

A systems biology model studying the role of cholesterol in Alzheimer's disease progression

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Abstract— A simplified network describing the interactions between the cholesterol and beta amyloid (A β) synthesis pathways was generated using information available from literature and the KEGG database. A system of non-linear differential equations was developed and modeled using several different initial conditions. Rate constants were approximated using ratio-metric data from the literature, or by fitting parameters to obtain a stable simulation within given biological constraints. Simulations demonstrated the importance of negative feedback control by cholesterol in the regulation of beta amyloid levels. Additionally, the effect of reduced LRP-1 levels and oscillatory cholesterol generation was studied.

I. INTRODUCTION

ALZHEIMER'S disease (AD) is the most common type of dementia, affecting 5.3 million people in the US alone [1]. AD is diagnosed post-mortem by the presence of diffuse amyloid beta (A β) plaques in the brain, as well as hyperphosphorylated tau proteins that are present in the axons of affected neurons. Several other changes in brain pathology have been noted in post-mortem analyses, including an increased level of neuroinflammation, deposition of A β in cerebral microvasculature, mitochondrial dysfunction and other metabolic abnormalities. Additionally, decreases in the expression pattern of the low density lipoprotein receptor-related protein (LRP-1) and the presence of the ApoE ϵ 4 allele have been linked to increased risk of AD.

Recent studies have shown that cholesterol levels in both the blood plasma and the brain may play a role in disease pathogenesis, though the exact role is not well understood. Several studies have suggested that high plasma cholesterol, due to hypercholesterolemia or heart disease, may lead to an increased deposition of A β , particularly in the neurovasculature [2,3]. Epidemiological studies have also shown that low levels of brain cholesterol are found in individuals with AD [4,5]. A recent study on the relationship between amyloid precursor protein (APP; precursor for A β) and cholesterol levels demonstrated that increased APP lead to a decrease in LRP-1 and cholesterol, with an increase in ApoE [6].

Although much information is known about the synthesis

and regulation of cholesterol in peripheral regions of the body due to its correlation with heart disease, cholesterol regulation in the brain is just starting to be understood, particularly with how it relates to AD. Given the stochastic nature of many biomolecules, in combination with the difficulty of directly studying interactions between different metabolic, proteomic and genomic networks in real-time, a systems biology modeling approach provides a unique opportunity to better understand the possible interactions between cholesterol and the proteins involved in AD. This paper describes the first known attempt to develop a model for the interactions between lipidomic and proteomic networks, particularly cholesterol, A β and ApoE, and to model the effect that these biomolecules have on AD progression.

II. MODEL GOALS & ASSUMPTIONS

In this paper, we present the first-ever systems biology model used to study the role of cholesterol in AD. This model was developed to help ask several questions: (1) What are the key nodes and regulatory points in the described network? (2) Does inhibition of BACE activity by cholesterol fit with the known data? (3) How does varying the expression levels of LRP-1 alter steady state levels of A β ?

A topological network describing the interactions between the simplified proteomic, lipidomic and metabolic pathways have been derived (Figure 1). The network was simplified to include only those molecules that are most relevant from a biological standpoint and directly relevant to the questions that we were trying to ask. There are 15 rate constants and a total of 17 molecules in the network. The degree of each major node was calculated by determining the total number of edges in and out of a node, including inhibitory interactions. The degree is listed in Table 1. The nodes with highest degree (AcetylCoA and cholesterol) were expected to play key roles in the overall behavior of the system. All molecules are assumed to reside in one of two compartments: the brain (limited concentration levels) and blood (infinite sink for any molecule being transported across the BBB). Within the brain, the cholesterol was subdivided between astrocytes and neurons, while the ApoE pool was subdivided into astrocytic and free ApoE.

