2013

A SYSTEMS BIOLOGY APPROACH FOR UNDERSTANDING INFLAMMATION IN THE GASTROINTESTINAL TRACT OF A CROHN'S PATIENT

Gigi Meyer
Virginia Commonwealth University

College of Humanities and Sciences
Virginia Commonwealth University

This is to certify that the thesis prepared by Gigi G. Meyer titled "A SYSTEMS BIOLOGY APPROACH FOR UNDERSTANDING INFLAMMATION IN THE GASTROINTESTINAL TRACT OF A CROHN'S PATIENT" has been approved by her committee as satisfactory completion of the thesis requirement for the degree of Master of Science in Mathematics

Dr. Rebecca Segal, Department of Applied Mathematics, College of Humanities and Sciences

Dr. Angela Reynolds, Department of Applied Mathematics, College of Humanities and Sciences

Dr. Rebecca L. Heise, Department of Biomedical Engineering

Dr. Dewey Taylor, Graduate Chair, Department of Mathematics and Applied Mathematics, College of Humanities and Sciences

Dr. David Chan, Acting Chair, Department of Mathematics and Applied Mathematics, College of Humanities and Sciences

Dr. James S. Coleman, Dean, College of Humanities and Sciences

Dr. F. Douglas Boudinot, Graduate Dean

June 17, 2013

A SYSTEMS BIOLOGY APPROACH FOR UNDERSTANDING INFLAMMATION IN THE GASTROINTESTINAL TRACT OF A CROHN'S PATIENT

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science Applied Mathematics at Virginia Commonwealth University.

by

GIGI GABRIELLE MEYER
Bachelor of Science, Virginia Polytechnic and State University, 2000
Masters Engineering, The Pennsylvania State University, 2005

Director: Rebecca A. Segal, Associate Professor
Department of Mathematics and Applied Mathematics

Virginia Commonwealth University
Richmond, Virginia
June 2013

Table of Contents:

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Abstract

A SYSTEMS BIOLOGY APPROACH FOR UNDERSTANDING INFLAMMATION IN THE GASTROINTESTINAL TRACT OF A CROHN'S PATIENT

By Gigi Gabrielle Meyer, Master of Science
A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.
Virginia Commonwealth University, 2013

Major Director: Rebecca A. Segal
Associate Professor, Department of Mathematics and Applied Mathematics

ABSTRACT: A system of ordinary differential equations is developed to model the effect of fatty acids on chronic intestinal inflammation that is typical of a Crohn's patient. Several murine studies have shown an anti-inflammatory response when specific polyunsaturated fatty acids are included regularly in the diet. It is believed that the fatty acids serve as a specific ligand that activates the Peroxisome Proliferator Activated Receptor (PPAR) which is located on multiple cell types that are active in the inflammatory response. The binding of the PPAR results in a suppression of the inflammatory pathway. Results of the model indicate a muted inflammatory response when fatty acids are added regularly to the diet in mild to moderate cases of Crohn's. Results of mathematical analysis show a stable fixed point with decreased inflammatory markers and pathogen levels when fatty acids are added regularly to the diet.

Chapter 1: Introduction and Motivation

Inflammation is a complex, multi-time scale process. It is the body's natural reaction to foreign invading pathogens or injury, and results in the removal of harmful stimuli, damaged cells or irritants, thereby aiding in the process of healing. This initial reaction is meant to protect the body. However, when inflammatory signals fail to resolve inflammation can become self-perpetuating and harmful to the body. Acute inflammation is the initial response to injury or infection; there is a rapid onset and it can quickly become severe. Chronic inflammation happens over an extended period of time, sometimes months or years. Several scenarios may lead to the state of chronic inflammation. The typical scenario is one in which there is a failure to eliminate the original stimulus that caused the acute inflammation. A second possible course to chronic inflammation is through the onset of an autoimmune response in which immune cells attack healthy tissue, by mistaking them for foreign material. A final possible trigger for chronic inflammation is the presence of a chronic and persisting irritant (Linder and Melby, 2012). Some diseases that are caused by chronic inflammation include asthma, tuberculosis, rheumatoid arthritis, chronic periodontitis, ulcerative colitis and Crohn's disease, chronic sinusitis, and chronic active hepatitis.

During acute inflammation three main processes occur: First, arterioles (small branches of arteries that lead to capillaries) dilate, which results in increased blood flow. Second, capillaries become more permeable, resulting in an increased rate of blood proteins and fluid moving into the interstitial spaces (between tissues). Third, neutrophil and macrophage immune cells move in to the interstitial spaces (Linder and Melby, 2012). The possible outcomes of acute

inflammation are 1) a healthy resolution (and inflammation is removed), 2) an abscess develops (and infection may persist), or 3) chronic inflammation.

The outcomes of chronic inflammation is the most severe of the "failed" outcomes, resulting in destruction of tissue, a thickening and scarring of connective tissue, and the possible death of cells and tissues. Common treatments used to control inflammation include non-steroidal anti-inflammatory drugs (NSAIDs), such as naproxen, ibuprofen and aspirin, are taken to alleviate pain caused by inflammation, and to counteract the cyclooxygenase (COX) enzyme which synthesizes prostaglandins that promote inflammation (Nordqvist, 2012). Corticosteroids are a class of steroid hormones that are anti-inflammatory by preventing phospholipid release, which blocks mechanisms involved in inflammation. Lastly, a newly developed medication is Immune Selective Anti-Inflammatory Derivatives (ImSAIDs), (Mathison et al., 2010). This therapy is a class of peptides that alter the activation and migration of immune cells that are involved in amplifying the response. Since these drugs do have side effects, the diet can be a useful tool to modify inflammation. Some foods have been shown to have anti-inflammatory properties; in particular, Omega-6 fish oils and tart cherry juice have been shown to reduce inflammation when ingested regularly (Sleigh et al., 2012). Anti-inflammatory diet modifications will be described in more detail in Chapter 2.

CHAPTER 2: BIOLOGY BACKGROUND

INFLAMMATORY BOWEL DISEASE (IBD)

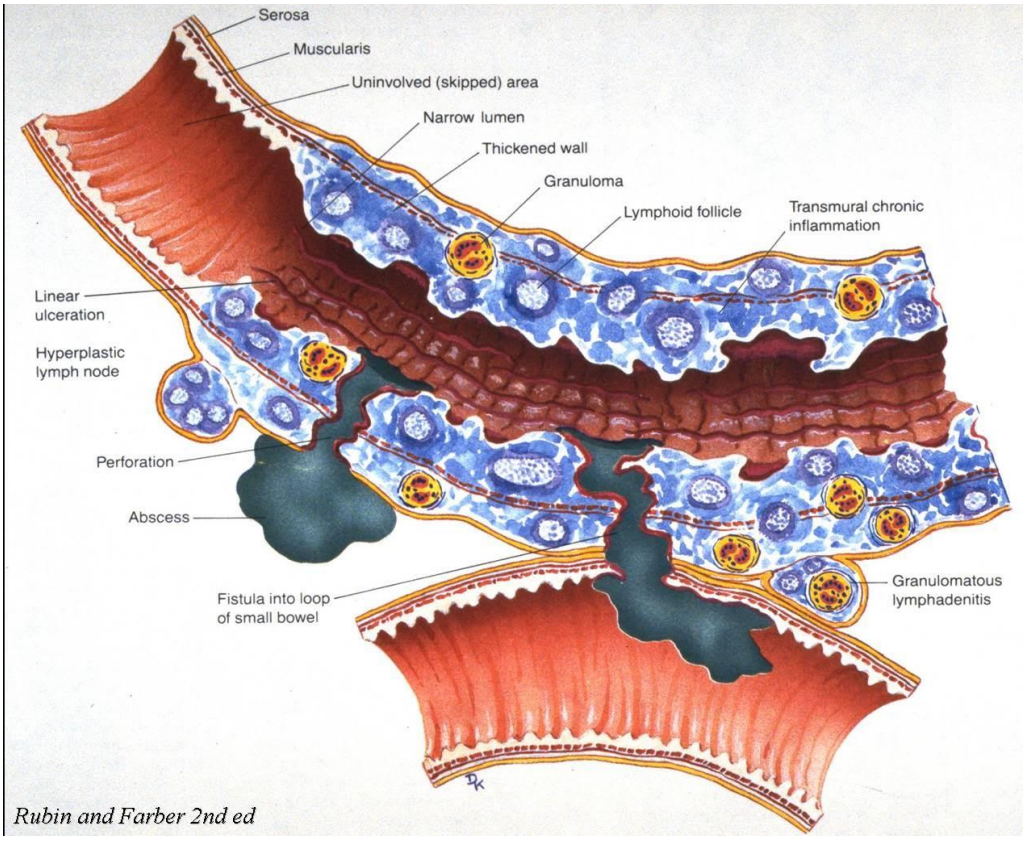
Inflammatory Bowel Disease (IBD) is characterized by chronic inflammation in response to bacteria in the lumen microflora, which results in lesions of the epithelial lining and lamina propria (Swidsinski et al., 2002). There are two common types of IBD – Ulcerative Colitis and Crohn's disease. An estimated 1.4 million individuals suffer from IBD in the U.S. (Herrinton et al., 2007).

In a healthy individual, immune cells of the gut are mostly inactive towards the populations of bacteria in the microflora. This tolerance is attributed to the presence of regulatory immune cells that are antagonistic to the inflammatory pathways. Inflammatory pathways triggered by small populations of pathogenic bacteria are quelled by the presence of these anti-inflammatory regulatory cells. However, in IBD the immune system mistakes food, bacteria, and other materials for foreign invading substances and will attack cells of the intestine. In a normal, healthy individual, invading bacteria are destroyed, and then regulatory cells stop the process. In IBD, the inability of the regulatory cells to stop the inflammation response and/or the overwhelming response to materials mistakenly identified as foreign invading substances causes the indiscriminate release of toxic peroxide anions, proteases, and oxygen / nitrogen radicals by pro-inflammatory immune cells. This leads to tissue damage.

Based on the process as described above, modeling inflammation for Crohn's disease is unique for two main reasons. First, in a sterile organ system, the immune processes are generally stopped when the pathogen population has been eliminated. However, in the intestine, antigens

that induce inflammation (bacterial microflora, food) cannot be entirely eliminated, and so if left unchecked, the collateral damage of the immunopathogenesis can become more harmful to the host than the invading bacteria (Wendelsdorf et al., 2010). Second, as intestinal tissues are damaged in this process, the epithelial barrier between the lumen and lamina propria allows bacteria to cross into the lamina propria, thereby inducing an additional positive feedback loop that magnifies the inflammation. Figure 1 (Rubin and Farber, 1995) shows a section of the intestine in Crohn's disease. The lamina propria is inflamed and swollen. There are also lesions in which bacteria from the lumen may leak in to the lamina propria, which further exacerbates the inflammation response.

Figure 1: The intestine in Crohn's disease (Rubin and Farber, 1995)**.**



Crohn's disease can involve any section of the gastrointestinal tract, but occurs most frequent at the end of the small intestine or at the beginning of the large intestine. Crohn's may affect all layers of the gastrointestinal tissue, and symptoms include persistent diarrhea, cramping abdominal pain, fever, rectal bleeding, and fatigue. A common complication is blockage of the intestine due to swelling and scar tissue, and sometimes ulcers within the intestinal tract that may become infected fistulas (Sohrabpour et al., 2010). As a result of these complications, an individual may be depleted of proteins, calories, and vitamins. Ulcerative colitis is limited to the large intestine, and only affects the top layers of the intestine. Up to 80% of patients with Crohn's eventually require surgery to remove sections of the intestine, which may not entirely cure the individual (Cosnes et al., 2011). In contrast, for Ulcerative colitis patients that are unresponsive to medication and require surgery to remove portions of the colon, the condition does not reoccur.

The Inflammation Pathway

The inflammation response specific to the intestinal tract involves many immune defense cells and pro- and anti-inflammatory chemical mediators that help to regulate the system from the onset to conclusion of an infection. Mast cells are typically the first responders to an invading pathogen, and also carry out some inflammatory-mediating functions. Mast cells are found in the connective tissue and mucous membranes of the body, and contain mediator granules of histamine, eosinophil chemotactic factor, neutrophil chemotactic factor, platelet activating factor, and cytokines IL-3, IL-4, IL-5, IL-6 and TNF-alpha (Kaiser, 2011). They are also capable of processing leukotriene and prostaglandins which act as pro-inflammatory mediators. See Table 1 for a description of each of these chemical mediators (Kaiser, 2011; Tilg et al., 2013).

Table 1: Inflammatory mediators released by the Mast Cell

Histamine	Pro-Inflammatory	Constriction of smooth muscles, vasodilation, increased mucous secretion.
Eosinophil chemotactic factor	Pro-Inflammatory	Recruits Eosinophils
Neutrophil chemotactic factor	Pro-Inflammatory	Recruits Neutrophils
Platelet activating factor (PAF)	Pro-Inflammatory	Activator and mediator of leucocyte functions – platelet aggregation, inflammation, and anaphylaxis.
TNF-α	Pro-Inflammatory	Recruits Neutrophils
IL-1	Pro-Inflammatory	Recruits Phagocytes, Lymphocytes
IL-3	Pro-Inflammatory	Recruits Monocytes and Dendritic Cells
IL-5	Pro-Inflammatory	Stimulates B cell growth, immunoglobulin secretion, eosinophil activation
Leukotrienes	Pro-Inflammatory	Cause prolonged constriction of smooth muscles.
Prostaglandins	Pro-Inflammatory	Increased vascular permeability constricts smooth muscles, magnify pain and induce fever.
IL-4	Anti-Inflammatory	Suppress IL-1 and TNF
IL-6	Anti-Inflammatory	Neutralizes effects of IL-1, induce release of IL-1 and TNF antagonists

Mast cells have pattern-recognition receptors (PRRs) on the surface of the cell that interact with pathogen-associated molecular patterns (PAMPs.) The PAMP of an invading pathogen will bind to a PRR, and mast cells will release granule contents. This promotes inflammation, and attracts other immune defense cells to the site of infection.

