ABSTRACT

WINDOLOSKI, KRISTEN ANN. Mathematical Modeling of Experimentally Induced Inflammation and Sepsis. (Under the direction of Mette S. Olufsen).

Inflammation is a highly prevalent attribute of both infectious and non-infectious medical conditions. While progress has been made in understanding how inflammation impacts these illnesses, there are still remaining mechanisms and connections that need to be uncovered and explored to develop effective treatments. Short-term inflammation can be replicated experimentally in an endotoxin challenge. During this, a transient immune response characterized by changes in immune mediators, body temperature, cardiovascular markers, hormone release, and pain perception is elicited from low-dose endotoxin given to healthy subjects. Prolonged inflammation, however, such as that seen in sepsis is challenging to measure and study, leaving gaps between experimental knowledge of inflammation and that which occurs during sepsis. To bridge this gap and gain further understanding of how multi-system mechanisms influence inflammation, this dissertation develops novel computational models integrated with experimental and clinical data that examine immune, cardiovascular, endocrine, and whole-body responses to both short-term and prolonged inflammation.

The significant accomplishments of this thesis include creating the first mathematical model of a continuous infusion of endotoxin and building the first mathematical model of immune-cardiovascular-hormonal-thermal-pain dynamics, both calibrated to experimental data. This research also identifies new multi-system connections. Furthermore, this work constructs and examines a new model of infection and sepsis, which can be connected downstream with the comprehensive multi-system model forming a novel mathematical model that better analyzes sepsis dynamics.

Analysis of the continuous infusion model suggests that the prolonged cytokine response seen in experimental data is due to an increase of anti-inflammatory cytokine production by immune cells and decrease in pro-inflammatory decay rates. Extensions to prolonged inflammation exhibit oscillating immune markers up to two days after 20-32 hours of continuous endotoxin administration.

The multi-system model reveals that the primary systemic impact of endotoxin lasts around 24 hours, but residual effects are present for up 10 days during repeated doses. Endotoxin administration timing is important for hormone release, where administration early or late in the day induces more pronounced and disrupted hormone rhythms. Increased endotoxin doses result in pronounced immune markers and body temperature, larger decreases in pain perception, blood pressure, and heart rate, and blunted hormone release and oscillations. Fur-
thermore, a continuous infusion shows similar immune marker, blood pressure, and hormone behavior, bringing the system on the verge of hypotension.

Finally, through parametric analysis, the mathematical model of infection mimics several clinical inflammation scenarios including an aseptic infection. It exhibits that an anti-inflammatory enhancer, such as vitamin C, switches the disease progression from a negative outcome to a healthy resolution depending on the timing and strength of administration. Coupling between the sepsis model and the multi-system model in the future will allow for examination of whole-body dynamics during sepsis, improved disease understanding, and evaluation of potential therapeutic treatments more directly.
Mathematical Modeling of Experimentally Induced Inflammation and Sepsis

by
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DEDICATION

To my Grampy, who lost his battle with sepsis.
Kristen Windoloski grew up in Easthampton, Massachusetts. Originally interested in working with young children, Kristen attended Fitchburg State University to pursue a degree in elementary education with a mathematics minor. However, the more math courses Kristen took, the more she became interested in the challenging subject. After a conversation with one of her math professors, she changed her major sophomore year and pursued her undergraduate degree in mathematics. The summer before her senior year, she attended a biostatistics program in Colorado. It was at that point that Kristen realized her passion for combining mathematics and biology, and she decided to apply for graduate school. She graduated from Fitchburg State University with top honors and a bachelors degree in mathematics and minors in social science and international studies. Over the summer, Kristen worked as a research assistant on a mathematical ecology project and then moved to North Carolina to pursue her Ph.D. in applied mathematics at North Carolina State University. Working under the advisement of Mette S. Olufsen, Kristen built her mathematical and physiological knowledge, and she also seized multiple professional development opportunities during her time in graduate school. Kristen will remain in Raleigh following the completion of her Ph.D.
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The induction of inflammation at a site in the human body, which can be physically characterized by swelling, redness, warmth, and pain [258], is a mechanism used to combat and resolve the invasion of pathogenic entities or damage from injury [174]. If present in healthy tissue or left unresolved, inflammation can cause the breakdown of tissue and damage to vital organs, potentially sending the body into a state of emergency [178]. Because of its harmful repercussions and prominence in many clinical applications such as autoimmune diseases (rheumatoid arthritis, systemic lupus erythematosus) [76, 268, 150, 166, 301], cardiovascular and gastrointestinal diseases (heart disease, inflammatory bowel disease) [104, 62, 219], and sepsis [77, 143, 178], inflammation has been the focus of scientific studies for decades. During that time, interactions between the immune system and others such as the cardiovascular, endocrine, and nervous systems were discovered [117, 237, 104, 34, 14], and these contributed to the complexity involved in grasping the mechanisms behind these clinical phenomena.

While progress has been made in understanding the role of inflammation in these conditions and their management and treatment, there are still gaps in fully comprehending the whole-body dynamics involved with inflammation. For example, sepsis is still not thoroughly understood as it lacks a universal biomarker and therapeutic target [126]. Sepsis is considered a leading cause of death worldwide [178] with an estimated 30 million people per year affected [16], a mortality rate of approximately 16-33% [74], and reports of over $20 billion in costs to
U.S. hospitals in 2011 [238]. Therefore, continued exploration of inflammation dynamics and its interactions with other systems in controlled and uncontrolled settings are needed to gain understanding of mechanisms that occur during inflammatory conditions.

One method to study the underlying mechanisms is mathematical modeling, which has been used to investigate immune [137, 66, 207] and multi-system [50, 10, 69] dynamics during inflammation. Such studies have explored responses to experimentally induced inflammation in humans and animals, simulated clinical inflammation scenarios, and suggested treatment regimes for inflammatory conditions. However, the transition from the typical experimental model of inflammation (a bolus administration of endotoxin to healthy subjects), which all inflammatory mathematical models calibrated to experimental data utilize, to clinical inflammation is a large leap. Bolus endotoxin administration elicits a short-term immune response while clinical inflammation is prolonged (over days, weeks, or even years) and sometimes enhanced. Thus, the development of a better translational mathematical model between experimental and clinical inflammation is needed to gain further insight into dynamics of clinical inflammation. Additionally, recent advances mapping the interactions of the immune system with other systems have identified pathways that have yet to be computationally implemented and explored. Thus, there is a gap in understanding the impact that these multi-system pathways have on the progression of inflammation. A comprehensive, multi-system model can then be combined with the translational immune model to explore the extensive dynamics during sepsis, using the model to predict the efficacy of potential therapeutic treatments.

In this thesis, we study the transition from short-term, experimentally controlled scenarios of inflammation to prolonged, clinical scenarios of inflammation. The primary goals are to:

- Construct and analyze a mathematical model of immune dynamics during a continuous infusion of endotoxin, a more representative model of prolonged inflammation than a bolus [130];
- Use the model to suggest potential mechanistic differences between a bolus and continuous infusion of endotoxin;
- Extrapolate the continuous infusion model to simulate long-term and enhanced inflammation;
- Formulate a mathematical model examining immune, cardiovascular, hormonal, thermal, and pain dynamics over hours and days;
- Create and analyze a mathematical model of sepsis with whole-body impact to study the efficacy of vitamin C treatment on sepsis patients.
Goals (i)-(iii) develop the first mathematical model of a continuous infusion of endotoxin calibrated to experimental data. This provides a novel model that can simulate prolonged inflammation similar to that in a clinical setting, which the traditional bolus model fails to do. Goal (iv) develops a new mathematical model investigating unexplored, multi-system impacts of inflammation not used in previous multi-system inflammatory models, and goal (v) lays the preliminary groundwork to construct an innovative, multi-system mathematical model of sepsis by translating the model in goal (i) to simulate an infection.

Results have been published in two manuscripts, [288] (Chapter 6) and [289] (not included in this thesis), one submitted manuscript (Chapter 5), and one article published in the SIAM News Blog [291] (not included in this thesis). Windoloski is the first author on all four. The author has one manuscript relating to sepsis dynamics (Chapter 7) in preparation.

1.1 Overview

This dissertation includes eight chapters, the first of which encompass the physiological background, modeling literature review and motivation, and mathematical background needed for the latter chapters. This is followed by two manuscripts (one submitted for review, one published) and one project with unpublished work. The outline of each of these chapters is as follows:

• **Chapter 2** discusses the physiological aspects of inflammation and the systems affected, including the immune system, cardiovascular system, hypothalamic-pituitary-adrenal (HPA) axis, and the interactions between these systems. The diagnosis, pathophysiology, and treatment of sepsis is also discussed.

• **Chapter 3** presents a review of mathematical models, specifically differential equations models, representing the physiological systems discussed in Chapter 2. A comparison of model elements and conclusions are discussed, and gaps where these models fall short are noted.

• **Chapter 4** reviews the mathematical and statistical background used in this thesis. Topics discussed include techniques of sensitivity analysis, subset selection, parameter estimation, uncertainty quantification, and statistical hypothesis testing.

• **Chapter 5** consists of the submitted manuscript “Characterization of differences in immune responses during bolus and continuous infusion endotoxin challenges using mathematical modeling” [290] by Windoloski, Janum, Berg, and Olufsen. The SIAM News
Blog article Windoloski and Olufsen [291] also discusses the motivation for this work. The manuscript presents the analysis of an immune response mathematical model calibrated to two sets of data driven by different endotoxin administration methods. Formulation and analysis of the resulting model and data are presented, and mean and subject-specific simulations are shown. The model is then used to simulate prolonged inflammation.

- **Chapter 6** consists of the published manuscript “A unified computational model of the human response to lipopolysaccharide-induced inflammation” [288] by Windoloski, Bangsgaard, Dobreva, Ottesen, and Olufsen. A short article about this work is also published in Windoloski et al. [289] (not included in this thesis). The study investigates immune-cardiovascular-hormonal-thermal-pain dynamics during an endotoxin challenge on multiple timescales and examines these dynamics during changes in endotoxin dosage, administration method, and administration timing.

- **Chapter 7** consists an unpublished study focused on the mathematical modeling of sepsis. Data from sepsis patients are included in the study. The structure of the model is analyzed through flow and parametric analysis. The impact vitamin C therapy on sepsis progression is also studied. Motivation for this study was also mentioned in the SIAM News Blog article [291].

- **Chapter 8** provides a discussion of the overall conclusions from the thesis as well as the direction of future work on this project.
This thesis focuses on increasing understanding of inflammation and its multi-system impacts during human experimental (via an endotoxin challenge) and septic scenarios. An endotoxin challenge administers a dose of endotoxin, an immunostimulant derived from the membrane of gram-negative bacteria [101], to healthy humans where it binds with toll-like receptors (TLR) such as TLR4 [180] to initiate a short-term response. An infection such as that during sepsis elicits a similar response [178], but it is long-lasting. Both initiations of the immune system provoke widespread reactions, including cardiovascular and hormonal changes.

This chapter describes the physiological background of the major components of the inflammatory response including the immune system, cardiovascular system, and hypothalamic-pituitary-adrenal (HPA) axis. Unless otherwise stated, the information in Section 2.1 (immune system) is taken from Murphy [174], Section 2.2 (cardiovascular system) is taken from Boron and Boulpaep [27], and section 2.3 (HPA axis) is taken from Guyton and Hall [95]. It is well-known in literature that the immune, cardiovascular, and endocrine systems are interconnected, so we discuss the physiological interactions between them in Section 2.4. Finally, we describe the diagnosis, pathophysiology, and care protocols for sepsis in Section 2.5.
2.1 Immune System

The immune system is the body’s line of defense protecting against foreign invaders such as bacteria and viruses, and it promotes the restoration of function and tissue during times of illness or injury. This defense and repair mechanism has two layers, which are referred to as the innate and the adaptive immune system (shown in Figure 2.1). Following a pathogen's penetration of multiple physical and chemical barriers in place to deter access to the body (green box in Figure 2.1), the innate immune system (purple box in Figure 2.1) initiates by activating various cells and chemicals. It responds to a threat in a matter of minutes to hours and its response can last up to several days, often capable of eliminating many pathogens on its own. There are, however, times when the innate immune system is not fully effective, and so it relies on the adaptive immune system (orange box in Figure 2.1) to complete the defense process. The adaptive immune response forms immunological memory with pathogens that it encounters, rids the system of the pathogen in a more effective manner, and evades severe disease if it appears again. As the body’s secondary immune response, the adaptive immune response takes longer to activate, typically hours or days, but it can provide lifelong protection [174].

Figure 2.1: A high level overview of immune response stages as a pathogen enters the body and activates both the innate (fast response) and adaptive (slow response) immune system.
2.1.1 Immune Cells

The elements of the immune response originate from the pluripotent hematopoietic stem cells shown in Figure 2.2, which are produced from bone marrow. The stem cells can differentiate into erythrocytes (red blood cells), platelets, and leukocytes (white blood cells), all of which circulate in the blood. Red blood cells deliver oxygen to tissues and organs via blood flow, platelets promote coagulation in the event of an injury, and leukocytes are the main cells guiding the immune system response. Leukocytes are divided into two categories, the common myeloid progenitor and the common lymphoid progenitor (third branch in Figure 2.2), which determine the type of cells to be formed. These entities can differentiate further to produce cells that migrate into tissues [174], such as macrophages and plasma cells also shown in Figure 2.2.

Figure 2.2: Immune cell differentiation from hematopoietic stem cells. The figure is used with permission from Gordon Betts et al. [90] under the Creative Commons Attribution license (CC BY). No changes were made to the original figure. The license can be found at https://creativecommons.org/licenses/by/4.0/.
Innate Immunity

The majority of cells that play a role in the innate immune response are generated from the myeloid lineage (left branch in Figure 2.2) and include polymorphonuclear leukocytes (granulocytes), monocytes, macrophages, mast cells, and dendritic cells. Aside from mast cells, all are considered phagocytes, which are cells that possess the ability to engulf a pathogen and break it down by releasing enzymes from the cell’s lysosome. This process, called phagocytosis, is one of the primary defenses of the innate immune system and results in the elimination of the pathogen [174].

One of the main myeloid-derived cells are granulocytes, which are derived from the myeloblast in Figure 2.2 that differentiates into either neutrophils, eosinophils, and basophils. Neutrophils are the most abundant type of white blood cell, and they are also the most important due to their phagocytic capabilities. The two other granulocytes, eosinophils and basophils, circulate in the blood in smaller amounts and are particularly useful because of their capability to eliminate larger pathogens such as parasites, which other immune cells are not able to ingest due to their smaller size [174].

Two additional phagocytic cells are monocytes and macrophages, which are derived from the monoblast in Figure 2.2. Macrophages are derived from monocytes, which circulate in the bloodstream and migrate into nearly all tissues. Once in the tissue, monocytes differentiate and mature into macrophages. While one of their main functions is phagocytosis, macrophages and monocytes in addition to neutrophils are also important inducers of inflammation, a key regulatory process for ensuring a successful and balanced immune response. Macrophages are subdivided into two types that influence the direction of inflammation during the immune response. The M1-type macrophage promotes inflammation, while the M2-type macrophage suppresses it [174].

The other two immune cells generated from the myeloid lineage are mast cells and dendritic cells (not shown in Figure 2.2). Mast cells mature in tissues and are involved in the protection of the body’s internal barrier and, like macrophages, monocytes, and neutrophils, they also promote inflammation. However, mast cells do not possess phagocytic abilities. Meanwhile, dendritic cells are phagocytes. While they engage in phagocytosis in their immature form, their primary function is to link the innate and adaptive immune responses. When an immature dendritic cell is activated by a pathogen and moves into tissue as a mature dendritic cell, it is able to present antigens from the pathogen on its structure. This action activates T lymphocyte (T cell) antigen receptors, and thus, initializes the adaptive immune response [174].
Adaptive Immunity

The antigen-specific cells known as T lymphocytes (T cells) and B lymphocytes (B cells) stem from the lymphoid lineage (right branch in Figure 2.2) and are synthesized by bone marrow. There are approximately one billion lymphocytes circulating in the human body. While B cells also mature in the bone marrow, T cells mature in the thymus, located just above the heart. Upon maturation, T and B cells circulate through the blood, lymph, and peripheral lymphoid organs. Inactivated lymphocytes remain in the lymphoid tissues until activated by an antigen, in which they then become effector lymphocytes [174].

The main function of T and B cells is to present antigen-specific receptors on their cell surfaces to bind with those specific antigens invading the system. Once a B cell antigen receptor is activated, B cells proliferate into plasma cells, as shown in Figure 2.2. Plasma cells generate antibodies, collectively called immunoglobulins (Ig), which are able to detect and neutralize antigens or send the antigen to macrophages to undergo phagocytosis. On the other hand, when the T cell antigen receptor is activated, T cells can differentiate into either cytotoxic T cells, helper T cells, or regulatory T cells. These daughter cells assist in the elimination of infected cells, send additional signals to help guide cell reproduction and responses, and ensure a balanced system response to antigen presence, respectively. B and T cells can also differentiate into memory cells (not shown in Figure 2.2), which are dormant cells that can transform into active lymphocytes once they encounter their specific antigen again. These cells are important in immunological memory and disease immunity [174].

2.1.2 Cytokines

The main mediators of the innate immune system are signaling proteins called cytokines, which are released from various immune cells specific to each cytokine (as shown in Figure 2.3) and dictate the actions of immune cells throughout the immune response. Cytokines are generally categorized as pro-inflammatory (stimulating inflammation) or anti-inflammatory (inhibiting inflammation), though there are cytokines and their interactions can vary depending on their location in the body and cellular origin [174].

Pro-Inflammatory Cytokines

One of the main early pro-inflammatory cytokines released during the immune response is tumor necrosis factor alpha, TNF-\(\alpha\), which is produced by many different cells including but not limited to macrophages, natural killer (NK) cells, T cells [174, 122], neutrophils, B cells, and monocytes [122] as shown in Figure 2.3. It is one of the main cytokines responsible for
the induction of fever during illness [43, 75] and the typical symptoms of inflammation at
the site of infection or injury such as swelling, redness, warmth, and pain [258]. Excessive
levels of plasma TNF-α have been shown to be present in patients with conditions such as
sepsis [98, 178, 220], rheumatoid arthritis [76, 268], inflammatory bowel disease [189], and
COVID-19 [48, 94]. Since TNF-α promotes inflammation, it recruits immune cells such as
neutrophils to tissues, which then produce more pro-inflammatory cytokines [174]. Two other
early pro-inflammatory cytokines released are interleukin one alpha and beta (IL-1α and IL-
1β). These cytokines are produced by macrophages, neutrophils, and monocytes [122]. IL-1
functions similarly to TNF-α by also contributing to the onset of fever [75] and promoting
physical symptoms of inflammation [118]. Together, these early pro-inflammatory cytokines
activate secondary pro-inflammatory cytokines such as IL-6, which can promote production
of anti-inflammatory cytokines to regulate their response or stimulation further inflammation
[75, 118].

Secondary pro-inflammatory cytokines include interleukin 6 (IL-6) and interleukin 8 (IL-8),
also known as CXCL8. These cytokines are produced during the mid to late innate immune

![Cytokine Network](image)

Figure 2.3: The cytokine network produced by various immune cells. The figure is used with
permission from Zhang and An [298].
response [174]. IL-6 is released by neutrophils, macrophages, T and B cells, dendritic cells, and monocytes, and IL-8 is released by neutrophils, NK cells, and monocytes [122]. IL-8 is a primary recruiter for neutrophils and inducer for cell movement [122]. IL-6, on the other hand, is a cytokine that exhibits both pro- and anti-inflammatory properties. It is a primary stimulator of the acute phase response [75, 118], which is a reaction that produces acute phase proteins in the liver and is involved in the induction of fever and increased circulation of neutrophils and other leukocytes [114]. On the contrary, IL-6 also suppresses the release of TNF-α and IL-1 [104], promotes the production of anti-inflammatory cytokines [116, 255], and self-limits its own production [75, 270]. It also acts as anti-inflammatory during its role when produced from muscle cells during exercise [194, 195]. Correlations with this cytokine and mortality in cardiovascular complications [104] and cancer [249] are seen in a clinical setting, and it is suggested that IL-6 plays a pivotal role in inflammatory conditions such as sepsis and COVID-19 [55, 122].

**Anti-Inflammatory Cytokines**

One of the main anti-inflammatory cytokines released during the inflammatory response is interleukin 10 (IL-10), which is produced by macrophages, monocytes, NK cells, T and B cells, and dendritic cells [122] as shown in Figure 2.3. This cytokine inhibits inflammation, invoking a negative feedback on the production of TNF-α, IL-1α, IL-1β, IL-6, and IL-8 as well as suppressing the ability of phagocytes to engage in phagocytosis and release cytokines [118]. Elevated concentrations of IL-10 have been present in those with inflammatory diseases such as sepsis [122]. Another anti-inflammatory cytokine is interleukin one receptor antagonist (IL-1ra), which is produced by monocytes [174], macrophages, and neutrophils [75, 174]. This cytokine counteracts the pro-inflammatory actions of IL-1α and IL-1β [75]. While IL-10 and IL-1ra act in the short-term, the cytokine transforming growth factor beta (TGF-β) acts on a slower time scale to inhibit the immune response and is more closely associated with the adaptive immune response [174]. Produced by monocytes, platelets, and T cells, TGF-β regulates T cell and NK cell production and activation [75] by stimulating production of regulatory T cells, which helps to limit inflammation [174].

In this thesis, we focus on investigating the dynamics of monocytes, pro-inflammatory cytokines TNF-α, IL-6, and IL-8, and anti-inflammatory cytokine IL-10. Since endotoxin is administered into the bloodstream during an endotoxin challenge, it comes in contact with immune cells circulating in the blood such as monocytes instead of those in tissues. Monocytes are critical cells in inflammatory response that activated during experimental and clinical inflammation and release cytokines to regulate that response, such as TNF-α, IL-6, and IL-8,
and IL-10. Though in this thesis we focus on the human response to inflammation, we acknowledge that the immune and cytokine responses between species can differ [230]. Specifically, Copeland et al. [56] compared murine and human responses to an equivalent-dose endotoxin challenge and saw that humans exhibited a quick induction of fever, increased heart rate, and decreased blood pressure that was not observed in mice. This proposes that the inflammatory cytokine responses seen in humans versus mice differ in how they impact physiological processes.

2.2 Cardiovascular System

The cardiovascular system, shown in Figure 2.5, is responsible for the transportation of blood, where the heart and vessels are its primary components. The heart acts as a pump that drives the movement of blood throughout the body via varying sized vessels. Through hormone and autonomic nervous system regulation, the cardiovascular system adapts to handle the stress of an individual’s everyday life [27].

2.2.1 The Heart

The heart provides the momentum for blood to flow through the body’s vessels and is made up of two sides. As shown in Figure 2.4, the left heart pumps oxygenated blood into the systemic circulation, whereas the right heart pumps deoxygenated blood into the pulmonary circulation [27]. Each side of the heart has an atrium (where the blood enters from the veins) and a ventricle (where the blood enters from the atrium and is then expelled into the arteries). The flow of blood between (i) the atrium and the ventricle and (ii) the ventricle and the arteries is controlled by valves. These valves ensure that the heart acts as a pump, receiving blood at low pressure and then ejecting it at high pressure following a build up of pressure during cardiac contraction [95]. To ensure conservation of volume, the left and right heart each output 5L of blood per minute [27].

2.2.2 The Circulation

Vessels that transport blood through the body make up the circulation, which has two parts: the systemic and the pulmonary circulation [27].

The systemic circulation (bottom half of Figure 2.5) transports oxygenated blood at high pressure (Figure 2.6c) from the left heart to the tissues and then carries deoxygenated blood at low pressure back to the right heart. As noted in Figure 2.6, the systemic circulation contains
approximately 84% of the total blood volume [90]. From the left heart, blood is pumped into the aorta, which successively branches into vessels of small diameter (Figure 2.6a). The result of this successive branching is that the combined area of all of the arteries increases significantly (Figure 2.6b). The smallest arteries (the arterioles) then branch into capillaries, which are the smallest vessels (with a diameter of 5-10 \( \mu \)m). The exchange of oxygen, nutrients, waste, and other molecules occurs in the capillary beds, and it is also where blood travels at the slowest velocity (Figure 2.6d). Figure 2.5 shows that the capillary beds merge into venules, which begins the process of carrying deoxygenated blood and waste to the right heart and then into the lungs. The venules then converge into successively larger veins that parallel the arterial network and complete the systemic circulation [27].

The pulmonary circulation (top half of Figure 2.5) begins at the right heart, where deoxygenated blood is pumped through a rapidly branching network of pulmonary arteries. The pulmonary arteries transport blood to the pulmonary capillary beds adjacent to the lungs, where waste exchange occurs during exhalation and oxygen intake occurs on inhalation. The oxygenated blood then travels through the pulmonary veins and back to the left heart, where the oxygenated blood is pumped into the systemic circulation. This significantly shorter pathway enables the right heart to conduct transport at a pressure significantly lower than the

Figure 2.4: The anatomy of the heart. Oxygenated blood is shown in red, and deoxygenated blood is shown in blue. The figure is used with permission from Gordon Betts et al. [90] under the Creative Commons Attribution license (CC BY). No changes were made to the original figure. The license can be found at https://creativecommons.org/licenses/by/4.0/.
2.2.3 Regulation

The cardiovascular system requires sophisticated regulation to satisfy the demands of the muscles and tissues during various activities. To do so, the cardiovascular system works in tandem with the hormone and central and autonomic nervous system to control heart rate, cardiac contractility, vessel constriction [27].

Long-term control (days to weeks) of the cardiovascular system is obtained from low-
pressure baroreceptors. These receptors exist in the cardiopulmonary vasculature and regulate blood volume [44]. Short-term control (seconds to minutes) is achieved by high-pressure sensors, called baroreceptors, that sense blood pressure changes in the carotid sinus (via the sinus nerve) and the aortic arch (via the vagus nerve) of the arteries, as shown in Figure 2.7. Baroreceptors send afferent signals via the sinus and vagal nerves to the medulla, cerebral cortex, or the hypothalamus in the brain. Upon processing these signals, efferent signals are sent via the sympathetic and parasympathetic neurons. Parasympathetic signaling is primarily conducted via the vagal nerve and affects the cardiovascular system by controlling heart rate, while sympathetic signaling affects the vasculature, heart rate, and cardiac contractility [27] as seen in Figure 2.7.

At rest, parasympathetic activity is high where neurons fire at about 80% of their maximum capacity and sympathetic firing is low at about 20% of their maximum capacity [283]. A drop in blood pressure (for example, in response to a postural change) causes an increase in afferent baroreceptor firing to the vagal nerve. The afferent signal is integrated in the brain.

Figure 2.6: Vessel diameter, total cross-sectional area of vessels, average blood pressure, and velocity characteristics of the cardiovascular system. The figure is used with permission from Gordon Betts et al. [90] under the Creative Commons Attribution license (CC BY). No changes were made to the original figure. The license can be found at https://creativecommons.org/licenses/by/4.0/.
The efferent response is a fast (one to two heartbeats) decrease in parasympathetic activity and a simultaneous (but slower) increase in sympathetic activity. Parasympathetic withdrawal elicits a fast increase in heart rate and sympathetic stimulation results in vasoconstriction, a further increase in heart rate, and an increase in cardiac contractility [44, 27, 95]. Thus, the parasympathetic nerve activity only impacts the heart, while the sympathetic activity impacts both the heart and the vasculature as shown in Figure 2.7 [95].

Inflammation can also impact cardiovascular regulation. It can alter baseline firing rates of parasympathetic and sympathetic neurons [129, 27] as well as the baroreflex sensitivity to changes in blood pressure. Rogausch et al. [211] observed increased baroreflex sensitivity following endotoxin administration, while reports of decreased baroreflex sensitivity during LPS-infusion of rats [203] and during other diseases such as hypertension, heart failure, and heart attack [44] have also been seen.

![Figure 2.7: Regulation of heart rate (HR) and blood pressure by baroreceptors. High-pressure baroreceptors in the carotid sinuses and aortic arch as well as low-pressure baroreceptors in the cardiopulmonary system relay changes in blood pressure to the central nervous system (CNS). These signals result in either increases or decreases in sympathetic (SNA) and parasympathetic nerve activity (Para-SNA), which impact the heart and vasculature. This figure is used with permission from Chapleau [44].](image-url)
2.3 Hypothalamic-Pituitary-Adrenal (HPA) Axis

The hypothalamic-pituitary-adrenal (HPA) axis is a hormone-secreting channel within the endocrine system constituting of the hypothalamus, the pituitary gland, and the adrenal glands as shown in Figure 2.8 (left panel). Both the hypothalamus and the pituitary glands are located in the brain and are connected via the pituitary stalk, which contains portal blood vessels. Hormones released by the hypothalamus dictate the actions of the pituitary gland, which has two compartments: the anterior pituitary and the posterior pituitary. The anterior pituitary releases a variety of hormones which regulate metabolic processes, and the posterior pituitary serves as a location where terminal nerves pass through to their end location on capillary beds. These nerve endings release hormones associated with water retention and perinatal function. The adrenal glands, composed of the adrenal medulla and the adrenal cortex, are found in the abdomen region just above the kidneys. The adrenal medulla is closely associated with the sympathetic nervous system, which releases norepinephrine and epinephrine controlling baseline activity (long-term effects). The adrenal cortex releases a category of hormones called corticosteroids, which encompasses mineralocorticoids, glucocorticoids, and androgens. Specifically, the hypothalamus secreting corticotrophin-releasing hormone (CRH), the anterior pituitary secreting adrenocorticotrophic hormone (ACTH), and the adrenal cortex secreting cortisol (a glucocorticoid) make up the HPA axis [95] as shown in Figure 2.8 (right panel).

Feedback Loop

Figure 2.8 (right panel) displays the regulation of the HPA axis pathway, which is based on feedback loops between the hypothalamus, the anterior pituitary, and the adrenal cortex. Activation of the hypothalamus originates from the nervous system in response to the 24-hour circadian rhythm and stressful events such as trauma, emotional changes, changes in molecular compositions, and other stimuli [27, 95]. The circadian rhythm's influence on the secretion of HPA axis hormones results in lower hormone secretion at night and higher hormone secretion during the day with frequent oscillatory behavior (ultradian rhythms) [95]. Activated neurons in the hypothalamus then produce hypothalamic-releasing hormones, one of which is CRH. CRH travels through the portal vessels in the pituitary stalk to the anterior pituitary and activates corticotrophes, which are anterior pituitary cells that release ACTH and account for approximately 20% of all cells in this pituitary sector [95]. When ACTH is produced, it can either exhibit a negative feedback on hypothalamic neurons [27] or progress in the bloodstream to the second layer of the adrenal cortex called the zona fasciculata [95]. Here, ACTH stimulates the adrenal cortex which produces the main glucocorticoid called cortisol. This makes up
approximately 95% of all glucocorticoids. The daily secretion of cortisol follows the circadian release of CRH. Upon release from the adrenal cortex, cortisol then exhibits a direct negative impact on both the hypothalamus and the anterior pituitary suppressing the production of CRH and ACTH respectively [95], as shown in Figure 2.8.

2.4 Multi-System Interactions

The three physiological systems (immune, cardiovascular, and endocrine) are known to impact each other, and this subsection discusses these interactions. Several of these connections are used to relate immune, cardiovascular, and endocrine system dynamics in Chapter 6 to further understanding of the multi-system impacts of inflammation. The remaining interactions presented here are considered potential connections to implement in future multi-system inflammation models as discussed in Chapter 8. Only system connections relating quantities studied in this thesis are shown Figures 2.9, 2.10, and 2.11. For the cardiovascular system

Figure 2.8: The hypothalamic-pituitary-adrenal (HPA) axis anatomy (left) and regulatory feedback loop (right). Green arrows represent stimulation, and red lines denote inhibition. This figure is used with permission from Gudmand-Hoeyer et al. [92]. Abbreviations: SCN, suprachiasmatic nucleus; PVN, paraventricular nucleus; CRH, corticotropin-releasing hormone; ACTH, adrenocorticotropic hormone; CORT, cortisol.
specifically, we investigate impacts on blood pressure, heart rate, nitric oxide production, and cardiac output. Nitric oxide is a vasodilator, and cardiac output is upregulated by heart rate. An increase in cardiac output results in an increase in blood pressure. However, changes in blood pressure also results in changes in heart rate. This is one example that displays the complexity of the interactions between the immune, cardiovascular, and endocrine systems, and it is very difficult to determine the exact pathways in which these systems communicate. The language used in literature is often vague. Specific pathways are sometimes not specified and the change in one quantity due to another (increases or decreases) are occasionally reported through general terms such as *regulate, impact, or modulate*, which complicates translating these connections to a mathematical model. Some interactions are also tissue or organ specific. This subsection reports these system couplings as they were described in literature. More work is needed to identify primary and secondary pathways of these mechanisms.

2.4.1 **Immune and cardiovascular interactions**

Immune-cardiovascular relationships corresponding to specific modeling components used in this thesis to describe general inflammation are shown in Figure 2.9, which include the cholinergic anti-inflammatory pathway [258, 193], TNF-α stimulation and IL-10 inhibition of nitric oxide production [47, 258, 218], TNF-α downregulation of cardiac output [258, 193], TNF-α upregulation of cardiac contractility which increases cardiac output [199], and TNF-α regulation of blood pressure [14].

Bidirectional immune and cardiovascular interplay is found across multiple pathways and can result in vascular and cardiac malfunction. A important interaction between the immune and cardiovascular systems is the cholinergic anti-inflammatory pathway. Afferent vagal nerves are stimulated by endotoxin or pro-inflammatory cytokines and increase efferent parasympathetic neurons signaling to release acetylcholine (ACh), which binds to ACh receptors on macrophages. This inhibits macrophage release of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-18 in cardiac and liver tissue [193, 258]. Since increased parasympathetic outflow results in decreased heart rate and TNF-α production, this interaction is shown in Figure 2.9 as heart rate stimulating TNF-α production. IL-10 production has been shown to not be impacted by this mechanism [258]. Tracey [258] also reports that TNF-α promotes nitric oxide (NO) production by immune cells, and Chesrown et al. [47], Salim et al. [218] suggest that IL-10 inhibits nitric oxide production as shown in Figure 2.9. Furthermore, Tracey [258] and Pavlov and Tracey [193] describe that increases in pro-inflammatory cytokine TNF-α also decreases cardiac output (shown in Figure 2.9) and increases vascular blockage and permeability.

There is also discussion of the ability of cytokines to affect cardiac contractility [14, 199].
Prabhu [199] suggests that pro-inflammatory cytokines (mainly TNF-α) impact cardiac contractility in two phases. The first phase is composed of an short immediate response where the heart engages in stronger contractions and is able to relax quicker, likely without changes to gene expression, but could result in either a positive or negative cardiac effect. We incorporate this impact into Figure 2.9 through the upregulation of cardiac output by TNF-α since stronger contractions increase stroke volume and thus, cardiac output. This could be due to cytokine modifications to the excitation-contraction (E-C) coupling responsible for the contraction of the heart and nitric oxide impacts [199]. Dal Lin et al. [62] reports that macrophages can stimulate electrical signaling to the atrioventricular (AV) node. The longer secondary phase ultimately characterized as cardiodepressant but consists of systolic and diastolic dysfunction that could be due to changes in gene expression, nitric oxide effects, reactive oxygen species production, and alterations in signaling [199].

Other cells thought to play a role in both immune and cardiac regulation include endothelial cells, cardiac fibroblasts, and pericytes. Endothelial cells are in close contact with immune cells and (i) play an important role in cell migration to the site of infection through endothelial adhesion and (ii) promote microthrombosis. They can also produce nitric oxide, and impairment of that ability can impact vascular regulation. Additionally, cardiac fibroblasts are regulators of both the innate and adaptive immune responses and pericytes promote the migration of immune cells to sites of infection and damage. Dysfunction in pericytes as well as endothelial cells can result in numerous cardiovascular effects such as stroke [62].

Immune and cardiovascular interactions are also seen in those with major heart problems.

![Diagram of connections between the immune and cardiovascular systems](image)

**Figure 2.9:** Diagram of connections between the immune and cardiovascular systems relating to concepts modeled in this thesis. Solid lines signify stimulation, and dotted lines signify inhibition.
such as myocardial infarction, heart disease, and heart failure, which can appear in patients with sepsis or be a preexisting condition that complicates sepsis [167, 119]. There is conflicting evidence about whether certain cytokines play a beneficial or harmful role, and this role can depend on a multitude of factors [14]. For example, Bartekova et al. [14] suggests that TNF-α can be either harmful or protective during myocardial infarction depending on which receptor it binds to. Evidence of additional effects of cytokines on cardiac function include a suppressive effect from pro-inflammatory cytokines produced in the myocardium that can induce left ventricular dysfunction [199], hypotension due to continuously elevated TNF-α and hypertension due to continuously low TNF-α levels as shown through up- and downregulation of blood pressure by TNF-α (shown in Figure 2.9), and ischemic tissue repair from cytokines such as IL-6, IL-8, and IL-10 [14].

2.4.2 Immune and hormone interactions

It is well-documented that there is bidirectional communication between inflammatory mediators and hormone secretion in the HPA axis, as shown in Figure 2.10. Communication from the immune system to the HPA axis consists of (i) direct stimulation of CRH and ACTH by pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) and anti-inflammatory cytokine IL-10 [22, 33, 117, 131, 237, 240], and (ii) simultaneous stimulation and inhibition of cortisol by anti-inflammatory cytokine IL-10 [117, 131, 237, 240], as demonstrated in Figure 2.10.

While all HPA axis levels (hypothalamus, pituitary, and adrenal glands) can produce their own cytokines and have cytokine receptors [237], a majority of studies propose that downstream production of cortisol from CRH and ACTH is the primary mechanism that cytokines use to influence production of HPA axis hormones. Various stimulation pathways have been suggested, including the stimulation of vagal afferent nerves which emit norepinephrine in the paraventricular nucleus (PVN) of the hypothalamus, entering the hypothalamus through a more permeable blood-brain barrier during infection or via active transport, impacting the median eminence (ME) nerve-endings in the hypothalamus, or affecting cerebral endothelial or glial cells which activate hypothalamic neurons through secondary processes [117, 237].

There is also evidence that ACTH and cortisol production can come from the direct activation of the anterior pituitary and the adrenal glands, respectively, by cytokines. Bernardini et al. [22] isolated hypothalami and anterior pituitary cells of rats and, after TNF-α administration, both CRH and ACTH concentrations increased in each cell type with dose-dependency. Silverman et al. [237] also observed ACTH and cortisol production with the removal of CRH genes in animals. In the adrenal cortex, pro-inflammatory cytokine receptors are present in all three regions, but only IL-1β and IL-6 stimulate cortisol production, while TNF-α produced in the
adrenal glands inhibits production [237] as shown in Figure 2.10. In reference to the impact that IL-10 has on the release of HPA axis hormones, IL-10 can be produced in hypothalamic, pituitary, and neural tissues [237, 240]. Evidence that IL-10 upregulates CRH and ACTH production (displayed in Figure 2.10) directly includes in-vitro IL-10 administration on pituitary tumor cells and splenocytes from mice producing elevated ACTH concentrations [240], and that IL-10 can activate the production of i) CRH by stimulating ME fragments in the hypothalamus and ii) ACTH by inducing pituitary cells to produce macrophage migration inhibitory factor (MIF) [237, 240]. Through the production of MIF, IL-10 stimulates cortisol increases via the regulatory feedback in the HPA axis (Figure 2.8). However, studies have shown that IL-10 also inhibits cortisol production [117, 237], which is included in Figure 2.10. For example, Koldzic-Zivanovic et al. [131] showed that mice with an IL-10 inhibitor exhibited higher cortisol concentrations than wild mice, even though ACTH levels between the two groups remained similar.

In turn, as shown in Figure 2.10, there is also evidence that the HPA axis directly impacts the production of inflammatory mediators through CRH upregulation of pro-inflammatory cytokines as well as stimulation of IL-6 and IL-10 by cortisol. Agelaki et al. [1] reported that in vitro testing on two types of murine macrophages during a CRH-enhanced endotoxin challenge resulted in increased concentrations of TNF-α, IL-1β, and IL-6. An additional in vivo experiment by Agelaki et al. [1] also promotes this notion, where the administration of a CRH antagonist restricted the production of LPS-induced cytokines TNF-α, IL-1β, and IL-6. In regards to cortisol, Hjemdahl et al. [104] reports that cortisol can activate production of IL-6 through the stimulation of norepinephrine and epinephrine. Additionally, Van der Poll et al. [262] reports suppressed concentrations of TNF-α and larger concentrations of IL-10 during an endotoxin challenge when hydrocortisone is administered just prior to LPS injection.

### 2.4.3 Cardiovascular and hormone interactions

There is evidence that the HPA axis influence cardiovascular mechanisms. Many studies imply that the influence of CRH on cardiovascular function is likely indirect via stimulation of cortisol production. However, as shown in Figure 2.11, suggested direct CRH links to cardiovascular function include stimulation of norepinephrine and epinephrine release (which increases heart rate and, subsequently, cardiac output and mean arterial pressure), regulation of vascular dilation through nitric oxide modulation, and production of vasodilation peptides by signaling to cardiomyocytes [34].

Similarly, most ACTH effects on cardiovascular function also arise from its stimulation of cortisol. For example, hypercortisolism due to excess ACTH production can lead to hypertension, endothelial dysfunction, cardiomyopathy, and other cardiovascular complications whereas
hypocortisolism due to an insufficient ACTH supply can lead to hypotension [34, 284]. Independent of cortisol production, ACTH has been suggested to impact cardiovascular function through multiple pathways including signaling directly to ACTH receptors on aortic endothelial cells to regulate blood pressure (as shown in Figure 2.11), impacting metabolism of fat tissue, and regulating adrenal vasculature and blood flow [34].

Figure 2.11 illustrates the impacts of hormone secretion on cardiovascular function, which stem from the production of glucocorticoids in the adrenal glands, particularly cortisol. Cortisol helps to maintain cardiovascular contractility by increasing tension of cardiac muscle. In terms of specific effects that cortisol has on the vasculature, it regulates endothelial function such as adhesion molecules, concentrations of IL-6 and IL-8 among other pro-inflammatory cytokines and chemokines, nitric oxide, and endothelial vasoconstrictor agents [34]. Specifically, the release of cortisol activates the endothelial glucocorticoid receptor (GR), which can suppress the release of molecules such as nitric oxide [34, 89, 88, 104, 284] and upregulate vasoactivators such as epinephrine, angiotensin II, and other catecholamines on smooth muscle and endothelial cells [34, 104] as shown in Figure 2.11. The result is an increase in blood pressure [34, 89, 104, 284], with some studies showing this particularly true for acute hypertension [34]. Goodwin and Geller [89] suggests that the hypertensive state could also be a result of mineralcorticoid receptor (MR) activation. However, there are studies that have found that lack of endothelial NO did not result in hypertension. An additional effect on vasculature includes suppressing cell migration and production during new vessel formation which downregulates endothelial repair [34].

**Figure 2.10:** Diagram of connections between the immune system and the HPA axis found in literature relating concepts modeled in this thesis. Solid lines signify stimulation, and dotted lines signify inhibition.
There is also evidence that glucocorticoids perpetuate direct and indirect actions on the heart. In vitro studies have found that administration of glucocorticoids to cardiomyocytes resulted in protection from apoptosis and damage, but could also result in hypertrophy. The receptor that glucocorticoids signal to may also influence its behavior on the heart. Glucocorticoid signaling via the GR is important for regulating electrical cardiac signaling and left ventricular function, and loss of the GR has resulted in hypertrophy and heart failure. Meanwhile, glucocorticoid signaling through the MR has resulted in elevated inflammation and fibrosis in addition to regulation of electrical signaling, and loss of MR can lead to cardiac malfunction and cardiomyocyte apoptosis [34]. Furthermore, Lee et al. [142] suggests that during times of threat, the hypothalamus directly communicates with the sympathetic nervous system to stimulate the release of cortisol and cause the adrenal glands to produce catecholamines, which can result in increase heart and respiratory rate as shown in Figure 2.11, with Hjemdahl et al. [104] suggesting that excessive glucocorticoids could also result in increased cardiac output. Figure 2.11 displays all of the connections between hormone release and the cardiovascular discussed in this section that are modeled in this thesis.

Figure 2.11: Diagram of connections between the cardiovascular system and the HPA axis found in literature relating concepts modeled in this thesis. Solid lines signify stimulation, and dotted lines signify inhibition.
2.5 Sepsis

This thesis focuses on the mathematical modeling of immune system relations, their impact on tissue and organ function during sepsis, and how septic conditions could be improved. Thus, we describe how sepsis is diagnosed, its pathophysiology, and its current course of treatment. Sepsis is medical emergency most recently defined as "life-threatening organ dysfunction caused by a dysregulated host response to infection" [238]. Liu et al. [148] notes that sepsis is one of the top causes of death in hospitals in the United States, with a global mortality rate of approximately 15%-33% [74]. Over the last several decades, cases of sepsis as well as cost of treatment has risen. Globally, Rudd et al. [215] has estimated that 49 million cases and approximately 11 million deaths due to sepsis occurred in 2017. Buchman et al. [32] estimated that the total cost of sepsis care for medicare patients rose from $27.7 to $41.5 billion between 2012 and 2018. The high mortality rate, prevalence of cases, and economic impact of sepsis make it a priority to develop early diagnostic tools and improved treatments to lower the length of hospital stays and, ultimately, death.

2.5.1 Diagnosis

Infants, the elderly, and individuals with preexisting health complications or immuno-compromised systems due to cancer, previous septic infection, or other reasons are particularly susceptible to sepsis. Furthermore, additional factors such as type and location of the initial infection, racial background, sex, and current medicinal intake are also influential in disease development and severity [42]. Criteria from Singer et al. [238] defines sepsis as a clinical syndrome in a patient that displays:

1. Evidence of a suspected or confirmed infection, verifiable by the quick Sequential Organ Failure Assessment (qSOFA),

2. Organ dysfunction, which is quantified as a 2+ point increase from the patient’s baseline SOFA score.

qSOFA and SOFA scoring variables are shown in Table 2.1. A subset of sepsis called septic shock is characterized as a form of sepsis with a higher mortality rate where individuals show irregularities in cardiovascular and metabolic function [238]. Literature suggests mortality rates between 20% - 55% for patients with septic shock [6, 16, 232]. The clinical criteria for its diagnosis from Singer et al. [238] includes individuals with sepsis who display:

1. Evidence of hypotension, quantified as mean arterial pressure (MAP) less than 65mmHg and the need for vasopressors to keep MAP above 65mmHg,
2. Evidence of hyperlactatemia, verified by serum lactate levels greater than 2 mmol/L.

Patients who are diagnosed with sepsis or septic shock are commonly admitted to a hospital's intensive care unit (ICU) for critical around-the-clock care [6, 178]. Although the criteria above are currently in practice to diagnosis sepsis and septic shock, there is no singular chemical biomarker that defines sepsis. This makes it difficult to diagnose and treat since the individual symptoms are present in many other diseases [42].

Table 2.1: Table of Sequential Organ Failure Assessement (SOFA) and quick SOFA (qSOFA) scoring systems for sepsis diagnosis based on Singer et al. [238]. Abbreviations: $F_{1O_2}$, fraction of inspired oxygen; GCS, Glasgow Coma Scale; MAP, mean arterial pressure; $P_{aO_2}$, partial pressure of oxygen.

<table>
<thead>
<tr>
<th>qSOFA (Quick SOFA) Criteria</th>
<th>Score</th>
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<tbody>
<tr>
<td>High respiratory rate ($\geq 22$ breaths/min)</td>
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<tr>
<td>Altered mental status (GCS$\leq 14$)</td>
<td></td>
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<tr>
<td>Low systolic blood pressure ($\leq 100$mmHg)</td>
<td></td>
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<tr>
<td><strong>Sequential Organ Failure Assessment (SOFA) Scoring</strong></td>
<td></td>
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<tr>
<td><strong>Measured Variable</strong></td>
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</tr>
<tr>
<td><strong>Respiratory</strong></td>
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<tr>
<td>$P_{aO_2}/F_{1O_2}$ (mmHg) $\geq 400$</td>
<td>&lt;400</td>
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<tr>
<td>$P_{aO_2}/F_{1O_2}$ (mmHg) $&lt; 400$</td>
<td></td>
</tr>
<tr>
<td>Coagulation</td>
<td></td>
</tr>
<tr>
<td>Platelets ($\times 10^3$/µL) $\geq 150$</td>
<td>&lt;150</td>
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<tr>
<td>Digestive (Liver Function)</td>
<td></td>
</tr>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>&lt;1.2</td>
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<tr>
<td>Cardiovascular</td>
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<tr>
<td>MAP (mmHg) $\geq 70$</td>
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<tr>
<td>Nervous</td>
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<tr>
<td>GCS Score</td>
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<tr>
<td>Renal</td>
<td></td>
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<tr>
<td>Creatinine (mg/dL)</td>
<td>&lt;1.2</td>
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<tr>
<td>Urine output (mL/d)</td>
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26
2.5.2 Pathophysiology

*Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa* have commonly been found in individuals with sepsis [178]. However, sepsis can be caused by any bacteria, fungi, parasite, or virus [109] and its stimulus is much more potent to the immune system than a typical infection [178]. Increased inflammatory stimuli can consequentially lead to the hyperactivation of the immune system and produce residual effects on other systems in the body that can result in the breakdown of cardiovascular, metabolic, neural, urinary, and endocrine function [238].

