MODELING THE PHARMACODYNAMICS OF rHuEPO, A BLOOD DOPING DRUG

by

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ABSTRACT

MODELING THE PHARMACODYNAMICS OF rHuEPO, A BLOOD DOPING DRUG

For this study, a model to analyze the dynamic effects of recombinant human erythropoietin (rHuEPO), a very popular and hard to detect blood doping drug, is constructed. rHuEPO is the synthetic version of erythropoietin (EPO) hormone which is responsible for the red blood cell production depending on the blood oxygen levels in the body. The usage of rHuEPO in competitions is prohibited by World Anti-Doping Agency (WADA). The personalized tests aiming to detect the usage of rHuEPO are mainly carried out by screening the various blood values that are affected by rHuEPO, which are the hematocrit (red blood cell count), hemoglobin concentration and reticulocyte count. Accordingly, red blood cell regulation structure and the pathways of rHuEPO in the body and their effects on red blood cells are included into the model. Various validation scenarios are conducted to show that the model is consistent with the relevant literature and data. Next, the model is run under two different disequilibrium conditions to reveal the base dynamic behaviors of the model. Our aim is to discuss the dynamic patterns for rHuEPO users and how these patterns differ for rHuEPO users and altitude trainers (a legitimate practice for enhancing blood oxygen capacity). Moreover, we want to analyze the masking of rHuEPO usage with altitude training. Thus, three scenarios are investigated: (1) sensitivity analysis with rHuEPO usage; (2) altitude training; (3) altitude training together with rHuEPO usage. According to the simulation results of our scenarios, various blood values have distinct dynamic behaviors in three distinct periods: before the competition during drug use, during the competition when drug provides competitive advantage, and after the competition. We conclude that these findings can provide a basis to design strong anti-doping tests. Our model can be personalized for individual athletes and hard-to-cheat personalized tests can be developed against rHuEPO use.

ÖZET

BİR KAN DOPİNG İLACI OLAN rHuEPO'NUN FARMAKODİNAMİĞİNİN MODELLENMESİ

Bu çalışmada, çok popüler ve tespit edilmesi zor bir kan doping ilacı olan, rekombinant insan eritropoietininin (rHuEPO) dinamik etkilerinin analiz edilmesi amacıyla bir model kurulmuştur. rHuEPO, vücuttaki kan oksijen seviyesine göre kırmızı kan hücresi üretiminden sorumlu olan eritropoietin (EPO) hormonunun yapay versiyonudur. Yarışmalarda rHuEPO kullanımı Dünya Anti-Doping Ajansı (WADA) tarafından yasaklanmıştır. rHuEPO kullanımının tespit edilmesi için yapılan kişiye özel testlerde; hematokrit, hemoglobin konsantrasyonu ve retikülosit sayımı gibi rHuEPO kullanımından etkilenen kan değerlerine bakılmaktadır. Bu nedenle, kırmızı kan hücresi düzenleme yapısı, rHuEPO'nun vücut içinde izlediği yol ve kırmızı kan hücreleri üzerindeki etkisi modele eklenmiştir. Model geçerliliğine yönelik çeşitli senaryo çıktıları incelenerek, modelin ilgili literatür ve veriler ile tutarlı olduğu gösterilmiştir. Daha sonra, modelin temel dinamikleri iki denge-dışı senaryo ile elde edilmiştir. Amacımız, rHuEPO kullanımının oluşturduğu değişik dinamik davranışları tartışmak ve rHuEPO kullanımının yüksek irtifa antrenmanından (yasal bir kan oksijen kapasitesi artırma yöntemi) farkını göstermektir. Ayrıca, rHuEPO kullanımının yüksek irtifa antrenmanı ile gizlenmeye çalışılması da tezde analiz edilmektedir. Bu amaçlara yönelik üç temel senaryo incelenmiştir: (1) rHuEPO kullanımı ile hassaslık analizi, (2) yüksek irtifa antrenmanı, (3) yüksek irtifa antrenmanıyla birlikte rHuEPO kullanımı. Üç senaryo simülasyon sonuçları, kan değerlerinin üç farklı günlerde farklı ve belirgin dinamik davranışları olduğunu göstermektedir: yarışmadan önce ilaç kullanılırken, yarışma esnasında ilacın avantajlı etkileri sürerken, ve yarışmadan sonra. Bu sonuçlar, güçlü doping testleri geliştirmek için önemli bir temel oluşturabilecektir. Modelimiz ileride atletler için kişiselleştirilebilecek ve rHuEPO kullanımına karşı, kandırılması zor, kişisel testler oluşturulabilecektir.

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ED_{50}	The Dose That Results in a 50% Increase In Bioavailability
E_{max}	Maximum Increase In Bioavailability
F_0	Minimum Absolute Bioavailability
F	Bioavailability
fr	The Ratio Of Bioavailable Dose That Goes Into Fast Absorption
K_m	EPO Level That Halves The Saturated Destruction
P50	Partial Pressure Of Oxygen When The Blood Is 50% Saturated
Vmax	Maximum Elimination Rate Of The Saturable Destruction

LIST OF ACRONYMS/ABBREVIATIONS

atm	Atmosphere
dL	Deciliter
EPO	Erythropoietin
fL	Femtoliter
gr	Gram
HIF	Hypoxia Inducible Factors
IU	International Unit
kU	Kilounits
m	Meter
mL	Milliliter
mmHg	Millimeters of Mercury
μmol	Micromole
mU	Milliunits
RBC	Red Blood Cell
rHuEPO	Recombinant Human Erythropoietin
SC	Subcutaneous
WADA	World Anti-Doping Agency

1. INTRODUCTION

Blood doping is used by athletes to boost the oxygen carrying capacity of their bodies. One of the most common methods of blood doping is the injection of a drug named *recombinant human erythropoietin (rHuEPO). Erythropoietin (EPO)* is a hormone that controls the production and survival of red blood cells. rHuEPO is the synthetic version of human erythropoietin and is mainly used as a therapeutic drug for anemia patients. However, athletes use rHuEPO to increase their red blood cell mass, thus, increasing the oxygen carrying capacity of the cardiovascular system [1]. This gives them unfair advantage in sports competitions such as running, cycling, swimming etc. Usage of rHuEPO or other red blood cell stimulating agents is prohibited by World Anti-Doping Agency (WADA).

In this study, we first aim to model the pharmacodynamics of rHuEPO in the body and its effects on red blood cells. Altitude training is another method used by athletes to stimulate red blood cell production so as to increase the oxygen carrying capacity of the cardiovascular system, boosting their performances [2]. Thus, our second aim is to analyze the effects of altitude on the dynamics of red blood cell production. By using our model, our final goal is to provide some insights for improving the process for detecting rHuEPO users (dopers) by exploring different scenarios involving doping drug usage and altitude training.