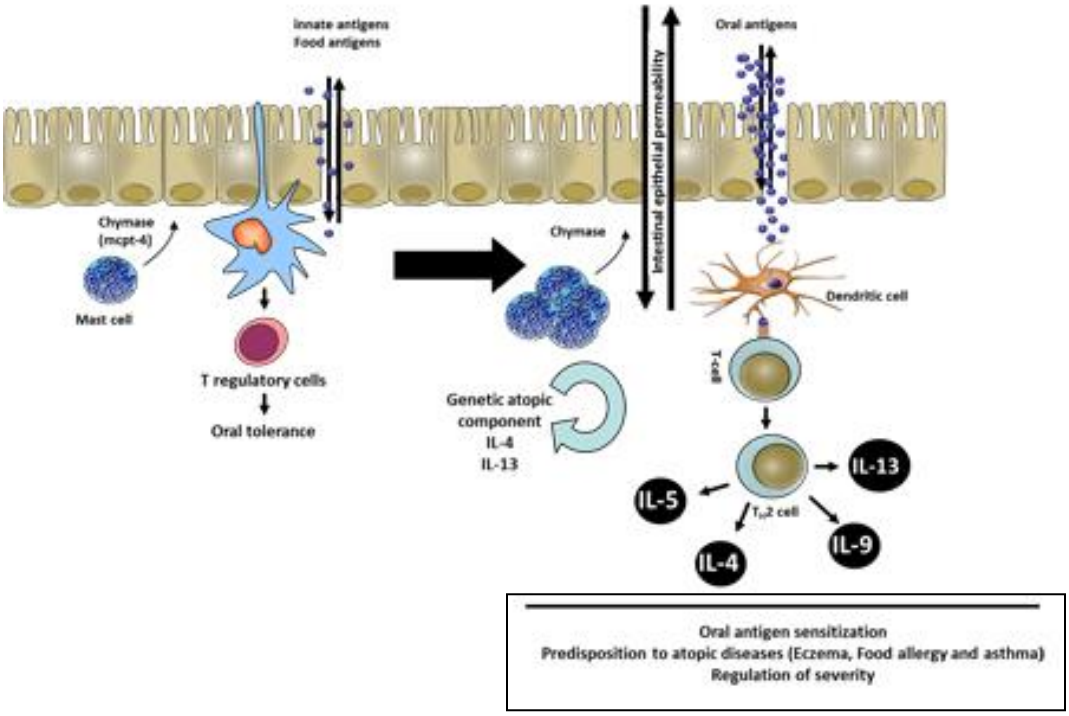
The next cells to react are dendritic cells, and are derived from monocytes in an immature form. Once the dendritic cell has engulfed antigens and is activated by pro-inflammatory cytokines, the

dendritic cell will migrate to regional lymph nodes. At this point, the dendritic cell will present antigens to the populations of naïve T-lymphocyte cells. Dendritic cells engulf microorganisms and other foreign materials and degrade the material with lysosomes; peptides from microbial proteins are then bound to a MHC-II molecule and put on the surface of the cell, where they are recognized by T-cell receptors and CD4 molecules on naïve T4-lymphocytes and T8-lymphocytes. These T-cells then become activated, proliferate, and differentiate into effector cells. Dendritic cells also produce pro-inflammatory cytokines. Monocytes respond to anti-inflammatory and pro-inflammatory cytokine signals (Mosser, 2003).

Monocytes reside in the blood, and when signaled, migrate from the blood and enter the tissue to become activated and differentiate into macrophages. The defense functions of macrophages are to kill microbes, infected cells, and tumor cells by phagocytosis. Once macrophages engulf microorganisms, they can become activated by T-helper lymphocytes, and produce an increased number of lysosomes. Like dendritic cells, peptides from engulfed and degraded microorganisms are bound to the MHC-II molecule and put on the cell surface of the macrophage to be recognized by T-cell receptors and CD4 molecules on effector T4-lymphocytes. This leads to the activation and differentiation of the macrophage. Type I macrophages are pro-inflammatory and function to release toxins that destroy surrounding pathogens; type II macrophages are anti-inflammatory and function to help heal the body. Cytokines TNF-α, IL-1, IL-6, and IL-8 are produced by macrophages; these chemicals induce fever, increase phagocytosis and energy output, promote sleep, activate resting T-lymphocytes, attract and activate neutrophils, and stimulate endothelial cells to replicate, form capillaries and fibroblasts that form connective scar tissue. Figure 2 (Hogan Lab, 2009) provides a spatial description of immune defense cells in the intestine; the figure displays the reactions of an

individual with a food allergy, comparable to that of a hypersensitive immune response in an individual with Crohn's.

Figure 2: Intestinal Immune Response from a food allergy (Hogan Lab, 2009).



THE PPAR RECEPTOR

It is believed that fatty acids serve as a specific ligand that activates the Peroxisome Proliferator Activated Receptor (PPAR), and that the binding of the PPAR results in a suppression of the inflammatory pathway. PPAR is a member of the nuclear receptor family of transcription factors. Nuclear receptors are a class of proteins found within cells that sense steroid and thyroid hormones and once bound by a specific signaler, have the ability to regulate gene expression. Nuclear receptors have the ability to directly bind to DNA and regulate the expression of adjacent genes. This regulation of gene expression by the nuclear receptors is generally only

possible when a specific ligand (a molecule that can affect receptor behavior) is present. The ligand, once bound to the nuclear receptor, will result in a conformational change of the receptor, which will cause activation and resulting up or down regulation of gene expression. For example, polyunsaturated fatty acids (PUFA) serves as a ligand to the PPAR receptor, and down-regulates with an anti-inflammatory effect. Omega-6 fatty acids are a type of PUFA, found in flaxseeds, walnuts, fish, and oils.

Studies have found a high concentration of the PPAR receptor in the upper gastrointestinal tract, and through the layers of luminal tissue. Due to the location and the prevalence of Crohn's disease in this region, and the regulatory activities described earlier in this section, PPAR receptor may play a fairly involved role in the disease. In one particular study, the abundance, distribution, and function of PPAR-γ were analyzed in murine epithelial cells taken from the small and large intestine (Su et al., 2007). The study found that receptor subtype PPAR-γ was located throughout the colon, with significantly more found in the proximal 1/3 of colon – or upper small intestine – and most intensely in luminal cells of the proximal colon. Lesser amounts of the receptor were found in the subluminal cells, which indicates that PPAR-γ is expressed in all cells of intestinal proximal crypts.

THE DIET

As mentioned earlier, many medications are available to treat chronic intestinal inflammation, but the side effects can be serious (increased intestinal irritation and inflammation, rashes and darkening of the skin, upset stomach, vomiting, diarrhea, muscle aches, abdominal pain, flu-like symptoms, headache, anxiety, insomnia – CCFA, 2010). Therefore, modifications to the diet may serve as an important tool in controlling the inflammatory response. Along with normal gut

microbiota, dietary foods are the most common luminal antigens in the bowel and may influence intestinal inflammation (Cabre and Domenech, 2012). Possible mechanisms by which dietary products have an influence are by having a direct antigenic effect, by alteration of gene expression, and by modulation of inflammatory mediators. High intakes of total fat, omega-3 fatty acids, and meat were associated with higher risk for developing IBD, while high vegetable and fruit intake were associated with a decreased risk (Hou et al., 2011) Diets high in refined sugars also were implicated in the development of Crohn's and Ulcerative Colitis (Hansen et al., 2011). A low-residue diet (i.e., devoid of insoluble fiber) is advised during flares of IBD.

As mentioned earlier, PUFA may play some anti-inflammatory role as a ligand to the PPAR receptor. Several studies have been conducted to measure the activity of the PPAR receptor in response to various types of PUFA. Conjugated Linoleic Acid (CLA), naturally found in milk, cheese, and other dairy products, has been found to suppress colonic inflammation and up-regulated PPAR-gamma (Bassaganya-Riera and Hontecillas, 2010.) Another study found similar effects with Punicic acid (Bassaganya-Riera et al., 2011). Table 2 gives a summary list of pro- and anti-inflammatory diet modifications (Drake, 2007).

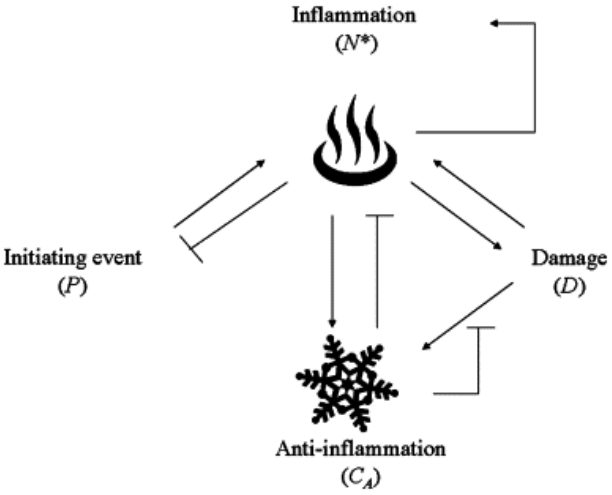
Table 2: Diet modifications

Pro-Inflammatory	Anti-Inflammatory
Trans Fatty Acids	Omega-6 Fatty Acids
Saturated Fatty Acids	Low Cholesterol Diets
High Glycemic Index Foods	Low Glycemic Index Foods
Excessive Alcohol	Dietary Fiber
Smoking	Arginine-rich foods (fish, nuts)

CHAPTER 3: MATHEMATICAL BACKGROUND

Due to its complex nature, the inflammatory response has attracted the interest of mathematical biologists, and several models of inflammation have been proposed. A reduced mathematical model of the inflammatory response that incorporates pro-inflammatory and anti-inflammatory elements has been shown to simulate a patient's "health" and "death" scenarios following acute inflammation (Reynolds et al., 2006). Figure 3 (Reynolds et al., 2006) represents the interactions provided in the reduced four-variable model. Arrows and bars represent up- and inhibition, or down-regulation in the inflammatory cascade. The balance of pro-inflammatory and anti-inflammatory processes and the overall effect on an infection and pathogen population is well suited for a dynamic model.

Figure 3: Interactions included in the four-variable model of acute inflammation (Reynolds et al., 2006).



In this model, components of the adaptive immune response, such as T cells and specific antibodies are not included. The reduced model consists of four differential equations, with dependent variables representing the levels of pathogen (P), activated phagocytes (N*) – such as

activated neutrophils, tissue damage (D) and anti-inflammatory mediators (C_A), such as cortisol and interleukin-10. A two-variable subsystem N* / P and N* / D are analyzed, using C_A as a parameter, and then these subsystems are combined to form a three-variable model. Finally, the C_A dynamics is incorporated into the system to create the four-variable model. The subsystem approach was used to ensure consistency with biological processes, and evaluated through simulations and bifurcation analysis.

Fixed points, also known as a critical points or equilibrium points, are calculated by setting the derivatives of each variable in the system to zero. Fixed points were calculated in Reynolds et al. (2006) subsystems to determine parameter ranges in which stability and bifurcations occurred. A bifurcation occurs in system behavior when a change in parameter value alters the number of fixed points or their stability. Reynolds et al. (2006) model finds three equilibrium points, corresponding to health state, aseptic, and septic death.

Reynolds et al. (2006) four variable model is useful because it is easier to analyze in context of immune pathology than a much larger system of differential equations, but the non-specific nature of the model may lose some of the actual biological processes involved. In contrast, a system of twenty-nine ordinary differential equations was proposed to model the immunopathology of Inflammatory Bowel Disease (Wendelsdorf et al., 2010). In this case, simulations were able to reveal a positive feedback loop between the inflammatory type-I macrophage and the anti-inflammatory type-II macrophage. The ability of the type-I macrophage to be removed from the site of infection, either by reverting back to an undifferentiated state, or to a type-I macrophage, helped to cease immunopathogenesis even as

bacteria continue to reside at the site of infection and are eliminated by other immune defense cells. Wendelsdorf et al. use three location compartments in their model – lumen (inner intestine), lamina propria (first underlying layer, or effector site), and mesenteric lymph node (deepest layer in intestinal wall, or inductive site.) Populations are tracked depending on whether they are in an effector or inductive site. By including these separate compartments, this model provides a useful method to track inflammation in each anatomical layer of the intestine.

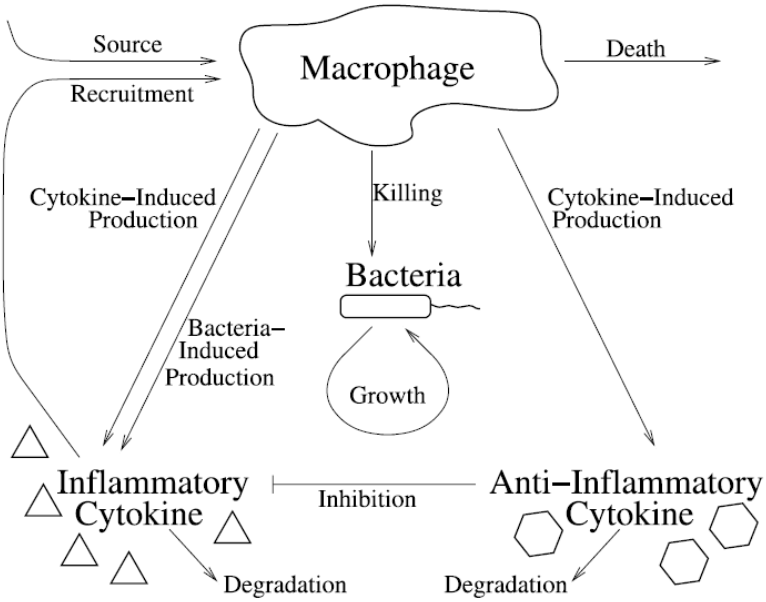
Although the 29-ODE system proposed by Wendelsdorf et al. extensively covers the range of immune cells involved in the anatomical compartments specific to Crohn's, the number of ODEs involved makes the set of equations hard to work with. The study does not identify any steady state, or conduct bifurcation analysis. Also, Wendelsdorf et al. mentions the PPAR-gamma receptor as a possible cause of the macrophage-switch activity but does not provide any direction for how to modify the Effector / Inductive macrophage equations to isolate or simulate this activity, as with the addition of PUFA.

Another modeling approach has been to detail the biological interactions that may cause progression from an acute to chronic state. Herald (2009) presents a system of four nonlinear ordinary differential equations specific to the dynamics of respiratory infections that initiate inflammation of the airway and lead to such conditions as cystic fibrosis and chronic obstructive pulmonary disease. This four-variable system describes the interactions of macrophages, populations of pro-inflammatory and anti-inflammatory cytokines, and bacteria (pathogens).

The focus of the model is to characterize the dynamics of chronic inflammation in the absence of pathogens. Specifically, to model (1) inclusion of anti-inflammatory cytokines to suppress chronic inflammation, (2) model whether the presence of non-aggressive bacteria can cause

chronic inflammation after an infection has been resolved, and (3) model the effectiveness of cytokine therapies on chronic inflammation. Figure 4 below shows the interactions of the four variables included in the Herald model. The model was able to simulate healthy behavior as well as chronic inflammation in the presence or absence of bacteria. After the introduction of bacteria, the model showed five possible outcomes: (1) uncontrolled bacterial growth, (2) clearance of bacteria with resolution of inflammation, (3) clearance of bacteria with chronic inflammation, (4) chronic bacterial infection with inflammation, and (5) oscillations in the bacterial population with inflammation.

Figure 4: Interaction of variables in the chronic inflammation model, (Herald, 2010.)



In comparison, both Herald and Wendelsdorf et al. track both pro- and anti-inflammatory cytokine populations, while Reynolds tracks an anti-inflammatory cytokine population and a general pro-inflammatory pathogenesis response. It could be that tracking the anti-inflammatory cytokine population is of more importance to studying the macrophage dynamics of the model than pro-inflammatory cytokines alone. The results of both Wendelsdorf, et al.'s and Herald's

models focus on this cytokine-macrophage dynamic; while Reynolds et al, 2006 has grouped a non-specific local immune response (mast cells, monocytes, undifferentiated macrophages, neutrophils) into a non-specific group that is taken to be at Quasi steady state and focused on the pathogen / phagocytic immune cell population, given by N* and treated as a combined neutrophil / type I macrophage combo. The general connecting theme of these models thus far has been to model the underlying dynamics of cytokine targets. However, it may be useful to also model possible inhibitors of pro-inflammatory cytokine production, such as the PPAR receptor in combination with the cytokines.

