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Research

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T-cell apoptosis. Tocounteract these effects, clinical studies have explored the possibility ofising antioxidant levels, with mixed results.

In this paper, 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.

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 pathogens. Moreover, upon production, these O_2^- mole-

O_2^-

cules are rapidly metabolized 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 [1,6,7].

Despite their positive role, reactive oxygen species can be

Infected CD4+

CD4+ T cells become infected at rate $(1 - \varepsilon)\beta xy$. 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- B 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

$$\beta(r) = \frac{\beta_{max}\, r}{\ldots}.$$

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 the Parameter Estimation section, 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 [36,37].

Analytical results

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

where $B = \alpha\lambda_a m + \lambda_m h_r h_a - \lambda_r p$. 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.

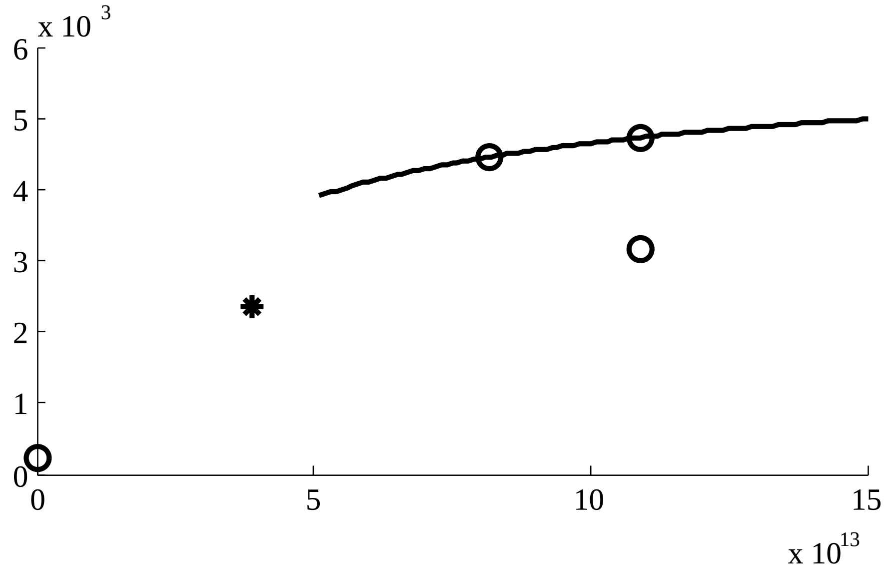


Figure 2 **β(r) curve**. The β(r) curve, denoted by the solid line, represents the rate of infection in the absence of drug therapy. The dashed line depicts the decrease in the infection rate due to HAART, the β(r)(1 - ε) curve. The dotted line represents the maximum rate of infection.

Using the next-generation matrix method from [38], we find the basic reproductive ratio to be

$$R_0 = \frac{\beta(r^d)(1-\varepsilon)\lambda_x}{d_x d_y}, \tag{6}$$

which makes intuitive sense since a single infected cell at the uninfected equilibrium will produce new infected cells at rate $\beta(r^d)(1 - \varepsilon)x^d$, 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:

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

This yields four eigenvalues

$$-d_x < 0, \tag{7}$$

$$d_y(R_0 - 1) \text{ and} \tag{8}$$

$$-\frac{p(r^d)^2 + \lambda_r + h_a r^d \pm \sqrt{(p(r^d)^2 + \lambda_r + h_a r^d)^2 - 4r^d(\lambda_r h_a + (r^d)^2 h_r p)}}{2r^d} < 0. \tag{9}$$

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

Parameter estimation

Developing reasonable (if uncertain) parameter estimates is one of the most difficult aspects of theoretical immu-

mean age to those of the Jaruga *et al.* study [28] (33.9 ± 1.6 vs 27 ± 9), the majority of whom were also IDUs [43]. Twelve months of treatment were shown, on average, to increase these patients' $CD4^+$ T cell counts from 231 ± 87 cells/ L to 345 ± 62 cells/ L, which is approximately the same level as in the HIV(+) groups in Jaruga *et al.* [28]. 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 ε set to be . 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 $\varepsilon = 0$. Using (6), we find R_0 at this equilibrium to be:

Given the parameter values in Table 1, this yields $\beta(r^*) =$ 0.00422. Since NF-κ

The parameter p, which should be constant for all individuals, is found using (4) at the control equilibrium, i.e., for $\gamma \neq 0$ and = 0:

From equation (4) at the HIV(+)P equilibrium,

We find our final parameter, k, from equation (3) at the HIV(+)P equilibrium:

Finally, from equation (3) at the HIV(+)V equilibrium:

