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Modeling cholesterol metabolism by gene expression profiling in the hippocampus†

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Abstract

An important part of the challenge of building models of biochemical reactions is determining reaction rate constants that transform substrates into products. We present a method to derive enzymatic kinetic values from mRNA expression levels for modeling biological networks without requiring further tuning. The core metabolic reactions of cholesterol in the brain, particularly in the hippocampus, were simulated. To build the model the baseline mRNA expression levels of genes involved in cholesterol metabolism were obtained from the Allen Mouse Brain Atlas. The model is capable of replicating the trends of relative cholesterol levels in Alzheimer's and Huntington's diseases; and reliably simulated SLOS, desmosterolosis, and Dhcr14/Lbr knockout studies. A sensitivity analysis correctly uncovers the Hmgcr, Idi2 and Fdft1 sites that regulate cholesterol homeostasis. Overall, our model and methodology can be used to pinpoint key reactions, which, upon manipulation, may predict altered cholesterol levels and reveal insights into potential drug therapy targets under diseased conditions.

Introduction

As information of biochemical pathways that compose entire organisms emerges,¹ there is a pressing need to understand how biochemical reactions produce metabolic profiles under a variety of physiological conditions.² Unfortunately, while much is known about multiple pathways and their stoichiometries, the reaction functions and the specific values of rate constants are not known.^{3,4} Measuring reaction rate constants is difficult since *in vitro* experiments can yield values that are different from the ones present in tissue.⁵ Furthermore, as the size of biochemical reactions being studied increases measuring each reaction becomes intractable. Computational methodologies used to determine rate constants usually

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consist of finding a set of reactions by minimizing the difference of the output of the simulation to a target metabolic profile^{6,7} or by modeling protein–protein interactions.³ However, pattern matching requires measuring several metabolites to be used as the target values and biophysical approaches require knowing the molecular structure of the enzymes. Thus, there is considerable need to develop approaches that can approximate or determine the reaction values without the need of large scale experiments that measure each reaction or use template matching.

Since mRNA expression is correlated with the density of enzymes then we assume that there has to be a mapping between the level of specific mRNA expression and the reaction rate constant in which its enzyme is involved. Under conditions of quasi-steady-state the production rate of a Michaelis–Menten enzymatic reaction depends on the concentration of total enzyme (known as $v_{\max} = k[E]_0$, where k is the rate of enzymatic complex to product and $[E]_0$ is the total concentration of the enzyme). However, the assumptions of a quasi-steady-state process in cells have been under question for the last several years.^{8–10} The high concentration of macro-molecules present in cells, known as molecular crowding, can affect the reaction rate constant of a biochemical process^{5,11,12} resulting in significantly different dynamics than expected from a classical reaction–diffusion approach.^{11–13} Under such diffusion limited conditions the rates of reactions are believed to differ significantly from those measured in a well-mixed environment.^{8,11,12} We decided to explore the essential effects of coupling enzymatic level expression with the speed of a reaction. Thus, we hypothesized that enzymatic expression resulted in a direct change in the kinetic rates of a biochemical reaction.

We decided to test the usefulness and validity of our hypothesis by building a biochemical model of cholesterol homeostasis in the brain. We concentrated on the hippocampus since cholesterol synthesis in this area is greatly affected by diseases such as Alzheimer's (AD),¹⁴ Huntington's (HD),¹⁵ Smith-Lemli-Opitz syndrome (SLOS),¹⁶ and desmosterolosis,¹⁷ resulting in neuron death or loss of function.^{18–21}

System wide *in situ* hybridization data for large numbers of genes has recently become available through the Allen Mouse Brain Atlas (AMBA, <http://www.brain-map.org>). Using the AMBA we obtained the expression levels and patterns in the hippocampus for all genes involved in the core cholesterol homeostasis process.²² Because mRNA expression based on *in situ* hybridization is a relative measurement the resulting reaction constants were normalized. Since the kinetic values were normalized and there is practically no concentration information of many of the metabolites involved in brain cholesterol production we decided to normalize the time and concentration of the model and examine the percent changes due to mRNA variations due to illnesses or genetic manipulations. When the percentage changes in mRNA expression observed in AD²³ were incorporated into our model, our results confirmed the reported cholesterol increases in both moderate and severe AD stages.²⁴ Similarly, when we incorporated the reported percentage changes due to HD²⁵ our model reproduced the reported increase in cholesterol concentration.²⁶ In addition, our simulations replicated SLOS, and knock out studies of Dhcr14, Lbr, and Dhcr24.^{27–29} A sensitivity analysis of the baseline cholesterol model suggested that Idi2, at the isoprenoid branch point, and Fdft1, downstream of this reaction, have a strong influence

on cholesterol production, as has been suggested by experiments.³⁰ Overall, the adult hippocampus cholesterol metabolism model replicated several sets of experimental evidence suggesting that our technical approach can be used to parameterize biochemical models based on mRNA expression patterns and our model can be used to pinpoint key reactions, which, upon manipulation, may adjust cholesterol levels and reinstate homeostasis under diseased conditions.