Herald presents the populations of macrophage, pro- and anti-inflammatory cytokines and pathogen present in alveoli in the equations shown below. A new model of inflammation with fatty acid diet modifications was developed from these equations, and is presented in the next chapter.

$$\frac{dM}{dt} = s + \frac{rC}{1+k_1 C} - m_d M$$

$$\frac{dC}{dt} = p_c BM + M\frac{aC}{(1+k_2 C)}\frac{1}{(1+k_3 A)} - c_d C$$

$$\frac{dA}{dt} = p_a CM - a_d A$$

$$\frac{dB}{dt} = gB - b_d MB$$

The pathogen population here is a generic stimulus that may trigger chronic inflammation, which can include pathogenic or non-pathogenic bacteria, viruses and pollutants. The pathogen growth rate here is exponential. Even in the absence of bacteria, the macrophage population is maintained through the source term, *s*. Once a pathogen has entered the respiratory tissue, the macrophage population will recruit more cells through pro-inflammatory cytokines that are signaled to fight the infection (Herald, 2009.) The population of pro-inflammatory cytokines

increases through pathogen-induced production and the positive feedback from the pro-inflammatory cytokine / macrophage loop.

Herald's 4-variable system above was chosen to be modified to a new 5-variable model of pro- and anti-inflammatory processes. It provides a good starting point for because many specific terms are included, yet the simplicity of the model allows for mathematical analysis. An additional macrophage term can be added to the system to represent Alternative-type macrophages, and the macrophage and cytokine equations can be modified to include the anti-inflammatory effects of fatty acids. The model is explained in further detail in Chapter 4: Model Development.

CHAPTER 4: MODEL DEVELOPMENT

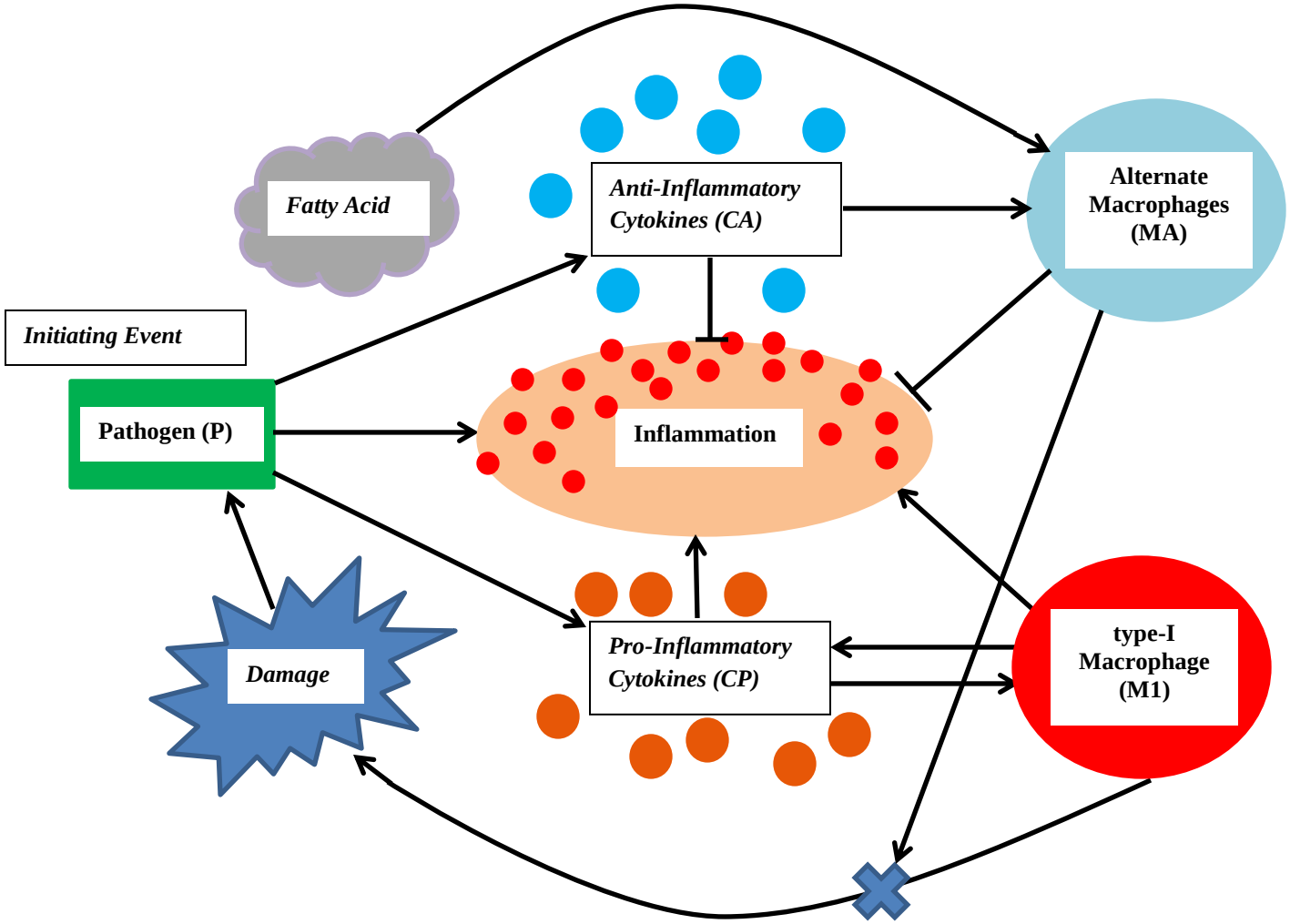
The model presented in this paper includes five ordinary differential equations that are specific to inflammation occurring in the lamina propria of a Crohn's patient. The presented system aims to describe the anti-inflammatory effects of alternate-type macrophages and dietary fatty acids as a possible treatment to inflammation in Crohn's. Table 3 below shows variable definitions for the model.

Table 3: Variable Definitions for the Chronic Inflammation Model

Variable	Units	Definition
M1	Cells	Lamina Propria type-1 macrophage population
MA	Cells	Lamina Propria type-II and type-III macrophage population
CP	Pg/ml	Pro-inflammatory cytokine concentration
CA	Pg/ml	Anti-inflammatory cytokine concentration
P	Cells	Bacterial / antigen population

Figure 5 is a diagram of the inflammatory and anti-inflammatory pathways in the model.

Figure 5: Inflammation Pathways of the 5-variable model. Arrow represent upregulation, bars represent inhibition.



MAST CELLS

Mast cells are unique in that they store preformed inflammatory mediators in their granules, poised for immediate release on activation. Like other immune cells, mast cells can also provide sustained synthesis and release of these products. Granules can also be re-generated for later cycles of degranulation from the same mast cell. Therefore, mast cells are deemed to be considerable contributors in this model.

The mast cell has powerful pro- and anti-inflammatory effects. Granules contain a variety of bioactive proteins that cause inflammation: neutrophil chemoattractants leukotriene B4, interleukin 8 (kerotinocyte-derived chemokine in rodents), macrophage inflammatory protein-2, and tumor necrosis factor (TNF) are all prestored and can be released within seconds of cell activation. In addition, histamine and serotonin will induce vascular permeability, which allows influx of fluid, immune cells, and proteins to the damaged tissue. Anti-inflammatory cytokines generated by mast cells include: IL-4, which drives the T-helper Lymphocyte type 2 (Th2) responses, which releases additional anti-inflammatory cytokines (Kaiser, 2011; Tilg et al., 2013).

The equation (1) below indicates that mast cells release both pro- and anti- inflammatory cytokines in the presence of pathogens. Mast cells are then considered to have released their store of granules are removed from the system. Here, pro-inflammatory cytokines are created at rate k_p and anti-inflammatory cytokines created at rate k_c.

$$*+P \xrightarrow{smt} MT+P$$
$$MT+P \rightarrow CA+CP+P$$

$$\frac{dMT}{dt} = smt*P - kp*MT*P - kc*MT*P \quad (1)$$

The mast cell activity happens very quickly compared to the other system dynamics and so is then taken to be at quasi-steady state:

$$0 = smt*P - k_p*MT*P - k_c*MT*P$$
$$smt*P = MT*P*(k_p+k_c)$$
$$MT = \frac{smt}{(k_p+k_c)}$$

This term is further simplified to a macrophage source term similar to that presented in the model by Herald (2009).

PRO-INFLAMMATORY CYTOKINES AND TYPE-I MACROPHAGES

The cytokines released by mast cells signal undifferentiated macrophages (monocytes) to migrate to the site and differentiate into type-I and alternate-type macrophages. First, pro-inflammatory cytokines represented by CP will signal a differentiation to the type I form with rate *a*. This is represented by a source term ($s1$) in the M1 equation below, and considered to be constant within the model area of the lamina propria. Dietary fatty acid is thought to have a suppressive effect on the differentiation of type-I macrophages and instead encourages alternate-type differentiation. Because of this, it is included in and represented by the anti-inflammatory cytokine population. Both types of macrophages are modeled with the same cell death rate μ_{M1}.

$$* \xrightarrow{s1} M1$$

$$CP \xrightarrow{\frac{r_1}{1+k_1*CP}} CP + M1$$

$$M1 \xrightarrow{\mu_{M1}} *$$

$$\frac{dM1}{dt} = s1 + \frac{r_1 * CP}{1 + k_1 * CP} - \mu_{M1} * M1 \qquad (2)$$

Even in the absence of cytokines, because of the positive $s1$ source term, the macrophages have a positive, non-zero steady state of $s1 - \mu_{M1} * M1$, or $M1 = \frac{s1}{\mu_{M1}}$. Biologically, this represents the background level of immune cells that are always present and ready to react to any invading pathogen.

Similar to the system modeled by Herald, r_1 represents the recruitment rate for type-1 macrophages in response to pro-inflammatory cytokine. k_1 is the saturation constant for pro-inflammatory cytokine-induced macrophage recruitment. The recruitment rate for a type-I macrophage response is stronger than the alternate-type macrophage response. The saturation constant k_1 determines how quickly the $\frac{r_1 * CP}{1 + k_1 * CP}$ term will grow. For example, a smaller value will generate faster macrophage recruitment.

Pro-inflammatory cytokines are first generated by type-1 macrophages when they come into contact with pathogens or antigens. They are also generated in the absence of pathogens or antigens through a positive-feedback loop with type-1 macrophages. Dietary fatty acids and anti-inflammatory cytokines are theorized to slow the release of pro-inflammatory cytokines by binding to the PPAR receptor of the type-I macrophage and inhibiting pro-inflammatory cytokine release. Cytokines have a relatively short half-life, μ_C.

$$P + M1 \xrightarrow{p_{cp}} CP + P + M1$$

$$M1 \xrightarrow{\frac{a*CP}{(1+k_2CP)(1+k_3CA)}} M1 + CP$$

$$CP \xrightarrow{\mu_C} *$$

The rate equation for pro-inflammatory cytokines is thus:

$$\frac{dCP}{dt} = p_{CP} * P * M1 + M1 * \frac{a * CP}{(1 + k_2 CP)(1 + k_3 CA)(1 + k_4 FA)} - \mu_C CP \quad (3)$$

DIETARY FATTY ACIDS

As discussed earlier, dietary fatty acids such as Butyrate, Conjugated Linoleic Acid and Punicic are theorized to have a beneficial effect in the health of Crohn's patient by suppressing inflammation caused by pathogens or dietary antigens. For the purpose of the model, it is theorized that fatty acid intake will have a direct anti-inflammatory effect. This is thought to occur though the actions of the PPAR receptor, blocking further recruitment of pro-inflammatory cells and cytokines (Mosser, 2002). Fatty acids are therefore added directly into the anti-inflammatory cytokine levels. This group inhibits pro-inflammatory cytokine release by type-1 macrophages and promotes differentiation to alternate-type macrophages. Parameter k_4 represents the fatty acid absorption rate of the fatty acid intake FA.

ANTI-INFLAMMATORY CYTOKINES AND ALTERNATE-TYPE MACROPHAGES

Anti-inflammatory cytokines are released when alternate-type macrophages come into contact with pro-inflammatory cytokines. For the anti-inflammatory cytokines CA we have:

$$CP + MA \xrightarrow{p_{ca}} CP + MA + CA$$
$$CA \xrightarrow{\mu_C} *$$

The rate equation for pro-inflammatory cytokines is thus:

$$\frac{dCA}{dt} = p_{CA} * CP * MA - \mu_C CA \qquad (4)$$

Next, the anti-inflammatory effects of type-II and type-III macrophages are combined into an "alternate" category. Fatty Acids are theorized to help recruit alternate-type macrophages by binding to PPAR receptors:

$$* \xrightarrow{s2} MA$$

$$CA \xrightarrow{\frac{r_2}{1+k_1*CA}} CA + MA$$

$$FA \xrightarrow{\frac{r_3}{1+k_4*FA}} FA + MA$$

$$MA \xrightarrow{\mu_{M1}} *$$

$$\frac{dMA}{dt} = s2 + \frac{(r_2 * CA + r_3 * FA)}{(1 + k_1 * CA)(1 + k_4 * FA)} - \mu_{M1} * MA \qquad (5)$$

PATHOGENS

The pathogen equation was modified from the one presented in the Herald (2009) paper. As discussed earlier, the intestinal lumen is host to a multitude of naturally occurring bacteria. Any bacteria that move past the epithelial barrier into the lamina propria become pathogen invaders. In Crohn's disease, the inflammatory response is often out of balance with the actual pathogen invasion that as occurred. Type-1 macrophages emit toxic peroxide anions, proteases, and oxygen / nitrogen radicals (NO) that cause damage to tissues surrounding it. A tissue-damage term was developed in Reynolds et al. (2006), model paper, and is included in the model here. In this system, damage that occurs to the epithelial tissues breaks the barrier between the intestinal lumen and the Lamina Propria. Since this lack of barrier allows many of the naturally-occurring bacteria to slip into the Lamina Propria site, the damage term has a positive effect on the growth of the pathogen. This has been added to the pathogen equation with *fd* (damage function.) Pathogens enter through breaks in the epithelial barrier at this rate. For the purposes of fixed point analysis, this term was treated as a constant rate, *dam*. This implies that damage will continue to occur, and provide a positive input for pathogens, no matter how small the population of type-I macrophages. However, in simulations, a function was used to emulate a non-zero background level of immune cells which does not cause damage. Damage will occur

only when type-I macrophage levels and resulting NO levels are high enough. Thus, the presence of type-I macrophages will not incur any damage at some normal threshold. The function used in simulations models damage as zero below this threshold. The anti-inflammatory cytokines and alternate-type macrophages help to repair tissue damage and subdue the inflammation. Therefore, a greater percentage of the anti-inflammatory cells in the system will decrease the damage that occurs. When pro-inflammatory type-1 macrophages make up a greater percentage of the system, damage to the tissues will occur. The damage function is shown below, where percentage c represents the balance necessary between type-1 macrophages, alternate-type macrophages and fatty acid in the system for damage to occur. If type-1 macrophages surpass this percentage difference, then damage occurs at a rate proportional to the population of type-1 macrophages in the system. Otherwise, no damage occurs.

$$fd = if\,(M1 - c*MA > 0)\ \ then\ \ (dam*M1)\ \ else(0)$$

The pathogen equation is presented below in the form used for fixed point analysis:

$$\frac{dP}{dt} = kpg*P - e_p*M1 + dam*(M1 - c*MA) \qquad (6)$$

Table 4 shows parameter definitions for the 5-variable model.

