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Reconciling conflicting clinical studies of ANTIOXIDANT SUPPLEMENTATION AS HIV THERAPY: A MATHEMATICAL APPROACH

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RECONCILING CONFLICTING CLINICAL STUDIES OF ANTIOXIDANT SUPPLEMENTATION AS HIV THERAPY: A MATHEMATICAL APPROACH

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Abstract

Small highly reactive molecules called reactive oxygen species (ROS) play a crucial role in cell signalling and infection control. However, high levels of ROS can cause significant damage to cell structure and function. Studies have shown that infection with the human immunodeficiency virus (HIV) results in increased ROS concentrations, which can in turn lead to faster progression of HIV infection, and cause $CD4^+$ T-cell apoptosis. To counteract these effects, clinical studies have explored the possibility of raising antioxidant levels, with mixed results. In this thesis, a mathematical model is used to explore this potential therapy, both analytically and numerically. For the numerical work, we use clinical data from both HIV-negative and HIV-positive injection drug users (IDUs) to estimate model parameters; these groups have lower baseline concentrations of antioxidants than non-IDU controls. Our model suggests that increases in $CD4^+$ T cell concentrations can result from moderate levels of daily antioxidant supplementation, while excessive supplementation has the potential to cause periods of immunosuppression. We discuss implications for HIV therapy in IDUs and other populations which may have low baseline concentrations of antioxidants.

Keywords: HIV, $CD4^+$ T cell, mathematical model, antioxidant supplementation, vitamins, reactive oxygen species, therapy, treatment

Statement of Co-Authorship

The work presented in Chapter 2 has been accepted for publication.

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List of Abbreviations

AIDS acquired immune deficiency syndrome

AZT azidothymidine (zidovudine)

DDE delay differential equation

H_2O_2 hydrogen peroxide

HAART highly active antiretroviral therapy

HIV human immunodeficiency virus

HIV(-) HIV-negative injection drug users

HIV(+) HIV-positive injection drug users

HIV(+)P HIV-positive injection drug users receiving a placebo

HIV(+)V HIV-positive injection drug users receiving vitamin supplementation

IDU injection drug user

NF-κB nuclear factor κB

nRTI nucleoside reverse transciptase inhibitor

nnRTI non-nucleoside reverse transciptase inhibitor

ODE ordinary differential equation

PDE partial differential equation

PI protease inhibitor

ROS reactive oxygen species

List of Symbols

a - concentration of antioxidants [molecules/μL]

α - rate of antioxidant supplementation [molecules μL^{-1} day^{-1}]

α_c - level of antioxidant supplementation at which the internal equilibrium undergoes a bifurcation [molecules μL^{-1} day^{-1}]

$\beta(r)$ - function describing the rate of infection of uninfected $CD4^+$ T cells [$(cell/\mu L)^{-1}$ day^{-1}]

b_0 - rate of ROS-independent infection of uninfected $CD4^+$ T cells, equivalent to $\beta(0)$ [$(cell/\mu L)^{-1}$ day^{-1}]

b_{max} - maximum rate of infection of uninfected $CD4^+$ T cells [$(cell/\mu L)^{-1}$ day^{-1}]

d_x - rate of uninfected $CD4^+$ T cell elimination from the system [day^{-1}]

d_y - rate of infected $CD4^+$ T cell elimination from the system [day^{-1}]

ϵ - effectiveness of drug therapy

h_a - natural rate of antioxidant decay [day^{-1}]

h_r - natural rate of ROS decay [day^{-1}]

k - rate of ROS production by infected cells [molecules $cell^{-1}$ day^{-1}]

$\hat{\lambda}_a$ - rate of dietary antioxidant uptake in control group [molecules μL^{-1} day^{-1}]

λ_a - rate of dietary antioxidant uptake in injection drug users [molecules μL^{-1} day^{-1}]

λ_r - natural rate of ROS production [molecules μL^{-1} day^{-1}]

λ_x - rate of $CD4^+$ T cell production by the thymus [cells μL^{-1} day^{-1}]

m - rate of ROS removal by reaction with antioxidants [$(molecule/\mu L)^{-1}$ day^{-1}]

p - rate of antioxidant removal by reaction with ROS [$(molecule/\mu L)^{-1}$ day^{-1}]

r - concentration of ROS [molecules/μL]

R_0 - basic reproductive ratio

x - concentration of uninfected $CD4^+$ T cells [cells/μL]

$x_{\max}$ - the maximum attainable stable equilibrium concentration of uninfected T cells [cells/μL]

x^d - disease free equilibrium [cells/μL]

$\hat{x}$ - equilibrium of controls [cells/μL]

x^* - equilibrium of HIV(-) [cells/μL]

x^p - equilibrium of HIV(+)P [cells/μL]

x^v - equilibrium of HIV(+)V [cells/μL]

y - concentration of infected $CD4^+$ T cells [cells/μL]

CHAPTER 1

Introduction

According to the World Health Organization (WHO), the human immunodeficiency virus (HIV) currently infects about 33 million people worldwide – with more than three-fifths residing in Africa – and millions more are indirectly affected (WHO 2008). Despite concerted efforts to improve global access to antiretroviral drugs, in 2005, only 20% of persons in low- and middle-income countries were receiving the required treatment (WHO 2006). Injection drug users form a particular group of interest due to the endemic nature of HIV infection in this population (KERR *et al.* 2007). According to the WHO, the global population of injection drug users (IDUs) consists of approximately 15.9 million people, of which 3 million are HIV-positive. The spread of the virus is particularly rampant in populations where injecting equipment is re-used and shared. Of the new HIV infections, one in ten is caused by the use of injection drugs. In Eastern Europe and Central Asia, 80% of all HIV infections can be attributed to drug use (WHO 2009).

The need for improved access to therapy and therapeutic options which support increased adherence is clear. Even amongst persons who have access to antiretrovial medications, two-thirds do not adhere to the treatment regimen (WHO 2003). Furthermore, in HIV-positive IDUs vitamin deficiencies have been impli-

cated in faster disease progression (TANG and SMIT 2000). In this thesis, the use of vitamin supplements in IDUs as a potiential HIV therapy is explored. A brief review of the revelant immunology, findings of clinical studies, and modelling techniques is provided below.

1.1 The immune system

A layered defense system protects the body from infection through three lines of increasing specificity. The first prevents foreign pathogens from entering the body and consists of the skin, mucous membranes, and secretions of the skin and mucous membranes. Pathogens that penetrate these surface barriers encounter the second line of defense which is also non-specific and innate. This defense mechanism (1) utilizes phagocytic white blood cells: i.e., neutrophils and macrophages which ingest invading organisms and (2) triggers an inflammatory response which shuts down protein synthesis in host cells, recruits immune cells to the area of infection, and promotes healing. The aforementioned immune cells, called lymphocytes, constitute the third line of defense, the adaptive immune system. This mechanism, consisting of B lymphocytes (B cells) and T lymphocytes (T cells), enables an efficient and selective immune response which eliminates a particular invader, or pathogen, from the body. Both B cells and T cells recognize pathogens by means of their plasma membrane-bound antigen receptors.

Upon infection of a cell by a pathogen, a fragment of this invader, called an antigen, is presented to nearby T cells. Two main types of T cells respond: cytotoxic T cells by initiating a sequence which results in the death of the infected cell, and helper T cells by activating and directing other immune system cells. The interactions between the infected cells and T cells are greatly enhanced by the presence of T cell surface proteins, CD8 and CD4, which are present on the

surface of most cytotoxic and helper T cells, respectively.

1.2 The human immunodeficiency virus

As a virus, HIV is dependent upon the replication machinery of cells for reproduction, specifically those that bear surface CD4 receptors. The life cycle of HIV can be divided into eight stages:

1. The virus attaches to the CD4 receptor of a potential host T cell. The $CD4^+$ T cell's coreceptors, CCR5 or CXCR4, interact with the virus' glycoproteins gp120 and gp41.
2. The encapsulated genetic material of the virus enters the cell.
3. The viral capsule, containing two viral RNA strands and three replication enzymes (integrase, protease, and reverse transcriptase), is uncoated.
4. Reverse transcriptase begins with the transcription of the viral RNA into a DNA double helix. Next, integrase cleaves a portion off of the 3' end of each strand of DNA, making it "sticky" and integrates it into the host cell's genome.
5. The machinery of the cell is used to multiply the viral genome: cell activation induces transcription of the DNA into messenger RNA.
6. The messenger RNA initiates the production of viral proteins which will be used to envelop new virus particles. In order to create an infectious virus, some of these proteins are cleaved into smaller core proteins by the viral protease.
7. The new virions are assembled: two RNA strands and three replication enzymes are surrounded by core proteins.

8. As they bud from the cell, new virions are encapsulated in host and viral proteins. Over time, this process eventually results in the death of the host cell through apoptosis (programmed cell death).

It is unfortunate that HIV uses the antigen-recognizing receptors of and targets the cells that are meant to fight infection. Whereas a healthy adult has a concentration within the range of 800-1200 $CD4^+$ T cells/μL plasma, this level is substantially decreased over several years as a result of HIV infection, the progression of which can be categorized by three phases. (1) During the initial phase, in the weeks following infection, patients develop a high viral load and flu-like symptoms. Their $CD4^+$ T cell concentrations drop and subsequently rise again, causing the viral load to decrease and the symptoms to disappear. (2) In the second phase of the disease, which can last for an average of ten years, patients generally remain asymptomatic. However, the virus continues to replicate and $CD4^+$ T cell levels progressively decline. (3) A patient enters the final phase of infection once the $CD4^+$ T cell concentration drops below 200 cells/μL, which is the definition of AIDS, and opportunistic infections become more common.

Throughout the first and second phases of infection, HIV-infected patients have a strong immune response. Nevertheless, as well as appropriating the immune cell's own machinery, the virus poses further challenges for the immune system, rendering it nearly impossible to eliminate: (1) the viral genome is encapsulated within the cell's DNA for the life of the cell and can therefore not be eliminated without killing the cell, and (2) each replication of the virus results in mutational changes which may allow the virus to escape immune surveillance. Both characteristics also pose problems for drug development and treatment.

Despite these challenges, certain drugs have been made available to interfere with four of the key steps in viral replication: fusion inhibitors which prevent viral entry into the host cell; nucleoside (nRTI) and non-nucleoside (nnRTI) reverse

transciptase inhibitors; integrase inhibitors which prevent the integration of the viral genome into a cell's DNA; and protease inhibitors (PI) which prevent the cleavage of core proteins causing uninfective virions to bud from the cell. When they were first used in monotherapy treatment, nucleoside reverse transcriptase and protease inhibitors were found to have limited benefits. Due to the rapid rate of random mutation of the virus, it was (and remains) extremely likely that a strain less vulnerable to the effects of these drugs would emerge in patients on monotherapy. Therefore, although a patient's viral load would decline for a period of time, pre-treatment levels could once again be observed within weeks or months because of drug resistance to any one drug. The development of different classes of drugs made combination therapy, the simultaneous usage of drugs from multiple classes, possible. Currently, it is recommended that this multidrug therapy, called highly active antiretroviral therapy (HAART), consist of two nRTIs and one nnRTI or one PI, and should commence in patients with symptomatic HIV or a $CD4^+$ T cell count below 350 cells/μL plasma (HAMMER *et al.* 2008).

For several reasons, this current line of HIV medications has been found wanting. First, with the annual price ranging from US$12,000 - 21,000 per person in the United States, US$22,110 in Switzerland, US$12,813 - 14,587 in Canada, and US$675 - 1622 in South Africa (FANG *et al.* 2007), these costly medications place a huge financial burden on individuals and governments alike and may not be sustainable on a global scale (RICHMAN *et al.* 2009). Happily, new initiatives are seeking to make certain generic HIV medications more affordable at $210 per patient per annum in low- to middle-income countries (KAISER DAILY HIV/AIDS REPORT 2009). Second, although the toxicity of new HAART medications is very low, prolonged treatment may result in an accumulation of toxicity. In the medical community, there is concern about the increased rates of heart disease, diabetes, liver disease, and cancers in treatment-receiving HIV-positive

individuals (RICHMAN *et al.* 2009). Third, patients can suffer from several side effects such as anxiety, fatigue, lipodystrophy, diarrhea, nausea, anorexia, insomnia (AMMASSARI *et al.* 2001), and depression (AMMASSARI *et al.* 2004) which have been linked to adherence rates substantially lower than the 95% required for optimal treatment. Fourth, drug resistance has developed in many patients and is a constant concern. Therefore, as a therapy to be used alongside a drug treatment regimen, the use of antioxidants has been proposed to potentially alleviate some concerns associated with HIV-infection and drug therapy.

1.3 Antioxidant supplementation as HIV therapy: Clinical studies

In examining possible therapeutic regimens for HIV-positive individuals, various mechanisms have been and continue to be studied by researchers from a wide range of disciplines. As we have seen, the pharmaceutical side is of particular interest. In this section, a potential therapy that has seemingly received less attention is briefly explored: the use of antioxidant supplementation. The relevant immunology is left for the following chapter.