Results

The objective of this study was to test our methodology of mapping enzymatic mRNA expression data to reaction rate constants. For this purpose we built a computer model of adult brain cholesterol production based on the expression levels of genes involved in cholesterol biosynthesis. We concentrated on the hippocampus since cholesterol homeostasis in this area of the brain is greatly affected by diseases such as AD and HD.

Fig. 1 shows a detailed diagram of cholesterol production, for simplicity the metabolite names have been indexed M1 to M52, their corresponding names can be found in Table S1 (ESI[†]). In brief, cholesterol synthesis starts with the generation of mevalonate, isoprenoid side-products and squalene.³¹ The post-squalene portion commits to sterol synthesis and leads to lanosterol production. The process branches into two alternate routes, both of them producing cholesterol. Cholesterol I, characterized by lanosterol to lathosterol synthesis, is the predominant pathway in adult neural tissues. Cholesterol I branches into cholesterol III which is characterized by production of desmosterol. Cholesterol III is most prominent during early brain development.³² Cholesterol II is characterized by zymosterol production.

We obtained the expression values of all genes involved in core cholesterol production in the hippocampus from the AMBA (see Methods) and plotted them in Fig. 2. The graph is divided by metabolic stage (synthesis and degradation) and pre/post-squalene transition, mediated by squalene epoxidase (Sqle). While reactions before squalene can be involved in other metabolic pathways,³¹ the post-squalene products are exclusively committed to cholesterol production. The overall pattern of cholesterol related genes indicates an apparent bottle-neck effect due to the relatively low expression of Lbr and Tm7sf2 (which produces DHCR14). The gene products of Lbr and Tm7sf2 have Dhcr14 enzyme activity, involved in a conversion step from lanosterol (M15) to cholesterol (M50). However, post-squalene genes downstream of Lbr and Tm7sf2 are expressed at much higher levels. Therefore, cholesterol could still be synthesized despite the low expression of Lbr and Tm7sf2 as long as high levels of substrate for these Dhcr14 enzymes are produced. In fact, Cyp51, the gene product of which acts on the Dhcr14 intermediary product, is expressed at an appropriately higher magnitude. In the post-squalene segment involved in the production of *S*-2,3-epoxysqualene (M14), had the highest expression at 100%. This *S*-2,3-epoxysqualene intermediate is used to synthesize lanosterol (M15), the initial steroidal precursor following the mevalonate pathway.^{33,34} In the degradation component, Cyp46a1, responsible for conversion of cholesterol to 24*S*-hydroxycholesterol (M51) and subsequent removal from neural tissue, was expressed at 100%. However, the other two degradation genes, Cyp27b1 and Ch25h, were not expressed, although Ch25h has been found in the hippocampal region in aged and AD human subjects.²³

Baseline model of cholesterol metabolism in the adult hippocampus

Using the network structure described in Fig. 1 and the expression values of cholesterol genes from Fig. 2 we built a mass-action model of cholesterol metabolism. This model includes the known enzymes and corresponding genes involved in cholesterol anabolism³⁵ and catabolism.^{27,36} As explained in Methods, we assumed a direct linear mapping between mRNA expression values to rate constants of metabolite production. We assigned $k_{f,b}$ values between 0 and 1, where 1 corresponds to the maximum expression value at 100%. The model consisted of 53 reactions (Table S2, ESI[†]), carried out by 24 different kinetic values (Table S3, ESI[†]), and produced 51 metabolites (Table S1, ESI[†]). Since no temporal restrictions were implemented the time evolution of the systems of equations is not directly mapped to actual time units. We ran all simulations for 1×10^6 a.u. (arbitrary units), which resulted in stable levels of almost all metabolites. The metabolites that did not reach a stable level were those that were end-products, for which downstream metabolism was not explicitly modeled, thus resulting in accumulation of metabolite. The reactants that are a result of basic biological functions such as ATP, NADPH, and O_2 were assumed to be constant for all conditions. Since the specific concentration of reactants is not known we assigned an initial arbitrary concentration of 0.1 (arbitrary units) for all reactants. Fig. 3 shows a plot of the value of the cholesterol metabolic profile at the end of the simulation.

Model validation

Our model differs from traditional approaches in that the reaction rate constants are given by the expression pattern of each gene.^{37,38} Therefore we did not run multiple simulations to tune the model to a specified metabolic profile. Validation of this type of model requires relative comparisons within the baseline metabolic profile and relative changes due to genetic or pharmacological manipulations.^{39,40}

Initially, the metabolic profile showed that the lanosterol–lathosterol products were found at higher concentrations than desmosterol (Fig. 3), a characteristic of the cholesterol pathway in the adult brain. Specifically, the average concentrations of lanosterol (M15), 4,4-dimethyl-5 α -cholesta-8,24-dien-3 β -ol (M34), 4- α -methylzymosterol (M41), and 5 α -cholesta-7,24- dien-3 β -ol (M47) was higher than the average concentration of 24,25-dihydrolanosterol (M16), and desmosterol (M32).⁴¹ Our model replicated this internal characteristic of cholesterol metabolism using a simplified enzymatic network approach and reaction rate constants that did not required tuning.