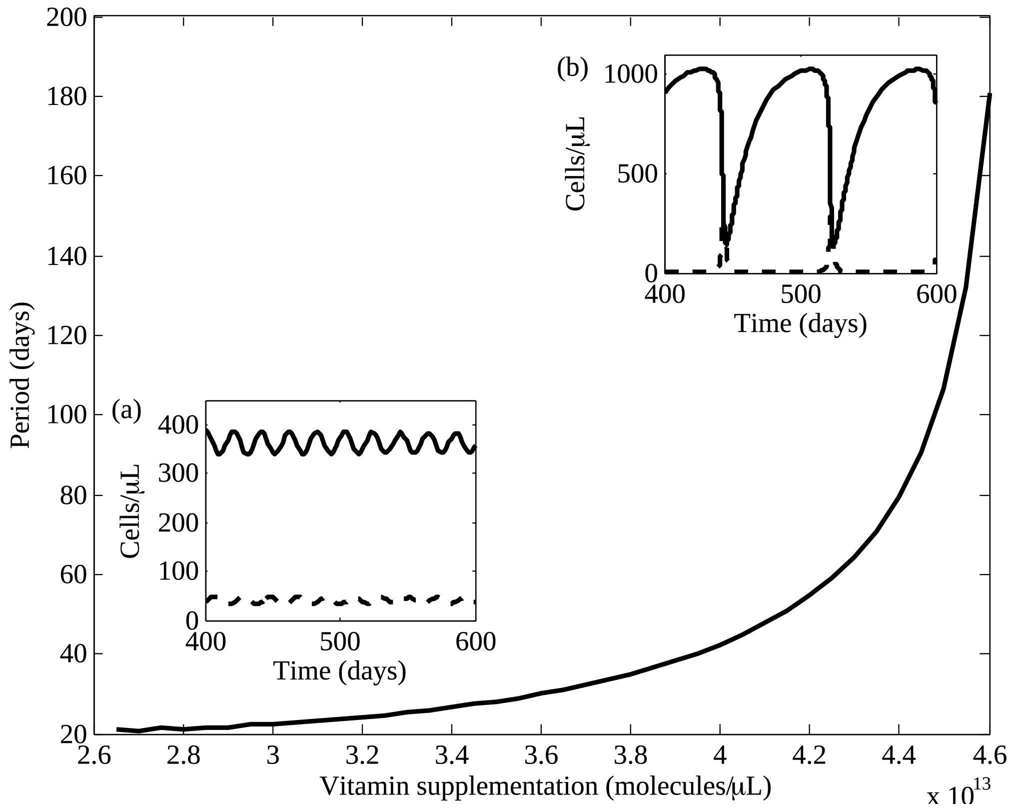
Using the parameters in Tables 1 and 2, the equilibria of our model were found analytically. At these parameter values and antioxidant supplementation levels, only one biologically meaningful internal equilibrium exists, and this equilibrium agrees well with the $CD4^+$ T cell and antioxidant concentrations in Jaruga *et al.* [28], as illustrated in Figure 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 [28]. 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/ L to 360 cells/ L. 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 400 cells/ L found for the HIV(+)V group in Jaruga *et al.* [28], 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 (MATLAB ®, The MathWorks Inc.)

observed in Figure 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 concentration of 3.7 cells/ L. In addition, the ROS concentration increases to an equilibrium value well beyond normal levels and the antioxidant concentration decreases. Note that in Figure 4b 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 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 3.5 cells/ L) is observed. However, as we noted in the discussion of Figure 3, we are unable to reach the clinical mean, x^v, found in Jaruga *et al.* [28]. Instead, the level of supplementation required for a mean $CD4^+$ count of 460 cells/ L, 116 mg/day, results in the oscillatory dynamics illustrated in Figure 5b.

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,



[illegible]. As the vitamin supplementation level increases beyond α_c = 2.63 × 10^{13} molecules/μL per day, changes in the dynamics of the limit cycles can be observed. Insets (a) and (b) show the limit cycle at α = 2.8 × 10^{13} and α = 4.45 × 10^{13}, respectively. In the insets, the uninfected and infected cell concentrations are respectively represented by the solid and dashed lines.

To investigate this difference further, we examined the extent to which x_{max} is sensitive to assumptions regarding our parameter values.

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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 performed a numerical bifurcation analysis as illustrated in Figure 6, increasing α until the stability of the internal equilibrium is lost.

We test for sensitivity in two ways. First, we examine the sensitivity of x_{max} to the parameter values from the literature which we initially assumed in the Parameter Estimation section and upon which further parameter estimates depend. In a second analysis, 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.

Sensitivity to initial parameter estimates

In this section, we vary five parameters which have a high degree of uncertainty in order 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 the Parameter Estimation section.

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 , the amount of antioxidants absorbed from the diet of control individuals. Note that, when changes, so do 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 8a, it may be observed that, as increases, our model predicts a reduction in r^v, while x_{max} increases only slightly: a 200% increase in 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 $_a$, the amount of antioxidants absorbed from the diet of IDUs, and find that x_{max} decreases modestly as $_a$ increases (Figure 8b). Note that, when $_a$ changes, so do 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 $_a < 0.048$ g

Despite these cascading changes to subsequently computed parameters in response to changes in , $_a$ or ε, we λ 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 [36]. In contrast, the predicted ROS concentration at = $_c$ 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.

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

rium via antioxidant supplementation. We found $x_{\max}$ to be relatively insensitive to moderate variation in five ini-

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

min 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.

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The authors declare that they have no competing interests.

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RDvG and LMW developed the model. RDvG analyzed the model, analytically and numerically, and produced all figures. RDvG and LMW interpreted the results. RDvG drafted the manuscript.

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