The hormone erythropoietin (EPO) is an acidic glycoprotein [3]. EPO is responsible for the red blood cell production in body. The existence of EPO ensures the survival of various stem cells in bone marrow and is responsible for their proliferation. These stem cells eventually become *erythrocytes* with the existence of EPO [4]. EPO is mainly produced in kidneys in response to low oxygen levels in blood (hypoxia) [5]. Recent studies show that, EPO responds to the arterial oxygen content and regulates the red blood cell level to match up to the oxygen needs of the body [6]. In response to hypoxia, *hypoxia inducible factors (HIF)* begin to form in EPO-producing cells in

kidney [7]. HIF bonds to the EPO producing genes in the DNA and create the signal for EPO production. With this, EPO level in blood starts to increase [4]. After some time (around 24 hours) the increased EPO level boosts the *reticulocyte* levels in blood, which are the precursors of erythrocytes [2]. These reticulocytes mature and become erythrocytes [8]. Erythrocytes and reticulocytes contain a special oxygen binding protein called *hemoglobin*. Thus, with the increase in erythrocyte level, the hemoglobin level increases and the arterial oxygen content increases, which means hypoxia is eliminated. This ensures adequate oxygen delivery to the tissues. With the normalization of arterial oxygen level, EPO level starts to decline and returns to its normal level [9]. The EPO gene was first cloned in 1983. This led to the production of the first synthetic EPO drug called the recombinant human erythropoietin (rHuEPO) [10]. This drug is mainly used for anemic patients or patients who suffer from various kidney conditions [1].

The connection between ascent to altitude and a rise in red blood cells was first shown in 1882 [4]. Arterial oxygen content is affected by the partial pressure of oxygen in the atmosphere. At high altitudes, the surrounding air is less dense than at sea level and, because of this, the partial pressure of oxygen is lower than at sea level. This lowers the arterial oxygen content and triggers an EPO response. Accordingly, there are different altitude training regimens available for athletes to increase the oxygen carrying capacity of their blood. One of our aims is to explore the difference between rHuEPO usage and altitude training.

Saturation diving involves diving for long periods of time. The divers stay under the sea for the whole diving period. This practice is used for underwater construction and research. During saturation diving, the divers breathe air with higher partial pressure of oxygen than at sea level. Because of this, the divers have increased arterial oxygen levels for the diving period. This is similar to the increase in the arterial oxygen level by rHuEPO injection. Recent studies on saturation diving reveal that, increased arterial oxygen level has a negative impact on blood EPO level [11, 12]. Accordingly, we include the results of these studies in the relevant equations of our model so as to

represent the effects of higher than normal arterial oxygen content on blood EPO level.

Usage of rHuEPO in competitions is banned since 1990. rHuEPO has a short half-life in human body; 8 to 24 hours based on the injection method. Because of this fact, the existence of rHuEPO cannot be detected in urine after several days from injection. Also, the fact that rHuEPO has almost the same molecule structure as EPO further complicates the testing procedures [13]. However, the indirect effects of rHuEPO injection on red blood cells can be observed for months. This fact led to a shift in testing paradigm of WADA. Previously, only the banned substances or their metabolites were searched for in the tests. The usage of rHuEPO as a doping drug resulted in new testing methods aiming to detect the indirect effects of the drug. WADA is currently developing a method called "Athlete's Biological Passport". This method involves testing athletes regularly to create a baseline for their various blood values, mainly the *hematocrit, hemoglobin concentration, reticulocyte count* and *OFF-hr Score* which is derived from *reticulocyte count* and *hemoglobin concentration*. If a shift occurs in any of these monitored values, the athlete can be accused of using rHuEPO [14]. Altitude training can also give results similar to rHuEPO usage making it difficult to reach definite conclusions. Our model and simulation experiments will explore dynamic consequences of rHuEPO usage, as well as altitude training, aiming to shed light on the issue.

2. PROBLEM DEFINITION AND RESEARCH OBJECTIVES

Blood doping is a popular doping approach. The most popular method of blood doping is the injection of rHuEPO (recombinant human erythropoietin) drug. Its popularity comes from the fact that it has a short half-life in human body and its chemical structure is very similar to natural EPO (erythropoietin) [13]. The anti-doping tests for rHuEPO is costly, time-consuming and easy to fool with clever rHuEPO injection regimens. For this reason, it is suggested to look for the various effects of rHuEPO usage on the blood values [13]. Thus, our main problem is modeling the pharmacodynamics of rHuEPO and the red cell production in human body so that we can conduct simulation experiments to investigate the dynamic consequences of rHuEPO usage. This problem is challenging and interesting to model since the red blood cell production in human body involves various delays, non-linearities and feedback loops.

Our first research objective, as mentioned above, is modeling the pharmacodynamics of rHuEPO in human body and its effects on red blood cells. This involves the inclusion of injection and absorption structures for rHuEPO and the problem-related structures for natural red blood cell production system.

Our second research objective involves simulation experiments with different dosages of rHuEPO to show how an rHuEPO user can fool the doping tests and to discuss the dynamic patterns for the blood values of rHuEPO users, which can provide useful information in developing strong rHuEPO tests. Next, we incorporate altitude training in our simulation experiments to find out about the differences between the effects of altitude training and rHuEPO usage on red blood cells. Altitude training is another red blood cell stimulation method which is currently allowed by WADA. Using our model, our aim is to explore the subtle dynamic differences in blood values between altitude training and rHuEPO usage to separate the two practices. It is important to

understand the source of increased oxygen carrying capacity in an athlete as altitude training is legitimate, but rHuEPO is not.

Final objective of our research involves the design of simulation experiments to analyze the masking of rHuEPO injection with altitude training, which is a complex case and is not easy to identify. We want to explore the dynamic differences between altitude training without drug usage and rHuEPO injection during altitude training so that we can detect the rHuEPO users.

Ultimately, we hope to provide some useful dynamic insights for doping agencies in developing improved testing methods.

3. RESEARCH METHODOLOGY

A system is composed of interacting interdependent variables that come together and form a unified structure. System dynamics methodology studies the dynamic problems that arise in systems which have complex interactions between their components and have hard to predict dynamic behaviors. Human body is such a system that has interacting subsystems such as the digestive system, respiratory system, nervous system etc. The main focus in our study is the usage of rHuEPO (recombinant human erythropoietin) drug and its interaction with the red blood cell production system. As mentioned before, the red blood cell production system has various non-linear interactions, feedback loops and time delays. Because of these properties, the prediction of the dynamic behaviors of the variables in the red blood cell production system is hard and not straight forward, making system dynamics methodology an excellent tool to study our problem. We thus apply system dynamics methodology in our study.

One of the main tools of system dynamics methodology is the causal loop diagramming. Causal loop diagrams describe the qualitative relationships between the variables in a system. They help in visualizing our mental models and in the discussion of the important feedback loops that govern the system. As an example, see the causal loop diagram of our model in the next chapter (see Figure 4.1).