The cholesterol model also replicated multiple knockout and genetic defect studies. When Dhcr14 reactions, associated with Lbr and Tm7sf2 genes, are knocked-out, the brain produces practically no cholesterol (M50).²⁸ We tested this condition in the model by independently setting the kinetic value of the Dhcr14, Lbr and Tm7sf2, reactions to zero. In cases of modeled single knockouts, the cholesterol levels did not change from baseline. However, when these Dhcr14 reactions were both set to zero, cholesterol levels dropped to null (Fig. 4A), indicating that both reactions are well below saturation levels, as suggested by *in vivo* studies.²⁸

Elimination of Dhcr24 in mice leads to undetectable cholesterol and dramatically increased desmosterol (M30) levels with age,²⁹ a condition known as desmosterolosis. In this case cholesterol production regresses to producing desmosterol (M32) which is the main sterol during early developmental stages (see Cholesterol III in Fig. 1).²⁹ Our model faithfully replicated this process. When the kinetic parameter of Dhcr24 was set to zero, cholesterol (M50) production decreased while desmosterol (M32) levels increased through the Cholesterol III pathway. Since desmosterol degradation mechanisms do not seem to compensate *in vivo* with desmosterolosis, our model also showed this product accumulating indefinitely (Fig. 4B).

SLOS is attributed to a mutation in the Dhcr7 gene that encodes the final enzyme responsible for brain cholesterol synthesis.^{16,42} The SLOS mutation lessens or eliminates the enzymatic functional properties of the DHCR7 protein. The loss of function due to DHCR7 reduction results in excessive accumulation of 7-dehydrocholesterol (M47) and a reduction of cholesterol (M48). 7-dehydrocholesterol is the immediate precursor to cholesterol and 27-hydroxy-7-dehydrocholesterol (M52).²⁷ We tested our model to mimic SLOS by performing a sensitivity analysis of the Dhcr7 baseline kinetic value by 3 orders of magnitude. As experimentally shown, 7-dehydrocholesterol accumulates as Dhcr7 decreases, as a direct consequence 27-hydroxy-7-dehydrocholesterol increases (Fig. 5A and B). In our model of SLOS, 7-dehydrocholesterol accumulates because the small rate of transformation to 27-hydroxy-7-dehydrocholesterol mediated by Cyp27b1 cannot compensate for the much higher rate of production of 7-dehydrocholesterol by SC5DL. Also consistent with *in vivo* SLOS conditions, experimental reduction of Dhcr7, in our model, resulted in a decrease in cholesterol production (Fig. 5C). In contrast, increases in the kinetic rate for the Dhcr7 reaction produced a saturation of cholesterol levels. This saturation effect on cholesterol production shown in Fig. 5C is due to a limitation in the production of 7-dehydrocholesterol mediated by Sc5dl, with a $k_f = 0.195$ that is less than one third of the basal Dhcr7 value.

Remarkably, without the need for tuning kinetic parameters, our simulations replicate basic genetic manipulations at multiple sites of the cholesterol synthesis pathway, suggesting that the strategy of mapping reaction rate constants with gene expression levels can describe the overall homeostatic behavior of cholesterol production in the brain.

Model sensitivity and robustness

Since the cholesterol model developed in the previous section could replicate the effects of strong (knockout) manipulations we decided to study the effects of small changes in reaction parameters on cholesterol production. A local sensitivity analysis was applied to the baseline model to determine potential points of regulation of cholesterol homeostasis,⁴³ see Methods. The result of such analysis is a time dependent evolution of the observed variable due to an infinitesimal perturbation in a rate constant. In our case we tested the response of cholesterol production to all kinetic parameters (Fig. 6). For clarity purposes we divided the analysis in the rate constants involved in the mevalonate branch (Fig. 6A), isoprenoid branch point (Fig. 6B), squalene synthesis (Fig. 6C), and Cholesterol I to III (Fig. 6D–F, respectively with Cholesterol III including cholesterol degradation). In general, perturbations in most kinetic

rates resulted in no changes in cholesterol expression. Those kinetic reactions that had an effect on cholesterol production showed transitory and long term influence.

In the mevalonate pathway (from M1–M6 in Fig. 1) we found that changes in all the kinetic rates resulted in transient changes in cholesterol production. The largest peak in sensitivity was from manipulation of Hmgcr (Fig. 6A). The enzymatic site of HMGCR in the cholesterol pathway (M5 to M6) is affected by statins, cholesterol synthesis inhibiting drugs that have been shown to have a negative correlation with incidence of Alzheimer's disease.^{44,45} The sensitivity analysis suggests that the acute effect of statins might differ from long term treatments.^{46,47}

Analysis of the isoprenoid branch point (M7–M10) revealed only one gene that resulted in a strong effect on cholesterol levels, Idi2. Interestingly, the effect of changes in Idi2 resulted in long lasting modulation of cholesterol production (Fig. 6B). However, since Idi2 controls a forward and backward reaction the net result is more modest, as expected with a freely reversible reaction.⁴⁸

