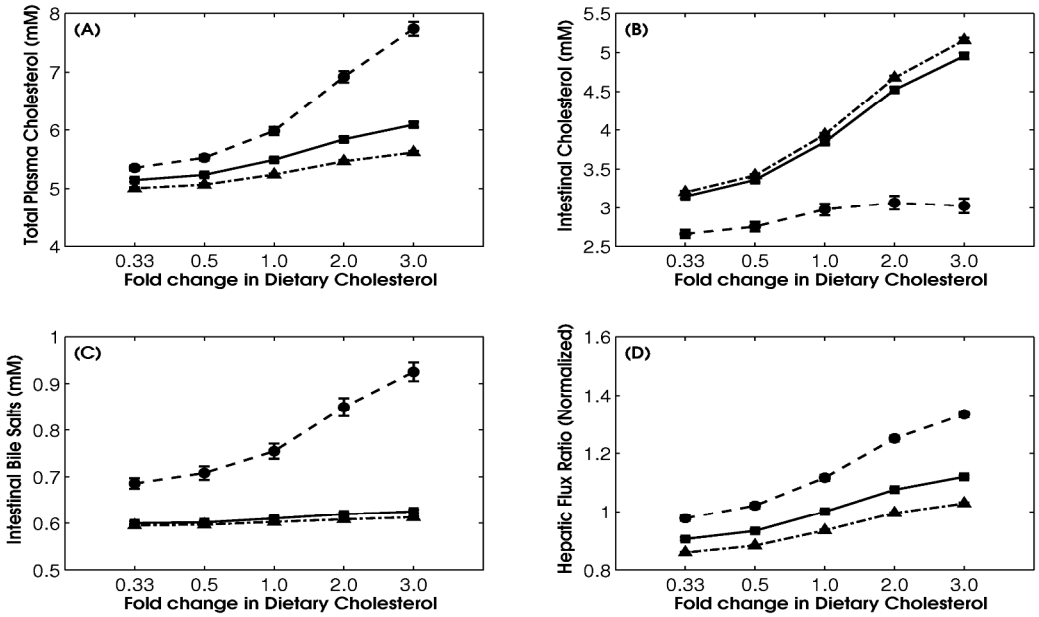

Figure 4 - Perturbation analysis of different feedbacks in the liver-intestine module. Removal of the HBS negative feedback increases the IBS concentration, thus increasing the flux of cholesterol absorption to the liver. This increases the total plasma cholesterol as well. Removal of the IBS positive feedback reduces the flux of cholesterol absorption responsible for its accumulation in the liver, which leads to a reduction in the hepatic and plasma cholesterol levels. Solid line with squares represents the normal phenotypic response, dashed line with circles represents absence of FB1, and dashed-dotted line with triangles represents absence of FB2 (as in Fig.1).

The steady state values are represented as the mean with a standard deviation of 1000 realizations through random multi-parametric perturbations. Under normal physiological levels, a three-fold increase in the dietary cholesterol (relative to about 304 mg/day) raises the plasma cholesterol to about 6 mM indicating the advent of hypercholesterolemia. This is accompanied by an increase in the intestinal cholesterol levels. However the bile salt levels do not increase on increasing the dietary cholesterol levels. Further, as expected the Hepatic flux ratio increases by about 15% in the range studied. Parametric perturbation resulted in a negligible change in the standard deviations indicating a robust behavior under physiological conditions.

On contrary,removal of the negative feedback on the synthesis of hepatic bile salts (FB1)leads to an increase in the plasma cholesterol levels, even for normal dietary cholesterol levels of 304 mg/day indicating hypercholesterolemia. In such an instance, reduction in the dietary cholesterol levels can help to maintain healthy plasma cholesterol levels.The perturbation is accompanied by a lower intestinal cholesterol due to higher flux towards hepatic cholesterol and hence, towards blood (See Fig. 4(B) and Fig. 4(D)). The removal of the negative feedback results in a loss of robustness indicated by higher standard deviation around the mean when parametric

perturbations are performed. This indicates that the negative feedback (FB1) is essential for a robust performance by the network.

Next, we analyzed the role of the positive feedback by intestinal bile salts on intestinal cholesterol absorption (FB2). Interestingly, on removing the FB2, the total cholesterol levels decrease even at higher dietary cholesterol levels with a maximum concentration of the blood cholesterol of 5.4 mM even when the dietary levels are 3 times that of the normal. This indicates that removal of the positive feedback essentially maintains the blood cholesterol levels independent of the dietary cholesterol levels in the range studied. This is also accompanied by a lower intestinal bile salts and the input/output flux ratio of hepatic cholesterol pool. The lower flux towards hepatic cholesterol due to absence of the feedback ensures higher cholesterol concentration in the intestine. The standard deviation due to parametric perturbation was the same as that for the normal physiological condition indicating that the feedback does not play a role in the robustness of the system. Therefore, the presence of the negative feedback (FB1) essentially controls the perturbation effects in the parameters.

Effect of multiplefeedbackson homeostasis

The previous results indicate that while negative feedback is essential for maintaining normal plasma cholesterol levels, the absence of positive feedback maintains lower cholesterol levels. Since the individual knockout demonstrates a contrast in the module's response, we explore the effect of simultaneous knockout of both the feedbacks (FB1 and FB2). On simultaneous removal of the feedback, the steady state levels for the plasma and intestinal cholesterol are lower than that observed for normal physiological conditions (See Fig.5). This indicates that the positive feedback dominates over the negative feedback. Although, in such a situation, the plasma cholesterol is low, the intestinal bile salts are higher than in the normal physiological conditions. Further, the absence of the negative feedback increases the standard deviation in the intestinal bile salt levels. Thus, the presence of the two feedbacks is essential for maintaining robust homeostasis of both plasma cholesterol and intestinal bile salt levels.