It has been elucidated that HIV-positive persons suffer from micronutrient deficiencies (STEPHENSON *et al.* 2006; MILLER 2003) which are caused by a combination of decreased nutrient intake, gastrointestinal malabsorption, increased nutritional requirements, and psychosocial factors (CARBONNEL *et al.* 1997; MILLER 2003). Extensive reviews of observational studies and intervention trials of such nutritional shortfalls in HIV-positive individuals not receiving HAART reveal that low serum concentrations of micronutrients such as thiamine, selenium, zinc, and vitamins A, B-3, B-6, B-12, C, D, and E have been independently linked to a weakened immune system and a higher risk of the following: vertical (mother-to-

child) transmission (FAWZI and HUNTER 1998), faster disease progression (FAWZI *et al.* 2005), low $CD4^+$ T cell counts, HIV-related diseases, and mortality (DRAIN *et al.* 2007). Intervention trials have shown that such persons can benefit from micronutrient supplementation (JIAMTON *et al.* 2003; DRAIN *et al.* 2007; MEHTA and FAWZI 2007).

Among their other benefits, certain micronutrients have antioxidant properties: carotenoids and vitamins A, C, and E (GROPPER *et al.* 2009). As such they remove potentially harmful reactive oxygen species (ROS) from the system. Heightened serum concentrations of ROS (GIL *et al.* 2003; ISRAËL and GOUGEROT-POCIDALO 1997; LI and KARIN 1999; SCHWARZ 1996), called oxidative stress, and lowered antioxidant concentrations (STEPHENSON *et al.* 2006) have been attributed to HIV infection. Since elevated ROS levels have been linked to more rapid HIV progression (BARUCHEL and WAINBERG 1992; SCHWARZ 1996), antioxidant supplementation has been suggested (GARLAND and FAWZI 1999; GIL *et al.* 2003) and studied (ALLARD *et al.* 1998; JARUGA *et al.* 2002; DE SOUZA JR. *et al.* 2005; DRAIN *et al.* 2007) as a potential, though contested, HIV therapy. Representative intervention trials are discussed below. In addition, a summary of the 2002 Jaruga *et al.* study can be found in Chapter 2.

Allard *el al.* find vitamin C and E supplementation to be a beneficial HIV therapy: The 1998 randomized, double-blind placebo-controlled trial by Allard *et al.* (1998) studied 49 stable HIV-positive patients who were on any combination of drug therapy. Participants were placed on a control diet two weeks prior to and for the duration of the study. A random assignment was made to one of two groups: the first received daily supplements of 800 IU of vitamin E and 1000 mg of vitamin C (two tablets); the second received matching placebo tablets. The characteristics of both groups were measured at the start of the study, finding no significant differences between them with respect to demographics, diet, drug therapy, CD4 count, and plasma HIV viral load. After three

months of supplementation, a significant drop in plasma viral load was observed in the group receiving vitamins, while the placebo group saw their viral loads rise. The authors concluded by emphasizing the benefits of vitamin supplements in viral load and oxidative stress reduction; indicating that although there was no difference observed between the groups regarding HIV-associated infections, this ought to be further studied; and noting the potential benefits of supplementation in the developing world (ALLARD *et al.* 1998).

de Souza *et al.* report limited benefits of vitamin E supplementation during HIV infection: With the aim of investigating the potential restoration capabilities of vitamin E on lymphocytes, de Souza *et al.* (2005) conducted a double-blind study on 29 HIV-positive, antiretroviral-receiving individuals in which patients were divided into two groups: the control group received a placebo, while the study group received 800 mg of daily vitamin E supplementation for six months. It was found that supplementation increased lymphocyte viability, but no significant differences in the $CD4^+$ T cell count, $CD8^+$ T cell count, $CD4^+/CD8^+$ ratio or viral load markers were observed (DE SOUZA JR. *et al.* 2005).

Concerns regarding antioxidant supplementation: It has been suggested that antioxidant supplementation may not be beneficial in all HIV-positive individuals (DRAIN *et al.* 2007). For example, although vitamin A supplementation reduced two-year mortality in infants who became HIV-infected in the late intrauterine or early postnatal period, it increased two-year mortality in infants infected more than six weeks after birth (HUMPHREY *et al.* 2006). The administration of vitamin A supplements to women has been implicated in increased vaginal viral shedding (no effect on risk was observed from vitamins B, C, and E) (FAWZI *et al.* 2004) and a heightened risk of mother-to-child HIV transmission (FAWZI *et al.* 2002). In addition, high doses of vitamin C supplementation have been shown to reduce the bioavailability of the protease inhibitor indinavir (SLAIN

et al. 2005). These findings, among others, undoubtedly necessitate concern, and have led authors to question the benefits of universal vitamin A supplementation for women in HIV-endemic areas (FAWZI *et al.* 2004; HUMPHREY *et al.* 2006). Despite these concerns, Fawzi *et al.* (2004) maintain that prenatal supplementation of vitamins B, C, and E should be continued due to their many reported positive effects on maternal and fetal health.

In summary, studies have shown a range of potential implications of antioxidant supplementation. Some have found reasons for concern, others have shown negligible effects, and still others have been very positive about the potential of antioxidant supplementation as a therapy for HIV-infected persons. Despite this range of opinions, the 2007 review by Drain *et al.* maintains that supplementation in persons not receiving HAART is clearly beneficial; however, there are not sufficient data to indicate whether the same can be said for persons receiving HAART.

Surrounding the treatment of HIV, there are still many things that need to be further studied. However, due to the nature of this virus and the possible ethical limitations of clinical studies that could arise, the potential for theoretical work is significant. In the next sections, mathematical modelling techniques are introduced and some basic HIV models are examined.

1.4 A brief review of early mathematical models

In the years following the discovery of HIV, research focusing on immunological and epidemiological aspects of the virus took off in a multitude of directions. By the late 1980s, early mathematical models were being constructed and analysed even before the required parameter estimates were available. Nevertheless, pertinent qualitative information could be gleaned from these simple models. In

particular, in 1989, Anderson and May used a mathematical model to examine whether the assumption that the infectivity of an HIV-positive individual remained constant throughout his or her lifetime was reasonable. It was found that this may not be the case. Seeking to utilize their mathematical tools to their greatest potential, Anderson and May underlined the importance of both an improved understanding of the immune system and reliable parameter measurements (ANDERSON and MAY 1989). Over the next two decades, mathematical models proved to be an indispensable tool, the results of just a few of which are detailed below. First, however, it is useful to review a very simple mathematical model of HIV infection.

The basic, in-host HIV model as presented and expanded upon in, for example, Nowak and Bangham (1996), Perelson *et al.* (1996), and Bonhoeffer *et al.* (1997) involves three populations: uninfected cells (x), infected cells (y), and free infectious virus particles, called virions (v):

$$\begin{aligned} \dot{x} &= \lambda - d_x x - \beta x v \\ \dot{y} &= \beta x v - d_y y \\ \dot{v} &= k y - c v, \end{aligned}$$

where λ is the rate of generation and d_x is the death rate of uninfected cells, d_y is the death rate of infected cells, k is the rate at which infected cells produce virions, and c is the viral clearance rate. The mass action β term simply denotes the rate at which uninfected cells become infected, accounting for the probability of a virion finding an uninfected cell, the rate of entry, and the probability of successful infection. As a system of ordinary differential equations, this model assumes that the interactions between the populations occur in a well-mixed compartment, such as the bloodstream. In so doing, spatial dynamics such as those that would occur in tissues are ignored, as is the stochastic variability between in-

fected individuals. Nevertheless, much information can be gleaned from a model such as this. For example:

Disease-free equilibrium: In the absence of infection, the system will reach the disease free equilibrium: $\hat{x} = \lambda/d_x$, $\hat{y} = 0$, and $\hat{v} = 0$.

Internal equilibrium: Infection will cause, in the long run, the internal equilibrium to be reached with $x^* = \frac{cd_y}{\beta k}$, $y^* = \frac{\lambda}{d_y} - \frac{cd_x}{\beta k}$, and $v^* = \frac{\lambda k}{cd_y} - \frac{d_x}{\beta}$.

Basic reproduction ratio: The basic reproduction ratio, which is simply the number of new infected cells caused by one infected cell immediately after the initial infection, can be determined as follows: (1) the total number of infectious virions produced by each infected cell is k/d_y, where $1/d_y$ is the average lifetime of infected cells, and (2) the probability of each virion infecting a cell is $\frac{\beta\hat{x}}{c}$, where, on average, each virion lives for $1/c$ days. Multiplication of these probabilities yields, $R_0 = \frac{\beta\hat{x}k}{cd_y}$. When $R_0 < 1$, each infected cell, on average, results in less than one infection. Therefore, the infection is eliminated. On the other hand, when $R_0 > 1$, the infection persists. This can also be observed in the above model when the internal equilibrium is re-written as $x^* = \frac{1}{R_0}\frac{\lambda}{d_x}$, $y^* = \frac{cd_x}{\beta k}(R_0 - 1)$, and $v^* = \frac{d_x}{\beta}(R_0 - 1)$.

In what follows it will be shown through a review of certain papers, that models extended from this very simple model have been especially useful in (1) predicting various aspects of in-host HIV infection dynamics, (2) furthering our understanding of HIV progression, i.e., rates of virion replication and $CD4^+$ T cell turnover, and (3) the design of drug therapies.

In 1993, Perelson *et al.* used a system of ordinary differential equations to model the in-host dynamics of an HIV-infected individual. Amongst their findings were three key results. First, the model suggested that there exists a critical level of infectious virion production per infected cell, N_{crit}; a production rate below this level would lead to the stability of the uninfected state. Second, for certain parameter values, a Hopf bifurcation resulting in oscillatory fluctuations

may be observed. Similar dynamics have also been found in Anderson and May (1989) and were alluded to above. Third, similarities between the time courses of the virions, latently infected T cells, and actively infected T cells led to quasi-steady-state analysis. To make the model as simple as possible, but no simpler (EINSTEIN 1934), Perelson *et al.* used this quasi-equilibrium to convert the system of four ODEs into one of two ODEs, explicitly measuring uninfected and latently infected $CD4^+$ T cell populations. They found that, although there was a noticeable decrease in the time needed to reach the steady state, the simpler system shared similar features with – and was therefore a good summary of – the full model (PERELSON *et al.* 1993).

Despite the vast quantities of papers published relating to HIV (over 55,000 articles published between 1981-1994), it was not until the back-to-back 1995 *Nature* publications by Ho *et al.* and Wei *et al.* that the rapidity of viral turnover was quantified. It was known that during the asymptomatic stage of the disease, a balance between virus production and clearance may be observed. Using this information, study participants were treated with a protease inhibitor (HO *et al.* 1995) or a reverse transcriptase inhibitor (WEI *et al.* 1995). The resulting, almost immediate, inhibition of infectious virion production, and the ability to actually quantify a patient's plasma viral load, led to the conclusion that virions in the plasma have a half-life of approximately 2.1 days (HO *et al.* 1995; WEI *et al.* 1995). This implies that the majority of circulating plasma virions are the result of a continuous cycle of new lymphocyte infection, viral replication, and cell turnover (WEI *et al.* 1995). Therefore, Ho *et al.* suggested that while this rapid lymphocyte regeneration may simply be the result of an activated immune system, it cannot last forever. Therefore, instead of aiming to continuously replenish $CD4^+$ T cells, treatment should prevent their destruction (HO *et al.* 1995).

Eight years earlier, to the excitement of the medical and HIV communities, the first drug, nucleoside reverse transcriptase inhibitor azidothymidine (AZT), had

been FDA-approved for HIV therapy and had resulted in a slower progression of the virus to AIDS amongst HIV-positive individuals. Over time, and after the approval of a few other drugs, it became apparent that monotherapy was not the solution for which all had hoped. The publication of the aforementioned 1995 papers helped to solidify the conclusion in a 1996 paper by Perelson *et al.* that these observations were inevitably the result of the rapid viral mutation rate. In addition, the conclusion by certain studies that a combination of AZT and either zalcitabine (ddC) or didanosine (ddI) (both nRTIs) delayed resistance to AZT (ANTONELLI *et al.* 1994; JABLONOWSKI 1995), led to the suggestion that potent antiretrovirals targeting different aspects of viral replication be used in concert (PERELSON *et al.* 1996). To arrive at these conclusions, the basic model presented above was modified to incorporate drug treatment:

$$\begin{aligned} \dot{x} &= \lambda - d_x x - (1-\epsilon)\beta x v \\ \dot{y} &= (1-\epsilon)\beta x v - d_y y \\ \dot{v} &= k y - c v, \end{aligned}$$

where ϵ is the effectiveness of the treatment. This multiplication of β by a factor which is dependent upon drug effectiveness continues to be used in different types of models (PERELSON and NELSON 1999; HEFFERNAN and WAHL 2005; RONG *et al.* 2007; KHALILI and ARMAOU 2008). Furthermore, the model presented by Perelson *et al.* (1996) was used to determine average values of other important parameters: the viral life-span, the number of virions produced per day, and the HIV-1 generation time.

That same year, a paper by de Jong *et al.* discussed the results of a clinical study in which patients were prescribed AZT therapy. Treatment resulted in an initial decrease in HIV viral load, but within one to three months, the concentration of wild-type virus had rebounded independent of drug resistance. An identical result

was observed in mathematical modelling, which showed that it was the result of an ever-increasing target cell concentration and an incomplete suppression of viral replication. In the absence of a drug-resistant mutation, the system stabilizes at a new equilibrium slightly above pretreatment levels, whereas mutation would result in a return to these levels (DE JONG *et al.* 1996).