The effect of statins on suppression of the mevalonate pathway and the isoprenoid branch point can result in suppression of farnesylpyrophosphate and geranyl–geranylpyrophosphate needed for synaptic plasticity.^{49–51} Therefore, post-isoprenoid metabolic sites of intervention can be considered as novel therapeutics to control cholesterol metabolism without the side effects associated with statins. The sensitivity analysis of the squalene synthesis segment (M11 to M14) uncovered a very strong dependence of cholesterol production on the value of Fdft1, the gene product of which, squalene synthase mediates production of squalene from farnesyl pyrophosphate. Although the value of the relative sensitivity due to Fdft1 was smaller than in Idi2, this process is not affected by a backward reaction. As in the Idi2 case, changes in Fdft1 resulted in sharp and prolonged modification of cholesterol production, corroborating the proposal that this reaction is a candidate target of intervention when brain cholesterol metabolism is defective, but also not without potential complications.⁵²

Cholesterol I and II pathways showed transient sensitivity mediated by Lbr and Tm7sf2 (Fig. 6D and E). Cholesterol III only showed sensitivity to changes in degradation (Fig. 6F), as expected from basic mass-action analysis (Fig. 1). Overall, the sensitivity analysis shows that Idi2 and Fdft1 are regulatory sites in the production of cholesterol that could have substantial long term effects, while multiple sites along the pathway have only transient effects.

We further tested the robustness of our model to changes in reaction rate constants of HMGCR and CYP46A1. These enzymes display important kinetic parameters that stabilize cholesterol levels.⁵³ The values of Hmgcr and Cyp46a1 were varied separately and simultaneously, to determine their effects on cholesterol levels. With independent simulations, their kinetic values were increased and decreased by three orders of magnitude. In each case the concentration levels of cholesterol, mevalonate, and 24-hydroxycholesterol were monitored for the effects of such manipulations. Only in the case when the kinetic rate constant controlled by Cyp46a1 decreased by a factor of 100 did cholesterol increase

indefinitely (not shown). This accumulation is clearly due to the abolishment of degradation of cholesterol. All other manipulations showed changes only of a few percentage points, thus confirming that our simulations were robust to parameter manipulation.

Cholesterol metabolism in Alzheimer's and Huntington's diseases

Given that our model was able to reproduce the effects of several genetic manipulations we next wanted to determine if the model could reveal cholesterol metabolism changes in AD. The experimental measurement of cholesterol in neural tissues presents challenges different from other tissues¹⁸ and is dependent on the cellular compartments sampled.²⁴ One group has shown age-related decrease in major membrane lipids is accelerated in individuals with AD,⁵⁴ while others have shown that an increase in plasma cholesterol is a risk factor for AD.⁵⁵ However, there is evidence that membrane cholesterol content may either indicate metabolic changes that lead to AD,⁵⁶ or could be a biomarker signaling a high magnitude of neuronal death.^{57–60}

Published microarray data from the CA1 region of the human hippocampus was used from cases with varied severity of AD determined by cognitive status: incipient (IAD), moderate (MAD), or severe (SAD).^{23,61} The baseline cholesterol gene expression values were modified by the percent changes found in the three independent microarrays of incipient, moderate, and severe AD (compare changes from baseline in Table S3, ESI[†]). The modified expression values were used to perform independent simulations for IAD, MAD, and SAD stages of cognitive loss (Table S2, ESI[†]). The metabolic profile from each simulation under conditions for the different AD stages (Fig. 7A) revealed cholesterol to have a small decrease for IAD ($\times 5\%$) with respect to the baseline model, whereas, both MAD and SAD showed increases of 31% and 38%, respectively. Quantitative comparisons are difficult to make when using the calculated concentration values from our model against experimental measurements of total tissue free cholesterol. However, increases in tissue free cholesterol are associated with increases in plasma membrane cholesterol.⁶² Free cholesterol levels of isolated frontal cortex membranes of human cases have been found to be slightly higher for mild AD, and are significantly and progressively elevated in the moderate and severe cases.²⁴ Thus, our modeling results can replicate the trend for increased neuronal cholesterol in AD patients as the illness progresses.

The predictive power of modeling resides in monitoring variables that are difficult to measure experimentally. Using computer modeling we can gain insight into changes in metabolic pathways otherwise difficult to measure experimentally. As seen in the case of the AD models, all levels of illness severity showed remarkable changes in the production of 7-dehydrodesmosterol and desmosterol (M31 and M32). Both of these metabolites increased in parallel (98%, 326% and 452% for 7-dehydrodesmosterol; 112%, 256%, 320% for desmosterol, for IAD, MAD, and SAD). Desmosterol and 7-hydrodesmosterol are generated in the cholesterol biosynthesis pathway III (Fig. 1), which contributes minimally to cholesterol production in the normal adult brain,³² showing a shift in cholesterol metabolic pathways as the severity of AD increases.