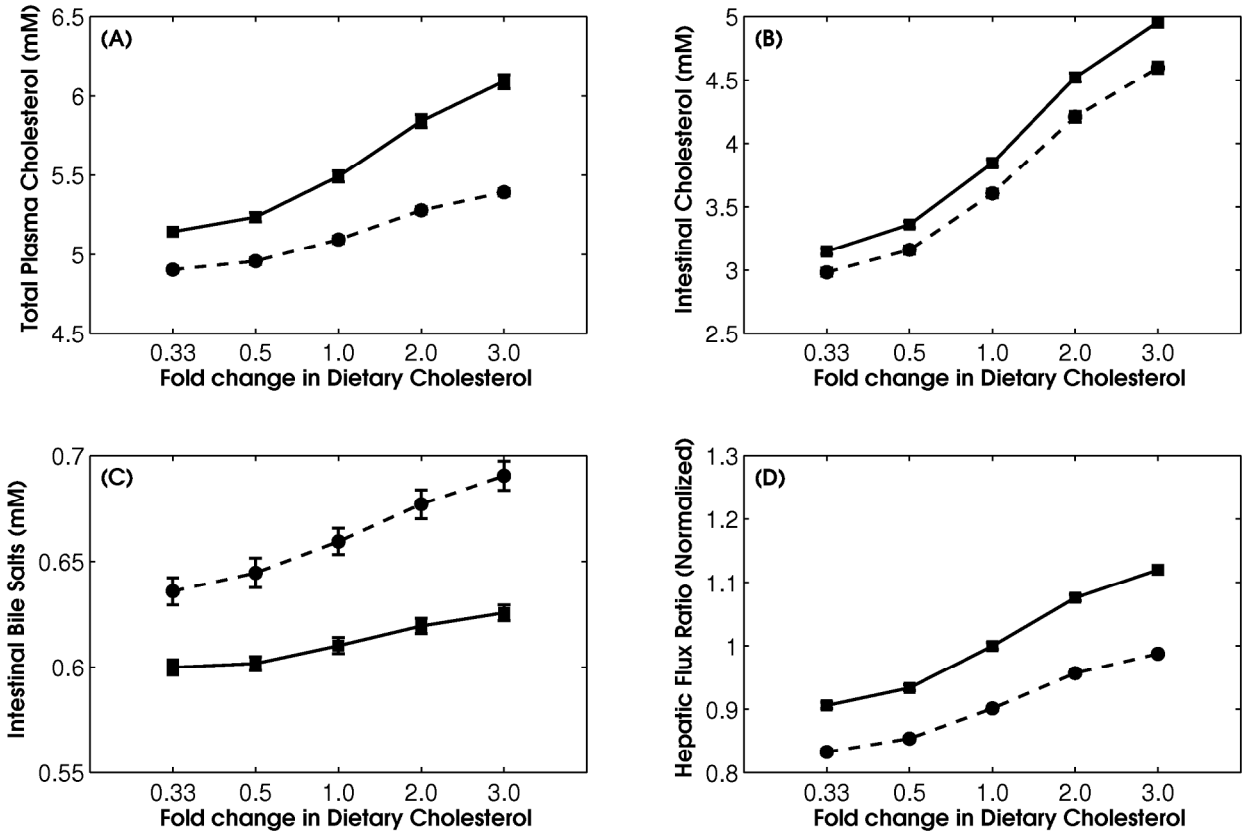

Figure 5 - Simultaneous removal of HBS and IBS feedbacks. Removal of the HBS negative feedback leads to cholesterol increase due to an uninhibited rise of IBS concentrations. The effect of high levels of IBS on cholesterol absorption flux can be countered by removing the positive feedback of IBS on this process. This brings back cholesterol levels to healthy limits.Solid line with squares represents the normal phenotypic response and dashed line with circles represents the simultaneous removal of FB1 and FB2 (as in Fig.1).

Perturbation in the bile excretion

In order to evaluate the significance of the functioning of these two feedbacks we lowered the bile salt excretion flux by reducing the parameter k4 (See the model equation in supplementary info). In such a scenario, the steady state plasma cholesterol is very high indicating hypercholesterolemia (See Fig.6(A)) However, the intestinal cholesterol levels marginally decrease, due to an increased flux towards hepatic cholesterol (See Fig.6(D)). On removing the two feedbacks along with the faulty bile excretion, the total plasma cholesterol levels are invariant to dietary cholesterol levels. Although healthy physiological levels are observed for the cholesterols, the intestinal bile salts levels show a 16-fold increase tothe normal physiological range. Thus, this loss of bile homeostasis indicates the significance of these two feedbacks. Further, as observed before, the absence of the feedbacks increases the standard deviations indicating a sensitive response to perturbations.