Further evidence of the significance of mathematical models to HIV research is provided in the highly-cited 1997 paper by Finzi *et al.*, which identified in-host HIV-1 reservoirs and cautioned against the termination of treatment in patients with no evidence of residual virus. Finzi *et al.* cite the 1997 mathematical paper by Perelson *et al.* as making the "first rational predictions of treatment times required for virus eradication." The Perelson *et al.* paper also receives credit for making note of the possibility of small, undetectable viral compartments, i.e., in the brain, which decay slowly (FINZI *et al.* 1997) and which directly affect the possibility of viral eradication from an infected person (PERELSON *et al.* 1997). Notably for the work presented in the following chapter, the paper finds evidence that, although only 2% of the target cells reside in the plasma and the remainder of infection occurs within the immune system organs (HO *et al.* 1995), the infected cells in the bloodstream are representative of the population throughout the body (PERELSON *et al.* 1997; HAASE *et al.* 1996). This is likely due to the fact that the concentrations of these cells in the plasma are proportional to those in the tissues. In addition, the paper determines a quantitative estimate of the half-lives of long-lived infected cells and of latently infected lymphocytes, along with the respective pool sizes of the two populations. It also estimates the length of treatment given a 100%-effective treatment regimen (PERELSON *et al.* 1997).

Thus far, only papers dealing with ordinary differential equations have been examined. While these models are sufficient for certain types of problems, they neglect certain temporal features, spatial patterns, and interpatient variability

that may be relevant to other problems. To address these aspects, delay differential equations, partial differential equations, and stochastic systems may be used.

First, to account for the time it takes between viral infection of a cell and the production of new virus particles, models using delay differential equations (DDEs) have been used in several papers (for example: Herz *et al.* (1996), Mittler *et al.* (1998), Culshaw and Shigui (2000), and Nelson and Perelson (2002)). They have been instrumental in estimating the half-life of free virions and the turnover rate of productively infected cells during treatment with PIs or RTIs (HERZ *et al.* 1996; NELSON and PERELSON 2002). DDEs have also been useful in the examination of delay induced oscillations (CULSHAW and SHIGUI 2000). This delay is of utmost importance when timing is of the essence, as in the cases indicated above, but may be neglected for many other features of viral dynamics (HERZ *et al.* 1996).

Second, systems of partial differential equations (PDEs) have been used to address temporal and spatial patterns of the cell. For example, PDEs may be used in problems which include varying virion production and cell death rates in the model, both of which depend upon the length of time that a cell has been infected (KIRSCHNER 1996; NELSON *et al.* 2004). PDEs have also been used by way of a finite element scheme to simulate spatial virion flow, T-cell interactions, and local HIV progression (GRAZIANO *et al.* 2008).

Third, where variability between patients is vital, stochastic models may be employed. Three examples are: (1) Using stochastic modelling, Tuckwell and Le Corfec (1998) investigated the early stages of HIV infection and obtained a distribution of the time required to reach a certain viral density. The latter could be useful for determining the probability of viral detection at a given point in time. (2) Heffernan and Wahl (2005) used Monte Carlo methods to estimate the natural

variability in T cell concentrations and viral load, and to determine, as a function of the number of virions to which an individual is initially exposed, the probability that this exposure becomes an established infection. (3) Khalili and Armaou (2008) examined optimal treatment strategies in early infection by predicting the response of the average patient using stochastic modelling techniques.

The drawback of these three classes of models is their complexity. In order to arrive at robust conclusions based on any mathematical model, it is crucial to keep the model as simple as possible, while retaining only those biological features that are relevant to the question at hand. To this end, a fairly simple mathematical model is presented in Chapter 2 with the aim of examining antioxidant supplementation as a potential therapy for HIV-infected patients. This is the first mathematical approach that has been applied to this intriguing question.

1.5 Scope of thesis

In this chapter, antioxidant supplementation in HIV patients has been introduced as a complementary therapy to current pharmaceutical lines of treatment. The need for more research in this area, especially for HIV-infected persons on HAART, has been outlined. The remainder of this thesis is therefore devoted to the development of a mathematical model which provides theoretical insight and reconciles the results of conflicting intervention trials. Written in manuscript style, the chapter in which this model is developed, Chapter 2, is completely self-contained and is organized as follows.

In Section 2.1, the relevant immunology is discussed. In particular, the reactive oxygen species pathway is reviewed, as is the mechanism whereby increased ROS concentrations yield increased viral replication. A dampening of this replication rate is shown to result from elevated concentrations of antioxidants. Furthermore,

a specific intervention trial, described by Jaruga *et al.* (2002) and showing the benefits of antioxidant supplementation, is introduced.

In Section 2.2, a novel in-host HIV model is developed for antioxidant supplementation which incorporates populations of uninfected cells, infected cells, antioxidants, and reactive oxygen species. In addition, the infectivity rate, β, is defined to be a function of a dynamic variable, which is different from the usual approach employed by other models.

The analytical results regarding the disease free equilibrium are presented in Section 2.3.

In Section 2.4, estimates of the parameter values are either obtained directly from the literature or are computed using the equilibrium concentrations reported in the Jaruga *et al.* study (2002).

In Section 2.5, the numerical results of the model are presented and most are shown to agree well with clinical results. One set of clinically measured equilibrium values, in the presence of antioxidant supplementation, cannot be obtained without entering a region of instability. Focusing on the region of instability, a maximum stable level of supplementation is found.

This maximum level is further investigated in Section 2.6, through sensitivity analysis. In this section, several parameters, for which there are high degrees of uncertainty, are varied. Furthermore, interpatient variability is examined.

A discussion of the results of Sections 2.5 and 2.6 can be found in Section 2.7 and the chapter is concluded.

Chapter 3 concludes this thesis and suggests directions for future work.

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CHAPTER 2

Reconciling conflicting clinical studies of antioxidant supplementation as HIV therapy: a mathematical approach

2.1 Introduction

Reactive oxygen species (ROS) are highly reactive byproducts of cellular respiration. As second messengers, they play an important role in cell signaling and in gene regulation (e.g., cytokine, growth factor, and hormone action and secretion; ion transport; transcription; neuromodulation; and apoptosis) (GLOIRE *et al.* 2006; LANDER 1997). ROS are also important for the normal function of the immune system; T cells both are influenced by and influence intracellular ROS levels. In particular, ROS play a positive role in the proliferation of T cells and immunological defence (GLOIRE *et al.* 2006; LANDER 1997; DEVADAS *et al.* 2002; HILDEMAN 2004; GROPPER *et al.* 2009).

A variety of reactive oxygen species are produced throughout the body. One particular species of interest, superoxide (O_2^-), is generated in two ways and for different reasons (GROPPER *et al.* 2009): (1) as an accidental result of incomplete electron transfers in the electron transport chain and (2) in activated white

blood cells with the purpose of destroying pathogens. Moreover, upon production, these O_2^- molecules are rapidly metabolised into hydrogen peroxide (H_2O_2), a mild oxidant, which further helps to destroy some pathogens. Intermediate concentrations of H_2O_2 (and certain other ROS) result in the activation of nuclear factor κB (NF-κB), a transcription factor that upregulates several cellular processes, including cell proliferation and apoptosis (GIL *et al.* 2003; GLOIRE *et al.* 2006; KHAN and WILSON 1995).

Despite their positive role, reactive oxygen species can be harmful. At normal ROS concentrations, cell function and structure are protected from destructive interactions with ROS by various defence mechanisms. These include the use of both enzymatic and nonenzymatic antioxidants, substances that significantly delay or prevent the oxidation of a given substrate. Non-enzymatic antioxidants obtained directly from the diet (i.e., glutathione, vitamins A, C and E, and flavenoids) decrease oxygen concentrations, remove catalytic metal ions and eliminate radicals from the system (GUTTERIDGE 1994; MARTINDALE and HOLBROOK 2002). Enzymatic antioxidants remove ROS from the system and are not consumed by the reaction. These enzymes, such as superoxide dismutases, catalase and glutathione peroxide, are naturally produced by the body (MARTINDALE and HOLBROOK 2002); oral supplements and injections are also available (MARTINDALE and HOLBROOK 2002). In addition, antioxidants repair oxidative damage, eliminate damaged molecules and prevent mutations from occurring (GUTTERIDGE 1994).

In the event that intracellular ROS levels increase moderately, cells respond by boosting antioxidant levels and by promoting proinflammatory gene expression (LI and KARIN 1999; SEN *et al.* 2000). There are two main functions of the resulting translated proteins: (1) signaling proteins activate the immune system by various cytokines, growth factors and chemokines, and (2) enzymes improve a cell's response to inflammatory, growth-stimulatory and apoptotic signals (SEN

et al. 2000). When ROS levels exceed a cell's antioxidant capacity, oxidative stress is reached; this has the potential to cause significant damage to DNA, proteins, and lipids and can induce apoptosis. In addition, conditions favourable for the pathogenesis of several diseases may be created (GLOIRE *et al.* 2006). Such high levels of ROS are generally the result of chronic and acute inflammatory diseases or environmental stress (LI and KARIN 1999).

Individuals infected by the human immunodeficiency virus (HIV) exhibit heightened serum concentrations of ROS (GIL *et al.* 2003; ISRAËL and GOUGEROT-POCIDALO 1997; LI and KARIN 1999; SCHWARZ 1996) and lowered antioxidant concentrations (STEPHENSON *et al.* 2006). The resulting oxidative stress affects disease progression in several ways. First, oxidative damage to $CD4^+$ T cells may impair the immune system's response to HIV (STEPHENSON *et al.* 2005). Second, the well-known hallmark of HIV, the depletion of $CD4^+$ T cell concentration in the plasma, is further exacerbated by oxidative stress-induced apoptosis. Third, increased HIV transcription leading to faster disease progression results from an increased activation of NF-κB (GIL *et al.* 2003). It has been found that while NF-κB activation is not absolutely necessary for viral replication, it accelerates the process 20-fold (HISCOTT *et al.* 2001; STEPHENSON *et al.* 2005; CHEN *et al.* 1997). Moreover, it has been suggested that NF-κB is itself activated by HIV (HISCOTT *et al.* 2001). It has been shown that this activation of NF-κB is inhibited by antioxidants (such as N-acetyl cysteine and pyrrolidine dithiocarbarnate) (KHAN and WILSON 1995).

Thus, the precise pathways by which ROS exacerbate HIV infection remain unclear, as does the mechanism by which antioxidants prevent exaggerated HIV transcription and T cell apoptosis. Although certain papers report negligible effects and advise that caution be exercised (see Bowie and ONeill (2002), Drain et al. (2007), and Israël and Gougerot-Pocidalo (1997)), select clinical studies have clearly demonstrated the benefits of antioxidant therapy for HIV-positive

patients (see Gil et al. (2003), Allard et al. (1998), Braunstein (2006), Ezimah et al. (2008), Jiamton et al. (2003), and Opara et al. (2007)). In particular, a clinical study conducted by Jaruga *et al.* (2002) demonstrated a clear benefit for antioxidant therapy in injection drug users when compared with the appropriate control group. In this study, samples were collected from a control group of 10 healthy volunteers, a group of 15 HIV-negative injection drug users (denoted HIV(-)) and a group of 30 asymptomatic HIV-positive injection drug users (denoted HIV(+)). The latter HIV-positive group was divided into two subgroups: one subgroup of 15 patients received a placebo (HIV(+)P), while the other received a daily supplement of 5000 units of vitamin A, 100 units of vitamin E and 50 mg of vitamin C (HIV(+)V). After six consecutive months of treatment, it was found that patients in groups HIV(-) and HIV(+)P had significantly lower blood plasma concentrations of vitamins A, C and E than the control group, while individuals in the HIV(+)V group had levels characteristic of the control group. In addition, while there was a lack of statistical significance, the $CD4^+$ T cell count for HIV(+)V individuals was 100 cells/μL higher than for those receiving a placebo. In conclusion, the authors of the study reaffirm that the combination of infection with HIV and lifestyle factors typical of injection drug users (for example, a diet which is not rich in antioxidants) may lead to oxidative stress, a potential factor in AIDS development.

In the sections which follow, a mathematical model is developed to investigate the use of antioxidants as a treatment strategy for HIV. We use clinical data from Jaruga *et al.* to estimate parameter values for both control and HIV(+) cases, and then test in detail the results of varying the level of antioxidant supplementation in the HIV(+)V group, largely through numerical bifurcation analysis. We also include an analysis of the sensitivity of our predictions to both parameter estimates and interpatient variability.

Note: Despite the benefits that can be obtained from antioxidant supplementa-

tion, we maintain that the need for accessible and affordable antiretrovirals in developing countries is of utmost importance and must not be neglected.

2.2 The model

As outlined in the Introduction, HIV-infected CD4$^+$ T cells can produce HIV virions via two ROS-independent pathways: either directly or through the activation of NF-κB. However, it has been shown that the combined effect of these pathways accounts for a mere one-twentieth of the total virion production (CHEN *et al.* 1997). The more substantial fraction of virion production has been attributed to ROS-activated NF-κB (CHEN *et al.* 1997). During HIV infection, immune cells (such as macrophages and neutrophils) are also activated, resulting in an increase in ROS generation. Thus, infected cells indirectly produce high levels of ROS, which in turn directly increase the production of virions by infected cells. Antioxidants can control this vicious cycle by reducing ROS concentrations.