**Immune Response**

When a pathogen invades the human body as shown in Figure 2.12, the innate immune system is activated by pattern-recognition receptors (PRRs) such as toll-like receptors, which recognize molecules called pathogen-associated molecular patterns (PAMPs) released by the invading pathogen [109, 42, 96, 256, 214, 178]. PRRs can be found on immune cells such as monocytes and macrophages [96]. Recognition of the pathogen activates phagocytic cells including monocytes, macrophages, neutrophils and natural killer (NK) cells [96, 109, 256, 214, 178], which attempt to eliminate the pathogen through phagocytosis. These activated immune cells also release large quantities of pro-inflammatory (TNF-α, IL-1, IL-6, IL-8, IL-18) [96, 109, 220] and anti-inflammatory cytokines [42]. The major distinction between sepsis and a regular infection is that the production of pro-inflammatory cytokines is amplified [109, 178, 220]. Commonly referred to as a cytokine storm as shown in Figure 2.12, the increased production of pro-inflammatory cytokines is due to the overstimulation of immune cells and the transcriptional pathway, NF-κB, by the pathogen [178]. Upon the innate immune system’s inability to clear the pathogen alone, the adaptive immune system initializes to produce lymphocytes including effector T cells which can further promote production of macrophages [109]. In addition to PAMPs, PRRs on monocytes and macrophages also detect molecules called damage-associated molecular patterns (DAMPs) that are released from damaged tissues [96], which is seen in Figure 2.12. When DAMPs are detected, large concentrations of high-mobility group beta one (HMGB-1) are released by liver cells, which can further promote cell death [109]. Recognition of PAMPs during sepsis can lead to damaged tissues, and thus the release of DAMPs in addition to PAMPs can turn a normally well-balanced immune response into a vicious, dysregulated inflammatory cycle [257].

The recognition of the pathogen also activates the complement system, which further stimulates inflammation and assists the body in discarding damaged particles. Additionally, pathogen recognition activates acute phase proteins in the liver, some of which promote the
inhibition of inflammation [220]. Furthermore, immune cells engage in endothelial adhesion, which promotes the efficiency of the immune response by providing a gateway for immune cells in the bloodstream to migrate to the pathogen site in the tissues [96].

The initial hyperinflammatory state of sepsis is closely followed by (and some suspect in tandem with) a hypoinflammatory state that exhibits considerable immunosuppression [42, 178, 220]. Immunosuppression can prevent the initial infection from being cleared or lead to repeat infections [109], and clinical therapies such as the administration of norepinephrine,

---

**Figure 2.12:** The initial cytokine storm during sepsis. When bacteria makes its way into the body, it releases PAMPS and DAMPS which bind to PRRs on the surface of immune cells, which activates the immune system. Immune cells then produce excessive levels of pro-inflammatory cytokines which help recruit other immune cells to the infection site. These overwhelming levels of cytokines can result in cellular death and organ dysfunction. This figure is used with permission from Tang et al. [252] under the Creative Commons Attribution license (CC BY). No changes were made to the original figure. The license can be found at https://creativecommons.org/licenses/by/4.0/.
phenylephrine, and hydrocortisone have been known to contribute to the hypoinflammatory state [256]. The immunosuppressive stage of sepsis focuses on impairing immune cell function, decreasing production of immune cells except regulatory T cells, and apoptosis of cells.

Overwhelming exposure to endotoxin during the hyperinflammatory stage can lead to a phenomena called endotoxin tolerance, in which immune cells like monocytes lose their ability to invoke inflammation [178, 256]. Torres et al. [256] suggests a reprogramming or change in gene expression of monocytes and macrophages could be at work during sepsis. Immunosuppressive behavior has major implications on immune cell functionality including decreased production of pro-inflammatory cytokines (TNF-α, IL-1α, IL-1β, IL-2, IL-6, IL-12, IL-18, IFN-γ) [96, 256], stagnant or increased production of anti-inflammatory cytokines (IL-1ra, IL-10, TGF-β) [109, 178, 256], suppression of dendritic cells [256], and the suppression and exhaustion of T cells and B cells [178, 256]. T cells are suppressed in part by myeloid-derived suppressor cells (MDSCs), which keep hold of the L-arginine needed for T cell energy [256]. T cell inhibition is also amplified due to the large pathogen load and resulting hyper- and hypo-inflammatory cytokines [178], and is influenced by IL-7 pathway, which is important for T cell maturation [42]. Apoptotic T and B cells also contribute to the inhibition of monocyte, macrophage, and dendritic cell function. When these dead cells are consumed by the phagocytes, it promotes the phagocytes to produce more anti-inflammatory cytokines [178].

In addition to suppression of immune cell production and function, the immunosuppressive state of sepsis also exhibits increased rates of apoptosis, particularly in adaptive immune cells such as dendritic cells, T and B cells, and NK cells [178, 214, 256] as shown in Figure 2.13. However, regulatory T cell production increases. The regulatory T cell has the ability to suppress functions of other inflammation-promoting immune cells, and exhibits pronounced expression of protein BCL-2 which allows the cell to escape apoptosis. The detection of DAMPs by immune cells also stimulates regulatory T cell production [256].

**Coagulation Response**

The hyperactivation of the immune response, the activation of the complement system, and mechanisms in the coagulation system all contribute to the coagulopathy and disseminated intravascular coagulation (DIC) commonly seen in sepsis patients [42, 96, 109, 178, 220] as shown in Figure 2.13. In addition to endothelial cells, immune cells such as monocytes release tissue factor (TF), which upregulates the coagulation cascade to produce increased magnitudes of thrombin, an enzyme that converts fibrinogen to fibrin. Increased circulation of fibrin results in an amplified number of blood clots when the fibrin mixes with platelets [96, 220]. Another factor contributing to coagulation dysregulation is a decrease in fibrinolysis, which is the
process that disintegrates existing blood clots [96, 109, 220]. This occurs due to the net effect of sustained elevated levels of pro-inflammatory cytokines (TNF-\(\alpha\) and IL-1\(\beta\)), which results in decreased levels of plasmin, the enzyme responsible for the breakdown of blood clots [96]. Furthermore, a decrease Protein C levels (which promote anti-coagulation and exhibit anti-inflammatory effects on immune cells and cytokines), and an increase in platelet activating factor, P-selectin, and von Willebrand factor (all important in platelet or immune cell adhesion) may also promote coagulopathy [96, 220].

These excessive responses by the immune and coagulation systems can result in significant inhibition to endothelial cell functions needed for regulation of hemostasis [220], and can lead to large-scale bleeding elsewhere due to low leftover concentrations of available platelets and other molecules needed for clotting. Moreover, the formation of microthrombi can lead to tissue ischemia and hypoxia, increased tissue permeability, and subsequent organ damage [96, 220] as exhibited in Figure 2.13.

**Organ Dysfunction**

The notable immune and coagulation responses previously discussed cascade into organ dysfunction in multiple systems (Figure 2.13) including the cardiovascular, metabolic, gastrointestinal, nervous, endocrine, and renal systems, which can lead to organ shutdown and death. Dysregulation in the microcirculation from the coagulatory response can impair blood flow [42], which can result in tissue ischemia and cellular hypoxia [220] and subsequently lead to organ hypoperfusion [96, 220]. Vasodilation due to inflammatory mediators, decreased stroke volume and cardiac output, increases in end diastolic and systolic volumes, and changes in heart rate also follow [96, 220]. The dysregulated inflammatory response also contributes to cardiac problems. Cardiomyopathy has been observed in individuals with sepsis. Increases in TNF-\(\alpha\) and IL-1\(\beta\) levels may lead to a decrease in and mitochondrial malfunctions of cardiac myocytes [96]. Additional cardiac implications during sepsis include capillary leak and shock [220].

Metabolic consequences of sepsis primarily focus on mitochondrial dysfunction of immune cells, which can also contribute to immunosuppression [96, 220, 256]. Impaired blood flow from cardiovascular dysfunction can cause cellular hypoxia due to inadequate oxygen delivery to cells, resulting in metabolic problems [42, 96, 220]. To provide a quick source of energy to immune cells to carry out their duties during infection, anaerobic glycolysis for ATP generation is increased, which results in amplified lactic acid levels [96, 256]. Furthermore, ATP levels decrease because of the immune production of reactive oxygen species (ROS) [96]. ROS can bind with increased levels of nitric oxide (NO), whose product (reactive nitrogen species - ROS)
inhibits the electron transfer chain (ETC) and can lead to further mitochondrial damage and mitochondrial apoptosis [109]. In an attempt to sustain immune cell energy, the body may also engage in gluconeogenesis, which produces amino acids from the breakdown of muscle [96]. Gyawali et al. [96] also reports the potential for individuals with sepsis to also enter a hyperglycemic state due to increased resistance of insulin, as seen in Figure 2.13.

In addition to cardiac and metabolic consequences, effects of sepsis on other systems can occur. Figure 2.13 shows that a change in mental status or encephalopathy can occur due to the breakdown of the blood-brain barrier from endothelial dysfunction, which can allow the pathogen, immune cells, or cytokines to enter [96, 256]. Encephalopathy could also be a result of

![Figure 2.13: A diagram of organ dysfunction during sepsis. The hyperinflammatory and immunosuppressive states at the start of sepsis cascade result in many areas of dysfunction throughout the body including cardiovascular, metabolic, neural, renal, and gastrointestinal problems. This figure is used with permission from Nedeva et al. [178] under the Creative Commons Attribution license (CC BY). No changes were made to the original figure. The license can be found at https://creativecommons.org/licenses/by/4.0/.](image-url)
tissue ischemia, which can cause dysfunction in cerebral regulation and impact cerebrovascular flow [256]. In the HPA axis, decreased concentrations of CRH, ACTH, and cortisol are emitted and can lead to adrenal insufficiency syndrome [109, 256]. In the gastrointestinal region, liver dysfunction is common resulting from the reduced elimination of bilirubin the liver. Also shown in Figure 2.13 is that increased permeability of the intestinal mucous membrane can lead to bacterial invasion and autodigestion by enzymes [96]. In the urinary system, kidney injury or failure due to decreased perfusion and renal apoptosis of tubule cells is common in sepsis patients [96, 220].

2.5.3 Treatments

An important step to recovery for sepsis patients is to immediately transport those that are severely-ill to the ICU. Quickly starting treatment is necessary for sepsis patients since it is known that delayed treatment greatly reduces chance of survival [74, 197]. Once a patient is diagnosed with sepsis, there are several steps that medical professionals take. In addition to intravenous fluid administration for typically the duration of care [74, 96], septic patients are given antibiotics to help fight the infection [42, 74, 197] and infected tissue or devices may be removed to aid in stopping the spread of infection [42, 74]. Evans et al. [74] suggests that early antibiotic administration is essential in reducing septic mortality, and that either an extended (>30 minutes) or a continuous infusion of antibiotics is recommended for optimal outcomes compared to the traditional bolus. If a patient is in septic shock and fluid resuscitation fails to increase the mean arterial pressure, the administration of vasopressors such as norepinephrine, vasopressin, dopamine, or epinephrine are given to help increase blood pressure [74, 96]. Additional supportive care measures are also sometimes implemented depending on the needs of the patient. If the organ systems targeted are the kidneys or the lungs, dialysis or respiratory assistance via a mechanical ventilator may be necessary. To aid in metabolic dysfunction, nutritional supplements and the administration of insulin may be needed [197].

While the above care tactics are standard in response to a septic patient, there is currently no accepted single treatment for sepsis or septic shock. Dozens of studies in recent years have explored a variety of treatments focused on different components of sepsis. These include

- anti-TNF therapy [205, 202, 152, 197],
- anti-IL-1 therapy [197],
- coagulation pathways [42, 197],
- immunoglobulins [197],
• endotoxin removal [42],

• the administration of levosimendan [42, 197] and amino acids [197],

• glucose control [42],

• nitric oxide therapy [197],

• corticosteroid administration [42].

Many of these therapies have shown either negative or inconclusive results. Those that have shown some benefit to sepsis patients remain controversial [42, 197]. Recently though, vitamins, particularly the intravenous administration of vitamin C, have been given significant consideration as a possible therapeutic target for sepsis patients, but studies are still being conducted to prove efficacy in large clinical settings [126, 197, 276]. Mathematical modeling can help aid in discovering and predicting the efficacy of proposed therapeutic treatments through in silico trials on virtual patient populations or through calibration of a model to septic data. It can also be used to increase understanding of the underlying dynamics that occur during sepsis, which can suggest target spots for therapeutic treatment development. Multi-system models in particular can be useful in these aspects to study treatment impacts across the multiple systems that sepsis affects.
CHAPTER 3

MODELING LITERATURE REVIEW

Mathematical modeling can provide insight into underlying mechanisms and dynamics of physical processes not observed in an experimental or clinical setting. In this thesis, we focus on modeling the inflammatory response and its interactions with other systems, in particular the cardiovascular system and HPA axis. We model these interactions from an immune system perspective since the initial stage of sepsis is marked by inflammation, which cascades into further downstream dysfunction in the body. In an effort to understand how previous authors have represented these systems, their interactions, and sepsis, we compile and review models published in literature. We focus on differential equations models, but acknowledge that there are others such as agent-based models that describe this phenomena. Differential equations models simulate homogeneous populations across continuous time-varying dynamics and are simple to execute, while agent-based models are more computationally expensive but can model heterogeneous behavior and incorporate stochasticity [111].

3.1 Inflammation Models

Mathematical models of the innate and adaptive immune systems have become prevalent in literature over the last two decades. Motivation for the early development of these was to
provide insight into the dynamics of the acute inflammatory response, which is complex and not fully understood, and hypothesize what areas to explore for therapies that could resolve inflammation in a septic-like scenario. Mathematical models of inflammation vary in both size and structure. Typically, small models of inflammation are made up of generalized immune response states whereas larger models specify particular components of the immune system (cells, cytokines, signaling pathways, etc.). This allows for a more focused and comprehensive study of the system’s dynamics and can be targeted for a specific infection. Some models also utilize cytokine experimental data from endotoxin challenges in humans or animals to calibrate or validate their models, whereas others are of a purely theoretical nature. An overview of inflammation models is given in Table 3.1.

Most inflammation models include the pathogen, cells (macrophages, monocytes, neutrophils), and pro- and anti-inflammatory cytokines. Simple models (consisting of three to

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Figure 3.1: (a) Simple inflammation model diagram. A stimulus (endotoxin, pathogen) activates pro-inflammatory and anti-inflammatory responses (immune cells, cytokines), which exhibit both positive and negative feedback on each other to create a regulated response and resolve inflammation. The models may also include external factors (damage) that can upregulate pro- and anti-inflammatory responses and be regulated by the anti-inflammatory response. (b) Complex inflammation model diagram. A stimulus (endotoxin, pathogen) activates a variety of pro- and anti-inflammatory immune cells (monocytes, macrophages, neutrophils, dendritic cells, T cells), producing pro- and anti-inflammatory cytokines (TNF-α, IL-6, IL-8, IL-10, T cell-released cytokines, chemokines) that can either promote or suppress the actions of the immune cells. Immune cells or the stimulus may promote the production of external immune factors (damage), which can exhibit positive feedback on the system and be regulated by cytokines. In (a) and (b), stimulation is represented by solid arrows and suppression is represented by circles with dotted lines.
four differential equations and shown in Figure 3.1a) lump cell and cytokine actions together. An example is the model by Kumar et al. [137]. This model analyzed fixed points and bifurcations to study the inflammatory response to a pathogen. The model reproduces several inflammation scenarios such as a healthy response to infection, persistent non-infectious inflammation, persistent infectious inflammation, recurrent infection, and immuno-deficiency. This three-state model consisted of only a pathogen, an early pro-inflammatory mediator (which encompassed effects from immune cells and cytokines), and a late pro-inflammatory mediator (which incorporated anti-inflammatory cytokine and hormonal effects as well as damaged tissue) and was the foundation for future inflammation models. To study the impact of a dynamic anti-inflammatory mediator within the system, Reynolds et al. [207] built upon the work by Kumar et al. [137] creating a four-state model consisting of a pathogen, a general pro-inflammatory term (summarizing the effect of phagocytes), a general anti-inflammatory term (summarizing the effect of cytokines and hormones), and a damage term describing the effects of inflammation on tissues. They analyzed the system's fixed points and bifurcations, showing that the pathogen growth rate and initial load as important bifurcation parameters, as the model was constructed from smaller subsystems. They concluded that the dynamic anti-inflammatory mediator is important for a successful immune response and that it is a potential focus site for treatment in systemic inflammatory diseases such as sepsis. Using an adapted version of the simple inflammation model in Reynolds et al. [207], Day et al. [66] investigated the phenomena of endotoxin tolerance and potentiation through repeated endotoxin administration, and also explored these phenomena through the analysis of fixed points and nullclines. Day et al. [66] concluded that timing, dosage, and type of endotoxin administration all played a role in the physiological system response. A larger generalized inflammation model was derived in Foteinou et al. [79], which had general pro- and anti-inflammation terms, but did not specify immune cells, cytokines, or chemokines. Instead, Foteinou et al. [79] created an eight-state model focused on including receptors and signaling complexes into the inflammatory response, and investigated the effect of therapy administration timing as well as the effects of timing and dosage amount of endotoxin on the system, similar to Day et al. [66]. Though a formal bifurcation analysis was not conducted, a change in the end-behavior of the system was observed when the initial endotoxin load was increased.

Inflammation models with a more complex, detailed representation of the immune response (shown in Figure 3.1b) incorporate the specific influence of immune cells, cytokines, or pathways and are generally represented by larger systems of equations. Roy et al. [213] and Brady et al. [29] proposed similar sized models studying the immune response to an endotoxin challenge. Both models included the cytokines TNF-α, IL-6, and IL-10. Roy et al. [213] calibrated their model to rat data and included dynamic terms describing phagocytic immune cells and
tissue damage, while Brady et al. [29] used human data and included other model terms such as monocytes and the cytokine IL-8 to study the correlation between normal and abnormal immune responses and heart rate variability. Unlike Roy et al. [213] and Brady et al. [29], Torres et al. [257] focused on solely modeling immune cells (macrophages and neutrophils) and also accounted for the polarization of macrophages. While the above mathematical models focused on the innate immune response, Su et al. [246] included components from the adaptive immune response and created a large system of partial differential equations that incorporates dendritic cells, macrophages, neutrophils, and T cells in addition to cytokine and chemokine states. Su et al. [246] reinforced the importance of dendritic cells and T cells on a successful immune response recovery.

The inflammation models presented here provide a good foundation for dynamically studying the immune response, but these models either (i) are too generalized to determine any tangible impact of an element on the system, (ii) omit the use of real data to calibrate their dynamics or use experimental data from a bolus endotoxin challenge, or (iii) incite a quickly decaying inflammatory stimulus not representative of prolonged inflammation. Thus, a specific inflammation model that utilizes data from a better representation of prolonged inflammation such as a continuous infusion of endotoxin [130] is needed to better study inflammation during systemic events like sepsis. We develop the first mathematical immune response model for this calibrated to experimental data in Chapter 5, which has the same level of detail and is based on the model by Brady et al. [29].

3.2 Circulation Models

Cardiovascular complications are common in many diseases such as hypertension, hypotension, and heart failure, and numerous studies have modeled cardiovascular components in order to gain understanding of these conditions. In this thesis, we focus particularly on the mathematical modeling of the circulation, as it is one of the primary points of impact of inflammation on the cardiovascular system. The circulation is generally modeled in two ways, and the method used is typically dependent upon the goal of the model. The first method utilizes a multi-dimensional model that explores blood flow in both time and space, where the spatial component is modeled in one, two, or three dimensions. This type of model is advantageous to use when studying blood flow in a particular location in the circulation [28]. Examples of these types of models are seen in Marsden and Esmaily-Moghadam [159], Colebank et al. [53], Bartolo et al. [15]. The second method focuses on the modeling of the circulation by lumping sections of the circulatory system into compartments and using an electrical circuit analogy to describe
Table 3.1: Table of inflammation models published in literature.

<table>
<thead>
<tr>
<th>Publication</th>
<th>Model States</th>
<th>Data</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Generalized Inflammation Models</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kumar et al. (2004)</td>
<td>Pathogen Early pro-inflammation Late pro-inflammation</td>
<td>None</td>
<td>Symptoms of sepsis appear in several inflammation scenarios. Treatment may differ depending on the state of a patient.</td>
</tr>
<tr>
<td>Reynolds et al. (2006)</td>
<td>Pathogen Pro-inflammation Anti-inflammation Tissue Damage</td>
<td>None</td>
<td>Anti-inflammation is important to return the system to homeostasis. Treatment for inflammatory conditions may want to focus on promoting anti-inflammation.</td>
</tr>
<tr>
<td>Day et al. (2006)</td>
<td>Pathogen Pro-inflammation Anti-inflammation Tissue Damage</td>
<td>Mice</td>
<td>Endotoxin administration time, dose amount, and type of endotoxin dose all influence the inflammatory response outcome during an endotoxin challenge.</td>
</tr>
<tr>
<td>Foteinou et al. (2009)</td>
<td>Endotoxin and complexes Receptors Pro-inflammation Anti-inflammation Energy</td>
<td>Human</td>
<td>Early intervention of therapy within 2-4 hours during unresolved inflammation can help recovery. Endotoxin administration time and dosing play a key role in the system differentiating between responses.</td>
</tr>
<tr>
<td><strong>Complex Inflammation Models</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Su et al. (2009)</td>
<td>Antigens Cells Cytokines Chemokines</td>
<td>None</td>
<td>The influence of dendritic cells and T cells on the immune response is important for a healthy resolution. Regulatory T cells help balance the system response from macrophages.</td>
</tr>
<tr>
<td>Roy et al. (2012)</td>
<td>Endotoxin Immune cells Cytokines IL-10 promoter Anti-inflammatory mediators Tissue damage</td>
<td>Rat</td>
<td>The immune response exhibits nonlinear behavior across multiple endotoxin challenge scenarios of varying dose amount.</td>
</tr>
<tr>
<td>Brady et al. (2018)</td>
<td>Endotoxin Immune cells Cytokines</td>
<td>Human</td>
<td>There's a proposed correlation between the immune response and heart rate variability (HRV). Individuals with abnormal immune responses had increased HRV, hypothesized to be from enhanced signaling from vagal nerves.</td>
</tr>
<tr>
<td>Torres et al. (2019)</td>
<td>Pathogen Inflammatory stimulus Immune cells</td>
<td>Mice</td>
<td>Apoptotic neutrophils provide significant stimuli that enacts the immune response. Anti-inflammatory macrophage dynamics corresponds to neutrophil response. Intervention timing when could incite chronic inflammation.</td>
</tr>
</tbody>
</table>
the flow between them. Here, blood flow is analogous to current, pressure to voltage, volume to charge, compliance to capacitance, and resistance remains the same in both applications. Compartmental modeling is advantageous when studying the circulatory system as a whole [28] and when investigating connections between the cardiovascular system and other systems [158]. The mathematical model of the cardiovascular system in this thesis uses compartmental modeling, so we focus on describing compartmental circulatory models for the remainder of this subsection.

 Compartmental models of the circulation in literature are subdivided into two types depending on the influence of the heart. Pulsatile circulation models incorporate the pulsation of the heart, so they can be used to study its beat-to-beat impact on the circulation, while non-pulsatile models ignore the beat-to-beat rhythm to study average effects of the heart on the circulation. Pulsatile models are insightful when studying the circulation on small time scales such as seconds or minutes, while non-pulsatile models are more appropriate for studying long-term cardiovascular behavior over hours or days [28, 158].

 Models such as Ursino [259], Mukkamala and Cohen [173], Heldt et al. [102], Olufsen et al. [183], Williams et al. [287], Marquis et al. [158] incorporate pulsatile effects of the heart through a time-varying elastance function, which make them more computationally expensive. Ursino [259] studied the baroreceptor control of arterial pressure by creating an eight compartment model consisting of the systemic and pulmonary circulations, the left and right heart, and signaling/effect pathways. To study the pulsatile behavior of non-invasive blood pressure, heart rate, and instantaneous lung volume, [173] modeled the systemic and pulmonary circulations

Figure 3.2: Diagrams of two compartmental cardiovascular models of blood flow. (a) Five compartment model from Williams et al. [287] (b) four compartment model from Grodins [91]. See the respective publications for detailed descriptions of each subfigure. These figures are used with permission from Williams et al. [287] and Grodins [91], respectively.
but did so using a six compartment model that encompassed the arteries and veins of both circulations as well as the left and right ventricles. Another model that incorporated pulsatile behavior of the systemic and pulmonary circulations was that by [102] who proposed a ten compartment model with left and right ventricles and compartments denoting the thorax, abdomen, and extremities of the body. This closed loop model was attached to an arterial baroreflex and cardiopulmonary reflex submodel, and the model showed the capacity to simulate short and long term cardiovascular responses to head up tilt (HUT) and lower body negative pressure. A similar sized model was the 11 compartment model created by Olufsen et al. [183], which predicted changes in arterial pressure and blood flow velocity between heartbeats when moving from sitting to standing. This model was coupled with an autonomic and cerebral regulation model, but only modeled the systemic circulation. Two additional pulsatile models are those in Williams et al. [287] (shown in Figure 3.2a) and Marquis et al. [158], which both use a five compartment model of the systemic circulation with compartments signifying the left ventricle, large (upper body) arteries and veins, and small (lower body) arteries and veins. The model by Williams et al. [287] focused on fitting arterial pressure changes during HUT using heart rate as an input, while Marquis et al. [158] created an identifiable model to make more accurate predictions and capture the uncertainty of the model results.

Other circulation models, including those by Grodins [91], Ursino et al. [260], Pstras et al. [201], Williams et al. [286], model blood flow using a non-pulsatile heart. As shown in Figure 3.2b, Grodins [91] proposes a four compartment model consisting of the left and right heart and in series systemic and pulmonary circuits. Using the Frank-Starling Law to describe the behavior of the heart and mass-balance equations to describe flow between compartments, this model simulates steady state conditions in the circulation and was influential in the development of later cardiovascular models [60]. To study dynamic patterns of cardiovascular quantities across small timescales after acute carotid sinus activation in subjects undergoing a vagotomy, Ursino et al. [260] created a six compartment model. The compartments included the left and right heart, systemic and pulmonary arteries, and systemic and pulmonary veins as well as used a mean filling pressure. More recently, Pstras et al. [201] adapted the model from Ursino et al. [260] to form a nine compartment model of the circulation encompassing the left and right heart, aorta, systemic and pulmonary arteries, systemic and pulmonary veins, the systemic capillaries, and the vena cava. This model investigated the response to the Valsalva maneuver, and showed that the effect of nonlinear pressure-volume relationships in the systemic veins is important to accurately model the Valsalva maneuver. Moreover, the non-pulsatile model by Williams et al. [286] investigated cardiovascular responses during HUT, showing the ability of mathematical models to predict immeasurable quantities of interest such as changes in cardiac contractility, vascular resistance, and arterial compliance. The lumped parameter
model consisted of five compartments representing the left ventricle, upper body systemic arteries and veins, and lower body systemic arteries and veins. The non-pulsatile model is obtained by integrating the pulsatile model also described in the publication at filling and ejection times, which produces an average behavior over the cardiac cycle. This thesis uses the cardiovascular model by Williams et al. [286], as it is ideal for studying dynamics over hours.

### 3.3 HPA Axis Models

The HPA axis, which constitutes a complex network of mechanisms that work in tandem to regulate the body’s secretion of hormones, is disrupted during stress disorders such as depression, alcoholism, and anorexia [4, 124]. To better understand what mechanisms play a role in the development of these conditions and recommend therapeutic treatments, mathematical models of hormone dynamics have been developed. As discussed in Chapter 2, the HPA axis strongly influences inflammation dynamics, which is why we study these types of models in this thesis. While numerous mathematical models of the HPA axis exist in literature, we focus on the most recent models and recognize that the same general structure exists for more seasoned models as well.

Mathematical models of the HPA axis consist of the three major hormones (CRH, ACTH, and cortisol) as dynamic variables [4, 11, 92, 124, 157, 273]. These models all had common relationships between the hormones as seen in Figure 3.3, where CRH stimulated the release of ACTH, and ACTH stimulated the release of cortisol. They also included negative feedback of cortisol onto the production of ACTH, and all but Markovic et al. [157] included negative feedback of cortisol onto CRH. Figure 3.3 also shows that several studies [11, 92, 157, 273] included the influence of the circadian rhythm on CRH production through either the utilization of the sine function [92, 157], Hill functions [11], or a constant term [273], while others [4, 124] did not include circadian influence.

Although the mathematical models share these commonalities, they have different directions and distinctive features such as additional states or pathways that set them apart. The model by Vinther et al. [273], dubbed the "minimal model", included only the interactions specified above but focused on the model’s capability to produce ultradian oscillations. Markovic et al. [157] studied the mechanisms of the HPA axis in response to acute and chronic stress, and included concentration of the adrenal hormone, aldosterone, as a dynamic variable in the model as shown in Figure 3.3. This component was stimulated by ACTH and inhibited the production of cortisol. The models by Andersen et al. [4] and Gudmand-Hoeyer et al. [92] built upon that of Vinther et al. [273] including additional feedback within the system and both
studied the structure of their model to gain insight into depression mechanisms related to hyper- and hypocortisolemia states. The model by Gudmand-Hoeyer et al. [92] also displayed both circadian and ultradian rhythms. Structure-wise, Andersen et al. [4] incorporated mechanisms of the hippocampus through the glucocorticoid receptor (GR) and mineralcorticoid receptor (MR) onto the CRH production, while Gudmand-Hoeyer et al. [92] included a dynamic state incorporating actions of the hippocampus and suprachiasmatic nucleus (SCN) onto the production of CRH, which is shown in Figure 3.3. The study by Bangsgaard and Ottesen [11] also examined depression, identifying parameters related to CRH production and the circadian rhythm as primarily influential in the transition to a hypercortisolemic state. Finally, the model by Karin et al. [124] added two dynamic states related to the functional mass of corticotrophs and adrenal cells as shown in Figure 3.3. These new states accounted for the system response

Figure 3.3: Elements of HPA axis models with stimulation denoted by arrows and inhibition by circles. Commonly included HPA axis model elements (black solid lines) are model states CRH, ACTH, and cortisol (Cort) usually with the influence of the circadian rhythm. These models feature downstream stimulation of CRH, ACTH, and cortisol, and include negative feedback of cortisol onto CRH and ACTH actions. Additional model elements in the literature are shown on the figure in different colors and line patterns. Markovic et al. [157] includes negative aldosterone feedback in the model (pink dashed lines), Andersen et al. [4] includes both positive and negative feedback on CRH from cortisol via different receptors (blue dotted lines), Gudmand-Hoeyer et al. [92] includes regulatory elements from the suprachiasmatic nucleus and hippocampus that have both positive and negative effects on CRH (green dashed-dotted lines), and Karin et al. [124] includes cells at the hypothalamic and adrenal levels that contribute to ACTH and cortisol production (yellow dashed-dotted-dotted lines).
to stress seen over weeks instead of the traditional time scale of days, which could help explain
the trends in ACTH and cortisol seen in stress-induced complications. In this thesis, we use
the model by Bangsgaard and Ottesen [11] since it incorporates all three HPA axis hormones
and models circadian and ultradian rhythms.

3.4 Multi-System Models

The immune system, cardiovascular system, and HPA axis work in conjunction to regulate the
body’s response to stimuli, and the dysregulation of one system can lead to significant dys-
function in the others such as in sepsis, high cholesterol, and hypertension. The relationships
between two or more of these systems have been examined through multiple mathematical
modeling studies shown in Figure 3.4 with varying model complexities. Because we are seeking
to construct a mathematical model that relates all three systems during an inflammation event
to gain understanding of their influence on inflammation and dysfunction progression, we
present several published multi-system mathematical models and describe the gaps that these
studies leave.

Models such as those in Malek et al. [156] and Bangsgaard et al. [10] study the interconnected
dynamics of the innate immune system and the HPA axis during an endotoxin challenge as
shown in Figure 3.4. To study the impact of bidirectional communication between the two
systems, Malek et al. [156] developed a small, five state system of delay differential equations
(DDEs) that encompassed components such as endotoxin, TNF-α, IL-6, ACTH, and cortisol. The
circadian rhythm was modeled using trigonometric functions. Dynamic CRH concentrations
were omitted in favor of a constant CRH stimuli in order to present a simplified HPA axis
submodel that could still produce circadian and ultradian oscillations. In contrast, the model
by Bangsgaard et al. [10] focused on improving model prediction capabilities to experimental
data and included dynamic concentrations of CRH in addition to ACTH and cortisol. The
circadian influence on CRH was modeled using Hill functions. The model by Bangsgaard et al.
[10] also incorporated a detailed immune submodel by including anti-inflammatory cytokines
IL-10 and TGF-β as well as phagocytic immune cells. The cytokine IL-6 was excluded from the
study.

Mathematical models such as Chow et al. [50] and Dobreva et al. [69] concentrated on
the interplay between inflammatory and cardiovascular dynamics, as shown in Figure 3.4. To
examine whether various shock scenarios could be produced by a singular system under dif-
ferent physiological conditions, Chow et al. [50] introduced a system of 13 ordinary differential
equations (ODEs) that included a detailed innate immune response (endotoxin, resting and
activated macrophages and neutrophils, cytokines TNF-α, IL-6, IL-10, and IL-12, a general inflammation inhibition term, and a tissue damage term) in addition to a subtle cardiovascular response (several nitric oxide elements and blood pressure). On the other hand, to predict optimal therapeutic treatments during sustained inflammation, Dobreva et al. [69] expanded on the work by Chow et al. [50] and proposed a complex 15-state DDE model with a detailed immune response (endotoxin, resting and activated monocytes, and cytokines TNF-α, IL-6, IL-8, and IL-10), a detailed cardiovascular response (nitric oxide, blood pressure, and heart rate) that modeled systemic arterial and venous blood flow, and body temperature and pain influences. While Dobreva et al. [69] focused on model optimization to subject-specific data, both models utilized experimental data from endotoxin challenges (mice data in Chow et al. [50] and human data in Dobreva et al. [69]).

There also exists mathematical models that study the dynamics of the interdependence between the immune system, cardiovascular system, and the HPA axis as shown in Figure 3.4. To study the impact of the circadian rhythm on inflammatory dynamics incorporated with autonomic nervous regulation, Scheff et al. [222] developed a complex mathematical model of

Figure 3.4: Diagram of multi-system models in literature. Red connections denote cardiovascular and immune system models, blue lines denote cardiovascular, immune, and HPA axis models, and black lines denote immune system and HPA axis models.
immune, endocrine, and heart rate interactions. The large system proposed involved equations describing transcriptional and signaling pathways of immune responses, cortisol and melatonin dynamics (with the absence of dynamic CRH and ACTH signaling), autonomic nervous system release of epinephrine, and heart rate variability (HRV). To investigate the changes in heart rate activity in response to autonomic dysfunction and study the anti-inflammatory and cardiovascular impact of multiple inflammation scenarios, Foteinou et al. [80] proposed a similar model describing the dynamics of immune response signaling and transcriptional pathways, cortisol signaling, epinephrine signaling, HRV, autonomic nerve signaling and outflow (including sympathetic and parasympathetic nerve activity), and heart rate.

While the models by Scheff et al. [222], Foteinou et al. [80] incorporated dynamics between the immune, cardiovascular, and endocrine systems, they focused on immune pathways/signaling and used general pro- and anti-inflammation response terms instead of specific cells and cytokines. However, Scheff et al. [222] did utilize experimental data from IL-1β and IL-10 to represent the pro- and anti-inflammatory responses. Their HPA axis models also did not include specific dynamics for CRH and ACTH, and the model by Foteinou et al. [80] omitted specific circadian influence on hormone release and did not produce ultradian oscillations in cortisol dynamics. Furthermore, while their cardiovascular components included heart rate [80], heart rate variability, and autonomic nervous system signaling, Scheff et al. [222], Foteinou et al. [80] neglected to dynamically model the circulation and blood pressure. Moreover, they excluded influences of body temperature and pain perception on dynamics as shown in Figure 3.4. Finally, while Foteinou et al. [80] utilized heart rate and parasympathetic function experimental data, they lacked use of experimental data for their immune and hormone states. Scheff et al. [222] incorporated experimental data from IL-1β, IL-10, cortisol, melatonin, epinephrine, and HRV. Based on these gaps within the multi-system models by Scheff et al. [222] and Foteinou et al. [80] as well as the lack of incorporation of all three systems in the models by Chow et al. [50], Malek et al. [156], Bangsgaard et al. [10], Dobrev et al. [69], there is a need for a multi-system model of immune, cardiovascular, and hormone dynamics that includes (i) an immune response model with prominent cells and cytokines involved in the inflammatory response, (ii) modeling of pre-cortisol hormones CRH and ACTH with evident circadian and ultradian influences, (iii) temperature and pain dynamics, (iv) modeling of the cardiovascular circulation and blood pressure, and (v) specific measurable quantities such as blood pressure and temperature that can easily be identified in a clinical setting. We address this need in Chapter 6 of this thesis by introducing a coupled model of immune, cardiovascular, endocrine, thermal, and pain dynamics.
3.5 Sepsis Models

As the prevalence and financial burden of sepsis has increased in recent years, numerous mathematical models studying sepsis have emerged. Though progress in understanding its pathophysiology has been made, sepsis still has a high mortality rate and viable treatments are not available. Therefore, mathematical modeling is a tool that can be used to increase insight into the dynamics of sepsis, validate current management methods, and test potential therapeutic treatments.

As shown in Table 3.2, mathematical models of sepsis range from fairly simple to very complex ODE systems that often incorporate the dynamics of interconnecting systems in the body. Vasilescu et al. [269] and Gillis et al. [86] proposed very small systems that look at the general immune system response during sepsis. To examine the correlation between mass-specific rate of blood circulation (SRBC) and sepsis patient outcome, Zuev et al. [302] created a four-state model, while Lin et al. [146] investigated the connection of insulin sensitivity to septic progression using a five-state glucose-insulin model. Moderate-sized ODE systems of sepsis of eight and nine states were used by Zhao et al. [300] and Tallon et al. [251] respectively.

Table 3.2: Table of sepsis models published in literature. This table include the number of differential equations (states) in each model, the physiological systems represented in those models (denoted by an X), and the type of sepsis data used in the publication.

<table>
<thead>
<tr>
<th>Publication</th>
<th>Number of States</th>
<th>Immune System</th>
<th>Cardiovascular System</th>
<th>Nervous System</th>
<th>Metabolic System</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>[302]</td>
<td>4</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>Human</td>
</tr>
<tr>
<td>[269]</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Human</td>
</tr>
<tr>
<td>[146]</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Human</td>
</tr>
<tr>
<td>[242][244]</td>
<td>18-19</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td>Rat</td>
</tr>
<tr>
<td>[234][233]</td>
<td>18</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td>None</td>
</tr>
<tr>
<td>[86]</td>
<td>3-4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>None</td>
</tr>
<tr>
<td>[164]</td>
<td>20+</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>Human</td>
</tr>
<tr>
<td>[293]</td>
<td>20+</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>Human</td>
</tr>
<tr>
<td>[300]</td>
<td>8</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td>Mice Rabbit Human</td>
</tr>
<tr>
<td>[251]</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Human</td>
</tr>
<tr>
<td>[46]</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>None</td>
</tr>
</tbody>
</table>
Tallon et al. [251] studied the connection between IL-10 and IL-18 during sepsis, while [300] evaluated the impact of a varying IL-10 treatment over the patient’s disease course.

Larger, more comprehensive ODE systems of sepsis progression are also seen in various studies such as those by Shi et al. [234, 233], Song et al. [242], Stojkovic and Obradovic [244], McDaniel et al. [164], Yamanaka et al. [293], Chen et al. [46]. To study whether the concentration of anti-inflammatory cytokines played a major role in sepsis progression, Shi et al. [234, 233] proposed a detailed acute inflammatory response model of sepsis specifying different innate and adaptive immune cells, cytokines, and sepsis biomarkers. Chen et al. [46] built upon the model by Shi et al. [234, 233] including the differentiation between monocyte-derived type 1 macrophages (M1 macrophage) and monocyte-derived type 2 macrophages (M2 macrophage) as well as adding additional interactions of CD4+ and CD8+ T cells. [242] and [244] built a large system focused on studying the innate immune system and metabolism, including metabolic products such as creatinine (CRT) and alanine aminotransferase (ALT). To study the interactions between the immune, cardiovascular, and nervous system during sepsis, [293] proposed a large coupled mathematical model of septic shock. The model used the simplified inflammatory response model from [207] while incorporating cardiovascular and sympathetic nervous system markers of septic shock, including vasodilation, increased vessel permeability, decreased stroke volume, and fatigue. To test septic treatments on virtual patients such as fluid administration and vasopressors, [164] created a comparably large system that incorporated elements of the immune, cardiovascular, and nervous systems. As shown in Table 3.2, several models used animal or human data to validate their sepsis predictions.

Of particular importance when modeling sepsis is the inclusion of at least one state that represents organ dysfunction, as this is an essential part of the current definition of sepsis as discussed in section 2.5. Though some models specified a particular quantity (cardiovascular or metabolic) as a sign of organ damage in the system, most models represent tissue and organ dysfunction with a non-dimensional damage state. This generalized state portrayed the overall well-being of the system, and an increase in damage signified a major decline in health of a septic individual. Although there were slight differences in how some mathematical models represented the change in damage (shown in Table 3.3), most models [50, 207, 302, 234, 233, 164, 293, 46] cited immune cells such as phagocytes or cytokines such as IL-6 as primary instigators of damage. Other promoters of damage include cardiovascular deviations [50, 293] or exogenous trauma [50]. Factors that provided an inhibitory effect on the progression of damage include the production of anti-inflammatory mediators and nitric oxide [207, 50, 164]. Mathematical models that included a damage term also have it exert either a positive or negative feedback on other model states. A positive feedback on immune cells (phagocytes) and anti-inflammatory mediators is observed in [207, 293, 50, 164] as well as on the production
of HMGB-1 in [234, 233, 46], which incited the production on monocytes (a phagocyte). An inhibitory effect of damage is also seen in [302], where damage decreases the immune cells’ ability to clear the pathogen.

In publications focused on mathematically modeling sepsis, authors often defined multiple clinically relevant scenarios of inflammation progressing from a healthy response to sepsis. As

Table 3.3: Table categorizing how tissue damage is modeled in literature and how it feeds back into the system.

<table>
<thead>
<tr>
<th>Model States</th>
<th>Influence Tissue Damage</th>
<th>Influence Other States</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stimulatory Effect</td>
<td>Inhibitory Effect</td>
</tr>
<tr>
<td>Pathogen</td>
<td>[302]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immune Cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>General Immune Cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phagocytes</td>
<td>[207]</td>
<td>[207]</td>
</tr>
<tr>
<td>Macrophages</td>
<td></td>
<td>[50]</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>[234]</td>
<td>[233]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immune Mediators</td>
<td></td>
<td></td>
</tr>
<tr>
<td>General Immune Mediators</td>
<td>[302]</td>
<td></td>
</tr>
<tr>
<td>Anti-Inflammatory Mediators</td>
<td>[207]</td>
<td>[207]</td>
</tr>
<tr>
<td>IL-6</td>
<td>[50]</td>
<td>[164]</td>
</tr>
<tr>
<td>HMGB-1</td>
<td></td>
<td>[234]</td>
</tr>
<tr>
<td>Cardiovascular and Other Influences</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Pressure</td>
<td>[50]</td>
<td></td>
</tr>
<tr>
<td>Stroke Volume</td>
<td>[293]</td>
<td></td>
</tr>
<tr>
<td>Nitric Oxide</td>
<td></td>
<td>[50]</td>
</tr>
<tr>
<td>External Trauma</td>
<td>[50]</td>
<td></td>
</tr>
</tbody>
</table>
shown in Table 3.4, a healthy infection response was categorized as elevation of the pathogen, immune system responders (cells, cytokines) and cardiovascular markers (respiratory rate, heart rate, and nitric oxide) along with a small decrease in blood pressure and minimal tissue damage. All states returned to normal levels quickly and without any permanent effects. On the other hand, sepsis was exhibited in mathematical modeling simulations as the elevation or clearance of the pathogen, elevated immune system responders (cells, cytokines), and elevated cardiovascular markers (respiratory rate, heart rate, and nitric oxide) along with a major drop in blood pressure, blood volume, stroke volume, energy production (metabolic effects), and significant tissue damage. These system changes do not recover without interventions, and even with treatment, may not lead to improvement.

While existing sepsis models provide a strong foundation to study the dynamics and potential treatments of sepsis, most them either (i) lack detailed immune response models [302, 269, 146, 86, 293, 300], which is important to study the beginning stages of sepsis and inflammation, (ii) lack cardiovascular components [269, 146, 242, 234, 233, 244, 86, 300, 251, 46], which is a significant source of dysfunction during sepsis, (iii) omit the influence of the HPA axis on dysregulation and dysfunction of the system altogether, or (iv) lack human septic data to validate their models [242, 234, 233, 244, 86, 46]. To address these shortcomings, we begin the preliminary construction of a sepsis model in Chapter 7 with immune dynamics validated with sepsis data. Future work will be focused on combining this preliminary sepsis model to the cardiovascular and HPA axis models presented in Chapter 6 to create a comprehensive model capable of capturing immune, cardiovascular, and hormone dysfunction during sepsis.
Table 3.4: Table summarizing clinically-relevant scenarios of inflammation from mathematical models in literature. Conclusions of these behaviors are taken from mathematical modeling simulations in Kumar et al. [137], Reynolds et al. [207], Zuev et al. [302], Song et al. [242], Shi et al. [234, 233], Stojkovic and Obradovic [244], Gillis et al. [86], McDaniel et al. [164], Yamanaka et al. [293], Zhao et al. [300], Chen et al. [46]. Abbreviations: BP, blood pressure; CV, cardiovascular; HR, heart rate; NO, nitric oxide; RR, respiratory rate.

<table>
<thead>
<tr>
<th>State Behavior</th>
<th>Healthy Response</th>
<th>Persistent Non-Infectious Inflammation</th>
<th>Persistent Inflammation</th>
<th>Recurrent Infection</th>
<th>Immuno-suppression</th>
<th>Sepsis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogen</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Oscillations of elevation without recovery</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevation without recovery</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immune (Cells, Cytokines)</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oscillations of elevation without recovery</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevation without recovery</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevation of pro-inflammatory mediators with recovery but elevation of anti-inflammatory mediators without recovery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Tissue Damage</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None or minor damage with recovery</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate tissue damage with recovery</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Major tissue damage without recovery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Cardiovascular (CV)/Metabolic</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None or minor CV changes (RR, HR, NO) with recovery</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated RR, HR, and NO without recovery</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lowered BP with recovery</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lowered BP without recovery</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decreased blood volume with recovery</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decreased blood volume without recovery</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decreased stroke volume without recovery</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decreased energy production without recovery</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>
This dissertation presents new mathematical models of short-term and prolonged inflammation. To calibrate the continuous infusion model in Chapter 5 to both mean and subject-specific data, we utilize sensitivity analysis, subset selection, and parameter estimation methods. We determine the level of uncertainty within our model by computing prediction and confidence intervals and parameter confidence. Finally, to analyze both the experimental data (cytokine peaks and peak timing) and our optimization results across two separate studies, we invoke statistical hypothesis testing. While there are multiple methods that can be used to conduct these types of analyses, this chapter describes the approaches used in this study.

Therefore, we present: (i) the least squares formulation for ordinary differential equations (ODE) models in section 4.1, (ii) sensitivity analysis methods that determine which parameters have the largest impact on system dynamics in section 4.1.1, (iii) subset selection methods such as the structured correlation method and the SVD-QR method, which identify parameters to estimate, in section 4.1.2 (iv) frequentist uncertainty quantification methods used to calculate parameter confidence intervals and model prediction and confidence intervals in section 4.2, and (v) methods of statistical hypothesis testing in section 4.3.
4.1 Parameter Estimation

We define a system of ordinary differential equations (ODEs) to take the form

\[ \frac{dx}{dt} = f(t, x(t), \theta) \]  
\[ y(t) = g(t, x(t), \theta) \]

with initial condition \( x(0) = x_0 \) where the state vector is given by \( x(t) = (x_1(t), x_2(t), \ldots, x_p(t))^T \), the parameter vector is given by \( \theta = (\theta_1, \theta_2, \ldots, \theta_q)^T \), and the model output is given by \( g(t, x(t), \theta) \).

To fit the mathematical model in equations (4.1) and (4.2) to experimental data, sensitivity analysis and subset selection methods are used to determine an identifiable set of sensitive parameters that can be estimated uniquely given the model and available data [182]. Parameters are then estimated using optimization that minimizes the least squares error between the model and the data. We assume that the model output \( g \) and experimental data \( Y \) at time \( t_i \) for \( 1 \leq i \leq N \) observations are related via the statistical model

\[ Y_i = g(t_i, x(t_i), \theta) + \epsilon_i, \]  

where \( \epsilon_i \sim \mathcal{N}(0, \sigma^2) \) with unknown variance \( \sigma^2 \) [12].

The inverse problem described above involves solving the following least squares formulation to find the optimal parameter vector \( \hat{\theta} \), which is given by

\[ \hat{\theta} = \arg \min_{\theta} J(\theta), \]  

where

\[ J(\theta) = \sum_{i=1}^{N} [g(t_i, x(t_i), \theta) - Y_i]^2. \]

The least squares cost \( J \) is minimized by finding parameter vector \( \theta \) that minimizes the distance between the model output, \( g(t_i, x(t_i), \theta) \), and the observed data, \( Y_i \), at time \( t_i \). Equation (4.5) can be more compactly written as

\[ J(\theta) = r^T r, \]  

which is the inner product of the residual \( r = [r_1, r_2, \ldots, r_N]^T \) where \( r_i = g(t_i, x(t_i), \theta) - Y_i \). The least squares cost in equations (4.5) and (4.6) assumes that all observations have equal importance. However, weights could be applied to treat some observations with higher importance than others [12]. Because the models presented in this dissertation incorporate parameters with physiologically-relevant quantities that operate on various levels of magnitude, we utilize
logarithmic scaling of the parameters, where

$$\hat{\theta} = \log(\theta).$$

(4.7)

The logged parameters $\hat{\theta}$ makes for easier analysis and computation of the system, and this parameter conversion works in the models here since all parameters are positive.