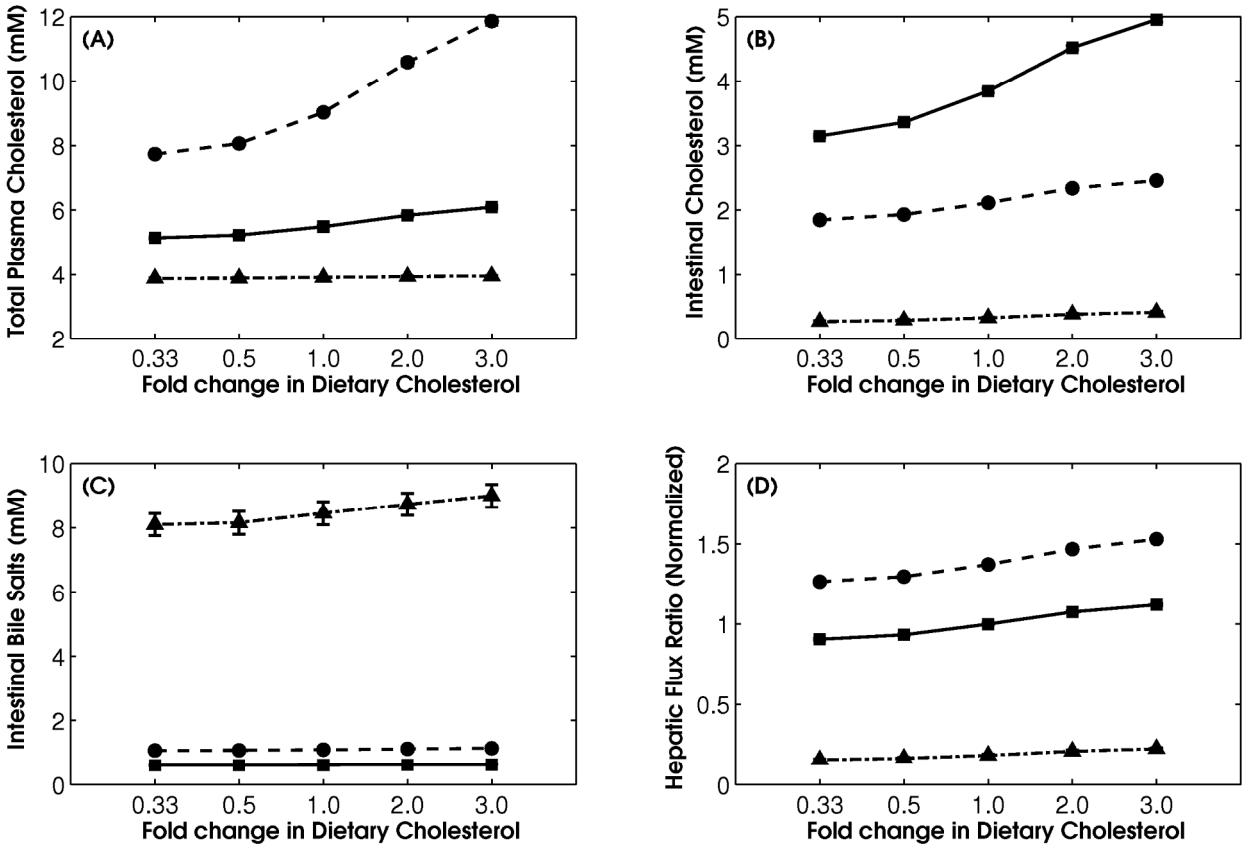

Figure 6 - Feedback perturbations in the scenario of faulty bile excretion. The importance of the HBS and IBS feedbacks is not apparent in figures (A), (B), (C), and (D) as far as cholesterol regulation is concerned. However, in figure (C), it is quite significant that absence of the feedbacks leads to a deregulation of bile homeostasis. Due to uninhibited synthesis of bile salts, an uncontrolled increase in bile concentrations are observed (dash-dot line), in contrary to the case where feedbacks exist (dotted line). A significant rise in standard deviation denotes a lack of robustness due to the removal of the feedbacks (figure d – dash-dot curve).Solid line with squares represents the normal phenotypic response, dashed line with circles represents a scenario of faulty bile excretion, and dashed-dotted line with triangles represents absence of FB1 and FB2 in the scenario of faulty bile excretion (as in Fig.1).

Role of the feedback on cholesterol excretion

Next, we eliminate the feedback from the intestinal bile salts on the excretion of the cholesterol from the intestine (FB3). It can be observed that the feedback does not significantly affect the physiological response (See Fig.7). The removal of the feedback, however, increases the variation in response to the parametric perturbations.

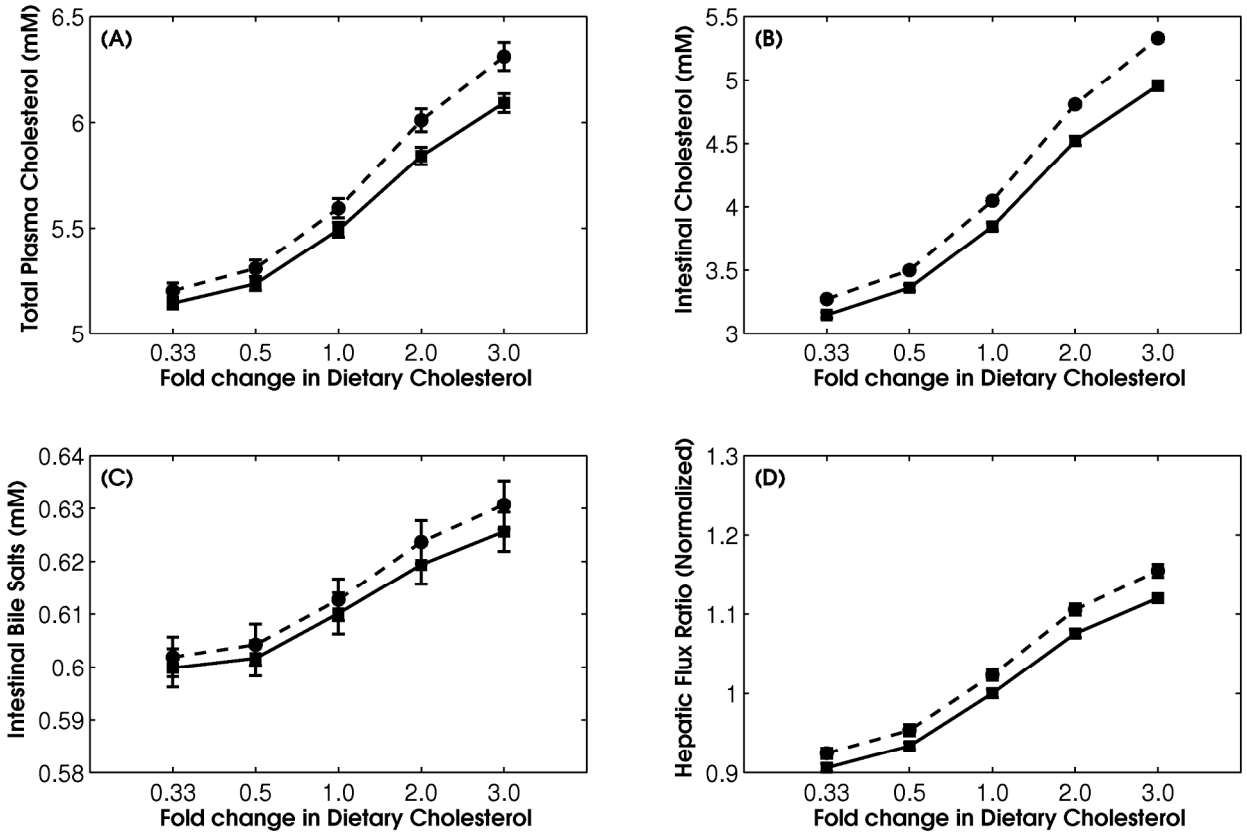

Figure 7 - Absent IBS positive feedback on cholesterol excretion. The impact of removal of this feedback is not significant and the cholesterol levels show little deviation from the normal values.Solid line with squares represents the normal phenotypic response, and dashed line with circles represents removal of FB3 (as in Fig.1).