The increases in 7-dehydrodesmosterol, desmosterol and cholesterol are not due to a change in the enzymes directly involved in their production. In fact, during AD the expression of

Dhcr7, the gene involved in desmosterol production, decreases as a function of the severity of the illness (from 0.65 in IAD to 0.47 in SAD, with 0.61 the value at baseline). In the case of 7-dehydrodesmosterol the Sc5dl value is slightly reduced as well (from 0.19 in IAD to 0.17 in SAD). Direct cholesterol production through Dhcr24 is unaffected by AD, an apparent discord since Dhcr24 is the Seladin-1 gene.⁶³ Thus, the overall changes observed in cholesterol production are a result of the emergent properties of the biochemical network.

The sensitivity analysis shown in Fig. 6 gives more insights into other possible points responsible for metabolic changes in AD. This analysis suggests that small changes in Idi2 and Fdft1 could account for the strong and sustained changes in cholesterol production. While Idi2 does not show any changes during AD, Fdft1 partially accounts for the observed changes in cholesterol production ($\times 10\%$ in IAD, to 20% in MAD and SAD). The other gene that could contribute significantly to the increase of cholesterol production in AD is Hsd17b7 which increases from 0.137 at baseline to 0.259 in SAD (an 89% increase). Hsd17b7 is involved in the large increase in zymosterol production seen in Fig. 7A, which then gets compensated by the decrease in Ebpl (from 0.835 in baseline to 0.649 in SAD), Sc5dl (from 0.195 in baseline to 0.167 in SAD), and Dhcr7 (from 0.614 in baseline to 0.466 in SAD), which results in cholesterol production in cholesterol pathway II. Finally, the other gene responsible for large increases in cholesterol production is Mvk that increases about 75% from baseline to SAD (see reaction ML5 through ML7 in Table S2, ESI†) and a lesser contribution from Fdps (30%), both of which are in the mevalonate synthesis pathway.

Huntington's disease is associated with early pathologies in the caudate nucleus in the adult brain^{64,65} and directly related to motor deficiencies; whereas, cognitive loss is associated with pathologies in the hippocampus.⁶⁶ Due to the lack of direct information regarding the effects of HD on cholesterol metabolism in the hippocampus we used microarray data of cholesterol metabolism genes from the caudate²⁵ to simulate HD changes in the adult mouse hippocampus (see Table S2 (ESI†) HD column). The simulations show that cholesterol increases by 120% of its baseline value (Fig. 7B). In this case our results are remarkably in agreement with recent published results of an HD transgenic mouse model that displays a 130% increase in cholesterol levels.²⁶ As opposed to the case in AD, during HD only one of the core cholesterol metabolism genes shows a notable increase, Fdft1, from 0.093 during baseline levels, to 0.124, a 33% increase that accounts for the observed change in cholesterol production. All the other genes used for the model vary by less than 10% from baseline values.

Cholesterol production is affected by Hmgcr manipulation, which is the site of statin interference presently targeted to lower cholesterol levels in the brain as a treatment for AD. However, recent reports suggest that statins also affect the production of molecules related to synaptic plasticity,⁶⁷ thus potentially causing a significant side effect to AD patients undergoing statin treatment.^{46,47} The metabolites produced at the isoprenoid branch point in the early cholesterol pathway are involved in synaptic plasticity,⁶⁸ emphasizing the importance of finding sites for intervention downstream of this point or multiple points at which a combined effect is not strong on the isoprenoids involved in synaptic plasticity. The sensitivity analysis in Fig. 6 shows that the isoprenoid and squalene sections of the pathway (Fig. 6B and C) are sensitive to small changes in Idi2 and Fdft1, respectively. More

importantly, rate changes in either of these reactions result in persistent changes in cholesterol production. In the squalene branch point, manipulations of Fdft1 show a strong influence in the production of cholesterol, with the advantage of being downstream from the isoprenoid branch point.

Finally, we determined the amount of change in Idi2 and Fdft1 expression from SAD necessary to re-establish baseline cholesterol levels. Fig. 8A shows a plot of cholesterol ratio with reference to normal baseline levels *versus* the ratio of modified-Idi2 to SAD-Idi2 value. A value of 1 in the ordinate corresponds to the baseline cholesterol level and in the abscissa to the value of Idi2 in SAD. The plot shows that the value of Idi2 has to be decreased by about 20% to recover baseline cholesterol levels. The same analysis for Fdft1 (Fig. 8B) shows that the activity of this gene has to be decreased by more than 60% to return to normal cholesterol concentrations. Since both sites could synergistically contribute to changing cholesterol levels we ran a parameter sweep of Idi2 and Fdft1 values to find the values that return cholesterol production to baseline levels (Fig. 8C). Interestingly, we found that while Fdft1 has to be reduced less than when modified alone (0.9 instead of 0.4 of the value of Fdft1 in SAD) the value of Idi2 had to be modified much more (0.55 instead of 0.8 of the original Idi2 SAD, black dot in Fig. 8C). The metabolic profile generated by the combination of changes in Fdft1 and Idi2 that recovered baseline cholesterol levels resulted in a consistent recovery, or normalization, of the entire baseline metabolic profile (Fig. 8D). In fact, calculating the least square distance between the baseline model and all the test metabolic profiles confirmed this normalization. The metabolic profile optimized only for matching cholesterol production coincided with the most normalized profile collectively. Thus, regulation of Idi2 and Fdft1 could be candidate targets to help in recovering normal hippocampal cholesterol metabolism in SAD cases. In fact, recent data suggest that modulating Fdft1 can regulate cholesterol levels without inhibiting isoprenoid synthesis seen with statins.³⁰