To best determine the parameter set $\theta$ that minimizes $J$, sensitivity analysis and subset selection methods are implemented. These methods determine which parameters to optimize and fix, and those that are optimized should be both sensitive and identifiable parameters. Sensitivity and identifiability analysis methods are discussed in the following subsections.

### 4.1.1 Sensitivity Analysis

Sensitivity analysis involves determining the influence of parameters on the model output and can be studied from a local or global perspective. *Local sensitivity analysis* quantifies the impact of a small parameter perturbation in the neighborhood of the nominal value on the model output, while *global sensitivity analysis* investigates the influence of parameters on the model output when parameter values are varied within a parameter space. Local sensitivity analysis examines each parameter individually, while global sensitivity analysis studies multiple influences at once [241, 181]. This dissertation uses local sensitivity analysis.

The local parameter sensitivity is given by the matrix

$$\chi = \frac{\partial y}{\partial \theta} = \begin{pmatrix} \frac{\partial y_{i1}}{\partial \theta_1} & \cdots & \frac{\partial y_{i1}}{\partial \theta_q} \\ \vdots & \ddots & \vdots \\ \frac{\partial y_{iN}}{\partial \theta_1} & \cdots & \frac{\partial y_{iN}}{\partial \theta_q} \end{pmatrix},$$

(4.8)

where $y_{ji} = g_j(t_i, x(t_i), \theta)$ for $1 \leq j \leq M$ and $M$ is the number of states in the model output. Each entry of $\chi$ quantifies how the model output of a specific state changes at a certain time with respect to a model parameter [182]. For ODE systems where an explicit solution is able to be obtained, sensitivities can be determined algebraically by the sensitivity equations. For smooth
functions, these can be derived by determining the following set of differential equations

\[
\frac{d}{d\theta} \left( \frac{\partial x}{\partial \theta} \right) = \frac{\partial}{\partial \theta} \left( \frac{dx}{dt} \right) = \frac{df}{dx} \frac{\partial x}{\partial \theta} + \frac{df}{d\theta}
\]

(4.9)

where \( \frac{df}{dx} \) and \( \frac{df}{d\theta} \) are the model and parameter Jacobian respectively, and \( \frac{\partial x}{\partial \theta} \) is found by differentiating the model in equation (4.1) with respect to the parameters [12, 181]. Solving the system of sensitivity equations in (4.9) along with the original system of ODEs in equation (4.1) would produce the sensitivities in \( \chi \). Thus, a system of ODEs with \( p \) states and \( q \) parameters results in solving a system of \( p \times q \) sensitivity equations [12].

For systems of ODEs without analytical solutions, it is necessary to compute the sensitivities numerically. The sensitivity for parameter \( \theta_k \) can be computed using automatic differentiation [73], the complex step method [160], or derivative approximation methods such as forward differences

\[
\frac{\partial y_{ji}}{\partial \theta_k} \approx \frac{g_j(t_i, x(t_i), \theta + he_k) - g_j(t_i, x(t_i), \theta)}{h},
\]

(4.10)

which uses a first-order Taylor series expansion, or central differences

\[
\frac{\partial y_{ji}}{\partial \theta_k} \approx \frac{g_j(t_i, x(t_i), \theta + he_k) - g_j(t_i, x(t_i), \theta - he_k)}{2h},
\]

(4.11)

which uses a second-order Taylor series expansion [37, 181]. In these approximation methods, \( e_k \) is the \( k \)th standard basis vector and, because the model in equation (4.1) was computed with a relative error tolerance \( \phi \), \( h = \sqrt{\phi} \) is the step size [198]. While central differences achieve higher accuracy in solutions than forward differences (\( O(h^2) \) versus \( O(h) \)), it is more computationally expensive. Central differences requires \( 2q \) function evaluations versus forward differences which requires \( q + 1 \) function evaluations [37]. Other methods for computing local sensitivities include the complex step method. This thesis utilizes forward differences to numerically approximate the sensitivity matrix. Since the functions analyzed in this thesis change relatively slowly, this method should provide us with reasonable results.

Because parameter values and model states may have different magnitudes and units, it can be appropriate to instead determine the relative sensitivity of the parameter given by

\[
\hat{\chi} = \frac{\partial y}{\partial \theta} \hat{\theta}
\]

(4.12)

where \( \hat{y} \) is some characteristic of \( y \) (mean, median, maximum). To compare the parameter sensitivities, a metric can be determined to quantify each of the time-varying sensitivities in \( \chi \).
This can be computed by taking the two-norm of each column of $\chi$, 

$$S_k = \|\chi(k)\|_2,$$  \hspace{1cm} (4.13)

for $1 \leq k \leq q$ parameters. The singular quantities, $S_k$, representing the sensitivity for each of the $q$ parameters can be ranked from largest to smallest (most sensitive to least sensitive) by dividing each $S_k$ by the maximal sensitivity [182]. Given that the integration tolerance of the ODE solver is $\rho$, the sensitive parameter error is $O(\sqrt{\rho})$. Thus, the threshold for sensitive parameters is set at $\phi = 10\sqrt{\rho}$ since the sensitivities are numerical approximations of the Jacobian [198]. Therefore, a group of $n$ sensitive parameters and $n - q$ insensitive parameters are formed. Parameters deemed insensitive are fixed at their nominal values, and parameters rendered sensitive proceed through additional analysis to determine if they can be estimated [182].

While local sensitivity analysis can be useful if the model's nominal parameter values are strong, it can be advantageous to conduct a global sensitivity analysis. Since the local sensitivity is based on nominal values, parameters with large uncertainties may not be valid near optimal values if the nominal value changes. However, global sensitivity analysis can uncover whether parameters categorized as insensitive locally remain insensitive. Global sensitivity analysis methods include Delayed Rejection Adaptive Metropolis (DRAM) [97]. We conduct a manual sampling of nominal parameter values from a distribution in Chapter 5 of this thesis to examine local sensitivity in a small region close to nominal values.

### 4.1.2 Subset Selection

Once a subset of sensitive parameters is determined, that subset is reduced to one that is also identifiable, meaning that the parameters can be found uniquely given available data. There are two types of identifiability: structural and practical identifiability.

**Structural Identifiability:** The model in equations (4.1) and (4.2) is defined as structurally identifiable if there exists a unique parameterization of the model for any given output. A specific parameter is considered globally structurally identifiable if $f(t, x(t), \theta_i) = f(t, x(t), \tilde{\theta}_i)$ implies $\tilde{\theta}_i = \theta_i$. If varying the parameter $\theta_i$ to $\tilde{\theta}_i$, which is in a small neighborhood near $\theta_i$, and $f(t, x(t), \tilde{\theta}_i) = f(t, x(t), \theta_i)$ implies $\tilde{\theta}_i = \theta_i$, then the parameter $\theta_i$ is called locally structurally identifiable. If all parameters within a model are structurally identifiable, then it follows that the model is structurally identifiable. If a parameter is not globally or locally structurally identifiable, then the parameter and the model are both considered structurally unidentifiable.
Structural identifiability of a model determines if model parameters can be uniquely identified given the model output and information (equations) about the states in the model. It focuses on the structure of the mathematical model, assuming it to accurately represent the system being modeled, and does not incorporate experimental data into its criteria. There are several \textit{a priori} methods (that use only the model) that can be used to determine structural identifiability including Lie group theory [285], power series expansion [169, 285], the implicit function theorem [169], differential geometry, numerical algebraic geometry [285], and a differential algebra approach involving the construction of input-output equations [169, 275, 285]. A disadvantage of many of these methods is that they are significantly expensive to compute and, therefore, can only be applied to very low-dimensional ODE systems [169, 285]. Thus, computationally inexpensive methods are a main focus of development currently. Joubert et al. [120] recently developed a reparameterization method for model structural unidentifiabilities that cut computational cost. \textit{A posteriori} methods (which utilize available data to determine unidentifiable parameters and only analyze local structural identifiability) have also emerged [285], such as the method of mean optimal transformations by Hengl et al. [103], profile likelihood by Murphy and Van der Vaart [175], Kreutz [135], and Bayesian Markov chain Monte Carlo (MCMC) sampling [285]. However, these methods can still be challenging to implement for large systems, and so continued work is needed to develop computationally inexpensive methods for structural identifiability.

Given that (i) structural identifiability methods cannot feasibly work on all types of models, (ii) structural identifiability assumes accurate model structures with errorless measurements whereas realistic models are a simplification of a biological system that incorporates noisy data [169], and (iii) many models only have portions of available data [182], there is a need address parameter identifiability under these conditions [169, 182]. This method of identifiability is called practical identifiability, and structural identifiability is a necessary but not sufficient condition for practical identifiability [182].

\textit{Practical Identifiability:} A technical definition of practical identifiability is not as readily agreed upon as with structural identifiability, so there are several proposed definitions in literature. Following the definition in Miao et al. [169], parameters are \textit{practically identifiable} if, given the model output \( g \) with measurement error \( \epsilon \sim (0, \sigma^2) \) where data \( y(t, x(t), \theta) = g(t, x(t), \theta) + \epsilon(t) \), then \( y\left(t, x(t), \hat{\theta}_i\right) = y(t, x(t), \theta) \) implies \( \hat{\theta}_i = \theta \). Wieland et al. [285] defines that a model and its data are \textit{practically identifiable} if the estimated parameters have finite confidence intervals.

Thus, a model is practically identifiable if its parameters can be uniquely determined given
the system output and data. While structural identifiability focuses on the structure of the mathematical model, practical identifiability also accounts for the experimental data [182]. A structurally identifiable parameter could be practically unidentifiable given noisy available data [169]. If a parameter is practically unidentifiable, then it could be made identifiable by incorporating additional data into the system. If that is not possible, then an alternative option is to reducing model complexity by fixing parameter values [285].

Because models in practice utilize experimental data, ensuring practical identifiability on parameters is important for obtaining accurate parameter estimates [285]. The two methods used in this thesis, the structured correlation method (SCM) and the singular value decomposition (SVD) QR method, are practical identifiability methods based on local analysis [182]. Other methods of identifiability include MCMC [169, 285], profile likelihood [285], principal component analysis (PCA), the orthogonal method, and the eigenvalue method [169].

**Structured Correlation Method**

The structured correlation method (SCM) utilizes the Fisher information matrix (FIM), defined as

\[ F = \chi^T \chi, \quad (4.14) \]

to determine correlations between sensitive parameters, where \( \chi \) is the sensitivity matrix in equation (4.8). The mathematical reasoning for this is that the SCM is based on the residual sum of squares between error-free measurements and first-order approximation of the model output. Let \( \theta^* \) be the nominal parameter vector. Then the first-order Taylor expansion of the model output at \( \theta \) near \( \theta^* \) is given by

\[ y(t_i, x(t_i), \theta) \approx y(t_i, x(t_i), \theta^*) + \frac{\partial y(t_i, x(t_i), \theta^*)}{\partial \theta} (\Delta \theta) \quad (4.15) \]

where \( \Delta \theta = \theta - \theta^* \) and \( 1 \leq i \leq N \) data points. Thus, the residual sum of squares is given by

\[ \text{RSS}(\Delta \theta) = \sum_{i=1}^{N} \left[ y(t_i, x(t_i), \theta^*) - \left. \left( y(t_i, x(t_i), \theta^*) + \frac{\partial y(t_i, x(t_i), \theta^*)}{\partial \theta} \right) \right|_{\theta = \theta^*} (\Delta \theta) \right]^2 \quad (4.16) \]

\[ = \sum_{i=1}^{N} \left[ \left. \frac{\partial y(t_i, x(t_i), \theta)}{\partial \theta} \right|_{\theta = \theta^*} (\Delta \theta) \right]^2 \quad (4.17) \]

\[ = (\chi \Delta \theta)^T (\chi \Delta \theta) \quad (4.18) \]

\[ = (\Delta \theta) F(\Delta \theta). \quad (4.19) \]
A minimum of $\text{RSS}(\Delta \theta)$ is $F \Delta \theta = 0$. If $F$ is nonsingular, then $\Delta \theta = 0$ is the unique solution ($\theta = \theta^*$) and, so, the parameters are identifiable locally at the parameter vector $\theta^*$. However, if $F$ is singular, then the solution $\Delta \theta = 0$ is not a unique solution and so the parameter vector $\theta$ is not locally identifiable at $\theta = \theta^*$. This analysis is only valid locally, and cannot infer global identifiability [169].

If $F$ is singular, then studying the columns of the sensitivity matrix, $\chi$, can determine which columns of $F$ are correlated. This corresponds to finding the correlations between parameters. To determine if $F$ is singular, its condition number can be computed, which is given by

$$\kappa(F) = \frac{\sigma_1}{\sigma_q},$$

where $\sigma_1$ and $\sigma_q$ are the largest and smallest singular values of the Fisher information matrix respectively. If the condition number is large, the matrix is considered singular or close to singular. This indicates that columns of the sensitivity matrix suspected to be correlated based on the model structure need to be manually removed to reduce the condition number before proceeding. Thus, a nonsingular matrix, $F$, would be remaining.

With $F$ nonsingular and columns $\chi_i$ and $\chi_j$ of the sensitivity matrix, the correlation between parameters $i$ and $j$ can be determined by computing the covariance matrix, $C$, where

$$C_{ij} = \frac{(F^{-1})_{ij}}{\sqrt{(F^{-1})_{ii}} \sqrt{(F^{-1})_{jj}}}. \quad (4.21)$$

Here, $\sqrt{(F^{-1})_{ii}}$ and $\sqrt{(F^{-1})_{jj}}$ represent the sample standard deviations of $\chi_i$ and $\chi_j$. If the covariance between parameters $\theta_i$ and $\theta_j$ is close to one ($|C_{ij}| \geq \delta$ where $\delta \approx 1$), then parameters $\theta_i$ and $\theta_j$ are correlated and therefore cannot both be estimated. If more than one pair of parameters are correlated, the pair with the largest correlation is selected and the parameter within that pair with the smallest sensitivity is removed from the subset. The process of computing the covariance matrix continues after each correlated parameter is removed until there are no correlations above the threshold $\delta$. The specific value of $\delta$ is set by the user [169, 182].

**Singular Value Decomposition and QR Factorization**

The singular value decomposition (SVD) followed by QR factorization method is a mathematical technique of subset selection where a set of identifiable parameters that can be estimated are determined by the numerical rank of the sensitivity matrix, $\chi$. The numerical rank is found by examining the computational accuracy of the singular values of $\chi$ and is used to reorder and partition the parameter vector $\theta$ into identifiable and unidentifiable subsets. It may also
be advantageous to perform the SCM after the SVD-QR method since the resulting parameter set may still contain correlations between parameters [28]. The steps of the SVD-QR method are detailed below from Olufsen and Ottesen [182], Pope et al. [198].

**SVD-QR Algorithm**

1. For a set of parameters, $\theta$, compute the sensitivity matrix, $\chi$, from equation (4.8).

2. Perform an SVD on $\chi$ such that $\chi = U \Sigma V^T$ where $U$ and $V$ are orthogonal matrices containing the left and right singular vectors of $\chi$, respectively, and $\Sigma$ is the diagonal matrix with nonnegative singular values $\sigma_i$ of $\chi$ arranged in decreasing order along the diagonal.

3. Determine the numerical rank, $\rho$, of $\chi$ by setting a threshold for the smallest allowable singular value based on the relative error tolerance of the ODE solver used in equation (4.8).

4. Partition the matrix $V$ into $V = [V_\rho \, V_{q-\rho}]$.

5. Find a permutation matrix, $P$, such that $V_\rho^T P = QR$ where $Q$ is an orthogonal matrix and $R$ is a matrix whose first $\rho$ columns form an upper triangular matrix with decreasing elements on the diagonal.

6. Using the permutation matrix $P$, reorder parameters such that $\hat{\theta} = P^T \theta$.

7. Partition $\hat{\theta} = [\hat{\theta}_\rho \, \hat{\theta}_{q-\rho}]$. The subset $\hat{\theta}_\rho$ is a set of identifiable parameters that can be estimated, and the parameters in $\hat{\theta}_{q-\rho}$ are to be fixed at their nominal values.

### 4.2 Uncertainty Quantification

Uncertainty quantification focuses on characterizing uncertainties in model parameter values, mean response, and future observations. Generating these intervals are important in the field of drug development as they can provide critical information about the reliability of model estimates surrounding drug effectiveness and adverse events [138].

The two approaches used for uncertainty quantification are frequentist and Bayesian methods. Frequentist methods are governed by assigning probability distributions to data and fixing parameters as an estimate of their true value, while Bayesian methods assign probability distributions to parameters and fix their data. Another important difference between the two is that Bayesian methods incorporate prior knowledge into their predictions as further data is observed, whereas frequentist methods only base their conclusions on the presently collected data [78]. In this thesis, we utilize frequentist methods of uncertainty quantification and discuss
them in detail using the asymptotic analysis described in Banks et al. [13], Seber and Lee [229], and Smith [241]. While Bayesian methods are outside the general scope of this thesis, they have the potential to make more accurate predictions due to their incorporation of prior knowledge, but they are also more computationally expensive to implement. Further detail on Bayesian methods can found in Haario et al. [97], Smith [241], Lye et al. [153].

4.2.1 Parameter Confidence Interval

Using asymptotic analysis, confidence intervals for estimated parameters in \( \theta \) can be derived. Recall that the Fisher information matrix, \( F \), is defined as \( F = \chi^T \chi \) with sensitivity matrix, \( \chi \), defined in equation (4.8). The measurement errors \( \epsilon_i \) for \( 1 \leq i \leq N \) data points defined in the statistical model (equation (4.3)) are assumed i.i.d. and \( \epsilon_i \sim \mathcal{N}(0, \sigma^2) \). If parameter vector estimate \( \hat{\theta} \) minimizes the cost function \( J \), which has sensitivity matrix \( \hat{S} \) and error variance estimator given by

\[
\hat{s}^2 = \frac{1}{N - \ell} J(\hat{\theta})
\]

for number of parameters estimated, \( \ell \), then the sampling distribution for \( \theta \) for large data (\( N \to \infty \)) is given by the multivariate normal distribution

\[
\theta \sim \mathcal{N}(\bar{\theta}, \sigma^2 F^{-1}),
\]

where \( \bar{\theta} \) is the true parameter set. Using \( \tilde{\theta} \approx \hat{\theta} \) and \( \sigma^2 F^{-1} \approx \hat{s}^2 \hat{F}^{-1} \) with \( \hat{F} = \hat{S}^T \hat{S} \), the parameter confidence interval for \((1 - \alpha)\) confidence level is given by

\[
\hat{\theta}_i \pm t_{N - \ell}^{\alpha/2} \hat{s} \sqrt{C_{ii}},
\]

where \( t_{N - \ell}^{\alpha/2} \) is the t-statistic with \( N - \ell \) degrees of freedom and \( C_{ii} \) is defined in equation (4.21) [229, 13, 241].

4.2.2 Prediction Interval

Model prediction intervals quantify the expected distribution with which a new future observation would fall. These prediction intervals are wider than model confidence intervals to account for the uncertainty and random variability in an unobserved measurement [138]. To generate a prediction interval for \( y \) at time \( t_i \) for \( 1 \leq i \leq N \), let

\[
y_i = g(t_i, x(t_i), \bar{\theta}) + \epsilon_i,
\]

where
where \( g(t, x(t), \tilde{\theta}) \) is the model output with true parameter set \( \tilde{\theta} \) and \( \epsilon_i \sim \mathcal{N}(0, \sigma^2) \) is the normally distributed error. We estimate \( y_i \) using the model prediction given by

\[
\hat{y}_i = g\left(t_i, x(t_i), \tilde{\theta}\right),
\]  

(4.26)

where \( \hat{\theta} \) is the optimal parameter set. As \( N \) gets large, \( \hat{\theta} \) becomes close to \( \tilde{\theta} \). Therefore, using a first-order Taylor series expansion of \( g \), we get that

\[
g\left(t_i, x(t_i), \hat{\theta}\right) \approx g\left(t_i, x(t_i), \tilde{\theta}\right) + (\nabla_{\theta}^T g_i)(\hat{\theta} - \tilde{\theta}),
\]  

(4.27)

where

\[
\nabla_{\theta}^T g_i = \begin{bmatrix}
\frac{\partial g(t_i, x(t_i), \hat{\theta})}{\partial \theta_1} & \cdots & \frac{\partial g(t_i, x(t_i), \hat{\theta})}{\partial \theta_q}
\end{bmatrix},
\]  

(4.28)

for \( q \) parameters in \( \hat{\theta} \). Hence, we have that

\[
y_i - \hat{y}_i \approx [g\left(t_i, x(t_i), \hat{\theta}\right) + \epsilon_i] - [g\left(t_i, x(t_i), \tilde{\theta}\right) + (\nabla_{\theta}^T g_i)(\hat{\theta} - \tilde{\theta})],
\]  

(4.29)

\[
y_i - \hat{y}_i = \epsilon_i - (\nabla_{\theta}^T g_i)(\hat{\theta} - \tilde{\theta}).
\]  

(4.30)

Thus,

\[
\mathbb{E}[y_i - \hat{y}_i] = \mathbb{E}[\epsilon_i] - (\nabla_{\theta}^T g_i)\mathbb{E}[\hat{\theta} - \tilde{\theta}] \approx 0,
\]  

(4.31)

and

\[
\text{var}[y_i - \hat{y}_i] \approx \text{var}[\epsilon_i] - \text{var}[(\nabla_{\theta}^T g_i)(\hat{\theta} - \tilde{\theta})],
\]  

(4.32)

\[
\approx \sigma^2 + \sigma^2(\nabla_{\theta}^T g_i)(\chi^T \chi)^{-1}(\nabla_{\theta} g_i),
\]  

(4.33)

\[
= \sigma^2(1 + v_0),
\]  

(4.34)

where \( v_0 = (\nabla_{\theta}^T g_i)(\chi^T \chi)^{-1}(\nabla_{\theta} g_i) \). So, \( y_i - \hat{y}_i \) is asymptotically defined as

\[
y_i - \hat{y}_i \sim \mathcal{N}(0, \sigma^2(1 + v_0)).
\]  

(4.35)

Because the estimator of the variance, \( \hat{s}^2 \) (defined in equation (4.22)), is independent of \( y_i \) and also asymptotically independent of \( \hat{\theta} \), then \( \hat{s}^2 \) is asymptotically independent of \( y_i - \hat{y}_i \). Therefore,

\[
\frac{y_i - \hat{y}_i}{s \sqrt{1 + v_0}} \sim t_{N-\ell},
\]  

(4.36)
asymptotically is the \( t \)-distribution with \( N - \ell \) degrees of freedom. Hence, the \((100 - \alpha)\%\) prediction interval for the model response \( y \) at \( t_i \) is given by

\[
\hat{y}_i \pm t_{N-\ell}^{\alpha/2} \hat{s} \sqrt{1 + \Gamma_i},
\]

where \( \Gamma_i = (\nabla^T \theta g_i) (\chi^T \chi)^{-1} (\nabla \theta g_i) \). \[229, 13, 241\]

### 4.2.3 Confidence Interval

Model confidence intervals quantify the model precision in estimating a statistic (the mean response in this thesis) based on the available information and data. As the number of observations becomes larger, the width of the confidence intervals becomes smaller since the estimated standard error shrinks according to the law of large numbers \[138\]. To generate a confidence interval for the mean response, consider

\[
\hat{y}_i = g(t_i, x(t_i, \hat{\theta})),
\]

to be an estimate of the mean response \( \bar{y}_i = g(t_i, x(t_i, \hat{\theta})) \). Thus,

\[
\mathbb{E}[\hat{y}_i] = \bar{y}_i,
\]

and by equation (4.27) we have

\[
\text{var}[\hat{y}_i] = \text{var}[g(t_i, x(t_i, \hat{\theta})],
\]

\[
= \text{var}[g(t_i, x(t_i, \theta))] + \text{var}[(\nabla^T \theta g_i) (\theta - \hat{\theta})],
\]

\[
\approx \text{var}[(\nabla^T \theta g_i)(\theta - \hat{\theta})],
\]

\[
= \sigma^2 (\nabla^T \theta g_i)(\chi^T \chi)^{-1}(\nabla \theta g_i),
\]

\[
= \sigma^2 \nu_0,
\]

where \( \nu_0 = (\nabla^T \theta g_i)(\chi^T \chi)^{-1}(\nabla \theta g_i) \). So, \( \hat{y}_i \) is asymptotically defined as \( \hat{y}_i \sim \mathcal{N}(\bar{y}_i, \sigma^2 \nu_0) \). Therefore, we asymptotically have that

\[
\frac{\hat{y}_i - \bar{y}_i}{\hat{s} \sqrt{\nu_0}} \sim t_{N-\ell},
\]
is the t-distribution with $N - \ell$ degrees of freedom. Hence, the (100-$\alpha$)% prediction interval for the model response $y$ at $t_i$ is given by

$$\hat{y}_i \pm t_{N-\ell}^{\alpha/2} \hat{s} \sqrt{\Gamma_i}, \quad (4.47)$$

where $\Gamma_i = (\nabla^T \theta g_i)(\chi^T \chi)^{-1}(\nabla \theta g_i)$ [229, 13, 241].

### 4.3 Statistical Hypothesis Testing

Hypothesis testing is a statistical method used to determine how often random chance gives an effect as large as observed in the data without any true impact occurring. Hypothesis testing involves a null and alternative hypothesis, typically denoted as $H_0$ and $H_A$ respectively. The null hypothesis is a statement about a population parameter, $\theta$, that indicates no real effect ($H_0 : \theta = \theta_0$), and the alternative hypothesis is a statement about a population parameter that there is a real effect. The alternative hypothesis can either be one-sided ($H_A : \theta > \theta_0$ or $H_A : \theta < \theta_0$) or two-sided ($H_A : \theta \neq \theta_0$). To determine whether to reject or fail to reject the null hypothesis, a test statistic and p-value are computed. The p-value, commonly denoted as $\alpha$, indicates the probability that only random chance would produce the observed test statistic. Common p-values include 0.1, 0.05, and 0.01. If the p-value for the hypothesis test falls below the set threshold, then the resulting test statistic is considered statistically significant. Thus, the test statistic would seldom occur by chance and the null hypothesis would be rejected. If the p-value is above the threshold, then the resulting test statistic would not be considered statistically significant and the null hypothesis would fail to be rejected [49].

In this thesis, we utilize the two-sample unpaired unequal variances $t$-test, a specific type of hypothesis test used when two independent samples of data have unequal variances. A brief discussion on other types of $t$-tests are also included below.

#### 4.3.1 T-Tests

A $t$-test is a hypothesis test that compares the mean of a population or populations, and it has the test statistic, $t$, that follows the t-distribution under the null hypothesis. The t-distribution is similar to the normal distribution since it takes the shape of a symmetric bell-shaped curve. However, it has thicker tails. As the degrees of freedom (the number of independent pieces of information available to calculate the test statistic) increase in the t-distribution due to increases in sample size, though, the tails get thinner and eventually approach the normal distribution [82] as shown in Figure 4.1. The following information is found in Chihara and
Hesterberg [49] and Freund and Wilson [82], but can also be found in many other statistics texts and articles.

There are one sample and two sample student $t$-tests. One-sample student $t$-tests determine if a population has the mean specified in the null hypothesis. It uses the $t$-statistic given by

$$t = \frac{\bar{X} - \mu}{\sqrt{s^2/n}},$$

where $\bar{X}$ is the sample mean, $\mu$ is the theorized population mean stated in the null hypothesis, $s^2$ is the sample variance, and $n$ is the sample size with $n - 1$ degrees of freedom.

A two-sample $t$-test determines whether two population means are equal. There are several types of two-sample $t$-tests depending on the independence, variance, and sample size of the two samples. If two samples are dependent (paired), meaning that a sample was repeated on the same group or two samples were matched, then the paired student $t$-test would be used. Its $t$-statistic is given by

$$t = \frac{\bar{d} - d_0}{\sqrt{s^2_{\bar{d}}/n}},$$

where $\bar{d}$ is the mean of the paired sample differences, $d_0$ is the hypothesized difference in

![Figure 4.1: Comparison of the $t$-distribution and normal distribution. The normal distribution ($\mu = 0, \sigma^2 = 1$) is given by the blue curve, and three Student-$t$ distributions are given by the black dashed lines ($\nu = 1$ df), blue dotted lines ($\nu = 4$ df), and red dashed lines ($\nu = 15$ df). Abbreviations: df, degrees of freedom.](image-url)
population means, \( s_d^2 \) is the estimated variance of the sample differences, and \( n \) is the number of pairs. The paired \( t \)-test has \( n - 1 \) degrees of freedom.

If two samples are independent (unpaired), meaning they consist of two different populations or groups, then the sample size and variance determine the \( t \)-statistic and degrees of freedom. The two-sample unpaired equal variances \( t \)-test (also called the pooled \( t \)-test) assumes equal variances among the two samples of size \( n_1 \) and \( n_2 \). The \( t \)-statistic is given by

\[
t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{(n_1-1)s_1^2 + (n_2-1)s_2^2}{n_1+n_2-2} \cdot \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}},
\]

where \( \bar{X}_1 \) and \( s_1^2 \) are the mean and variance of sample one, \( \bar{X}_2 \) and \( s_2^2 \) are the mean and variance of sample two, and there are \( n_1 + n_2 - 2 \) degrees of freedom. If the sample sizes are the same \( (n = n_1 = n_2) \), then equation (4.50) simplifies to

\[
t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{s_1^2/n_1 + s_2^2/n_2}},
\]

with \( 2n - 2 \) degrees of freedom.

The two-sample unpaired unequal variances \( t \)-test, also known as Welch’s \( t \)-test, is used to compare the means of two samples when those samples have unequal variances and potentially unequal sizes. The \( t \)-statistic is given by

\[
t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{s_1^2/n_1 + s_2^2/n_2}};
\]

with degrees of freedom, \( df \), given by

\[
df = \frac{(s_1^2/n_1 + s_2^2/n_2)^2}{(s_1^2/n_1)^2 \cdot n_1 - 1 + (s_2^2/n_2)^2 \cdot n_2 - 1}.
\]

The two-sample unpaired unequal variances \( t \)-test does not use pooled variances as done in the two-sample unpaired equal variances (pooled) \( t \)-test (equation (4.50)). Furthermore, the two-sample unpaired unequal variances \( t \)-test is thought to be a more powerful but less known tool than the student’s \( t \)-test. It better controls type I (false positive) and type II (false negative) errors, but still performs reasonably well even if variances are similar as long as the underlying assumption of normality is still held [216]. The two-sample unequal variances \( t \)-test is used in Chapter 5 of this dissertation.
The study “Characterization of differences in immune responses during bolus and continuous infusion endotoxin challenges using mathematical modeling” has been submitted for publication to *Experimental Physiology*. The author’s contributions include calibration and statistical analysis of data, determining the continuous infusion portion of the model, obtaining all nominal model fits for mean and subject data, conducting all sensitivity analysis, subset selection, optimization, and uncertainty quantification model results for mean and subject-specific data, executing statistical analysis on optimized parameters, performing simulation results for perturbations in endotoxin infusion duration and dose, and being the primary writer of the manuscript. The motivation for this work is also discussed in a SIAM News Blog [291]
5.1 Abstract

Endotoxin administration is commonly used to study the inflammatory response, and though traditionally given as a bolus injection, it can be administered as a continuous infusion over multiple hours. Several studies hypothesize that the latter better represents the prolonged and pronounced inflammation observed in conditions like sepsis. Yet, very few experimental studies have administered endotoxin using both strategies, leaving significant gaps in determining the underlying mechanisms responsible for their differing immune responses. We use mathematical modeling to analyze cytokine data from two studies administering a 2 ng/kg dose of endotoxin, one as a bolus and the other as a continuous infusion over four hours. Using our model, we simulate the dynamics of mean and subject-specific cytokine responses as well as the response to long-term endotoxin administration. Cytokine measurements reveal that the bolus injection leads to significantly higher peaks for IL-8, while IL-10 reaches significantly higher peaks during continuous administration. Moreover, the peak timing of all measured cytokines occurs later in the continuous infusion. We identify three model parameters that significantly differ between the two administration methods. Monocyte activation of IL-10 is greater during the continuous infusion, while recovery rates of TNF-α and IL-8 are faster for the bolus injection. This suggests that a continuous infusion elicits a stronger, longer-lasting systemic reaction through increased stimulation of monocyte anti-inflammatory mediator production and decreased recovery of pro-inflammatory catalysts. Furthermore, our continuous infusion model exhibits prolonged inflammation with recurrent peaks resolving within two days during long-term (20-32 hours) endotoxin administration.

5.2 Introduction

Endotoxin (lipopolysaccharide, LPS) derived from gram-negative bacteria’s outward membrane [101] is an immunostimulant administered to healthy subjects as an experimental procedure to study the inflammatory response [247]. This type of experiment, referred to as an endotoxin challenge, has allowed insight into mechanisms and treatments of inflammation events such as rheumatoid arthritis [171, 166, 150], systemic lupus erythematosus [301], cancer [72, 105, 295], Alzheimer’s disease [239, 59, 2], and sepsis [77, 81, 235, 143].

In an endotoxin challenge, LPS can be administered as a bolus (instantaneous) injection
[56, 52, 115], a continuous infusion over several hours [21], or a combination of the two [130] in both humans and animals [8]. The response is an increase in pro- (TNF-α, IL-1β, IL-6, IL-8) and anti- (IL-10, IL-1ra) inflammatory cytokines, immune cells, body temperature, heart rate, blood pressure, and hormone levels [87, 8, 52, 115]. The peak of each measured quantity and the time it takes to reach the peak vary depending on a host of controllable (administration method and total dose administered) and uncontrollable (individual variation due to genetics, sex, and health status) factors.

Taudorf et al. [254] performed an endotoxin challenge in healthy men administering 0.3 ng/kg of LPS as a bolus and a continuous infusion over 4 hours. They found that the administration method significantly affects TNF-α, IL-6, and neutrophil production rates. These measured quantities peaked earlier and had larger magnitudes during the bolus administration than the continuous infusion. Kiers et al. [130] compared immune responses to 1 and 2 ng/kg bolus doses of LPS in addition to a 1 ng/kg bolus followed by a 3 ng/kg continuous infusion over 3 hours. This study showed a significant difference in mean cytokine concentrations, flu-like symptoms (headache, nausea, shivering, pain), temperature, and heart rate increases between the bolus-only and the bolus plus continuous infusion dose. Cytokines responses (TNF-α, IL-6, IL-8, IL-10) reached significantly higher peak levels, and subjects exhibited prolonged elevated flu-like symptoms during the bolus plus continuous infusion method. These results demonstrate that continuous infusion initiates a more durable and occasionally more pronounced impact on the immune response during the incitement of inflammation.

Although experimental studies are suitable for investigating the effects of endotoxin administration method, they do not provide insight into why differing dynamics are observed. This is where the power of mathematical modeling of physiological systems can be applied. Simulations with mathematical models can highlight the underlying mechanisms of disease, aid in disease diagnosis, test and validate treatments and predict patient trajectory and mortality. Numerous mathematical models of inflammation have been developed over the last two decades. Kumar et al. [137], Day et al. [66], Reynolds et al. [207] developed small but novel mathematical models, highlighting their ability to reproduce inflammation scenarios of clinical relevance and potential to predict treatment strategies. Several models built upon this foundation by adding specific immune cells and cytokines activated during the inflammatory response [50, 213, 79, 246, 190, 29, 257]. Others created detailed model incorporating feedback from other physiological entities such as the cardiovascular system, nervous system, the hypothalamic-pituitary-adrenal (HPA) axis, pain perception, and thermal responses [222, 80, 156, 10, 69, 288]. Several models were calibrated to experimental data or validated in specific patients. Some used data from a bolus endotoxin challenge in animals (mice or rats) [50, 66, 213, 190, 257] while others used bolus data from human subjects [79, 222, 80, 156, 10, 29, 69, 288]. These studies
demonstrate the need for computational inflammation models that (i) utilize experimental data from a continuous infusion of endotoxin and (ii) investigate the mechanisms behind response differences observed during variations in endotoxin administration method.

Recent experimental studies [130, 266] propose that a continuous endotoxin infusion is more appropriate to study the prolonged system response during systemic inflammation and sepsis. To provide more insight into understanding what immune signaling components are impacted during the switch from a bolus to continuous infusion, we study the inflammatory response to continuous infusion of endotoxin through the lens of a mathematical model. Doing so provides (i) newfound insight into the response differences between a bolus and continuous administration of endotoxin, (ii) a better mathematical representation to study the dynamics of sepsis, and (iii) a better model to investigate treatments of inflammatory conditions since the continuous infusion prolongs the exposure window for treatment testing.

We present a novel inflammatory response mathematical model predicting innate cytokine responses (TNF-α, IL-6, IL-8, IL-10) to a 2 ng/kg bolus and four-hour continuous infusion of endotoxin with experimental data from Janum et al. [115] and Berg et al. [21]. The model structure is rigorously explored through sensitivity and identifiability analysis, and parameter estimation calibrates the model to mean and subject-specific cytokine data. We compare each study’s cytokine data to characterize larger endotoxin doses than compared in previous literature and develop statistical uncertainty bounds for the optimal mean model. This hypothesis-generating study suggests mechanisms responsible for varying immune dynamics observed in bolus and continuous infusion experimental studies via statistical analysis of optimized model parameters. We propose that the transition from a bolus to continuous infusion impacts physiologically-relevant components related to IL-10 activation by monocytes and TNF-α and IL-8 degradation rates. Moreover, we use our continuous infusion model to investigate the system response to perturbations in infusion duration and total endotoxin dose administered. This illustrates its ability as a clinically-realistic in silico model that can simulate prolonged and pronounced responses.

5.3 Methods

5.3.1 Ethical approval

The current work utilized experimental data from two published studies by Berg et al. [21] and Janum et al. [115]. The study by Berg et al. [21] was approved by the Scientific Ethical Committee of Copenhagen and Frederiksberg Municipalities in Denmark. The data from Berg et al. [21] was made available by Berg (coauthor of this study). The study by [115] received approval
for the experimental protocol by the Regional Committee on Health Research Ethics and the Regional Monitoring Board, and the study followed the protocols listed in the Declaration of Helsinki. Individual data from Janum et al. [115] was made available by Janum (coauthor of this study) and Mehlsen (coauthor of Janum et al. [115]). All participants from both studies gave their written and oral consent.

5.3.2 Experimental data

The experiments by Berg et al. [21] and Janum et al. [115] administered the same total dose of endotoxin (2 ng/kg) to healthy study participants, one as a continuous infusion and one as a bolus injection. Mean and subject-specific cytokine data were measured and used to calibrate our mathematical model.

The study from Berg et al. [21] investigated the effects of an increase in mean arterial pressure on cerebral autoregulation; it included nine healthy male participants aged 21-25. All study participants were subject to physical examination. Data were only included from subjects with normal blood work and cardiovascular markers. Participants did not take medication, had a typical medical history, were non-sedentary, and infection-free at least four weeks before the study. The study by Janum et al. [115] was designed to investigate the connection between pain and the innate immune system reaction in 20 male athletes aged 18-35. All study participants had a healthy weight, were non-smokers, and had no signs of illness two weeks prior to the study day. Pre-screening activities involved a review of each subject’s medical history, a physical examination, and laboratory work.

In Berg et al. [21], participants were subject to a 4-hour continuous infusion of 2 ng/kg (0.5 ng/kg/hr) of endotoxin (Batch G2 B274, US Pharmacopeial Convention, Rockville, MD, USA) administered via an antecubital catheter. In contrast, the study participants in Janum et al. [115] received a 2 ng/kg bolus endotoxin dose (Lot EC-6, National Institutes of Health, Bethesda, MD, USA) via a peripheral intravenous catheter following two hours of baseline stabilization. In Berg et al. [21], blood samples were taken hourly for the first 4 hours following the start of endotoxin administration and 2 hours after completed endotoxin administration. Measurements from Janum et al. [115] were taken hourly, starting two hours before endotoxin administration and continuing for six hours following administration. To capture peak response, this study analyzed an additional blood sample taken 1.5 hours after LPS administration. Endotoxin administration and cytokine measurement times for each study are shown in Figure 5.1. Janum et al. [115] used ELISA (Meso Scale Discovery, Rockville, Maryland, USA) and Berg et al. [21] used SECTOR Imager 2400 (Meso Scale Diagnostics, Gaithersburg, MD, USA) to determine concentrations of TNF-\( \alpha \), IL-6, IL-8, and IL-10.
Subjects 1, 2, 6, 8, and 9 from the continuous infusion study were missing one cytokine measurement, and subject 4 was missing four measurements. Of these, subjects 4, 8, and 9 were missing baseline concentration measurements of IL-6. Subject 4 also did not have a baseline concentration of IL-10. Figure 5.2 shows data from both studies, identifying outliers from both

Figure 5.1: Administration times for the bolus (black arrow) [115] and continuous infusion (red arrow) [21]. Times that measurements were taken for each study are denoted by the black (bolus) and red (continuous infusion) bars.

Figure 5.2: Box and whisker plots of the continuous infusion ($m = 9$) [21] and bolus ($n = 20$) [115] data. Black boxplots above the symbol ‘B’ represent bolus data, and gray boxplots above the symbol ‘C’ represent continuous infusion data. The red cross symbol denotes abnormal responses (outliers) from each study. This figure is generated using MATLAB code adapted from Danz [64].
data sets. Because the immune response exhibits significant variation in individual responses to stimuli, we considered these outlying data points abnormal but not unrealistic.

Because of small sample sizes, the influence of abnormal data points on the average cytokine response is significant. Therefore, we calculated the mean cytokine response after removing outlying data points outside the $1.5 \times \text{IQR}$ range, where the IQR encompasses the 25th and 75th percentile of data for each endotoxin data set. Because of the small number of study participants, we only removed abnormal (outlying) measurements instead of that individual's entire cytokine profile. The whisker length was set at MATLAB’s default ($1.5 \times \text{IQR}$) since our data was approximately normally distributed. We used the mean of the bolus and continuous infusion data to calibrate our mathematical model. In the remainder of this study, we refer to this as the mean bolus or continuous endotoxin administration.

Mean and subject-specific cytokine characteristics are reported in Table 5.1. Primary pro- and anti-inflammatory cytokines TNF-α and IL-10 had higher mean peak concentrations during continuous infusion, while secondary cytokines IL-6 did not depend on the administration method, but IL-8 had a higher peak value for bolus injection. With the continuous infusion, peak concentrations were later for all measured cytokines. Individual subject concentrations from both studies displayed a considerable variation in cytokine responses, where TNF-α,

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Study</th>
<th>TNF-α</th>
<th>IL-6</th>
<th>IL-8</th>
<th>IL-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD) Baseline</td>
<td>Bolus</td>
<td>1.02 (0.155)</td>
<td>0.911 (0.314)</td>
<td>3.01 (0.767)</td>
<td>0.204 (0.0864)</td>
</tr>
<tr>
<td>Concentration (pg/mL)</td>
<td>Continuous</td>
<td>6.24 (1.38)</td>
<td>0.610 (0.159)</td>
<td>2.96 (0.648)</td>
<td>4.20 (1.53)</td>
</tr>
<tr>
<td>Peak of Mean Concentration (pg/mL)</td>
<td>Bolus</td>
<td>326</td>
<td>702</td>
<td>714</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Continuous</td>
<td>532</td>
<td>707</td>
<td>456</td>
<td>136</td>
</tr>
<tr>
<td>Peak of Subject Concentration (pg/mL)</td>
<td>Bolus</td>
<td>60-1297</td>
<td>351-1856</td>
<td>522-1124</td>
<td>20-105</td>
</tr>
<tr>
<td></td>
<td>Continuous</td>
<td>212-1293</td>
<td>303-1335</td>
<td>170-683</td>
<td>57-376</td>
</tr>
<tr>
<td>Peak Timing of Mean Concentration (pg/mL)</td>
<td>Bolus</td>
<td>1.5</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Continuous</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Peak Timing of Subject Concentration (pg/mL)</td>
<td>Bolus</td>
<td>1.5-2</td>
<td>2-3</td>
<td>1.5-3</td>
<td>2-3</td>
</tr>
<tr>
<td></td>
<td>Continuous</td>
<td>3-4</td>
<td>4</td>
<td>3-4</td>
<td>3-6</td>
</tr>
</tbody>
</table>
IL-6, and IL-8 had a higher variance with bolus injection. Cytokine baseline values reported in Table 1 are all within the normal range [23], but the continuous infusion study has higher baseline values for TNF- and IL-10 than the bolus study. We hypothesize this variation could stem from (i) natural differences in cytokine levels in healthy individuals, which are known to vary markedly [253], (ii) different study protocols between the two groups such as the impact of invasive procedures like catheterization on physiological stress, or (iii) different experimental time courses of the studies, which may have given rise to systematic error in relation to the assaying method.

5.3.3 Data calibration

To compare the cytokine responses from the two studies, we adjusted the bolus data so that both studies had the same baseline concentration. This was done by determining the difference ($\bar{d}_0^j$) between the mean bolus ($\bar{b}_0^j$) and continuous infusion ($\bar{c}_0^j$) baseline

$$\bar{d}_0^j = \bar{b}_0^j - \bar{c}_0^j$$

for cytokine $j = \{TNF, IL6, IL8, IL10\}$ and then shifting the concentrations by

$$\hat{b}_i^j(k) = b_i^j(k) - \bar{d}_0^j,$$

where $b_i^j(k)$ is the original cytokine concentration $j$ at time $i$ for the $k$th bolus participant ($1 \leq k \leq 20$). The adjusted cytokine concentration is denoted by $\hat{b}_i^j(k)$. Figure 5.3 displays the mean and subject-specific continuous infusion and bolus data.

5.3.4 Mathematical model

Our mathematical model (Figure 5.4) adapted from our previous studies [29, 69, 288] predicting dynamics of the innate immune response to endotoxin included a system of seven ordinary differential equations (ODEs) with 45 parameters. The equations characterized the time-varying endotoxin dose, the number of resting and activated monocytes, and concentrations of pro- and anti-inflammatory cytokines. Below we briefly describe the model and refer to Brady [28] for a detailed derivation of the equations.

**Endotoxin:** The equation determining the endotoxin concentration ($E$, ng/kg) was adapted from Brady et al. [29], Dobreva et al. [69] to account for a continuous infusion. This formulation is similar to that in Windoloski et al. [288] and motivated by Day et al. [66]. The endotoxin rate
of change was given by

\[
dE dt = \begin{cases} 
D_h - k_E E & t \leq D_{ad} \\
-k_E E & t > D_{ad}
\end{cases},
\]

(5.1)

Figure 5.3: Mean and subject-specific continuous infusion and bolus data. Red circles denote the continuous infusion data \((m = 9)\) connected by solid lines, and black squares denote the bolus data \((n = 20)\) connected by dotted lines. Thin lines represent subject-specific responses and thick vertical lines denote mean (SD).

Figure 5.4: Cytokine interactions. Endotoxin administration recruits (activates) monocytes from a large pool of resting monocytes \((M_R)\). The active monocytes \((M_A)\) upregulates production of pro- \((\text{TNF-\(\alpha\)}, \text{IL-6}, \text{and IL-8})\) and anti- \((\text{IL-10})\) inflammatory cytokines. \text{TNF-\(\alpha\)} generates positive feedback on monocyte, IL-6, and IL-8 production. IL-6 exhibits anti-inflammatory properties through self-regulation, downregulation of TNF-\(\alpha\), and upregulation of IL-10. IL-10 downregulates all cytokine production and the activation of monocytes. Solid black lines represent stimulation, and dotted black lines represent inhibition.
where $D_h$ (ng/kg/hr) was the endotoxin dose administered per hour, $D_{ad}$ (hr) the dosing administration duration, and $k_E$ (hr$^{-1}$) the endotoxin decay rate. The 2 ng/kg continuous infusion was administered over 4 hours so $D_h = 0.5$, $D_{ad} = 4$, and $E(0) = 0$, whereas $D_h = 0$, $D_{ad} = 0$, and $E(0) = 2$ for the bolus injection.

**Monocytes:** During the endotoxin challenge, resting monocytes circulating in the blood are activated, upregulating cytokine production. This process regulates inflammation via positive and negative feedback [212]. The resting ($M_R$) and activated ($M_A$) monocytes (number of cells, noc) were found from

$$\frac{dM_R}{dt} = k_{MR}M_R \left(1 - \frac{M_R}{M_\infty}\right) - H_M^U(E)\left(k_M + k_{MTNF}H_M^U(TNF)\right)H_M^D(IL10)M_R \quad (5.2)$$

$$\frac{dM_A}{dt} = H_M^U(E)\left(k_M + k_{MTNF}H_M^U(TNF)\right)H_M^D(IL10)M_R - k_{MA}M_A, \quad (5.3)$$

where $k_{MR}$ (hr$^{-1}$) denoted the regeneration rate and $M_\infty$ (noc) the carrying capacity for the resting monocytes. The activated monocytes were upregulated by endotoxin [212] at rate $k_M$ (hr$^{-1}$) and inflammatory cytokine TNF-$\alpha$ at rate $k_{MTNF}$ (hr$^{-1}$). They were also downregulated by anti-inflammatory cytokine IL-10 [136]. This process was activated relative to the resting monocytes. The increase in activated monocytes caused an identical decrease in the resting monocytes. Finally, the activated monocytes decayed at rate $k_{MA}$ (hr$^{-1}$).

In equations (5.2)-(5.3), the upregulation or downregulation of state $Y$ by state $X$ was as

$$H_Y^U(X) = \frac{X^h}{\eta_{YX}^X + X^h}, \quad H_Y^D(X) = \frac{\eta_{YX}^h}{\eta_{YX}^X + X^h},$$

where $h$ represents the steepness of the curve and $\eta_{YX}$ the half-maximum value.