Effect of Increased Bile Transport

These studies indicate that the positive regulation of intestinal bile salts over absorption (FB2) plays a more significant role thanthat over the excretion (FB3). This motivated the idea for a control strategy that involves reducing intestinal bile salt concentrations to reduce liver and plasma cholesterol levels. The strategy is implemented by boosting the rate of bile salt transport from intestine to liver, which depletes the intestinal bile salt concentration.

Figure 8 shows the steady state response, when the bile transport parameter from intestine to liver is increased by 1.5-fold. This results in reduced blood cholesterol levels. The reduced intestinal bile concentration causes a decrease in the intestinal cholesterol absorption, as seen in the hepatic flux ratio (See Fig.8(D)). As this is an outflow for intestinal cholesterol pool, the intestinal cholesterol levels go up. The decrease in absorption can be directly correlated to a

decreased flux towards blood. Thus, the above strategy of lowering the intestinal bile transport is a possible mechanism to regulate plasma cholesterol levels.

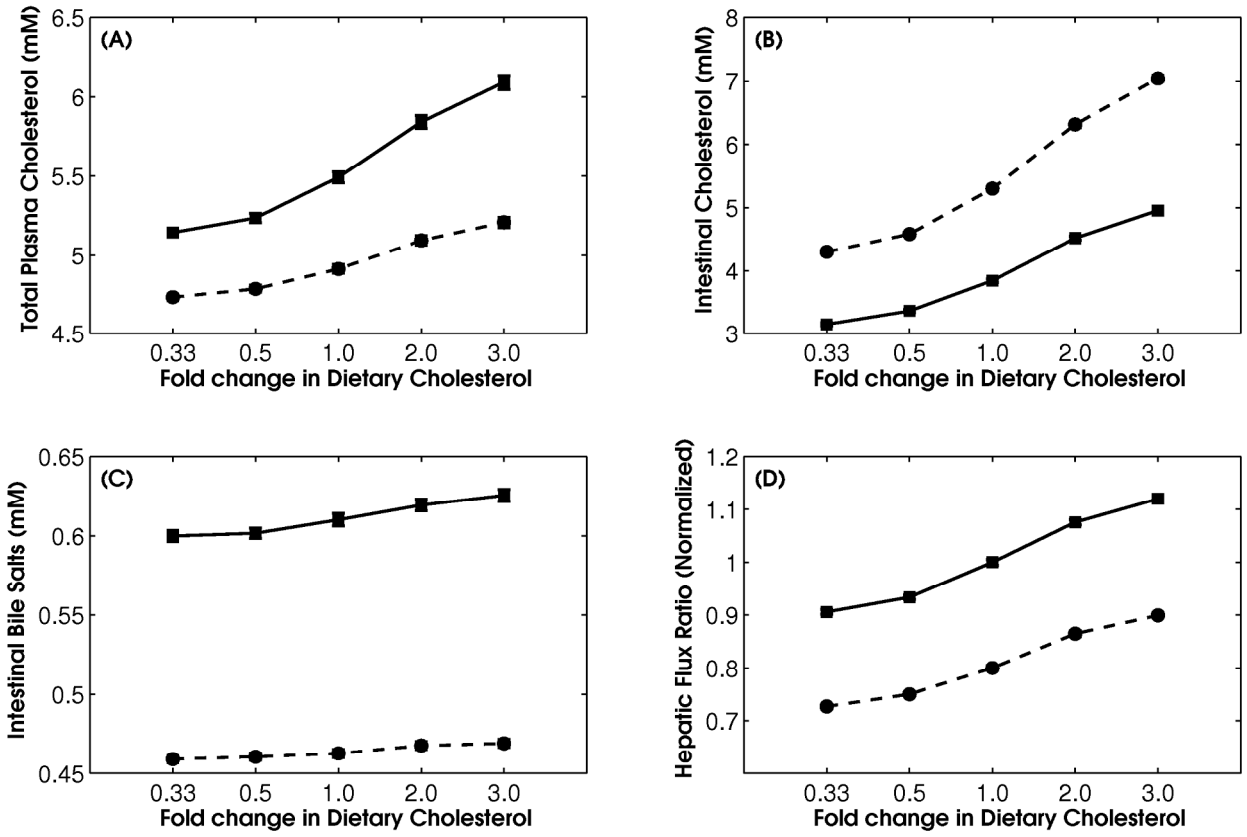

Figure 8 - Effect of Increased Bile Transport on Cholesterol Homeostasis. The increased bile transport reduces intestinal bile salts thus reducing the excretion and absorption fluxes. Since absorption is affected more significantly, cholesterol returning to the liver is reduced, thus resulting in healthier plasma cholesterol values. The reduction in flux values leads to an accumulation of cholesterol in the intestine depicted in figure (B). Solid line with squares represents the normal phenotypic response, and dashed line with circles represents the scenario with increased bile transport.

To test the efficacy of such a control strategy, a scenario of familial hypercholesterolemia (FH) was induced in the model. FH is a genetically acquired disease in which patients suffer from high cholesterol levels due to faulty Low-Density Lipoprotein (LDL) receptor metabolism[36]. This causes a buildup of cholesterol levels in the blood which is linked to a high risk of coronary heart disease[9]. In the model, FH was induced by reducing the rate of hepatic LDL receptor synthesis (k_{hrs}) to 0.8-fold the original value. In this case, the plasma cholesterol levels increased to 6.25 mM from 5.5mM under normal dietary cholesterol levels indicating FH. Under FH, if the intestinal bile transport is lowered, the plasma cholesterol levels reduce back to healthy limits (See Fig.9(A)). As seen before, the intestinal cholesterol levels increase from 3.9 mM to 6.9 mM

(See Fig.9(B)). Thus, the healthy physiological homeostasis is regulated through the intestinal bile transport in coordination with the feedbacks.