Stock-flow diagramming is another important tool of the system dynamics methodology. Stock-flow diagrams include the quantitative relationships in a system and they are usually constructed based on qualitative relationships depicted by causal loop diagrams. These diagrams have three main components: stocks, flows and convertors, where convertors stand for auxiliary variables and parameters. Stocks represent important accumulations in a system and, thus, these variables have inertia; they cannot be changed instantaneously, their movement is gradual. They are key elements in creation of the endogenous dynamics of a system. They can only be changed by their flows. Flow variables represent how stocks are accumulated and depleted over time;

the sum of all flows connected to a stock give its net rate of change over time. If we hypothetically freeze time, flows (production, death rates...) would be undefined and they would cease to exist. However, stocks (inventory, population...) would still be meaningful. The rest of the intermediate variables in a model are represented by convertors. Parameters are also represented by convertors and they can potentially become variables depending on the boundaries of the problem.

In a stock-flow model, visually the stocks are typically shown as a rectangle, the flows are shown as pipes flowing in and out of stocks. The convertors are either shown by circles or they are merely represented with variable names without a shape attached to them. As an example, see the stock-flow diagram of our model in the next chapter (see Figure 4.2). The stocks have the general conservation equation:

$$\frac{d(Stock)}{dt} = Inflows - Outflows \tag{3.1}$$

that represents their rate of change.

In system dynamics methodology, after the model is completed, its validity is thoroughly tested by structural and behavior (output) tests. Once the model validity is established with a satisfactory level of confidence, research problem can be addressed by a series of simulation experiments by making use of the model.

4. OVERVIEW OF THE MODEL

Human body uses EPO to respond to changes in oxygen levels by regulating the red blood cell levels. The major factors and interactions in our model that represent this regulation are summarized in Figure 4.1, which shows the causal relations between the most important variables in our system. If a change in a variable at the tail of the arrow causes a change in the same direction on the variable at the arrowhead, then this is represented with a "+" sign at the arrowhead. The opposite of this case (a change in opposite direction) is represented as a "-" sign at the arrowhead.

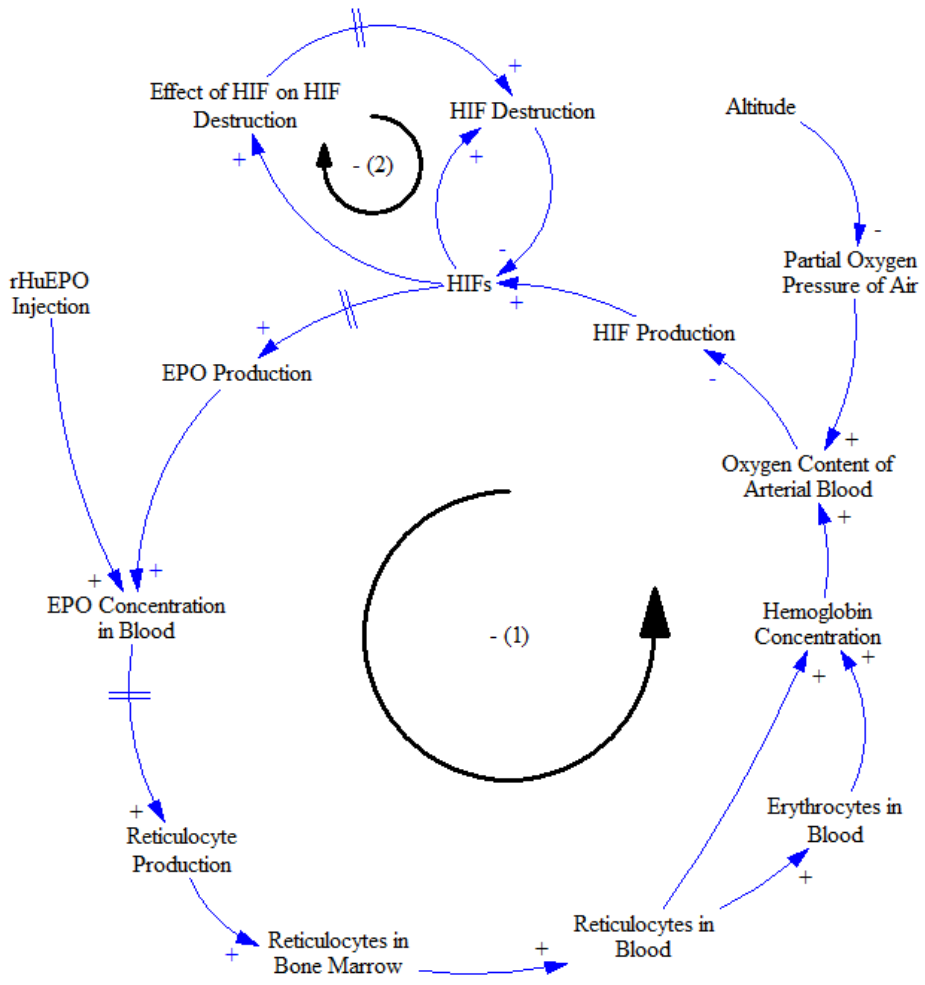


Figure 4.1. The simplified causal loop diagram displaying the major interactions.

The sign of a feedback loop is the arithmetic product of all signs around the loop. There are two fundamentally different types of loops: (+) loops are self-reinforcing, unstable in their dynamics. In isolation, they produce either increasingly increasing, or increasingly declining dynamics. On the other hand, (-) loops are equilibrium-seeking, they are stable. In isolation, they either increase or decrease in a stable way so as to seek an equilibrium level. One final symbol used in causal-loop diagrams is two parallel lines (//) to indicate that there is a significant time delay on a cause and effect link.

The most important causal loop in our model is the balancing loop numbered 1. This loop controls the natural red blood cell levels in the human body. Our model captures the two situations that the oxygen content of arterial blood responds to: (1) a decrease in partial pressure of oxygen in air through a change in altitude; (2) a decrease in hemoglobin concentration. Either way, the lowered *Oxygen Content of Arterial Blood* increases *HIF (hypoxia inducible factor)* level. HIF molecules are normally destroyed in the presence of oxygen. However, lowered oxygen level allows them to survive and increases their production rate [3]. Increasing *HIF* level triggers *EPO (erythropoietin) Production*. The newly produced EPO hormones then flow into the blood stream where they go into the bone marrow and facilitate the proliferation and the growth of the *Reticulocytes in Bone Marrow*. These reticulocytes are then released into the blood stream where they mature and become erythrocytes. With the arrival of new reticulocytes and new erythrocytes, the *Hemoglobin Concentration* in the blood increases, expanding the oxygen capacity of the blood. The increased capacity allows more oxygen molecules to enter the bloodstream. With this, *Oxygen Content of Arterial Blood* increases. As an external (input) variable, *rHuEPO (recombinant human erythropoietin) Injection* directly increases the *EPO Concentration in Blood*. Loop 2 represents the negative feedback on HIF by itself. Increasing HIF level starts to produce various enzymes that degrade HIF molecules after a delay [3, 15]. The effect of these enzymes is represented as *Effect of HIF on HIF Destruction*. As the number of HIF molecules starts to increase, with a time delay, the *Effect of HIF on HIF Destruction* increases the *HIF Destruction*. Increasing *HIF Destruction* has a negative effect on HIF level. This balancing loop lowers the EPO production during

long hypoxic (low blood oxygen level) periods by lowering the HIF level.