III. MODEL EQUATIONS

A system of non-linear differential equations was developed to represent the described network. The majority of metabolic reactions were described by direct conversion

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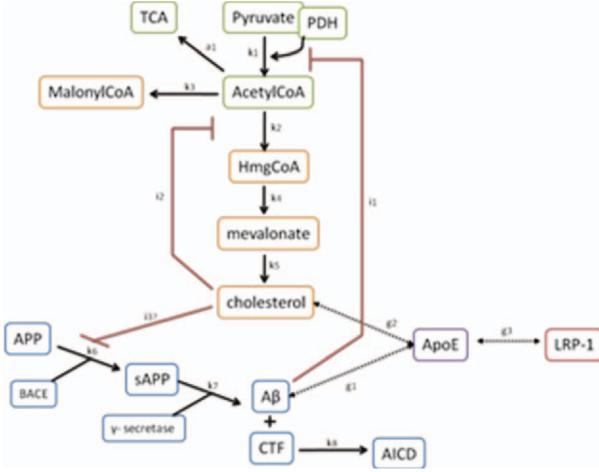


Figure 1: Network topology for mixed proteomic-lipidomic network to study AD. Precursor molecules (green boxes), lipidomic molecules (orange), and proteomic molecules (blue, purple and red) are described. Inhibitory interactions are given by a red line with a blunted bar, metabolic reactions between precursor and product are described by solid black lines, and binding interactions between molecules are given by dotted, black lines. Acronyms used: **APP**: amyloid precursor protein; **ApoE**: apolipoprotein E; **AICD**: APP intracellular domain; **Aβ**: beta amyloid; **CTF**: C-terminal fragment; **HmgCoA**: 3-hydroxy-3-methylglutaryl-coenzyme A; **LRP-1**: low density lipoprotein receptor-related protein; **PDH**: pyruvate dehydrogenase; **TCA**: the citric acid cycle

rates from precursor to product molecule. Inhibitory interactions within metabolic reactions or between metabolic, proteomic or lipidomic sub-networks were described by Michaelis-Menten-like rate kinetics. Neuronal cholesterol levels were maintained at a constant value throughout simulations via feedback control. This feedback mechanism also affected cholesterol levels in astrocytes, the level of ApoE in both astrocytes and in the interstitial space, and synthesis levels of HmgCoA by astrocytes, demonstrating the highly interdependent nature of cholesterol and ApoE concentrations in different cell types within the brain. The following sections describe these equations in further detail.

Metabolic Interactions:

Metabolic interactions encompass all interactions represented by green boxes in Figure 1. AcetylCoA is generated from pyruvate via action of pyruvate dehydrogenase (PDH). The rate of this reaction has been shown to be influenced by the concentration of Aβ (Hoshi 1996). Since the concentration of PDH is believed to be constant during AD, the rate equation was modified to be independent of PDH concentration:

$$\frac{dACoA}{dt} = k_1 \left(\frac{1}{1 + \alpha[A\beta]} \right) [pyruvate] - k_2[ACoA] - a_1[ACoA] \quad (1)$$

A small fraction of this AcetylCoA (k_2) will be converted to HmgCoA. Conversion of HmgCoA to mevalonate is the rate-limiting step in cholesterol synthesis, and is negatively inhibited by cholesterol (negative feedback loop). When neuronal cholesterol (choIN) is below the

TABLE I
DEGREE OF KEY NODES

Symbol	Quantity
AcetylCoA	6
HmgCoA	3
Mevalonate	2
Cholesterol	4
Aβ	3
ApoE	3
LRP-1	2

AcetylCoA has the highest degree, followed by cholesterol.

threshold value, all HmgCoA that is synthesized is used to make mevalonate:

$$\frac{dHmgCoA}{dt} = k_2[ACoA] - k_4 \left(\frac{1}{1 + \gamma[cholN]} \right) [HmgCoA] \quad (2)$$

When neuronal cholesterol levels are normal, there is relative excess of HmgCoA, which can then be used for other metabolic processes or degraded:

$$\frac{dHmgCoA}{dt} = k_2[ACoA] - k_4 \left(\frac{1}{1 + \gamma[cholN]} \right) [HmgCoA] - d_6[HmgCoA] \quad (3)$$

Lipidomic Reactions & Interactions:

Mevalonate is the precursor to cholesterol; the cholesterol synthesis pathway actually has 19 steps between mevalonate and cholesterol, however, these steps have been considered trivial for the goals of this model and the average production rate for cholesterol has been used:

$$\frac{dmev}{dt} = k_4 \left(\frac{1}{1 + \gamma[cholN]} \right) [HmgCoA] - k_5[mev] \quad (4)$$