To model these processes, we propose a system of differential equations which consists of four populations: uninfected CD4$^+$ T cells (x), infected CD4$^+$ T cells (y), reactive oxygen species (r) and antioxidants (a):

$$\frac{dx}{dt} = \lambda_x - d_x x - \beta(r)(1-\epsilon)xy \tag{2.1}$$

$$\frac{dy}{dt} = \beta(r)(1-\epsilon)xy - d_y y \tag{2.2}$$

$$\frac{dr}{dt} = \lambda_r + ky - mar - h_r r \tag{2.3}$$

$$\frac{da}{dt} = \lambda_a + \alpha - par - h_a a, \tag{2.4}$$

where $\beta(r)$ is a positive, increasing function.

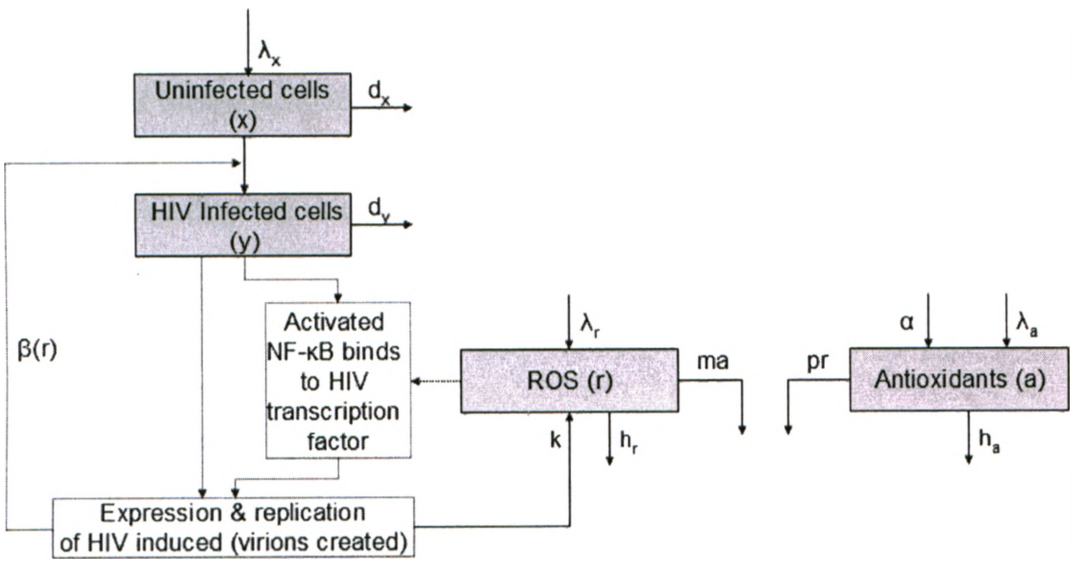

Figure 2.1: Schematic diagram of the model. Reactive oxygen species, while not the sole means for transcription of HIV, directly increase the transcription rate. This results in an increased infection rate $\beta(r)$.

Uninfected CD4$^+$: CD4$^+$ T cells are produced by the thymus at constant rate λ_x, are eliminated from the system at per capita rate d_x and become infected through mass action kinetics at rate $\beta(1-\epsilon)xy$, where the infection rate β is a function of r, described below, and ϵ is the effectiveness of drug therapy.

Infected CD4$^+$: CD4$^+$ T cells become infected at rate $\beta(1-\epsilon)xy$. Infected cells are removed from the system at per capita rate d_y.

Reactive oxygen species: ROS are naturally produced at constant rate λ_r. In the event of infection, ROS are also produced by infected cells at a rate proportional to the number of infected CD4$^+$ T cells, ky. ROS are eliminated from the system by reacting with antioxidants at rate mar and through all other processes, including reactions with NF-κB and other molecules, such as enzymes, at decay rate $h_r r$.

Antioxidants: Antioxidants are introduced into the system via dietary intake at constant rate λ_a. Plasma antioxidant levels may be supplemented therapeutically at constant rate α. Antioxidants have natural decay rate $h_a a$. Since a large fraction of antioxidants are regenerated after reaction with ROS, we define a new rate of antioxidant consumption, par, where p is much smaller than m.

Infectivity: To capture ROS-activated transcription in our model, we would like $\beta(r)$ to be a saturating, increasing function of r. For simplicity, we choose a Michaelis-Menten equation. Therefore, we take

$$\beta(r) = b_0 + \frac{r(b_{\max} - b_0)}{r + r_{\text{half}}}. \tag{2.5}$$

While several other forms of $\beta(r)$ might be equally reasonable, this expression provides a good fit to the (limited) data derived from clinical studies (the "ROS-

absent", $\beta(r^*)$ and $\beta(r^p)$ points described in Section 2.4, and illustrated in Figure 2).

Note: Many standard HIV models also incorporate an explicit virion population. While virions are not directly modelled in our system, the vital role that they play is not neglected: since they are in quasi-equilibrium with the infected cells, the concentration of virions in the system is roughly proportional to that of the infected cells (NOWAK and MAY 2000; PERELSON *et al.* 1993).

2.3 Analytical Results

Evaluating for the equilibria yields one biologically meaningful disease-free equilibrium:

$$\begin{aligned} x^d &= \frac{\lambda_x}{d_x} \\ y^d &= 0 \\ r^d &= (-B + \sqrt{B^2 + 4ph_a\lambda_r h_r})/2h_r p \\ a^d &= (\lambda_r - h_r r^d)/mr^d \end{aligned}$$

where $B = \lambda_a m + \alpha m + h_r h_a - \lambda_r p$. Thus, when $\lambda_r > h_r r^d$, or whenever the production rate of ROS exceeds their overall removal rate, an HIV-negative individual will exhibit a balanced ROS-antioxidant equilibrium.

Using the next generation matrix method from (VAN DEN DRIESSCHE and WATMOUGH 2002), we find the basic reproductive ratio to be

$$R_0 = \frac{\beta(r^d)(1-\epsilon)\lambda_x}{d_x d_y}, \tag{2.6}$$

which makes intuitive sense since a single infected cell at the uninfected equilib-

rium will produce new infected cells at rate $\beta(r^d)(1-\epsilon)x^d$, for mean lifetime $1/d_y$. (We note that in practice, ϵ is almost always zero in this situation.)

We next examine stability of the disease-free equilibrium using the following Jacobian:

$$\begin{pmatrix} -d_x & -R_0 & 0 & 0 \\ 0 & d_y(R_0-1) & 0 & 0 \\ 0 & k & \frac{-\lambda_r}{r^d} & -mr^d \\ 0 & 0 & -\frac{p(\lambda_r - h_r r^d)}{mr^d} & -h_a - pr^d \end{pmatrix}$$

This yields four eigenvalues

$$-d_x < 0, \tag{2.7}$$

$$d_y(R_0 - 1) \text{ and} \tag{2.8}$$

$$-\frac{p(r^d)^2 + \lambda_r + h_a r^d \pm \sqrt{(p(r^d)^2 + \lambda_r + h_a r^d)^2 - 4r^d(\lambda_r h_a + (r^d)^2 h_r p)}}{2r^d} < 0. \tag{2.9}$$

Therefore, the disease-free equilibrium is stable when $R_0 < 1$ (from (2.8)).

In addition to the disease-free equilibrium, two biologically meaningful internal equilibria exist; we omit their analytical expressions here since their complicated form offers little insight. Instead, following parameter estimation, we complete a bifurcation analysis of all three biologically meaningful equilibria in Section 2.5. We note that our model, and the analytical results described up to this point, could be generalized to other factors that are produced in proportion to infected T cells (ky term), increase the in-host transmission rate ($\beta(r)$ term) and can be counteracted through mass action kinetics by some exogenous factor (mar term). However in the next section we estimate parameters specific to ROS and antioxidants, and further numerical results are thus specific to this case.

2.4 Parameter Estimation

Developing reasonable (if uncertain) parameter estimates is one of the most difficult aspects of theoretical immunology, and yet can be an extremely worthwhile endeavor (SMITH? 2008). In the Tables and subsections which follow, we describe our estimates for both control (HIV(-)) and HIV-positive parameters. We examine the sensitivity of our main results to these estimates in Section 6.

The model described above includes a total of four populations and 15 parameters. Estimates of six of these parameters (λ_x, d_x, d_y, λ_a, h_a and ϵ) were directly obtained from the literature and can be found in Table 2.1. We use R_0 and the seven clinically measured equilibrium levels from Table 2.3 to deduce the other parameters (see Table 2.2), except for α, which we will vary to investigate therapy. Throughout this section and the numerical work which follows, we will use units of cells per μL plasma (for x and y) or molecules per μL plasma (for a and r). In estimating parameters related to the population r, we specifically examine the reactive oxygen species hydrogen peroxide as it has been shown to play an important role in the activation of HIV transcription. Moreover, since ascorbic acid (vitamin C) has been cited as a key H_2O_2 scavenging antioxidant (KARYOTOU and DONALDSON 2005), we use it as our antioxidant for the purpose of parameter estimation.

In this section and the work which follows we also refer to four cases of the infected equilibrium, which differ only in their parameter values. Specifically, we denote (1) the uninfected, control diet case with a "hat" (i.e. $\hat{x}$), (2) the uninfected, IDU case with an asterisk (i.e. x^*), (3) the infected, placebo case with a superscript "p" (i.e. x^p) and (4) the infected, vitamin supplementation case with a superscript "v" (i.e. x^v) (see Table 2.3). These populations correspond to the healthy control, HIV(-), HIV(+)P and HIV(+)V groups of Jaruga *et al.*, respectively (JARUGA *et al.* 2002).

Table 2.1: Parameter estimates from the literature.

Parameter	Value	Reference
λ_x	60.76 cells μL^{-1} day^{-1}	(KRAKOVSKA and WAHL 2007)
d_x	0.057 day^{-1}	(RIBEIRO *et al.* 2002)
d_y	1 day^{-1}	(DI MASCIO *et al.* 2004)
$h_r + m\hat{a}$	5.99×10^7 day^{-1}	(half life = 1 ms) (RETH 2002)
$\hat{\lambda_a}$	5.47×10^{13} molecules μL^{-1} day^{-1}	(GROPPER *et al.* 2009)
λ_a	2.74×10^{13} molecules μL^{-1} day^{-1}	estimated from (GROPPER *et al.* 2009)
h_a	0.0347 day^{-1}	(half life = 8 - 40 days, choose 20 days) (HICKEY and ROBERTS 2004)
R_0	4.5	estimated from (NOWAK and MAY 2000)
ϵ	$\frac{1}{3}$	estimated from (MANFREDI and CHIODO 2000)

Table 2.2: Parameter estimates.

Parameter	Value
b_0	0.000211 $(\text{cell}/\mu L)^{-1}$ day^{-1}
$b_{\max}$	0.00621 $(\text{cell}/\mu L)^{-1}$ day^{-1}
r_{half}	3.57×10^{13} molecules μL^{-1}
h_r	1.66×10^7 day^{-1}
λ_r	1.86×10^{21} molecules μL^{-1} day^{-1}
k	1.49×10^{19} molecules $cell^{-1}$ day^{-1}
m	1.27×10^{-6} $(\text{molecule}/\mu L)^{-1}$ day^{-1}
α	variable molecules μL^{-1} day^{-1}
p	5.04×10^{-14} $(\text{molecule}/\mu L)^{-1}$ day^{-1}

2.4.1 Literature estimates for $\hat{\lambda}_a$ and λ_a

It has been recommended that dietary vitamin C intake for all persons exceed 200 mg per day (LEVINE *et al.* 1996). Seventy-eight percent or about 160 mg per day of this amount is absorbed by the approximately 10 L volume of plasma and extracellular space (GRAUMLICH *et al.* 1997). This corresponds to an antioxidant introduction rate in the control group, $\hat{\lambda}_a$, of 5.47×10^{13} molecules μL^{-1} day^{-1} ($\frac{0.16\ g}{10 \cdot 10^6\ \mu L\ day} \times 6.022 \cdot 10^{23}\ \frac{molecules}{mol} \times \frac{1}{176.14}\ \frac{mol}{g}$). In order to account for the fact that injection drug users (IDUs) may have a smaller vitamin C intake, we set the amount of dietary vitamin C absorbed in groups HIV(-), HIV(+)P and HIV(+)V to be 80 mg/day which yields $\lambda_a = 2.74 \times 10^{13}$ molecules μL^{-1} day^{-1}. Both of these estimates have a high degree of uncertainty since the pharmcokinetics and bioavailability of ascorbic acid are complex (GRAUMLICH *et al.* 1997). These parameter values will be examined in the sensitivity analyses to follow.