Our model mainly consists of five sectors: blood-cells sector, oxygen content sector, HIF sector, EPO sector and rHuEPO-injection sector. A simplified stock-flow diagram can be found in Figure 4.2. The blood-cells sector of our model represents the production and aging structure of red blood cells. Reticulocytes are produced with the effect of *EPO Concentration*. They mature into erythrocytes and die. The oxygen sector takes on from this point. The hemoglobin level of blood is obtained from the number of erythrocytes and reticulocytes. Then, based on the altitude, the partial pressure of oxygen in arteries and the oxygen saturation in arteries are calculated. Altitude also affects the plasma volume of blood. The oxygen content of arterial blood is calculated using the oxygen saturation in arteries and total hemoglobin level [16]. Any change in this content leads to a change in HIF level. In the EPO sector, changing levels of HIF lead to the production of EPO. Changing levels of HIF also influence their own HIF destruction outflow, which is depicted in the HIF sector. rHuEPO-injection sector captures the subcutaneous (SC) injection of rHuEPO. The change in *EPO Concentration* affects the reticulocyte production, thus, closes the loop. *EPO Concentration* also affects the maturation time of reticulocytes in blood [17]. Some amount of EPO goes to peripheral tissues and, after some delay, re-enters into the blood stream. A more technical explanation of the model will be given in Chapter 5.

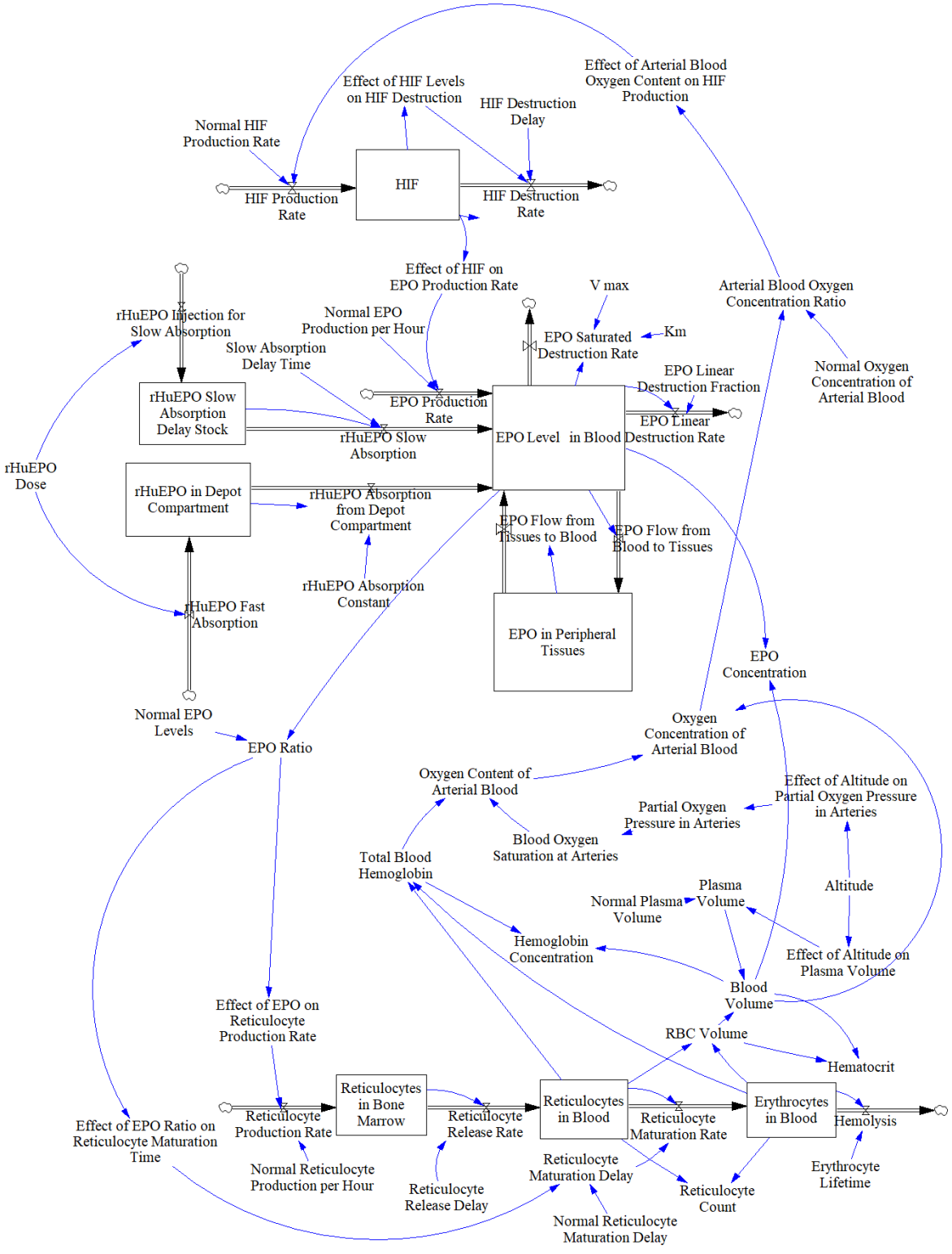


Figure 4.2. The simplified overall model (stock-flow) diagram.

5. DESCRIPTION OF THE MODEL

As mentioned before, our model consists of five sectors. (1) Blood-cells sector includes three stocks: *Reticulocytes in Bone Marrow*, *Reticulocytes in Blood* and *Erythrocytes in Blood* (Figure 5.1). These represent the related cell levels in blood and their units are "cells". The blood-cells structure also has an information delay for the effect of EPO (erythropoietin) on reticulocyte maturation delay. (2) Oxygen-content sector does not have any important stocks. However, it has several information delays formulated with the *SMOOTH* function of Vensim software (Figure 5.4). (3) The HIF (hypoxia inducible factor) sector essentially represents the production and destruction of the *HIF* stock (Figure 5.7). (4) EPO sector has the *EPO Level in Blood* and *EPO in Peripheral Tissues* stocks (Figure 5.10). These represent the EPO levels in blood and tissues respectively. Their units are "International Units (IU)". One IU of EPO exerts the same red cell production stimulating activity in rodents as 5 μmol cobaltous chloride [18]. The *EPO Level in Blood* stock is then converted into milliunits (mU) and divided by the *Blood Volume* to find the *EPO Concentration* in blood which is usually represented as mU/mL. (5) Finally, the rHuEPO (recombinant human erythropoietin)-injection sector consists of *rHuEPO Dose for Fast Absorption*, *rHuEPO in Depot Compartment*, *rHuEPO Dose for Slow Absorption* and three material delay stocks: *rHuEPO Delay Stock1*, *rHuEPO Delay Stock2* and *rHuEPO Delay Stock3* (Figure 5.12). The units for all these stocks are IU. The absorption of rHuEPO after subcutaneous injection happens in two parts: fast absorption and slow absorption. Fast absorbed part first waits in a depot compartment and, after a delay, mixes into the blood stream. The slow absorbed part has a lag time of around 12 hours before it mixes into the blood stream [17]. The structure of five sectors of the model defined above, their stocks, other main variables and formulations will be described in Section 5.2 in more detail.