Table 4: Parameter Definitions for the Chronic Inflammation Model

Parameter	Units	Definition	Parameter Value
$s1, s2$	cells / hour	Source term for the lamina propria macrophage population	0.0125 - 0.08 (Reynolds)
r_1	Cells / ((pg/ml)*hour)	Recruitment rate for type-1 macrophages in response to pro-inflammatory cytokine	0.01 (Reynolds)
r_2	Cells / ((pg/ml)*hour)	Recruitment rate for alternate-type macrophages in response to anti-inflammatory cytokine	0.008 (estimated)

r_3	Cells / ((pg/ml)*hour)	Dietary intake / absorption rate for fatty acids	0.0001 – 0.0005 (estimated)
μ_M	1 / hour	Natural rate macrophages leave due to death or migration	0.002 (Reynolds)
a	Cells / hour	Auto-induction rate of pro-inflammatory cytokine production	0.002 (estimated)
p_{cp}	(pg/ml) / (cells^2 * hour)	Bacterial induced inflammatory cytokine production rate	0.01 (estimated)
p_{ca}	(pg/ml) / (cells^2 * hour)	Production of anti-inflammatory cytokine	0.04 (Reynolds)
μ_C	1 / hour	Half-life of cytokines	0.02 (estimated)
k_1	1 / (pg/ml)	Saturation constant for pro-inflammatory cytokine induced macrophage recruitment	0.7 – 0.9 (estimated)
k_2	1 / (pg/ml)	Saturation constant for production of pro-inflammatory cytokine through auto induction	0.7 – 0.9 (estimated)
k_3	1 / (pg/ml)	Saturation constant for inhibition of anti-inflammatory cytokines	0.7 – 0.9 (estimated)
k_4	1 / (pg/ml)	Saturation constant for fatty acid binding	0.5 – 0.8 (estimated)
kpg	1 / hour	Bacterial growth rate	0.021 – 2.44 (Reynolds)
e_p	1 / (cells * hour)	Bacterial death rate due to macrophages	0.6 (Reynolds)
c		Threshold at which damage occurs.	0.8 – 1.2 (estimated)
FA	(pg/ml)	Dietary Fatty Acid Intake	0 – 2 (estimated)
Dam	M1-c*MA	Damage produced by type-I macrophages	0.35 – 0.5 (estimated)

The parameters in the table above were estimated or drawn from values given in the Reynolds et al. (2006) inflammation model.

In order to identify the sensitive parameters in the model which lead to chronic inflammation, the system was non-dimensionalized. This allows the relative sizes of the variables and parameters of the model to be better understood. The non-dimensionalized system is shown below, and the new parameters are shown in Table 5. Non-dimensionalized variables are given by (*m1*, *ma*, *cp*, *ca*, *p*). Herald's (2009) original scaled values were used in these equations, with the exception

of the alternate-type macrophage and pathogen equations, and additional scaled parameters used in the type-I macrophage and cytokine equation.

$$\frac{dm1}{d\tau} = 1 + \alpha \frac{cp}{(1+\beta * cp)} - m1 \qquad (6)$$

$$\frac{dcp}{d\tau} = p * m1 + \delta * cp\left[\frac{\psi * m1}{(1+cp)(1+ca)(1+f_{ab})} - 1\right] \qquad (7)$$

$$\frac{dma}{d\tau} = 1 + \alpha 2 \frac{(ca + f_{ut})}{(1+\beta 2 * ca + f_{ab})} - ma \qquad (8)$$

$$\frac{dca}{d\tau} = \xi * cp * ma - \delta * ca \qquad (9)$$

$$\frac{dp}{d\tau} = \kappa * \left[p * (\phi - m1) + \gamma * (m1 - c * ma)\right] \qquad (10)$$

Table 5: Non-dimensionalized parameters

Scaled Parameter	Equivalent from Table 4	Value
α	$\frac{r1}{s1 * k2}$	0.6 - 1.2
β	$\frac{k1}{k2}$	1
δ	$\frac{\mu_c}{\mu_m}$	10
ψ	$\frac{a * s1}{\mu_c * \mu_m}$	0.5 - 4
f_{ab}	$fa * k4$	0 – 1.5
$\alpha 2$	$\frac{r2}{s2 * k3}$	0.4 – 1
$\beta 2$	$\frac{k1}{k3}$	1
f_{ut}	$\frac{r3}{r2} k3 * fa$	0 - 1

ξ	$\frac{p_{ca} * s2 * k3}{k2 * \mu_m^{\ 2}}$	0.8 - 2
κ	$\frac{e_p * s1}{\mu_m^{\ 2}}$	20 - 50
ϕ	$\frac{kpg * \mu_m}{e_p * s1}$	0.01 - 2
γ	$\frac{dam * k2 * p_{cp} * s1}{e_p * \mu_m^{\ 2}}$	0.5 – 2

The non-dimensionalization process provides a reduction in the total number of variables present in the equations. This allows for a more straightforward analysis of the system.

CHAPTER 5: MATHEMATICAL ANALYSIS

3-VARIABLE MODEL STEADY STATES IN THE ABSENCE OF DIETARY FATTY ACID

The model was first simplified to analyze the effect of the damage term, γ, in a low or absent fatty acid diet. In the absence of a regular supply of dietary fatty acids, the terms f_{ab} and f_{ut} are set to zero. A steady state analysis is outlined below to determine the value of variables over time. By setting derivatives to zero, the variables are no longer changing.

The steady state value for anti-inflammatory cytokines is then:

$$ca = \frac{\xi * cp * ma}{\delta}.$$

Using this to solve for alternative-type macrophages at steady state, we have:

$$ma = \frac{1}{2*\xi + cp*(2*\alpha 2 - \beta 2)}.$$

The non-dimensional parameter $\beta 2$ is a ratio of the saturation constants, can be taken to be close to 1. If $\alpha 2$ is taken to be close to 1, the sum of the terms $2*\xi + cp*(2*\alpha 2 - \beta 2)$ is positive and leads to a population of *ma* that is less than 1, that continues to decline as *cp* grows. Therefore, although the anti-inflammatory cytokines may continue to persist if *cp* is high, ultimately the system reduces to the 3-equation model:

$$\frac{dm1}{d\tau} = 1 + \alpha \frac{cp}{(1+\beta * cp)} - m1 \qquad (11)$$

$$\frac{dcp}{d\tau} = p * m1 + \delta * cp\left[\frac{\psi * m1}{(1+cp)} - 1\right] \qquad (12)$$

$$\frac{dp}{d\tau} = \kappa * \left[p * (\phi - m1) + \gamma * m1\right] \qquad (13)$$

Solving for the steady states, we find that:

$$m1 = 1 + \alpha * \frac{cp}{(1 + \beta * cp)}$$

$$p = \frac{\gamma * m1}{(m1 - \varphi)} = \frac{\gamma * \left(1 + \frac{\alpha * cp}{(1 + \beta * cp)}\right)}{1 + \frac{\alpha * cp}{(1 + \beta * cp)} - \varphi}$$

After substituting in the values of *m1* and *p* above, the steady state for the *cp* equation is the roots of the equation below:

$$(-\delta\beta^2 + \delta\beta\alpha + \delta\beta^2\phi)cp^4 +$$
$$(-\gamma\alpha^2 - \delta\psi\alpha\phi\beta - \delta\psi\alpha^2 - \gamma\beta^2 + \delta\alpha - 2\delta\beta + \delta\beta^2\phi + \delta\beta\alpha + \delta\psi\beta^2 - 2\gamma\beta\alpha - \delta\psi\beta^2\phi + 2\delta\phi\beta - \delta\beta^2)cp^3 +$$
$$(-2\gamma\alpha - \delta + \delta\phi - \delta\psi\alpha\phi - \gamma\beta^2 - 2\gamma\beta - \gamma\alpha^2 + \delta\alpha - 2\delta\psi\phi\beta + 2\delta\psi\beta + 2\delta\phi\beta - 2\delta\beta - 2\gamma\beta\alpha)cp^2 +$$
$$(\delta\phi - \gamma - \delta - 2\gamma\alpha + \delta\psi - \delta\psi\phi - 2\gamma\beta)cp - \gamma = 0$$

Because of the complexity of the *cp* equation, above, it helps to start by trying to put bounds on some of the parameters. First, it is helpful to note that to be biologically relevant, all the parameters and variables here are greater than or equal to zero. With this restriction, any initial condition will result in the type-I macrophage equation with $m1 \geq 1$. In the pathogen equation, the parameter ϕ is the ratio of bacterial growth to macrophage kill rate. Larger values indicate a more aggressive, quickly growing pathogen that is unmanageable to the type-I macrophage population. It determines bacterial sensitivity, and controls whether an infection can be cleared or will become chronic. The parameter γ is a ratio of the damage produced by the different types of macrophages. In order to solve for a steady state value of *p* greater than or equal to zero, we must have $\phi < m1$.

By substituting in the values of *m1* and *p* into the cytokine equation (12), we have:

$$\frac{dcp}{d\tau} = \frac{\gamma * \left(1 + \frac{\alpha * CP}{(1+\beta * CP)}\right)}{1 + \frac{\alpha * CP}{(1+\beta * CP)} - \varphi} * \left(1 + \alpha * \frac{cp}{(1+\beta * cp)}\right) + \delta * cp \left[\frac{\psi * \left(1 + \alpha * \frac{cp}{(1+\beta * cp)}\right)}{(1+cp)} - 1\right]$$

The parameter δ is the ratio of cytokine death rate to macrophage death rates and was taken to be $\delta \approx 10$. After some rearrangement, we have:

$$\frac{dcp}{d\tau} = \frac{1 + cp * (1 + \alpha * \gamma)}{((1-\phi) + cp * (1 + \alpha - \phi))} * (1 + cp * (1+\alpha)) + 10cp * \left[(\psi - 1) + cp * (\psi + \psi\alpha - 2) - cp^2\right]$$

Note that the term on the right of this equation is negative if $\psi \le 1$ and $\psi(1+\alpha) \le 2$. Using this, we can put bounds on *cp* by setting $(1+\alpha) \le \frac{2}{\psi}$.

$$\frac{dcp}{d\tau} = \frac{1 + cp * \left(1 + \left(\frac{2}{\psi} - 1\right) * \gamma\right)}{\left(1 + \frac{2}{\psi} * cp\right) - \phi * (1 + cp)} * \left(1 + cp * \frac{2}{\psi}\right) + 10cp * \left[(\psi - 1) - cp^2\right]$$

Since γ includes a multiplier of $\frac{1}{\mu_m{}^2}$, then $\gamma >> 1$. In order to maintain a value of $cp \ge 0$, then the term on the left must be positive and greater than or equal:

$$\frac{1 + cp * \left(1 + \left(\frac{2}{\psi} - 1\right) * \gamma\right)}{\left(1 + \frac{2}{\psi} * cp\right) - \phi * (1 + cp)} * \left(1 + cp * \frac{2}{\psi}\right) \ge 10cp * \left[(\psi - 1) - cp^2\right]$$

Note that $\psi \le 1$, so $1 + cp * \left(1 + \left(\frac{2}{\psi} - 1\right) * \gamma\right)$ is positive.

From the denominator we determine that $cp > \frac{(\phi - 1)}{\left(\frac{2}{\psi} - \phi\right)}$ is required to maintain a biologically relevant state, given $\psi(1+\alpha) \leq 2$.

The *m1* steady state becomes:

$$m1 \geq 1 + \alpha * \frac{\frac{(\varphi - 1)}{\left(\frac{2}{\psi} - \varphi\right)}}{1 + \beta * \frac{(\varphi - 1)}{\left(\frac{2}{\psi} - \varphi\right)}} = 1 + \frac{\alpha * (\varphi - 1)}{\frac{2}{\psi} - \varphi + \beta * (\varphi - 1)}$$

$$p = \frac{\gamma * m1}{(m1 - \phi)}$$

Varying the parameter ϕ was found to have significant impact on the model. Since ϕ determines bacterial sensitivity it will ultimately determine whether an infection is cleared or becomes chronic; a low value of ϕ signifies a weaker, non-aggressive pathogen, and a high value of ϕ signifies an aggressive, resistant pathogen. The following parameter values were chosen to study the states that occur when varying ϕ: $\gamma = 1, \psi = 2, \alpha = 1, \delta = 10, \beta = 1, \kappa = 30.$ While the first three of these parameters may change depending on the conditions of the system, the last three represent basic biological functions. To simplify the analysis, these values are taken to be constant and $\delta = 10, \beta = 1, \kappa = 30$ will be used for the remainder of this analysis.

By varying values of ϕ in the three-equation model above, the following changes in stability were found using Maple. The eigenvalues for the system were computed with the different parameter values and were analyzed for stability criteria.