2.4.2 Finding x^p, y^p, x^v and y^v

The clinical data in Table 2.3 give only the sum of $CD4^+$ T cells, $x^p + y^p$ and $x^v + y^v$. To find each term independently, we combine equation (2.1) and equation (2.2), at equilibrium,

$$\lambda_x - d_x x - d_y y = 0, \tag{2.10}$$

where λ_x, d_x and d_y are known. Thus, for the HIV(+)P case, we find $x^p = 317$ and $y^p = 43$. Likewise, for the HIV(+)V case, we find $x^v = 423$ and $y^v = 37$.

2.4.3 Estimating the function $\beta(r)$ and ϵ

The Jaruga *et al.* study which we use to estimate certain parameters was comprised of HIV-negative individuals and patients on highly active antiretroviral therapy (HAART). Since HAART reduces the rate of infection in an HIV-positive individual, we consider the effectiveness of this therapy in our model, denoted by ϵ. To estimate this parameter, we use the results of a study by Manfredi *et al.* which examined a group of individuals of a similar mean age to those of the Jaruga *et al.* study (33.9 ± 1.6 (MANFREDI and CHIODO 2000) vs 27 ± 9 (JARUGA *et al.* 2002)), the majority of whom were also IDUs (MANFREDI and CHIODO 2000). Twelve months of treatment were shown, on average, to increase these patients' $CD4^+$ T cell counts from 231 ± 87 cells/μL to 345 ± 62 cells/μL, which is approximately the same level as in the HIV(+) groups in Jaruga *et al.*. Using the concentration of $CD4^+$ T cells before and during therapy as a proxy to estimate effectiveness, and assuming that this effectiveness has reached equilibrium after twelve months, we set ϵ to be $1 - \frac{231}{345} \approx \frac{1}{3}$. We note that this overall measure of the effectiveness of therapy includes pharmacological effectiveness, as well as the adherence of the IDU group.

We are ultimately interested in modelling the three IDU populations, HIV(-), HIV(+)P and HIV(+)V. Therefore, we take R_0 to be defined at the HIV(-) case where $\epsilon = 0$. Using (2.6), we find R_0 at this equilibrium to be:

$$R_0 = \frac{\beta(r^*)\lambda_x}{d_x d_y}.$$

Given the parameter values in Table 2.1, this yields $\beta(r^*) = 0.00422$. Since NF-κB activation results in a 20-fold increase in HIV transcription (CHEN *et al.* 1997), we let $\beta(r^*) = b_0 + 19b_0$ and thus $b_0 = 0.000211$. From the disease-free IDU equilibrium we therefore have two points with which to fit the $\beta(r)$ curve, $\beta(r^*)$ and $\beta(0)$. A third point is obtained from the HIV(+)P equilibrium. In this

case, since $y \neq 0$, $d_y = 1$ and $\beta(r)(1-\epsilon) = \frac{1}{x}$ at equilibrium (equation (2.2)), $\beta(r^p) = \frac{1}{x^p(1-\epsilon)} = 0.00473$.

These three points on the $\beta(r)$ curve allow us to fit the two other free parameters, yielding, $b_{\max} = 0.00621$ and $r_{\text{half}} = 3.57 \times 10^{13}$. This fixes the function $\beta(r)$ (see Fig. 2.2) which models the rate of infection in the absence of drugs. A second curve modelling the effect of therapy, $\beta(r)(1-\epsilon)$, can be used to iteratively estimate a further free parameter h_r. The procedure we use is to estimate a value of h_r, then follow through the steps described in subsection 2.4.4. This allows for numerical estimates of four more parameters, and ultimately yields an estimate for r^v, the concentration of ROS at the HIV(+)V equilibrium. We then iteratively adjust our initial estimate of h_r such that $\beta r^v(1-\epsilon) = 1/x^v$ lies along the dashed curve in Figure 2.2. This procedure yields $h_r = 1.66 \times 10^7$ day^{-1}.

2.4.4 Estimating λ_r, p, m and k

Given that $m\hat{a} + h_r = 5.99 \times 10^7$ day^{-1} (Table 2.1), knowing $\hat{a}$ and assuming h_r, we directly compute $m = 1.27 \times 10^{-6}$. In addition, since $y = 0$ at the uninfected equilibrium, equation (2.3) at equilibrium yields

$$\begin{aligned} \lambda_r &= (m\hat{a} + h_r)\hat{r} \\ &= 1.86 \times 10^{21} \text{ molecules } \mu\text{L}^{-1} \text{ day}^{-1}. \end{aligned}$$

We assume that λ_r, the rate at which ROS are naturally produced, is constant for all individuals. In contrast, λ_a represents the dietary influx of antioxidants, and we thus assume that λ_a is constant for the HIV(-) and HIV(+) groups, but may differ for the control group. Therefore, we use a^* and r^* to find λ_a for the

Table 2.3: Equilibrium populations from the literature.

Parameter	Value	Reference
Healthy control group		
$\hat{x}$	1,066 cells/μL	(MOHRI *et al.* 2001)
$\hat{r}$	51.5 ± 4.95 μM	(AL-GAYYAR *et al.* 2007)
$\hat{a}$	56.8 ± 4.5 μM	(JARUGA *et al.* 2002)
HIV(-) (IDU)		
x^*	1,066 cells/μL	(MOHRI *et al.* 2001)
a^*	12.1 ± 1.8 μM	(JARUGA *et al.* 2002)
HIV(+)P (IDU)		
$x^p + y^p$	360 cells/μL	(JARUGA *et al.* 2002)
y^p	43 cells/μL	from equation (2.10)
a^p	8.2 ± 1.8 μM	(JARUGA *et al.* 2002)
HIV(+)V (IDU)		
$x^v + y^v$	460 cells/μL	(JARUGA *et al.* 2002)
y^v	37 cells/μL	from equation (2.10)
a^v	49.0 ± 5.0 μM	(JARUGA *et al.* 2002)

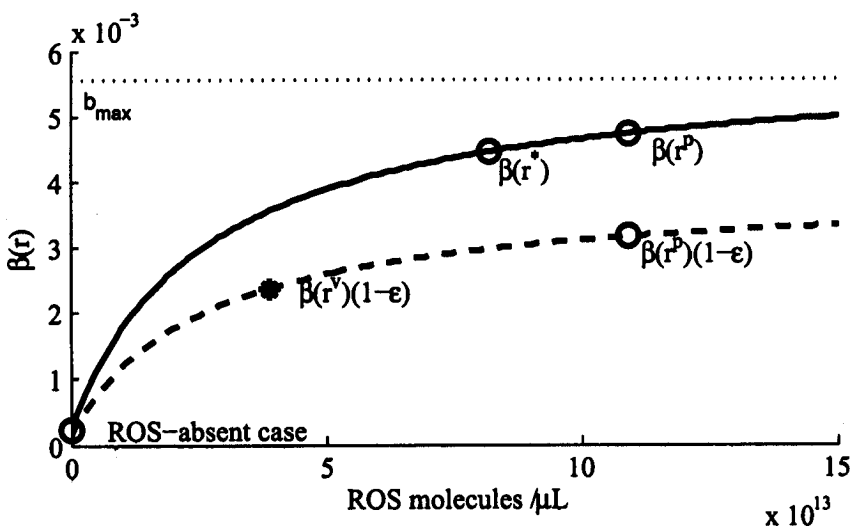

Figure 2.2: The $\beta(r)$ curve, denoted by the solid line, represents the rate of infection in the absence of drug therapy. The dashed line depicts the decrease in the infection rate due to HAART, the $\beta(r)(1-\epsilon)$ curve. The dotted line represents the maximum rate of infection.

IDU groups. Thus, we must first find r^*. Using (2.3) at equilibrium,

$$\begin{aligned} r^* &= \frac{\lambda_r}{ma^* + h_r} \\ &= 7.20 \times 10^{13} \text{ molecules}/\mu\text{L or } 119.56\mu\text{M}. \end{aligned} \tag{2.11}$$

The parameter p, which should be constant for all individuals, is found using (2.4) at the control equilibrium, i.e., for $y = 0$ and $\alpha = 0$:

$$\begin{aligned} p &= \frac{\hat{\lambda}_a - h_a \hat{a}}{\hat{r}\hat{a}} \\ &= 5.04 \times 10^{-14} \text{ (molecules}/\mu\text{L)}^{-1} \text{ day}^{-1}. \end{aligned}$$

From equation (2.4) at the HIV(+)P equilibrium,

$$\begin{aligned} r^p &= \frac{\lambda_a - h_a a^p}{ma^p} \\ &= 1.09 \times 10^{14} \text{ molecules}/\mu\text{L or } 181.00\mu\text{M}. \end{aligned} \tag{2.12}$$

We find our final parameter, k, from equation (2.3) at the HIV(+)P equilibrium:

$$\begin{aligned} k &= \frac{mr^p a^p + h_r r^p - \lambda_r}{y^p} \\ &= 1.49 \times 10^{19} \text{ molecules cell}^{-1} \text{ day}^{-1}. \end{aligned}$$

Finally, from equation (2.3) at the HIV(+)V equilibrium:

$$\begin{aligned} r^v &= \frac{\lambda_r + ky^v}{ma^v + h_r} \\ &= 4.45 \times 10^{13} \text{ molecules}/\mu\text{L or } 73.89\mu\text{M}. \end{aligned} \tag{2.13}$$

2.5 Numerical Results

Using the parameters in Tables 2.1 and 2.2, the equilibria of our model were found analytically. At these parameter values and antioxidant supplementation levels, only one biologically meaningful internal equilibrium exists, and this equilibrium agrees well with the $CD4^+$ T cell and antioxidant concentrations in Jaruga *et al.*, as illustrated in Figure 2.3. In the first two columns, we compare the control individuals with the HIV-negative IDUs whose lifestyle, including a poorer diet, is a closer control to the HIV-positive IDUs in the Jaruga *et al.* study. As expected, a significant increase in ROS and decrease in antioxidant concentrations is observed in the HIV(-) group. Furthermore, in the presence of HIV infection and absence of antioxidant treatment these trends continue – the concentrations of ROS and antioxidants further increase and decrease, respectively, in the HIV(+)P group. This is combined with a sizable drop in the total $CD4^+$ T cell concentration from 1066 cells/μL to 360 cells/μL. With daily antioxidant supplementation of approximately 116 mg, the antioxidant concentrations increase and ROS concentrations decrease, but neither quite reach the levels observed in control individuals. Although at this level of supplementation the analytically predicted equilibrium does reach the $CD4^+$ T cell equilibrium of 460 cells/μL found for the HIV(+)V group in Jaruga *et al.*, it is important to note that this equilibrium point is unstable, as described in greater detail below.

Before examining the benefits and limitations of vitamin supplementation, we test our analytical results using numerical integration (ode23s, MATLAB®, The MathWorks Inc.) for HIV-negative IDUs who subsequently become infected with HIV. In the absence of vitamin supplementation, such an individual would display trends similar to those observed in Figure 2.4: an initially healthy concentration of $CD4^+$ T cells is followed, upon infection, by a sharp decline in the number of uninfected $CD4^+$ T cells which eventually equilibrates at a significantly lower

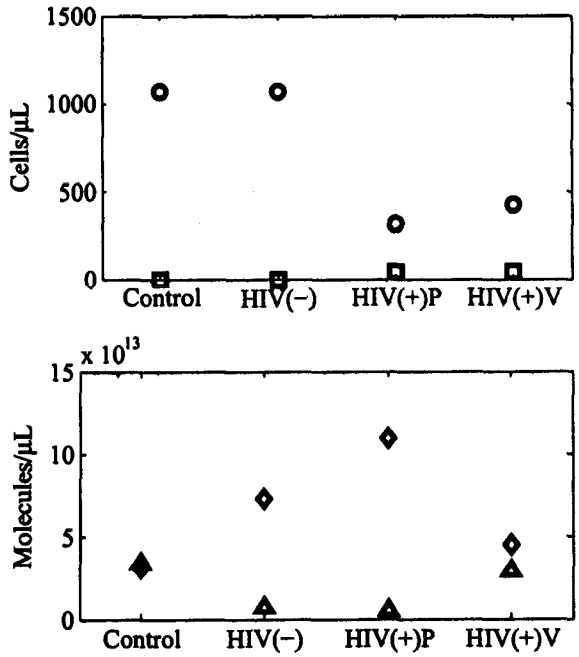

Figure 2.3: The analytical results for the control, HIV(-) and HIV(+)P groups. We include the unstable equilibrium point of the HIV(+)V group at a total vitamin C supplementation level of approximately 116 mg/day. The respective levels of infected cells are denoted with circles, infected cells with squares, ROS with diamonds and antioxidants with triangles.