All cholesterol synthesis occurs in astrocytes. This cholesterol must then be bound to ApoE and transported from astrocytes to neurons, at a rate t_1 . Astrocytic cholesterol is also degraded at an extremely slow rate (half-life of 5 years):

$$\frac{dcholA}{dt} = k_5[mev] - t_1[ApoE_A] - d_2[cholA] \quad (5)$$

Proteomic Interactions:

When neuronal cholesterol is at or above the threshold value, there is no need to transport cholesterol to neurons; in this case, the middle term is trivial. The concentration of ApoE in astrocytes would also vary similarly (birth minus death minus amount transported):

$$\frac{dapoE_A}{dt} = b_a - t_1[ApoE_A] - ([apoe_A] - [cholA]) \quad (6)$$

Neuronal cholesterol levels vary depending on the amount of cholesterol being transferred from neurons; cholesterol also degrades very slowly (same half-life as astrocytic cholesterol):

$$\frac{dchol_N}{dt} = t_1[ApoE_A] - d_2[cholN] \quad (7)$$

The ApoE that is used to transport cholesterol from astrocytes to neurons is often recycled back to the cell surface and, in combination with ApoE that is generated by neurons, can be used to transport A β from the brain to the blood in a two-step process of A β binding to ApoE, followed by transport across the blood-brain barrier via LRP-1:

$$\begin{aligned} & \frac{dApoE}{dt} \\ &= b_n - k_{f1}[Abeta][apoe] \\ &+ \left(\frac{k_{f2} + k_{f3}}{[Abeta] + \frac{(k_{f2} + k_{f3})}{k_{f1}}[LRP]} \right) [LRP][Abeta][apoe] \\ &+ t_1[ApoE_A] - d_5[apoe] \end{aligned} \quad (8)$$

When neuronal cholesterol levels are above threshold, there is only trivial amounts of cholesterol-laden ApoE being transferred to neurons, and thus, very minimal recycling of ApoE back to the surface to be part of the free ApoE pool. The amount of total LRP in the system is assumed to stay at a constant value.

APP Processing:

APP is processed into A β via a two-step process by action of beta-secretase, followed by cleavage by gamma-secretase. No difference has been made between the 40 and 42 amino acid length forms. Beta-secretase activity is believed to be regulated by levels of neuronal cholesterol. In this model, we have used the evidence presented by Cramer et al to introduce an inhibitory effect of cholesterol on beta-secretase activity:

$$\frac{dAPP}{dt} = b_1[APP] - k_6[APP] \left(\frac{1}{1 + \beta[cholN]} \right) - d_3[APP] \quad (9)$$

Beta amyloid is produced from this cleavage, but is also degraded via endocytosis or binding and removal by ApoE/LRP-1:

$$\frac{dAbeta}{dt} = k_7[sAPP] - endo[Abeta] - k_{f1}[Abeta][apoe] - k_t[Aeta][LRP] \quad (10)$$

Rate constants for generation and degradation of most key molecules were approximated using data from the literature. Forward binding rates, interaction rates and the strength of inhibition were all approximated and fitted to

create a stable simulation environment. All rates have units of x/day^{-1} and can be given upon request.

IV. RESULTS

The above model was implemented and simulated in Matlab. The system of non-linear differential equations was solved using the ODE45 function. Simulations were initialized to values that might be seen in an adult brain, and were run for 20,000 days, or approximately, 55 years.

A. Standard Simulation Run

A simulation using all rate constants as described in Table 1 was run as a reference. Convergence was reached for all simulated molecules within the first 6000 days of the simulation. Only APP continued a slowly upward trend during the entirety of the simulation, increasing from an initial value of 1000 to a final value of 1183. Not shown is AcetylCoA levels, which converged from 10,000 to 8845.

B. Effect of increased APP and A β generation

Several simulations were run to study the effect that increased initial concentrations of APP or increased generation of A β have on the system as a whole. In two cases (increased initial APP concentration and 25% increased A β generation rate) led to significant increases in the concentration of APP, and subsequently increased levels of A β .