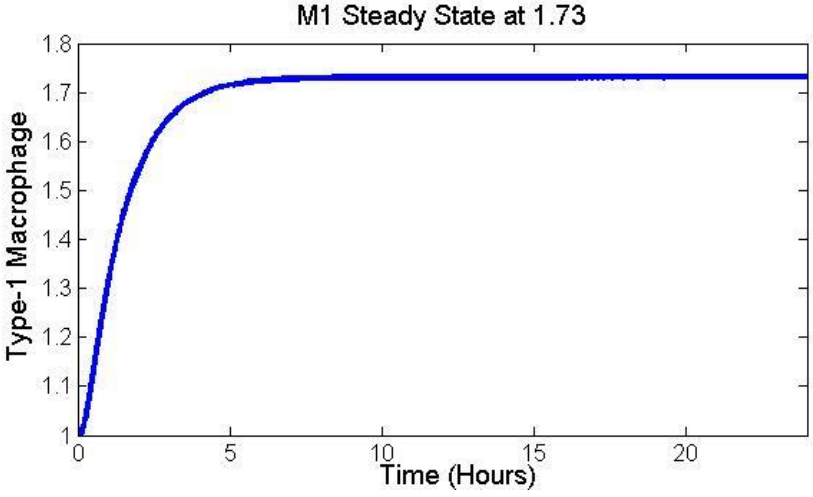
Table 6: Steady States for the 3-Variable Model in the Absence of Fatty Acids

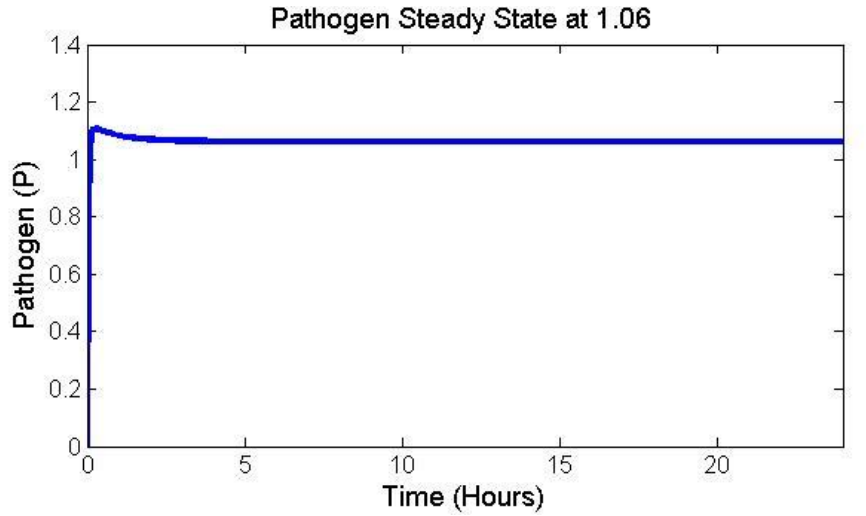
Values of ϕ	Eigenvalues	Stable Fixed Point (*m1, cp, p*)
Low: $0.01 < \phi < 0.5$	All negative real part	Stable, (1.73, 2.71, 1.06) at $\phi = 0.1$
Medium: $0.5 < \phi < 1.5$	All negative real part	Stable, (1.76, 3.25, 3.12) at $\phi = 1.2$
High: $1.5 < \phi < 2.0$	All negative real part	Stable, (1.88, 7.58, 22.57) at $\phi = 1.8$
$\phi \geq 2$	None	No fixed points

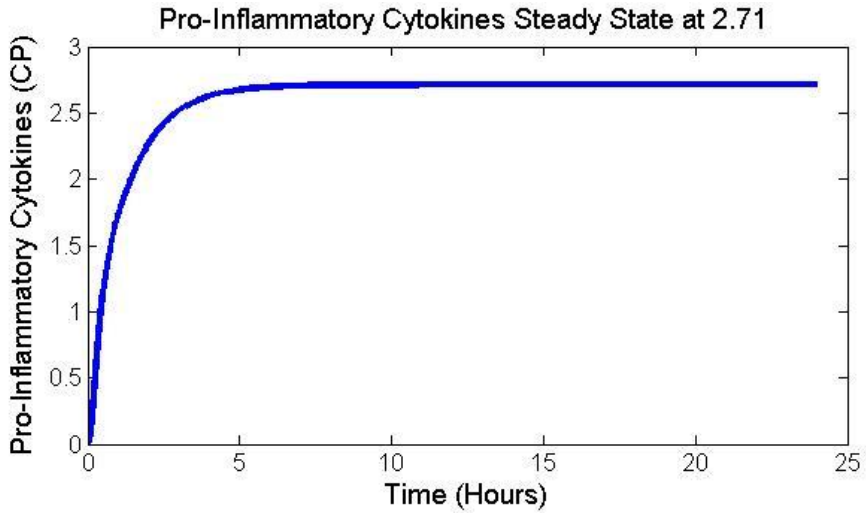
The general trend found in the analysis is that as ϕ increases, the steady state values for *m1*, *cp*, and *p* all also increase. However, as ϕ is increased towards a value of 2, *m1* maxes out near a value just less than 2, while *cp* and *p* will increase towards infinity. If $\phi \geq 2$, there is no equilibrium solution and *cp* and *p* will grow unbounded.

Simulations in XPP with parameter values $\phi = 0.1, \gamma = 1, \psi = 2, \alpha = 1, \delta = 10, \beta = 1, \kappa = 30$ and initial conditions $(m1, cp, p) = (1, 0, 0)$ will result in the fixed point (1.73, 2.71, 1.06) found for $\phi = 0.1$ in Table 6. A time scale of hours is used in each simulation. Thus, the fixed point for $\phi = 0.1$is reached within 12 hours. Pathogen levels reach the steady state value of 1.06 within 1 hour in the simulation below.

Figure 6: Steady States for the 3-Variable System in the Absence of Fatty Acids with $\phi = 0.1$ reaches steady state at (1.73, 2.71, 1.06).

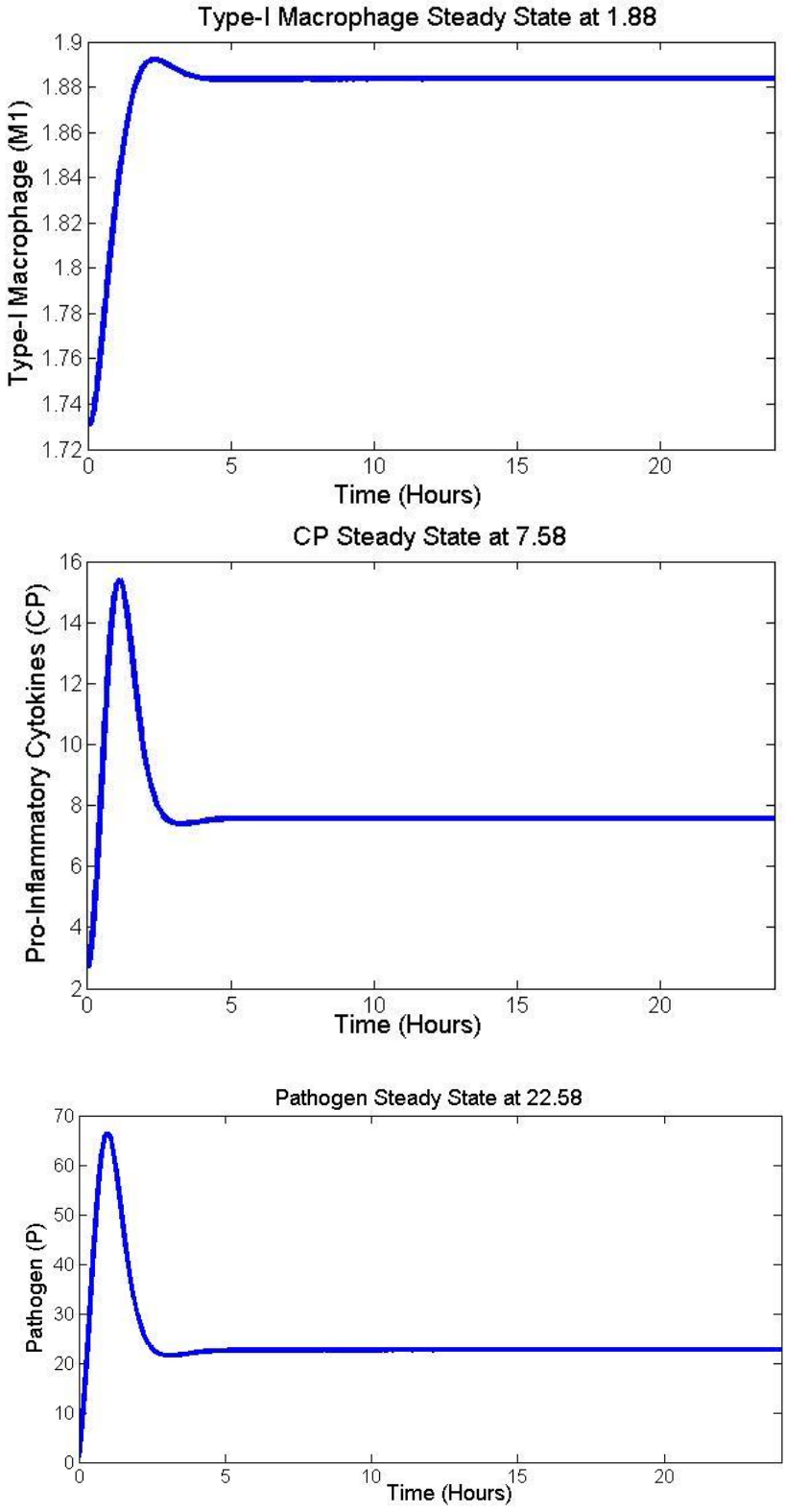






By increasing ϕ, the state of chronic inflammation also increases. The following simulation uses ϕ = 1.8, and uses an initial condition of the last fixed point $(m1, cp, p) = (1.73, 2.71, 1.06)$. The solution is now drawn towards the new fixed point of (1.88, 7.58, 22.57) shown in Table 6. Notice the spike in pathogens and inflammatory cytokines that is managed by the type-I macrophage, but does not return to previous lower fixed points. This is to be expected from such an aggressively growing and resistant strain of pathogen given with ϕ = 1.8. The fixed point contains higher steady state values of type-I macrophage, pro-inflammatory cytokine and pathogen.

Figure 7: Steady States for the 3-Variable System in the Absence of Fatty Acids with $\phi = 1.8$ reaches steady state at (1.88, 7.58, 22.57).

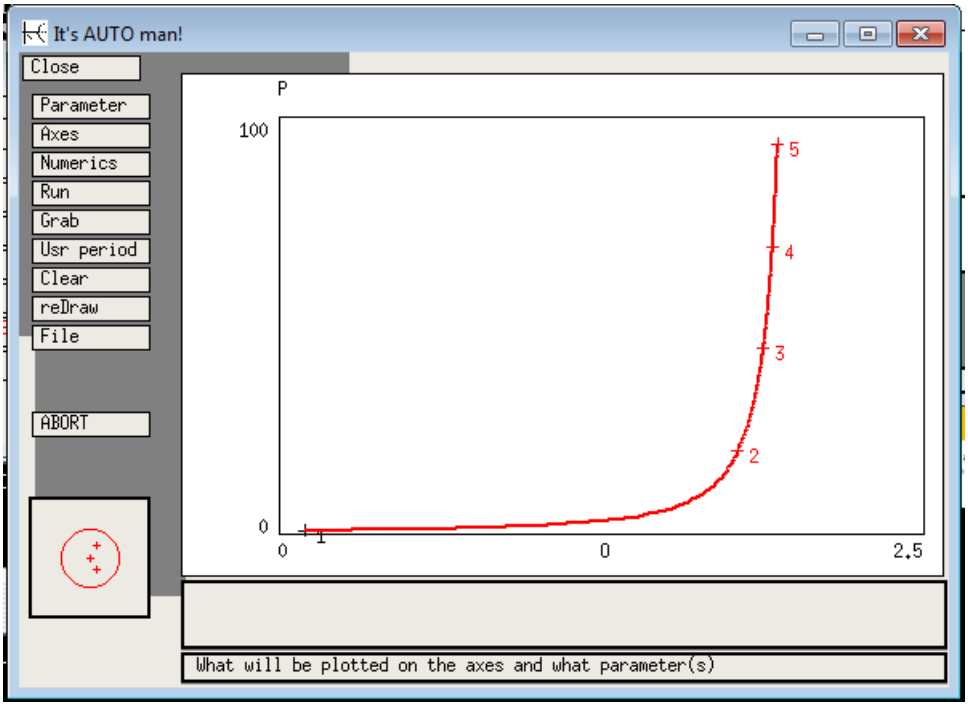


Simulations run with the damage function $fd = if\,(M1 > c)then(dam * M1)else(0)$, where $dam = \gamma$ will result in the same fixed points found above, given an initial condition for *m1* > c. This leads us to the conclusion that the model has different states of chronic inflammation. We can define these as the "Health" state, which is characterized by low type-I macrophage, low pathogen and inflammatory cytokine and occurs with a low damage / γ value; a "Low Chronic" state which is characterized by low but persistent levels of type-I macrophage which causes damage and thereby supporting a positive pathogen population; and a "High Chronic" which is characterized by higher levels of type-I macrophage, and very high levels of inflammatory cytokine and pathogen. Figure 6 above shows a "Low Chronic" state, and Figure 7 demonstrates a "High Chronic." Model health states that occur in the presence and absence of fatty acids are further explained in Chapter 6, Results.

BIFURCATION ANALYSIS

A bifurcation is any major qualitative change in the dynamical behavior of a system in response to a parameter value. The figure below shows how *p* increases in response to increasing values of ϕ.

Figure 8: Bifurcation diagram - *p* increases in response to increases in ϕ.



In Herald's (2009) paper, a bacterial state only occurs where the bacterial population can withstand the immune response ($\phi > 1$). However, in this study the damage / leakage term will always produce a state with some level of pathogen present with $\gamma > 0$.

Biologically, the change in fixed points in response to ϕ represent the low and high chronic states of chronic inflammation mentioned earlier. In the "low" state, $\phi < 0.5$ and the immune response is able to manage the pathogen in the system and minimize damage that occurs from higher levels of *m1*. The "low" state is shown by fixed point #1 in Figure 8. In the "high" inflamed state for $\phi > 1.5$, the immune system has much more *cp* circulating, producing more *m1*, resulting in damage and pathogens leaking in. This is represented by the region of fixed points 2 – 5 in Figure 8. A bifurcation diagram of type-I macrophage *m1* responding to increasing values of ϕ has very similar dynamics in which low and high chronic states are represented.

3-Variable Reduced Model in the Absence of Damage

Next, it is important to analyze the system when the damage term, γ, is small. If γ=0, this would effectively reduce the three-equation system to the one studied by Herald (2009). Four equilibrium states were found in Herald's (2009) analysis: One healthy state, two inflamed states in the absence of pathogens (one high, one low state), and one chronically infected state. Whenever $\gamma > 0$, though, there will always be some pathogen present, and the only likely states are the high and low chronic state found earlier. Therefore, a function was used to study the effect of γ in simulations. Ultimately, we are most interested in how the addition of fatty acids, and modifying the anti-inflammatory response of the system, will minimize the damage caused by *m1* cells and the resulting pathogens that leak in.

There are some interesting changes in stability when γ=0. Since *p* is zero at steady state, varying ϕ no longer has any effect on the fixed points. However, given the set of parameters $\phi = 0.1, \gamma = 0, \psi = 2, \alpha = 1, \delta = 10, \beta = 1, \kappa = 30$, there are 2 fixed points. The fixed point (1, 0, 0) is unstable, and the fixed point (1.71, 2.41, 0) is stable. The system produces a third unstable fixed point at (0.31,-0.41,0), but this is not biologically relevant.

5-Variable Model Steady States

5-Variable Model Steady States in the Absence of Fatty Acids

Next, the entire five-equation non-dimensional system was analyzed, with the fatty acid input set to zero. This models a diet low or absent in fatty acids:

$$\frac{dm1}{d\tau} = 1 + \alpha \frac{cp}{(1 + \beta * cp)} - m1$$

$$\frac{dcp}{d\tau} = p*m1 + \delta*cp\left[\frac{\psi*m1}{(1+cp)(1+ca)} - 1\right]$$

$$\frac{dma}{d\tau} = 1 + \alpha 2\frac{(ca)}{(1+\beta 2*ca)} - ma$$

$$\frac{dca}{d\tau} = \xi*cp*ma - \delta*ca$$

$$\frac{dp}{d\tau} = \kappa*\left[p*(\phi - m1) + \gamma*(m1 - c*ma)\right]$$

The following parameter values were chosen to study the states that occur when varying ϕ: $\gamma = 1, \psi = 2, \alpha = 1, \delta = 10, \beta = 1, \kappa = 30, \beta 2 = 1, \alpha 2 = 0.75, \xi = 1.2, c = 0.8.$ The first 6 parameters are the same as those used in the previous analysis. The table below shows the new five-equation system dynamics. The "Low" state can be described as a mild flare up in a Crohn's patient, while the "High" state would refer to a major flare up that may require hospitalization for a Crohn's patient.