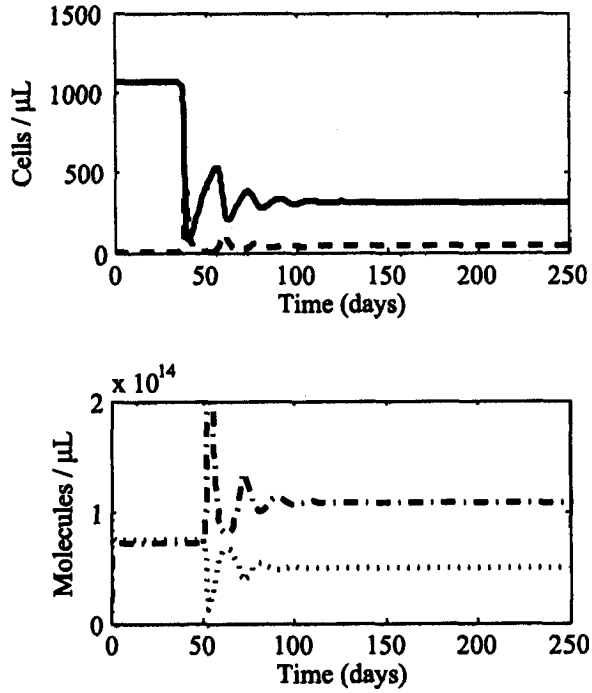

Figure 2.4: An initially uninfected IDU who subsequently becomes infected. Here we observe a significant drop in uninfected $CD4^+$ T cell levels (solid line) characteristic of the infection. An equilibrium is eventually reached where $x = 317$ cells/μL, $y = 43$ cells/μL, $r = 1.09 \times 10^{14}$ molecules/μL and $a = 4.94 \times 10^{12}$ molecules/μL. The concentration of infected cells is represented by the dashed line and ROS by the dashed-dotted line. For clarity the time course of antioxidants has been scaled; the dotted line plots $10a$.

concentration of 317 cells/μL. In addition, the ROS concentration increases to an equilibrium value well beyond normal levels, and the antioxidant concentration decreases. Note that in Figure 2.5 the antioxidant concentration is scaled by a factor of ten so that these trends can be more clearly observed.

Next we examine the behaviour of our model when patients are given moderate daily vitamin supplementation. For this case, our model suggests that an HIV-positive IDU's T cell count can increase, with a concomitant reduction of ROS. However, the magnitude and nature of these changes are dependent upon the level of supplementation. Notice, for example, the outcomes of two different supplementation levels in Figure 2.5. When we supplement the diet with 58 mg of absorbed antioxidants per day, an increase in the level of uninfected $CD4^+$ T cells (to 345 cells/μL) is observed. However, as we noted in the discussion of Figure 2.3, we are unable to reach the clinical mean, x^v, found in Jaruga *et al.*. Instead, the level of supplementation required for a mean $CD4^+$ count of 460 cells/μL, 116 mg, results in the oscillatory dynamics illustrated in Figure 2.5.

We further investigate this interesting behaviour through numerical bifurcation analysis, substituting our parameter values into the analytically-determined eigenvalues of the Jacobian. Using the vitamin supplementation level, α, as a bifurcation parameter, we observe that increasing α causes an increase in the concentration of uninfected cells and a decrease in ROS concentrations, as expected (Figure 2.6). However, there exists a critical vitamin supplementation level, $\alpha_c = 2.63 \times 10^{13}$ molecules/μL per day (approximately 78 mg/day), at which the internal equilibrium undergoes a supercritical Hopf bifurcation: the stable internal equilibrium for $\alpha < \alpha_c$ becomes a stable limit cycle for $\alpha > \alpha_c$ (Figure 2.6). Further analysis reveals three additional bifurcations at values of $\alpha > \alpha_c$, however these are of little clinical relevance.

These bifurcation diagrams also confirm what we found in Section 2.3: the disease-

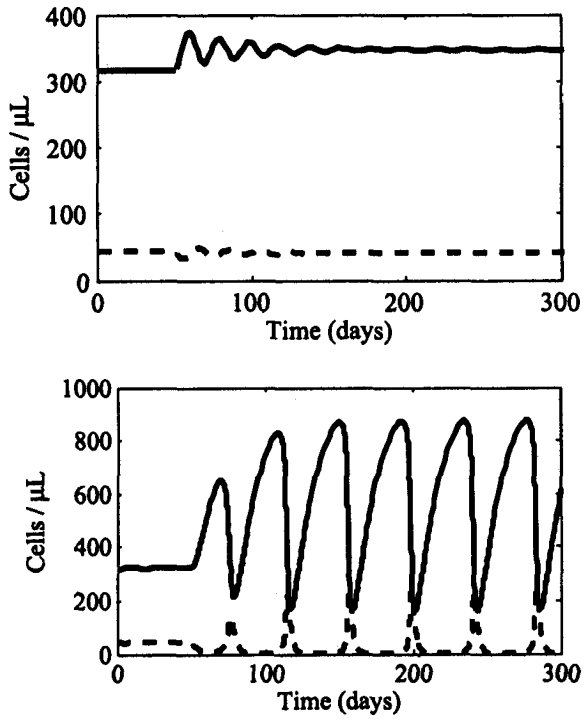

Figure 2.5: Uninfected (solid line) and infected (dashed line) cell concentration for an initially infected IDU who begins vitamin supplementation on day 50. In (a), a stable equilibrium results from a supplement of $\alpha = 2.0 \times 10^{13}$ molecules/(μL day) which corresponds to 58 mg of daily vitamin C supplementation. In (b), a periodic cycle appears when $\alpha = 4.0 \times 10^{13}$ molecules/(μL day), corresponding to 116 mg of daily vitamin C supplementation.

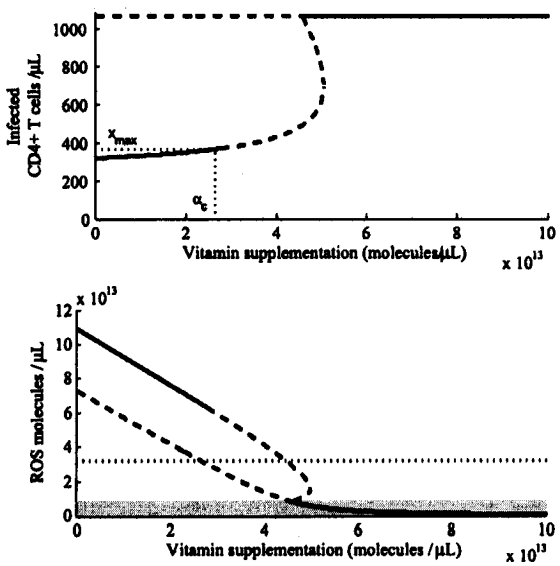

Figure 2.6: Bifurcation diagrams of our model of the uninfected T cells and ROS. A solid line implies a stable equilibrium and a dashed line implies an unstable equilibrium. In (a), we indicate the maximum attainable stable concentration of uninfected cells, $x_{\max}$ (dotted black line). The dotted horizontal line in (b) indicates the ROS level observed in non-IDU control individuals. We denote the ROS concentration for which the disease-free equilibrium becomes stable with the grey box.

free equilibrium is stable when $R_0 < 1$. This occurs when $r < 8.16 \times 10^{12}$ molecules/μL per day (shaded region in Figure 2.5), with $\alpha \geq 4.59 \times 10^{13}$ molecules/μL per day, or a total supplementation level greater than 134 mg/day. Our model therefore suggests that there exists a supplementation level at which an HIV(+) individual could theoretically clear all infected cells in plasma. Yet, this only occurs when the concentration of ROS is well below normal levels, and would therefore not be physiologically possible.

The behaviour of the limit cycle is further examined, in the regime $\alpha > \alpha_c$, by integrating our system numerically for 600 days and measuring the time between the last two peaks. As shown in Figure 2.7, when vitamin supplementation levels increase above α_c, the period of the oscillations increases dramatically. Interestingly, as α changes, so does the behaviour of the limit cycle, depicted in the insets of Figure 2.7. For supplementation levels close to α_c, the oscillation is moderate, with symmetrical peaks and troughs. Higher levels of α, on the other hand, result in severe oscillations, characterised by extended intervals of high $CD4^+$ T cell counts followed by sharp, short-lived periods in which the patient is in an immunocompromised state. Regardless of the shape of these oscillations, a therapeutic regimen which causes repeated periods of immunosuppression would not be clinically advisable. Thus our model predicts the existence of a maximum vitamin supplementation level, α_c, beyond which further supplementation might be detrimental.

To better understand this threshold behaviour, we look at $x_{\max}$, which we define to be the maximum attainable stable equilibrium concentration of uninfected T cells, that is, the equilibrium value of x when $\alpha = \alpha_c$ (Figure 2.6). Using the parameter values as indicated in Section 2.4, $x_{\max} = 369$ cells/μL, which falls short of the mean value $x^v = 423$ cells/μL reported in Jaruga *et al.*. To investigate this difference further, we examined the extent to which $x_{\max}$ is sensitive to assumptions regarding our parameter values.

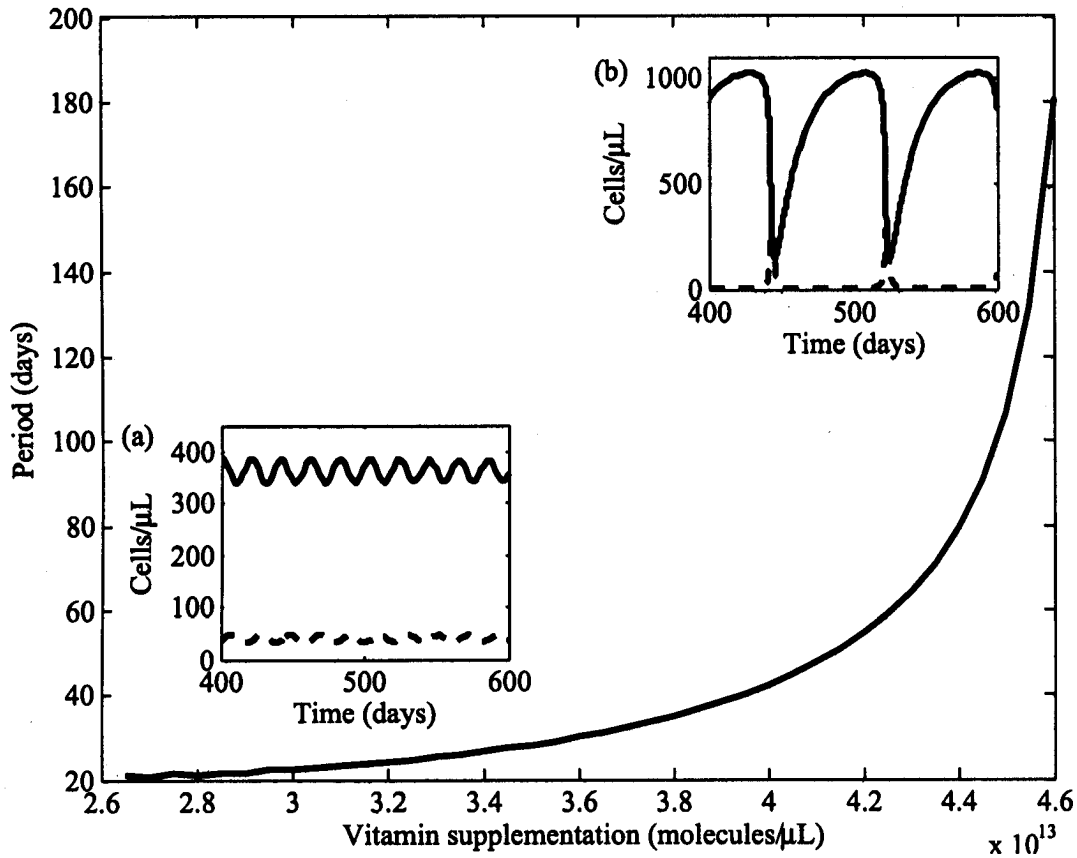

Figure 2.7: The period of the limit cycle as a function of vitamin supplementation levels. As the levels change, so do the dynamics of the limit cycles. Insets (a) and (b) show the limit cycle at $\alpha = 2.8 \times 10^{13}$ and $\alpha = 4.45 \times 10^{13}$, respectively. In the insets, the uninfected and infected cell concentrations are respectively represented by the solid and dashed lines.

2.6 Sensitivity Analysis

We examine the sensitivity of our model to several parameters for which our assumed values have a high degree of uncertainty, or which may display significant interpatient variability. In particular, we look at how the maximum attainable uninfected $CD4^+$ T cell concentration, $x_{\max}$, changes as a result of varying parameters. In each case, to compute $x_{\max}$ we perform a numerical bifurcation analysis as illustrated in Figure 2.6, increasing α until the stability of the internal equilibrium is lost.

We test for sensitivity in two ways. First, in Section 2.6.1, we examine the sensitivity of $x_{\max}$ to the parameter values from the literature which we initially assumed in Section 2.4, and upon which further parameter estimates depend. Second, in Section 2.6.2, we look at the sensitivity of $x_{\max}$ to interpatient parameter variation. In both sections, we examine the trends in $x_{\max}$ as well as the corresponding concentrations of infected T cells, ROS, and antioxidants when a parameter of interest is varied.

2.6.1 Sensitivity to initial parameter estimates

In this section, we vary five parameters which have a high degree of uncertainty to test the overall sensitivity of our results to these assumed parameter values. In cases where the values of other parameters depend on these initial estimates, we subsequently recompute all other dependent model parameters, using the method described in Section 2.4.

Dietary antioxidant intake of the controls: First, due to the natural variability surrounding the diet of control individuals and the uncertainty regarding the amount of antioxidants absorbed, we vary $\hat{\lambda}_a$, the amount of antioxidants

absorbed from the diet of control individuals. Note that when $\hat{\lambda}_a$ changes, our estimates of parameters h_r, m, k, $b_{\max}$, r_{half} and p. In addition, equilibria r^*, r^p and r^v were altered. From Figure 2.8a, it may be observed that as $\hat{\lambda}_a$ increases, our model predicts a reduction in r^v, while $x_{\max}$ increases only slightly: a 200% increase in $\hat{\lambda}_a$ causes a 21% increase in $x_{\max}$.