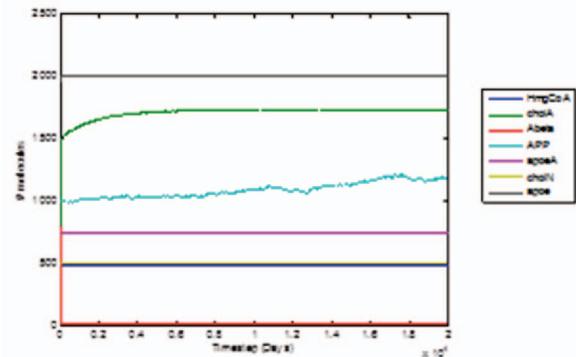


Figure 2: Reference simulation. Standard simulation showing a subtle increase in the levels of APP. Only trivial increases in A β are noticed.

C. Effect of sinusoidal input functions

Steroids and other molecules derived from cholesterol often have a sinusoidal generation rate related to circadian rhythms. To study the possible effect of oscillatory cholesterol input on the system, we varied cholesterol generation by introducing a periodic generator function. The period of the sinusoid was approximately one day, while the magnitude of the function was varied from 0 to 1 (normal range), 0.25 to 1 (assumes a basal generation rate), and 0 to 2 or 4 (assuming an increase in the cholesterol production). Figure 4 displays simulation results.

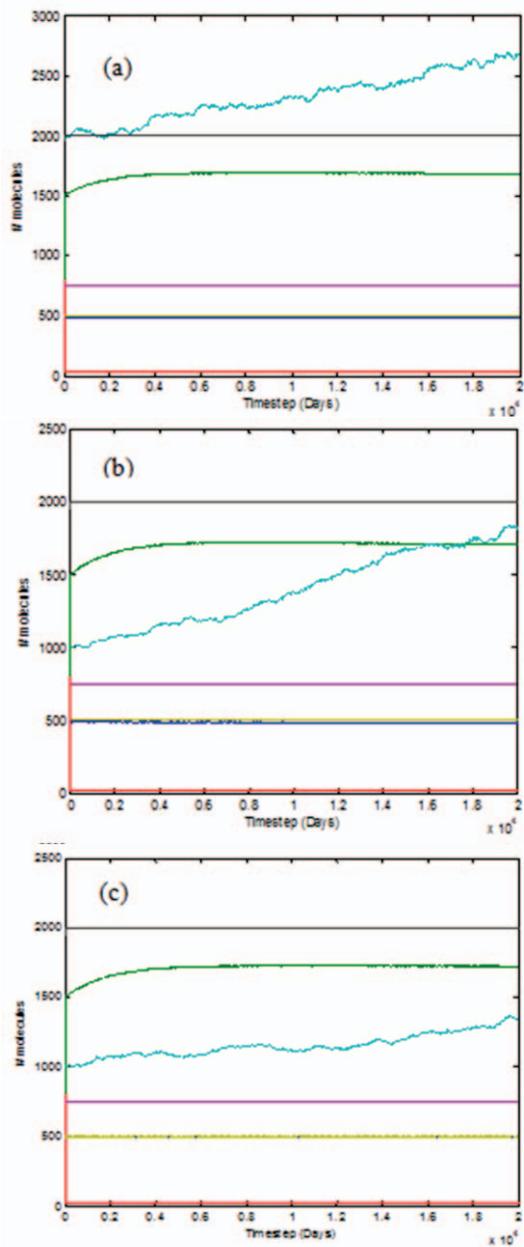


Figure 3: Effect of Increased Initial APP concentration or increased A β cleavage. (a) Initial APP levels were doubled, leading to a significant increase in the amount of APP and A β present in the system. HmgCoA levels began to drop slightly as A β levels started to increase. Final APP level: 2672; final A β level: 40 (b) A β generation rates were increased by 25%, leading APP levels to nearly double. Ironically, A β levels did not change significantly. Final APP level: 1821; final A β level: 27 (c) A β generation rates were increased by 12.5%, leading APP levels to increase slightly. A β levels did not change significantly. Final APP level: 1345; final A β level: 20