Table 7: Steady States for the 5-Variable Model in the Absence of Fatty Acids

Values of ϕ	**Eigenvalues**	**Stable point (M1,MA,CP,CA,P)**
Low: $0 < \phi < 0.5$	All negative real part	Stable, (1.64, 1.15,1.76, 0.24, 0.47) at ϕ = 0.1
Medium: $0.5 < \phi < 1.5$	All negative real part	Stable, (1.67, 1.16, 2, 0.28, 1.58) at ϕ = 1.2
High: $1.5 < \phi < 2.0$	All negative real part	Stable, (1.84, 1.34, 5.23, 0.84, 19.34) at ϕ = 1.8
$\phi \geq 2$	None	No fixed points

Note that in the absence of fatty acids, the anti-inflammatory cytokine population is much less than the pro-inflammatory cytokine population.

5-VARIABLE MODEL STEADY STATES IN THE PRESENCE OF FATTY ACIDS

Adding fatty acids to the system ($f_{ab} = 1, f_{ut} = 0.8$) produces the following changes:

Table 8: Steady States for the 5-Variable Model in the Presence of Fatty Acids

Values of ϕ	**Eigenvalues**	**Stability (M1,MA,CP,CA,P)**
Low: ϕ = 0.1	2 Fixed Points: Negative and positive; all negative	Unstable, (1.05, 1.3, 0.05, 0.01, 0); Stable, (1.18, 1.3, 0.23, 0.04, 0.13)
Medium: ϕ = 1.2	2 Fixed Points: Negative and positive; all negative	Unstable, (1.04, 1.3, 0.04, 0, 0); Stable, (1.44, 1.33, 0.79, 0.13, 1.57)
High: ϕ = 1.8	2 Fixed Points: Negative and positive; all negative	Unstable, (1.04, 1.3, 0.04, 0.01, 0); Stable, (1.83, 1.43, 4.91, 0.84, 22.29)
$\phi \geq 2$	Negative and positive	Unstable, (1.04, 1.3, 0.04, 0.01, 0) at ϕ = 2

Notice that there is a significant suppressed immune response (lower *m1*, *cp* values) and decreased pathogen population when fatty acids are added to the system at the lowest value of ϕ. As ϕ increases, however, any protective effect fatty acids may provide the system from pathogens diminishes and total pathogen population rises slightly. Since ϕ represents the pathogen growth rate and resistance to the immune response, this is to be expected. Low levels of ϕ represent a non-aggressive pathogen or antigen that has leaked into the system, possibly of the variety normally found in the gut. The fatty acids help to protect the system by suppressing a chronic inflammatory response. If an aggressive variety of pathogen leaked in, the suppressive effect of the fatty acids could be counter-productive. However, the presence of fatty acids in the system does help the model manage values of $\phi \geq 2$. The model will not blow up as it would for $\phi \geq 2$ and no fatty acids included in the system. This analysis indicates that fatty acids would be

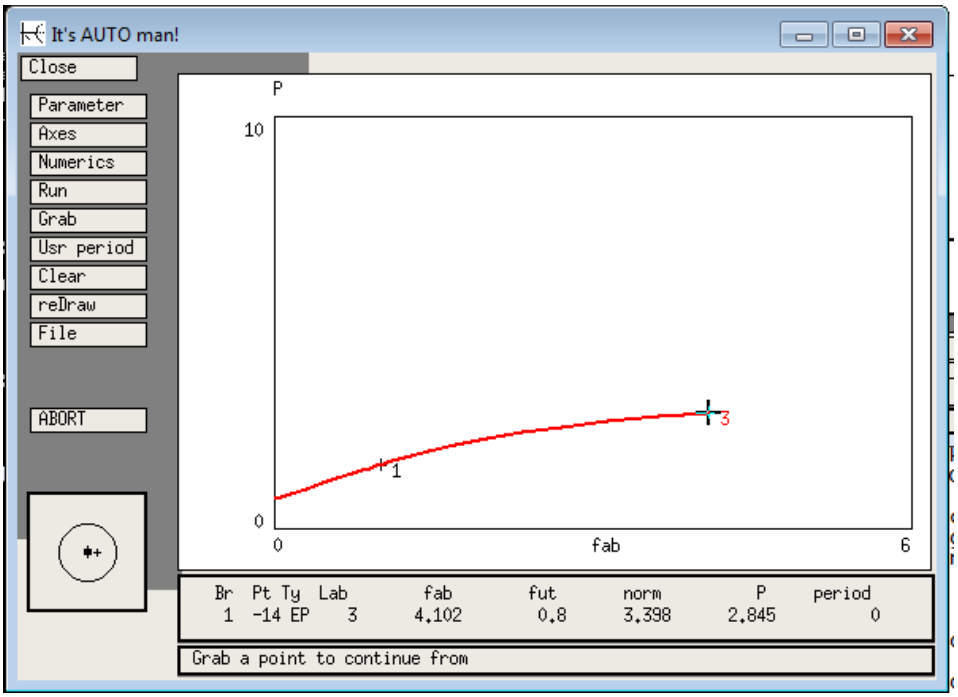
most beneficial to a Crohn's patient if added regularly to the diet before an inflammatory episode, or during a low-chronic state. If a patient were to add fatty acids during a high chronic inflammation state, it could be more detrimental than helpful. These scenarios will be further reviewed in Chapter 6: Results.

BIFURCATION ANALYSIS

The 5-variable model with fatty acids included shows the most interesting dynamics. Based on the results shown in Table 8, a bifurcation occurs with the addition of ($f_{ab} = 1, f_{ut} = 0.8$). The addition of the fatty acid produces a change in both number of fixed points and stability at different values of ϕ. The unstable fixed points have a much lower population of inflammatory markers and pathogen.

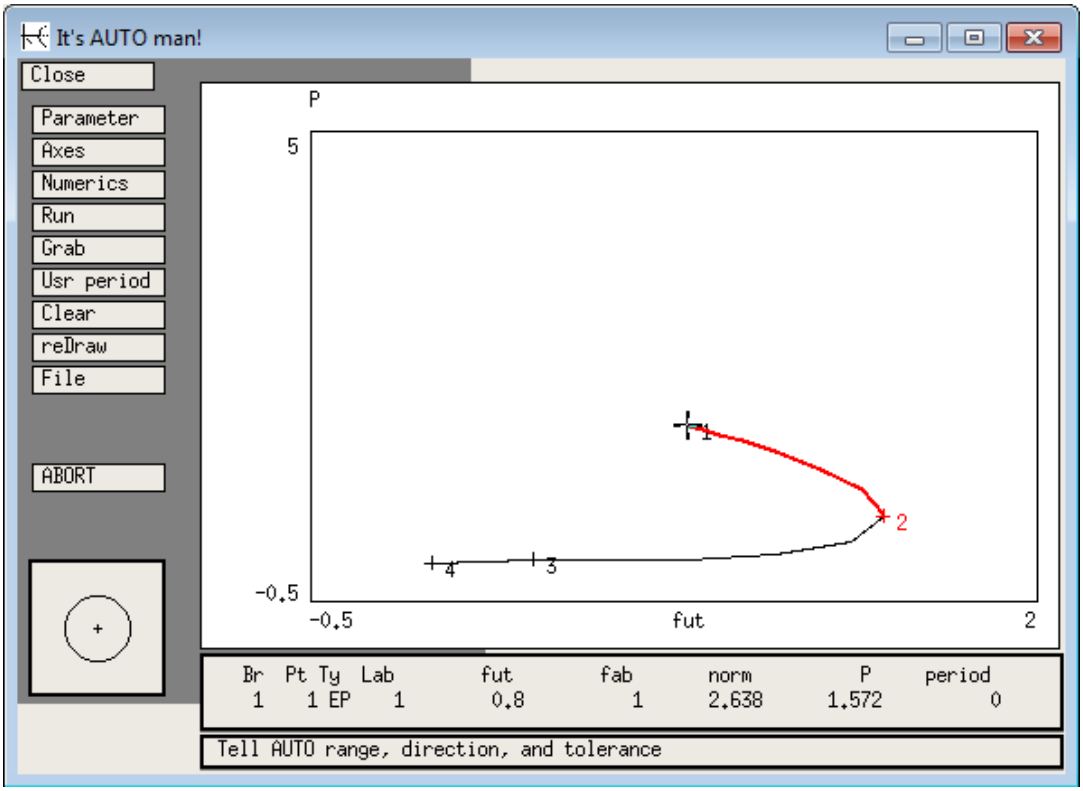
The bifurcation diagrams below start from the fixed point at (1.44, 1.33, 0.79, 0.13, 1.57) with ϕ = 1.2 and fatty acids included. The following shows *p* population changing with parameter *fab* (fatty acid absorption). Based on the figure, the value of *fab* = 1 gives a stable steady state with the best possible (low) value of pathogen. Earlier analysis has shown that in the absence of fatty acids ($f_{ab} = 0, f_{ut} = 0$) a higher inflamed state will occur.

Figure 9: Bifurcation diagram - *p* increases in response to increase in *fab*.



Although an increase in fatty acid absorption parameter does not help to decrease the pathogen population, it appears that the fatty acid utilization parameter (*fut*) will. Figure 10 below shows a saddle node bifurcation of *p* with changing *fut*. Increasing *fut* to a value of 1.473 will result in a lower total pathogen population of 0.51 (point #2 in the diagram.) Points #3 and #4 are not biologically relevant since they have slightly negative values of *p*. For values of *fut* less than 1.473, stable fixed points occur for $p > 0$ and unstable fixed points occur where $p < 0$. There are no fixed points for values of $fut > 1.473$.

Figure 10: Bifurcation diagram – *p* decreases in response to increase in *fut*.



CHAPTER 6: RESULTS

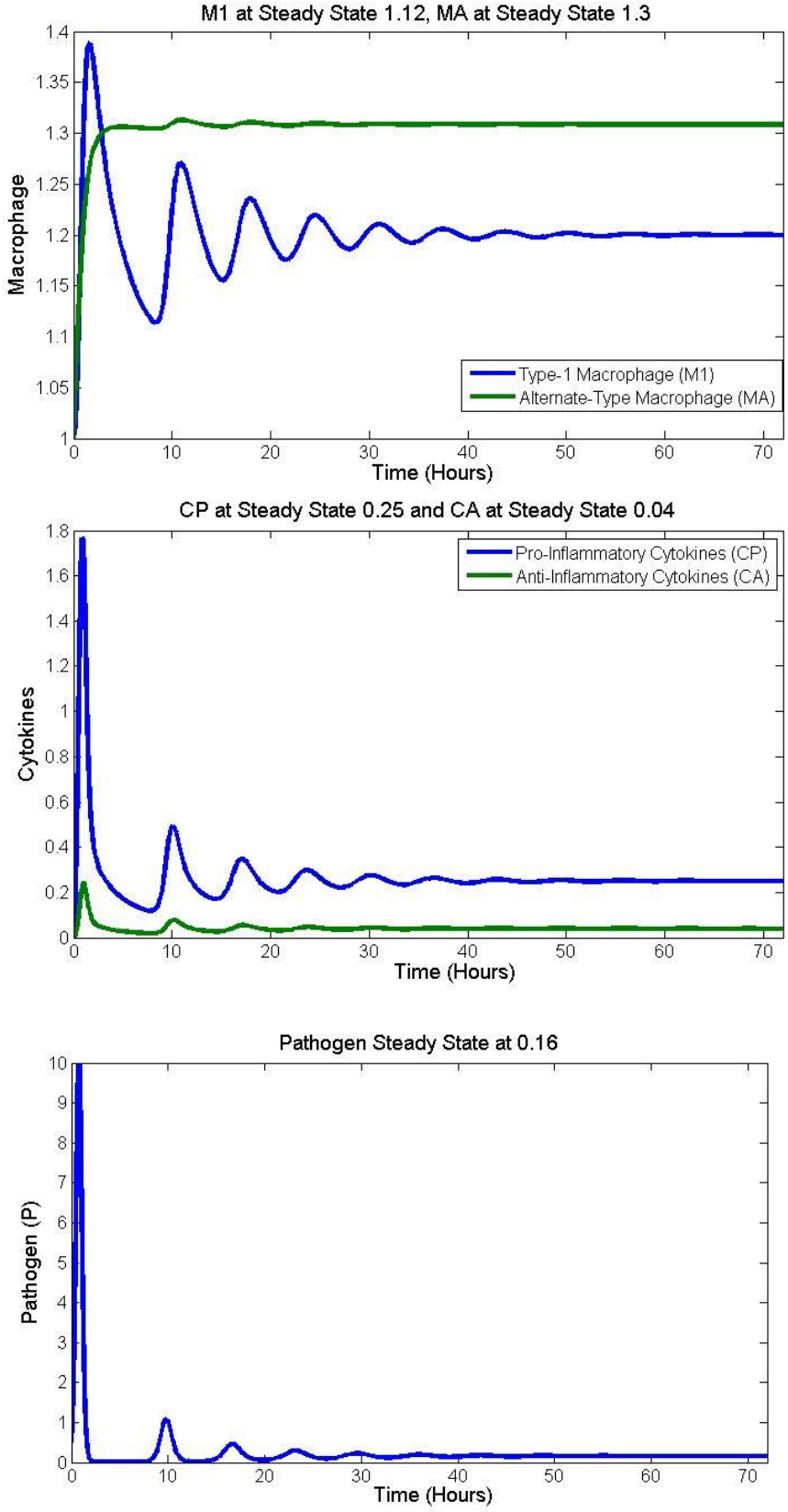
THE NORMAL HEALTH STATE AND THE LOW CHRONIC STATE

The normal Health State is characterized by a resolved infection; pathogens are driven to zero or near zero; anti-inflammatory markers *m1* and *cp* have reduced levels that do not cause damage. The Health State can be achieved when the damage parameter $\gamma = 0$. This will occur when type-I macrophages are at a normal threshold that does not cause damage.

PATHOGENS ARE INTRODUCED IN A HIGH FATTY ACID DIET

The following simulations show an infection returning to the Health State (*m1, ma, cp, ca, p*) = (1.12, 1.3, 0.25, 0.04, 0.16) with initial conditions (*m1, ma, cp, ca, p*) = (1, 1, 0, 0, 0.5). Fatty acids have been included in this model. The oscillations seen are due to *m1's* response to the spike in *p*, and the fatty acid / anti-inflammatory effect dampening this response. These two forces oscillation back and forth until an equilibrium is reached. The pathogens are initially seen to spike in population, but within 72 hours (3 days) the infection has been cleared, and inflammatory cells return to normal levels. The threshold at which damage will occur has been set to *m1* = 1.4, using the damage function *fd* described in earlier sections.