Discussion

We developed a technique that linearly mapped mRNA expression patterns to reaction rate constants. We used this technique to model brain cholesterol biosynthesis and degradation. Although, the simulation of knockout manipulations can be explained from the network structure, the relative final metabolite levels in all other manipulations were a result of the specific values of the derived rate constants. We used the model to predict the metabolic profile changes of multiple diseases, including AD and HD. Sensitivity analyses showed that cholesterol production is transiently dependent on changes in the isoprenoid section⁶⁹ and that a reaction mediated by Fdft1 during squalene synthesis can have long lasting effects on cholesterol production.³⁰

Approximating rate constants in metabolic networks

Determining the kinetic rates of large numbers of biochemical reactions based on metabolic and gene expression patterns is a difficult problem in systems biology. Our approach assumed that the rate of production, not the metabolite concentration, is dependent on gene

expression. Thus, as mRNA are produced and, presumably, enzymes are translated in the cell the probability of a reaction varies.

Our model is based on the general expression of genes in the hippocampus; therefore, our simulations are not applicable to individual cells but tissues. The dynamical process of gene regulatory networks was not explicitly modeled here and could significantly modify the results. Furthermore, the relationship between mRNA expression and protein translation could be non-trivial, as it is in the case of stable mRNA and short lived proteins. Nevertheless, our implementation, as any other simulation strategy, is a simplified version of the processes taking place in a real tissue or individual cells. For simplicity we assumed that higher values of mRNA expression linearly translate to faster reaction rate constants, however, this might not be the case in all situations. More research on the effects of the actual structure of cells^{11,70–72} on biochemical reaction–diffusion is needed to understand how large scale metabolic reactions take place in cells and tissues.

Independently of the underlying biophysical foundations of our model, the practical results from our approach are that the normalized kinetic values replicate experimental results and there is no need to train the model to a specific metabolic profile or known protein shape. Deriving reaction rate constants from test-tube experiments, or inducing their value computationally does not warranty finding a set of reactions that can replicate multiple normal and disease conditions. This is due to the presence of local minima that arise in such systems.^{2,3} Thus, since our rates are derived directly from mRNA expression we hypothesize that our relative levels of rate constants represent the relationships actually present in the real system. Further research would be to rescale the time and concentration variables to match experimental results that track temporal changes in metabolites. However, for this type of application more knowledge of metabolite concentration would be needed.

Regulatory sites of cholesterol production

Extensive literature implicates the deregulation of cholesterol homeostasis, accumulation of precursor metabolite, or compromise of supplies to side products from cholesterol synthesis as major contributors to neurodegenerative diseases by causing neuron functional loss. Statins, which are HMG-CoA reductase inhibitors, are now being considered as potentially therapeutic measures in some neurodegenerative diseases such as AD.⁷³ However, the levels of specific inhibition on downstream intermediary metabolites brought on by statins have not been extensively studied.⁷⁴

We found three main regulatory sites in the cholesterol network, Hmgcr, Idi2, and Fdft1. Hmgcr and Idi2 are involved in pre-isoprenoid branch point processes. Cholesterol showed transitory sensitivity to manipulation of either gene expression resulting in temporal concentration changes that returned to baseline values at long simulation times. Our evidence from the Hmgcr sensitivity analysis supports the idea that other compensatory factors play a role in the long term efficacy of statins to sustain a decreased cholesterol biosynthesis.^{75,76} In contrast, Fdft1 was involved in the production of squalene which is after the isoprenoid branch point. The side products of the isoprenoid branch are associated with molecules involved in synaptic plasticity, thus it is important to find cholesterol

regulatory sites after the isoprenoid branch point. Recent evidence suggests that our model actually predicts correctly the Fdft1 regulatory site.³⁰

Our objective was not to model the complete cholesterol homeostasis network during HD or AD; however, our validated model did reveal several characteristics of these diseases. The cholesterol changes in our HD model quantitatively matched the experimental evidence.²⁶ These results contrast with recent reports from another group^{18,65,77} asserting the hypothesis that cholesterol biosynthesis and levels decrease in human HD. However, Valenza and coworkers have also shown a direct correlation among mRNA levels for the Hmgcr gene, HMGCR protein content, and enzyme activity as they change over the progression of the HD pathology in their transgenic mice.^{18,78}