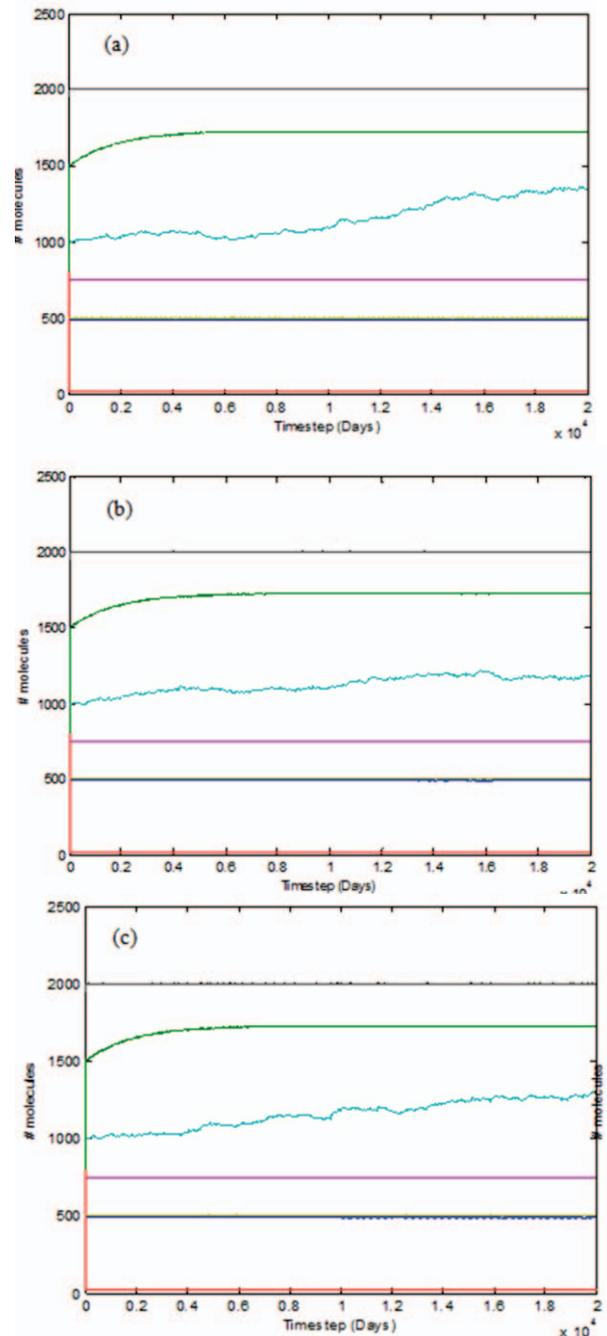
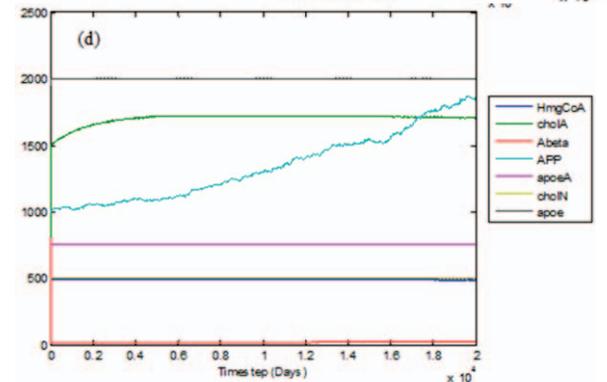


Figure 4: (right) Effect of Periodic Cholesterol Generation. (a) Sinusoidal generation of cholesterol assuming a basal generation rate. Magnitude of generation rate varies from 0.25 to 1. $k_5 = 0.375 \cdot \sin(2\pi t - \pi/2) + 0.625$. Final APP: 1349; final A β : 20. (b) Magnitude of generation rate varied from 0 to 1. $k_5 = 0.5 \sin(2\pi t - \pi/2) + 0.5$. Final APP: 1187; final A β : 18. (c) Magnitude of generation rate varied from 0 to 2. $k_5 = \sin(2\pi t - \pi/2) + 1$. Final APP: 1297; final A β : 19. (d) Magnitude of generation rate varied from 0 to 4. $k_5 = 2 \sin(2\pi t - \pi/2) + 2$. Final APP: 1858; final A β : 27.