**Inflammatory mediators:** Activated monocytes upregulate cytokines, signaling proteins that promote or suppress inflammation [174]. Cytokines that stimulate inflammation, called pro-inflammatory, include TNF-$\alpha$, IL-6, and IL-8 [118]. TNF-$\alpha$ is an early pro-inflammatory mediator responsible for the induction of fever [174] and recruitment of other pro-inflammatory cytokines [118]. IL-6 is a secondary pro-inflammatory mediator primarily involved in the induction of the liver acute phase response [118], although it can exhibit anti-inflammatory properties as well [255]. IL-8 is a late pro-inflammatory mediator mainly responsible for recruiting neutrophils to the target site [24]. Monocytes also release anti-inflammatory cytokines, particularly IL-10, to counteract pro-inflammatory responses and provide a balanced immune response [118]. These four cytokines are essential components of the innate immune response.
Their interactions can be predicted by

\[
\frac{d \text{T} \text{N} \text{F}}{dt} = k_{\text{T} \text{N} \text{F} \text{M}} H_{\text{T} \text{N} \text{F}}^D (\text{I} \text{L} \text{6}) H_{\text{T} \text{N} \text{F}}^D (\text{I} \text{L} \text{10}) M_A - k_{T \text{N} \text{F}} (\text{T} \text{N} \text{F} - w_{T \text{N} \text{F}}) \tag{5.4}
\]

\[
\frac{d \text{I} \text{L} 6}{dt} = \left( k_{6M} + k_{6T \text{N} \text{F}} H_{I \text{L} 6}^U (\text{T} \text{N} \text{F}) \right) H_{I \text{L} 6}^D (\text{I} \text{L} \text{6}) H_{I \text{L} 6}^D (\text{I} \text{L} \text{10}) M_A - k_6 (\text{I} \text{L} 6 - w_6) \tag{5.5}
\]

\[
\frac{d \text{I} \text{L} 8}{dt} = \left( k_{8M} + k_{8T \text{N} \text{F}} H_{I \text{L} 8}^U (\text{T} \text{N} \text{F}) \right) H_{I \text{L} 8}^D (\text{I} \text{L} \text{10}) M_A - k_8 (\text{I} \text{L} 8 - w_8) \tag{5.6}
\]

\[
\frac{d \text{I} \text{L} 10}{dt} = \left( k_{10M} + k_{106} H_{I \text{L} 10}^U (\text{I} \text{L} 6) \right) M_A - k_{10} (\text{I} \text{L} 10 - w_{10}). \tag{5.7}
\]

In equation (5.4), TNF-\(\alpha\) was activated by monocytes [118] at rate \(k_{T \text{N} \text{F} \text{M}}\) (pg (mL hr noc)\(^{-1}\)) and downregulated by IL-6 and IL-10 [255]. In equation (5.5), IL-6 was activated by monocytes and TNF-\(\alpha\) [118] at rates \(k_{6M}\) (pg (mL hr noc)\(^{-1}\)) and \(k_{6T \text{N} \text{F}}\) (pg (mL hr noc)\(^{-1}\)), and downregulated by itself [270] and IL-10 [118]. Similarly in equation (5.6), IL-8 was activated by monocytes and TNF-\(\alpha\) at rates \(k_{8M}\) (pg (mL hr noc)\(^{-1}\)) and \(k_{8T \text{N} \text{F}}\) (pg (mL hr noc)\(^{-1}\)), and downregulated by IL-10 [118]. In equation (5.7), IL-10 was activated by monocytes and IL-6 at rates \(k_{10M}\) (pg (mL hr noc)\(^{-1}\)) and \(k_{106}\) (pg (mL hr noc)\(^{-1}\)), [174, 116]. Cytokines decayed to their baseline concentrations \(w_i\) (pg (mL)\(^{-1}\)) at rate \(k_i\) (hr\(^{-1}\)) for \(i = \{T \text{N} \text{F}, 6, 8, 10\}.

**Model summary:** The mathematical model described above was an ODE system of the form

\[
\frac{dX}{dt} = f(t, X; \theta), \tag{5.8}
\]

where \(X \in \mathbb{R}^7\) denoted the time-varying states \(X = \{E, M_R, M_A, T \text{N} \text{F}, I \text{L} 6, I \text{L} 8, I \text{L} 10\}\) determining endotoxin (\(E\)), monocytes (resting \(M_R\) and activated \(M_A\)), TNF-\(\alpha\), IL-6, IL-8, and IL-10 concentrations. The model parameters \(\theta \in \mathbb{R}^{45}\) are listed in Table 5.2 with subsections indicating what state the parameters belong to.

To fit the mathematical model to the experimental data, we minimized the least squares cost function, \(J\), given by

\[
J = r^T r, \quad r = [r_{T \text{N} \text{F}} \quad r_{I \text{L} 6} \quad r_{I \text{L} 8} \quad r_{I \text{L} 10}], \tag{5.9}
\]

where \(r_k\) is the residual vector for each state \(k = \{T \text{N} \text{F}, I \text{L} 6, I \text{L} 8, I \text{L} 10\}\) and

\[
r_k = \frac{1}{\sqrt{N}} \left( \left[ y_{1}^{k} \ldots y_{N}^{k} \right] - y_{data}^{k} \right). \tag{5.10}
\]
In equation (5.10), \( N \) refers to the number of data points for each state \( k \), \( y^k_i = g(t_i, X_k(t_i); \theta) \) denotes the model output for the state \( k \) at time \( t_i \), \( 1 \leq i \leq N \), and \( y^k_{data} \) is the associated data. The least squares cost \( J \) was minimized using the nonlinear optimization solver, \textit{fmincon}, from MATLAB (MathWorks Inc., Natick, MA, USA). Upper and lower parameter bounds were set by multiplying and dividing the parameters’ nominal value by a factor of four.

### 5.3.5 Nominal parameters

Nominal parameter values were taken from Brady [28] except for cytokine baseline concentrations (\( w_{TNF}, w_6, w_8 \) and \( w_{10} \)), which were set to the mean continuous infusion and bolus data. We manually adjusted nominal parameters affecting peak timing to account for the observation (Figure 5.2) that the timing of cytokine activation depends on the administration method. To support convergence of the gradient-based optimizer used to estimate identifiable model parameters, we further improved the nominal model fit to the peak magnitudes of cytokine profiles by scaling the peak concentration of state \( i = \{TNF, IL6, IL8, IL10\} \), denoted \( X_i \), using the technique from Windoloski et al. [288] where

\[
X_i = \alpha \tilde{X}_i, \tag{5.11}
\]

for the scaling factor \( \alpha \) and desired peak concentration \( \tilde{X}_i \). We substituted equation (5.11) into the ODE for state \( X_i \) giving

\[
\frac{dX_i}{dt} = f(t, \theta, X_i, X_j) \quad \Rightarrow \quad \frac{d(\alpha \tilde{X}_i)}{dt} = f(t, \theta, \alpha \tilde{X}_i, X_j)
\]

for states \( j \neq i \). Therefore,

\[
\frac{d\tilde{X}_i}{dt} = \frac{1}{\alpha} f(t, \theta, \alpha \tilde{X}_i, X_j). \tag{5.12}
\]

The scaling factor \( 1/\alpha \) was distributed to each term on the right side of the ODE, scaling the associated parameters in each term. State \( X_i \) was also scaled when it was upregulating another state variable, \( Y \), as

\[
H_Y^U(X_i) = H_Y^U(\alpha \tilde{X}_i) = \frac{(\alpha \tilde{X}_i)^h}{\eta_Y^{hX_i} + (\alpha \tilde{X}_i)^h} \quad \Rightarrow \quad H_Y^U(\tilde{X}_i) = \frac{\tilde{X}_i^h}{\left(\frac{\eta_Y^{hX_i}}{\alpha}\right)^h + \tilde{X}_i^h}.
\]

Thus, half-saturation values were scaled by \( 1/\alpha \). A similar approach was applied for downregulation functions. Baseline cytokine parameters (\( w_i \)) were also scaled. Table 5.2 lists the nominal parameters for the continuous infusion and bolus mean model.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Unit</th>
<th>Nominal Value (C)</th>
<th>Nominal Value (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_h$</td>
<td>$E$ administered pr hour</td>
<td>ng (kg hr)$^{-1}$</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>$D_{ad}$</td>
<td>$E$ administered duration</td>
<td>hr</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>$k_E$</td>
<td>$E$ decay rate</td>
<td>hr$^{-1}$</td>
<td>1.01</td>
<td>1.01</td>
</tr>
<tr>
<td>$k_{MB}$</td>
<td>Regeneration rate</td>
<td>hr$^{-1}$</td>
<td>0.006</td>
<td>0.006</td>
</tr>
<tr>
<td>$k_{MA}$</td>
<td>Activated decay rate</td>
<td>hr$^{-1}$</td>
<td>2.51</td>
<td>2.51</td>
</tr>
<tr>
<td>$k_{MTNF}$</td>
<td>Activation rate by $TNF$</td>
<td>hr$^{-1}$</td>
<td>9.000</td>
<td>8.650</td>
</tr>
<tr>
<td>$k_M$</td>
<td>Activation rate by $E$</td>
<td>hr$^{-1}$</td>
<td>0.041</td>
<td>0.041</td>
</tr>
<tr>
<td>$\eta_{ME}$</td>
<td>Upregulation half max of $E$</td>
<td>ng (kg)$^{-1}$</td>
<td>2</td>
<td>3.3</td>
</tr>
<tr>
<td>$\eta_{M10}$</td>
<td>Downregulation half max of $IL10$</td>
<td>pg (mL)$^{-1}$</td>
<td>4.394</td>
<td>3.884</td>
</tr>
<tr>
<td>$\eta_{MTNF}$</td>
<td>Upregulation half max of $TNF$</td>
<td>pg (mL)$^{-1}$</td>
<td>222.222</td>
<td>140.845</td>
</tr>
<tr>
<td>$h_M$</td>
<td>Upregulation exp of $E$ on $M$</td>
<td>non dim</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>$h_{M10}$</td>
<td>Upregulation exp of $IL10$ on $M$</td>
<td>non dim</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>$h_{MTNF}$</td>
<td>Upregulation exp of $TNF$ on $M$</td>
<td>non dim</td>
<td>3.16</td>
<td>3.16</td>
</tr>
<tr>
<td>$M_0$</td>
<td>Carrying capacity</td>
<td>noc</td>
<td>30000</td>
<td>30000</td>
</tr>
<tr>
<td>$k_{TNF}$</td>
<td>Decay rate</td>
<td>hr$^{-1}$</td>
<td>0.6</td>
<td>1</td>
</tr>
<tr>
<td>$k_{TNFM}$</td>
<td>Activation rate by $M$</td>
<td>pg (mL hr noc)$^{-1}$</td>
<td>1.333</td>
<td>0.845</td>
</tr>
<tr>
<td>$\eta_{TNF10}$</td>
<td>Downregulation half max of $IL10$</td>
<td>pg (mL)$^{-1}$</td>
<td>17.576</td>
<td>15.536</td>
</tr>
<tr>
<td>$\eta_{TNF6}$</td>
<td>Downregulation half max of $IL6$</td>
<td>pg (mL)$^{-1}$</td>
<td>560</td>
<td>560</td>
</tr>
<tr>
<td>$h_{TNF}$</td>
<td>Upregulation exp of $TNF$ on $M$</td>
<td>non dim</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>$w_{TNF}$</td>
<td>Baseline concentration</td>
<td>pg (mL)$^{-1}$</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>$k_6$</td>
<td>Decay rate</td>
<td>hr$^{-1}$</td>
<td>0.66</td>
<td>0.66</td>
</tr>
<tr>
<td>$k_{6M}$</td>
<td>Activation rate by $M$</td>
<td>pg (mL hr noc)$^{-1}$</td>
<td>0.81</td>
<td>0.81</td>
</tr>
<tr>
<td>$k_{6TNF}$</td>
<td>Activation rate by $TNF$</td>
<td>pg (mL hr noc)$^{-1}$</td>
<td>0.81</td>
<td>0.81</td>
</tr>
<tr>
<td>$\eta_{610}$</td>
<td>Downregulation half max of $IL10$</td>
<td>pg (mL)$^{-1}$</td>
<td>35.152</td>
<td>31.071</td>
</tr>
<tr>
<td>$\eta_{66}$</td>
<td>Downregulation half max of $IL6$</td>
<td>pg (mL)$^{-1}$</td>
<td>560</td>
<td>560</td>
</tr>
<tr>
<td>$\eta_{6TNF}$</td>
<td>Upregulation half max of $TNF$</td>
<td>pg (mL)$^{-1}$</td>
<td>411.11</td>
<td>260.563</td>
</tr>
<tr>
<td>$h_{610}$</td>
<td>Downregulation exp of $IL10$ on $IL6$</td>
<td>non dim</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>$h_{66}$</td>
<td>Downregulation exp of $IL6$ on $IL6$</td>
<td>non dim</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>$h_{6TNF}$</td>
<td>Upregulation exp of $TNF$ on $IL6$</td>
<td>non dim</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>$w_6$</td>
<td>Baseline concentration</td>
<td>pg (mL)$^{-1}$</td>
<td>0.610</td>
<td>0.610</td>
</tr>
<tr>
<td>$k_{8}$</td>
<td>Decay rate</td>
<td>hr$^{-1}$</td>
<td>0.66</td>
<td>0.66</td>
</tr>
<tr>
<td>$k_{8M}$</td>
<td>Activation rate by $M$</td>
<td>pg (mL hr noc)$^{-1}$</td>
<td>0.509</td>
<td>0.789</td>
</tr>
<tr>
<td>$k_{8TNF}$</td>
<td>Activation rate by $TNF$</td>
<td>pg (mL hr noc)$^{-1}$</td>
<td>0.509</td>
<td>0.789</td>
</tr>
<tr>
<td>$\eta_{810}$</td>
<td>Downregulation half max of $IL10$</td>
<td>pg (mL)$^{-1}$</td>
<td>17.576</td>
<td>15.536</td>
</tr>
<tr>
<td>$\eta_{86}$</td>
<td>Downregulation half max of $IL6$</td>
<td>pg (mL)$^{-1}$</td>
<td>560</td>
<td>560</td>
</tr>
<tr>
<td>$\eta_{8TNF}$</td>
<td>Upregulation half max of $TNF$</td>
<td>pg (mL)$^{-1}$</td>
<td>411.11</td>
<td>260.563</td>
</tr>
<tr>
<td>$h_{810}$</td>
<td>Downregulation exp of $IL10$ on $IL6$</td>
<td>non dim</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>$h_{86}$</td>
<td>Downregulation exp of $IL6$ on $IL6$</td>
<td>non dim</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>$h_{8TNF}$</td>
<td>Upregulation exp of $TNF$ on $IL6$</td>
<td>non dim</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>$w_8$</td>
<td>Baseline concentration</td>
<td>pg (mL)$^{-1}$</td>
<td>2.959</td>
<td>4.175</td>
</tr>
<tr>
<td>$k_{10}$</td>
<td>Decay rate</td>
<td>hr$^{-1}$</td>
<td>0.66</td>
<td>0.66</td>
</tr>
<tr>
<td>$k_{10M}$</td>
<td>Activation rate by $M$</td>
<td>pg (mL hr noc)$^{-1}$</td>
<td>0.019</td>
<td>0.017</td>
</tr>
<tr>
<td>$k_{106}$</td>
<td>Activation rate by $IL6$</td>
<td>pg (mL hr noc)$^{-1}$</td>
<td>0.019</td>
<td>0.017</td>
</tr>
<tr>
<td>$\eta_{10}$</td>
<td>Upregulation half max of $IL6$</td>
<td>pg (mL)$^{-1}$</td>
<td>560</td>
<td>560</td>
</tr>
<tr>
<td>$\eta_{106}$</td>
<td>Upregulation exp of $IL6$ on $IL10$</td>
<td>non dim</td>
<td>3.88</td>
<td>3.88</td>
</tr>
<tr>
<td>$w_{10}$</td>
<td>Baseline concentration</td>
<td>pg (mL)$^{-1}$</td>
<td>4.239</td>
<td>3.747</td>
</tr>
</tbody>
</table>

Table 5.2: Parameter descriptions, units, and nominal values for the continuous infusion (C) and bolus injection (B) endotoxin models.
Most nominal parameter values used for model calibration to the individual data were set to the mean optimal values except for the initial cytokine concentrations ($w_{TNF}$, $w_6$, $w_8$ and $w_{10}$), which were set to the individual’s cytokine value at baseline. For subjects missing measurements at time zero, we scaled their concentration after one hour based on values from subjects where data were available; IL-6 and IL-10 baseline values were set at 50% and 75% of their concentrations at hour one. The scaling analysis was also applied to the individual subjects because of the significant individual variation in cytokine responses between study participants. To reduce the number of scaled parameters in the subject-specific optimizations, we only scaled cytokines with scaling factor $\alpha < 0.9$ or $\alpha > 1.1$.

5.3.6 Sensitivity analysis and subset selection

The highly nonlinear mathematical model had seven states and 45 parameters. Because of its structure [28, 29] and the quantity of data, we selected a parameter subset from the rate constants to estimate. We first conducted a local relative sensitivity analysis as described in Olufsen and Ottesen [182] on the mean continuous infusion response using the residual vector in equation (5.9). The sensitivity matrix $\chi$ was given by

$$\chi = \frac{\partial r}{\partial \log(\theta)} = \frac{\partial y}{\partial \theta} \cdot \frac{\theta}{\max(y_{data})},$$  

(5.13)

where $y = g(t, X(t), \theta)$ was the model output at time $t$, $\theta$ the nominal parameter set, and $y_{data}$ the mean continuous infusion data. We approximated the $(i, j)$ entry in the submatrix $\chi_k$ using forward differences, where

$$\chi = \begin{bmatrix} \chi_{TNF} & \chi_{IL6} & \chi_{IL8} & \chi_{IL10} \end{bmatrix}^T.$$

(5.14)

For submatrix $X_k$, elements $\chi_{ij}$ were given by

$$\chi_{ij} = \frac{g(t_i, X_k(t_i), \theta + he_j) - g(t_i, X_k(t_i), \theta)}{h} \cdot \frac{\theta}{\max(y_{data}^k)},$$

(5.15)

where $\phi = 10^{-8}$ was the solver tolerance, $h = \sqrt{\phi}$ the step size [198], and $e_j$ the basis vector in the $j$th direction. We ranked relative sensitivities by computing the two-norm of each column of $\chi$, obtaining a single sensitivity per parameter. We repeated the sensitivity analysis by simulating 100 runs sampling parameters from a uniform distribution varying $\pm 30\%$ around the parameter’s nominal value to study effects due to perturbations in parameter values.

Sensitive rate constants were used to select an identifiable parameter subset that can
be estimated. We utilized two practical identifiability techniques, the structured correlation method (SCM) and the SVD-QR method [169, 182]. The SCM used the Fisher-information matrix $F = \chi^T \chi$. We checked the condition number to ensure that $F$ had an inverse and calculated $G = F^{-1}$. The matrix $G$ was used to determine the pairwise parameter covariance $C_{ij}$ by

$$C_{ij} = \frac{G_{ij}}{\sqrt{G_{ii} G_{jj}}}, \quad (5.16)$$

where $(i, j)$ refers to $\theta_i$ and $\theta_j$. Parameter pairs for which $|C_{ij}| > 0.9$ were considered correlated. The parameter set with the largest correlation was selected. The parameter within that set with the smallest relative sensitivity was removed from the parameter set, and the process was repeated until there were no correlated parameters.

The SVD-QR method used singular value decomposition (SVD) to determine identifiable parameters. This method decomposed the sensitivity matrix $\chi = U \Sigma V^T$ where $U$ and $V$ contained the left and right singular vectors of $\chi$, and $\Sigma$ contained the singular values $\sigma$ of $\chi$. The largest $k$ singular values of $\chi$ were determined by $\sigma(k) \geq 10 \sqrt{\phi}$ where $\phi$ was the ODE solver tolerance. The first $k$ columns of the right singular vectors, $V_k$, were extracted from $V$ and used to find a permutation matrix $P$ such that $V_k^T P = QR$, where $Q$ was an orthogonal matrix and $R$ was an upper triangular matrix. The permutation matrix $P$ was then used to reorder the parameter vector $\theta$ as

$$\hat{\theta} = P^T \theta. \quad (5.17)$$

The first $k$ parameters of $\hat{\theta}$ were considered identifiable.

The subset selection methods determine identifiable parameters near the nominal values. To ensure that optimal values were also identifiable, we investigated the parameter convergence for each subset. We conducted 20 optimizations for each subset with nominal parameters drawn from a uniform distribution of $\pm 10\%$ of each estimated parameter’s nominal value. All other parameters were fixed. For each of the 20 runs, we calculated the coefficient of variation ($Cov$) for each estimated parameter $\theta_i$ where

$$Cov(\theta_i) = \frac{\sigma_i}{\bar{\theta}_i}. \quad (5.18)$$

We denoted $\bar{\theta}_i$ as the mean and $\sigma_i$ as the standard deviation of the estimated parameter $\theta_i$. Parameters in each subset with $Cov \geq 0.1$ were identified. The least sensitive parameter was removed from the set, and this process was repeated until all estimated parameters in each subset had a $Cov < 0.1$. The resulting parameter subsets were considered sensitive and identifiable and were used for parameter estimation.
5.3.7 Statistical methods

For each parameter subset, the goodness of fit was computed using the coefficient of determination (R²) [70], the corrected Akaike information criterion (AICc) [35], and the Bayesian information criterion (BIC) [227]. Details of these measurements are provided in Section A.1 of Appendix A.

Additionally, we constructed parameter and model confidence and prediction intervals using the frequentist approach detailed in Seber and Wild [228], Banks et al. [12], Smith [241]. Parameter confidence intervals for optimized parameter \( \tilde{\theta}_i \) were computed as

\[
\tilde{\theta}_i \pm t_{N-q}^{\alpha/2} \sqrt{\Sigma_{ii}},
\]

where \( N \) was the total number of data points, \( q \) was the number of parameters that were estimated, \( t_{N-q}^{\alpha/2} \) was the \( t \)-value from the student’s \( t \)-distribution for confidence level \( 1 - \alpha \) with \( N - q \) degrees of freedom, and the variance estimator matrix \( \Sigma \) was given by

\[
\Sigma = (\chi^T(\tilde{\theta})V^{-1}\chi(\tilde{\theta}))^{-1}.
\]

We defined \( \chi \) similarly to equations (5.14) and (5.15) where

\[
\chi(\tilde{\theta}) = \begin{bmatrix} \chi_{TNF}(\tilde{\theta}) & \chi_{IL6}(\tilde{\theta}) & \chi_{IL8}(\tilde{\theta}) & \chi_{IL10}(\tilde{\theta}) \end{bmatrix}^T
\]

and the \((i, j)\) element of submatrix \( \chi_k(\tilde{\theta}) \) with \( k \in \{TNF, IL6, IL8, IL10\} \) was approximated using forward differences given by

\[
\chi_{ij}(\tilde{\theta}) = \frac{g(t_i, X_k(t_i), \tilde{\theta} + he_j) - g(t_i, X_k(t_i), \tilde{\theta})}{h}.
\]

Here, \( \tilde{y}^k_i = g(t_i, X_k(t_i), \tilde{\theta}) \) was the optimal model output with optimal parameter vector \( \tilde{\theta} \) for cytokine state \( k \) at time \( t_i \) for \( 1 \leq i \leq N_k \) where \( N_k \) was the number data points for cytokine \( k \), and \( h \) and \( e_j \) were defined as in equation (5.15). The diagonal variance matrix \( V \) was given by

\[
V = \text{diag}(\sigma_{TNF}, \sigma_{IL6}, \sigma_{IL8}, \sigma_{IL10}),
\]

where \( \sigma_k \) was a diagonal matrix of size \( N_k \times N_k \) with entries

\[
\frac{1}{N_k - q} \left( r_k^T r_k \right), \quad r_k = [\tilde{y}^k_1 \ldots \tilde{y}^k_{N_k}] - y^k_{data}
\]
with $y_{data}^k$ defined in equation (5.10). The asymptotic prediction interval for cytokine $k$ at time $t_i$ was given by

$$\text{PI}_i^k = \tilde{y}_i^k \pm t_{N_k-q_k}^{a/2} s_k \sqrt{1 + G_{i k}^T \left( \chi_{k}^T(\tilde{\theta}) \chi_{k}(\tilde{\theta}) \right)^{-1} G_{i k}}$$

(5.25)

and the confidence interval by

$$\text{CI}_i^k = \tilde{y}_i^k \pm t_{N_k-q_k}^{a/2} s_k \sqrt{G_{i k}^T \left( \chi_{k}^T(\tilde{\theta}) \chi_{k}(\tilde{\theta}) \right)^{-1} G_{i k}}.$$  

(5.26)

We defined $\tilde{y}_i^k$ and $N_k$ as in equation (5.22), $q_k$ was the number of estimated parameters that impacted cytokine state $k$, and $t_{N_k-q_k}^{a/2}$ was the $t$-value for confidence level $1 - \alpha$ with $N_k - q_k$ degrees of freedom. $\chi_{k}(\tilde{\theta})$ was given by (5.21) and (5.22), but columns in $\chi_{TNF}(\tilde{\theta})$, $\chi_{IL6}(\tilde{\theta})$, and $\chi_{IL10}(\tilde{\theta})$ corresponding to IL-8 parameters were eliminated since they did not impact those state variables (see Figure 5.4). Entries of these columns were approximately zero and made $F_k = \chi_{k}^T(\tilde{\theta}) \chi_{k}(\tilde{\theta})$ singular unless removed. The matrix $G_{i k}^T$ was defined as

$$G_{i k}^T = \left( \frac{\partial \tilde{y}_i^k}{\partial \theta_1} \ldots \frac{\partial \tilde{y}_i^k}{\partial \theta_{q_k}} \right),$$

(5.27)

which was $i$th row of the submatrix $\chi_{k}(\tilde{\theta})$, and the variance estimator $s_k^2$ was given by

$$s_k^2 = \frac{1}{N_k-q_k} r_k^T r_k,$$

(5.28)

with $r_k$ in equation (5.24). Due to the small number of data points per cytokine, we generated pseudodata to compute uncertainty intervals. Data points at eight and twelve hours were set by quartering the cytokine concentration at six hours and returning the cytokine to the baseline value. Then, a piecewise cubic spline interpolation was performed from $t = 0 - 12$ hours.

Statistical data analysis included a two-sample unequal variances t-test ($\alpha = 0.05$) on the continuous infusion and bolus data before data calibration to compare their maximal concentrations and peak timing statistically. Abnormal cytokine responses (outliers in Figure 5.2) were omitted from the data sampled to conduct the hypothesis test, and the data distributions were approximately normal. A two-sample unequal variances t-test ($\alpha = 0.05$) was also performed on the set of optimized parameters from subject-specific optimizations to determine statistically significant differences in parameter values between the two administration methods. Parameter values that were outliers within their data set were not included in the data sampled to conduct the hypothesis test. The distributions of the optimal parameter values were approximately normal.
5.4 Results

5.4.1 Data

Statistical comparison (Table 5.3) of the continuous infusion and bolus injection data show a significantly smaller concentration of IL-8 \((p = 0.00147)\) and larger concentration of IL-10 \((p = 0.00200)\) with continuous infusion. The peak concentration for TNF-\(\alpha\) \((p = 0.0809)\) and IL-6 \((p = 0.702)\) did not statistically differ significantly between the two studies, but the time to peak cytokine concentration was significantly longer for all cytokines during the continuous infusion study: TNF-\(\alpha\), IL-6, and IL-8 \((p < 0.0001)\) and IL-10 \((p = 0.00695)\).

5.4.2 Sensitivity analysis and subset selection

Single and repeated sensitivity analysis (Figure 5.5) highlights the system’s dependence on endotoxin, activated monocytes, TNF-\(\alpha\), and IL-10 dynamics. The system’s most sensitive parameters are the growth or decay of these states, where \(k_{TNF_M}\) (growth rate of TNF-\(\alpha\) by monocytes) and \(k_{MA}\) (activated monocyte decay rate) have the most significant impact on the model. This can be explained by endotoxin and activated monocytes promoting the activation of cytokines, where TNF-\(\alpha\) and IL-10 are the main cytokines that upregulate and downregulate other states. The least sensitive rate constant is \(k_{MR}\), the regeneration rate for resting monocytes. Given that our study administers a finite dose of endotoxin that does not deplete the resting monocytes before the system can recover, it is reasonable that this parameter has a minute effect on the system. The single and repeated sensitivity analysis results exhibit similar behavior with minor variations in the order of sensitivity. This observation and careful scaling of nominal parameter values provides a good foundation for choosing identifiable subsets among the sensitive parameters.

Table 5.3: Statistical significance \((\alpha = 0.05)\) of data attributes between the continuous infusion \((m\) subjects) and bolus \((n\) subjects) studies in Berg et al. [21] and Janum et al. [115]. Subjects with abnormal responses (outliers) for either quantity were omitted from the sample.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Maximal Concentration</th>
<th>Time to Maximal Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-(\alpha)</td>
<td>(p = 0.0809 (m = 9, n = 19))</td>
<td>(p &lt; 0.0001 (m = 9, n = 19))</td>
</tr>
<tr>
<td>IL-6</td>
<td>(p = 0.702 (m = 9, n = 18))</td>
<td>(p &lt; 0.0001 (m = 9, n = 18))</td>
</tr>
<tr>
<td>IL-8</td>
<td>(p = 0.00147 (m = 9, n = 19))</td>
<td>(p &lt; 0.0001 (m = 9, n = 19))</td>
</tr>
<tr>
<td>IL-10</td>
<td>(p = 0.00200 (m = 8, n = 17))</td>
<td>(p = 0.00695 (m = 8, n = 18))</td>
</tr>
</tbody>
</table>
The parameter $k_{MR}$ is insensitive, removed from the subset, and fixed at its nominal value. Identifiability analysis using the SCM and SVD-QR method is performed on the remaining 15 sensitive rate constants. Because different identifiability analysis methods are not guaranteed to produce the same results [28], we generate three parameter subsets, two using only the SCM and one using the SVD-QR followed by the SCM. Results show that all rate constants for monocytes, IL-6, IL-8, and IL-10 cannot be uniquely estimated. Therefore, the sensitive rate constants are split into two subsets, one that includes monocyte-activated growth rates and the other that contains cytokine-activated growth rates. The SCM performed on each of these subsets results in the two subsets

$$S_1 = \{k_E, k_{MA}, k_{MTNF}, k_{TNF}, k_{TNFM}, k_6, k_{6TNF}, k_8, k_{8TNF}\}$$
$$S_2 = \{k_{MA}, k_M, k_{TNF}, k_{TNFM}, k_6, k_{6M}, k_8, k_{8M}, k_{10}, k_{10M}\}.$$  

The third subset is found by performing SVD-QR followed by the SCM on all 15 rate constants, which results in the subset

$$S_3 = \{k_{MA}, k_{TNF}, k_{TNFM}, k_6, k_{6M}, k_8, k_{8M}, k_{10M}\}.$$  

The identifiability and convergence of the above parameter subsets are checked numerically using the coefficient of variation method, enabling us to reduce the subsets further, obtaining

![Figure 5.5: Ranked sensitivities. Local sensitivities, scaled by the maximum sensitivity, are denoted by yellow crosses. Boxplots of scaled relative sensitivities are generated from $n = 100$ local sensitivity analysis simulations. Values are scaled by the maximum average sensitivity. Black dots denote outliers.](image-url)
three sensitive and identifiable parameter subsets

\[ s_1 = \{ k_{MA}, k_{MTNF}, k_{TNF}, k_{TNFM}, k_6, k_8, k_{8TNF} \} \]
\[ s_2 = \{ k_{MA}, k_M, k_{TNF}, k_{TNFM}, k_6, k_{8M}, k_9, k_{10}, k_{10M} \} \]
\[ s_3 = \{ k_{MA}, k_{TNF}, k_{TNFM}, k_8, k_{8M}, k_{10M} \}. \]

Note that \( S_2 = s_2 \), indicating that the subset \( S_2 \) was identifiable.

### 5.4.3 Parameter estimation and uncertainty quantification

Model fit for the mean continuous infusion data (\( R^2 \), AICc, BIC, and least squares cost \( J \)) for subsets \( s_1, s_2, \) and \( s_3 \) are reported in Table 5.4. Subset \( s_3 \) has the lowest AICc and BIC values, but the \( R^2 \) value and least squares cost did not differ significantly between the three subsets. Given the significance of the AICc and BIC values, we conduct the remaining simulations using \( S_{Final} = s_3 \) including

\[ S_{Final} = \{ k_{MA}, k_{TNF}, k_{TNFM}, k_8, k_{8M}, k_{10M} \}. \]

The mean continuous infusion model exhibits later activation of monocytes and cytokines compared to the bolus injection model (Figure 5.6). As a result, the main pro- and anti-inflammatory cytokines TNF-\( \alpha \) and IL-10 have larger peak concentrations. The immune resolution time during the continuous infusion model is approximately ten to twelve hours, whereas the mean bolus model is only six to eight hours. Comparison of model fits by the coefficient of determination (\( R^2 \)) for each cytokine reveal that TNF-\( \alpha \) and IL-6 are fitted better by the bolus model, while the continuous infusion model better predicts IL-8 and IL-10. Differences are minor, though, specifically for IL-8 and IL-10. The unobserved model states \( E, M_R, \) and \( M_A \) align with dynamics suggested or observed in both experimental and modeling studies, indicating that

Table 5.4: Goodness of fit measurements for the optimized subsets \( s_1, s_2, \) and \( s_3 \). The coefficient of determination is denoted as \( R^2 \), AICc represents the corrected Akaike information criterion, BIC the Bayesian information criterion, and \( J \) the least squares cost.

<table>
<thead>
<tr>
<th>Subset Estimated</th>
<th>Number of Parameters</th>
<th>Average ( R^2 )</th>
<th>AICc</th>
<th>BIC</th>
<th>( J )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( s_1 )</td>
<td>7</td>
<td>0.915</td>
<td>15.4</td>
<td>16.6</td>
<td>0.0602</td>
</tr>
<tr>
<td>( s_2 )</td>
<td>9</td>
<td>0.923</td>
<td>24.7</td>
<td>22.5</td>
<td>0.0466</td>
</tr>
<tr>
<td>( s_3 )</td>
<td>6</td>
<td>0.913</td>
<td>11.1</td>
<td>13.3</td>
<td>0.0547</td>
</tr>
</tbody>
</table>
we captured appropriate time course dynamics for these states. We use an endotoxin decay rate almost identical to that in Chow et al. [50] and the resting monocyte depletion follows a similar time course to that reported in Kiers et al. [130]. Furthermore, the resting monocyte recovery time course for our model (shown in Figure A.36 in Appendix A) is similar to the resting period between trial days in the study by Janum et al. [115], which suggests that it could take at least 4 weeks for complete immune recovery.

We generated $N = 61$ data points for each cytokine to determine the mean data and model uncertainty using confidence level $(1 - \alpha)$ with $\alpha = 0.05$. Confidence bounds on the optimal parameters from the continuous infusion and bolus mean model responses are given in Table 5.5. The upper and lower bounds remain within the physiological values (positive parameters close to their nominal value) except for $k_{10M}$, which has a negative lower bound. Prediction and confidence intervals on the optimal mean model are shown in Figure 5.7. Both prediction and confidence intervals for the bolus (Figure 5.7b) are tighter than those for the continuous infusion model (Figure 5.7a), indicating the variability of mean measurements and model output is larger in the continuous infusion data. This is plausible, given the sample sizes of the two studies. The lower bound for the prediction intervals of both dose types extends into negative cytokine values, which is mathematically but not physiologically appropriate. The negative portion of the lower bound could be omitted to provide a positive lower bound of the prediction interval. If additional data were available or computationally generated for these cytokines, then the prediction bounds would be tighter and, eventually, nonnegative. Similar uncertainty results (seen in Figures A.37-A.43 in Appendix A) were obtained using Bayesian inference with the Delayed Rejection Adaptive Metropolis (DRAM).

Figure 5.6: Optimized model fit to mean continuous infusion and bolus data estimating $S_{Final}$. The mean continuous infusion fit is marked by red solid lines, and the mean (SD) of the data by red circles and error bars. The mean bolus fit has black dotted lines, and black triangles and error bars denote the mean (SD) of the data.
We fit the model to the subject-specific cytokine profiles from the continuous infusion \((m = 9)\) and bolus injection \((n = 20)\) studies by estimating the parameters in \(S_{Final}\). Results for continuous infusion subject 1 and bolus injection subject 16 are shown in Figure 5.8, and dynamics for the remaining subjects are presented in Figures A.1-A.29 in Appendix A. Results show that our model captures varying cytokine responses to the same total dose of endotoxin for both administration methods. While individual peak cytokine concentrations and peak timing differ from that in the mean response, the model (shown in Figures 5.8a and 5.8b) is sufficiently robust to capture variation in data. This is evidenced by high \(R^2\) values for all

![Figure 5.7: 95% prediction and confidence intervals for the mean (A) continuous infusion and (B) bolus responses. Mean and SD data points are marked by circles and error bars. Prediction intervals are the yellow dashed-dotted lines, and confidence intervals are the blue dotted lines.](image-url)
The mean and standard deviation for subject-specific optimal and scaled parameters are listed in Table 5.6, and a boxplot of the optimized subject-specific parameter values are shown in Figures 5.9A and B. We observe similar median parameter values for the continuous infusion and bolus subject-specific optimizations for parameters $k_{MA}$, $k_{TNFM}$, and $k_{8M}$. For parameters $k_{MA}$, $k_{TNFM}$, and $k_{10M}$, there is a larger variance in the continuous infusion than the bolus injection. Optimized parameter values denoted as outliers in Figures 5.9A and B correspond to subjects 3, 5, and 9 from the continuous infusion study and subjects 3, 9, 13, 14, and 20 from the bolus study. These subjects all had abnormal endotoxin responses (at least one outlying data point in Figure 5.2). Boxplots of all scaled subject-specific parameters are shown in Figure 5.9C and all subject-specific parameter values are listed in Table 5.6.

Statistical comparison of the continuous infusion and bolus optimized parameters show that $k_{TNF}$ and $k_8$ ($p < 0.0001$) are significantly larger during the bolus injection, indicating the TNF-α and IL-8 decay faster during the bolus dose. Additionally, $k_{10M}$ ($p = 0.0142$) was significantly larger during the continuous infusion, implying that monocyte activation of IL-10 is more pronounced during a continuous infusion of endotoxin. As a result, the continuous infusion had a significantly larger activation response of IL-10 by monocytes and substantially smaller TNF-α and IL-8 degradation rates. Parameters $k_{MA}$ ($p = 0.465$), $k_{TNFM}$ ($p = 0.106$), and $k_{8M}$ ($p = 0.0615$) were not significantly different between the two administration methods, as reported in Table 5.6. Abnormal responses denoted as outliers in Figure 5.9 were not included in the sample from each study.

Table 5.5: Optimal 95% parameter confidence bounds for the mean continuous infusion and bolus model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Continuous Infusion (Optimal Value ± Bound)</th>
<th>Bolus (Optimal Value ± Bound)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{MA}$</td>
<td>$3.49 \pm 0.0994$</td>
<td>$2.67 \pm 0.0787$</td>
</tr>
<tr>
<td>$k_{TNF}$</td>
<td>$0.423 \pm 0.132$</td>
<td>$1.40 \pm 0.118$</td>
</tr>
<tr>
<td>$k_{TNFM}$</td>
<td>$1.39 \pm 0.0696$</td>
<td>$0.998 \pm 0.0398$</td>
</tr>
<tr>
<td>$k_8$</td>
<td>$0.386 \pm 0.119$</td>
<td>$0.686 \pm 0.100$</td>
</tr>
<tr>
<td>$k_{8M}$</td>
<td>$0.613 \pm 0.193$</td>
<td>$0.746 \pm 0.163$</td>
</tr>
<tr>
<td>$k_{10M}$</td>
<td>$0.0365 \pm 0.127$</td>
<td>$0.0150 \pm 0.124$</td>
</tr>
</tbody>
</table>
5.4.4 Infusion perturbations

We use the optimal mean continuous infusion model to study the response to a longer duration of inflammation and enhanced immune stimulation. Figure 5.10A shows the model response when 2 ng/kg of endotoxin is given continuously over 4, 8, 12, and 24 hours. The infusion duration impacts peak cytokine concentrations and the response's resolution time. Peak concentrations declined and occurred later as the infusion duration increased. Cytokine concentrations returned to baseline approximately 12, 16, 20, and 36 hours following the infusion start for the 4, 8, 12, and 24-hour continuous infusions. The system exhibits oscillatory behavior when the infusion is extended to 24 hours. The increase of anti-inflammatory cytokine IL-10 around 10 hours combats the initial pro-inflammatory response of TNF-α to decline around

![Diagram showing continuous and bolus injections with correlations and fitting lines](image)

Figure 5.8: Optimal model responses for (A) subject 1 from the continuous infusion study and (B) subject 16 from the bolus study.
12 hours. However, because the endotoxin is still being administered, it rebounds a second
time once IL-10 levels begin to decline. Following the termination of endotoxin administration,
the monocytes are no longer activated, and as a result, the inflammatory markers return to
baseline. This recurrent inflammatory behavior transpires when endotoxin is administered for
20 to 32 hours, after which the stimulation from the endotoxin is not strong enough to induce a

Table 5.6: Subject-specific parameter values as mean (SD) and estimated parameter p-values
with significance level $\alpha = 0.05$. Estimated parameters are marked in bold and remaining
parameters were scaled from their nominal values. Mean (SD) values are computed using
subject parameter values, including abnormal responses. P-values were calculated by removing
the abnormal responses prior to hypothesis testing, with $m$ subjects from the continuous
infusion and $n$ subjects from the bolus study being included.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Continuous Infusion</th>
<th>Bolus</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{MA}$</td>
<td>3.88 (1.06)</td>
<td>3.59 (0.587)</td>
<td>$p = 0.465(m = 9, n = 20)$</td>
</tr>
<tr>
<td>$\eta_{M_{10}}$</td>
<td>5.69 (3.38)</td>
<td>5.72 (2.79)</td>
<td></td>
</tr>
<tr>
<td>$\eta_{M_{TNF}}$</td>
<td>249 (152)</td>
<td>152 (114)</td>
<td></td>
</tr>
<tr>
<td>$k_{TNF}$</td>
<td>0.642 (0.274)</td>
<td>1.78 (0.406)</td>
<td>$p &lt; 0.0001(m = 9, n = 19)$</td>
</tr>
<tr>
<td>$k_{TNFM}$</td>
<td>2.25 (1.56)</td>
<td>1.44 (0.948)</td>
<td>$p = 0.106(m = 9, n = 19)$</td>
</tr>
<tr>
<td>$\eta_{TNF_{10}}$</td>
<td>22.8 (13.5)</td>
<td>22.9 (11.2)</td>
<td></td>
</tr>
<tr>
<td>$\eta_{TNF_{6}}$</td>
<td>674 (356)</td>
<td>686 (346)</td>
<td></td>
</tr>
<tr>
<td>$w_{TNF}$</td>
<td>7.22 (5.09)</td>
<td>6.86 (5.22)</td>
<td></td>
</tr>
<tr>
<td>$k_{6M}$</td>
<td>0.975 (0.516)</td>
<td>0.992 (0.500)</td>
<td></td>
</tr>
<tr>
<td>$k_{6TNF}$</td>
<td>0.975 (0.516)</td>
<td>0.992 (0.500)</td>
<td></td>
</tr>
<tr>
<td>$\eta_{610}$</td>
<td>45.5 (27.1)</td>
<td>45.8 (22.3)</td>
<td></td>
</tr>
<tr>
<td>$\eta_{66}$</td>
<td>674 (356)</td>
<td>686 (346)</td>
<td></td>
</tr>
<tr>
<td>$\eta_{6TNF}$</td>
<td>461 (280)</td>
<td>280 (211)</td>
<td></td>
</tr>
<tr>
<td>$w_{6}$</td>
<td>0.658 (0.484)</td>
<td>0.831 (0.690)</td>
<td></td>
</tr>
<tr>
<td>$k_{8}$</td>
<td>0.439 (0.127)</td>
<td>0.719 (0.174)</td>
<td>$p &lt; 0.0001(m = 8, n = 19)$</td>
</tr>
<tr>
<td>$k_{8M}$</td>
<td>0.733 (0.331)</td>
<td>0.941 (0.386)</td>
<td>$p = 0.0615(m = 8, n = 19)$</td>
</tr>
<tr>
<td>$k_{8TNF}$</td>
<td>0.542 (0.202)</td>
<td>0.884 (0.154)</td>
<td></td>
</tr>
<tr>
<td>$\eta_{810}$</td>
<td>22.8 (13.5)</td>
<td>22.9 (11.2)</td>
<td></td>
</tr>
<tr>
<td>$\eta_{8TNF}$</td>
<td>461 (280)</td>
<td>280 (211)</td>
<td></td>
</tr>
<tr>
<td>$w_{8}$</td>
<td>5.03 (5.73)</td>
<td>3.33 (1.07)</td>
<td></td>
</tr>
<tr>
<td>$k_{10M}$</td>
<td>0.0518 (0.0339)</td>
<td>0.0238 (0.012)</td>
<td>$p = 0.0142(m = 7, n = 19)$</td>
</tr>
<tr>
<td>$k_{106}$</td>
<td>0.0250 (0.0148)</td>
<td>0.0251 (0.012)</td>
<td></td>
</tr>
<tr>
<td>$\eta_{106}$</td>
<td>674 (356)</td>
<td>686 (346)</td>
<td></td>
</tr>
<tr>
<td>$w_{10}$</td>
<td>5.82 (5.04)</td>
<td>6.280 (3.24)</td>
<td></td>
</tr>
</tbody>
</table>
pronounced response (Figure A.35 in Appendix A). This simulation also shows that the system takes approximately 21-23 days to recover (Figure A.36 in Appendix A) relative to the resting monocyte population returning to the baseline value.

Figure 5.10B displays the model response for a 4-hour continuous endotoxin infusion of 2, 4, 8, and 16 ng/kg. The total endotoxin dose impacts peak cytokine concentrations and the immune resolution time. Larger doses of endotoxin result in earlier, greater peak cytokine concentrations, which occur approximately 1.5-2 hours before peak cytokine concentrations for smaller endotoxin doses. Additional simulations increasing both the duration of the continuous infusion and the total dose of endotoxin are shown in Figures A.30-A.34 in Appendix A.

Figure 5.9: (A) Boxplots of subject-specific optimized parameters from the bolus ‘B’ (black, \( n = 20 \) subjects) and continuous ‘C’ (blue, \( m = 9 \) subjects) administration models. Associated p-values are listed next to each parameter, (B) zoomed in boxplot of optimized parameter \( k_{10M} \) from (A), and (C) boxplots of subject-specific scaled parameters. On all plots outliers are denoted by the red cross. Parameters considered statistically significant (\( \alpha = 0.05 \)) include \( k_{TNF} \) (\( p < 0.0001 \)), \( k_8 \) (\( p < 0.0001 \)), and \( k_{10M} \) (\( p = 0.0142 \)). Parameters not statistically significant include \( k_{MA} \) (\( p = 0.465 \)), \( k_{TNFM} \) (\( p = 0.106 \)), and \( k_{8M} \) (\( p = 0.0615 \)). This figure is generated using MATLAB code adapted from Danz [64].
Figure 5.10: Continuous infusion mean model response when (A) 2 ng/kg of endotoxin is administered as a 4 (red solid lines), 8 (yellow dashed-dotted lines), 12 (light blue dotted lines), and 24-hour (dark blue dashed lines) continuous infusion, and (B) 2 (red solid lines), 4 (yellow dashed-dotted lines), 8 (light blue dotted lines), and 16 ng/kg (dark blue dashed lines) of endotoxin is administered as a 4-hour continuous infusion.
5.5 Discussion

This study uses hypothesis-generating mathematical modeling to compare bolus and continuous administration of LPS. The model is calibrated to data from Berg et al. [21] and Janum et al. [115]. Data analysis reveals that IL-10 has a significantly higher peak for the continuous dose, while the peak IL-8 concentration is higher with the bolus injection. For the 2 ng/kg four-hour continuous dose, peak cytokine concentrations occur significantly later than in the bolus dose. The model predicts that this delayed behavior continues when the dose is given over a longer time. Model parameter analysis provide insight into what processes may change with administration methods. Our results suggest that the continuous infusion of endotoxin increases the monocyte production rate of anti-inflammatory cytokine IL-10 and decreases the clearance rates of significant pro-inflammatory markers TNF-\(\alpha\) and IL-8. Our continuous infusion model is crucial as it can replicate characteristics of clinical inflammation associated with prolonged elevation of immune markers when endotoxin infusion is extended and pronounced cytokine responses when the endotoxin dosage is increased. Interestingly, administration over 20 and 32 hours produces double cytokine peaks indicating inflammation recurrence. Finally, we found that it takes over 20 days before the resting monocytes have reached the same level as before the stimulus.

Our findings agree with observations in Kiers et al. [130] comparing a 2 ng/kg bolus response to a 1 ng/kg bolus followed by a 3 ng/kg continuous infusion. A bolus injection followed by a continuous infusion showed higher IL-10 production and prolonged symptoms due to an extended elevation of cytokines. They also reported a significantly higher production of TNF-\(\alpha\), IL-6, and IL-8 with a bolus injection plus continuous infusion. Our study exhibited an increased TNF-\(\alpha\) production, though results were not statistically significant, likely due to the small number of subjects and high variance between subjects. IL-8 had significantly lower peaks during the continuous infusion (Figure 5.6). Our findings also agree with the partial conclusion of Taudorf et al. [254], who reported that (i) the release of cytokines TNF-\(\alpha\) and IL-6 occurred significantly later with the continuous infusion compared to a bolus injection and (ii) TNF-\(\alpha\) and IL-6 concentrations were significantly larger for the bolus dose. For our study, IL-6 was higher for the bolus dose but again, results were not significant. Taudorf et al. [254] also reported larger neutrophil concentrations that peaked earlier with the bolus dose. Our study did not account for neutrophil dynamics, a component that could be added in future studies. Differences in our findings could be a result of low endotoxin dosage in Taudorf et al. [254] and unequal total endotoxin dosing in Kiers et al. [130]. Overall, our findings implicate that continuous stimulation of the system over hours could promote a more significant anti-inflammatory response to counteract prolonged levels of pro-inflammatory cytokines, leading
to lower maximal concentrations of secondary cytokines such as IL-8.