The simulation results from incipient, moderate, and severe stages of AD qualitatively replicate experimental data.^{24,74} However, the accumulation of cholesterol in AD is highly likely to vary from region to region.^{55,79} Nevertheless, our cholesterol network reproduces the sensitivity found in the Hmgcr and Fdft1 sites. Furthermore, the simulations strengthen the argument for increased neuronal cholesterol in moderate and severe AD. Our combination of techniques and analysis supports the hypothesis that cholesterol increases as a function of AD development and that recovery of baseline levels can be achieved by regulation in the Fdft1 site, where inhibitors are known to be selective for lowering cholesterol in neurons without affecting isoprenoid synthesis.^{80,81}

In our simulations we only included the most immediately related genes in cholesterol biosynthesis and degradation. Expanding the metabolic network to regulatory genes would probably result in the discovery of more regulatory sites by using a similar sensitivity analysis. A finer grained model could include micro-RNAs, *e.g.*, miR-33,^{82,83} that affect cholesterol homeostasis and are changed in diseases, *e.g.*, AD.⁸⁴ Therefore, the linkage of gene transcription level to kinetic rates in combination with sensitivity analysis of the biochemical network can be a powerful technique to determine regulatory sites in metabolic reactions.

Summary

The adult hippocampus cholesterol metabolism model replicated several sets of experimental evidence, from several human genetic disorders, knockout mice, and AD and HD. Our proposed technique of using gene expression to model reaction rate constants in biochemical pathways and sensitivity analysis can determine the effects of subtle and knockout changes in cholesterol production. Extension of the model including the regulatory and downstream metabolic reactions should result in more detailed and quantitative predictions on cholesterol homeostasis during normal and disease states.

Materials and methods

Modeling of biochemical reactions

Baseline gene expression data used in this study was obtained from the AMBA (Seattle, WA, <http://www.brain-map.org/>). A detailed explanation of how expression values are calculated can be found in the AMBA website (<http://mouse.brain-map.org/pdf/>

[InformaticsDataProcessing.pdf](#)). We have previously established that all genes necessary for cholesterol production are present in the hippocampus, particularly centered in the neuronal layers.²² The reported values of expression intensity from the AMBA ranges from 0 to 100 (Fig. 2). We re-normalized these values between $E \in (0-1]$, see below. We assumed a one-substrate linear mass-action mechanism (eqn (1))

$$S \xrightleftharpoons[k_b]{k_f} P \quad \frac{d[P]}{dt} = k_f[S] - k_b[P] \quad (1)$$

where S is the substrate; $k_f = E_f$ and $k_b = E_b$, are the forward and backward rate constants. Similarly, E_f and E_b are the normalized expression level values of the enzymes involved in producing P and S . This model can be extended to a two-substrate catalysis system (eqn (2))

$$A+B \xrightleftharpoons[k_b]{k_f} C \quad \frac{d[C]}{dt} = k_f[A][B] - k_b[C] \quad (2)$$

Cholesterol synthesis model

While there is a significant amount of experimental data on the mRNA expression levels of most genes involved in cholesterol production in the liver, evidence on the biochemical control of brain cholesterol homeostasis in baseline and diseased states is slowly emerging.^{15,85–87} The core biochemical pathway of cholesterol synthesis is well characterized (see Fig. 1), consisting of 51 enzymatic reactions,³⁵ see Table S2 (ESI†). For degradation, we modeled two additional reactions, Cyp46a1 and Cyp27b1 (reactions D1 and D2 in Table S2, ESI†) based on available AMBA gene expression data, bringing the total of modeled reactions to 53. The core cholesterol metabolic reaction set is controlled by 24 genes (Table S4, ESI†) and corresponding enzymes (Table S3, ESI†). The gene names are designated by lower case letters and their corresponding enzymes are in capital letters except for Tm7sf2 whose gene product is an isozyme of DHCR14. The core pathway produces 52 different metabolites (Table S1, ESI†). The network model was generated from existing pathway information on cholesterol biosynthesis (see *superpathway of cholesterol biosynthesis* in <http://biocyc.org/> and *steroid biosynthesis* in <http://www.genome.jp/kegg>) and known enzymatic steps for neuronal catabolism of cholesterol and 7-dehydrocholesterol for removal from the brain.^{27,62}

We obtained mRNA expression levels on all genes for enzymes essential in cholesterol production and degradation in brain tissue from the AMBA and used those values to set up enzymatic reaction constants. We assigned the reaction constants in the range of [0–1], with 1 being the maximum expression level (e.g., Hmgcs1, Sqle, and Cyp46a1). While mostly all known cholesterol reactions favor a forward reaction, the Idi2 reaction (ML8 in Table S2, ESI†) has equally reversible properties. The equilibrium of Idi2 favors dimethylpyrophosphate (M10 in Table S1, ESI†), but the reversibility of the isomerase reaction demonstrated direct formation of the reactant, isopentyl-pyrophosphate (M9 in Table S1, ESI†).⁴⁸ Therefore, we set $k_f = k_b$.