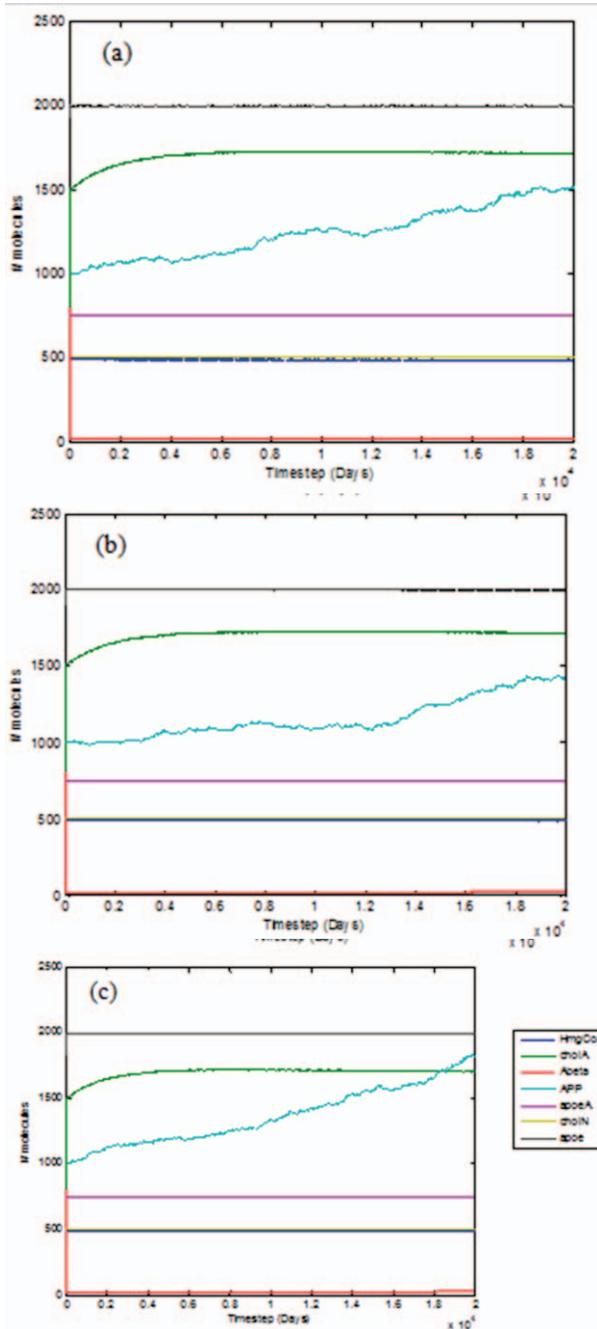


Figure 5:(below) Effect of Decreased LRP: (a) LRP levels decreased by 25%. Final APP: 1520; final A β : 23. (b) LRP levels decreased by 50%. Final APP: 1431; final A β : 22. (c) LRP levels decreased by 75%. Final APP: 1828; final A β : 29.

D. Effect of decreased LRP

LRP-1 levels have been shown to be decreased in individuals with AD. To study this, we varied the LRP levels from 25% to 75% of levels used in the reference simulation. Figure 5 displays these results.

E. Effect of decreased neuronal cholesterol

Recently, a lot of interest has been paid to the effect of cholesterol on APP processing and A β levels in the brain. Although treatment with a statin is now being studied as a

possible treatment paradigm, there are a multitude of conflicting results. In these simulations, the effect of decreased neuronal cholesterol on the system was studied. Specifically, the effect of a decreased initial cholesterol concentration, as well as a decrease in the transfer of cholesterol from astrocytes to neurons, was studied. Results are presented in Figure 6.