Figure 11: Pathogens introduced in a high fatty acid diet

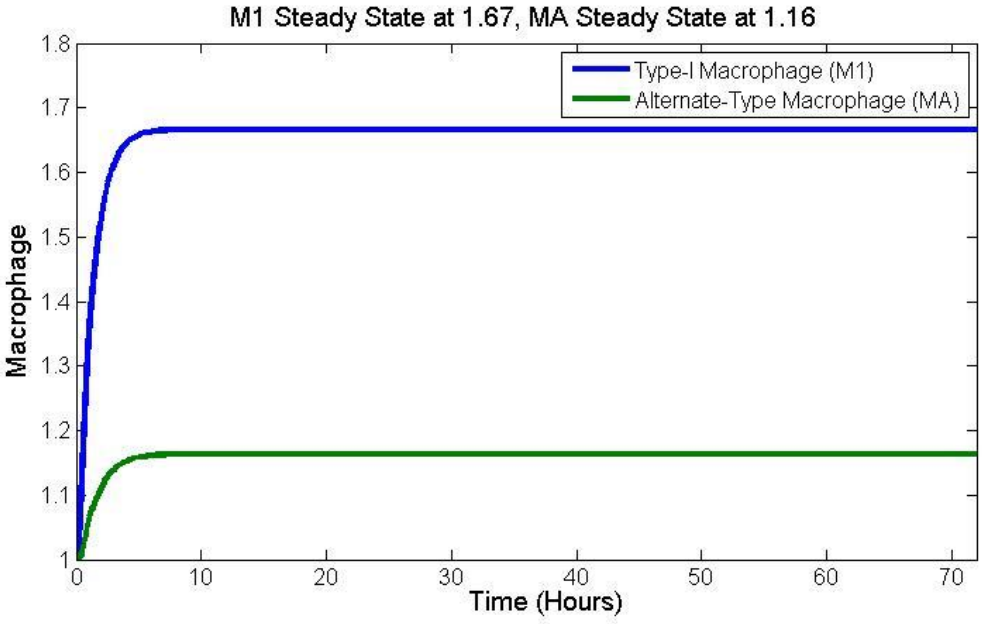


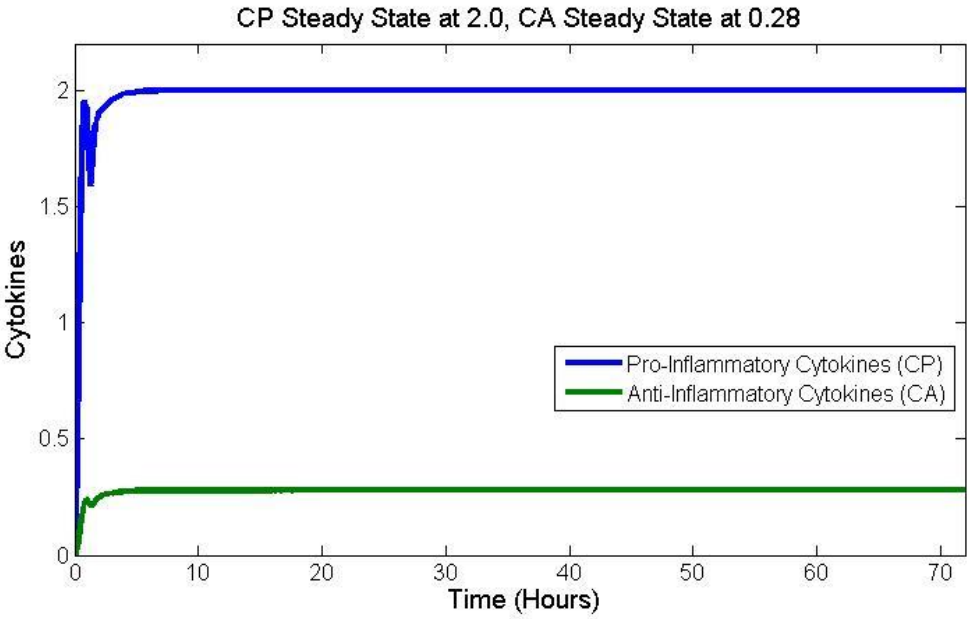
Type I macrophages and combined alternate type macrophages will return to steady-state background levels of 1. The background levels allow the body to quickly mount an immune response if pathogens are introduced to the system. Cytokines return to a near - zero steady state, since epithelial cells, mast cells, and macrophages are not reacting to a pathogen stimuli. The presence of fatty acids in the system encourages a higher alternate macrophage steady state.

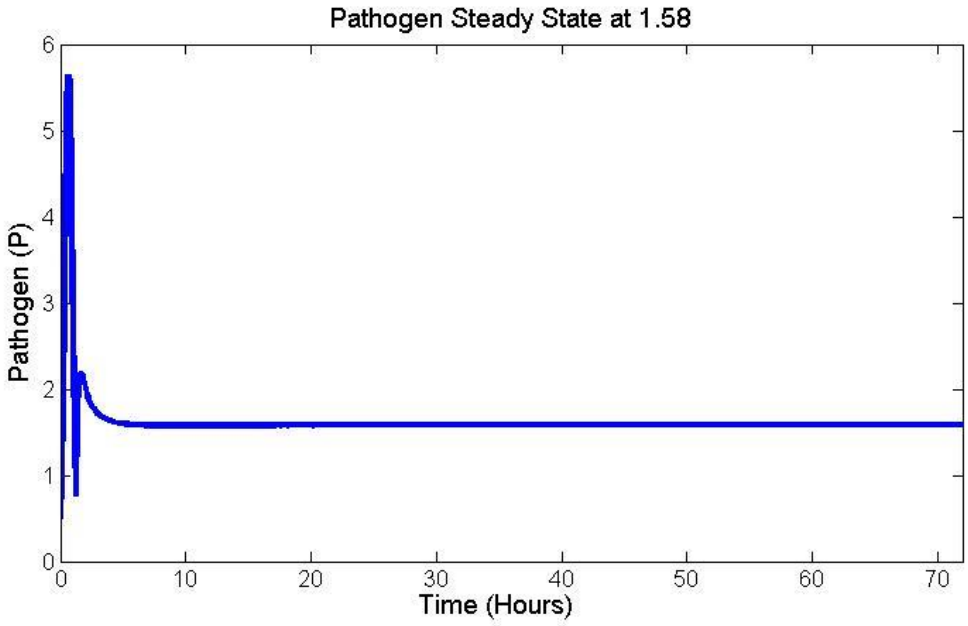
PATHOGENS ARE INTRODUCED IN A LOW FATTY ACID DIET

A low, or no fatty acid diet will produce a higher type-I macrophage steady state. The same simulation is run with fatty acids set to zero. The higher type-I macrophage steady state triggers damage and increases pathogen population. Within 24 hours, this has resulted in a low chronic state with steady state (*m1*, *ma*, *cp*, *ca*, *p*) = (1.67, 1.16, 2, 0.28, 1.58.)

Figure 12: Pathogens introduced in a low fatty acid diet

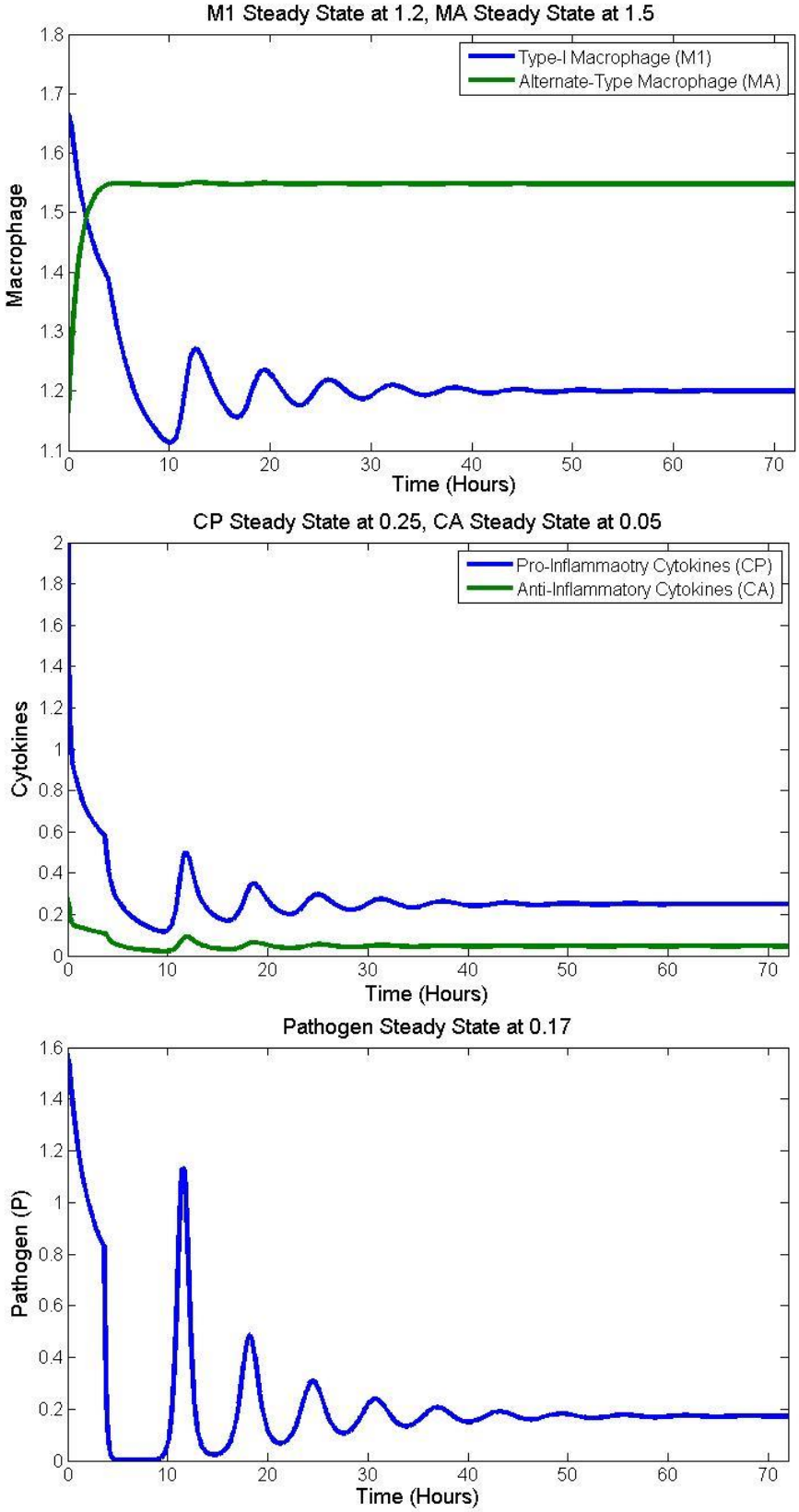






Re-starting the simulation with the ending chronic infection state as the new initial conditions but now with fatty acids added back in was able to force the steady states back to normal levels. Note: A higher value of *fut* was required ($fab = 1,\ fut = 1.45$) than if fatty acid was included initially at ($fab = 1,\ fut = 0.8$).

Figure 13: Introducing fatty acids during a low-chronic state



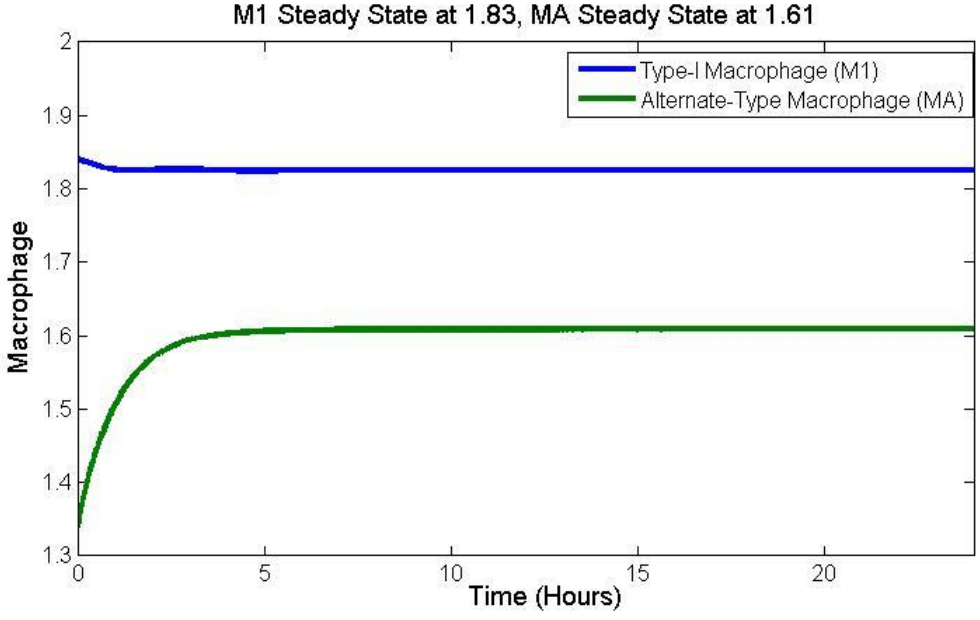
THE HIGH CHRONIC STATE

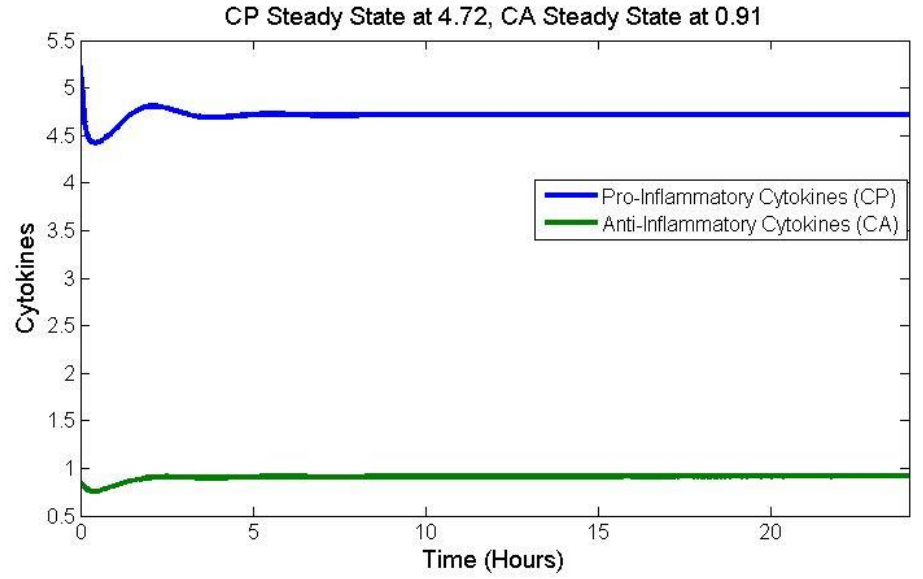
EFFECTS OF A ADDING A HIGH FATTY ACID DIET

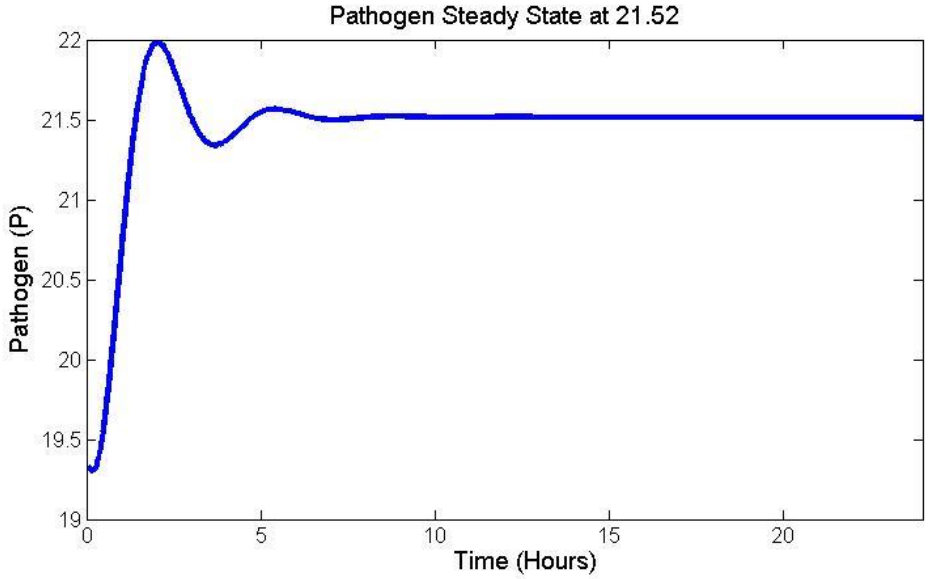
The simulations above show the health state, and a low chronic state that occurs in the absence of fatty acids in the diet. The next set of simulations starts with a high chronic state and introduces the same fatty acid diet combo ($fab = 1, fut = 1.45$) that was able to resolve the low chronic state. The high chronic steady state was found with fatty acid set to zero, and then added again for the initial conditions: (*m1, ma, cp, ca, p*) = (1.84, 1.34, 5.23, 0.84, 19.34).