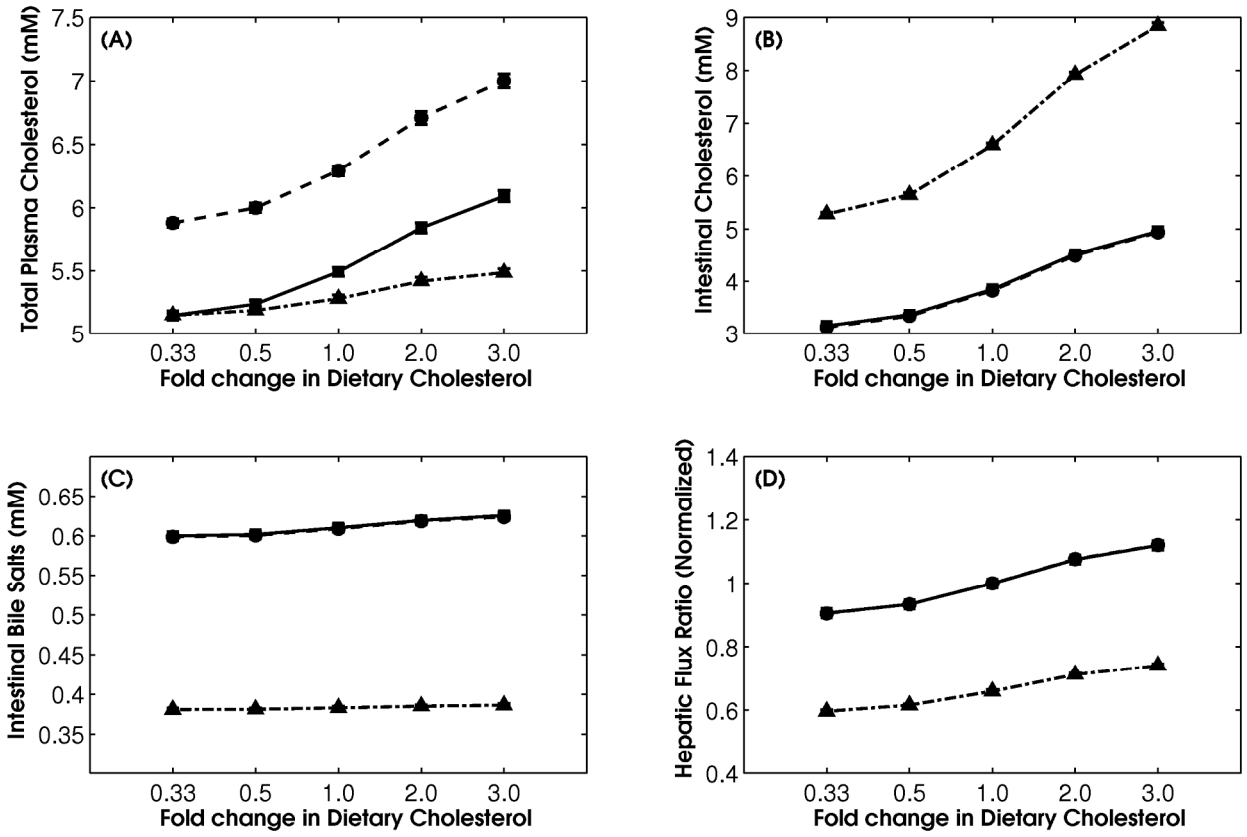

Figure 9 - Significance of Increased Bile Transport as a therapeutic method. The method of Increased Bile Transport to bring down cholesterol levels is implemented in the scenario of familial hypercholesterolemia. This method helps control cholesterol levels bringing them down to healthy limits. Solid line with squares represents the normal phenotypic response, dashed line with circles representsFamilial Hypercholesterolemia, and dashed-dotted line with triangles represents increased bile transport in a scenario of Familial Hypercholesterolemia.

Effect of parameter sensitivity

The positive feedback by intestinal bile salts on the hepatic cholesterol absorption is represented by a Hill equation with a Hill coefficient of n=2.5. The role of sensitivity was studied by making the feedback less sensitive by assuming the Hill coefficient value to be sub-sensitive (with n=0.7). Figure 10 (A) plots the reaction rates of the hepatic cholesterol absorption rate for the two Hill coefficients values (dash line for n=0.7 and solid line for n=2.5) and the cholesterol excretion rate (dash-dot line) at various intestinal bile salt conditions. It can be noted that for a sensitive positive feedback response, the excretion is higher than the Hepatic cholesterol absorption rate at low concentrations of IBS. This results in lower plasma cholesterol (See phase-

plane plot in Fig.10(B)). If the sensitivity is lowered (i.e. n=0.7), under similar situation, the response shifts to a higher plasma cholesterol level due to higher absorption rate over the excretion rate (See Fig.10(B)). A complete reversal in the response is observed at higher IBS concentrations (See Fig.10(C)). The results demonstrate the role of IBS concentrations and sensitivity of the positive feedback regulation on the steady state plasma cholesterol levels.

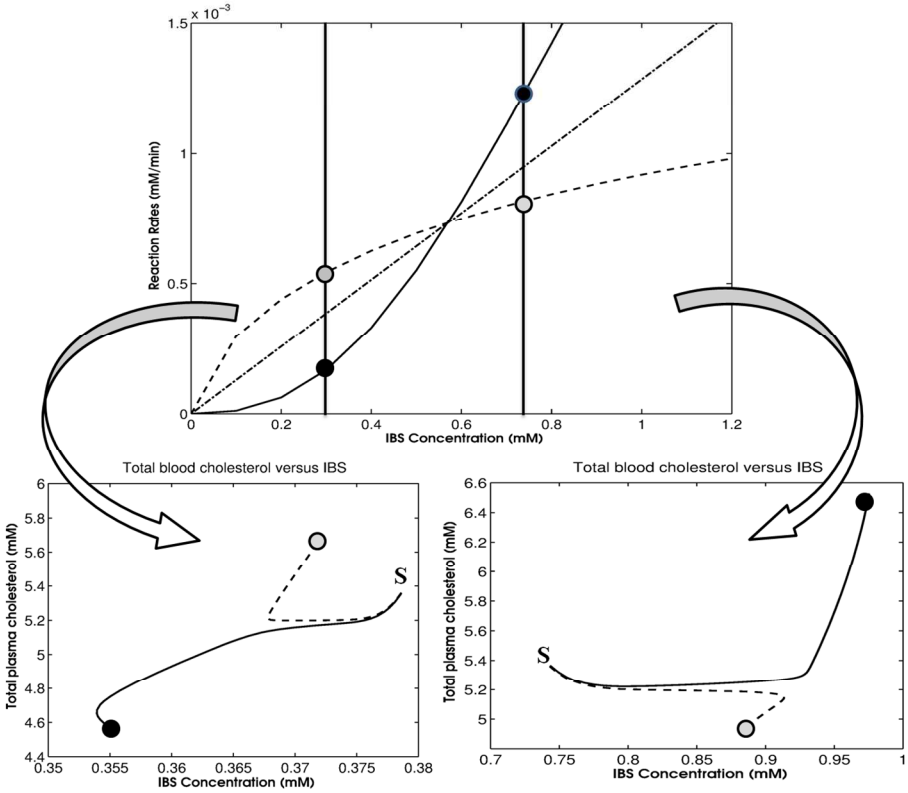

Figure 10 - Flip Response due to varying sensitivity in the intestinal cholesterol absorption. Sold line represents cholesterol absorption with n=2.5, dashed line represents absorption with n=0.7, and dashed-dotted line represents the reaction rate of cholesterol absorption.