There was one gene, Nsdhl, with complete sets of images on AMBA but no reported mRNA expression level data sets. Lacking the AMBA value we derived our own expression number

with the following method. We assumed that the rate constants along consecutive downstream reactions are preserved between mouse and human brains. First, we obtained the gene expression for *Nsdhl* (H_q) and the gene for the immediately prior enzyme reaction, *Sc4mol*, (H_p) from human brain data (http://telethon.bio.unipd.it/bioinfo/HGXP_170/index.html). Second, we calculated the ratio of H_q to H_p ($R_f = H_q/H_p$). Finally, the predicted enzymatic mRNA mouse expression was calculated by $M_q = R_f \times M_p$; where M_p is the immediately prior enzyme reaction, *Sc4mol*.

For the kinetic parameters of AD we used available micro-array data from cholesterol biosynthesis and degradation markers.²³ We applied the percent changes from age matched controls to incipient, moderate, and severe AD cases to our baseline mRNA expression values in order to derive disease state kinetic parameters. For HD we calculated the kinetic parameters from fold changes provided by microarray data (Table S3; (ESI[†]) Hodges *et al.*, 2006). Eqn (3) was used to derive the HD kinetic parameters (E_{ka}) from the fold changes (F_k) and baseline expression (E_{kb}) provided by the AMBA.

$$E_{ka} = E_{kb} 2^{F_k} \quad (3)$$

The original biochemical network model was assembled in COPASI (www.copasi.com). After testing with baseline values for the reactions, the model was exported in SBML. All test models used to generate the data sets presented in this study were simulated after import of the SBML file into the SimBiology toolbox in Matlab (Natick, MA). We performed a sensitivity analysis (Fig. 6), based on Ingalls and Sauro (2003),⁴³ using an algorithm included in the SimBiology toolbox.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Schematic diagram of the cholesterol synthesis and degradation pathways. Metabolites M1 to M52 are described in Table S1 (ESI[†]). The enzyme encoding genes are indicated in each reaction. The grey arrows indicate enzymatic reactions carried out by Dhcr24.

Fig. 2.

Adult hippocampus cholesterol expression profile. The expression profile of the adult mouse hippocampus plots the 24 genes involved in cholesterol metabolism. The gene expression intensity values were obtained from *in situ* hybridization data collected from the Allen Mouse Brain Atlas

Fig. 3.

Final concentration of metabolites calculated from the cholesterol pathway model described in Fig. 1. The value of the metabolite indicates the name of the metabolite found in Table S1 (ESI[†]). Simulation time was 1×10^6 arbitrary time units. The first metabolite that feeds the reaction was not plotted.

Fig. 4.

The cholesterol model replicates knockout and desmosterolosis conditions. (A) Production of cholesterol is stopped only when both *Tm7sf2* and *Lbr* are knocked out of the model. (B) The decrease of cholesterol level in desmosterolosis, due to *Dhcr24* knockout, is reproduced by the model as well as the indefinite accumulation of desmosterol. In the model desmosterol accumulates because we are not explicitly modeling its metabolism similar to the human condition.

Fig. 5.

The cholesterol model replicates SLOS disease which is due to mutations in Dhcr7 that decrease enzyme activity. As the Dhcr7 reaction decreases by three orders of magnitude, 7-dehydrocholesterol and 27-hydroxy-7-dehydrocholesterol increase (A and B) and cholesterol (C) decreases.

Fig. 6.

Sensitivity analysis of cholesterol model shows strong influences of Idi2 and Fdft1 in cholesterol regulation. The sensitivity analysis was performed by using the algorithm proposed by Ingalls and Sauro, 2003. In most cases, changes in rate constants did not result in modifications in cholesterol production (not shown). In the mevalonate pathway (A), Hmgcr showed the strongest response, which was transitory. The isoprenoid and squalene synthesis (B and C) showed strong and constant influence. The rest of the pathway (Cholesterol I–III), showed transitory sensitivity (D–F). The negative sensitivity shown in

(F) comes from the trivial case of changing the rate constant of cholesterol degradation reactions.

Fig. 7. Predicting the metabolic profiles of Alzheimer's and Huntington's diseases. (A) Percent difference between baseline cholesterol metabolic profile and three different progressive stages of Alzheimer's disease (incipient, moderate, and severe). (B) Percent difference between baseline cholesterol metabolic profile and Huntington's disease. The metabolite numbers correspond to the names in Table S1 (ESI[†]).

Fig. 8. Recovering baseline cholesterol metabolic profile. (A) Using the severe Alzheimer's disease model we modified the value of enzyme produced by Idi2 (O) to recover the value of cholesterol in the baseline model (•). (B) Using the severe Alzheimer's disease model as a starting point, we modified the value of the enzyme produced by Fdft1 (O) to recover the value of cholesterol in the baseline model (•). (C) We tested the model to a systematic change of Idi2 and Fdft1 to recover the value of cholesterol in the baseline model (•). The values found for Idi2 and Fdft1 were different for those determined in A or B. (D) The metabolic profile calculated using the modifications determined in C is the closest (in mean squared difference) to the baseline metabolic profile from all the metabolic profiles calculated in the mesh.