A significant contribution of this study is the statistical analysis of optimal parameter distributions between the two administration methods, which has not been examined in earlier works. Results show that the activation rate of IL-10 by monocytes was significantly larger, and the TNF-α and IL-8 decay rates were substantially lower in the continuous infusion versus the bolus injection. These results suggest that continual endotoxin infusion amplifies the monocyte production of IL-10 and dulls the resolution of pro-inflammatory cytokines TNF-α and IL-8. Because endotoxin is rapidly degraded in the liver [161], a bolus of endotoxin results in short-lived exposure to resident macrophages in the liver while a continuous infusion elicits a longer, more steady exposure that could allow for adaptive mechanisms to set in, such as decreased cytokine clearance. Therefore, given that TNF- and IL-8 are mainly cleared by receptor-mediated endocytosis and renal elimination [163, 297, 206, 19], reduced receptor expression from the continuous endotoxin exposure could result in lower clearance rates of TNF- and IL-8. While our results suggest physiological differences in processes relating to stimulation and degradation rates, where a continuous infusion may allow mechanisms to adapt that are otherwise not present during the short-lived bolus administration, our model is a simplification of the immune system. There are physiological processes and mechanisms that are not explicitly modeled in our study, so the impact of those processes may be accounted for in our model parameters. Hence, additional studies are needed to verify our results.

Kiers et al. [130] indicates that a continuous infusion of endotoxin is a more probable model of prolonged inflammation in conditions like sepsis, where a hyperinflammatory state (often referred to as a cytokine storm) is accompanied by an immunosuppressive phase with elevated anti-inflammation levels [178, 256]. Thus, stimulation by a continuous infusion of endotoxin may exhibit mild but clear signs of a prolonged pro-inflammatory response and a hyperactive anti-inflammatory response, similar to dynamics observed in sepsis and supporting the hypothesis of Kiers et al. [130]. We note, however, that LPS studies cannot fully represent the dynamics of sepsis since LPS stimulates the toll-like receptor four (TLR-4) pathway [296] whereas inflammation during sepsis can be stimulated via multiple pathways [299].

Model analysis demonstrated the reliance of dynamics on the endotoxin, monocyte, TNF-α, and IL-10 states. These constituents encompass primary elements of the inflammatory response - immune cells that respond to stimuli and the main pro- and anti-inflammatory cytokines that modulate the response strength [118]. Thus, it is plausible that these components strongly dictate immune dynamics. It is also reasonable that the least influential component is the monocyte regeneration rate since the challenges analyzed here are from short-lived low-dose endotoxin exposure and the monocyte pool is not depleted prior to endotoxin clearance. However, we suspect that this parameter is important for the infusion perturbations (Figures
9B and A.30-A.34) since these extrapolations of the model result in depleted resting monocyte levels. Additional work remains to study the sensitivity of $k_{MR}$ in these scenarios. Furthermore, if simulating a pathogenic insult, we suspect that the influence of this parameter on system dynamics would significantly increase to clear an infection of much greater magnitude than is safely observed in an endotoxin challenge.

In Figure 5.9, several parameter values were marked as outliers for continuous infusion and bolus subject-specific model fits. These parameters correspond to subjects from both studies that exhibited abnormal cytokine responses for at least one of the measured cytokines. Given this, we hypothesize that these subjects may experience a more severe response or even enhanced complications to a clinical inflammation event. While this requires further investigation, it could be explored in silico by mathematical modeling.

We also explore variations of endotoxin infusion duration and total dose in Figure 5.10. A comparable simulation was conducted in Windoloski et al. [288] on a bolus endotoxin model where the total dose was increased, representing the administration of more potent immune stimuli that cannot safely be given to humans and the stimuli strength of clinical infection. Both our study and Windoloski et al. [288] observed enhanced cytokine production, but the current study showed a lack of significant dose-response in cytokines except in IL-10. Experimental studies such as Suffredini et al. [248] and Lipcsey et al. [147] studied the impact of endotoxin dose strength in both humans and pigs, respectively, and both studies observed significant cytokine increases as dose increased. However, these studies are not directly comparable to the current study due to differences in study population, endotoxin type, or administration method. We suggest that the weak dose-response for the pro-inflammatory cytokines in our study could be due to a slow monocyte regeneration rate, where the supply of resting monocytes is depleted too low from the initially larger insult and cannot activate enough monocytes to mount a comparable response. Since our study is hypothesis-generating, examining the impact of monocyte regeneration could be a probable future direction to verify our results.

Our simulations extending the continuous infusion duration correspond to the clinical scenario of continual systemic aggravation by inflammatory stimuli. In this case, the model produces up to approximately 36 hours of elevated immune markers depending on the length of the endotoxin infusion. It also displays attributes similar to endotoxin tolerance, a clinical phenomenon related to a reduced response to endotoxin after initial exposure [282], through the appearance of multiple decreasing cytokine peaks when continual endotoxin administration is given across 20 to 32 hours. Oscillations in cytokine concentrations also arise for 24 and 36-hour infusions when the total endotoxin dose is increased from 2 ng/kg to 4, 8, and 16 ng/kg (Figures A.33-A.34 in Appendix A), showing that the system can produce fluctuating behavior for prolonged periods of inflammation if the stimuli are large enough. These cytokine oscilla-
tions that occur could also be clinically-relevant with reference to recurrent infections where
the system returns close to baseline before peaking again. In a clinical setting, however, the
inflammatory stimulus is a live pathogen whose concentration would also fluctuate, compared
to an endotoxin challenge where the administration of endotoxin is constant until cessation of
infusion. This model of prolonged inflammation can be used to study inflammatory dynamics
over longer periods and test or validate treatments for inflammatory conditions given the
longer endotoxin exposure window.

5.5.1 Limitations

A primary limitation is that our model is calibrated to two data sets from Berg et al. [21] and
Janum et al. [115]. Although both administered a total dose of 2 ng/kg of endotoxin and had
similar experimental protocols, the endotoxin was sourced from different vendors and was
conducted on a different set of individuals. Thus, the studies are not statistically independent.
While using data from two separate studies is a limitation, we accounted for this using modeling
techniques for accurate comparison. Furthermore, while there are currently no dose-response
comparisons for a 2 ng/kg endotoxin dose with these two sources, there are two studies that
administer a 0.3 ng/kg bolus endotoxin dose using them. Taudorf et al. [254] utilizes Batch G2
B274 (The United States Pharmacopeial Convention, Inc., Rockville, MD) and Andreasen et al.
[5] uses Lot EC-6 (US Pharmacopeial Convention, Rockville, MD, USA). Both studies produce
very similar cytokine peaks and dynamic timing. TNF- peaks at approximately 15 pg/mL two
hours after endotoxin administration and IL-6 peaks around 35 pg/mL three hours after endo-
toxin administration. While these two endotoxin batches produce similar dynamics, utilizing
additional endotoxin challenge data or, more ideally, comparing the immune responses during
both endotoxin administration strategies on the same subjects using the same endotoxin batch
would yield the best results. Our study prompts the need for future endotoxin studies using the
same endotoxin batch in a randomized crossover design to measure the cytokine responses in
the same population using the two different dose regimes.

Furthermore, there is widespread individual variation in the human immune response,
which is evidenced by the individual subject data used in this study (Figures 5.2 and 5.3).
However, this is not uncommon. It is well-known that immune responses vary between indi-
viduals due in part to uncontrollable factors such as genetics, age, sex, seasonal and circadian
influences, and environmental effects [30]. Additionally, only a few studies administer large
endotoxin doses (such as 2 ng/kg as used here) as both a bolus and a continuous infusion.
While Taudorf et al. [254] administers 0.3 ng/kg of endotoxin as a bolus and a continuous
infusion, the cytokine concentrations are notably lower than those from a larger dose [133, 115]
and near or below reported concentrations in septic patients [40, 292, 21]. Torres et al. [256] suggests that most patients are likely in the immunosuppressive stage of sepsis upon hospital admittance, so we suspect initial inflammation levels could be higher than reported in sepsis studies. Therefore, data from a 2 ng/kg endotoxin challenge likely yield more realistic cytokine concentrations as observed in sepsis and should be used in our study.

Another limitation is that we do not have enough data to validate our endotoxin perturbation results on the continuous infusion model. Experimental data for a continuous infusion of larger endotoxin doses in humans is not seen in literature except in Kiers et al. [130] (who administers a total of 4 ng/kg of endotoxin) since larger doses of endotoxin are considered unsafe [8]. Safety may also play a role in the lack of experimental studies administering endotoxin for an extensive time beyond 4 hours. There are animal studies such as that by Castegren et al. [41] that conduct longer infusions, but contrast in the physiological makeup between animals (mice, pigs) and humans [187] could result in different responses between species than is observed during our longer infusion. The oscillatory, recurring behavior that we do observe, however, has been seen in humans during urinary tract infections, yeast infections, and children's ear infections [106, 9, 25]. Further analysis of the model and verification against experimental studies would be needed to determine whether this behavior is a product of the model structure or a consequence of extended endotoxin infusions.

Another limitation is that, although our mathematical model is highly nonlinear and complex, there are direct elements of the immune response (cells such as macrophages and neutrophils, cytokines such as IL-1β and TGF-β, and signaling pathways such as the NF-κB pathway) and other sources of immune regulation (cardiovascular, nerve, hormonal, metabolic) that are not included here. Although Janum et al. [115] reports that IL-1β was measured in the bolus study, its concentrations were not detectable. Our previous work [69] explored interactions of immune, cardiovascular, thermal, and pain responses during a bolus endotoxin challenge, and Windoloski et al. [288] expanded on that model to include hormonal regulation. Including these additional factors in the model dynamics could provide clearer insight into processes that activate at different speeds or strengths when the endotoxin challenge administration method is varied between a bolus and continuous infusion. A deeper understanding of continuous infusion dynamics could provide a better translational mathematical model of systemic inflammation such as sepsis, encompassing multi-organ dynamics.

5.5.2 Future work

Further investigation of this work involves additional examination of how initial conditions impact the model dynamics, which could include performing a multi-objective optimization
to determine the impact of fixed parameters on the different administration methods. We also plan to expand our study of continuous infusion dynamics to include immune interactions with other systems, such as the cardiovascular and neuroendocrine systems, thermal, pain, and metabolic regulation, building upon the work in Windoloski et al. [288]. These components are well-known to impact immune response and regulation [170, 104, 128, 115, 267, 69], and studying how a continuous infusion affects these elements can enhance understanding of clinically prolonged inflammation events. Additionally, while we simulate longer infusions in this study, we do not have human experimental data to verify these predictions. Future directions could focus on replicating the pig study from Castegren et al. [41] to investigate the dynamics of longer endotoxin infusions. Furthermore, while an endotoxin challenge attempts to mimic the dynamics of a clinical-level immune insult, its duration is finite. It cannot simulate the extensive effects of an actual infection or trauma. Therefore, mathematical modeling can extrapolate dynamics from controlled environments to clinical relevance by looking at the impact of age, smoking, diabetes, or cancer on immune responses. Additional future directions of this study focus on transitioning our endotoxin immune response model to a model of sepsis, a life-threatening condition involving hyperactive immune responses and subsequent organ failure that is still not fully understood [178]. Much research has been focused on identifying a universal biomarker and treatment of sepsis, but one has yet to be accepted within the scientific community [42]. However, recent progress has proposed several candidates, including administering vitamin C [126, 276] to sepsis patients. Adapting our current model to a model of sepsis could help improve our understanding of the mechanisms of sepsis and provide insight into the efficacy of potential sepsis treatments.

5.6 Conclusion

To enhance understanding of potential mechanisms impacting immune responses to endotoxin, we devised a physiologically-based mathematical model simulating mean and subject-specific dynamics in human volunteers exposed to continuous and bolus endotoxin administration. Comparison of subject-specific optimized parameter values revealed significant differences in the monocyte activation rate of IL-10 and recovery rates of pro-inflammatory cytokines TNF-α and IL-8. This suggests that increased IL-10 activation by monocytes and slower recovery rates of pro-inflammatory cytokines could play a role in the more pronounced anti-inflammatory response and smaller secondary cytokine response seen in the continuous infusion data. Additionally, these factors likely influence the system's elongated and more gradual response to the endotoxin, as seen by the statistically significant later peak concentration
times of all cytokines during the continuous infusion. Individuals with abnormal cytokine responses also reported statistically outlying optimal parameter values, suggesting their responses to a clinical infection could result in enhanced (outlying) reactions and complications. Simulations of the continuous infusion for a longer duration or increased dose amount display the model's capability to predict immune responses to prolonged inflammation or more potent inflammatory stimuli. Future directions of this work focus on verifying the hypothesized differing mechanisms between endotoxin administration methods through experimental crossover studies, including a whole-body response model to study the immune mechanisms occurring during a continuous infusion, and translating this model to study clinically observed inflammation in sepsis patients.
The study “A unified computational model of the human response to lipopolysaccharide-induced inflammation” was published in *Mathematics Online First Collections* by Springer, Cham in 2023. Author contributions include performing research on system couplings, providing input on model formulation, digitizing thermal data from literature, conducting model calibration to experimental data, performing all model simulations, formulation and management of code, and writing the manuscript. A short discussion article on this work was also published [289] (article not included in this thesis), and additional discussion of the multi-system pathways is included in Chapter 8. The model parameters are reported in Appendix B.
6.1 Abstract

This study develops a unified model predicting the whole-body response to endotoxin. We simulate dynamics using differential equations examining the response to a lipopolysaccharide (LPS) injection. The model tracks pro- and anti-inflammatory cytokines (TNF-\(\alpha\), IL-6, IL-10), concentrations of corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), and cortisol in the hypothalamic-pituitary-adrenal (HPA) axis. Daily hormonal variations are integrated into the model by including circadian oscillations when tracking CRH. Additionally, the model tracks heart rate, blood pressure, body temperature, and pain perception. Studied quantities function on timescales ranging from minutes to days. To understand how endotoxin impacts the body over this vast span of timescales, we examine the response to variations in LPS administration methods (single dose, repeated dose, and continuous dose) as well as the timing of the administration and the amount of endotoxin released into the system. We calibrate the model to literature data for a 2 ng/kg LPS bolus injection. Results show that LPS administration during early morning or late evening generates a more pronounced hormonal response. Most of the LPS effects are eliminated from the body 24 hours after administration, the main impact of inflammation remains in the system for 48 hours, and repeated dose simulations show that residual effects remain more than 10 days after the initial injection. We also show that if the LPS administration method or total dosage is increased, the system response is amplified, posing a greater risk of hypotension and pyrexia.

6.2 Introduction

The body has a wealth of regulatory mechanisms controlling vital functions that operate on timescales that differ by a factor of 10\(^7\), ranging from milliseconds (action potentials) to years (aging). Studying the effects of diseases on these timescales can be challenging, even for a well-defined event such as the inflammatory response to a low-dose endotoxin challenge (typically achieved by administering lipopolysaccharides (LPS)). The immune system is complex, and its response to a pathogenic threat entering the body through an external or internal wound varies significantly depending on the pathogen type, the degree of infection, the host’s age, sex, and ethnicity [83]. The body responds to the threat by activating local and systemic (innate) signaling cascades to remove the pathogen.

Most studies examining inflammatory signaling cascades focus on the short-term response (6-8 hours) using a combination of experimental and computational approaches examining dynamics in both animals and humans. In both species, inflammation can be stimulated by low-dose LPS administration. The effects have been studied both experimentally [52, 115, 168]
and computationally [10, 69, 196, 274] as this stimulus provides an excellent controlled model of the inflammatory cascade. But detailed experimental studies mapping inflammatory signaling pathways have found significant differences between animals and humans [71, 209, 265]. In addition to the immune response, pathogens impact dynamic signaling within the endocrine hypothalamic-pituitary-adrenal (HPA) axis, vascular systems, temperature regulation, and pain perception threshold [83], which display hourly, daily, monthly, and yearly variations. Long-term variations (monthly and yearly) are significant for chronic inflammation, but controlling the experimental environment is challenging. To address this challenge, we focused on developing a unified mathematical model examining the hourly and daily whole-body response to LPS, accounting for ultradian and circadian variation.

The immune, hormonal, and cardiovascular systems have historically been studied individually and often at different timescales. Mathematical modeling of the inflammatory cascade has been investigated on the timescale of hours using either models that lump inflammation components into broad categories (such as general pro-inflammatory and anti-inflammatory states) [66, 79, 137, 207] or more detailed models including specific immune response cells or cytokines [29, 50, 190].

Cardiovascular dynamics are typically studied over seconds or minutes to predict flow to a specific organ [53] or examine the control of blood flow in response to a challenge, such as the Valsalva maneuver [204]. While these models provide excellent predictions of hemodynamics, they do not address how these predictions vary daily, weekly, or monthly. Moreover, cardiovascular dynamics studies typically exclude influences from other systems, even though it is well known that the immune and hormonal systems impact dynamics. For example, the formation of atherosclerotic lesions involves an immune response [100, 45], and inflammation developing into sepsis depends on vagal responses [39, 278]. Additionally, elevated cortisol levels during stress result in increases in heart rate and anti-inflammatory reactions [151], and the transition to an advanced disease state is often accompanied by noticeable physiologic immune responses. Furthermore, morbidity is transformed into comorbidities often due to couplings by compromised immune or endocrine systems [84].

Previous studies have investigated the coupling of stress to inflammation [10, 156], and inflammation to cardiovascular dynamics, temperature, and pain perception [69, 222]. However, mathematical model coupling interactions between inflammation, stress, cardiovascular, pain, and thermal dynamics have yet to be investigated. Therefore, our study is the first to develop a mathematical model, henceforth denoted as the unified model (depicted in Figure 6.2) mapping the LPS response to the immune cascade, the HPA axis, and the cardiovascular system as well as temperature and pain dynamics on a timescale of hours to days. The unified model has several components: (1) an inflammation model that tracks concentrations of
tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), and interleukin 10 (IL-10) as well as resting and activated monocytes released in response to LPS; (2) an endocrine HPA axis model tracking concentrations of corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), and cortisol; (3) a cardiovascular model predicting heart rate, nitric oxide concentrations, vascular resistance, blood flow, and blood pressure using a circulation model integrated with a simple autonomous nerve system model; (4) a temperature model; and (5) a pain perception model. These systems operate on multiple timescales but are modeled on the timescale of hours.

Figures 6.1 and 6.2 show the coupling between the models including the stress hormone, cortisol, having a stimulating effect on heart rate, autonomous nerve system signaling affecting CRH and cytokine production, and inflammation affecting heart rate, temperature, and the HPA axis hormones. To test the validity of our unified model, we fit dynamics to data from Janum et al. [115] and Clodi et al. [52].

6.3 Methods

To understand how daily (ultradian and circadian) rhythms and stress impact inflammation and how inflammation impacts cardiovascular dynamics, we develop a unified model (shown in Figures 6.1 and 6.2) integrating and adapting Dobreva et al.‘s inflammatory-cardiovascular-temperature-pain model [69] and Bangsgaard et al.‘s inflammatory-HPA axis model [10]. The unified model is calibrated to data from human studies administrating a low dose of LPS. Below, we describe the data used for model calibration and each model component. Model parameter values, units, and initial conditions for all state variables are listed in the Appendix, Table B.1.

6.3.1 Data

Data are extracted from the studies by Clodi et al. [52] and Janum et al. [115]. We report data of importance for constructing the unified model, but these manuscripts also include data not used in our study. In brief, Clodi et al. [52] examines the immune response to oxytocin, while Janum et al. [115] investigates the connection between pain perception and the immune response with and without nicotine. For details on these studies, we refer to their original manuscripts.

These two experimental studies measure the human response to a low dose of endotoxin (2 ng/kg). Results are reported at least hourly for 6 hours after LPS administration. The studies were approved by the respective Institutional Review Boards, and all subjects consented to
participate. The study by Clodi et al. [52] was approved by the Institutional Review Board at the University of Vienna, Austria, and the study by Janum et al. [115] by the Regional Committee on Health Research Ethics and the Regional Data Monitoring Board at the University of Copenhagen, Denmark. The study by Clodi et al. [52] analyzes data from 10 male participants aged 20 to 40 years, and Janum et al. [115] analyzes data from 20 male participants aged 18 to 35 years. Participants were screened for abnormal health conditions for both studies and excluded if on any medication.

Both studies include measurements of pro-inflammatory cytokines TNF-α and IL-6, but

![Diagram](image-url)

**Figure 6.1:** Diagram showing interactions between the immune system (yellow), the HPA axis (pink), and the cardiovascular system (red and dark blue) during an endotoxin challenge. A bolus or continuous LPS dose is administered, prompting the activation of immune cells and the secretion of pro- and anti-inflammatory cytokines. LPS administration instigates the release of pro-inflammatory cytokines, stimulating the HPA axis to produce CRH, ACTH, and cortisol. Cortisol exhibits negative feedback on CRH and ACTH and positive feedback on anti-inflammatory cytokine IL-10 and heart rate. The cytokine production also causes an increase in body temperature (light blue), which upregulates heart rate. The heart rate exhibits positive feedback on the pro-inflammatory cytokine TNF-α. Additionally, LPS administration inhibits the pain perception threshold (green), which upregulates peripheral vascular resistance. The latter affects nitric oxide production, which is upregulated by TNF-α and downregulated by IL-10. The stimulation between elements is denoted by solid black lines and inhibition by dotted lines.
Janum et al. [115] also report measurements for IL-10. Despite administering the same dose, the two studies’ average inflammatory response varies significantly. The IL-6 data from Janum et al. [115] and Clodi et al. [52] peaks at approximately two and three hours, respectively. While these differences can be attributed to variations in the inflammatory response between individuals or be a result of differences in blood sample assaying [7], we also note that these blood samples were only collected hourly. Therefore, peak IL-6 concentrations could have occurred between the hourly blood samples, which would result in similar peak times.

Both studies report measurements of temperature, and Janum et al. [115] also reports pain perception threshold, heart rate, and blood pressure data. The study by Clodi et al. [52] mentions heart rate measurements, but values are not reported. However, Clodi et al. [52] does report ACTH and cortisol concentrations. Figure 6.3 shows the data used for model calibration. While it is ideal to use data from one single study when constructing a mathematical model, there is not an endotoxin study (to our knowledge) that reports all the desired quantities for each of our submodels.

![Figure 6.2: Model schematic. The inflammation model (yellow) tracks resting and activated monocytes as well as pro- (TNF-α and IL-6) and anti- (IL-10) inflammatory cytokines. The HPA axis model (pink) tracks CRC, ACTH, and cortisol. The cardiovascular model (red) tracks blood pressure and flow. This model is coupled with an autonomic control model predicting heart rate, nitric oxide (orange), and peripheral vascular resistance. In addition, we include a pain perception (green) and temperature (blue) model. Stimulation (upregulation) is marked by solid lines and inhibition (downregulation) by dotted lines.](image)
6.3.2 Inflammation model

Endotoxin administration. This study examines the response to low bolus and continuous LPS \((E, \text{ng/kg})\) stimuli. Similar to our previous study \([69]\) and the studies by Day et al. \([66]\) and Kadelka et al. \([121]\), we assume that LPS decays exponentially at the rate \(k_E (\text{hr}^{-1})\) once administered. Therefore, we let

\[
\frac{dE}{dt} = \begin{cases} 
  k_d - k_E E, & \text{for } t \leq t_{cd} \\
  -k_E E, & \text{for } t > t_{cd}
\end{cases}
\] (6.1)

For most simulations, the total dose administered is \(E_T = 2 \text{ ng/kg}\). If the stimulus is given as a bolus injection, \(E(0) = E_T\) and \(t_{cd} = 0\) (hr). For a continuous infusion, \(E(0) = 0\) and \(t_{cd}\) denotes the time over which the total dose \(E_T\) is administered. Here, \(k_d = E_T / t_{cd}\) (ng/kg·hr) is the amount of LPS administered each hour. Note, \(k_E\) has the same value independent of how the dose is administered (as bolus injection or continuous infusion).

Inflammation cascade. The response to endotoxin stimulation is a cascade of events, including monocyte (number of cells - abbreviated \(noc\)) activation and pro- and anti-inflammatory cytokine production. This study tracks TNF-\(\alpha\) (\(TNF\)), IL-6 (\(IL6\)), and IL-10 (\(IL10\)) concentrations (pg/mL) over time (hr). Equations are set up following the interactions shown in Figure 6.4

![Graphs showing data](image)

Figure 6.3: Data from Clodi et al. \([52]\) and Janum et al. \([115]\) (mean ± SE). Data from Clodi et al. \([52]\) are shown in dark gray and data from Janum et al. \([115]\) are shown in light gray.
using the same approach as our previous studies [10, 29, 69].

Endotoxin administration activates monocytes \((M_A, noc)\) at a rate \(k_M\) (hr\(^{-1}\)) recruited from the resting monocyte \((M_R, noc)\) population [26]. The monocyte recruitment is upregulated by TNF-\(\alpha\) at a rate \(k_{MTNF}\) (hr\(^{-1}\)) and down-regulated by IL-10 [136, 162]. The resting monocytes are regenerated at a rate \(k_{MR}\) (hr\(^{-1}\)) until the baseline level of resting monocytes, \(M_\infty\) (noc), is reached. The activated monocytes decay at a rate \(k_{MA}\) (hr\(^{-1}\)) without stimulation. Therefore, \(M_R\) and \(M_A\) dynamics are given by

\[
\frac{dM_R}{dt} = k_{MR}M_R \left(1 - \frac{M_R}{M_\infty}\right) - H_M^U(E)(k_M + k_{MTNF}H_M^U(TNF))H_M^D(IL10)M_R
\]

\[
\frac{dM_A}{dt} = H_M^U(E)(k_M + k_{MTNF}H_M^U(TNF))H_M^D(IL10)M_R - k_{MA}M_A.
\]

In the above equations and throughout this study, upregulation (stimulation) is denoted by \(H_Y^U(X)\) and downregulation (inhibition) by \(H_Y^D(X)\). These stimuli are modeled by Hill functions of form

\[H_Y^U(X) = \frac{X^h}{X^h + \eta_{XY}^h},\]

\[H_Y^D(X) = \frac{\eta_{XY}^h}{X^h + \eta_{XY}^h},\]

where \(\eta_{XY}\) denotes the half-saturation value of variable \(X\) and \(h\) the Hill exponent determining the steepness of the effect on \(Y\).

Activated monocytes stimulate TNF-\(\alpha\) production at a rate \(k_{TNFM}\) (pg/mL·hr·noc) [261]. Both IL-6 and IL-10 downregulate TNF-\(\alpha\) production, and TNF-\(\alpha\) naturally decays to baseline level \(w_{TNF}\) (pg/mL) at rate \(k_{TNF}\) (hr\(^{-1}\)) [51, 224, 243]. Activated monocytes stimulate IL-6 production at a rate \(k_{6M}\) (pg/mL·hr·noc), and TNF-\(\alpha\) stimulates IL-6 production at a rate \(k_{6TNF}\) (pg/mL·hr·noc) [243]. Moreover, IL-6 exhibits anti-inflammatory properties, including downregulation of itself, and IL-6 naturally decays to baseline level \(w_6\) (pg/mL) at a rate \(k_6\) (hr\(^{-1}\)) [270].

In addition to the inflammatory feedback, the cardiovascular system modulates pro-inflammatory cytokines. Typically, an inflammatory event increases heart rate \((HR, \text{bpm})\) above its baseline value \((HR_b, \text{bpm})\), which in turn impacts TNF-\(\alpha\) production at a rate \(k_{TNFH}\) (bpm\(^{-1}\)) [118, 193]. When the heart rate is at the baseline level, it does not impact TNF-\(\alpha\) production. Therefore,
pro-inflammatory cytokines TNF-α and IL-6 are determined by

\[
\frac{dT_{NF}}{dt} = k_{TNF\,M} H_{TNF}^D (IL6) H_{TNF}^D (IL10) (1 + k_{TNF\,HR} (HR - HR_b)) M_A - k_{TNF} (TNF - w_{TNF})
\]

\[
\frac{dIL6}{dt} = (k_{6M} + k_{6TNF} H_{1L6}^U (TNF)) H_{IL6}^D (IL6) H_{IL6}^D (IL10) M_A - k_6 (IL6 - w_6).
\]

The anti-inflammatory cytokine IL-10 is stimulated by activated monocytes at a rate of \(k_{10M}\) (pg/mL-hr-noc) and upregulated by IL-6 at the rate \(k_{106}\) (pg/mL-hr-noc) [36, 116]. IL-10 decays to baseline level \(w_{10}\) (pg/mL) at the rate \(k_{10}\) (hr\(^{-1}\)). In addition, cortisol (\(F\), µg/dL) has anti-inflammatory properties [132, 149], and its influence on IL-10 dynamics is modeled by upregulation of IL-10 production at the rate \(k_{10F}\) (pg/mL-hr-noc). Thus, the equation for IL-10 is given by

\[
\frac{dIL10}{dt} = (k_{10M} + k_{106} H_{1L10}^U (IL6) + k_{10F} H_{1L10}^U (F)) M_A - k_{10} (IL10 - w_{10}).
\]

Figure 6.4: Inflammatory model. LPS (E) stimulates monocyte activation (\(M_A\)), which upregulates pro- (TNF-α and IL-6) and anti- (IL-10) inflammatory cytokines. The cytokines regulate one another through positive and negative feedback. Note that IL-6 is pro- and anti-inflammatory, downregulating itself and TNF-α but upregulate IL-10. Additionally, the inflammatory response is regulated by the HPA axis and heart rate. Solid lines denote stimulation (upregulation) and dotted lines inhibition (downregulation).
6.3.3 HPA axis model

We briefly summarize the HPA axis model equations below, but a detailed description can be found in [10, 184] with further background in [4, 11, 92, 156, 107]. The HPA axis model is represented by three differential equations tracking concentrations of CRH \((C, \text{pg/mL})\), ACTH \((A, \text{pg/mL})\), and cortisol \((F, \mu g/dL)\) as

\[
\begin{align*}
\frac{dC}{dt} &= k_{CR} R(t) H_C^D(F)C + k_{CTNF} TNF - k_C(C - C_b) \\
\frac{dA}{dt} &= k_{AC} H_A^D(F)C + k_{ATNF} H_A^U(TNF) - k_A A \\
\frac{dF}{dt} &= k_{FA} H_F^D(IL10)A^2 - k_F F.
\end{align*}
\]

The release of CRH by the hypothalamus is influenced by the circadian rhythm, denoted by the time-dependent function \(R(t)\) (discussed in further detail below), at the rate \(k_{CR} \text{ (hr}^{-1}\)) and is also stimulated by the presence of TNF-\(\alpha\) through the rate \(k_{CTNF} \text{ (hr}^{-1}\)) \([18, 278]\). Cortisol downregulates CRH, and CRH decays to a baseline level \(C_b\) (pg/mL) at rate \(k_C\) (hr\(^{-1}\)). Stimulation of the pituitary gland by CRH leads to production of ACTH at the rate \(k_{AC} \text{ (hr}^{-1}\)) while cortisol downregulates this process \([277, 279]\). ACTH production is also upregulated by TNF-\(\alpha\) at the rate \(k_{ATNF} \text{ (pg/mL-hr)}\) \([18]\), and ACTH levels decay at a rate of \(k_A\) (hr\(^{-1}\)). Finally, cortisol is downregulated by the anti-inflammatory cytokine IL-10 and stimulated from the adrenal glands by ACTH at rate \(k_{FA} \text{ (\mu g/mL/pg-dL-hr)}\) \([112]\). In the absence of stimulation, cortisol levels decay at the rate \(k_F\) (hr\(^{-1}\)) \([10]\). A schematic of the HPA axis model is presented in Figure 6.5.

The time-dependent function \(R(t)\) (non-dimensional, n.d.) denotes an enforced exogenous circadian rhythm (the body’s 24-hour cycle) that impacts mental, physical, and behavioral processes in humans \([3, 177]\). It is modeled by

\[
R(t) = \left( \frac{t_m^k}{t_m^k + \alpha^k} \cdot \frac{(T_{24} - t_m)^\ell}{(T_{24} - t_m)^\ell + \beta^\ell + \varepsilon} \right) N_c
\]

as the product of an upregulation Hill function with a half-saturation value \(\alpha\) (min) and Hill exponent \(k\) (n.d.) and a downregulation Hill function with half-saturation value \(\beta\) (min) and Hill exponent \(\ell\) (n.d.). In the Hill functions, \(t_m\) (min) denotes the time in minutes during the 24 hour cycle shifted by the value \(\delta\) (min), i.e. \(t_m = (60t - \delta)\) modulo \(T_{24}\), with \(T_{24} = 60 \cdot 24 = 1440\) min as the cycle length (24 hours converted to minutes). Finally, \(\varepsilon\) (n.d.) denotes the base value of the circadian rhythm, and \(N_c\) (n.d.) is a scaling factor.
6.3.4 Cardiovascular model

Given the timescale of hours, the cardiovascular system and its control (shown in Figure 6.6) are modeled as non-pulsatile [286]. The transport model tracks changes in systemic volume ($V$, mL), pressure ($p$, mmHg), and flow ($q$, mL/hr) in the arteries and veins. Feedback from this and the model's control tracks changes in heart rate ($HR$, bpm) and peripheral vascular resistance ($R_s$, mmHg·hr/mL). The cardiovascular model consists of four compartments: the large arteries ($la$), small arteries ($sa$), small veins ($sv$), and large veins ($lv$). We make a note that while we model the cardiovascular section where $t$ is in hours, dimensional analysis can be used to model flow and, subsequently, resistance where $t$ is in seconds and heart rate where $t$ is in minutes, which is how these quantities are usually computed. The correct analysis would produce the same results as seen here in our study.

Cardiovascular transport. The system dynamics are modeled using a hydrodynamic analog to a resistor-capacitor (RC) circuit. Voltage is analogous to pressure, current to flow, capacitance to compliance (or elastance, $E$ (mL/mmHg), which is the reciprocal of compliance), while resistance $R_s$ refers to resistance in both formulations. A system of differential equations is

![Figure 6.5: HPA axis model. CRH (C) production is stimulated by the body's natural circadian rhythm (modeled by the function $R(t)$) and TNF-α. CRH and TNF-α also stimulate ACTH (A) production, which in turn stimulates cortisol (F) production. Cortisol is downregulated by IL-10 and exhibits negative feedback on both CRH and ACTH. Stimulation (upregulation) is denoted by solid lines and inhibition (downregulation) by dotted lines.](image-url)

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obtained by ensuring conservation of volume, given by

\[
\frac{dV_{i\alpha}}{dt} = Q - q_a \\
\frac{dV_{s\alpha}}{dt} = q_a - q_s \\
\frac{dV_{s\nu}}{dt} = q_s - q_v \\
\frac{dV_{l\nu}}{dt} = q_v - Q,
\]

where \( V_i \) (mL) denotes the total volume of compartment \( i \in \{l\alpha, s\alpha, s\nu, l\nu\} \), \( Q \) (mL/hr) is the cardiac output (flow) through the heart, and \( q_j \) (mL/hr), \( j \in \{a, s, \nu\} \) denotes the flow between the compartments. Flow is related to pressure via Ohm’s law, given by

\[
q_j = \frac{p_{out} - p_{in}}{R_j}, \tag{6.2}
\]

where \( R_j \) (mmHg·hr/mL) is the resistance between the \( j \)’th compartments. Pressure is related to volume via the pressure-volume equation

\[
V_i - V_{un, i} = E_i(p_i - p_{un, i}), \tag{6.3}
\]

where \( E_i \) is the compartment elastance, and \( V_{un, i} \) and \( p_{un, i} \) denote the unstressed volume and pressure, respectively. Cardiac output is determined by \( Q = HR \cdot V_{str} \), where \( HR \) (bpm) is the heart rate and \( V_{str} \) (mL/beat) is the stroke volume. Following the derivation in our previous study [286], stroke volume is given by

\[
V_{str} = V_{ED} - V_{ES} = -\left( \frac{p_{l\alpha}}{E_{Max}} - \frac{p_{l\nu}}{E_{Min}} \right), \tag{6.4}
\]

where \( V_{ED} \) and \( V_{ES} \) denote the end-diastolic and end-systolic volume (mL), \( p_{l\alpha} \) and \( p_{l\nu} \) are the systemic arterial and systemic venous pressures (mmHg), and \( E_{Max} \) and \( E_{Min} \) are the maximum (end-diastolic) and minimum (end-systolic) elastance (mmHg/mL).

**Cardiovascular control.** Since we analyze dynamics over hours rather than seconds, we ignore the fast response (within seconds) to sudden changes in blood pressure. The longer-term effects (on the order of minutes to hours) include inflammatory, cortisol, and temperature modulation of pseudo-steady levels of noradrenaline and acetylcholine regulating heart rate and vascular resistance via the sympathetic and parasympathetic systems. In addition, vascular
resistance is modulated by nitric oxide (NO), an effective vasodilator.

Heart rate is upregulated by changes in temperature ($T$, °C), blood pressure ($p_{la}$, mmHg), and cortisol ($F$). The rostral raphe region of the medulla oblongata has temperature-regulating sympathetic neurons located close to the cardiac-related sympathetic neurons. Therefore, an increase in body temperature triggered by inflammation upregulates heart rate via sympathetic activation and parasympathetic inhibition [38, 58, 176]. Similarly, cortisol upregulates heart rate via an increase in noradrenaline from the adrenal glands [110, 142, 226], while blood pressure impacts heart rate via two mechanisms [57, 125, 179, 294]. If the blood pressure is higher than its baseline, heart rate increases [179], but if blood pressure falls below the resting value, commonly observed in patients with active inflammation [186], heart rate is also upregulated.

![Cardiovascular model diagram](image)

**Figure 6.6:** The cardiovascular model. It predicts pressure $p$ (mmHg), flow $q$ (mL/hr), and volume $V$ (mL) in four compliant compartments representing the systemic large arteries ($la$), small arteries ($sa$), large veins ($lv$), and small veins ($sv$). Flow through the heart $Q$ (mL/hr) is predicted as a function of heart rate ($HR$, bpm) and stroke volume ($V_{str}$, mL/beat). The control model regulates heart rate as a function of blood pressure, cortisol ($F$, µg/dL), and body temperature ($T$, °C). Peripheral vascular resistance ($R_s$, mmHg·hr/mL) is regulated in response to pain perception ($P$, kPa) and nitric oxide (N, n.d.).
To include these effects, the change in heart rate is predicted as

$$\frac{dHR}{dt} = \frac{1}{\tau_H} \left( k_H (HR_M - HR_b) H_{HR}^U (T - T_b) H_{HR}^U (F) f(p_{la}) - (HR - HR_b) \right), \quad (6.5)$$

where $\tau_H$ (hr$^{-1}$) and $k_H$ (n.d.) are rate constants, $HR_M$ (bpm) is the maximal heart rate, $HR_b$ (bpm) is the baseline heart rate, $T$ is temperature ($^\circ$C), $T_b$ ($^\circ$C) is the baseline temperature, and the control responding to changes in blood pressure $f(p_{la})$ is given by

$$f(p_{la}) = \begin{cases} 
H_H^U (p_{lab} - p_{la}), & p_{la} \leq 100 \text{ mmHg} \\
H_H^D (p_{la} - p_{lab}), & p_{la} > 100 \text{ mmHg},
\end{cases}$$

where $p_{la}$ is the arterial blood pressure predicted by the cardiovascular model and $p_{lab}$ (mmHg) is the baseline arterial blood pressure.

**Peripheral vascular resistance**, $R_S$, is primarily regulated by pain [155, 217] and nitric oxide [144]. It increases with an increase in the rate of change of pain perception threshold $\Gamma = \frac{dP}{dt}$ (kPa/hr) with half saturation value $\eta_{RP}$ (kPa/hr) and growth rate $k_{RP}$ (mmHg/mL). Additionally, nitric oxide ($N$, n.d.) is a well-known vasodilator [144, 165, 218, 221], and so the presence of nitric oxide decreases vascular resistance at a rate of $k_{RN}$ (mmHg/mL). Elevated levels of $R_S$ return to baseline $R_{sb}$ (mmHg/hr/mL) at rate $k_R$ (hr$^{-1}$). These effects give

$$\frac{dR_S}{dt} = k_{RP} \frac{\Gamma^2}{\Gamma^2 + \eta_{RP}^2} - k_{RN} N - k_R (R_S - R_{sb}), \quad (6.6)$$

where nitric oxide ($N$) is determined by

$$\frac{dN}{dt} = k_{NM} H_N^U (TNF(t-\kappa)) H_N^D (IL10(t-\kappa)) M_A - k_N N,$$

accounting for the 2-4-hour delay in nitric oxide production in response to the inflammatory event. Activated monocytes stimulate nitric oxide production via inducible NO synthase [165]. It is upregulated by TNF-$\alpha$ and downregulated by IL-10 [47, 218]. The production rate is determined by the constant $k_{NM}$ ((hr-noc)$^{-1}$). The delay in NO stimulation and suppression from cytokine concentrations TNF-$\alpha$ and IL-10 is determined by $\kappa$. In the absence of stimulation, nitric oxide levels decay to baseline at the rate $k_N$ (hr$^{-1}$).

We note that in the remaining model simulation figures (Figures 6.7–6.12), vascular resistance $R_S$ is reported in seconds instead of hours. This was done to decrease the magnitude of the $R_S$ solution and was calculated by dividing the $R_S$ solution by 3600 (converting hours to
Temperature and pain

**Temperature.** The production of pro-inflammatory cytokines TNF-α and IL-6 stimulates and sustains [54, 141, 224] a fever while anti-inflammatory cytokine IL-10 [99, 188] helps to regulate body temperature. Therefore, the body temperature $T$ (°C) changes in response to endotoxin as

$$\frac{dT}{dt} = k_{TNF} H_T(TNF - w_{TNF}) + k_{IL6} H_T(IL6 - w_6) - k_{IL10} H_T(IL10 - w_{10}) - k_T(T - T_b),$$

where $k_{TNF}$, $k_{IL6}$, and $k_{IL10}$ describe the rate (°C/hr) at which TNF-α, IL-6, and IL-10 affect body temperature, respectively. Temperature returns to baseline level $T_b$ (°C) at the rate $k_T$ (hr⁻¹). As mentioned above, TNF-α and IL-6 upregulate temperature while IL-10 downregulates temperature. Each Hill function is shifted by the baseline cytokine value so that when cytokines are at the baseline level, they do not stimulate a change in body temperature. The equation is adapted from the more complex form in [69]. Temperature dynamics are shown in Figure 6.2.

**Pain.** Several studies have investigated the connection between immune response and pain perception [140, 271]. In response to either exposure to endotoxin or the cytokine cascade resulting from endotoxin detection, pain receptors (nociceptors) become activated [20, 115, 280]. We predict pain perception using the formulation from Dobreva et al. [69] resulting in

$$\frac{dP}{dt} = -k_{PE} E P - k_P(P - P_b).$$

The presence of endotoxin ($E$, ng/kg) decreases the pain perception threshold, $P$ (kPa), at the rate $k_{PE}$ (kg/ng-hr). As the endotoxin is eliminated from the system, $P$ returns to baseline value $P_b$ (kPa) at the recovery rate $k_P$ (hr⁻¹).

**Model calibration**

The inflammatory-cardiovascular model originates from Dobreva et al. [69] and the HPA axis model originates from Bangsgaard et al. [10]. Combining these into a unified model requires adjustments and re-calibration of the model parameters. The unified model uses modified computer code from the study by Dobreva et al. [69] augmented with a modified version of the integrated HPA model from Bangsgaard et al. [10].
We use cytokine, temperature, and hormonal data from Clodi et al. [52], and cardiovascular and pain perception data from Janum et al. [115]. The decision to use cytokine data from Clodi et al. [52] instead of Janum et al. [115] is due to the sequence used to couple the submodels. We first calibrated the inflammation model (uncoupled from the cardiovascular model) to the Clodi et al. [52] data and then coupled the inflammation model to the HPA axis model. Given that the Clodi et al. [52] study had both cytokine and hormonal data, we used the cytokine data from Clodi et al. [52] for model calibration. This involved coupling TNF-$\alpha$ with CRH and ACTH and IL-10 with cortisol. Each coupling was done successively to select the relevant parameter values. We also had to scale TNF-$\alpha$ and IL-6 down to the appropriate concentrations reported by Clodi et al. [52]. This required scaling several TNF-$\alpha$ and IL-6 related parameters. Next, we coupled the inflammatory-HPA axis model with the cardiovascular-temperature-pain model from Dobreva et al. [69] in a similar scaffolding manner. Once the models were coupled, as shown in Figure 6.2, influential parameters were manually adjusted to fit the model to the data.

6.4 Results

We examined the unified model's response to endotoxin dosing type, amount, and timing. The latter is of particular interest for exploring dynamics at different timescales: the fast cardiovascular response (minutes), the intermediate inflammatory, the HPA axis response (hours), and the slow response of the circadian rhythm (days), while exploration of the model's response to variations in endotoxin amount and dosing methods are of clinical relevance [8, 66, 130, 254].

All simulations used a total dose of 2 ng/kg (the dose used by both Clodi et al. [52] and Janum et al. [115]) except the simulation analyzing the impact of the total dose. Simulations are depicted over three to four 24-hour cycles, including up to two 24-hour cycles before the LPS administration and two 24-hour cycles post-LPS. Supplemental simulations (https://kwindoloski.wordpress.ncsu.edu/) show results on longer and shorter time intervals.

6.4.1 Single LPS administration - base simulation

We first simulate the effect of a bolus dose of 2 ng/kg of LPS given at time $t = 37.5$ hours (1:30 pm on day 2). Figure 6.7 shows the data and the model output. We depict dynamics for one full cycle ($t = 0$ to $t = 24$ hours) before LPS administration and two full cycles following dispensation, totaling four 24-hours cycles.

Figure 6.7 shows that the inflammation arising from the LPS stimulus causes a rapid increase in cytokine levels in the first hours following the injection. In agreement with previous studies [10, 50, 69], most of the inflammatory response returns to baseline after about 6 hours. The
same does not apply to the HPA axis hormones CRH, ACTH, and cortisol, which are strongly
affected by ultradian oscillations emerging from the circadian forcing. ACTH follows cytokine
dynamics timing, but CRH and cortisol are elevated for at least 24 hours following injection,
after which they normalize. Temperature and heart rate also have a fast response; they recover
in about 10 hours, but nitric oxide, resistance, pain perception, and blood pressure take about
three 24-hour cycles to recover fully.

In this simulation, we also see an initial blood pressure increase followed by a blood pressure
decrease below baseline. The initial increase is caused by a pain perception decrease and a
vasoconstriction increase. The blood pressure drop is a result of the heart rate normalizing at a
much faster rate than nitric oxide. This behavior is not captured explicitly in the blood pressure
data since blood pressure measurements are only taken for six hours following endotoxin
administration. However, decreases in blood pressure measurements below baseline have
been observed in other endotoxin challenge studies [134, 154, 221]. Thus, the mechanisms
included in our model and evidence from other experimental studies lead us to believe that
this behavior is plausible.

In summary, the model generates an initial increase in pro- and anti-inflammatory cytokine
levels, hormone levels, body temperature, nitric oxide, blood pressure, heart rate, and vascular
resistance. Elevation of IL-6 induces a slight fever, causing a drop in resistance, blood pressure,
and pain perception threshold below baseline levels.

### 6.4.2 Timing of LPS administration

To study the effect of LPS injection timing, we selected four dispensing times aligned with
critical cortisol values over a 24-hour cycle. Times selected (shown in Figure 6.8(a)) are at \(t = 2\)
hours (low cortisol just before the circadian increase), \(t = 7\) hours (the highest level of cortisol
caused by the circadian and ultradian effects), \(t = 11.9\) hours (the ultradian oscillation valley
during declining circadian activation), and \(t = 21.8\) hours (just after circadian activation where
the cortisol level is low). These times correspond to 2:00 am, 7:00 am, 12:00 pm, and 10:00 pm.
We denote these as *early morning*, *morning*, *noon*, and *late evening*. Similar to the calibration
simulation discussed above, this simulation uses the 2 ng/kg endotoxin dose.

Figures 6.8(a) and (b) show that the resting and activated monocytes primarily shift the
inflammatory response to the right. TNF-\(\alpha\) and IL-10 exhibit slightly higher peaks when LPS is
administered in the morning or at noon. However, the administration time mainly impacts HPA
axis hormones. ACTH and cortisol have a significant spike right after the LPS administration. If
administered in the morning, the peak is during the upslope of the circadian wave, dampening
the ultradian oscillations, while administering it in the early morning or late evening increases
Figure 6.7: Simulation generated by solving the unified model with parameters calibrated to data. Results are depicted for four 24-hour cycles. LPS is administered at time $t = 37.5$ hours (marked by vertical dotted lines). Results at times before LPS injection are marked by solid orange lines, while solid black lines are used for results at times after LPS injection. Data from Clodi et al. [52] are shown in dark gray and data from Janum et al. [115] in light gray (mean ± SE).
the ultradian oscillations. However, these states return to their baseline after 24 hours. Another interesting observation is the effect of LPS administration time on CRH. When administered in the morning or at noon, LPS timing has a minimal effect on CRH. Yet, when LPS is administered in the early morning or late evening, CRH concentration spikes within 8 hours to much higher levels than seen in Figure 6.8. As with ACTH and cortisol, CRH returns to its baseline after 24 hours.