Discussions

Apart from targeting some key enzymes of the biosynthesis pathway[16,37] (statins), not much has been proposed as an alternative measure to control cholesterol metabolism. Moreover, the statin therapy does not account for the actual causes of cholesterol increase, rather focusing on just the biosynthesis pathway.Thus, there exists a potential in elucidating different therapeutic mechanisms that can maintain cholesterol levels in the normal range by targeting the source of hypercholesterolemia. In our study, a control systems approach to understand the cholesterol metabolism was applied on a mechanism-based kinetic model based on the information available from models in literature[38–44]. This system engineering approach deals with interactions between

several modules in the network and the effect of each module on the overall performance of the network. In our study we simulated the integrated model to obtain insights on the role of the major feedback mechanisms in the entero-hepatic cholesterol and bile metabolism. We also ascertain the effect of perturbation in the bile transport mechanism as a potential control point in cholesterol homeostasis. The major insights of the feedback perturbations in the model are discussed below.

Role of individual feedbacks on cholesterol homeostasis

In this study we have analyzed the importance of various feedback regulations in the entero-hepatic bile transport module on the overall cholesterol homeostasis. Identifying the mechanisms responsible for elevated cholesterol consequently allows us to suggest therapeutic strategies to bring cholesterol levels back to the healthy range.The knockout of the negative feedback on hepatic bile salts enhanced the effect of the positive feedback thereby increasing the plasma cholesterol levels. It has been reported that hypercholesterolemia is observed in patients suffering from the defects in the FXR gene[45,46] that is responsible for employing the negative feedback mechanism[47,48]. Further the analysis also highlighted the significance of the feedback mechanisms to maintain robustness in the system by demonstrating higher standard deviation to the parametric perturbations on removing the feedback. The knockout of the feedback of intestinal bile salts reduced the absorption flux for the intestine thereby reducing the cholesterol levels both in liver and plasma. However, the knockout of the positive feedback on excretion of the intestinal cholesterol was not significant relative to the feedback on the absorption process.

Role of multiple feedbacks on cholesterol homeostasis

The simultaneous knockout of positive feedback of IBS and negative feedback of HBS demonstrated no significant difference in plasma cholesterol as compared to the normal. The cholesterol absorption into the liver is positively regulated by the intestinal bile salts. When the bile salts increase uncontrollably due to the absence of the HBS negative feedback, the flux to the liver increased causing an increase in the cholesterol levels. Hence, removal of the positive regulation of IBS on cholesterol absorption can help counter the pro-atherogenic effects of removing the negative regulation on bile synthesis. Therefore, the negative regulation of HBS and positive regulation of IBS exhibit a balance in action i.e. there is a need for both to exist together, and if one is perturbed, the other needs to be perturbed as well so as to maintain homeostasis. Thus, the intestinal cholesterol absorption plays a crucial role in determining the

cholesterol levels in the body at steady state. As more refined therapeutic strategies are investigated to control cholesterol, drugs can be designed to target this particular process and direct the net flux of cholesterol transport either towards the liver or excretion, effectively controlling the increase or decrease of cholesterol.

Role ofbile excretion

Since the collective removal of positive and negative feedbacks does not demonstrate any significant change in cholesterol levels, a pertinent question arises regarding the roles of the two feedbacks to regulate the cholesterol homeostasis. To explore this, a perturbation in the bile excretion was simulated. The simulations demonstrated an increase in cholesterol levels due to an increase in the bile salt concentrations, (See Fig. 6). In the perturbed bile excretion case, removal of the two feedbacks restores the plasma cholesterol levels. Essentially, the knockout of both the feedbacks results in a decoupling of the cholesterol and bile pathways in the liver-intestine module, which eliminates the regulatory effect of bile on cholesterol levels. However, the removal of these feedbacks causes an increase in the bile salt levels. Moreover, the increased standard deviations in bile levels indicate a loss of robustness in the bile homeostasis. Thus, the joint action of positive feedback of IBS and negative feedback of HBS ensure a control on the bile salt concentrations in the body and hence, are necessary for bile homeostasis.

Effect of Increased Bile Transport

The difference in regulatory strengths of IBS on absorption and excretion processes motivated the idea of a control strategy in which cholesterol levels can be controlled by altering the concentration of intestinal bile salts. This was implemented by increasing the rate of bile transport from intestine to liver, thereby reducing the IBS concentration. As shown in Figure 8, this results in a lower Input-Output Hepatic flux ratio, leading to a reduction of cholesterol pool in the liver, and a subsequent reduction in plasma cholesterol. Similar effects can be obtained by decreasing the liver to intestine bile transport. Due to reduced rates of absorption and excretion, the net outflow of cholesterol from the intestine is decreased, resulting in an accumulation of intestinal cholesterol. However, the accumulation is not that significant and can be mitigated by increasing the dietary fiber content which increases the excretion of intestinal cholesterol. To test the efficacy of this strategy, the increase in bile transport was implemented in a case of Familial Hypercholesterolemia. In a hypercholesterolemia case due to genetic defects, the bile transport perturbation resulted in restoration of normalcy in cholesterol levels, (See Fig. 9). This suggests

that increased bile transport from intestine to liver can be used as a control strategy to alleviate hypercholesterolemia.

A review article by Charach et al.[49] raises the point that improper excretion could possibly lead to a cholesterol build up thus causing coronary diseases associated with it. In fact, a popularly suggested cure for lowering cholesterol involves including flaxseed in the patient's diet. This seed has a high dietary fiber content which binds to the cholesterol in the intestine preventing its absorption and directing it to the excretion system. Other remedies like Chinese green tea and dietary plant proteins too lower the cholesterol content by increasing its excretion. Similar to a purge performed in a chemical plant to balance a build-up in the concentration of certain compounds, metabolic processes in a healthy human too work towards balancing a cholesterol build-up by purging.