Dietary antioxidant intake of IDUs: For reasons similar to those posed above, we secondly analyse the sensitivity of λ_a, the amount of antioxidants absorbed from the diet of IDUs, and find that $x_{\max}$ decreases modestly as λ_a increases (Figure 2.8b). Note that when λ_a changes, our estimates of parameters h_r, m, k, $b_{\max}$ and r_{half}. Equilibria r^*, r^p and r^v were altered as well. This restricts the range we can examine; when $\lambda_a < 0.048$ g day^{-1} the positivity of certain parameter values is lost. Importantly, close to the lowest possible value of λ_a we are able to replicate the HIV(+)V Jaruga *et al.* results, that is, $x^v + y^v = 460$. Again, a very modest change is observed: a 220% parameter increase results in a 21% decrease in $x_{\max}$.

Drug effectiveness: Thirdly, we vary drug effectiveness due to our uncertainty surrounding its estimate, and its dependence upon the treatment regimen. When ϵ changes, our estimates of parameters h_r, m, k, R_0, $b_{\max}$ and r_{half}. Equilibria r^* and r^v were altered as well. In Figure 2.9a, an increasing ϵ is shown to yield a decreasing $x_{\max}$, although again $x_{\max}$ is moderately sensitive to this parameter: a 31% increase in ϵ causes a 14% decrease in $x_{\max}$. Note that at higher values of ϵ than illustrated in Figure 2.9a the stability of the internal equilibrium is lost, whereas at lower values positivity of certain parameters is lost. This restricted range of ϵ only applies to our estimates of mean drug effectiveness for the IDU group in the Jaruga *et al.* study; interpatient variation in ϵ is possible over a much wider range, as described in detail in Section 2.6.2.

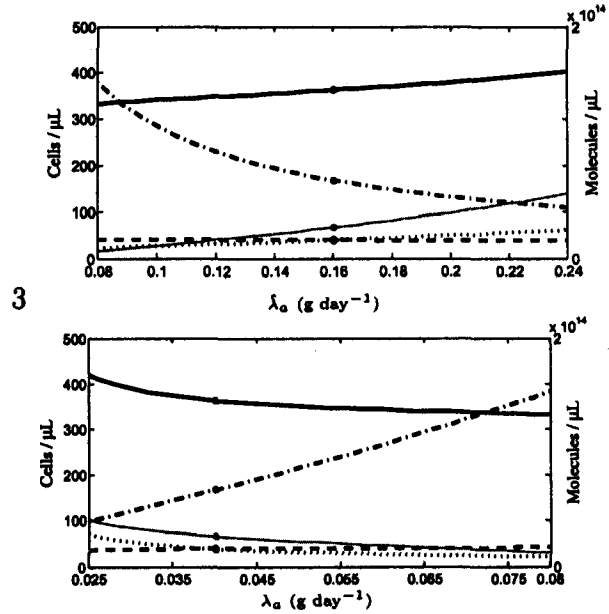

Figure 2.8: Sensitivity analyses of (a) $\hat{\lambda}_a$ and (b) λ_a. The equilibrium concentration of uninfected cells is represented by the solid line, infected cells by the dashed line, ROS by the dashed-dotted line and antioxidants by the dotted line. We also include the level of antioxidant supplementation, α_c (thin, grey line). Levels obtained at the default parameters values (x^v, y^v, r^v and a^v) are indicated by dots.

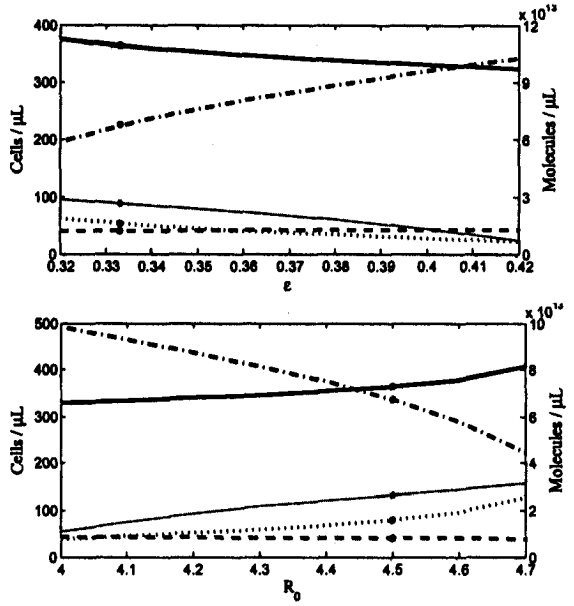

Figure 2.9: Sensitivity analyses of (a) ϵ and (b) R_0. The concentration of uninfected cells is represented by the solid line, infected cells by the dashed line, ROS by the dashed-dotted line and antioxidants by the dotted line. We also include the level of antioxidant supplementation, α_c (thin, grey line). Levels obtained at the default parameter values are indicated by dots.

Basic reproductive ratio: Fourthly and similarly, since there is uncertainty surrounding the value of R_0, the results of a range of parameter values are analysed. Note that when R_0 changes, our estimates of parameters h_r, m, k, R_0, $b_{\max}$ and τ_{half}. Equilibria r^* and r^v were altered as well. We observe in Figure 2.9b that as R_0 increases 17%, $x_{\max}$ increases 24%, and therefore find that $x_{\max}$ is somewhat sensitive to changes in R_0. Values of R_0 lying below the range presented in Figure 2.9b cause the disease-free equilibrium to regain stability, whereas those that are higher result in negative parameter values.

ROS removal: Finally, since the removal rate of ROS is extremely rapid and is therefore difficult to compute, we analyse the system for varying removal rates, $h_r + m\hat{a}$. We find that our results are completely insensitive to changes in $h_r + m\hat{a}$ (data not shown), since the values of the subsequently computed parameters, namely h_r, m and k, exactly compensate for this change.

Despite these cascading changes to subsequently computed parameters in response to changes in $\hat{\lambda}_a$, λ_a or ϵ, we find that $x_{\max}$ is fairly insensitive. However, the value of $x_{\max}$ is somewhat sensitive to our initial assumption of the in-host R_0 for HIV, which is interesting given that the value of this parameter is not well known (NOWAK and MAY 2000). In contrast, the predicted ROS concentration at $\alpha = \alpha_c$ is very sensitive to our initial assumptions regarding these parameters. We are able to replicate clinical results under the assumption that the IDU group has a very low dietary intake of antioxidants, corresponding to 48 mg absorbed per day.

2.6.2 Sensitivity to interpatient variability

In this section we quantify the sensitivity of our model to interpatient variation for several parameter values. Unlike in the previous section where dependent

parameter values were recalculated in response to variation in an assumed parameter, here we only vary the parameter of interest and hold all other parameters constant, except α which we vary to find α_c as before.

Drug effectiveness: Our first parameter of interest is drug effectiveness since ϵ varies from patient to patient due to differences in HIV progression and levels of adherence. As anticipated, our model is sensitive to the level of effectiveness, with $x_{\max}$ (solid line) rising with increasing effectiveness (Figure 2.10a). Furthermore, our model suggests that increasing a patient's drug effectiveness from $\frac{1}{3}$ to 0.7 is sufficient to drive the plasma concentration of infected cells to undetectable levels, as is observed in aggressive HAART (FINZI *et al.* 1997). Increased drug effectiveness also results in a reduction in the level of antioxidant supplementation required to realize $x_{\max}$ and increases the chance of oversupplementation. Thus our model predicts, interestingly, that antioxidant supplementation should be reduced in patients who exhibit strong adherence, although some level of supplementation would continue to be beneficial.

Basic reproductive ratio: Secondly, we test the sensitivity of our results to R_0, since this parameter could also display interpatient variability due to differences in immunocompetence, disease progression and other factors. As we observe in Figure 2.10b, although the concentration of ROS decreases, $x_{\max}$ is relatively insensitive to changes in R_0 over an extremely wide range: an increase from 0 to 35 results in a mere 3% increase in $x_{\max}$.

Natural ROS production: In the formulation of our model, we made the assumption that the natural rate of ROS production, λ_r, was the same for all individuals. Therefore, we thirdly examine the effect of a varying interpatient λ_r. In Figure 2.11, it may be observed that despite an increasing λ_r, ROS concentrations (dashed-dotted line) decrease and therefore $x_{\max}$ (solid line) increases.

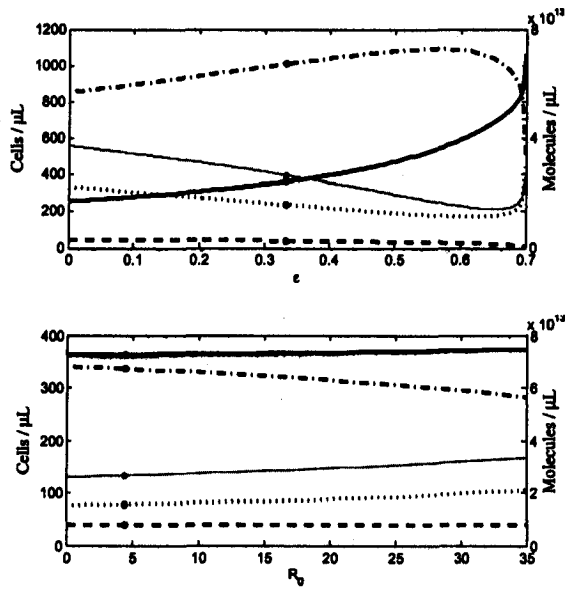

Figure 2.10: Sensitivity analyses of (a) ϵ and (b) R_0 for interpatient variability. The concentration of uninfected cells is represented by the solid line, infected cells by the dashed line, ROS by the dashed-dotted line and antioxidants by the dotted line. We also include the level of antioxidant supplementation, α_c (thin, grey line). Levels obtained at the default parameter values are indicated by dots.

This trend can be attributed to significant increases in antioxidant supplementation levels (thin, grey line); as λ_r increases, higher values of α_c are possible without losing the stability of the equilibrium. However, since physiological constraints would presumably impose some limit on the degree of the vitamin supplementation possible, we set a maximum antioxidant supplementation level of 2.0×10^{14} molecules μL^{-1} day^{-1}, which is approximately 586 mg/day, absorbed into the bloodstream. The quantitative value of this limit has been chosen arbitrarily to illustrate the qualitative effects of the physiological limit which presumably exists.

Thus, the increases in x_{max} continue until α reaches our imposed maximum, which in this example occurs when $\lambda_r = 5.65 \times 10^{21}$ molecules μL^{-1} day^{-1}. Further increasing λ_r, combined with a constant α level, results in a significantly increasing ROS concentration which causes x_{max} to decrease. We address this interesting qualitative prediction further in the Discussion.

Dietary antioxidant intake of IDUs: Lastly, we examine the effect of a varying dietary antioxidant intake and find that our results are insensitive to this variation, the only change being an alteration in the vitamin supplementation level required to achieve x_{max} (data not shown).

2.7 Discussion

We have developed and analysed a simple model of the interactions between $CD4^+$ T cells, reactive oxygen species and antioxidants. Verifying the results of various clinical studies, our model predicts that moderate levels of antioxidant supplementation in HIV-positive IDUs can lead to an increase in uninfected $CD4^+$ T cell concentrations. However, our model also suggests that excessive

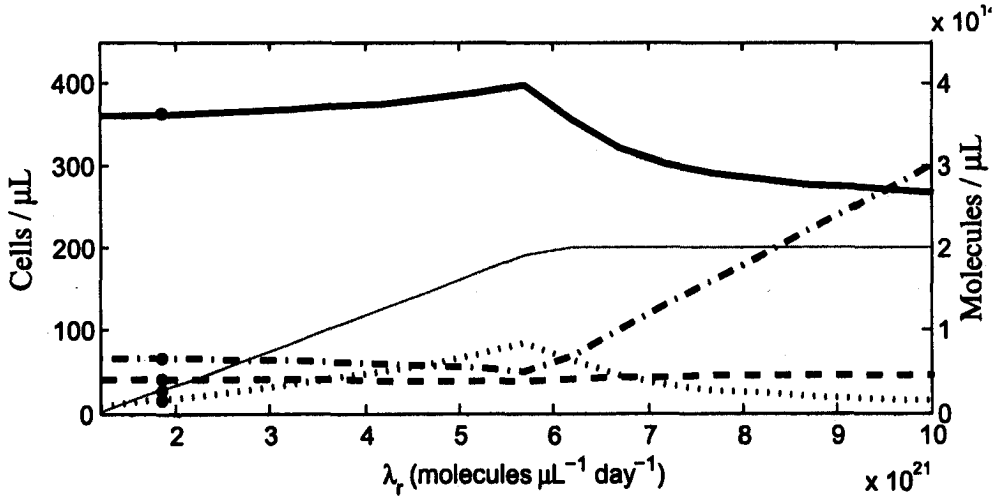

Figure 2.11: Sensitivity analyses of λ_r for interpatient variability. The concentration of uninfected cells is represented by the solid line, infected cells by the dashed line, ROS by the dashed-dotted line and antioxidants by the dotted line. We also include the level of antioxidant supplementation, α_c (thin, grey line). Levels obtained at the default parameter value are indicated by dots. Stability is lost for lower values of λ_r than those illustrated.

supplementation could cause fluctuating T cell concentrations in these individuals. For example, consider the limit cycle in Figure 2.5: in this case, a patient's immunological response is periodically compromised – characterized by a low concentration of uninfected $CD4^+$ T cells – leaving the individual vulnerable to opportunistic infections.