The cardiovascular state blood pressure exhibits a slightly larger drop if LPS is administered at noon, while vascular resistance and nitric oxide are unaffected by the injection time. The heart rate peak remains similar during the different injection times, but the return to the baseline is partially stunted when LPS is administered early or late evening. Finally, pain perception and temperature are shifted with the administration times.

### 6.4.3 Repeated LPS administration

A few studies have investigated the impact of repeated LPS administrations on the immune response by mathematical modeling [10, 66, 69, 210], but either not coupled to the cardiovascular dynamics or not accounting for ultradian and circadian variation. To understand these effects, we examine what happens when two 1 ng/kg LPS doses are administered repeatedly. Chosen so that the total dose remains the same as in our previous investigations, the first dose is administered at a fixed time ($t_1 = 13.5$ hours) and the second dose is given at times $t_2 = 14.5, 19.5, 25.5, \text{ and } 37.5$ hours corresponding to 1 hour, 6 hours, 12 hours, and 24 hours after the first LPS dose, respectively. Simulation results, shown in Figure 6.9, reveal that the inflammation states have pronounced peaks when the second dose is given up to six hours following the first dose. However, the second reaction is almost suppressed if the second dose is given after cytokine levels return to baseline values.

The HPA axis displays a rapid and pronounced ACTH peak reaching 100 pg/mL. Most LPS effects on ACTH have worn off by 4-6 hours after the second dose, but some effects remain for more than 48 hours. The cortisol dynamics are similar ACTH except when the repeated administration is close to the first dose. In this case, the rapid peak is modest, and normalization happens faster. The repeated dose causes elevated ultradian oscillation in the next circadian cycle. A more pronounced response in CRH is observed if the repeated dose of LPS is given while CRH is low compared to cases where CRH is elevated. When the second LPS dose is administered 12 or 24 hours after the first dose, the repeated administration almost normalizes the ultradian oscillations in CRH and, thus, ACTH and cortisol.

The cardiovascular state blood pressure drops below 100 mmHg when the second dose is given when blood pressure is high (up to 6 hours after the first dose), causing hypotension.
If the second dose is given after blood pressure returns to baseline, we see a modest blood pressure increase with no significant drops below baseline. When the second dose is delivered while the heart rate is high, the heart rate increases to a slightly higher peak value. In contrast, if a second dose is administered after heart rate returns to baseline, smaller increases in heart rate occur. The response in nitric oxide follows that of cytokines, except that the decay to baseline is significantly slower (approximately 48 hours). Resistance shows a modest peak due to the repeated injections. When the second dose is given up to 6 hours after the first dose, the resistance drops by up to 50% of its baseline value, followed by a slow recovery (approximately 48 hours). If the resistance returns to baseline before the repeated dose, we see an increase in resistance without the following drop below baseline. The temperature has a similar response to the repeated dose as the cytokines. The pain perception threshold dramatically drops when the repeated injection is given close to the first one, corresponding an increased pain sensitivity. However, the pain perception threshold exhibits a smaller drop the later the repeated dose is given, and its levels return to baseline in less than 24 hours.

These results demonstrate that effects persist even if the second dose is administered 24 hours after the first dose. Therefore we added additional simulations, administering the second dose 4, 9, 14, and 19 24-hour cycles following the first LPS dose (shown in Figure 6.10). These results allow us to investigate how long it will take before the effect of the first dose has worn off and when the impact of the second dose is similar to that of the first dose. The simulation results shown in Figure 6.10 demonstrate that the system takes approximately nine 24-hour cycles to repeat the dynamics from the first LPS dose. Cytokine levels, body temperature, pain perception threshold, nitric oxide, and cardiovascular markers in Figure 6.10(a) replicate the system’s initial response to 1 ng/kg of LPS after nine 24-hour cycles. For the HPA states, we see approximately the same response as with the initial 1 ng/kg dose when the second dose is administered about nine 24-hour cycles later. We also see a more pronounced response in the system after nine 24-hour cycles because the resting monocyte population is being regenerated to a larger monocyte pool than when the first dose was administered (populating up to its carrying capacity $M_\infty$). Therefore, more activated monocytes enter the system and produce an increased inflammatory response which cascades to other model states.

### 6.4.4 Effect of dose in single LPS injection

The model described in this paper is calibrated to data from studies administering a 2 ng/kg bolus dose of LPS. However, the stimulation of the system is much stronger when diseases such as sepsis elicit an immune response. Experimentally, substantially higher doses of LPS are not safe, but any dose can be administered computationally. Therefore, we investigate the
system dynamics for higher LPS doses. To ensure that we stay within the region for which the model is developed, we study the effect of 2, 4, 8, and 16 ng/kg bolus injections. Figure 6.11 shows the simulation results.

More monocytes are activated as the dose increases. The larger LPS doses cause a slight increase in TNF-α levels and a significant increase in IL-6 and IL-10 levels. Increases occur faster with the larger doses, and TNF-α returns to baseline faster. For the HPA states, the short-term CRH response is suppressed for larger LPS doses. Moreover, the next cycle is slightly affected. We also note that the immediate ACTH peak is not affected, but the next cycle is. Cortisol has a short-term peak response for low doses but is suppressed for larger LPS doses. However, the next cortisol cycle is markedly affected only for low doses.

The cardiovascular state blood pressure and vascular resistance show more prominent initial peaks and then plunges below 100 mmHg for the higher doses, causing hypotension. The recovery time is not affected by the dose. We also note that the resistance falls slightly more with the higher doses, while the peak heart rate response decreases and returns to baseline faster. Finally, the nitric oxide level increases with approximately the same recovery time. For the remaining states, peak temperature slightly increases with the larger doses, and the pain perception threshold dramatically decreases, but with the same recovery time.

### 6.4.5 Continuous LPS administration

When endotoxin (LPS), an immuno-stimulant, is injected into the body, it elicits an immune response, eliminating the endotoxin from the system. Studies have suggested that a single bolus dose is insufficient for mimicking realistic inflammatory reactions seen during systemic inflammation as it does not consider that immuno-stimulants, whether from an infection or an injury, aggravate the system for an extended period \[130, 266, 191, 254\]. Therefore, we examine the system's response to a continuous LPS infusion using the same total dosage (2 ng/kg). In our view, this is a better representation of the inflammatory response in diseases such as sepsis.

The continuous infusion is simulated using the second branch of the endotoxin equation (6.1). The initial dose, \(E(0)\), is set to zero and we include a constant production over a set time interval \(t_{cd}\). This is combined with exponential decay, which eliminates the endotoxin from the system. We apply a continuous dose over four hours, making the administered concentration 0.5 ng/kg per hour in the body. The model response is shown in Figure 6.12.

As expected, the endotoxin concentration changes from a decreasing exponential curve rapidly approaching zero to a distributed reaction to the LPS infusion approaching 0.5 ng/kg. For the inflammation states, the response to LPS is delayed. Moreover, peak values are higher than those observed for the single dose, particularly the late pro- and anti-inflammatory
states. After end-infusion, the cytokine states return to their baseline after about 10 hours. The monocyte response is delayed, and the drop in resting monocytes and the corresponding peak in activated monocytes is pronounced after end-infusion. The monocytes approach the baseline level with the same speed as after the single bolus simulation.

For the HPA states, the ultradian CRH peaks reach lower values, and the frequency is slightly increased compared to a single bolus administration. The ACTH response is similar to that of a single bolus, but it is delayed with a change in ultradian frequency while the peak value remains unchanged. The initial cortisol reaction is more suppressed and delayed than for the single bolus simulation, while the ultradian frequency and amplitudes are like those for CRH.

The cardiovascular states behave similarly to the single dose, except the peak value is delayed for all states. Blood pressure changes more than the other states. Its peak is lower, and it drops significantly below 100 mmHg. This trend is repeated for resistance, increasing less and dropping more than for a single dose. NO production is increased, but it returns to baseline at the same time as the bolus dose. The pain perception threshold also returns to baseline at the same time as for a single dose.

6.5 Discussion

We have developed a unified model integrating submodels for the immune system, the endocrine HPA axis, the cardiovascular system, temperature, and pain perception. The unified model integrates complex dynamic features acting on multiple timescales. The novelty involves the development of a platform for studying how coupled submodels impact dynamics over 24 hours. Previous studies have examined how stress impacts inflammation [10], and how inflammation impacts cardiovascular dynamics, temperature, and pain [69, 80, 223, 164, 293]. Still, to our knowledge, this is the first study coupling all five systems to examine the response to endotoxin.

Mathematical models analyzing physiological systems are typically studied in isolation as it is challenging to parameterize and calibrate an integrated model [123]. The same applies to many in vitro experimental studies [231]. Since the ultimate goal is to understand in vivo dynamics, more work is needed to develop coupled models.

This study examines the human responses to an endotoxin challenge in silico. Specifically, we look at the effect of injected or infused LPS doses, which is also a typical experimental test setup [8, 31]. The advantage is that the LPS effects are relatively short-lived [83] and tolerated by humans and animals [225], making it an ideal controlled environment to learn about system dynamics. Moreover, numerous experimental results provide data we can use to calibrate the
proposed model [52, 115].

We focus on examining dynamics up to 48 hours past LPS administration, varying the dose, timing, and administration method. A single bolus dose results in a relatively fast cardiovascular and inflammatory response independent of the administration time, while the HPA-axis hormones are sensitive to administration time. ACTH and cortisol rapidly increase after dispensing LPS, and the next circadian cycle is perturbed. CRH is also susceptible to the administration time; the effect is most pronounced when the administration coincides with the lowest cortisol levels. These results reveal that it takes more than 24 hours before the impact of LPS is cleared from the system. This is important as most in vivo LPS experiments only examine the response over 6-8 hours [52, 56, 83, 115]. However, a few recent studies have examined feedback over longer timescales that display results up to 72 hours, but without accounting for circadian or ultradian rhythms [31, 67]. A few recent studies have examined the effects of the circadian clock [127, 200], but these do not address how inflammation interacts with the other subsystems studied here. These results demonstrate that to compare results among subjects, it is essential to conduct experiments at the same time during the day [127, 200].

If the first bolus injection is followed by a second (repeated) injection, our simulations show that if the repeated dose is given within 6 hours, the effect is amplified; but if it is given later, the response is suppressed. Our additional investigation where the second dose was given several 24-hour cycles following the first LPS dose shows that it takes the system approximately ten 24-hour cycles after the initial dose to act as a new independent dose. This emphasizes our earlier point that the initial effects of an LPS dose are present in the body longer than 24 hours. This finding agrees with results reported by Patel et al. [192] examining monocyte regeneration in a study administering 2 ng/kg LPS. Their results show that by day 7, monocyte numbers had returned to steady-state values.

The effect of the bolus injection is mainly dependent on the size of the dose. The higher the dose, the higher the response, except for heart rate, CRH, and cortisol, which all show an opposite dependence. As discussed in our previous studies [29, 69], this model does not include a tissue damage component. Therefore, we do not observe a phase transition as reported in several previous modeling studies [50, 233] or the recent review by Minucci et al. [172]. While tissue damage is essential for simulating an infection caused by a wound or surgery, it is not relevant for a controlled LPS study. Typical doses given to humans are between 2-4 ng/kg [31], and it has been reported that doses of 1-2 µg [68] are lethal. Therefore, we limited the dose to 16 ng/kg, significantly above the limit administered in controlled trials but well below the lethal limit. Another consideration was administering a dose for which the physiological pathways included in the model still are valid. Our results show that for 16 ng/kg, blood pressure dropped to ~100 mmHg, which is approaching clinically-defined hypotension. More work is needed to
test if a higher dose puts the response below this limit.

A continuous infusion over four hours with the same total LPS dose as in the 2 ng/kg single bolus dose shows distributed and amplified effects compared to a single bolus. Exceptions are pain perception and cortisol, where the outcome is less than for a single dose, as well as heart rate and ACTH, where the effect is comparable. For CRH, ACTH, and cortisol, the ultradian frequency increases for the continuous infusion compared to the single bolus injection. Again, the longer infusion time causes a significant drop in blood pressure. Still, more work is needed to examine if the same dose administered over a longer time would cause a higher blood pressure drop or if it is necessary also to increase the total dose.

Results reported here bring new insight into how the systems couple. The model proposed here includes primary coupling between five subsystems. While numerous studies have examined parts of these [10, 29, 69, 80, 223, 164, 293], to our knowledge, this pilot study devising a unified model is the first to explore connections between these subsystems.

**Limitations.** This study devised a unified model calibrated to data from low dose LPS injections [52, 115]. Results were obtained by combining submodels from our previous studies [10, 69]. This approach has two limitations (1) we do not have a single data set measuring all states, and (2) coupling between the two models can be challenging to validate. Since we did not have a single set of data, we calibrated our model to the cytokine, hormonal, and temporal data from Clodi et al. [52] and the cardiovascular and pain data from Janum et al. [115]. However, it should be noted that cytokine data could be taken from either study since it was measured in both studies. Therefore, more work is needed to set up experimental studies measuring all required quantities in response to a single LPS dose and examine how the coupling strength between each submodel impacts dynamics.

Moreover, even though each submodel is "simple," (we only include two pro- and one anti-inflammatory cytokine, three HPA-axis hormones, and four compartments in the systemic circulation), the combined unified model is highly complex. It has 18 state variables and more than 100 parameters. Therefore, in addition to model calibration against data from a single experiment, more work is needed to study the sensitivity and uncertainty of model predictions.

Sensitivity analysis can reveal what parameters are the most influential, and if combined with subset selection, it is possible to determine what parameters can be identified uniquely given available data. The latter is advantageous for improving model validation. This analysis should focus on detailed scrutiny of parameters informing coupling between each submodel, mainly since each submodel has been analyzed in detail in earlier studies [10, 29, 69]. Finally, adding uncertainty quantification could benefit predictions to relate parameters to significant variation often observed in experimental measurements of dynamic quantities studied here.
However, more work is needed to examine the response to other types of infections, mainly since LPS mimics a bacterial infection that does not replicate in the body. Therefore, results reported here noting that it takes more than 48 hours before the LPS has cleared should be scrutinized if the model is generalized to investigate the effects of fungal or viral infections. The same applies to other types of infection, including inflammation associated with surgery, systemic infection like sepsis, chronic inflammation from autoimmune diseases, the mutation in cells giving rise to a pathogenic reaction, smoking, aging, or other immune modulators.

6.6 Conclusion

This study develops a unified model coupling inflammatory, HPA axis, and cardiovascular dynamics. Results show that infection generated by administration of low dose endotoxin (LPS) takes at least 48 hours to clear and that dosing type, amount, and timing affect dynamics. In particular, the repeated dose has a significant effect demonstrating that it takes about 10 days before the second dose is not influenced by the first. Another important finding is that several manipulations cause a substantial drop in blood pressure towards hypotension, a significant risk for patients. This effect is seen if the dose is administered over a longer time via a repeated injection or a continuous infusion, or if an increase in endotoxin dose is given.
Figure 6.8: Model simulations examining the timing of LPS administration. The solid black line denotes the model simulation before LPS administration. Black asterisks mark when LPS is administered (at $t = 2, 7, 11.9$, and $21.8$ hours). Post-LPS dynamics are depicted by orange solid lines ($t = 2$), red dashed lines ($t = 7$), blue dotted lines ($t = 11.9$), and green dashed-dotted lines ($t = 21.8$). (a) times (marked with dotted vertical lines) at which LPS is administered within a normal 24-hour cortisol cycle. (b) Inflammatory and cardiovascular simulations. (c) HPA axis simulations.
Figure 6.9: Repeated LPS dose: Model simulations when 1 ng/kg is administered at \( t_1 = 13.5 \) hours and 1 ng/kg is administered at \( t_2 \). The solid orange line denotes the model simulation before the first LPS dose. The vertical dotted line denotes when the first LPS dose is given \((t_1 = 13.5)\). The solid black line denotes the model dynamics between the first and second LPS doses. Black asterisks denote when the second LPS dose is given. The second LPS dose is administered at times \( t_2 = 14.5, 19.5, 25.5, \) and 37.5 hours. Model dynamics after the second dose of LPS is administered are given by purple solid lines \((t_2 = 14.5)\), red dashed lines \((t_2 = 19.5)\), blue dotted lines \((t_2 = 25.5)\), and green dashed-dotted lines \((t_2 = 37.5)\). (a) Inflammatory and cardiovascular simulations. (b) HPA axis simulations.
Figure 6.10: Repeated LPS dose further investigation: Model simulations when 1 ng/kg is administered at $t_1 = 13.5$ hours and 1 ng/kg is administered at $t_2$. The solid orange line denotes the model simulation before the first LPS dose. The vertical dotted line denotes when the first LPS dose is given ($t_1 = 13.5$). The solid black line denotes the model dynamics between the first and second LPS doses. Black asterisks denote when the second LPS dose is given. The second LPS dose is administered 4, 9, 14, and 19 24-hour cycles following the first LPS dose. Model dynamics after the second dose of LPS is administered are given by purple solid lines ($t_2 = 120.5$), red dashed lines ($t_2 = 240.5$), blue dotted lines ($t_2 = 360.5$), and green dashed-dotted lines ($t_2 = 480.5$). (a) Model simulations for non-HPA axis states. (b) Model simulations for HPA axis states.
Figure 6.11: Model simulations when the total amount of LPS administered as a bolus dose varies. The solid orange line denotes the model simulation before LPS administration. The vertical dotted line indicates when the LPS was given \((t = 13.5\) hours). Post-LPS model dynamics are shown by solid black lines \((2\, \text{ng/kg})\), red dashed lines \((4\, \text{ng/kg})\), blue dotted lines \((8\, \text{ng/kg})\), and green dashed-dotted lines \((16\, \text{ng/kg})\). (a) Inflammatory and cardiovascular simulations. (b) HPA axis simulations.
Figure 6.12: Model simulations for continuous LPS administration. We simulate a 2 ng/kg infusion over 4 hours. The solid orange lines denote results before LPS administration. The vertical dotted lines mark when the LPS is given (at $t = 13.5$ hours). The solid black lines show results for a 2 ng/kg bolus injection, and the dotted blue lines show results with continuous infusion.
7.1 Introduction

Sepsis is defined as “life-threatening organ dysfunction from a dysregulated host response to infection” [238], as discussed in detail in Section 2.5. Mathematical models of sepsis (reviewed in Section 3.5) range from simple, generalized models to very complex models involving multi-system dynamics, and several utilize clinical sepsis data to calibrate or validate their models. Since the motivation for the work in this dissertation is to bridge the gap between experimental models of inflammatory dynamics and clinical models of sepsis, we develop and analyze a new, intermediate mathematical model of infection and sepsis based on current sepsis pathophysiology using the continuous infusion model presented in Chapter 5. The new model contains a specific immune system response, which some existing sepsis models lack [302, 269, 146, 86, 293, 300]. However, this is important for studying and mitigating the early septic trajectory. Another novelty is the use of human sepsis data to validate the septic outcome, which is omitted in several sepsis models [242, 234, 233, 244, 86, 46]. Our preliminary model focuses on the inflammatory response, but since the structure is similar to our previous models,
it would be easy to integrate the model with the multi-system model in Chapter 6. This would generate a comprehensive sepsis model that can provide insight into the complex dynamics of sepsis in the immune, cardiovascular, and endocrine systems.

To transition the continuous LPS infusion model in Chapter 5 to predict the response to infection and sepsis, we incorporate its monocyte and cytokine dynamics but substitute the endotoxin equation with that for an infectious pathogen. We also connect the system to a dynamic damage component that measures the impact that inflammation on tissue and organ function, a key feature of sepsis. To examine the end-behavior of the new components, we conduct a flow analysis of the pathogen and damage equations. The end-behavior is studied computationally through a parametric analysis of the model, demonstrating the model’s ability to bifurcate to multiple clinical inflammation scenarios including a healthy infection response, aseptic infection (persistent non-infectious inflammation), persistent infectious immunosuppression, and recurrent infection. Our aseptic model output is compared to singular cytokine measurements from sepsis patients reported in Berg et al. [21]. Finally, we use our model to gain understanding of sepsis progression and resolution by exploring the effectiveness of a treatment that induces additional anti-inflammatory strength, such as the newly suggested therapeutic treatment of intravenous vitamin C administration [126]. Analysis of this treatment shows a nonlinear relationship between treatment administration time and dose administered. Later administration times require significantly larger dose increases to be successful at reversing negative outcomes. The motivation for this work is also discussed in a SIAM News Blog [291] (article not included in this thesis).

7.2 Data

To validate our mathematical model, we utilize cytokine data from the published study by Berg et al. [21], examining static and dynamic cerebral autoregulation in 16 clinically-diagnosed intensive care unit (ICU) patients (14 male, 2 female aged 40-74) with late-stage sepsis. All subjects had an infection at the time of study. Ethical approval was given by the Scientific Ethical Committee of Copenhagen and Frederiksberg Municipalities, and followed the Declaration of Helsinki. Patient consent to participate was awarded orally and in writing by both the patient’s next of kin and medical personnel. Study inclusion criteria included being (i) 18-75 years of age, (ii) diagnosed with severe sepsis or septic shock according to the guidelines in Levy et al. [145] within the last 72 hours, and (iii) inserted with a radial artery catheter. Evidence of (i) neural trauma or infection, cerebrovascular disease, or pregnancy, and (ii) arterial hypertension excluded participants from the study.
All patients were mechanically ventilated, and 11 of them were in septic shock. 14 patients used at least one sedative (propofol, fentanyl, and/or remifentanil), and four died within 30 days of the study. Measurements for each patient included standard sepsis-related scores (APACHE II, SOFA, Ramsay, GCS), a blood sample, cardiovascular markers, and cognitive status. The site and type of infection were also identified. During the study, a single measurement was taken from each patient. That measurement occurred either (i) within 24 hours (10 patients), (ii) between 24 and 48 hours (3 patients), or (iii) between 48 and 72 hours (3 patients) after sepsis diagnosis. The singular measurements of cytokine data (TNF-α and IL-6) from each of the 16 sepsis patients are shown in Figure 7.1. While TNF-α measurements between patients are relatively succinct between 2 and 28 pg/mL, there is large variation in IL-6 measurements between patients, ranging from 29—16,419 pg/mL. Both cytokine plots in Figure 7.1 also display that cytokine levels are higher in patients who were more recently diagnosed with sepsis (in the earlier stages of disease) whereas measurements taken later have lower concentrations.

### 7.3 Mathematical Model

The initial presence of an infectious agent is one of the diagnostic criteria of sepsis [238], and it is responsible for initiating the inflammation that progresses to sepsis. Once the system is stimulated, monocytes become activated and work to eliminate the pathogen. To create an effective response, the monocytes elicit pro- and anti-inflammatory cytokines. While the exact cytokine pathways can vary depending on the organ or tissue they reside in (as described in

![Figure 7.1: Cytokine data reported in Berg et al. [21] from 16 ICU severe sepsis and septic shock patients. Each patient had a single measurement taken for each cytokine, which are denoted by the black dots. The IL-6 plot (right) is presented on a logarithmic scale due to the magnitude of cytokine concentrations.](image-url)
section 2.4), there are common interactions between them. Moreover, diagnosis of sepsis and septic shock involve the presence of cardiovascular, neural, respiratory, metabolic, nervous, and renal dysfunction [42, 178, 96]. Therefore, incorporation of a pathogenic component, monocyte dynamics, cytokine influences, and a dynamic term representing tissue and organ dysfunction is critical to include in our mathematical model to appropriately capture sepsis dynamics.

The mathematical model presented in this chapter is adapted from the immune response model in Windoloski et al. [290] (Chapter 5). While that study examines the short-term immune response to endotoxin during a continuous infusion, the current study focuses on transitioning from a short-term response to a clinically prolonged response. Thus, to capture this response, we adapt the endotoxin equation in Windoloski et al. [290] to an equation describing the dynamic behavior of a pathogenic insult. We also incorporate a dynamic damage term describing the lumped effects of the pathogen and inflammation markers on body tissue and function. The mathematical model consists of a system of eight ordinary differential equations and 56 parameters. Model states include a pathogen (\( P \), unitless), resting and activated monocytes (\( M_R \) and \( M_A \), number of cells - noc), cytokines TNF-\( \alpha \), IL-6, IL-8, and IL-10 (\( T N F, I L_6, I L_8, I L_10 \), pg/mL), and tissue damage (\( D \), unitless). The relationships between model states are shown in Figure 7.2 and described below.

Throughout our model, we utilize Hill functions to predict the gradual stimulation or inhibition of state \( Y \) by state \( X \). The upregulation (stimulation) function is given by

\[
H^U_Y(X) = \frac{X^h}{\eta^h_{YX} + X^h}
\]

and the downregulation (inhibition) function is given by

\[
H^D_Y(X) = \frac{\eta^h_{YX}}{\eta^h_{YX} + X^h}
\]

The constant, \( \eta_{YX} \), represents the half maximum value of state \( X \) and the exponent, \( h \), determines the rate of increase or decrease of the function.

**Infectious Agent**

The initial driving force that instigates the immune response in our model is the presence of a pathogen. As modeled in Kumar et al. [137], Reynolds et al. [207], Shi et al. [234, 233], Yamanaka et al. [293], Chen et al. [46], the pathogen follows logistic growth with growth rate \( k_{PG} \) (hr\(^{-1}\)) and maximum concentration \( P_\infty \) (\( P \) units). Its destruction is due to the phagocytic properties of immune cells [174]. Non-specific inflammation models such as Kumar et al. [137], Reynolds
et al. [207], Zuev et al. [302], Yamanaka et al. [293] use a generalized pro-inflammation term (which includes phagocytes) to model this interaction, while more detailed studies utilize neutrophils and macrophages [234, 233, 164, 46]. Here, we represent the phagocytic destruction of the pathogen using monocytes, which are immature macrophages [174] that exhibit multiple roles during sepsis including pathogen clearance [292]. The monocytes eliminate the pathogen at the rate $k_{PM}$ ($(\text{noc-hr}^{-1})$, and the clearance strength is dependent on the number of activated monocytes and magnitude of the pathogen. Therefore, the change in the pathogen concentration is represented by

$$\frac{dP}{dt} = k_{PG} P \left(1 - \frac{P}{P_{\infty}}\right) - k_{PM} M_A P.$$  \hspace{1cm} (7.1)

Note that equation (7.1) resembles the structure of the endotoxin equation in [290] during a continuous infusion. However, instead of the constant infusion of 0.5 ng/kg/hr of endotoxin in the first term, we utilize the term $k_{PG} P \left(1 - \frac{P}{P_{\infty}}\right)$ to symbolize the dynamic behavior of a multiplying pathogen concentration. Furthermore, the second term in equation (7.1) is also in the endotoxin equation in [290]. The difference is that in this pathogen equation, we incorporate feedback from the monocytes onto the clearance of the stimuli whereas the endotoxin equation only incorporates the natural decay of the endotoxin in the system. Other pathogen equations

![Diagram](image_url)

Figure 7.2: Diagram of the mathematical model of infection. Stimulation is represented by solid lines, and inhibition is represented by dotted lines.
that could be explored in the future include

\[
\frac{dP}{dt} = k_{PG}P \left( 1 - \left( \frac{P}{P_\infty} \right)^2 \right) - k_{PM}M_A P
\]

or

\[
\frac{dP}{dt} = k_{PG}P^2 \left( 1 - \frac{P}{P_\infty} \right) - k_{PM}M_A P.
\]

**Immune Response**

The immune response is initiated when a pathogen is detected in the body. This involves the activation of monocytes, which assist in eliminating the pathogen. We determine the transition of resting monocytes \(M_R\) to activated monocytes \(M_A\) that migrate towards the site of infection to engage in phagocytosis in equations (7.2) and (7.3). Resting monocytes become activated through the detection of the pathogen or release of damaged particles, which activates monocytes through the recognition of PAMPs (pathogen-associated molecular patterns) or DAMPs (damage-associated molecular patterns) \([178]\) respectively. The activation of monocytes by the pathogen occurs at the rate \(k_{MP}\) (hr\(^{-1}\)). Major pro-inflammatory mediator, TNF-\(\alpha\), also stimulates the activation of monocytes at a rate \(k_{MTNF}\) (hr\(^{-1}\)) while IL-10 inhibits stimulation by the pathogen. The activation of monocytes by DAMPs occurs at the rate \(k_{MD}\) (hr\(^{-1}\)), and activated monocytes deplete at the rate \(k_{MA}\) (hr\(^{-1}\)). The resting monocyte pool is regenerated at the rate \(k_{MR}\) (hr\(^{-1}\)) up to the maximal monocyte concentration \(M_\infty\). Therefore, the equations describing the resting and activated monocyte dynamics are given by

\[
\frac{dM_R}{dt} = k_{MR}M_R \left( 1 - \frac{M_R}{M_\infty} \right) - \left( H^U_M(P) \left( k_{MP} + k_{MTNF}H^U_M(TNF) \right) H^U_M(IL10) + k_{MD}H^U_M(D) \right) M_R
\]

\(7.2\)

\[
\frac{dM_A}{dt} = \left( H^U_M(P) \left( k_{MP} + k_{MTNF}H^U_M(TNF) \right) H^U_M(IL10) + k_{MD}H^U_M(D) \right) M_R - k_{MA}M_A.
\]

\(7.3\)

Due to the damage that can occur from sustained inflammation during an infection, equations (7.2) and (7.3) differ from the monocyte equations during an endotoxin challenge in Chapters 5 and 6. The endotoxin challenge only stimulates a short-term immune response, which is not elicited for long enough to generate significant systemic dysfunction.

Once monocytes become activated, they produce pro- and anti-inflammatory cytokines that then feed back to promote or inhibit monocyte activation. Equations (7.4) - (7.7) describing
cytokine interactions remains unchanged from our previous studies [29, 69, 288, 290], so we refer to those manuscripts and Chapter 5 for a detailed description of these equations.

\[
\frac{dT\text{NF}}{dt} = k_{TNFM}H_{TNF}^D(II6)H_{TNF}^D(II10)M_A - k_{TNF}(T\text{NF} - w_{TNF}) \tag{7.4}
\]

\[
\frac{dII6}{dt} = \left(k_{6M} + k_{6TNF}H_{II6}^U(T\text{NF})\right)H_{II6}^D(II6)H_{II6}^D(II10)M_A - k_6(II6 - w_6) \tag{7.5}
\]

\[
\frac{dII8}{dt} = \left(k_{8M} + k_{8TNF}H_{II8}^U(T\text{NF})\right)H_{II8}^D(II10)M_A - k_8(II8 - w_8) \tag{7.6}
\]

\[
\frac{dII10}{dt} = \left(k_{10M} + k_{106}H_{II10}^U(II6)\right)M_A - k_{10}(II10 - w_{10}). \tag{7.7}
\]

**Tissue Damage**

**Motivation**

To capture the systemic effects that prolonged inflammation during sepsis has on the body, we incorporate a dynamic damage term in the model. Because our model portrays a snapshot of the immune response and does not incorporate other influences such as cardiovascular, hormonal, or metabolic impacts, we focus the stimulation of damage directly from inflammation. Two prominent cytokines associated with damage and dysfunction during sepsis are TNF-α and IL-6, and so these cytokines incite damage in our model.

TNF-α levels can be excessive in individuals with sepsis. Evidence suggests that high TNF-α concentrations has residual effects including an increase in nitric oxide production (which induces vasodilation) [193, 258, 14], vascular and tissue permeability [193, 258, 96, 220], thrombosis [96, 193, 258], decreased cardiac output [193, 258], and impacts on cardiac contractility [14, 199]. The downstream effects of these cardiovascular malfunctions can result in metabolic dysfunction [96, 42]. Therefore, TNF-α is a prominent trigger for tissue and organ damage. While other models have implemented cardiovascular impacts into their damage equations, our model is the first to directly use TNF-α.

The cytokine IL-6 has also been implicated as a correlative variable in sepsis-related complications and mortality [122], and it has been used in the models by Chow et al. [50] and McDaniel et al. [164] to promote systemic damage. It is an important initiator of the acute phase response [118, 75] and can also activate the coagulation system in response to inflammation, which can lead to endothelial damage and increased thrombosis [108]. This can cascade to further cardiovascular and metabolic complications. Therefore, IL-6 can also be an instigator of systemic
damage in the system.

Equation

We define the damage state variable, \( D \), to be a unitless quantity summarizing the tissue and organ dysfunction that results from inflammation (see section 2.5 for organ dysfunction in sepsis). This state takes on values between zero (healthy) and one (deceased), where the quantity \( D_{\text{max}} < 1 \) (unitless) is the threshold after which damage becomes irreversible. In the absence of cytokine stimulation and based on the work by Reynolds [208], the damage equation is constructed so that there are three equilibrium points at \( D = 0, D = D_{\text{max}}, \) and \( D = 1 \), where \( D = 0 \) and \( D = 1 \) are stable and \( D = D_{\text{max}} \) is unstable (see Figure 7.5b and section 7.4 for stability analysis). Thus, if \( D \) is below the damage threshold \( D = D_{\text{max}} \), then the system can recover to \( D = 0 \). Otherwise, the systemic damage continues to grow until the system eventually succumbs to the damage at \( D = 1 \). Therefore, with repair rate \( k_D \) (hr\(^{-1}\)), the damage equation begins with the form

\[
\frac{dD}{dt} = k_D D(1 - D)(D_{\text{max}} - D). \tag{7.8}
\]

Next, we consider the elements in the model that invoke tissue and organ dysfunction. As previously discussed, these include cytokines TNF-\( \alpha \) and IL-6. In Chapter 5, the activation timing and the timing that the peak TNF-\( \alpha \) and IL-6 concentrations occur differs. Thus, these two entities may impact the damage dynamics distinctively due to their time-varying differences. To study their individual impacts on damage progression in sepsis, we build three damage equations, two that investigate how TNF-\( \alpha \) and IL-6 impact systemic damage independently (equations (7.9) and (7.10)), and one studying their combined impact on the system (equation (7.11)). The equation studying their combined impact is used for the remainder of the study unless otherwise noted.

The incitement of damage from cytokines TNF-\( \alpha \) and IL-6 are incorporated into the damage equation through the growth terms \( k_{D_{\text{TNF}}}(1 - D)H_D^U(TNF - w_{TNF}) \) and \( k_{D_6}(1 - D)H_D^U(IL6 - w_6) \), respectively. Here, the undamaged tissue and organs (represented by \( 1 - D \)) become damaged by TNF-\( \alpha \) and IL-6 at rates \( k_{D_{\text{TNF}}} \) and \( k_{D_6} \) (hr\(^{-1}\)), respectively. A similar formulation is seen in the models by Chow et al. [50], Reynolds [208], and McDaniel et al. [164]. Also note that concentrations of TNF-\( \alpha \) and IL-6 must be above their baseline values \( w_{TNF} \) and \( w_6 \) in order to initiate any systemic damage, and that the inputs of these two upregulation functions \( (TNF - w_{TNF} \) and \( IL6 - w_6) \) will not become negative due to the structure of equations (7.4) and (7.5). Thus, the terms representing the independent impacts of TNF-\( \alpha \) and IL-6 are
combined with the repair dynamics in equation (7.8) to give the damage equations

\[
\frac{dD}{dt} = (1 - D) \left( k_{D_{TNF}} H_D^U (TNF - w_{TNF}) - k_D D (D_{max} - D) \right),
\]

(7.9)

and

\[
\frac{dD}{dt} = (1 - D) \left( k_{D_{IL6}} H_D^U (IL6 - w_{IL6}) - k_D D (D_{max} - D) \right).
\]

(7.10)

The stimulation terms for TNF-α and IL-6 are then combined to create a damage equation with integrated cytokine impacts on systemic damage given by

\[
\frac{dD}{dt} = (1 - D) \left( k_{D_{TNF}} H_D^U (TNF - w_{TNF}) + k_{D_{IL6}} H_D^U (IL6 - w_{IL6}) - k_D D (D_{max} - D) \right).
\]

(7.11)

### 7.4 Flow Analysis

We now focus on analyzing the ability of the mathematical model of infection given by equations (7.1)-(7.7) and (7.11) to bifurcate to clinical inflammation scenarios. To obtain preliminary insight into the different systemic states in the model that could be observed, we analytically study the pathogen and damage equations. Using techniques from Strogatz [245], we determine the equilibrium points and their stability in the isolated pathogen and damage ODEs. While this dynamical systems terminology is ideally restricted to studying the steady states of the full ODE system, we define these terms here to represent the end-behavior of the pathogen or damage when other dynamic states are held constant. Thus, we caution the reader that while this flow on the line analysis is only strictly valid for dynamics of a single equation, we analyze the decoupled system when examining the impact of flow.

#### 7.4.1 Pathogen

The steady state solutions for equation (7.1) are given by

\[
\frac{dP}{dt} = k_{PG} P \left( 1 - \frac{P}{P_\infty} \right) - k_{PM} M_A P = 0,
\]

\[
P \left( k_{PG} \left( 1 - \frac{P}{P_\infty} \right) - k_{PM} M_A \right) = 0,
\]
so the first equilibrium point is given by

\[ P = 0 \]  \hspace{1cm} (7.12)

and the second equilibrium point is given by

\[ k_{PG} \left( 1 - \frac{P}{P_{\infty}} \right) - k_{PM} M_A = 0, \]
\[ k_{PG} \left( 1 - \frac{P}{P_{\infty}} \right) = k_{PM} M_A, \]
\[ 1 - \frac{P}{P_{\infty}} = k_{PM} M_A, \]
\[ P = P_{\infty} \left( 1 - \frac{k_{PM}}{k_{PG}} M_A \right). \]  \hspace{1cm} (7.13)

The existence, location, and stability of these two equilibrium points depend on the relation between \( k_{PG}, k_{PM}, \) and time-varying state \( M_A. \) To determine stability, we rewrite the differential equation in (7.1) as

\[
\begin{align*}
\frac{dP}{dt} &= k_{PG} \left( 1 - \frac{P}{P_{\infty}} \right) - k_{PM} M_A P = 0 \\
\frac{dP}{dt} &= k_{PG} P - k_{PG} P^2 - k_{PM} M_A P = 0 \\
\frac{dP}{dt} &= \left( -\frac{k_{PG}}{P_{\infty}} \right) P^2 + (k_{PG} - k_{PM} M_A) P = 0.
\end{align*}
\]

This equation is a quadratic with at most 2 real roots (given by equations (7.12) and (7.13)) and will be oriented downward since \(-\frac{k_{PG}}{P_{\infty}} < 0.\) To determine the number of equilibrium points and their stability, we investigate three cases shown in Figure 7.3.

1. As shown in Figure 7.3a, if \( k_{PG} - k_{PM} M_A < 0 \) then \( 1 < \frac{k_{PM}}{k_{PG}} M_A \) and the equilibrium point in equation (7.13) would be negative. Therefore, the downward oriented parabola would have two roots given by equations (7.12) and (7.13) where \( \frac{dP}{dt} < 0 \) when \( P < P_{\infty} \left( 1 - \frac{k_{PM}}{k_{PG}} M_A \right) \) and \( P > 0, \) and \( \frac{dP}{dt} > 0 \) when \( P_{\infty} \left( 1 - \frac{k_{PM}}{k_{PG}} M_A \right) < P < 0. \) Hence, \( P = P_{\infty} \left( 1 - \frac{k_{PM}}{k_{PG}} M_A \right) \) is unstable and \( P = 0 \) is stable, and so the pathogen (which is always nonnegative) will be eliminated from the system.

2. As shown in Figure 7.3b, if \( k_{PG} - k_{PM} M_A > 0 \) then \( 1 > \frac{k_{PM}}{k_{PG}} M_A \) and the equilibrium point in
equation (7.13) would be positive. Therefore, the downward oriented parabola would have two roots given by equations (7.12) and (7.13) where \( \frac{dP}{dt} < 0 \) when \( P > P_\infty \left( 1 - \frac{k_{PM}}{k_{PG}} M_A \right) \) and \( P < 0 \), and \( \frac{dP}{dt} > 0 \) when \( 0 < P < P_\infty \left( 1 - \frac{k_{PM}}{k_{PG}} M_A \right) \). Hence, \( P = 0 \) is unstable and \( P = P_\infty \left( 1 - \frac{k_{PM}}{k_{PG}} M_A \right) \) is stable, and so the pathogen (which is always nonnegative) will grow to \( P = P_\infty \left( 1 - \frac{k_{PM}}{k_{PG}} M_A \right) \).

3. As shown in Figure 7.3c, if \( k_{PG} - k_{PM} M_A = 0 \) then

\[
\left( -\frac{k_{PG}}{P_\infty} \right) P^2 + (k_{PG} - k_{PM} M_A) P = \left( -\frac{k_{PG}}{P_\infty} \right) P^2,
\]

and so \( P = 0 \) is the only equilibrium point. Since the parabola is downward oriented, then \( \frac{dP}{dt} < 0 \) for \( P \neq 0 \). Therefore, \( P = 0 \) is half-stable, and so the pathogen (which is always nonnegative) will be eliminated from the system.

If \( M_A \) approaches its baseline value of zero (\( M_A \to 0 \)), then the second equilibrium point is

\[
P \to P_\infty
\]

(7.14)

and case 2 above (Figure 7.3b) applies since \( P \) is defined by equation (7.1). Here, \( P = P_\infty \) is stable and \( P = 0 \) is unstable, as shown in Figure 7.4a since \( \frac{dP}{dt} < 0 \) when \( P < 0 \) and \( P > P_\infty \) and \( \frac{dP}{dt} > 0 \) when \( 0 < P < P_\infty \). Because a healthy resolution of an infection would indicate that the

![Figure 7.3](image-url)

**Figure 7.3:** Flow on the line of \( P \) where (a) \( k_{PG} - k_{PM} M_A < 0 \), (b) \( k_{PG} - k_{PM} M_A > 0 \), and (c) \( k_{PG} - k_{PM} M_A = 0 \). The red circles represent the equilibrium points. An unfilled circle represents an unstable equilibrium, a half-filled circle represents a half-stable equilibrium, a filled circle represents a stable equilibrium. Black arrows indicate the direction of flow.
pathogen is eliminated from the system ($P = 0$ is stable) and levels of activated monocytes returned to baseline, we adapt the equation for $\frac{dP}{dt}$ to consist of only the decay part of equation (7.1) when minute levels of pathogen are still circulating ($P \leq 0.05$), so

$$\frac{dP}{dt} = -k_{PM}M_A P. \quad (7.15)$$

This ensures that the pathogen becomes eliminated when its concentration is small (5% of its initial condition as in Kumar et al. [137]) and that $P = 0$ is stable as shown in Figure 7.4b. We also note that, with the condition stated in equation (7.15), $\frac{dP}{dt}$ is discontinuous. Therefore, we formally define the pathogen equation as

$$\frac{dP}{dt} = k_{PG}P \left(1 - \frac{P}{P_\infty}\right) - k_{PM}M_A P$$

when $P > 0.05$ and

$$\frac{dP}{dt} = -k_{PM}M_A P.$$

when $P \leq 0.05$.

Figure 7.4: Flow on the line of $P$. (a) Flow when $\frac{dP}{dt}$ is given in equation (7.1) when $M_A = 0$ and (b) flow when $\frac{dP}{dt}$ is given in equation (7.15). The red circles represent the equilibrium points. An unfilled circle represents an unstable equilibrium, a filled circle represents a stable equilibrium. Black arrows indicate the direction of flow.
7.4.2 Damage

The steady state solutions for equation (7.11) are given by

\[
\frac{dD}{dt} = 0 \\
(1 - D)(k_{DTNF} H_D^U(TNF - w_{TNF}) + k_{D6} H_D^U(1L6 - w_6) - k_D D(D_{max} - D)) = 0
\]

so the first equilibrium point is

\[ D = 1. \tag{7.16} \]

The two other equilibrium points are given by

\[
k_{DTNF} H_D^U(TNF - w_{TNF}) + k_{D6} H_D^U(1L6 - w_6) - k_D D(D_{max} - D) = 0, \\
k_D D^2 - k_D D_{max} D + (k_{DTNF} H_D^U(TNF - w_{TNF}) + k_{D6} H_D^U(1L6 - w_6)) = 0,
\]

\[
\Rightarrow D = \frac{k_D D_{max} \pm \sqrt{k_D^2 D_{max}^2 - 4k_D (k_{DTNF} H_D^U(TNF - w_{TNF}) + k_{D6} H_D^U(1L6 - w_6))}}{2k_D}.
\]

This can be rewritten as

\[
D = \frac{k_D D_{max}}{2k_D} \pm \sqrt{\frac{k_D^2 D_{max}^2 - 4k_D (k_{DTNF} H_D^U(TNF - w_{TNF}) + k_{D6} H_D^U(1L6 - w_6))}{2k_D}},
\]

\[
D = \frac{D_{max}}{2} \pm \sqrt{\frac{1}{4} D_{max}^2 - \frac{k_{DTNF} H_D^U(TNF - w_{TNF}) + k_{D6} H_D^U(1L6 - w_6)}{k_D}},
\]

\[ D = \delta \pm \sqrt{\alpha - \epsilon - \beta} \tag{7.17} \]

where \( \alpha = \frac{1}{4} D_{max}^2, \beta = \frac{k_{D6}}{k_D} H_D^U(1L6 - w_6) \), and \( \delta = \frac{D_{max}}{2} \), and \( \epsilon = \frac{k_{DTNF}}{k_D} H_D^U(TNF - w_{TNF}) \). The existence, location, and stability of the equilibrium points given by equations (7.16) and (7.17) are dependent upon the relationship between \( \alpha, \beta, \delta, \) and \( \epsilon \). To analyze the equilibrium points and the direction of flow for \( D \), we first rewrite equation (7.11) in polynomial format

\[
\frac{dD}{dt} = (1 - D)(k_{DTNF} H_D^U(TNF - w_{TNF}) + k_{D6} H_D^U(1L6 - w_6) - k_D D(D_{max} - D))
\]

\[
= k_{DTNF}(1 - D)H_D^U(TNF - w_{TNF}) + k_{D6}(1 - D)H_D^U(1L6 - w_6) - k_D D(1 - D)(D_{max} - D)
\]

\[
= -k_D D^3 + (k_D D_{max} + k_D) D^2 - (k_D D_{max} + k_{DTNF} H_D^U(TNF - w_{TNF}) + k_{D6} H_D^U(1L6 - w_6)) D + \frac{k_{DTNF} H_D^U(TNF - w_{TNF}) + k_{D6} H_D^U(1L6 - w_6)}{D}.
\]
Since \(-k_D < 0\), then \(\frac{dD}{dt}\) has a cubic shape with up to three real roots where \(\frac{dD}{dt} \to \infty\) as \(D \to -\infty\) and \(\frac{dD}{dt} \to -\infty\) as \(D \to \infty\). Given this, we can investigate the three relationships between \(\alpha, \beta\) and \(\epsilon\) to determine existence, location, and stability of equilibrium points.

1. As shown in Figure 7.5a, if \(\alpha < \epsilon + \beta\), then equation (7.17) has two imaginary roots. Thus, the only real equilibrium point would be at \(D = 1\). This equilibrium point would be stable.

2. As shown in Figure 7.5b, if \(\alpha = \epsilon + \beta\), then equation (7.17) has one repeated root at

\[
D = \delta \pm \sqrt{\alpha - (\epsilon + \beta)} = \delta \pm \sqrt{0} = \delta = \frac{D_{\text{max}}}{2}.
\]

Thus, there are two equilibrium points at \(D = \frac{D_{\text{max}}}{2}\) and \(D = 1\). Since \(0 < D_{\text{max}} < 1\), then

![Figure 7.5: Flow on the line of D.](image)

Figure 7.5: Flow on the line of \(D\). (a) Flow when \(\alpha < \epsilon + \beta\), (b) flow when \(\alpha = \epsilon + \beta\), (c) flow when \(\alpha > \epsilon + \beta\) and \(\delta = \sqrt{\alpha - (\epsilon + \beta)}\), and (d) flow when \(\alpha > \epsilon + \beta\) and \(\delta > \sqrt{\alpha - (\epsilon + \beta)}\) where \(\alpha = \frac{1}{4}D_{\text{max}}^2\), \(\beta = \frac{k_D}{k_D}H_D^U(\overline{IL6} - \overline{w_6})\), and \(\delta = \frac{D_{\text{max}}}{2}\), and \(\epsilon = \frac{k_{DTNF}}{k_D}H_D^U(TNF - \overline{w_{TNF}})\). The red circles represent the equilibrium points. An unfilled circle represents an unstable equilibrium, a half-filled circle represents a half-stable equilibrium, a filled circle represents a stable equilibrium. Black arrows indicate the direction of flow.
\[
\frac{D_{\text{max}}}{2} < 1, \text{ and so by the shape of the cubic function, } D = \frac{D_{\text{max}}}{2} \text{ is half-stable and } D = 1 \text{ is stable.}
\]

3. If \( \alpha > \epsilon + \beta \), then there are three subcases:

(a) As shown in Figure 7.5c, if \( \delta = \sqrt{\alpha - (\epsilon + \beta)} \), then this implies that \( \epsilon + \beta = 0 \) since \( \sqrt{\alpha} = \delta \). If \( \epsilon + \beta = 0 \), then this implies that both \( \epsilon = 0 \) and \( \beta = 0 \) since these are both nonnegative functions. Hence, TNF-\( \alpha \) and IL-6 returned to their baseline concentrations. Therefore,

\[
D = \delta \pm \sqrt{\alpha - (\epsilon + \beta)} = \delta \pm \sqrt{\alpha} = \delta \pm \delta
\]

\[\implies D = 0, D = 2\delta = D_{\text{max}}. \quad (7.18)\]

Hence, there are three equilibrium points at \( D = 0, D = D_{\text{max}}, \text{ and } D = 1 \). By the shape of the cubic function dictating \( \frac{dD}{dt} \), we have that \( D = 0 \) and \( D = 1 \) are stable and \( D = D_{\text{max}} \) is unstable.