Although the addition of fatty acids does lower the type-I macrophage population, pathogen levels do not respond as before. In this case, adding fatty acids at such a high chronic state would be more detrimental to a patient than beneficial because it dampens the inflammatory process which allows the infection to gain an even stronger foothold.

Figure 14: Introducing fatty acids during a high-chronic state







These results indicate that 1) fatty acids regularly added to the diet will help protect an individual prone to intestinal inflammation from entering into a low chronic state and stay within a health state; and 2) fatty acids added regularly to the diet of an individual already within a low chronic state return to a health state. Finally, adding fatty acids to the diet of an individual already in a high chronic state is not advised. Adding fatty acids could be detrimental to the immune response fighting a high pathogen population. If antibiotics or medications were to lower the

high chronic state to a lower chronic state, then fatty acids could safely be added to bring the individual back to a health state.

CHAPTER 7: CONCLUSIONS

Type-1 macrophages are activated from undifferentiated macrophages by TNF and also secrete TNF (Mosser, 2003.) The punicic acid murine trial performed by Bassaganya-Riera (2011) shows that when mice are fed a diet high in punicic acid, values for TNF are significantly different than levels found in control mice, and monocyte chemoattractant protein shows diminished values compared to control, although not found to be statistically significant. Thus, in modeling the effects of a specific fatty acid diet fed to patients of IBD or Crohn's, we would expect to see a lower recruitment of macrophages, a lower level of type-1 macrophages, and lower levels of pro-inflammatory cytokines produced by the type-1 macrophages, since all these processes are repressed by the activated PPAR receptor in the presence of appropriate fatty acid ligands. Further, as shown in the murine study by Bassaganya-Riera, the diminished levels of type-I macrophages leads to a lowered incidence of sores and intestinal damage. Therefore, although the total type-I macrophage population is suppressed and leads to a slower kill rate of pathogens, the resulting decrease or lower incidence of intestinal tissue damage ensures that fewer pathogens will infiltrate damaged tissues from the intestinal lumen. Also, chronic inflammation is unlikely to occur in this scenario as pathogens are slowly but methodically removed and the inflammatory response is controlled.

Many models of inflammation have been presented in the literature, but few have included the anti-inflammatory effects of alternate-type macrophages and the dynamics that control their levels in the system. Several studies have documented the anti-inflammatory effects of PPAR-gamma on Monocytes and macrophages (Ricote et al., 1998), (Jiang et. al., 1998) and found significant amounts of the PPAR-gamma receptor on Neutrophils, T lymphocytes, B

lymphocytes, NK cells, Dendritic cells, Eosinophils and mast cells (Sugiyama et al., 2000), (Szeles et al., 2007). It may be that anti-inflammatory cytokine / ligand population dynamics are just as important to the system (and health of the individual) as the pro-inflammatory cytokines (which perpetuate chronic inflammation.)

The 5-variable model developed in this study included the dynamics of fatty acids on PPAR receptors of inflammatory cells and was able to simulate the results described in the studies by Bassaganya-Riera and Ricote. Simulations demonstrated that fatty acids included regularly in the diet will help protect an individual from entering into a chronically inflamed state, and will help an individual already in a low chronically inflamed state return to a health state. However, results indicated that fatty acids should not be added to the diet of an individual already in a high chronic inflamed state, as this would be detrimental to the immune response in place.

CHAPTER 8: OPEN QUESTIONS AND DISCUSSION

Each model gives a part of the puzzle, but none give a full picture. In the context of studying the anti-inflammatory effects of PPAR-gamma, it may be prudent to track ongoing undifferentiated and differentiated macrophage populations since studies have documented the anti-inflammatory effects of PPAR-gamma on monocytes and macrophages (Ricote et al., 1998), (Jiang et. al., 1998) and found significant amounts of the PPAR-gamma receptor on neutrophils, T lymphocytes, B lymphocytes, NK cells, dendritic cells, eosinophils and mast cells (Sugiyama et al., 2000), (Szeles et al., 2007).

In light of the fact that none of the models presented earlier specifically make any allocation for the dynamics of the PPAR-gamma receptor in the equations for mast, monocyte, macrophage cells, it would be interesting to include this dynamic, most likely at the point of differentiation (and population growth) of these cells, and see if the results found by each model would be the same. If the PPAR-gamma receptor activity is tracked, then an anti-inflammatory cytokine and possibly a separate variable for up-regulating ligands specific to PPAR-gamma should also be tracked. It may be that anti-inflammatory cytokine / ligand population dynamics will be found to be more important to the system (and health of the individual) then the pro-inflammatory cytokines (which perpetuate chronic inflammation.)

List of References

List of References

Barnes, 2009. The cytokine Network in acute Obstructive Pulmonary Disease. American Journal Respiratory Cell and Molecular Biology, 41(6): 631-638.

Bassaganya-Riera, J., Hontecillas, R., 2010. Dietary CLA and n-3 PUFA in inflammatory bowel disease. Current Opinions Nutritional and Metabolic Care, 13: 569-573.

Bassaganya-Riera, J., DiGuardo, M., et al., 2011. Activation of PPAR-gamma and delta by dietary punicic acid ameliorates intestinal inflammation in mice. British Journal of Nutrition, 106: 878-886.

Cabre, E., Domenech, E., 2012. Impact of environmental and dietary factors on the course of inflammatory bowel disease. World Journal of Gastroenterology 18 (29), 3814-3822.

Cosnes, J., et al., 2011. Epidemiology and Natural History of Inflammatory Bowel Diseases. Gastroenterology, 140(6) , 1785–1794.

Cush JJ. Baylor Research Institute, pilot study on tart cherry and osteoarthritis of the knees, 2007.

Crohn's and Colitis Foundation of America (CCFA), 2010. Understanding IBD medications and side effects. www.ccfa.org

Deeb, S.S., Fajas, L., Nemoto, M. et al., 1998. A Pro12Ala substitution in PPAR-gamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. Nat Genet, 20: 284-287.

Drake, V.J., 2007. Two Faces of Inflammation. Linus Pauling Research Newsletter, Oregon State University, Spring/Summer 2007.

Hansen TS., Jess T., Vind I., Elkjaer M., Nielson MF., Gamborg M., Munkholm P., 2011. Environmental factors in inflammatory bowel disease: a case-control study based on a Danish inception cohort. J Crohns Colitis 5, 577-584.

Herald, M.C., 2009. General Model of Inflammation. Bulletin of Mathematical Biology, 72: 765-779.

Herrinton LJ., Liu L., Lafata JE., Allison JE., Andrade SE., Korner EJ., Chan KA., Platt R., Hiatt D., O'Connor S., 2007. Estimation of the period prevalence of inflammatory bowel disease among nine health plans using computerized diagnoses and outpatient pharmacy dispensings. Inflammatory Bowel Disease, 13 (4): 451-61.

Hogan Lab, Groschwitz KR, Ahrens R, et al, 2009. Mast cells regulate homeostatic intestinal epithelial migration and barrier function by a chymase/Mcpt4-dependent mechanism. *Proc Natl Acad Sci U S A* 106(52): 22381-22386.

Hou, JK., Abraham B., El-Serag H., 2011. Dietary Intake and risk of developing inflammatory bowel disease: a systematic review of the literature. Am J Gastroenterology 106, 563-573.

Jiang, C., Ting AT., Seed B., 1998. PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. Nature, 391(6662): 82-86.

Jiang, J., Gajalakshmi V., Wang, J., et al., 2005. Influence of the C161T but not Pro12Ala polymorphism in the peroxisome proliferator activated receptor –gamma on colorectal cancer in an Indian population. Cancer Science, 96:507-512.

Kaiser, Gary E., 2011. Biology 230: Microbiology E-Text Lectures. Ch. 4-5.

Linder, T., Melby, A., 2012. Conjoint 401-403 Lecture Notes, Acute Inflammation. University of Washington: http://courses.washington.edu/conj/.

Mathison, R. et al., 2010. Salivary gland derived peptides as a new class of anti-inflammatory agents: review of preclinical pharmacology of C-terminal peptides of SMR1 protein. Journal of Inflammation, **7**:49.

Mosser, David M., 2002. The many faces of macrophage activation. Journal of Leukocyte Biology, 73(2): 209-212.

Nordqvist, C., 2012. "What Is Inflammation? What Causes Inflammation?." *Medical News Today*. MediLexicon, Intl., 31 Jul. 2012. http://www.medicalnewstoday.com/articles/248423.php.

Reynolds A., Rubin J., Clermont G., Day, J., Vodovotz, Y., Ermentrout, G.B., 2006. A reduced mathematical model of the acute inflammatory response: I. Derivation of model and analysis of anti-inflammation. J. Theoretical Biology, 242, 220-236.

Ricote, M., Li AC., Willson, TM., Kelly CJ., Glass CK., 1998. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. Nature, 391(6662): 79-82.

Rubin, E., Farber, J., 1995. Essential Pathology 2nd Edition.

Sheppach W., Luehrs H., Melcher R., Gostner A., Schauber J., Kudlich T., Weiler F., Menzel T., 2004. Anti-inflammatory and anti-caricinogenic effects of dietary fibre. Clin Nutr Suppl, 1 (2), 51-58.

Shrestha, U.K., Karimi, O., Crusius, J.B., Zhou, F., Wang, Z., Chen, Z., Bodegraven, A., Xiao, J., Morre, S., Wang, H., Li, J., Xia, B., 2009. Distribution of peroxisome-proliferator activated receptor-gamma polymorphisms in Chinese and Dutch patients with inflammatory bowel disease. Inflammatory Bowel Diseases, 16 (2): 312-319.

Sleigh, AE, Kuehl KS, Elliot DL ., 2012. Efficacy of tart cherry juice to reduce inflammation among patients with osteoarthritis. American College of Sports Medicine Annual Meeting. May 30, 2012.

Sohrabpour, et. al., 2010. Current Therapeutic Approaches in Inflammatory Bowel Disease. Current Pharmaceutical Design, 16, 3668-3683

Su, W., Bush, C.R., Necela, B.M., Calcagno, S.R., Murray, N.R., Fields, A.P., Thompson, E.A., 2007. Differential expression, distribution, and function of PPAR-gamma in the proximal and distal colon. Physiol Genomics, 30: 342-353.

Sugiyama, H., Nonaka, T., Kishimoto, T., Kormoriya, K., Tsuji, K., Nakahata, T., 2000. Peroxisome proliferator-activated receptors are expressed in human cultered mast cells: a possible role of these receptors in negative regulation of mast cell activation. European Journal of Immunology, 30(12): 3363-3370.

Swidsinski, A., Ladhoff, A., Pernthaler, A., Swidsinski, S., Loening-Baucke, V., Ortner, M., Weber, J., Hoffmann, U., Schreiber, S., Dietel, M., Lochs, H., 2002. Mucosal flora in the inflammatory bowel disease. Gastroenterology 122 (1), 44-54.

Szeles, L., Torocsik, D., Nagy, L., 2007. PPAR-gamma in immunity and inflammation: cell types and diseases. Biochimica et Biophysica Acta. 1771(8): 1014-1030.

Tilg,, H., Trehu,E., Atkins, MB., Dinarello, CA., Mier, JW., 2013. Interleukin-6 (IL-6) as an anti-inflammatory cytokine: induction of circulating IL-1 receptor antagonist and soluble tumor necrosis factor receptor p55. Blood 1994 83: 113-118.

Wendelsdorf, K., Bassaganya-Riera, J., Hontecillas, R., Eubank, S., 2010. Model of colonic inflammation: Immune modulatory mechanisms in inflammatory bowel disease. J. Theoretical Biology, 264, 1225-1239.

APPENDICES

XPP CODE for 3-Variable System:

```
par alpha=1
par Beta=1
par delta=10
par phi=2
par k=30
par O=0.1
par gamma=1

% par c=1
% par dam=0.1

dM1/dt=1+(alpha*CP/(1+Beta*CP))-M1
dCP/dt=P*M1+delta*CP*((phi*M1/(1+CP))-1)
dP/dt=k*(P*(O-M1)+gamma*m1)
% gamma=if(M1>c)then(dam)else(0)
% c corresponds to the percentage difference that will give damage.
@ total=48, dt=0.01, xhi=100, bound=100000
Done
```

XPP CODE for 5-Variable System:

```
par alpha=1
par alpha2=0.75
par Beta=1
par Beta2=1
par s2=1
par fut=0.8
par fab=1
par delta=10
par phi=2
par S=1.2
par k=30
par O=1.2
par c=0.8
par dam=1
par a=1.2


dM1/dt=1+(alpha*CP/(1+Beta*CP))-M1
dMA/dt=s2+(alpha2*(CA+fut)/(1+Beta2*CA+fab))-MA
dCP/dt=P*M1+delta*CP*((phi*M1/((1+CP)*(1+CA)*(1+fab)))-1)
dCA/dt=S*CP*MA-delta*ca
dP/dt=k*(P*(O-M1)+gamma*(m1-c*ma))
gamma=if(M1>a)then(dam)else(0)
% c corresponds to the percentage difference that will give damage.
@ total=30, dt=0.01, xhi=100, bound=100000
Done
```