In an effort to understand this periodic behaviour, we take a closer look at the system dynamics when the level of antioxidant supplementation is above the critical level, α_c, in Figure 2.12. In this figure, populations during the limit cycle are rescaled to facilitate comparison, while white vertical bars delineate the peak and trough concentrations of infected T cells. The most direct result of an increase in antioxidant supplementation is first an increase in the antioxidant concentration (dotted line) and a decrease in ROS (dashed-dotted line). These two effects produce a concomitant increase in uninfected cells (solid, black line) and reduction in infected cells (dashed line). As the concentration of uninfected cells increases, the infection rate per infected cell ($\beta(r)x$, grey line), reaches high levels, allowing both infected cell and ROS concentrations to increase sharply. These increases are short lived in part because of the extremely short half-life of ROS, and due to a rapid reduction in $\beta(r)x$. As ROS and infected cell concentrations plummet, the cycle is allowed to repeat. One hypothesis is that when the level of antioxidant supplementation is too high, the infection rate $\beta(r)x$ reaches too high a peak to allow for a stable equilibrium.

Regardless of its cause, the appearance of a limit cycle in our model could explain why some clinical studies show no improvement in patients' average $CD4^+$ T cell concentrations: it is plausible that high supplementation levels could cause fluctuating T cell counts which are then sensitive to the details of measurement timing, leading to the conclusion that antioxidant supplementation has no immunological benefit for HIV-positive patients.

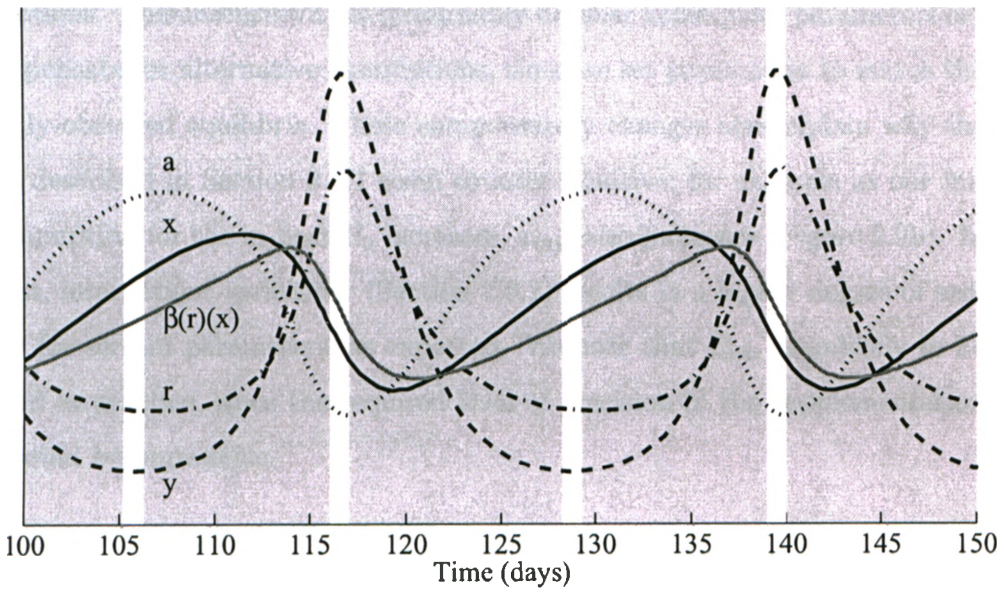

Figure 2.12: A closer look at the dynamics of the stable limit cycle. The concentration of uninfected cells is represented by the solid black line, infected cells by the dashed line, ROS by the dashed-dotted line and antioxidants by the dotted line, each rescaled for comparison. The solid grey line denotes $\beta(r)x$. White vertical bars delineate maxima and minima in infected cell concentrations.

Since antioxidant supplementation levels above a critical level, α_c, have the potential to pose difficulties for patients, we turn our attention to the stable equilibria obtained when $\alpha < \alpha_c$. We examined in particular the maximum concentration of uninfected CD4$^+$ T cells, $x_{\max}$, which could be obtained in principle as a stable equilibrium via antioxidant supplementation. We found $x_{\max}$ to be relatively insensitive to moderate variation in five initial parameter estimates, particularly when subsequent parameter estimates were changed as a result of these alternative assumptions. This insensitivity is presumably because subsequent parameters act to compensate for alternative assumptions, since we set parameters to match the clinically-observed equilibria. These compensatory changes also explain why the results described in Section 2.6.1 seem counter-intuitive; for example as our initial assumption for the in-host R_0 increases, $x_{\max}$ also increases (Figure 2.9b). In contrast, interpatient variability (Section 2.6.2) results in a higher degree of sensitivity for certain parameters, as expected. We note that $x_{\max}$ is unlikely to be achieved in practice, since the required level of precision in the supplementation level would be impossible.

Interestingly, our sensitivity analysis revealed that even when our initial parameter estimates were varied, the mean T cell count observed by Jaruga *et al.* after six months of antioxidant therapy was higher than any stable equilibrium value predicted by our model, except when considering exceptionally low values of λ_a. In the region of instability, however, values equivalent to the clinical data were frequently observed. For example, in Figure 2.13 we present an example in which the sum of uninfected and infected CD4$^+$ T cells at six months, 479 cells/μL, exceeds the 460 cells/μL found in Jaruga *et al.*. This was achieved with our default parameters and antioxidant supplementation of 2.87×10^{13} molecules/μL per day, or about 84 mg of absorbed antioxidants per day. This outcome is anecdotal and highly dependent upon the amount of vitamin C absorbed; however, it illustrates the potential sensitivity of clinical results to the details of measurement timing.

To further investigate the benefits of antioxidant supplementation, we hope that future work could see the model extended to include appropriate pharmacokinetics of antioxidants. In its present form, our model considers α to remain constant over time. If we included the full dynamics of antioxidant concentrations after an oral dose, including varying the antioxidant decay rate with plasma concentration (HICKEY and ROBERTS 2004), we predict that the oscillatory behaviour observed here would be exacerbated. Either standard pharmacokinetic modeling (WAHL and NOWAK 2000) or impulsive differential equations (SMITH and WAHL 2004) could be used to examine such effects.

It would also be interesting to explore the effects of enzymatic antioxidants – glutathione peroxidase and catalase, for example. Both of these enzymes are used in the elimination of hydrogen peroxide (H_2O_2), but are not consumed by these reactions. Their short half-lives (less than 10 minutes) (DESHMUKH *et al.* 1997; CARPENTER *et al.* 2001), however, could further exacerbate the variability already observed in the simple model.

In conclusion, while antioxidant supplementation may not be a long term solution for HIV-positive IDUs, our model suggests that moderate doses of antioxidants may temporarily boost uninfected $CD4^+$ T cell concentrations. This might enable HIV-positive individuals to lengthen the interval before costly drugs with severe side effects become necessary. These results could have implications for infected individuals in HIV-endemic areas, since dietary antioxidant intake depends on the availability of adequate antioxidant-rich produce. Moreover, where access to antiretroviral therapy is limited or non-existent due to economic constraints, a significantly more affordable vitamin supplementation therapy could potentially provide some limited benefit. Of course we emphasize that this in no way reduces the need for accessible and affordable antiretrovirals in developing countries.

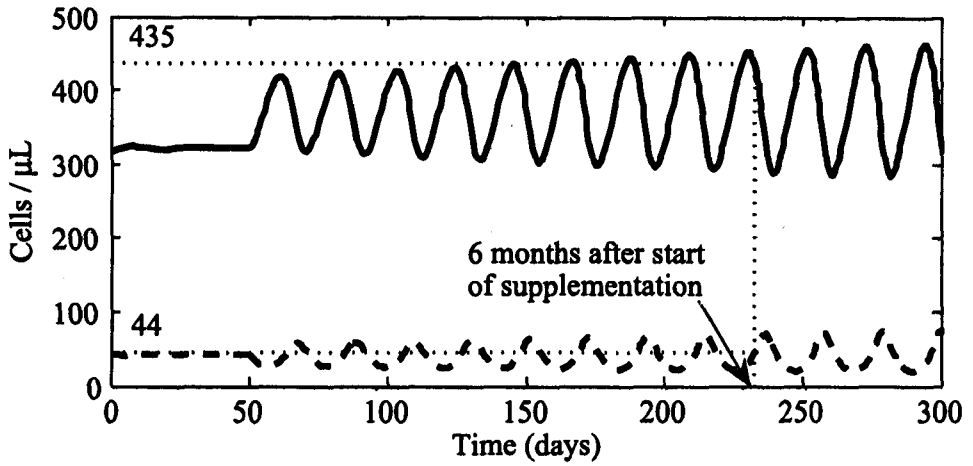

Figure 2.13: The oscillatory dynamics of the system when 84 mg of the daily vitamin supplement is absorbed. We see that six months after the start of supplementation, we reach $CD4^+$ T cell levels observed in the Jaruga *et al.* study. The concentration of uninfected cells is represented by the solid black line, infected cells by the dashed line.

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CHAPTER 3

Summary and Future Work

The aim of this thesis was to develop a model that would provide insight into the possibility of antioxidant supplementation as a complementary therapy for HIV-infected individuals, with a specific focus on injection drug users. Analysis of the resulting model suggested that moderate antioxidant supplementation can be beneficial for such persons; however, due to the oscillations predicted with excessive supplementation, the potential for detrimental effects remains. Therefore, the model is able to reconcile the different findings of clinical studies as presented in Chapter 1. The results of this research could have implications for persons in developed countries – making a case for a well-balanced, nutritious diet and, if necessary, antioxidant supplementation – as well as for those in developing countries where antioxidant-rich produce may not be readily available.

In order to arrive at these conclusions, a simple model was presented, the simplicity of which is, as usual, both a strength and a potential weakness. The system of only four ordinary differential equations lends itself more easily to analysis; however, even with the simple model, analytical results regarding the stability of the internal equilibrium provided no insight and the critical value of the bifurcation parameter could not be obtained analytically. Moreover, there are few enough parameters that values could be either acquired directly from the literature or

estimated using the equilibrium population sizes reported in clinical trials. This might not be possible with a more extensive model. Yet, the basic model may have neglected or over-simplified certain details which may have been important.

In what follows, three directions for future work are presented.

Realistic pharmacokinetics: The model used in this thesis can be extended to further investigate the benefits of antioxidant supplementation. In particular, it would be useful to incorporate realistic antioxidant pharmacokinetics into the system as has been done in models for drug therapy. For example, Wahl and Nowak (2000) included a $C(t)$-dependent viral replication rate in the standard HIV model, where $C(t)$ denoted the drug concentration as a function of time. Later, Smith and Wahl (2004) used impulsive differential equations to simulate therapy. Using this method, dose times correspond to instantaneous increases in drug concentration, and are followed by exponential decay. Both of these methods could be used to model antioxidant supplementation, where an additional biological feature could be the inclusion of a variable antioxidant decay rate that is dependent upon the plasma concentration (HICKEY and ROBERTS 2004).

Enzymatic antioxidants: It would also be interesting to explore the effects of enzymatic antioxidants – glutathione peroxidase and catalase, for example. Both of these enzymes are used in the elimination of hydrogen peroxide (H_2O_2) by lowering the activation energy required, and thereby increasing the rate of (catalysing) a reaction. Glutathione peroxidase catalyses reactions between H_2O_2 and the nonenzymatic antioxidant, glutathione, to produce water. Catalase speeds up reactions between H_2O_2 molecules which result in the production of water and oxygen. As enzymes, glutathione peroxidase and catalase are not consumed by these reactions; however, they have short half-lives (less than 10 minutes) (DESHMUKH *et al.* 1997; CARPENTER *et al.* 2001), which could further exacerbate the variability already observed in the simple model.

Antioxidant supplementation incorporated into drug therapy models: In 2008, Magombedze *et al.* analysed a model in which three of the HIV viral life-cycle stages are explicitly incorporated, namely: entry of the virus into an uninfected $CD4^+$ T cell, transcription of the viral RNA into DNA, and the production of new viral proteins. This model is further extended to include the effects of fusion inhibitors, reverse transcriptase inhibitors, and protease inhibitors. Including antioxidant supplementation in a model such as this could prove insightful.

In conclusion, the results of this thesis, along with those of clinical trials, suggest that antioxidant supplementation could be an important complement to existing pharmaceutical regimens in certain HIV-infected patients. The observations made herein – a decrease in oxidative stress-induced HIV replication in HIV-positive IDUs due to moderate antioxidant supplementation – can potentially be extended to a much wider population group. It may be hypothesized that such supplementation could also be used to delay the initiation of drug treatment in persons around the world. It is apparent, then, that alternative therapies should continue to be studied. Evidently mathematics has much to contribute in this regard.

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