(b) As shown in Figure 7.5d, if \( \delta > \sqrt{\alpha - (\epsilon + \beta)} \), then there are three positive equilibrium points at \( D = \delta - \sqrt{\alpha - (\epsilon + \beta)}, D = \delta + \sqrt{\alpha - (\epsilon + \beta)}, \text{ and } D = 1 \). Since \( 0 < D_{\text{max}} < 1 \), then \( 0 < \delta - \sqrt{\alpha - (\epsilon + \beta)} < \delta + \sqrt{\alpha - (\epsilon + \beta)} < 1 \). Therefore, by the shape of the cubic function, we have that \( D = \delta - \sqrt{\alpha - (\epsilon + \beta)} \) and \( D = 1 \) are stable and \( D = \delta + \sqrt{\alpha - (\epsilon + \beta)} \) is unstable.

(c) The case where \( \delta < \sqrt{\alpha - (\epsilon + \beta)} \) is not possible since \( \epsilon + \beta \) will always be nonnegative and

\[
\sqrt{\alpha - (\epsilon + \beta)} < \sqrt{\alpha} = \delta.
\]

Thus, no additional cases of equilibrium points will be obtained from this condition. Note that this means that \( D = 1 \) will never be a double root.

A similar analysis can be done on the damage equations in (7.9) and (7.10).

### 7.5 Parametric Analysis

A bifurcation analysis on the mathematical model (equations (7.1)-(7.7) and (7.11)) is the ideal method for examining the impact of parameter changes on solution behavior of the ODE system. However, given the complexity of the system in this chapter, we conduct a preliminary
parametric analysis to computationally infer solution behavior prior to a formal analysis. This parametric analysis displays the model's ability to bifurcate from a healthy infection response to clinical inflammation states such as an aseptic infection (persistent non-infectious inflammation), persistent infectious immunosuppression, and recurrent infection. This is performed by perturbing each parameter individually by a factor of 10 while fixing all other parameters at their nominal value. Thus, only one parameter was changed from its nominal value during each simulation. A subset of the resulting perturbations (organized by the type of inflammation they represent) are shown and discussed in the following subsections. A description of the captured inflammation states (and the septic infection state which is not captured by the current model) is shown in Figure 7.6 along with parameters that result in these states when perturbed. The results are related to the flow analysis described in section 7.4 in Table 7.2. Next steps involve relating these results to clinical findings.

7.5.1 Healthy Infection Response

To initialize our mathematical model of infection, we calibrate it to simulate a healthy response to infection (Table 3.4) which has the following characteristics as stated in Figure 7.6:

- The pathogen is cleared,
- Monocytes and cytokines return to baseline values,
- Minimal to no damage occurs. Any damage inflicted is repaired.

This is the common healthy response to infection, as observed in other mathematical models such as Kumar et al. [137], Reynolds et al. [207], McDaniel et al. [164].

Model Calibration

Initial conditions for the cytokine states are taken from mean cytokine concentrations of healthy individuals reported in Guo et al. [93]. Initial conditions for the resting and activated monocytes are set as in Chapters 5 and 6, and the pathogen is initially set to one as in Reynolds et al. [207]. Nominal parameters for cytokine equations (7.4-7.7) are taken from the optimal mean continuous infusion parameter set in Chapter 5, and several half-maximum and exponent parameters are manually adjusted. The majority of resting and activated monocyte nominal parameters are taken from the continuous infusion model in Chapter 5, Chow et al. [50], and McDaniel et al. [164], and most parameters for the pathogen and tissue damage equations are taken from Chow et al. [50], McDaniel et al. [164], and Reynolds et al. [207]. The remaining
parameters are manually set. For recovery from infection to a healthy state, we examine all three
damage equations. We first calibrate our model to a healthy response by using the damage
equation in equation (7.10). Then, the TNF-α component is added to create the damage
equation (7.11). Parameters used to simulate a healthy response to infection are shown in Table
7.1. The model was solved numerically using the MATLAB solver ode15s.

Figure 7.6: Diagram of the clinical inflammation states discussed in this work, including the
septic infection state which was not captured by the model. For each inflammation state,
the characteristic dynamics of the model are described. Also, the perturbed parameters that
were increased (higher) or decreased (lower) from their nominal value are stated. Each single
parameter perturbation listed was able to independently result in the inflammation state
while all other model parameters (including the others listed) remained at their nominal
value. The parameter denoted in red resulted in a model state where damage was repaired but
inflammatory markers remained high.
Model Response

The healthy response to infection is shown in Figure 7.7. The pathogen grows for approximately four hours before monocytes are activated. This causes a sharp increase in the production of pro- and anti-inflammatory mediators for about five hours after infection initiation, and these concentrations peak around hour six. As these entities increase between hours four and six, the pathogen is being destroyed by the activated monocytes. As the pathogen is eliminated from the system, the number of activated monocytes declines, which subsequently results in a decline in cytokine concentrations. During the peak of the monocytes and cytokines, a very minor amount of damage also begins to accrue around five hours after infection onset and increases until around hour seven or eight. After which, the small amount of damage inflicted within the system begins to recover. The pro-inflammatory cytokines TNF-α, IL-6, and IL-8 return to their healthy baseline concentrations approximately 45 hours after the onset of infection, while anti-inflammatory cytokine IL-10 recovers around 35 hours after infection. The minor damage caused by the infection is resolved about 24 hours after its onset.

Table 7.2 relates these results to the flow analysis derived in Section 7.4. The pathogen equilibrium point corresponds to Figure 7.4b since \( k_{PG} - k_{PM} M_A > 0 \), and so the pathogen is moving towards at \( P = 0 \), which is stable. The damage equation flows toward \( D = 0 \) as in Figure

Figure 7.7: A healthy model response to infection for 2 days post-infection onset. Immune cells and cytokines are able to clear the pathogen from the system and return to baseline values in a timely manner without causing much damage to the system. The damage that occurred is resolved within 2 days of infection.
7.5c since \( \alpha > \epsilon + \beta \) and \( \delta = \sqrt{\alpha - (\epsilon + \beta)} \). In this case, the model solution did not surpass the unstable equilibrium point at threshold \( D_{\text{max}} \), and so it returned to the stable equilibrium point at \( D = 0 \).

These results show that the model operates within reasonable cytokine concentrations and dynamics such as those seen in endotoxin challenge studies used in this thesis (continuous infusion endotoxin study from Berg et al. [21] and bolus endotoxin study from Janum et al. [115]). Using Table 5.1 from Chapter 5, we observe that TNF-\( \alpha \) concentrations peak in range shown in these studies, IL-6 peaks slightly lower than both studies, IL-8 in the range of the continuous infusion study, and IL-10 peaks in the range of the bolus study. The cytokines dynamics in our infection model display a small increase in concentration until four hours after infection onset, at which time their rate of increase becomes much larger. While a similar behavior is observed in the LPS studies, it occurs earlier. The bolus study by Janum et al. [115] exhibits this increase two hours after LPS administration and the continuous infusion study by Berg et al. [21] shows this around hour three after infusion begins. Peak timing for these cytokines occurs approximately four to five hours later in the infection response shown in Figure 7.7 than in the endotoxin studies, and similar peak timing are observed in other models of infection [207, 233, 164]. Furthermore, the recovery time of the healthy response back to baseline is also similar to that in the healthy infection response observed in McDaniel et al. [164].

**Damage Equation Influence**

We also investigate how the stimulation of systemic damage is influenced separately by TNF-\( \alpha \) and IL-6. Using damage equations in (7.9) and (7.10) with parameters from Table 7.1 results in the damage term dynamics shown in Figure 7.8. When IL-6 is the only stimulation term, the system displays a marginally later activation time of damage than when TNF-\( \alpha \) is the only damage-stimulating term (4.5 vs. 4.25 hours after infection onset). However, the total amount of damage caused to the system differs between the two stimulation terms. A higher magnitude of damage results from TNF-\( \alpha \) than IL-6 alone (0.0616 vs. 0.0274), and the maximal damage occurs later during TNF-\( \alpha \) only stimulation compared to IL-6 only stimulation (8 vs. 7 hours). Equations (7.9) and (7.10) both result in similar damage recovery times around 48 hours, but the rate at which both of these states recover differs. The IL-6 only stimulation results in a slower recovery than the TNF-\( \alpha \) only stimulation relative to their maximal damage. Therefore, we suspect that the impact of these model states on damage dynamics is more than simply reparameterizing the equation. The other model states (not shown) in equations (7.1)-(7.7) exhibit minor differences in peak concentration and peak timing when TNF-\( \alpha \) only and IL-6
only damage equations are utilized.

### 7.5.2 Aseptic Infection (Persistent Non-Infectious Inflammation)

Perturbation of several parameters (rate constants, half maximum values, and exponents) result in the system displaying *aseptic infection (persistent non-infectious inflammation)*. As shown in Figure 7.6, this is considered a state with the following characteristics:

- The pathogen is eliminated \((P = 0)\),
- Monocytes and cytokines remain significantly elevated \((M_A \gg 0, TNF \gg w_{TNF}, IL6 \gg w_6, IL8 \gg w_8, IL10 \gg w_{10})\),
- Critical organ and tissue damage occurred and resulted in a negative outcome \((D = 1)\).

The above description can considered a scenario in sepsis since its definition in Singer et al. [238] specifies the initial presence of infection and evidence of organ dysfunction. A similar response is also observed in mathematical models from Kumar et al. [137] and Reynolds et al. [207]. The work by Kumar et al. [137] shows the appearance of the persistent non-infectious inflammation state through increasing the initial pathogen load. The work by Reynolds et al. [207] shows that the appearance of this state, which they dubbed “aseptic death”, can arise through changes in the pathogen growth rate, where aseptic death is a stable state until the pathogen growth rate reaches 1.755, after which only the septic state (same as aseptic state except for the pathogen is non-zero) is stable. However, they also show that changes in the initial pathogen load and anti-inflammatory mediator can lead to an aseptic steady state.

![Figure 7.8: Individual damage dynamics for IL-6 (blue dotted line) and TNF-\(\alpha\) (red dashed line), and their combined influences (solid black line).](image-url)
Perturbations in the pathogen growth rate did not result in an aseptic or septic scenario in our model.

**Rate Constant Perturbations**

Scenarios of persistent non-infectious inflammation arising from perturbations in model rate constants are shown in Figure 7.9. The direction of perturbation (either increased or decreased from their nominal value) is also given in Table 7.2. Decreases in the activated monocyte \( k_{MA} = 0.349, 1.14 \) and IL-6 \( k_6 = 0.066 \) decay rates and increases in the monocyte activation rates of TNF-\( \alpha \) \( k_{TNFM} = 7.65 \) and IL-6 \( k_{6M} = 6.28 \) and the TNF-\( \alpha \) damage rate \( k_{DTNF} = 0.688 \) result in variable elevated levels of activated monocytes and cytokines, an eradicated pathogen, and maximal levels of damage inflicted on the system. It takes up to 20 hours to eliminate the infection, and 40 hours to about 6 days to inflict enough severe dysfunction that death occurs. Large TNF-\( \alpha \) levels resulting from a decrease in \( k_{MA} \) and increase in \( k_{TNFM} \) lead to a quick deterioration of the system (40 hours) whereas large concentrations of IL-6 resulting from a decrease in \( k_6 \) and increase in \( k_{6M} \) lead to slower deterioration (around 3 days). This is plausible given the separate distinct impact that TNF-\( \alpha \) and IL-6 have on the magnitude of damage (Figure 7.8).

In these simulations, a large sustained level of TNF-\( \alpha \) from decreases in \( k_{MA} \) result in high levels of anti-inflammatory cytokine IL-10, which is likely a combative move by the system to balance pro- and anti-inflammatory responses during inflammation as seen in literature [250, 236]. However, large concentrations of IL-6 result in lower concentrations of TNF-\( \alpha \), IL-6, and IL-10. We hypothesize that this reaction occurs due to the downregulation of TNF-\( \alpha \) by IL-6, which in turn results in lower production of IL-8. This may also suppress TNF-\( \alpha \) to the point where it cannot activate enough monocytes to result in IL-10 increases. All model states reach their steady state within 9 days of infection onset, and Figure 7.9 displays that different rate constant values result in multiple equilibrium points within the system.

Table 7.2 relates these results to the flow analysis derived in section 7.4. The pathogen corresponds to Figure 7.3a for all of the discussed rate constants above since \( k_{PG} - k_{PM} M_A < 0 \) for these simulations. Therefore, all scenarios were heading towards the only physiologically-relevant equilibrium point at \( P = 0 \), which is stable. The damage equation flows toward \( D = 1 \) as in Figure 7.5a for the above perturbations in rate constants \( k_{MA}, k_{TNFM}, k_6 \) and \( k_{6M} \) where \( D = 1 \) is the only equilibrium point since \( \alpha < \epsilon + \beta \). The perturbation in \( k_{DTNF} \) corresponds to Figure 7.5d where \( \alpha > \epsilon + \beta \) and \( \delta > \sqrt{\alpha - (\epsilon + \beta)} \). In this case, the model solution surpassed the unstable equilibrium point at \( \delta + \sqrt{\alpha - (\epsilon + \beta)} \) and continued to the stable equilibrium point at \( D = 1 \).
Figure 7.9: Model simulations resulting in persistent non-infectious inflammation from perturbations in rate constants. (a) Model simulations for the first 48 hours of infection. (b) Model simulations for 10 days since the onset of infection. All parameters remain fixed at their nominal value except for the specified perturbed parameter.
Half-Maximum and Exponent Perturbations

While single perturbations in the rate constants above result in variable steady-state concentrations of cytokines, there are several half-maximum and exponent values that, when perturbed, settle to the same cytokine concentration in the aseptic infection state. As shown in Figure 7.10, the system displays two distinct equilibrium points. Both eliminate the pathogen from the system and result in elevated cytokine concentrations. However, individual decreases in the parameters $\eta_{D6}$, $h_{D6}$, $\eta_{DTNF}$, and $h_{DTNF}$ come to steady state at one set of cytokine values, while a decrease in $h_{MD}$ results in lower cytokine concentrations. Additionally, the decrease in $h_{MD}$ results in recovered systemic damage while perturbations in the other parameters result in irreversible damage.

Table 7.2 relates these results to the flow analysis derived in Section 7.4. As with the rate constants, the pathogen corresponds to Figure 7.3a for all of the discussed half-maximum and exponent parameters above since $k_{PG} - k_{PM}M_A < 0$ for these simulations. All scenarios were heading towards the equilibrium point at $P = 0$, which is stable. The damage equation flows toward $D = 1$ as in Figure 7.5a for the above perturbations in parameters $\eta_{D6}$, $h_{D6}$, $\eta_{D}TNF$ and $h_{DTNF}$ where $D = 1$ is the only equilibrium point since $a < \epsilon + \beta$. The perturbation in $h_{MD}$ corresponds to Figure 7.5d where $a > \epsilon + \beta$ and $\delta > \sqrt{a-(\epsilon + \beta)}$. In this case, the model solution does not surpass the unstable equilibrium point at $D = \delta + \sqrt{a-(\epsilon + \beta)}$ and so its trajectory moves towards the stable equilibrium point at $D = \delta - \sqrt{a-(\epsilon + \beta)}$.

Characteristic Aseptic Infection and Comparison to Clinical Data

While we ideally would select a model simulation were all states remain non-zero to represent sepsis, the model does not capture that the septic infection state as in Figure 7.6. Thus, we select an aseptic infection (persistent non-infectious inflammation) simulation from Figure 7.9 to represent sepsis, compare our model to clinical data, and administer treatment. We select the simulation where $k_6$ was perturbed due to its resulting large concentrations of IL-6 and low concentrations of TNF-$\alpha$, which are commonly seen in sepsis patients [63, 122]. We omit selecting any half-maximum or exponent parameters for this representative simulation since changing these parameters can make the Hill functions flat. The results are shown in Figure 7.11, where $k_6 = 0.066$ and all other parameters remain fixed at their nominal values as given in Table 7.1.

We compare our aseptic simulation to the range of TNF-$\alpha$ and IL-6 data reported in Berg et al. [21] (discussed earlier in this chapter). The model does well at representing septic concentrations of IL-6, as IL-6 is within the specified data range on Figure 7.11. However, it produces higher levels (about 50 pg/mL more) of TNF-$\alpha$ than the range of concentrations in the data.
Figure 7.10: Model simulations resulting in persistent non-infectious inflammation from perturbations in up- and downregulation hill function half-maximum values and exponents. (a) Model simulations for the first 24 hours of infection. (b) Model simulations for 15 days since the onset of infection. All parameters remain fixed at their nominal value except for the specified perturbed parameter.
Berg et al. [21] states that all patients were mechanically ventilated during the study. Also, a vast majority of them were given at least one vasoactive drug (epinephrine, norepinephrine, or both) and had intravenous fluid administration since they were in septic shock. These vasoactive drugs and the sedative drug (propofol) used on some of the sepsis patients have been known to cause decreases in TNF-\( \alpha \) concentrations [264, 263, 113]. We suggest that since there is no management or treatment of the disease was administered in the model shown in Figure 7.11, that it’s possible that the true TNF-\( \alpha \) concentrations would be higher than reported in the data from Berg et al. [21]. If we wanted to get the model in its current form to produce TNF-\( \alpha \) such that it’s in the range of the data from [21], we could utilize the parameter scaling technique that was implemented in Chapters 5 and 6. This would scale multiple parameters associated with TNF-\( \alpha \), but would allow us to adjust the concentration to match the given data set.

The model simulation shows an initial increase in the pathogen levels for the first 3.5 hours after infection onset, and then the pathogen is eliminated 6 hours later. The increase in the pathogen prompts the activation of monocytes, which reach their first peak around 5 hours and their second peak around 30 hours. This elicits an increase in cytokines, with TNF-\( \alpha \) and IL-8 reaching their first peaks around 6 hours and IL-6 and IL-10 reaching their first peaks about 7 hours after infection initiation. All four cytokines then retreat before peaking for a second time at approximately 24, 27, 40, and 34 hours, respectively. It takes monocytes and cytokines about 10 days to reach their steady state values. In the meantime, systemic damage begins to amount around 5 hours after the infection begins. About 31 hours after the onset of infection, the damage level reaches the point (\( D = 0.8 \)) where any damage incurred cannot be reversed, and total damage (death) occurs around 84 hours (3.5 days) after the infection began. The timeline to death for our sepsis model is probable given that it’s suggested individuals with sepsis can succumb within 24 hours [61], and Daviaud et al. [65] studied one septic shock population where 32% of individual died within 3 days of being admitted to the ICU.

### 7.5.3 Persistent Infectious Immunosuppression

Variations in parameter rate constants, half-maximum values, and exponents of hill functions also result in *persistent infectious immunosuppression* shown in Figures 7.12 and 7.13. This clinically-relevant state shows the opposite behavior of that described in the persistent non-infectious inflammation scenario, where the immune response is too suppressed to eliminate the pathogen. Thus, as shown in Figure 7.6, it is characterized by the following:

- Elevated pathogen levels (\( P > 0 \)),
- Low levels of monocytes and cytokines (\( M_A > 0, TNF > w_{TNF}, IL6 > w_6, IL8 > w_8 \)),

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IL10 > w_{10},

- Minimally elevated systemic damage ($D \approx 0$).

A state such as this could occur in a patient who is immunocompromised from a recent infection or other comorbidities, making it challenging for the body to respond to an infection. Immunosuppression has been observed in individuals with a range of clinical conditions including HIV, cancer, organ and bone-marrow transplants, hemodialysis, and autoimmune diseases [139]. The immunosuppression state is similar to the severe immuno-deficiency state observed in the model by Kumar et al. [137]. In their system, they generate this state by decreasing the activation rate of inflammatory mediators by the pathogen. This results in elevated pathogen and late inflammatory mediators (including summarized damage on the system) levels but a low production of early inflammatory mediators. However, the level of damage seen in Kumar et al. [137]'s model contrasts with the damage dynamics seen in our model. We suspect that, in clinical practice, continued elevated damage would result in compounding problems for the individual, which they may succumb to as time progresses. The clinical observation of immunosuppression can also be related to the phenomena called endotoxin tolerance, in which a blunted immune response is seen in experimental settings during repeated endotoxin administration [17]. This response has also been studied computationally via mathematical

![Graphs showing immune response](image)

Figure 7.11: Sepsis model simulation where $k_6 = 0.066$. All other parameters remain fixed at their nominal value. This simulation runs for 10 days after the onset of infection, and the IL-6 y-axis is presented on a logarithmic scale. The data ranges from Berg et al. [21] are denoted by the shaded purple box for cytokines TNF-$\alpha$ and IL-6. Note that the data from Berg et al. [21] was collected when subjects had an infection present.
modeling in Day et al. [66] (mice) and Windoloski et al. [288] (humans).

Rate Constant Perturbations

Immunosuppression can be induced by increasing the rate constants representing the activated monocyte decay rate \( k_{MA} = 19.2, 34.9 \) and the TNF-\( \alpha \) decay rate \( k_{TNF} = 2.33 \) as shown in Figure 7.12. Both simulations for \( k_{MA} \) show the pathogen reaching its carrying capacity. While the smaller value of \( k_{MA} \) elicits an initially large response to the pathogen in terms of monocyte and cytokine activation, its response is unable to combat the pathogen in full. Thus, activated monocytes retreat to a steady low level, which results in low levels of cytokines and minor damage which recovers around after 4-5 days later. The larger value of \( k_{MA} \) exhibits a significantly smaller initial bout to combat the pathogen before retreating to its low elevated state after 4-5 days. This response elicits no systemic damage, as does the blunted response during the \( k_{TNF} \) simulation. All three rate constants show the system diverting to three separate equilibrium points based on the level of activated monocytes.

The equilibrium points for the pathogen and damage equations that result from these perturbations in rate constants are shown in Table 7.2 and Figures 7.3b and 7.5d respectively. Since the pathogen equation satisfies the condition where \( k_{PG} - k_{PM} M_A > 0 \) in all of these cases and \( M_A > 0 \), then Figure 7.3b applies and the pathogen trajectory will move towards the stable equilibrium point at \( P = P_{\infty} \left( 1 - \frac{k_{PM}}{k_{PG}} M_A \right) \). The damage equation follows that of Figure 7.5d since the cytokine levels are really low, which results in \( \alpha > \epsilon + \beta \). However, since cytokine levels have not returned to baseline, then \( \epsilon + \beta > 0 \) and so \( \delta > \sqrt{\alpha - (\epsilon + \beta)} \). Thus, \( D \) is attracted to the stable equilibrium point at \( D = \delta - \sqrt{\alpha - (\epsilon + \beta)} \).

Half-Maximum and Exponent Perturbations

Immunosuppressive behavior can also be induced by changing half-maximum saturation parameters and Hill function exponents. Results of this analysis are shown in Figure 7.13. Increases in the half-maximum values related to the upregulation of monocytes by TNF-\( \alpha \) \( (\eta_{MTNF} = 1222) \) and its exponent \( (h_{MTNF} = 22) \), the exponent of the upregulation of the monocytes by the pathogen \( (h_{MP} = 4.13) \), and the exponent of the downregulation of monocytes by IL-10 \( (h_{M10} = 2.75) \), and a decrease in the half-maximum value of the downregulation of TNF-\( \alpha \) by IL-6 \( (\eta_{TNF6}) \) result in this behavior. While the pathogen appears to establish a steady steady at multiple different pathogen levels, the resting and activated monocytes and IL-10 converge to the same equilibrium point for all simulations. The pro-inflammatory cytokine states and the tissue damage all converge to the same equilibrium point, except for the perturbation simulation for \( \eta_{TNF6} \), which has a smaller steady state. These perturbations in parameters
result in equilibrium points corresponding to the flow analysis in Figures 7.3b and 7.5d, as with the rate constant perturbations.

### 7.5.4 Recurrent Infection

Perturbations in parameter rate constants, half-maximum values, and exponents also result in recurrent infection states of the model. As shown in Figure 7.6, this state is characterized by the following:

- Oscillating levels of pathogen,
- Oscillating levels of monocytes and cytokines,
- Oscillating levels of damage.

Recurrent infections are observed often in women with urinary tract infections (UTIs) [106] and yeast infections [25] and children with otitis media [9], sinusitis [9], and pneumonia [185]. The

![Figure 7.12: Model simulations resulting in persistent infectious immunosuppression from perturbations in rate constants. Model simulations show dynamics for 10 days since the onset of infection. All parameters remain fixed at their nominal value except for the specified perturbed parameter.](image)
Figure 7.13: Model simulations resulting in persistent infectious immunosuppression from perturbations in up- and downregulation hill function half-maximum values and exponents. (a) Model simulations for the first 5 days of infection. (b) Model simulations for 10 days since the onset of infection. All parameters remain fixed at their nominal value except for the specified perturbed parameter.
frequency of their recurrences can vary depending on the individual and condition. However, recurrent infections have appeared computationally in models of inflammation, such as that in Kumar et al. [137] and Gillis et al. [86]. In Kumar et al. [137], the parameter that initiated the activation of late inflammatory mediators was decreased, resulting in recurrent behavior. They do report, though, that changes in all five of their model parameters could induce this behavior.

**Rate Constant Perturbations**

Perturbations of rate constants that result in recurrent infections are shown in Figure 7.14, where the system can exhibit both shorter and longer oscillatory periods. Figure 7.14a shows oscillations in all model states that repeat approximately every 24 hours when parameters involving the activation of monocytes by TNF-α ($k_{MTNF} = 2.93$) and the activation of TNF-α by monocytes ($k_{TNFM} = 0.765$) are decreased and the activation of IL-10 by monocytes ($k_{10M} = 0.283$) is increased. The system is not able to completely clear the infection, and so inflammatory cells and mediators elevate when the pathogen regains strength. As a result, the system exhibits low to medium levels of damage, which recovers within 24 hours.

Figure 7.14b displays oscillatory behavior created through perturbations in rate constants relating to the decrease in the phagocytosis rate of monocytes ($k_{PM} = 0.0016$), increase of the pathogen growth rate ($k_{PG} = 0.975$), and increase in activated monocyte decay rate ($k_{MA} = 11.3$). Both perturbations for $k_{PG}$ and $k_{PM}$ result in growth of the pathogen to saturation due to a depletion of the resting monocyte pool. The resting monocytes can slightly regenerate to elicit an immune response that can reduce the infection levels, but the pool of resting monocytes is too low to fully clear the pathogen. This cycle continues approximately every 2 weeks. The perturbation relating to $k_{MA}$ displays large, fast oscillations for the first 100 hours of infection before the pathogen steadily oscillates below saturation. This leads to recurring peaks of monocytes and cytokines as well as minimal ongoing systemic damage.

The simulations in Figures 7.14 could represent a patient who recently overcame illness, but got reinfected again and cannot fully fight the infection. As mentioned in Kumar et al. [137], we suspect that this behavior can result in compounding damage as time continued depending upon the condition, but more work needs to be done to investigate this behavior. Given that the flow analysis described in section 7.4 relies on states being held constant, those results do not apply for the recurrent infection scenario. True bifurcation analysis would explore the emergence of limit cycles here.
Half-Maximum and Exponent Perturbations

Figure 7.15 displays the system’s bifurcation to a recurrent infection state when half-maximum values (Figure 7.14a) and exponents (Figure 7.14b) of hill functions are perturbed. Figure 7.14a shows that the oscillatory behavior occurs when the half-maximum value of the pathogen upregulating monocytes ($\eta_{MP} = 116$) is increased, and IL-10 and IL-6 downregulating TNF-$\alpha$ ($\eta_{TNF10} = 1.76$ and $\eta_{TNF6} = 81.3$) are decreased. Recurrent infections in these scenarios occur approximately every 32, 24, and 14 hours, respectively, and the magnitude of the oscillations also decrease respectively between the perturbations. Minor amounts of tissue damage occurs but recovers during every bout with the pathogen. Figure 7.15b displays that this recurrent infection behavior also occurs during perturbations of the hill exponents relating to the increase of the pathogen activation of monocytes ($h_{MP} = 2.44$) and the downregulation of monocytes by IL-10 ($h_{M10} = 1.63$), and the decrease of the downregulation of TNF-$\alpha$ by IL-6 ($h_{TNF6} = 0.2$). Recurrent infections here occur approximately every 12, 22, and 30 hours, respectively. Interestingly, although the model simulation for the perturbation in $h_{MP}$ has the largest pathogen levels, it shows the lowest peaks in all cytokines except TNF-$\alpha$.

7.6 Vitamin C Treatment

7.6.1 Motivation and Protocol

Numerous clinical studies have attempted to identify a universal biomarker and therapeutic treatment of sepsis, but none have been proven successful [197, 126] aside from antibiotic treatment and supportive care measurements such as fluid and vasopressor administration [74]. Several recent studies [96, 126, 281, 276] suggest that intravenous administration of vitamin C may be a successful therapeutic treatment of sepsis. Vitamin C concentrations are typically very low for severely-ill sepsis patients, and low vitamin C levels have been shown to correlate with poor recovery [126, 276]. Compared to many other candidates for sepsis treatment, vitamin C is a micronutrient that humans regularly consume in food, and it is generally safe with little to no toxicity. Potential side effects include heightened iron absorption, pro-oxidant impacts, blood glucose difficulties, and tissue and kidney crystal deposits, but these consequences have not been observed in high-dose vitamin C controlled trials. Vitamin C impacts multiple sepsis-related channels, but because of the scope of our model, we focus on its ability to promote anti-inflammatory feedback through inhibiting pro-inflammatory pathways [276].

Since vitamin C exhibits anti-inflammatory actions, we administer it into the system via parametric enhancement to the production of the anti-inflammatory cytokine, IL-10, during
Figure 7.14: Model simulations resulting in recurrent infectious inflammation from perturbations in rate constants. (a) Model simulations for the first 5 days of infection that have short temporal oscillations. (b) Model simulations for the first 40 days of infection that have long temporal oscillations. All parameters remain fixed at their nominal value except for the specified perturbed parameter.
Figure 7.15: Model simulations resulting in recurrent infectious inflammation from perturbations in up- and downregulation hill function (a) half-maximum values and (b) exponents for 5 days since the onset of infection. All parameters remain fixed at their nominal value except for the specified perturbed parameter.
the characteristic aseptic infection case (Figure 7.11). Specifically, because the parameter $k_{10M}$ (monocyte activation rate of IL-10) is the most sensitive IL-10 parameter from the continuous infusion model analysis in Chapter 5 (see Figure 5.5), we classify the increase of $k_{10M}$ at a specified time as the impact of intravenous vitamin C treatment on the system. We select six values of $k_{10M}$ to sample from, ranging from its nominal value to 20 times that. The change in $k_{10M}$ is then applied at different points in the disease progression: 8, 12, 16, 20, and 24 hours after the onset of infection. While the above administration protocols are not explicitly aligned with dose concentrations from literature, we select administration times that correspond to early, mid, and late sepsis progression to explore literature claims [197, 74] that delayed treatment vastly reduces chance of survival in sepsis patients.

### 7.6.2 Results

Results (Figures 7.16-7.20) show that both the administration timing and dose (strength of $k_{10M}$) impact the efficacy of the treatment. The later the treatment is started, the higher vitamin C dose ($k_{10M}$ increase) is needed for any systemic damage to repair and return to a healthy state as shown in Figure 7.21. This figure shows an exponential relationship between the treatment administration time and the strength of the dose needed to recover. Figures 7.16 and 7.17 display the impact of vitamin C treatment starting at 8 and 12 hours after infection onset respectively. Both simulations show that an approximately 7-fold increase in $k_{10M}$ is needed for the system to successfully repair any damage. The system's cytokines return to their normal levels between four to eight days and four to 13 days for the treatment beginning at 8 and 12 hours after infection onset respectively. Figure 7.18 displays the impact of vitamin C treatment beginning 16 hours after the onset of infection. The dose needed for effective recovery is a 10-fold increase in $k_{10M}$ from its nominal value, and it took about four to eight days to recover. The 7-fold increase in $k_{10M}$ (dose) that was successful during earlier administration times slowed the progress of the damage by approximately 3 days, but it did not reverse the trajectory of the system.

Figure 7.19 exhibits the system response to vitamin C treatment administered 20 hours after infection onset. The system requires a 17-fold increase in $k_{10M}$ to recover, occurring seven to nine days after the infection began. Finally, we note that the current vitamin C treatment administered 24 hours after infection onset (Figure 7.20) is not strong enough to cause the system to change trajectory from a negative outcome. The simulations presented here support the notion that the anti-inflammatory properties of intravenous vitamin C administration have an advantageous effect on sepsis progression. Additionally, this work supports the belief that the chance for survival from sepsis increases the earlier that treatment is sought, and delaying
treatment by a few hours can result in an undesirable outcome [197, 74]. Furthermore, our work suggests that the relationship between the treatment administration time and strength of treatment is nonlinear, as shown in Figure 7.21. Linearly increasing the dose amount of vitamin C for more progressed stages of sepsis will not provide enough strength to reverse the negative outcome, and treatment will have to be more aggressive. Additional research needs to be conducted to determine whether other studies have investigated this phenomena in the experimental or clinical setting.

7.7 Discussion

This chapter introduces a new, preliminary immune model of infection derived from a well-analyzed immune response model from a continuous infusion of endotoxin in Chapter 5 and compared against clinical cytokine data from Berg et al. [21]. To our knowledge, the model is the first that directly links an experimental model of inflammation with a clinical model of inflammation. Through parametric analysis, we simulate several clinically-relevant scenarios of inflammation (shown in Figure 7.6) including aseptic infection (persistent non-infectious inflammation), persistent infectious immunosuppression, and recurrent infection. We found

![Graphs showing simulation results](image)

Figure 7.16: Model simulation of vitamin C treatment administered 8 hours after the onset of infection. The original septic model trajectory is given by the solid black line, and colored lines specified in the legend signify the changes in parameter $k_{10M}$. 
that one parameter, $k_{MA}$ (the rate activated monocytes decay), is important for the system's end-behavior. Variations in this parameter can generate all three clinical inflammation scenarios presented, making it a parameter of interest once a formal bifurcation analysis is conducted on the model. Other parameters whose perturbations resulted in two of the three clinical inflammation scenarios were $k_{TNFM}$ (the monocyte activation rate of TNF-α), $\eta_{TNF6}$ (half-maximum value of IL-6 downregulating TNF-α), and $h_{M10}$ (the exponent corresponding to the downregulation of monocytes by IL-10). These parameters could also be important to formally investigate in a bifurcation analysis.

Furthermore, we investigate the impact that intravenous vitamin C (through its anti-inflammatory properties via perturbation of the IL-10 activation by monocytes parameter) has on an aseptic outcome of the system. The results support literature [126, 276] that suggest that intravenous vitamin C can divert the system towards recovery instead of remaining on an aseptic death trajectory. Our results depict that the timing and strength of administration both play a critical role in determining the outcome of infection, and that the relationship between administration time and minimal effective dose is nonlinear. However, further research is needed to compare these results with findings in literature.

Figure 7.17: Model simulation of vitamin C treatment administered 12 hours after the onset of infection. The original septic model trajectory is given by the solid black line, and colored lines specified in the legend signify the changes in parameter $k_{10M}$. 
### Limitations and Future Work

The work presented in this chapter introduces a preliminary model of infection, but much remains to be done regarding the structure of the model, the clinical data used to validate the model, and the analysis conducted on the model.

#### Model Structure

While our model captures several clinically-relevant scenarios of inflammation including aseptic infection, the model does not capture a septic infection as shown in Figure 7.6. This is a model state where the pathogen, monocytes, cytokines, and damage remain elevated, and this scenario is depicted in other mathematical models such as Reynolds et al. [207] and [164]. Thus, future work should be focused on improving the model’s structure such that a septic infection scenario arises.

We suggest that changes to our pathogen and damage equation may result in the creation of this septic infection state. Comparing our model to the model by Reynolds et al. [207], we see that there is an additional term in the pathogen equation. By assuming that the local response reaches a quasi-steady state, Reynolds et al. [207] incorporates this local response to

![Graphs showing model simulation](image)

**Figure 7.18:** Model simulation of vitamin C treatment administered 16 hours after the onset of infection. The original septic model trajectory is given by the solid black line, and colored lines specified in the legend signify the changes in parameter $k_{10M}$. 

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the pathogen into their pathogen equation as an upregulation-type term that’s a function of pathogen and depletes the growth of itself. This self-inhibiting mechanism could result in the creation of the septic behavior state that is not observed in our model. A similar term is used in the blood pathogen equation in McDaniel et al. [164]. This term could also prove important for ensuring a healthy recovery in our model without the use of pathogen equation (7.15) when the pathogen becomes very small. Furthermore, the pathogen equation in Reynolds et al. [207] includes inhibition of the inflammatory mediator’s strength to eliminate the pathogen by the anti-inflammatory mediators. This could also play a role.

In regards to the damage equation, the one presented in this chapter was inspired by the structure of the tissue integrity equation in Reynolds [208]. However, further review of the model suggests that the parameter, $D_{\text{max}}$, is only the threshold for maximal damage when inflammatory mediators have returned to baseline and is not always an unstable equilibrium point. Thus, we suggest that an adaptation of the damage equation to the form of that in McDaniel et al. [164], where the term $D_{\text{max}} - D$ is included in each equation term and, thus, an equilibrium point. Additionally, our model does not contain any inhibition processes on the cytokine upregulation of damage. Reynolds et al. [207] utilizes the anti-inflammatory mediator and McDaniel et al. [164] implements nitric oxide as damage inhibitors, so the inclusion of an

Figure 7.19: Model simulation of vitamin C treatment administered 20 hours after the onset of infection. The original septic model trajectory is given by the solid black line, and colored lines specified in the legend signify the changes in parameter $k_{10M}$. 

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inhibition term in our damage equation may change the end-behavior of our solution towards a septic state.

Figure 7.20: Model simulation of vitamin C treatment administered 24 hours after the onset of infection. The original septic model trajectory is given by the solid black line, and colored lines specified in the legend signify the changes in parameter $k_{10M}$.

Figure 7.21: Plot showing the time that the parameter $k_{10M}$ was perturbed (treatment administered) versus the lowest value of $k_{10M}$ (dose amount) needed for the model to recover to a healthy state instead of aseptic death.
The model proposed here only examines immune dynamics during infection with one additional collective term representing the infection's effects on the whole body. In reality, there are many mechanisms and systems that are impacted during a sepsis event, as described in section 2.5. Therefore, future work should focus on (i) developing a more detailed immune model with additional specific pathways including adaptive immunity, and (ii) coupling our model of infection with the multi-system model presented in Chapter 6. Whereas blood samples that measure cytokine levels take time to analyze if drawn in a sepsis patient, markers of sepsis such as blood pressure, heart rate, and body temperature are easily measurable and readily available, making it even more beneficial to couple the mathematical model of infection with the multi-system model that includes those markers. Including additional systems such as the cardiovascular and endocrine systems would also be beneficial to assessing the impacts therapeutic treatments on sepsis progression. The current infection model implements intravenous vitamin C administration through the induction of increased anti-inflammatory mediator production. However, given that our current model of infection only includes several immune components, it cannot simulate the multi-system impacts that vitamin C has on the human body such as the production of cortisol and vasopressin, preservation of blood flow in the capillaries, and regulation nitric oxide dependent pathways [276]. Thus, the circulatory support and hormone production involved with vitamin C treatment could be better represented and studied through a more comprehensive mathematical model of infection.

Finally, the current model implements vitamin C administration by arbitrarily adjusting the level of IL-10 production by monocytes. However, this is difficult to translate to clinical needs. Thus, future work should focus on administering vitamin C dynamically through an equation similar to the continuous infusion of endotoxin in equation 5.1. This type of administration would allow concentrations based on literature to be administered into the system for a set amount of time, which would increase efficacy accuracy in the prediction and directly relate the mathematical model to clinical doses.

Data and Analysis

This chapter derives equilibrium points and their stability using flow on the line analysis from Strogatz [245]. However, this analysis assumes that the dynamic states are held constant, which, in reality, they are not. While this is a limitation, the flow on the line analysis still provided useful insight into the dynamics of the model and how the end-behavior switched in the different clinical scenarios. We also utilize clinical sepsis data from Berg et al. [21] to validate our aseptic model simulation with only one data point from each individual was measured during the study. However, patients from that study were severely infected, so they were not aseptic as in our
model simulation, and the lack of time course cytokine concentrations for each of the patients was not possible. Also, while the data was measured in the same window after sepsis diagnosis, it is still challenging to determine temporal trends from that since each individual was likely in a different state of sepsis progression and immune responses are known for wide variability between individuals. Therefore, future work should focus on obtaining temporal clinical data for sepsis to use for model validation. Additional research into experimental or clinical data for other scenarios of inflammation produced by the model would also be insightful to validate those predictions.

The work presented here introduces a preliminary model of infection, and so additional analysis on the system should be conducted to gain further understanding of its dynamics. While a parametric analysis was conducted to get insight into individual parameter impacts on solution behavior, a full bifurcation analysis should be implemented. This could be done using MATCONT, a MATLAB software package used to find equilibrium points and bifurcations numerically, or XPPAUT, a bifurcation software created by Bard Ermentrout (University of Pittsburgh). Analysis through either of these programs would allow us to identify bifurcation points within the system and the type of bifurcation. This could help determine physiological parameters within the model that cause a switch to septic-like states or other clinical scenarios of inflammation, which could help guide treatment development. Effective use of this software requires that the user has insight into the parameters in the system that might result in bifurcations, so the parametric analysis conducted in this chapter or a parametric analysis conducted on the model of infection coupled with the multi-system model could be used to narrow down bifurcation parameters. Other mathematical models of inflammation and sepsis [137, 207, 233, 164] have investigated the impact of initial conditions on the bifurcation of the system, particularly that of the pathogen. While not explored here, this analysis would also be interesting to conduct on our system to determine how initial conditions play a role in infection outcome.
### Table 7.1: Model parameters, values, descriptions, units, and sources. Abbreviations: UR, upregulation; DR, downregulation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nominal Parameters</th>
<th>Description</th>
<th>Unit</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{PG}$</td>
<td>0.3</td>
<td>Pathogen growth rate</td>
<td>hr$^{-1}$</td>
<td>[207]</td>
</tr>
<tr>
<td>$P_{so}$</td>
<td>$20 \times 10^5$</td>
<td>Max pathogen population</td>
<td>P (cc$^{-1}$)</td>
<td>[207]</td>
</tr>
<tr>
<td>$k_{PM}$</td>
<td>0.005</td>
<td>Monocyte phagocytosis rate of pathogen</td>
<td>hr$^{-1}$</td>
<td>Manually set</td>
</tr>
<tr>
<td>$k_{MR}$</td>
<td>0.05</td>
<td>Resting monocyte repopulation rate</td>
<td>hr$^{-1}$</td>
<td>[50]</td>
</tr>
<tr>
<td>$k_{MA}$</td>
<td>3.491</td>
<td>Activated monocyte decay rate</td>
<td>hr$^{-1}$</td>
<td>[290]</td>
</tr>
<tr>
<td>$k_{MTNF}$</td>
<td>9.000</td>
<td>Monocyte activation rate by TNF-$\alpha$</td>
<td>hr$^{-1}$</td>
<td>[290]</td>
</tr>
<tr>
<td>$k_{MP}$</td>
<td>0.041</td>
<td>Monocyte activation rate by pathogen</td>
<td>hr$^{-1}$</td>
<td>[290]</td>
</tr>
<tr>
<td>$k_{MD}$</td>
<td>0.05</td>
<td>Monocyte activation rate by damage</td>
<td>hr$^{-1}$</td>
<td>[164]</td>
</tr>
<tr>
<td>$\eta_{MP}$</td>
<td>15</td>
<td>Half max of pathogen UR monocytes</td>
<td>P units</td>
<td>Manually set</td>
</tr>
<tr>
<td>$\eta_{M10}$</td>
<td>4.394</td>
<td>Half max of IL-10 DR monocytes</td>
<td>pg (mL)$^{-1}$</td>
<td>[290]</td>
</tr>
<tr>
<td>$\eta_{MTNF}$</td>
<td>222.222</td>
<td>Half max of TNF-$\alpha$ UR monocytes</td>
<td>pg (mL)$^{-1}$</td>
<td>[290]</td>
</tr>
<tr>
<td>$\eta_{MD}$</td>
<td>0.75</td>
<td>Half max of damage UR monocytes</td>
<td>non dim</td>
<td>[164]</td>
</tr>
<tr>
<td>$h_{MP}$</td>
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<td>Exponent of pathogen UR of monocytes</td>
<td>non dim</td>
<td>Manually set</td>
</tr>
<tr>
<td>$h_{M10}$</td>
<td>0.5</td>
<td>Exponent of IL-10 DR of monocytes</td>
<td>non dim</td>
<td>Manually set</td>
</tr>
<tr>
<td>$h_{MTNF}$</td>
<td>4</td>
<td>Exponent of TNF-$\alpha$ UR of monocytes</td>
<td>non dim</td>
<td>Manually set</td>
</tr>
<tr>
<td>$h_{MD}$</td>
<td>1</td>
<td>Exponent of damage UR of monocytes</td>
<td>non dim</td>
<td>Manually set</td>
</tr>
<tr>
<td>$M_{\infty}$</td>
<td>30000</td>
<td>Monocyte carrying capacity</td>
<td>noc</td>
<td>[290]</td>
</tr>
<tr>
<td>$k_{TNF}$</td>
<td>0.423</td>
<td>TNF-$\alpha$ decay rate</td>
<td>hr$^{-1}$</td>
<td>[290]</td>
</tr>
<tr>
<td>$k_{TNF10}$</td>
<td>1.391</td>
<td>TNF-$\alpha$ activation by monocytes rate</td>
<td>pg (mL.hr noc)$^{-1}$</td>
<td>[290]</td>
</tr>
<tr>
<td>$\eta_{TNF10}$</td>
<td>17.576</td>
<td>Half max of IL-10 DR TNF-$\alpha$</td>
<td>pg (mL)$^{-1}$</td>
<td>[290]</td>
</tr>
<tr>
<td>$\eta_{TNF6}$</td>
<td>250</td>
<td>Half max of IL-6 DR TNF-$\alpha$</td>
<td>pg (mL)$^{-1}$</td>
<td>Manually set</td>
</tr>
<tr>
<td>$h_{TNF10}$</td>
<td>0.3</td>
<td>Exponent of IL-10 DR of TNF-$\alpha$</td>
<td>non dim</td>
<td>Manually set</td>
</tr>
<tr>
<td>$h_{TNF6}$</td>
<td>2</td>
<td>Exponent of IL-6 DR of TNF-$\alpha$</td>
<td>non dim</td>
<td>Manually set</td>
</tr>
<tr>
<td>$w_{TNF}$</td>
<td>2.896</td>
<td>Baseline TNF-$\alpha$ concentration</td>
<td>pg (mL)$^{-1}$</td>
<td>[93]</td>
</tr>
<tr>
<td>$k_6$</td>
<td>0.66</td>
<td>IL-6 decay rate</td>
<td>hr$^{-1}$</td>
<td>[290]</td>
</tr>
<tr>
<td>$k_{6M}$</td>
<td>0.81</td>
<td>IL-6 activation by monocytes rate</td>
<td>pg (mL.hr noc)$^{-1}$</td>
<td>[290]</td>
</tr>
<tr>
<td>$k_{6TNF}$</td>
<td>0.81</td>
<td>IL-6 activation by TNF-$\alpha$ rate</td>
<td>pg (mL.hr noc)$^{-1}$</td>
<td>[290]</td>
</tr>
<tr>
<td>$\eta_{610}$</td>
<td>25</td>
<td>Half max of IL-10 DR IL-6</td>
<td>pg (mL)$^{-1}$</td>
<td>Manually set</td>
</tr>
<tr>
<td>$\eta_{66}$</td>
<td>200</td>
<td>Half max of IL-6 DR itself</td>
<td>pg (mL)$^{-1}$</td>
<td>Manually set</td>
</tr>
<tr>
<td>$\eta_{6TNF}$</td>
<td>250</td>
<td>Half max of TNF-$\alpha$ UR IL-6</td>
<td>pg (mL)$^{-1}$</td>
<td>Manually set</td>
</tr>
<tr>
<td>$h_{610}$</td>
<td>1</td>
<td>Exponent of IL-10 DR of IL-6</td>
<td>non dim</td>
<td>[290]</td>
</tr>
<tr>
<td>$h_{66}$</td>
<td>1</td>
<td>Exponent of IL-6 DR of itself</td>
<td>non dim</td>
<td>[290]</td>
</tr>
<tr>
<td>$h_{6TNF}$</td>
<td>2</td>
<td>Exponent of TNF-$\alpha$ UR of IL-6</td>
<td>non dim</td>
<td>[290]</td>
</tr>
<tr>
<td>$w_6$</td>
<td>1.331</td>
<td>Baseline IL-6 concentration</td>
<td>pg (mL)$^{-1}$</td>
<td>[93]</td>
</tr>
<tr>
<td>$k_8$</td>
<td>0.386</td>
<td>IL-8 decay rate</td>
<td>hr$^{-1}$</td>
<td>[290]</td>
</tr>
<tr>
<td>$k_{8M}$</td>
<td>0.613</td>
<td>IL-8 activation by monocytes rate</td>
<td>pg (mL.hr noc)$^{-1}$</td>
<td>[290]</td>
</tr>
<tr>
<td>$k_{8TNF}$</td>
<td>0.509</td>
<td>IL-8 activation by TNF-$\alpha$ rate</td>
<td>pg (mL.hr noc)$^{-1}$</td>
<td>[290]</td>
</tr>
<tr>
<td>$\eta_{810}$</td>
<td>25</td>
<td>Half max of IL-10 DR IL-8</td>
<td>pg (mL)$^{-1}$</td>
<td>Manually set</td>
</tr>
<tr>
<td>$\eta_{8TNF}$</td>
<td>200</td>
<td>Half max of TNF-$\alpha$ UR IL-8</td>
<td>pg (mL)$^{-1}$</td>
<td>Manually set</td>
</tr>
<tr>
<td>$h_{810}$</td>
<td>1.5</td>
<td>Exponent of IL-10 DR of IL-8</td>
<td>non dim</td>
<td>[290]</td>
</tr>
<tr>
<td>$h_{88}$</td>
<td>3</td>
<td>Exponent of TNF-$\alpha$ UR of IL-8</td>
<td>non dim</td>
<td>[290]</td>
</tr>
<tr>
<td>$w_8$</td>
<td>3.529</td>
<td>Baseline IL-8 concentration</td>
<td>pg (mL)$^{-1}$</td>
<td>[93]</td>
</tr>
<tr>
<td>$k_{10}$</td>
<td>0.4</td>
<td>IL-10 decay rate</td>
<td>hr$^{-1}$</td>
<td>[290]</td>
</tr>
<tr>
<td>$k_{10M}$</td>
<td>0.037</td>
<td>IL-10 activation by monocytes rate</td>
<td>pg (mL.hr noc)$^{-1}$</td>
<td>[290]</td>
</tr>
<tr>
<td>$k_{106}$</td>
<td>0.019</td>
<td>IL-10 activation by IL-6 rate</td>
<td>pg (mL.hr noc)$^{-1}$</td>
<td>[290]</td>
</tr>
<tr>
<td>$\eta_{106}$</td>
<td>100</td>
<td>Half max of IL-6 DR IL-10</td>
<td>pg (mL)$^{-1}$</td>
<td>Manually set</td>
</tr>
<tr>
<td>$h_{106}$</td>
<td>3.68</td>
<td>Exponent of IL-6 UR of IL-10</td>
<td>non dim</td>
<td>[290]</td>
</tr>
<tr>
<td>$w_{10}$</td>
<td>0.053</td>
<td>Baseline IL-10 concentration</td>
<td>pg (mL)$^{-1}$</td>
<td>[93]</td>
</tr>
<tr>
<td>$k_{106}$</td>
<td>0.125</td>
<td>Damage activation by IL-6 rate</td>
<td>hr$^{-1}$</td>
<td>[164]</td>
</tr>
<tr>
<td>$k_{DTNF}$</td>
<td>0.125</td>
<td>Damage activation by TNF-$\alpha$ rate</td>
<td>hr$^{-1}$</td>
<td>[164]</td>
</tr>
<tr>
<td>$k_D$</td>
<td>0.15</td>
<td>Damage recovery rate</td>
<td>hr$^{-1}$</td>
<td>[164]</td>
</tr>
<tr>
<td>$\eta_{106}$</td>
<td>300</td>
<td>Half max of IL-6 UR damage</td>
<td>pg (mL)$^{-1}$</td>
<td>Manually set</td>
</tr>
<tr>
<td>$h_{106}$</td>
<td>4</td>
<td>Exponent of IL-6 UR of damage</td>
<td>non dim</td>
<td>Manually set</td>
</tr>
<tr>
<td>$h_{DTNF}$</td>
<td>4</td>
<td>Exponent of TNF-$\alpha$ UR of damage</td>
<td>non dim</td>
<td>Manually set</td>
</tr>
<tr>
<td>$D_{\text{max}}$</td>
<td>0.8</td>
<td>Irreversible damage threshold</td>
<td>non dim</td>
<td>[302, 208]</td>
</tr>
</tbody>
</table>
Table 7.2: Table of perturbed parameters resulting in a healthy infection, aseptic infection (persistent non-infectious inflammation), persistent infectious immunosuppression, and recurrent infection.