5.1. Assumptions

The model is built around a healthy male person. Initial stock values for blood cells and some parameters are chosen accordingly. The time unit of the model (for all variables and parameters) is hours. Time horizon in simulation experiments ranges between 3 to 84 days (72 to 2000 hours), depending on the purpose of the experiment and relevant data.

The *HIF* stock is a representative, conceptual stock. The enzymes and factors in human body change rapidly. The exact values of these enzymes are of no interest for this model. The model is only interested in HIF's effect on its own destruction and EPO production. HIF has a conceptual unit of "factor".

It is assumed that every reticulocyte cell matures into erythrocytes. There is no death of reticulocyte cells in the model. This is not the case in real life. However, this assumption would not have a major impact in our findings since the death rate of reticulocytes is much smaller than their maturation rate in a healthy person [8]. For this reason, the death rate of reticulocytes can be ignored for our purposes.

The arterial oxygen content is found only by obtaining the oxygen level bound to hemoglobin. The oxygen dissolved in other parts of the blood stream is ignored as it is a relatively small amount [16].

According to studies, increasing EPO level reduces the plasma volume, therefore, reduces the total blood volume [19]. However, this effect of EPO is minimal, so the model assumes no relationship between EPO level and the plasma volume.

Hemoglobin molecules require iron to be synthesized. Therefore, the iron supply can be a limiting factor for red blood cell production [20]. The model assumes the necessary iron is supplied for red blood cell production. It is also assumed that there are adequate number of stem cells for reticulocyte production at all times.

5.2. Technical Description of the Model

5.2.1. Blood-Cells Sector

The blood-cells sector represents the production, maturation and the death of red blood cells in the body. The main production site of the red blood cells is the bone marrow [8]. The presence of EPO ensures that the stem cells in the bone marrow grow and become reticulocytes. Then these reticulocytes are released into the blood stream where they mature into erythrocytes. The stock-flow diagram of blood-cells sector can be found in Figure 5.1.

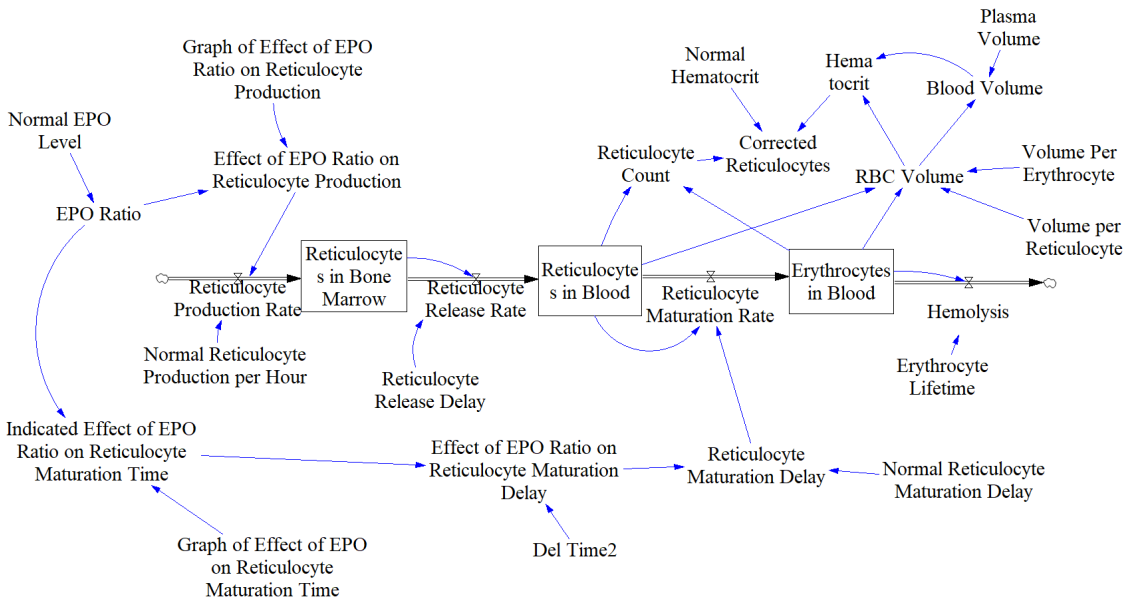


Figure 5.1. The diagram of blood-cells sector.

This sector starts with the production of reticulocytes:

$$\begin{aligned} \textit{Reticulocyte Production Rate} = \\ \textit{Normal Reticulocyte Production per Hour} \\ \times \textit{Effect of EPO Ratio on Reticulocyte Production} \end{aligned} \quad (5.1)$$

The value for *Normal Reticulocyte Production per Hour* is taken from a study [8]. The production flow of reticulocytes is controlled by the *EPO Ratio* variable. This variable is calculated as *EPO Level in Blood* divided by *Normal EPO Level*. The graphical function for the *Effect of EPO Ratio on Reticulocyte Production Rate* can be found in Figure 5.2.

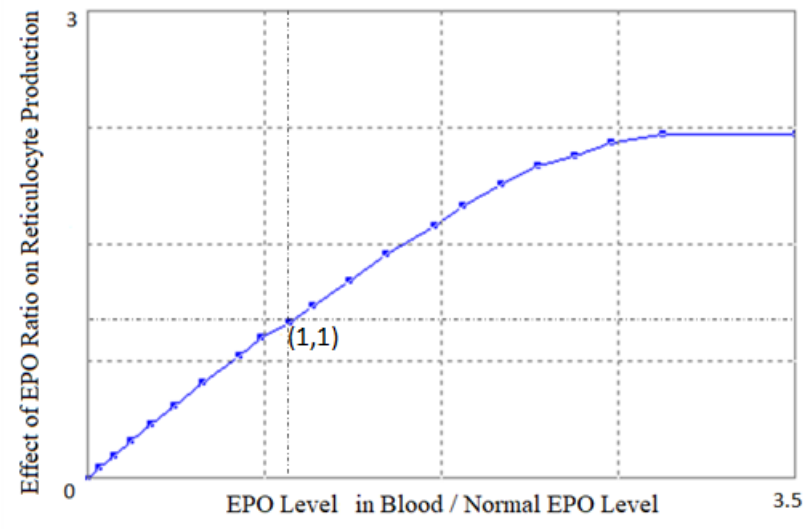


Figure 5.2. The effect of EPO ratio on reticulocyte production.

According to the effect graph, as *EPO Level in Blood* increases from its normal level, reticulocyte production rate starts to increase. However, after some value of EPO, the receptors of the stem cells in bone marrow are filled and cannot accept any more EPO. To represent this, the effect function saturates and stays constant. As *EPO Level in Blood* decreases from its normal level, reticulocyte production rapidly decreases. When the *EPO Level in Blood* comes close to zero, reticulocyte production stops.