V. DISCUSSION

A. Effect of increasing APP and/or A β generation

Increasing the initial concentration of APP led to a significantly increased concentration of APP in the long-term (2672 versus 1183), as well as a nearly double increase in A β (40 versus 18). This seems to imply that, at least in this model, there are few mechanisms keeping the APP levels in check, and if a significant increase in APP occurs during the formative years, it may have significant negative consequences years later.

Increasing the generation rate of A β by 25% led to a significant increase in the amount of APP in the system. However, the levels of A β only increased moderately (27 versus 18). It is a bit unexpected that the APP levels would increase so dramatically without an equally significant increase in A β levels. This could be explained by the fact that A β cleavage is a relatively uncommon event in the first place, and that a critical level of APP must be achieved before significant changes in A β are also observed.

This seems to be a bit counterintuitive and not necessarily in agreement with biological data, since APP levels are not significantly increased in AD, and increases in A β are thought to be due more to decreased clearance rather than increased generation. Further studies on this effect, as well as refinement of the model to prevent the increase in APP that is seen over time even in the standard reference simulation.

B. Effect of sinusoidal input functions

Oscillatory generation of astrocytic cholesterol led to significant changes in the system output only in the case where the magnitude of the generation rate constant was varied from 0 to 4. This would correlate to ‘uncalibrated’ generation of cholesterol by astrocytes. This increased level of cholesterol in astrocytes led to an increase in the amount of APP and a moderate increase in A β , which was unexpected given the inhibitory regulation that is supposed to exist between cholesterol and APP processing by β -secretase. Since neuronal cholesterol levels stay approximately constant, and it is these levels that affect APP processing, it may be possible that the increased APP may be due to other factors that have not been directly studied here. It is also interesting to note that the degradation rate for APP was highly sensitive to even small changes from the chosen value could lead to chaos (data not shown). This in itself may provide some insight into why APP levels may respond to such small changes in other molecules in the system.

C. Effect of decreased LRP

Decreasing LRP-1 levels led to an increase in APP as well as A β . This was the expected result since LRP-1 is necessary to clear A β from the brain. Future computational models should further subdivide the LRP into neuronal and endothelial to make an even more accurate model.

D. Effect of decreased neuronal cholesterol

Decreasing the initial value of neuronal cholesterol did not change the outcome of the system for any of the molecules studied. This was expected, given that a strong feedback loop was used to keep neuronal cholesterol within tight bounds. Future simulations should study the effect of this feedback to get a better idea of its importance in maintaining the system within the realm of stability. Decreasing the rate of transfer of cholesterol from astrocytes to neurons significantly decreased the levels of APP and A β . Such a situation might arise if ApoE did not carry cholesterol as well, or if fewer LRP receptors were present on neurons. However, it is interesting to note that even with a decreased efficiency of transfer, neuronal cholesterol levels were maintained at approximately constant levels. Just as in the study of the effects of oscillatory cholesterol production by astrocytes, the sensitivity of the APP reaction may be the cause of the observed increase in APP and A β .

VI. CONCLUSION

In this paper, we have presented one of the first systems biology models for Alzheimer's disease. We have studied in computational detail the interactions between cholesterol, ApoE, LRP-1 and A β . Simulation results showed several unexpected phenomena, including a decrease in APP and A β when the transfer rate of cholesterol between astrocytes and neurons is decreased. These results show that cholesterol and LRP levels play significant roles in the development of increased A β levels in the brain, one of the key markers for AD. Future models should study these effects in further detail and incorporate aspects of inflammation that are believed to play a role in AD pathogenesis.

REFERENCES

- [1] Alzheimer's Association (2007), "Alzheimer's Fact Sheet", http://www.alz.org/national/documents/PR_FFfactsheet.pdf, Accessed March 7, 2011.
- [2] Refolo, L. M., B. Malester, et al. (2000). "Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model." *Neurobiol Dis* 7(4): 321-31.
- [3] Puglielli, L., R. E. Tanzi, et al. (2003). "Alzheimer's disease: the cholesterol connection." *Nat Neurosci* 6(4): 345-51.
- [4] Ledesma, M. D. and C. G. Dotti (2006). "Amyloid excess in Alzheimer's disease: what is cholesterol to be blamed for?" *FEBS Lett* 580(23): 5525-32.
- [5] Elias, P. K., M. F. Elias, et al. (2005). "Serum cholesterol and cognitive performance in the Framingham Heart Study." *Psychosom Med* 67(1): 24-30.
- [6] Liu, Q., C. V. Zerbinatti, et al. (2007). "Amyloid precursor protein regulates brain apolipoprotein E and cholesterol metabolism through lipoprotein receptor LRP1." *Neuron* 56(1): 66-78.