<table>
<thead>
<tr>
<th>Perturbed Parameter</th>
<th>Parameter Change from Nominal Value</th>
<th>Corresponding Pathogen Flow</th>
<th>Corresponding Damage Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy Response to Infection</td>
<td><em>(P</em> cleared, <em>M</em> and cytokines to baseline, <em>D</em> repaired)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N/A</td>
<td>N/A</td>
<td>Figure 7.4b</td>
</tr>
<tr>
<td>Persistent Non-Infectious Inflammation</td>
<td><em>(P</em> cleared, <em>M</em> and cytokines elevated, severe <em>D</em>)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>k</em>_MA</td>
<td>0.349</td>
<td>Decreased</td>
<td>Figure 7.3a</td>
</tr>
<tr>
<td><em>k</em>_MA</td>
<td>1.135</td>
<td>Decreased</td>
<td>Figure 7.3a</td>
</tr>
<tr>
<td><em>k</em>_D</td>
<td>0.066</td>
<td>Decreased</td>
<td>Figure 7.3a</td>
</tr>
<tr>
<td><em>η</em>_D6</td>
<td>97.5</td>
<td>Decreased</td>
<td>Figure 7.3a</td>
</tr>
<tr>
<td><em>η</em>_D_TNF</td>
<td>220</td>
<td>Decreased</td>
<td>Figure 7.3a</td>
</tr>
<tr>
<td><em>h</em>_MD</td>
<td>0.325</td>
<td>Decreased</td>
<td>Figure 7.3a</td>
</tr>
<tr>
<td><em>h</em>_D6</td>
<td>0.4</td>
<td>Decreased</td>
<td>Figure 7.3a</td>
</tr>
<tr>
<td><em>h</em>_D_TNF</td>
<td>1.3</td>
<td>Decreased</td>
<td>Figure 7.3a</td>
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<tr>
<td><em>k</em>_6</td>
<td>6.28</td>
<td>Increased</td>
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<tr>
<td><em>k</em>_D_TNF</td>
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<td>Increased</td>
<td>Figure 7.3a</td>
</tr>
<tr>
<td><em>η</em>_M_TNF</td>
<td>1222</td>
<td>Increased</td>
<td>Figure 7.3a</td>
</tr>
<tr>
<td><em>h</em>_M_P</td>
<td>4.13</td>
<td>Increased</td>
<td>Figure 7.3a</td>
</tr>
<tr>
<td><em>h</em>_M_10</td>
<td>2.75</td>
<td>Increased</td>
<td>Figure 7.3a</td>
</tr>
<tr>
<td><em>h</em>_M_TNF</td>
<td>22</td>
<td>Increased</td>
<td>Figure 7.3a</td>
</tr>
<tr>
<td>Persistent Infectious Immunosuppression</td>
<td><em>(P</em> nonzero, <em>M</em> and cytokines, and <em>D</em> slightly elevated)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>η</em>_TNF6</td>
<td>25</td>
<td>Decreased</td>
<td>Figure 7.3b</td>
</tr>
<tr>
<td><em>k</em>_MA</td>
<td>19.2</td>
<td>Increased</td>
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</tr>
<tr>
<td><em>k</em>_MA</td>
<td>34.9</td>
<td>Increased</td>
<td>Figure 7.3b</td>
</tr>
<tr>
<td><em>k</em>_TNF</td>
<td>2.33</td>
<td>Increased</td>
<td>Figure 7.3b</td>
</tr>
<tr>
<td><em>η</em>_M_TNF</td>
<td>1222</td>
<td>Increased</td>
<td>Figure 7.3b</td>
</tr>
<tr>
<td><em>h</em>_MP</td>
<td>4.13</td>
<td>Increased</td>
<td>Figure 7.3b</td>
</tr>
<tr>
<td><em>h</em>_M_10</td>
<td>2.75</td>
<td>Increased</td>
<td>Figure 7.3b</td>
</tr>
<tr>
<td><em>h</em>_M_TNF</td>
<td>22</td>
<td>Increased</td>
<td>Figure 7.3b</td>
</tr>
<tr>
<td>Recurrent Infection</td>
<td><em>(P</em>, <em>M</em>, cytokines, and <em>D</em> oscillating)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>k</em>_FM</td>
<td>0.0016</td>
<td>Decreased</td>
<td>N/A</td>
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<tr>
<td><em>k</em>_M_TNF</td>
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<td>N/A</td>
</tr>
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<td><em>k</em>_TNF</td>
<td>0.765</td>
<td>Decreased</td>
<td>N/A</td>
</tr>
<tr>
<td><em>η</em>_TNF6</td>
<td>81.3</td>
<td>Decreased</td>
<td>N/A</td>
</tr>
<tr>
<td><em>η</em>_TNF</td>
<td>1.76</td>
<td>Decreased</td>
<td>N/A</td>
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<td><em>h</em>_TNF6</td>
<td>0.2</td>
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<td>N/A</td>
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<tr>
<td><em>k</em>_PG</td>
<td>0.973</td>
<td>Increased</td>
<td>N/A</td>
</tr>
<tr>
<td><em>k</em>_MA</td>
<td>11.3</td>
<td>Increased</td>
<td>N/A</td>
</tr>
<tr>
<td><em>k</em>_SM</td>
<td>0.283</td>
<td>Increased</td>
<td>N/A</td>
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<td><em>η</em>_MP</td>
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<td>Increased</td>
<td>N/A</td>
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<tr>
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<td>Increased</td>
<td>N/A</td>
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<tr>
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<td>1.63</td>
<td>Increased</td>
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The immune system, which encompasses a complex network of cell and cytokine mediators as well as external interactions with other systems, is a primary component of multiple clinical diseases. However, the dynamics of inflammation brought on by the immune system are still not fully understood in these conditions, especially in sepsis. Experimental studies and modeling literature have examined the impact of short-term inflammation through the administration of a bolus of endotoxin, but inflammation observed in the clinical setting is prolonged. Mathematical models have also studied interactions of the immune system with other systems, but these models fail to incorporate either comprehensive multi-system dynamics, experimental data, or connections noted in literature into their models. Therefore, the aim of the research presented here is to gain further understanding of inflammatory dynamics by

1. Exploring the connection and binding the gap between experimental knowledge and clinical observations of inflammation through mathematical modeling,

2. Examining multi-system connections of inflammation to analyze their impact on system dynamics.

The integration of these two goals lays the foundation for a comprehensive model of sepsis and its interaction with other biological systems, which can assist in understanding its complex
disease dynamics and aid in discovering a therapeutic treatment. The major accomplishments of this thesis include:

- Creating the first mathematical model of a continuous infusion of endotoxin calibrated to experimental data, which better describes the response to sustained inflammatory stimuli than current models in literature. This model aids in reducing the gap between the traditional short-term bolus endotoxin models of inflammation and clinically-observed prolonged inflammation in conditions such as sepsis. This work was submitted for publication, and the author was involved in the formulation of the mathematical model and implemented all its analysis and simulations.

- Constructing a complex mathematical model of immune, cardiovascular, endocrine, thermal, and pain dynamics to study inflammation and its whole body impacts. This is the first model developed that incorporates these multi-system dynamics into one network, and it also is the first immune-cardiovascular-hormone model that includes a detailed immune component, a compartmental model of the circulation with easily measurable markers such as heart rate and blood pressure, and a comprehensive model of the HPA axis with circadian and ultradian influences. This work was published, and the author was involved in the formulation of the model and performed all of its analysis and simulations.

- Designing a mathematical model of infection and sepsis formulated from the continuous infusion model presented in this thesis, which, to our knowledge, is the first to provide a direct link between an experimental and clinical model of prolonged inflammation. This model presents the foundation for a novel model of sepsis which, when incorporated with the multi-system model presented in this thesis, can allow for further insight into the complex, multi-system dynamics of the condition. The model would also include easily measurable markers of sepsis such as blood pressure, heart rate, and body temperature, which is important to clinicians. The author lead the model formulation and conducted all analysis and simulations of the model.

**Transition from a Bolus to a Continuous Infusion Experimental Model**

This work (i) addresses the differences seen in experimental data between a bolus and continuous infusion of endotoxin, and (ii) formulates a mathematical model of a continuous infusion of endotoxin that better represents prolonged inflammation. We show through statistical comparison of experimental cytokine data that significantly larger concentrations of IL-10 and significantly smaller concentrations of IL-8 as well as later peak timing for all cytokines occurs.
during a continuous infusion compared to a bolus. Through the development and analysis of a mathematical model describing endotoxin, monocyte, and cytokine dynamics to a continuous infusion of endotoxin, we suggest that these differences between administration methods are due to an increase in monocyte activation of IL-10 and decreases in decay rates of TNF-α and IL-8 during the continuous infusion. Our results also show that our mathematical model can be perturbed by increasing endotoxin administration duration or the total dose of endotoxin to produce a prolonged or more pronounced response, similar to that seen in clinical scenarios.

Since the main limitation of this work is that it utilizes two different sets of data, future work focuses on calibrating the model to bolus and continuous infusion experimental data from one large-dose (∼2 ng/kg) endotoxin study. Additionally, the model exhibited oscillating behavior during continuous stimulation for 20-32 hours. Thus, future work could also be aimed at using the model to study recurrent infections that result in cyclic patterns of inflammation.

**Multi-System Interactions**

This work develops a multi-system mathematical model of inflammation to study the interactive dynamics between the systems and their impact over time scales from hours to days. We examine the system interactions in this novel model by analyzing how the network responds to variations in endotoxin administration methods (bolus, repeated bolus, continuous infusion), timing, and total dose (simulating lethal amounts not able to be given to humans), which are relevant to clinically observed inflammation. Our results show that most endotoxin effects resolve within 24 hours of administration, though some effects linger for 48 hours and up to 10 days. These simulations suggest that scientists should monitor experimental subjects for longer in order to capture these prolonged system impacts. Our model also exhibits more pronounced cytokine concentrations, blood pressure, and body temperature during larger endotoxin doses or continuous administration of endotoxin, which suggests a borderline damaging inflammatory response for more potent stimuli or a longer exposure to stimuli as seen in some clinical scenarios. Furthermore, endotoxin administration timing primarily impacts hormone concentrations, with early morning or late evening administration resulting in an exacerbated hormone response, reiterating the importance that experimental studies should be conducted at the same time of day.

The immune response involves a complex network of reactions that influence function and regulation throughout the body, as described in detail in the physiological background of this thesis (Chapter 2), and the correspondence between entities in the network may vary depending on the location in the body and disease type. However, it is challenging to discern the true pathways of these interactions from the current knowledge in literature. While we
address and incorporate some of these pathways in the multi-system model presented in Chapter 6, there are additional possible links between these systems that may provide a more realistic representation. Figure 8.1a displays the connections currently implemented in our multi-system model, and Figure 8.1b shows connections discussed in Chapter 2 that are not currently implemented in the model but are suggested in physiological literature. Additional investigation into which are true pathways and which are secondary pathways that are already implemented in the model should be conducted. It can be challenging to determine direct and indirect pathways since many studies simply observe changes in quantities. For example, some studies report that TNF-$\alpha$ regulates both cardiac output and blood pressure [258, 193, 14], but the exact pathways that these mechanisms occur are unclear and may be intertwined.

Additional analysis could also be conducted to gain insight into important physiological parameters and connections between systems that largely influence system dynamics. For example, a formal local and global sensitivity analysis, subset selection routine, and parameter

![Diagram of connections between the immune system, cardiovascular system, and HPA axis. (a) Diagram of connections already implemented in the multi-system model from Chapter 6 (red), and (b) diagram of additional connections described in Chapter 2 not included in the multi-system model (black). Solid lines signify stimulation, and dotted lines signify inhibition.](image)

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estimation regime performed on the model could result in more accurate model predictions. Moreover, quantifying the uncertainty on the coupled model could allude to parameter variations responsible for the wide range of bodily responses seen in experimental and clinical data as well as potential therapeutic treatments that target these physiological components. While the multi-system model is currently calibrated to experimental data from a bolus endotoxin challenge, future work should focus on calibrating the model to a continuous infusion of endotoxin as done with the immune-only model in Chapter 5. This would allow further insight into the multi-system dynamics at play during a continuous infusion, and provide a better whole-body translational model from experimental to clinical inflammation.

Transition from Experimental to Clinical Inflammation

This work transitions the well-analyzed continuous infusion mathematical model to a preliminary model of infection. Our results show that the model can produce several clinically-relevant scenarios of inflammation including a healthy response, persistent non-infectious inflammation (including sepsis), immunosuppression, and recurrent infection. Increases and decreases in the parameter signifying the monocyte decay rate from the healthy state were able to produce all three inflammation scenarios, indicating that this could be an important parameter to study during a formal bifurcation analysis. Perturbation of the parameter associated with the activation rate of TNF-\(\alpha\) by monocytes also displays similar impacts and could be important to study. The simulation of vitamin C treatment during disease progression stages via perturbing the monocyte activation rate of IL-10 shows that the anti-inflammatory properties of the vitamin C treatment are effective at reversing the septic trajectory depending on the timeliness of the intervention and the strength of the dose.

Our model, however, failed to produce a septic state where the pathogen, monocytes, cytokines, and damage remain elevated, which was observed in the models by Reynolds et al. [207] and McDaniel et al. [164]. Differences between the pathogen and damage equations in these two models may account for our model’s failure to bifurcate to that state, so more investigation should be put into these equations. While the damage equation was based on the structure of the tissue integrity equation from Reynolds [208], the equation should be restructured as in McDaniel et al. [164] so that the irreversible damage threshold is always an unstable equilibrium point. Moreover, the work on the infection and sepsis model was preliminary, so an in-depth examination of the system's bifurcation structure should be performed to gain insight into physiological processes that cause the switch in the system from a healthy to septic state.

Finally, the infection and sepsis model could be improved to investigate its whole-body
impact. Sepsis induces a multi-system effect, so it would be relevant to couple the multi-system model with this sepsis model. Hormonal and cardiovascular mechanisms are key regulatory components of the body, so investigating their time-varying dynamics during a septic infection has the potential to provide further understanding of how sepsis evolves in patients. While cardiovascular components have been included in previous sepsis models [302, 164, 293], we are not aware of sepsis models with HPA axis dynamics or models that couple cardiovascular, hormone, and immune components during a septic infection. Additionally, the inclusion of adaptive immunity and measurements of metabolic function are also potential components that could add to the practicality of the sepsis model. Adaptive immunity elements such as T cells are also activated during the early stages of sepsis [109] and play a role in the immunosuppression phase, where their functions are inhibited and their cell death promotes anti-inflammatory cytokine production [178, 256]. Adaptive immunity components have been included in sepsis models [234, 233, 46] and are important for studying the immune response over days instead of hours. Given that cardiovascular and metabolic function are both used as markers for the clinical diagnosis of septic shock [238], incorporation of these two components could provide the opportunity to further study the distinction between sepsis and septic shock patients. Based on the extensive literature review in Chapter 3, there is a lack of mathematical models of sepsis that incorporate immune, hormonal, cardiovascular, thermal, pain, and metabolic functions. This provides a window of opportunity for our previously studied models to be used in tandem to examine in depth sepsis dynamics from a mathematical modeling perspective. The whole-body model could then be used to test or validate up and coming sepsis treatments for patient improvement, such as intravenous high-dose vitamin C treatment which could be more accurately modeled in a complex multi-system model.
REFERENCES


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APPENDICES
This appendix includes supporting information submitted for the manuscript “Characterization of differences in immune responses during bolus and continuous infusion endotoxin challenges using mathematical modeling” presented in Chapter 5. In Section A.1, we detail the mathematical formulations used to compute the goodness of fit measurements for model optimization that are reported in Chapter 5. These include the coefficient of determination, the corrected Akaike information criterion (AICc), and the Bayesian information criterion (BIC). Section A.2 reports the subject-specific optimization simulations from the continuous infusion (red) and bolus (black) mathematical models reported in Chapter 5. Each individual simulation shown in this section (Figures A.1-A.29) shows the optimal model output with that subject’s cytokine data plotted (either from the continuous infusion [21] or bolus study [115]).

Section A.3 reports simulations (Figures A.30-A.34) of the continuous infusion model where the infusion duration and total endotoxin dose administered were increased simultaneously. The continuous infusion was given as a 4, 8, 12, 24, and 36-hour infusion, and a total endotoxin dose of 2, 4, 8, and 16 ng/kg was administered. Figure A.35 displays a 2 ng/kg infusion of endotoxin over 18, 20, 32, and 36 hours. This simulation displays that the oscillatory cytokine
behavior arises when the 2 ng/kg infusion is given between a 20 and 32 hour span. After this range, the oscillations are die out due to lowered endotoxin stimuli within the system. Figure A.36 shows a temporal extension of Figure 9A in the main manuscript when a 2ng/kg dose of endotoxin was administered as a 4, 8, 12, and 24-hour infusion. This figure exhibits that it takes the system approximately 23 days when given shorter doses of endotoxin (4 and 8-hour infusions) and about 21 days (3 weeks) for longer doses of endotoxin (12 and 24-hour infusions) for the resting monocyte population to return to its baseline concentrations. All simulations in Section A.3 were generated using the optimal mean model parameters from the 4-hour continuous infusion in Chapter 5. Section A.4 shows Bayesian uncertainty results using the Delayed Rejection Adaptive Metropolis (DRAM) algorithm. We generate prediction and credible intervals for the model and also report the DRAM parameter chains, correlations, and distributions. We compare these DRAM results to the frequentist uncertainty quantification results shown in Chapter 5.

### A.1 Goodness of fits

We calculated three goodness of fit measurements for each parameter subset in the main manuscript by computing the coefficient of determination ($R^2$) [70], the corrected Akaike information criterion (AICc) [35], and the Bayesian information criterion (BIC) [227]. The coefficient of determination for cytokines $k \in \{TNF, IL6, IL8, IL10\}$ was given by

$$R^2_k = 1 - \frac{SSE_k}{SST_k}.$$ 

$SSE_k$ was the sum of squared error of cytokine $k$ and was given by

$$SSE_k = \sum_{i=1}^{N} (y^k_{\text{data}}(t_i) - \tilde{y}_i^k)^2$$

where $y^k_{\text{data}}(t_i)$ was the data for cytokine $k$ at time $t_i$ for $1 \leq i \leq N$ and $\tilde{y}_i^k = g(t_i, X_k(t_i), \tilde{\theta})$ was the optimized model output for cytokine $k$ at time $t_i$. $SST$ was the sum of squared error given by

$$SST_k = \sum_{i=1}^{N} (y^k_{\text{data}}(t_i) - \bar{y}_{\text{data}})^2$$
where $\bar{y}^k_{data}$ was the mean of the observed data from cytokine $k$. Larger $R^2$ values indicated a better explanation of the data by the model. The AICc and BIC accounted for the number of parameters being estimated. Smaller values of the AICc and BIC indicated a balance between model fit and the number of estimated parameters. AICc and BIC were calculated as

$$AICc = 2\ln(J) + 2\rho + \frac{2\rho(\rho + 1)}{M - \rho - 1}$$

and

$$BIC = 2\ln(J) + \rho \ln(M)$$

where $J$ was the least squares cost as defined in equation (9) in the main manuscript, $\rho$ the number of estimated parameters, and $M$ the total number of data points used in model calibration for each parameter subset.

### A.2 Subject-specific optimizations

![Graphs showing model fit for subject 1 from the continuous infusion subject-specific optimization.](image)

Figure A.1: Model fit for subject 1 from the continuous infusion subject-specific optimization.
Figure A.2: Model fit for subject 2 from the continuous infusion subject-specific optimization.

Figure A.3: Model fit for subject 3 from the continuous infusion subject-specific optimization.

Figure A.4: Model fit for subject 4 from the continuous infusion subject-specific optimization.
Figure A.5: Model fit for subject 5 from the continuous infusion subject-specific optimization.

Figure A.6: Model fit for subject 6 from the continuous infusion subject-specific optimization.

Figure A.7: Model fit for subject 7 from the continuous infusion subject-specific optimization.
Figure A.8: Model fit for subject 8 from the continuous infusion subject-specific optimization.

Figure A.9: Model fit for subject 9 from the continuous infusion subject-specific optimization.

Figure A.10: Model fit for subject 1 from the bolus subject-specific optimization.
Figure A.11: Model fit for subject 2 from the bolus subject-specific optimization.

Figure A.12: Model fit for subject 3 from the bolus subject-specific optimization.

Figure A.13: Model fit for subject 4 from the bolus subject-specific optimization.
Figure A.14: Model fit for subject 5 from the bolus subject-specific optimization.

Figure A.15: Model fit for subject 6 from the bolus subject-specific optimization.

Figure A.16: Model fit for subject 7 from the bolus subject-specific optimization.
Figure A.17: Model fit for subject 8 from the bolus subject-specific optimization.

Figure A.18: Model fit for subject 9 from the bolus subject-specific optimization.

Figure A.19: Model fit for subject 10 from the bolus subject-specific optimization.
Figure A.20: Model fit for subject 11 from the bolus subject-specific optimization.

Figure A.21: Model fit for subject 12 from the bolus subject-specific optimization.

Figure A.22: Model fit for subject 13 from the bolus subject-specific optimization.
Figure A.23: Model fit for subject 14 from the bolus subject-specific optimization.

Figure A.24: Model fit for subject 15 from the bolus subject-specific optimization.

Figure A.25: Model fit for subject 16 from the bolus subject-specific optimization.
Figure A.26: Model fit for subject 17 from the bolus subject-specific optimization.

Figure A.27: Model fit for subject 18 from the bolus subject-specific optimization.

Figure A.28: Model fit for subject 19 from the bolus subject-specific optimization.
A.3 Endotoxin perturbations

Figure A.30: 4-hour continuous infusion of 2 ng/kg (red solid lines), 4 ng/kg (yellow dash-dotted lines), 8 ng/kg (light blue dotted lines), and 16 ng/kg (dark blue dashed lines) of endotoxin using the optimal mean model parameters.
Figure A.31: 8-hour continuous infusion of 2 ng/kg (red solid lines), 4 ng/kg (yellow dash-dotted lines), 8 ng/kg (light blue dotted lines), and 16 ng/kg (dark blue dashed lines) of endotoxin using the optimal mean model parameters.

Figure A.32: 12-hour continuous infusion of 2 ng/kg (red solid lines), 4 ng/kg (yellow dash-dotted lines), 8 ng/kg (light blue dotted lines), and 16 ng/kg (dark blue dashed lines) of endotoxin using the optimal mean model parameters.
Figure A.33: 24-hour continuous infusion of 2 ng/kg (red solid lines), 4 ng/kg (yellow dash-dotted lines), 8 ng/kg (light blue dotted lines), and 16 ng/kg (dark blue dashed lines) of endotoxin using the optimal mean model parameters.

Figure A.34: 36-hour continuous infusion of 2 ng/kg (red solid lines), 4 ng/kg (yellow dash-dotted lines), 8 ng/kg (light blue dotted lines), and 16 ng/kg (dark blue dashed lines) of endotoxin using the optimal mean model parameters.
Figure A.35: 2 ng/kg continuous infusion for 18 (red solid lines), 20 (yellow dash-dotted lines), 32 (light blue dotted lines), and 36 hours (dark blue dashed lines) using the optimal mean model parameters.

Figure A.36: 2 ng/kg continuous infusion for four (red solid lines), eight (yellow dash-dotted lines), 16 (light blue dotted lines), and 24 hours (dark blue dashed lines) using the optimal mean model parameters. Monocytes return to baseline about 3 weeks after the infusion start for large doses of endotoxin.
### A.4 Delayed Rejection Adaptive Metropolis (DRAM)

We compare the asymptotic (frequentist) measures of uncertainty in Figure 7 of the main manuscript (prediction and confidence intervals) with Bayesian measures of uncertainty (prediction and credible intervals). Frequentist methods establish uncertainty by fixing parameters and assigning probabilities to data and Bayesian methods determine uncertainty through a single data set by assigning probability distributions to parameters [78]. We generate Bayesian inference results using a sampling method called Delayed Rejection Adaptive Metropolis (DRAM), which combines two Metropolis-Hastings Markov chain Monte Carlo (MCMC) algorithms (Delayed Rejection and Adaptive Metropolis). Additional details of DRAM can be found in Haario et al. [97], Smith [241], Lye et al. [153].

DRAM was run for 200,000 samples with a 40,000 burn-in period. Prediction and credible intervals for the mean bolus and continuous infusion models are shown in Figure A.37. These results are similar to the frequentist prediction and confidence intervals generated in Figure 7 of the original manuscript. The credible interval is the Bayesian form of a frequentist confidence interval. It is defined as the range in which 95% of the posterior distribution lies, or the range of values that a parameter lies within with probability 0.95 [49]. The prediction intervals for both uncertainty methods produce similar sized intervals for both models, with the frequentist prediction intervals being slightly wider than those produced by DRAM. The credible intervals for both the bolus and continuous infusion models are wider than the confidence intervals from the frequentist approach, specifically between hours zero and eight. However, the tails of both the Bayesian credible and frequentist confidence intervals are of similar width.

The parameter chains, correlations, and distributions for the DRAM simulations are shown in Figures A.38-A.43. The parameter chains show the convergence of DRAM following the burn-in period (marked by the vertical black line), where the parameter chain for the bolus model (Figure A.38) exhibits wider bounds for all optimized parameters except for $k_{TNFM}$ compared to the continuous infusion model chain (Figure A.39), which is about the same width as $k_{TNFM}$ in the continuous infusion. The difference in parameter chain characteristics is hypothesized to be due to the sensitive and identifiable parameter subset being derived from the continuous infusion model instead of the bolus model, leading to a larger variation in bolus model results. The DRAM parameter correlations are shown for the bolus model in Figure A.40 and the continuous model in Figure A.41. The densities for the continuous infusion look well distributed with possible linear correlations between parameters $k_{MA}$ and $k_{TNFM}$, but this interestingly did not impact the parameter distributions shown in Figure A.43. The DRAM parameter correlations for the bolus model (Figure A.40) have wider, more scattered distributions and a similar linear trend between parameters $k_{MA}$ and $k_{TNFM}$. Additionally, the
parameter correlations show that some parameters may be hitting their preset bounds, which is observed in the parameter chains for the bolus model (Figure A.38).

Finally, the optimized parameter distributions are shown in Figures A.42 and A.43. All parameter distributions for both the bolus and continuous infusion models are approximately normally distributed, with the parameter distributions from the bolus model exhibiting slightly skewed behavior with longer tails. The parameter distributions from the continuous infusion model (Figure A.43) show the optimal parameter value (denoted by the black line) close to the peak of the distribution curve, while the bolus model parameter distributions (Figure A.42) have slightly off-peak optimal values for parameters $k_{MA}$ and $k_8$ and further off-peak optimal value for parameter $k_{TNF}$. As previously discussed, we attribute the off-peak optimal values of several of the bolus parameters due to the optimized subset being generated from the continuous infusion. Furthermore, we suspect that the off-center optimal value for $k_{TNF}$ occurred because that parameter is the least sensitive of all optimized parameters (shown in Figure 5 in the original manuscript). Therefore, it can vary more than the other parameters with less of an impact on the model output.
Figure A.37: DRAM prediction (light gray) and credible (dark gray) intervals for the bolus (a) and continuous infusion (b) mean model response. Blue data points on (a) are from Janum et al. [115] and black points are generated data. Blue data points on (b) are from Berg et al. [21] and black points are generated data.
Figure A.38: DRAM parameter chains for the optimized parameter set $S_{Final}$ for the bolus model. 200,000 simulations were run with a burn-in period of 40,000. The black line represents the end of the burn-in period.

Figure A.39: DRAM parameter chains for the optimized parameter set $S_{Final}$ for the continuous infusion model. 200,000 simulations were run with a burn-in period of 40,000. The black line represents the end of the burn-in period.
Figure A.40: DRAM parameter correlations for the optimized parameter set $S_{Final}$ for the bolus model. 200,000 simulations were run with a burn-in period of 40,000.

Figure A.41: DRAM parameter correlations for the optimized parameter set $S_{Final}$ for the continuous infusion model. 200,000 simulations were run with a burn-in period of 40,000.
Figure A.42: DRAM parameter distributions for the optimized parameter set $S_{Final}$ for the bolus model. 200,000 simulations were run with a burn-in period of 40,000. The black line represents the bolus optimized parameter values reported in Table 5.5.

Figure A.43: DRAM parameter distributions for the optimized parameter set $S_{Final}$ for the continuous infusion model. 200,000 simulations were run with a burn-in period of 40,000. The black line represents the continuous infusion optimized parameter values reported in Table 5.5.
This appendix contains a table with the parameters and initial conditions used for the unified computational model described in Chapter 6.

Table B.1: Model parameters and initial conditions. Parameters with reference ∼ were scaled from their values reported in Bangsgaard et al. [10] and Dobreva et al. [69] to match the appropriate variable concentration in the model, parameters with * indicates that the parameter was manually adjusted, and parameters with ∼* were both scaled and manually adjusted.

<table>
<thead>
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<th>Parameter</th>
<th>Meaning</th>
<th>Value</th>
<th>Unit</th>
<th>Reference</th>
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<td>$k_E$</td>
<td>Endotoxin decay rate</td>
<td>1.08</td>
<td>hr$^{-1}$</td>
<td>[69]</td>
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<td>$k_M$</td>
<td>$E$ activation rate of monocytes</td>
<td>0.0414</td>
<td>hr$^{-1}$</td>
<td>[69]</td>
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<td>$k_{MTNF}$</td>
<td>TNF-$\alpha$ activation rate of monocytes</td>
<td>8.65</td>
<td>hr$^{-1}$</td>
<td>[69]</td>
</tr>
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<td>$k_{MR}$</td>
<td>$M_R$ regeneration rate</td>
<td>6×10$^{-3}$</td>
<td>hr$^{-1}$</td>
<td>[69]</td>
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<tr>
<td>$M_\infty$</td>
<td>Monocyte carrying capacity</td>
<td>3×10$^4$</td>
<td>noc</td>
<td>[69]</td>
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<td>$k_{MA}$</td>
<td>$M_A$ decay rate</td>
<td>2.51</td>
<td>hr$^{-1}$</td>
<td>[69]</td>
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<td>$\eta_{ME}$</td>
<td>Half-max of $E$ upreg $M_A$</td>
<td>3.3</td>
<td>ng/kg</td>
<td>[69]</td>
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<td>pg/mL</td>
<td>[69]</td>
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<td>n.d.</td>
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<td>n.d.</td>
<td>[69]</td>
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<td>n.d.</td>
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<td>$k_{TNFM}$</td>
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<td>Exp. of IL-10 downreg TNF-α</td>
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<td>Rate of IL-6 production due to $M_A$</td>
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<td>Half-max of IL-6 downreg IL-6</td>
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<tr>
<td>$η_{IL6}$</td>
<td>Half-max of TNF-α upreg IL-6</td>
<td>77.1 pg/mL</td>
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<tr>
<td>$h_{IL610}$</td>
<td>Exp. of IL-10 downreg</td>
<td>0.25 n.d.</td>
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<tr>
<td>$h_{IL66}$</td>
<td>Exp. of IL-6 downreg IL-6</td>
<td>0.25 n.d.</td>
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<tr>
<td>$h_{IL6}$</td>
<td>Exp. of TNF-α upreg IL-6</td>
<td>0.25 n.d.</td>
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<tr>
<td>$k_{MA}$</td>
<td>Rate of IL-10 production due to $M_A$</td>
<td>0.0105 pg/(mL hr noc)</td>
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</tr>
<tr>
<td>$k_{MA}$</td>
<td>Rate of IL-10 production due to IL-6</td>
<td>0.0191 pg/(ml hr noc)</td>
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<tr>
<td>$h_{MA}$</td>
<td>Rate of IL-10 production due to $F$</td>
<td>0.01 pg/(mL noc hr)</td>
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</tr>
<tr>
<td>$k_{IL10}$</td>
<td>IL-10 decay rate</td>
<td>0.834 hr$^{-1}$</td>
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<tr>
<td>$w_{10}$</td>
<td>IL-10 baseline amount</td>
<td>0.235 pg/mL</td>
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<tr>
<td>$η_{IL10}$</td>
<td>Half-max of IL-10 downreg IL-6</td>
<td>140 pg/mL</td>
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<tr>
<td>$η_{IL10}$</td>
<td>Half-max of IL-6 upreg IL-10</td>
<td>0.8 pg/mL</td>
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<td>$h_{IL10}$</td>
<td>Exp. of IL-6 upreg IL-10</td>
<td>3.68 n.d.</td>
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<tr>
<td>$h_{IL10}$</td>
<td>Exp. of F upreg IL-10</td>
<td>10 n.d.</td>
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**HPA axis model**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{CR}$</td>
<td>Cir. rhy. rate of C production</td>
<td>$4.10 \times 10^{11}$ hr$^{-1}$</td>
</tr>
<tr>
<td>$η_{CF}$</td>
<td>Half-max of $F$ downreg C</td>
<td>$2.39 \times 10^{-5}$ µg/dL</td>
</tr>
<tr>
<td>$h_{CF}$</td>
<td>Exp. of $F$ downreg C</td>
<td>2 n.d.</td>
</tr>
<tr>
<td>$k_{CTNF}$</td>
<td>Rate of C production by TNF-α</td>
<td>0.160 hr$^{-1}$</td>
</tr>
<tr>
<td>$k_C$</td>
<td>C decay rate</td>
<td>1.92 hr$^{-1}$</td>
</tr>
<tr>
<td>$C_b$</td>
<td>Baseline C level</td>
<td>0.06 pg/mL</td>
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<tr>
<td>$k_{AC}$</td>
<td>Rate of A production by C</td>
<td>$1.42 \times 10^{6}$ hr$^{-1}$</td>
</tr>
<tr>
<td>$η_{FA}$</td>
<td>Half-max of $F$ downreg A</td>
<td>$5.62 \times 10^{-6}$ pg/mL</td>
</tr>
<tr>
<td>$h_{FA}$</td>
<td>Exp. of $F$ downreg A</td>
<td>1 n.d.</td>
</tr>
<tr>
<td>$k_{ATNF}$</td>
<td>Rate of A production by TNF-α</td>
<td>100 pg/(mL hr)</td>
</tr>
<tr>
<td>$η_{ATNF}$</td>
<td>Half-max of TNF-α upreg A</td>
<td>33.33 pg/mL</td>
</tr>
<tr>
<td>$h_{ATNF}$</td>
<td>Exp. of TNF-α upreg A</td>
<td>2 n.d.</td>
</tr>
<tr>
<td>$k_A$</td>
<td>A decay rate</td>
<td>0.96 hr$^{-1}$</td>
</tr>
<tr>
<td>$k_{FA}$</td>
<td>Rate of $F$ production by $A$</td>
<td>0.0255 µg mL/(pg dL hr)</td>
</tr>
<tr>
<td>$η_{F10}$</td>
<td>Half-max of IL-10 downreg $F$</td>
<td>10 pg/mL</td>
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<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
<th>Units</th>
<th>Notes</th>
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<tbody>
<tr>
<td>$h_{F,10}$</td>
<td>Exp. of IL-10 downreg $F$</td>
<td>1</td>
<td>n.d.</td>
<td>*</td>
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<tr>
<td>$k_F$</td>
<td>$F$ decay rate</td>
<td>1.56</td>
<td>hr$^{-1}$</td>
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<tr>
<td></td>
<td>Circadian Rhythm</td>
<td></td>
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<tr>
<td>$\alpha$</td>
<td>Upregulation half-max</td>
<td>300</td>
<td>min</td>
<td>[10]</td>
</tr>
<tr>
<td>$k$</td>
<td>Upregulation exp.</td>
<td>5</td>
<td>n.d.</td>
<td>[10]</td>
</tr>
<tr>
<td>$\beta$</td>
<td>Downregulation half-max</td>
<td>950</td>
<td>min</td>
<td>[10]</td>
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<tr>
<td>$l$</td>
<td>Downregulation exp. at $t = \beta$</td>
<td>6</td>
<td>n.d.</td>
<td>[10]</td>
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<tr>
<td>$\varepsilon$</td>
<td>Baseline circadian rhythm level</td>
<td>0.01</td>
<td>n.d.</td>
<td>[10]</td>
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<tr>
<td>$N_c$</td>
<td>Scaling factor</td>
<td>1.92</td>
<td>n.d.</td>
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<tr>
<td>$\delta$</td>
<td>Circadian clock time shift</td>
<td>70</td>
<td>min</td>
<td>*</td>
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<tr>
<td></td>
<td>Cardiovascular circulation model</td>
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<tr>
<td>$R_a$</td>
<td>Arterial resistance</td>
<td>686</td>
<td>mmHg hr/mL</td>
<td>*</td>
</tr>
<tr>
<td>$R_{sb}$</td>
<td>Baseline peripheral resistance</td>
<td>3713</td>
<td>mmHg hr/mL</td>
<td>*</td>
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<tr>
<td>$R_v$</td>
<td>Venous resistance</td>
<td>9.72</td>
<td>mmHg hr/mL</td>
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<tr>
<td>$E_{la}$</td>
<td>Large artery elastance</td>
<td>0.791</td>
<td>mmHg/mL</td>
<td>*</td>
</tr>
<tr>
<td>$E_{sa}$</td>
<td>Small artery elastance</td>
<td>3.92</td>
<td>mmHg/mL</td>
<td>*</td>
</tr>
<tr>
<td>$E_{sv}$</td>
<td>Small vein elastance</td>
<td>0.132</td>
<td>mmHg/mL</td>
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<tr>
<td>$E_v$</td>
<td>Large vein elastance</td>
<td>0.0217</td>
<td>mmHg/mL</td>
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<tr>
<td>$E_{Max}$</td>
<td>Maximum elastance</td>
<td>3.20</td>
<td>mmHg/mL</td>
<td>*</td>
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<tr>
<td>$E_{Min}$</td>
<td>Minimum elastance</td>
<td>0.0265</td>
<td>mmHg/mL</td>
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<tr>
<td></td>
<td>Cardiovascular control model</td>
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<tr>
<td>$k_H$</td>
<td>Rate of change of HR</td>
<td>0.25</td>
<td>n.d.</td>
<td>*</td>
</tr>
<tr>
<td>$\tau_H$</td>
<td>HR time constant</td>
<td>0.791</td>
<td>hr</td>
<td>*</td>
</tr>
<tr>
<td>$H_M$</td>
<td>Maximum HR</td>
<td>190</td>
<td>bpm</td>
<td>[85][115]</td>
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<tr>
<td>$H_b$</td>
<td>Baseline HR</td>
<td>60.4</td>
<td>bpm</td>
<td>[115]</td>
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<tr>
<td>$p_{lab}$</td>
<td>Baseline BP</td>
<td>118</td>
<td>mmHg</td>
<td>[115]</td>
</tr>
<tr>
<td>$\eta_{HT}$</td>
<td>Half-max of $T$ upreg HR</td>
<td>0.354</td>
<td>°C</td>
<td>*</td>
</tr>
<tr>
<td>$h_{HT}$</td>
<td>Exp. of $T$ upreg HR</td>
<td>2</td>
<td>n.d.</td>
<td>[69]</td>
</tr>
<tr>
<td>$\eta_{HF}$</td>
<td>Half-max of $F$ upreg HR</td>
<td>5</td>
<td>µg/dL</td>
<td>*</td>
</tr>
<tr>
<td>$h_{HF}$</td>
<td>Exp. of $F$ upreg HR</td>
<td>4</td>
<td>n.d.</td>
<td>*</td>
</tr>
<tr>
<td>$\eta_{HP}$</td>
<td>Half-max of BP regulating HR</td>
<td>24.6</td>
<td>mmHg</td>
<td>[69]</td>
</tr>
<tr>
<td>$h_{HP}$</td>
<td>Exp. of BP regulating HR</td>
<td>4</td>
<td>n.d.</td>
<td>[69]</td>
</tr>
<tr>
<td>$k_{NM}$</td>
<td>$M_A$ production rate of NO</td>
<td>0.002</td>
<td>(hr noc)$^{-1}$</td>
<td>[69]</td>
</tr>
<tr>
<td>$k_N$</td>
<td>NO decay rate</td>
<td>0.045</td>
<td>hr$^{-1}$</td>
<td>[69]</td>
</tr>
<tr>
<td>$\eta_{NTNF}$</td>
<td>Half-max of TNF-α upreg NO</td>
<td>39.6</td>
<td>pg/mL</td>
<td>~</td>
</tr>
<tr>
<td>$\eta_{N10}$</td>
<td>Half-max of IL-10 downreg NO</td>
<td>4</td>
<td>pg/mL</td>
<td>[69]</td>
</tr>
<tr>
<td>$h_{NTNF}$</td>
<td>Exp. of TNF-α upreg NO</td>
<td>2</td>
<td>n.d.</td>
<td>[69]</td>
</tr>
<tr>
<td>$h_{N10}$</td>
<td>Exp. of IL-10 downreg NO</td>
<td>0.4</td>
<td>n.d.</td>
<td>[69]</td>
</tr>
<tr>
<td>$k_{RP}$</td>
<td>Rate of $R_S$ stimulation by $P$</td>
<td>13.0</td>
<td>mmHg/mL</td>
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<tr>
<td>$k_{RN}$</td>
<td>Rate of $R_S$ inhibition by NO</td>
<td>0.8</td>
<td>mmHg/mL</td>
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</tr>
<tr>
<td>$k_R$</td>
<td>$R_S$ recovery rate</td>
<td>4.28</td>
<td>hr$^{-1}$</td>
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<tr>
<td>$\eta_{RP}$</td>
<td>Half-max of $P$ upreg $R_S$</td>
<td>230</td>
<td>kPa/hr</td>
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<tr>
<td>$h_{RP}$</td>
<td>Exp. of $P$ upreg $R_S$</td>
<td>2</td>
<td>n.d.</td>
<td>[69]</td>
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<td>---------</td>
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</table>

**Pain model**

<table>
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<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{PE}$</td>
<td>Rate of change of $P$ due to $E$</td>
<td>0.2</td>
<td>kg/(ng hr)</td>
</tr>
<tr>
<td>$k_P$</td>
<td>Rate of $P$ recovery</td>
<td>0.15</td>
<td>hr$^{-1}$</td>
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<tr>
<td>$P_b$</td>
<td>Baseline $P$ level</td>
<td>781</td>
<td>kPa</td>
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</tbody>
</table>

**Temperature model**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_b$</td>
<td>Baseline temperature</td>
<td>36.2</td>
<td>°C</td>
</tr>
<tr>
<td>$k_T$</td>
<td>$T$ recovery rate</td>
<td>0.7</td>
<td>hr$^{-1}$</td>
</tr>
<tr>
<td>$k_{TTNF}$</td>
<td>Rate of $T$ upregulation by TNF-$\alpha$</td>
<td>1</td>
<td>°C/hr</td>
</tr>
<tr>
<td>$k_{T6}$</td>
<td>Rate of $T$ upregulation by IL-6</td>
<td>1.9</td>
<td>°C/hr</td>
</tr>
<tr>
<td>$k_{T10}$</td>
<td>Rate of $T$ downregulation by IL-10</td>
<td>0.2</td>
<td>°C/hr</td>
</tr>
<tr>
<td>$\eta_{TTNF}$</td>
<td>Half-max of TNF-$\alpha$ upreg $T$</td>
<td>130</td>
<td>pg/mL</td>
</tr>
<tr>
<td>$\eta_{T6}$</td>
<td>Half-max of IL-6 upreg $T$</td>
<td>140</td>
<td>pg/mL</td>
</tr>
<tr>
<td>$\eta_{T10}$</td>
<td>Half-max of IL-10 downreg $T$</td>
<td>40</td>
<td>pg/mL</td>
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<tr>
<td>$h_{TTNF}$</td>
<td>Exp. of TNF-$\alpha$ upreg $T$</td>
<td>1</td>
<td>n.d.</td>
</tr>
<tr>
<td>$h_{T6}$</td>
<td>Exp. of IL-6 upreg $T$</td>
<td>1</td>
<td>n.d.</td>
</tr>
<tr>
<td>$h_{T10}$</td>
<td>Exp. of IL-10 downreg $T$</td>
<td>1</td>
<td>n.d.</td>
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**Initial Conditions**

<table>
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<tr>
<th>Variable</th>
<th>Model State</th>
<th>Value</th>
<th>Unit</th>
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<tbody>
<tr>
<td>$E$</td>
<td>Endotoxin</td>
<td>0</td>
<td>ng/kg</td>
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<tr>
<td>$M_R$</td>
<td>Resting monocytes</td>
<td>28200</td>
<td>noc</td>
</tr>
<tr>
<td>$M_A$</td>
<td>Activated monocytes</td>
<td>0</td>
<td>noc</td>
</tr>
<tr>
<td>$TNF$</td>
<td>TNF-$\alpha$</td>
<td>0.466</td>
<td>pg/mL</td>
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<tr>
<td>$IL6$</td>
<td>IL-6</td>
<td>0.262</td>
<td>pg/mL</td>
</tr>
<tr>
<td>$IL10$</td>
<td>IL-10</td>
<td>0.234</td>
<td>pg/mL</td>
</tr>
<tr>
<td>$C$</td>
<td>CRH</td>
<td>1.368</td>
<td>pg/mL</td>
</tr>
<tr>
<td>$A$</td>
<td>ACTH</td>
<td>8.872</td>
<td>pg/mL</td>
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<tr>
<td>$F$</td>
<td>Cortisol</td>
<td>1.590</td>
<td>µg/dL</td>
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<tr>
<td>$V_{la}$</td>
<td>Large artery volume</td>
<td>146.647</td>
<td>mL</td>
</tr>
<tr>
<td>$V_{sa}$</td>
<td>Small artery volume</td>
<td>25.876</td>
<td>mL</td>
</tr>
<tr>
<td>$V_{sv}$</td>
<td>Small vein volume</td>
<td>28.162</td>
<td>mL</td>
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<tr>
<td>$V_v$</td>
<td>Large vein volume</td>
<td>159.943</td>
<td>mL</td>
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<td>$HR$</td>
<td>Heart rate</td>
<td>60.335</td>
<td>bpm</td>
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<tr>
<td>$R_S$</td>
<td>Vascular resistance</td>
<td>1.040</td>
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</tr>
<tr>
<td>$N$</td>
<td>Nitric oxide</td>
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<tr>
<td>$P$</td>
<td>Pain perception threshold</td>
<td>781.5</td>
<td>kPa</td>
</tr>
<tr>
<td>$T$</td>
<td>Temperature</td>
<td>36.22</td>
<td>°C</td>
</tr>
</tbody>
</table>

upreg = upregulating, downreg = downregulating, exp = exponent