The reticulocytes then stay in the bone marrow for around three to four days [8]. The flow *Reticulocyte Release Rate* is calculated as follows:

$$Reticulocyte\ Release\ Rate = \frac{Reticulocytes\ in\ Bone\ Marrow}{Reticulocyte\ Release\ Delay} \tag{5.2}$$

This flow represents the release of reticulocytes into the blood stream. They stay around one to two days in blood and mature into erythrocytes [8]. This maturation is affected by *EPO Level in Blood*. As *EPO Level in Blood* increases, the maturation delay of the reticulocytes also increases. This effect is a delayed effect and is modeled as an information delay using the *SMOOTH* function of Vensim software. The graphical function for the *Effect of EPO Ratio on Reticulocyte Maturation Delay* can be found in Figure 5.3. The presence of EPO increases the maturation time to as high as around six days [17].

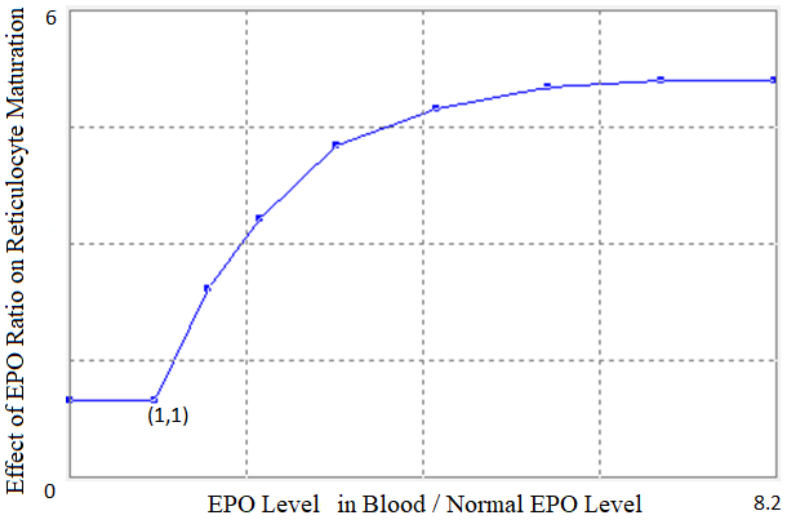


Figure 5.3. The effect of EPO ratio on reticulocyte maturation delay.

The reticulocytes then mature and become erythrocytes through the flow *Reticulocyte Maturation Rate*:

$$Reticulocyte\ Maturation\ Rate = \frac{Reticulocytes\ in\ Blood}{Reticulocyte\ Maturation\ Delay} \tag{5.3}$$

The erythrocytes have a lifetime of around 120 days [8]. The *Hemolysis* flow represents this. *Hemolysis* refers to the destruction of erythrocytes. The equation for

it is as follows:

$$Hemolysis = \frac{Erythrocytes\ in\ Blood}{Erythrocyte\ Lifetime} \tag{5.4}$$

Various blood values are also calculated in this sector. *Reticulocyte Count* is the percentage of reticulocytes in blood with respect to the total of red blood cells (reticulocytes and erythrocytes) and calculated as such in the model. *Hematocrit* is the volume ratio of red blood cells to the total blood volume. *Hematocrit* is also calculated in this part by finding the volume of reticulocytes and erythrocytes in blood. The volume of a reticulocyte is around 115 fL whereas the volume of erythrocytes is around 80 fL to 100 fL [21]. There is also the *Corrected Reticulocytes* variable which corrects the *Reticulocyte Count* variable by the change in *Hematocrit*. The equations for *Reticulocyte Count*, *Hematocrit* and *Corrected Reticulocytes* are given below.

$$Reticulocyte\ Count = \frac{Reticulocytes\ in\ Blood}{(Erythrocytes\ in\ Blood\ +\ Reticulocytes\ in\ Blood)} \tag{5.5}$$

$$\begin{aligned} RBC\ Volume = {} & Erythrocytes\ in\ Blood \times Volume\ Per\ Erythrocyte \\ & + Volume\ per\ Reticulocyte \times Reticulocytes\ in\ Blood \end{aligned} \tag{5.6}$$

$$Hematocrit = \frac{RBC\ Volume}{Blood\ Volume} \tag{5.7}$$

$$Corrected\ Reticulocytes = Reticulocyte\ Count \times \frac{Hematocrit}{Normal\ Hematocrit} \tag{5.8}$$

5.2.2. Oxygen Content Sector

In the oxygen content sector, the arterial oxygen content is calculated based on several variables. The stock-flow diagram of this sector can be found in Figure 5.4.

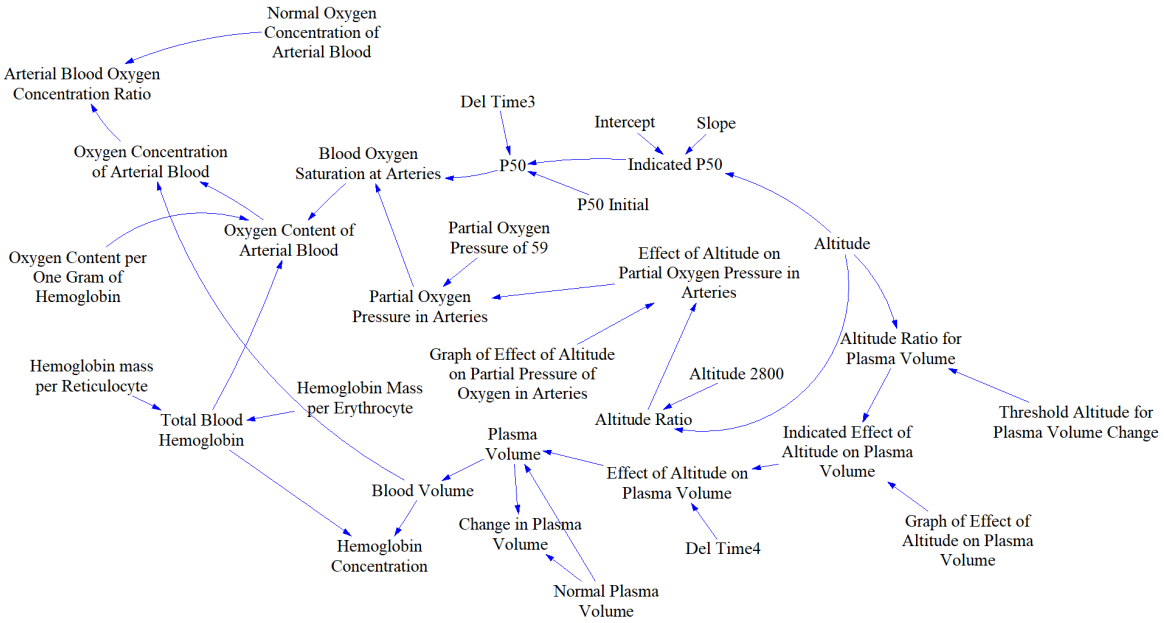


Figure 5.4. The diagram of oxygen sector.