Effect of feedback sensitivity

The Increased Bile transport trends displayed a dependence on the sensitivity of positive feedback on the intestinal cholesterol absorption. The intestinal cholesterol pool is maintained by three major fluxes - cholesterol excretion, absorption into the liver (outflows) and biliary cholesterol release from the liver (inflow). The balance of the intestinal cholesterol pool is governed mainly by the nature of kinetics of cholesterol excretion and cholesterol absorption. Here in the model, the excretion process is represented by mass action kinetics whereas the positive regulation of bile salts on the absorption process is represented by a Hill function. So as to assess the importance of the sensitivity of the positive regulation, we varied the Hill function from sub-sensitive to sensitive and observed the variation in plasma cholesterol. Interestingly, it was noted that there was a flip in the response in cholesterol levels the Hill function was varied from sensitive and sub-sensitive regulation (See Fig. 10(B)). In the sub-sensitive case, increasing the bile transport showed an increase in the plasma cholesterol, contrary to that observed for the sensitive case, where a substantial decrease was noted. This was essentially due to the relatively higher rate in the case of sub-sensitive regulation of absorption, which is higher to that of excretion flux that follows mass action kinetics. However, in the sensitive case, the mass action kinetics of the excretion process would dominate over the sensitively-regulated cholesterol absorption in the range of intestinal bile salts less than its own half saturation constant for the positive regulation (i.e. its Km value). Therefore, the differences in the kinetic nature of the absorption and excretion rates of intestinal cholesterol and its regulation by IBS can be exploited

to obtain the desired levels of cholesterol. It is expected that in a large population with different lifestyles, the parameter space for a flux ($K_{m,n}$) may vary in a certain range. Hence, difference in kinetics of these fluxes can lead to different response in a population.

Summary

The overall rigorous analysis of the hepato-intestinal bile transport systems demonstrates a significant diversity in the way it can regulate the plasma cholesterol levels. In case of feedback studies, the negative feedback on bile synthesis tightly controls the bile salts to ensure that liver cholesterol does not accumulate upon itself. The positive feedback on the intestinal cholesterol absorption maintains a balance between the amount of cholesterol leaving the system and that returning to the liver and perturbing this balance can shift the system in either direction. The positive and the negative feedbacks display a tender balance in their role, since disturbing one requires disturbing the other as well for a subsequent entry into healthy limits.The combined absence of both, while aiding the control of cholesterol homeostasis, is detrimental to bile salt homeostasis giving us an indication of their action. The therapeutic use of modifying the bile transport between liver and intestine can be seen in the resultant reduction in plasma cholesterol levels. This method was shown to be extremely efficient in controlling Familial Hypercholesterolemia. The balance of the fluxes in the intestinal cholesterol transport plays an even greater importance in such a perturbation, since the sensitivity of the positive feedback determines the homeostatic state of the system. The current study highlights the significance of the feedbacks and the excretion rates in the cholesterol homeostasis. The parameters space influencing these mechanisms would play a significant role in maintaining the homeostatic state. Perturbations in the parametric space may thus shift the homeostatic behavior. The heterogeneity in the parametric space in human population may indicate the susceptibility or the robustness of an individual towards hypercholesterolemia. The study indicates that regulating these mechanisms may be possible therapeutic targets.

References

1. Dietschy, J. M., Turley, S. D. & Spady, D. K. Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans. *J. Lipid Res.***34,** 1637–59 (1993).

2. Altmann, S. W.*et al.* Niemann-Pick C1 Like 1 protein is critical for intestinal cholesterol absorption. *Science***303,** 1201–4 (2004).

3. Dawson, P. A. & Rudel, L. L. Intestinal cholesterol absorption. *Curr. Opin. Lipidol.***10,** (1999).

4. Levy, E. *et al.* Intestinal cholesterol transport proteins: an update and beyond. *Curr. Opin. Lipidol.***18,** (2007).

5. Wang, D. Q.-H. Regulation of Intestinal Cholesterol Absorption. *Annu. Rev. Physiol.***69,** 221–248 (2007).

6. Lichtenstein, A. H. Intestinal Cholesterol Metabolism. *Ann. Med.***22,** 49–52 (1990).

7. Amarenco, P., Labreuche, J. & Touboul, P.-J. High-density lipoprotein-cholesterol and risk of stroke and carotid atherosclerosis: a systematic review. *Atherosclerosis***196,** 489–96 (2008).

8. Solberg, L. a. & Strong, J. P. Risk factors and atherosclerotic lesions. A review of autopsy studies. *Arterioscler. Thromb. Vasc. Biol.***3,** 187–198 (1983).

9. Austin, M. a, Hutter, C. M., Zimmern, R. L. & Humphries, S. E. Familial hypercholesterolemia and coronary heart disease: a HuGE association review. *Am. J. Epidemiol.***160,** 421–9 (2004).

10. Scandinavian Simvastatin Survival Study Group. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet***344,** 1383–1389 (1994).

11. Shepherd, J. *et al.* Prevention of Coronary Heart Disease with Pravastatin in Men with Hypercholesterolemia. *N. Engl. J. Med.***333,** 1301–1308 (1995).

12. Ott, D. B. & Lachance, P. A. Biochemical controls of liver cholesterol biosynthesis. *Am. J. Clin. Nutr.***34,** 2295–306 (1981).

13. Thomas, J., Shentu, T. P. & Singh, D. K. Cholesterol : Biosynthesis , Functional Diversity , Homeostasis and Regulation by Natural Products.

14. Silbernagel, G. *et al.* Cholesterol synthesis is associated with hepatic lipid content and dependent on fructose/glucose intake in healthy humans. *Exp. Diabetes Res.***2012,** 361863 (2012).

15. Reihnér, E. *et al.* Influence of pravastatin, a specific inhibitor of HMG-CoA reductase, on hepatic metabolism of cholesterol. *N. Engl. J. Med.***323,** 224–8 (1990).

16. Bauer, D. C. HMG CoA reductase inhibitors and the skeleton: a comprehensive review. *Osteoporos. Int.***14,** 273–82 (2003).