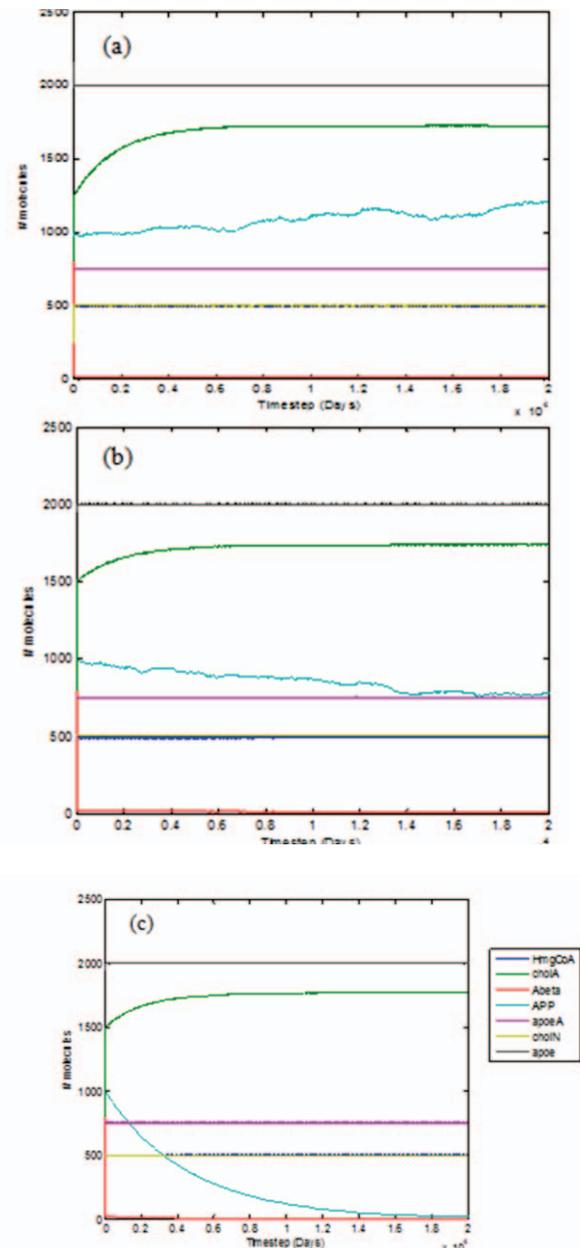


Figure 6: Effect of Decreased Neuronal Cholesterol: (a) Initial neuronal cholesterol decreased by $\frac{1}{2}$. Final APP: 1207; final A β : 18. (b) Cholesterol transfer rate decreased by 50%. Final APP: 781; final A β : 12. (c) Cholesterol transfer rate decreased by 90%. Final APP: 14; final A β : 0.

- [7] Hoshi, M. et al (1997). "Nontoxic Amyloid β Peptide 1-42 Suppresses Acetylcholine Synthesis." *J.Biol.Chem.* 272(4):2038-2041.
- [8] Bjorkhem, I. and S. Meaney (2004). "Brain cholesterol: long secret life behind a barrier." *Arterioscler Thromb Vasc Biol* 24(5): 806-15.
- [9] Rosa, P. and A. Fratangeli (2010). "Cholesterol and synaptic vesicle exocytosis." *Commun Integr Biol.* 2010 Jul-Aug; 3(4): 352-353.
- [10] Crameri, A., E. Biondi, et al. (2006). "The role of seladin-1/DHCR24 in cholesterol biosynthesis, APP processing and Abeta generation in vivo." *Embo J* 25(2): 432-43.