The first important variable is the *Total Blood Hemoglobin*. It is the sum of the hemoglobin content of reticulocytes and erythrocytes. Studies report around 27 gr/dL of hemoglobin in reticulocytes and around 33 gr/dL of hemoglobin in erythrocytes [21]. These values are then converted into gr/cells and the *Total Blood Hemoglobin* is found in grams. In most blood tests, the hemoglobin concentration is reported. The formula for *Hemoglobin Concentration* is given below. The unit of *Hemoglobin Concentration* is gr/dL.

$$Hemoglobin\ Concentration = \frac{Total\ Blood\ Hemoglobin}{Blood\ Volume} \tag{5.9}$$

To calculate the *Oxygen Concentration of Arterial Blood*, we need the *Blood Oxygen Saturation Percentage in Arteries*. Hemoglobin is the main oxygen transport molecule in the blood. A single hemoglobin molecule has four oxygen binding sites. Oxygen binds to the hemoglobin in a cooperative manner meaning that; if one molecule of oxygen is already bound to hemoglobin, the second oxygen molecule can be bound to

hemoglobin more easily. This relationship is represented in the sigmoid shaped oxygen hemoglobin dissociation curve (Figure 5.5). This curve relates the partial pressure of oxygen in the blood to the oxygen saturation. This curve can be expressed as Hill's exponent model [22].

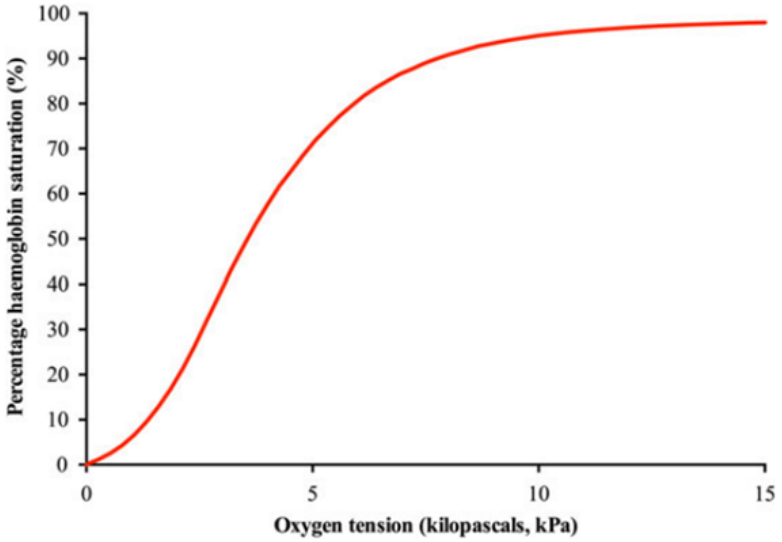


Figure 5.5. The standard human oxygen hemoglobin dissociation curve from [16].

In our model, the saturation is calculated based on the following formula:

$$Blood\ Oxygen\ Saturation\ in\ Arteries = \frac{(\frac{Partial\ Pressure\ of\ Oxygen\ in\ Arteries}{P50})^{2.7}}{1 + (\frac{Partial\ Pressure\ of\ Oxygen\ in\ Arteries}{P50})^{2.7}} \quad (5.10)$$

P50 is the partial pressure of oxygen when the blood is 50% saturated. The constant 2.7 is the Hill coefficient for normal blood [22]. It represents the cooperativity between oxygen molecules and hemoglobin. Partial oxygen pressure is affected by the altitude change. As the altitude increases, the partial pressure of oxygen in air decreases, which in turn decreases the partial pressure of oxygen in the arteries. This relationship is modeled based on the real data reported in a study [23]. Inserting the *Partial Pressure of Oxygen* to the above formula will give the *Blood Oxygen Saturation in Arteries*.

The oxygen hemoglobin dissociation curve (Figure 5.5) can shift to the right or to the left based on various factors [16]. A left shift indicates a higher affinity for oxygen and causes P50 to decrease. A right shift on the other hand lowers the affinity for oxygen and increases the P50 value. The normal P50 value for humans is 26.7 mmHg [24]. This curve is affected from changes in altitude after a delay. This delayed effect is represented in our model and is validated in model validation section.

Finally, we have all the necessary variables to calculate the *Oxygen Content of Arterial Blood*:

$$\begin{aligned} \textit{Oxygen Content of Arterial Blood} = \textit{Total Blood Hemoglobin} \times 1.34 \\ \times \textit{Blood Oxygen Saturation in Arteries} \end{aligned} \quad (5.11)$$

The number 1.34 represents the oxygen content for one gram of hemoglobin. This equation gives the oxygen content of blood in mL. The *Oxygen Content of Arterial Blood* is then divided by *Blood Volume* to find the *Oxygen Concentration of Arterial Blood*, which is in mL/dL.

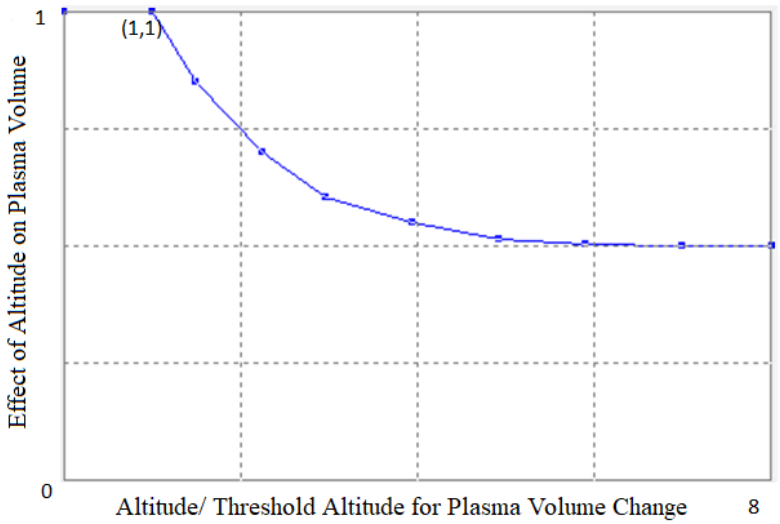


Figure 5.6. The effect of altitude on plasma volume.

Altitude also affects the blood plasma volume. As altitude increases, the plasma volume starts to decrease after some delay. The graphical function for the *Effect of Altitude on Plasma Volume* can be found in Figure 5.6. Again, the validation for this effect is carried out in Chapter 6 of this thesis.

5.2.3. HIF Sector

The HIF sector represents the production of hypoxia inducible factors in response to the changes in arterial oxygen content. Their main purpose is to carry the transcriptional message for EPO hormone from nucleus to ribosomes. The stock-flow diagram of this sector can be found in Figure 5.7.