17. Temel, R. E. *et al.* Hepatic Niemann-Pick C1-like 1 regulates biliary cholesterol concentration and is a target of ezetimibe. *J. Clin. Invest.***117,** 1968–78 (2007).

18. Sehayek, E. *et al.* Biliary cholesterol excretion: a novel mechanism that regulates dietary cholesterol absorption. *Proc. Natl. Acad. Sci. U. S. A.***95,** 10194–10199 (1998).

19. Dikkers, A. & Tietge, U.-J. Biliary cholesterol secretion: more than a simple ABC. *World J. Gastroenterol.***16,** 5936–45 (2010).

20. Meissner, M. *et al.* Bile acid sequestration normalizes plasma cholesterol and reduces atherosclerosis in hypercholesterolemic mice. No additional effect of physical activity. *Atherosclerosis***228,** 117–23 (2013).

21. Casdorph, H. R. Hypercholesteremia. Treatment with cholestyramine, a bile acid sequestering resin. *Calif. Med.***106,** 293–295 (1967).

22. Lofland, H. B., Clarkson, T. B., Clair, R. W. S. & Lehner, N. D. M. Studies on the regulation of plasma cholesterol levels in squirrel monkeys of two genotypes. *J. Lipid Res.***13,** 39–47 (1972).

23. Bhat, B. G. *et al.* Inhibition of ileal bile acid transport and reduced atherosclerosis in apoE-/- mice by SC-435. *J. Lipid Res.***44,** 1614–1621 (2003).

24. Post, S. M., de Crom, R., van Haperen, R., van Tol, A. & Princen, H. M. G. Increased fecal bile acid excretion in transgenic mice with elevated expression of human phospholipid transfer protein. *Arterioscler. Thromb. Vasc. Biol.***23,** 892–897 (2003).

25. Demirezen, E. M. & Barlas, Y. A simulation model for blood cholesterol dynamics and related disorders. in *27th Int. Conf. Syst. Dyn. Soc. Albuquerque, New Mex. USA sn***26,** 30 (2009).

26. Mc Auley, M. T., Wilkinson, D. J., Jones, J. J. L. & Kirkwood, T. B. L. A whole-body mathematical model of cholesterol metabolism and its age-associated dysregulation. *BMC Syst. Biol.***6,** 130 (2012).

27. Brown, L., Rosner, B., Willett, W. W. & Sacks, F. M. Cholesterol-lowering effects of dietary fiber: a meta-analysis. *Am. J. Clin. Nutr.***69,** 30–42 (1999).

28. Hegsted, D. M., Ausman, L. M., Johnson, J. A. & Dallal, G. E. Dietary fat and serum lipids: an evaluation of the experimental data. *Am. J. Clin. Nutr.***57,** 875–883 (1993).

29. Kritchevsky, D. Metabolic effects of dietary fiber. *West. J. Med.***130,** 123–7 (1979).

30. Mensink, R. P., Zock, P. L., Kester, A. D. M. & Katan, M. B. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *Am. J. Clin. Nutr.***77,** 1146–55 (2003).

31. Schonfeld, G. *et al.* Effects of dietary cholesterol and fatty acids on plasma lipoproteins. *J. Clin. Invest.***69,** 1072–80 (1982).

32. Mardones, P. *et al.* Hepatic cholesterol and bile acid metabolism and intestinal cholesterol absorption in scavenger receptor class B type I-deficient mice. *J. Lipid Res.***42,** 170–80 (2001).

33. Yu, L. *et al.* Expression of ABCG5 and ABCG8 Is Required for Regulation of Biliary Cholesterol Secretion. *J. Biol. Chem.***280,** 8742–8747 (2004).

34. Forker, E. L. Mechanisms of Hepatic Bile Formation. *Annu. Rev. Physiol.***39,** 323–347 (1977).

35. Russell, D. W. THE ENZYMES, REGULATION, AND GENETICS OF BILE ACID SYNTHESIS. *Annu. Rev. Biochem.***72,** 137–174 (2003).

36. Marais, a D. Familial hypercholesterolaemia. *Clin. Biochem. Rev.***25,** 49–68 (2004).

37. Buhaescu, I. & Izzedine, H. Mevalonate pathway: a review of clinical and therapeutical implications. *Clin. Biochem.***40,** 575–84 (2007).

38. Bhattacharya, B. S. Mathematical Modelling of Low Density Lipoprotein Metabolism. Intracellular Cholesterol Regulation. (2011).

39. Mazer, N. a & Carey, M. C. Mathematical model of biliary lipid secretion: a quantitative analysis of physiological and biochemical data from man and other species. *J. Lipid Res.***25,** 932–53 (1984).

40. Pas, N. C. A. van de. A physiologically based kinetic model for the prediction of plasma cholesterol concentrations in mice and man. (2011).

41. Pearson, T. *et al.* Mathematical modelling of competitive LDL/VLDL binding and uptake by hepatocytes. *J. Math. Biol.***58,** 845–80 (2009).

42. Ratushny, A. V. & Likhoshvai, V. A. Mathematical modeling of the gene network controlling homeostasis of intracellular cholesterol. *This issue* (2006).

43. Sy, J. A Model of Cholesterol Metabolism and Transport. (2008).

44. Lu, J., Hübner, K., Nanjee, M. N., Brinton, E. a & Mazer, N. a. An in-silico model of lipoprotein metabolism and kinetics for the evaluation of targets and biomarkers in the reverse cholesterol transport pathway. *PLoS Comput. Biol.***10,** e1003509 (2014).

45. T. Lundasen. Studies on the hormonal regulation of bile acid synthesis. (2007).

46. Pullinger, C. R. *et al.* Human cholesterol 7alpha-hydroxylase (CYP7A1) deficiency has a hypercholesterolemic phenotype. *J. Clin. Invest.***110,** 109–17 (2002).

47. Chiang, J. Y. L. Bile acids: regulation of synthesis. *J. Lipid Res.***50,** 1955–66 (2009).

48. LU, T. Molecular Basis for Feedback Regulation of Bile Acid Synthesis by Nuclear Receptors. *Mol. Cell***6,** 507–515 (2000).

49. Charach, G., Rabinovich, A., Argov, O., Weintraub, M. & Rabinovich, P. The role of bile Acid excretion in atherosclerotic coronary artery disease. *Int. J. Vasc. Med.***2012,** 949672 (2012).