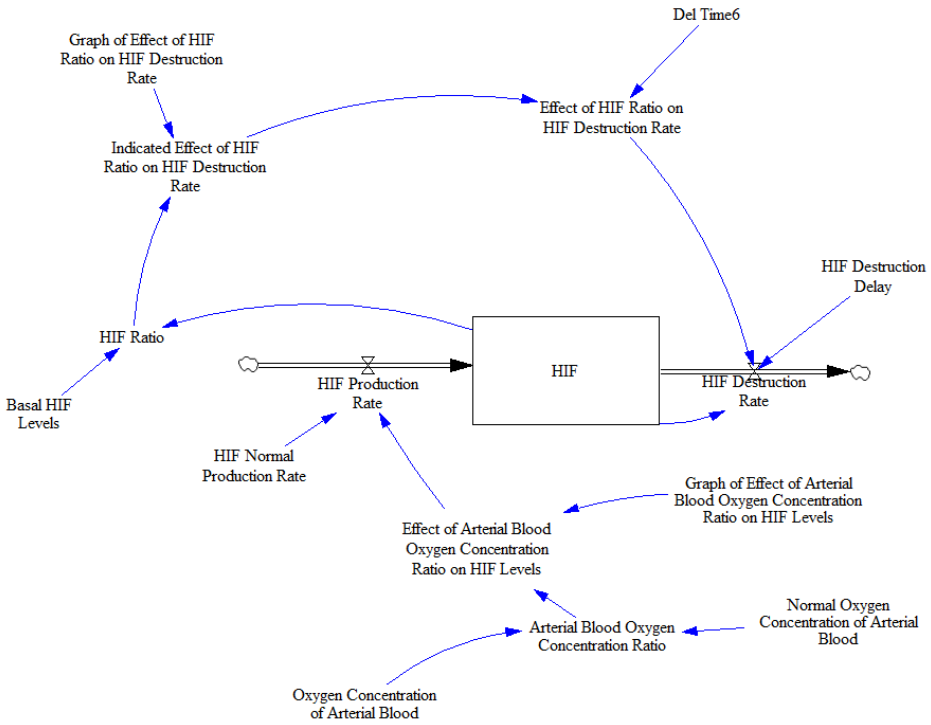


Figure 5.7. The diagram of HIF sector.

This sector starts with the calculation of the *Arterial Oxygen Concentration Ratio*, which is the ratio of *Oxygen Concentration of Arterial Blood* to *Normal Oxygen Concentration of Arterial Blood*. This ratio then affects the *HIF Production Rate*. The

equation for *HIF Production Rate* is given below.

$$\begin{aligned} &\textit{HIF Production Rate} = \textit{HIF Normal Production Rate} \\ &\qquad \times \textit{Effect of Arterial Oxygen Concentration Ratio on HIF Levels} \end{aligned} \tag{5.12}$$

As the oxygen concentration decreases from its normal value, HIF production increases. As discussed before, HIF is a representative, conceptual variable. The graphical function for the effect of arterial blood concentration ratio on HIF levels can be found in Figure 5.8. According to the graph, decreasing arterial blood concentration ratio increases HIF production. However, this increase in HIF production is bounded and the effect of decreasing arterial blood concentration ratio saturates. This is consistent with the fact that HIF is a transcriptional factor and its supply is limited. Also, an increase in oxygen levels above their normal values causes HIF level to decline and eventually go to zero.

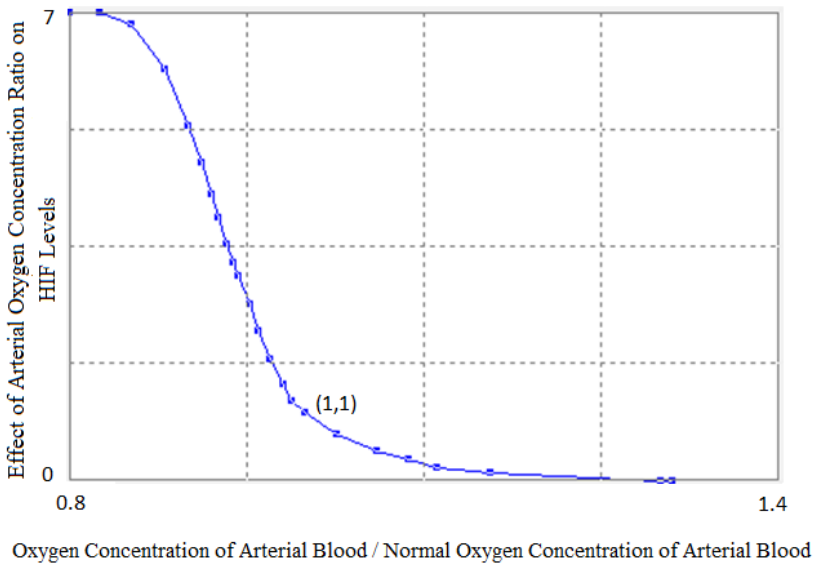


Figure 5.8. The effect of arterial oxygen concentration ratio on HIF levels.

When the HIF level starts to increase from its normal level, after a delay, various enzymes that degrade HIF are produced in human body. In our model, we first start

by calculating HIF Ratio by dividing *HIF* with *Basal HIF Levels*. This ratio has an effect on *HIF Destruction Rate*. The formula for *HIF Destruction Rate* is given below.

$$HIF\ Destruction\ Rate = \frac{HIF}{HIF\ Destruction\ Delay} \times Effect\ of\ HIF\ Ratio\ on\ HIF\ Destruction\ Rate \quad (5.13)$$

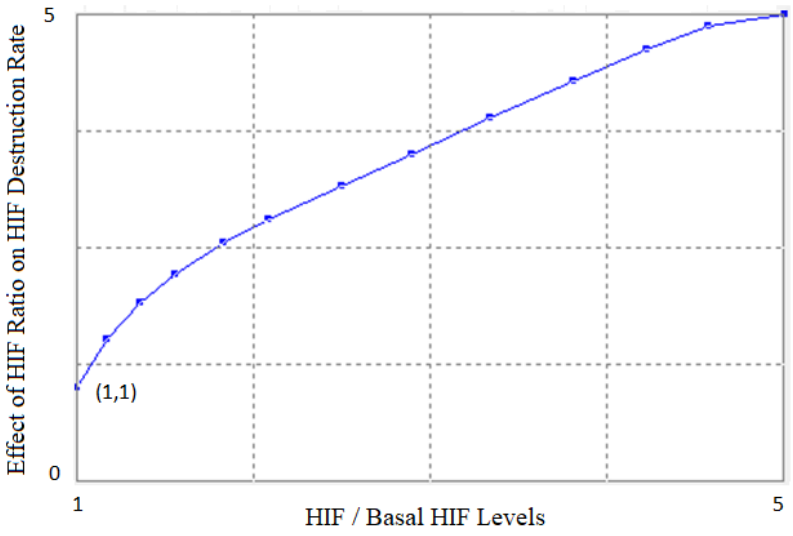


Figure 5.9. The effect of HIF ratio on HIF destruction rate.

The graphical function for the *Effect of HIF Ratio on HIF Destruction Rate* can be found in Figure 5.9. As *HIF Ratio* increases, the *HIF Destruction Rate* also increases. However, the effect saturates after some value since these degrading enzymes are not infinite in human body. Moreover, there is a time delay before this effect takes place, which is formulated using the *SMOOTH* function of Vensim software.

5.2.4. EPO Sector

This sector represents pathways of EPO in the body, from its production to its destruction. The stock-flow diagram of this sector can be found in Figure 5.10.

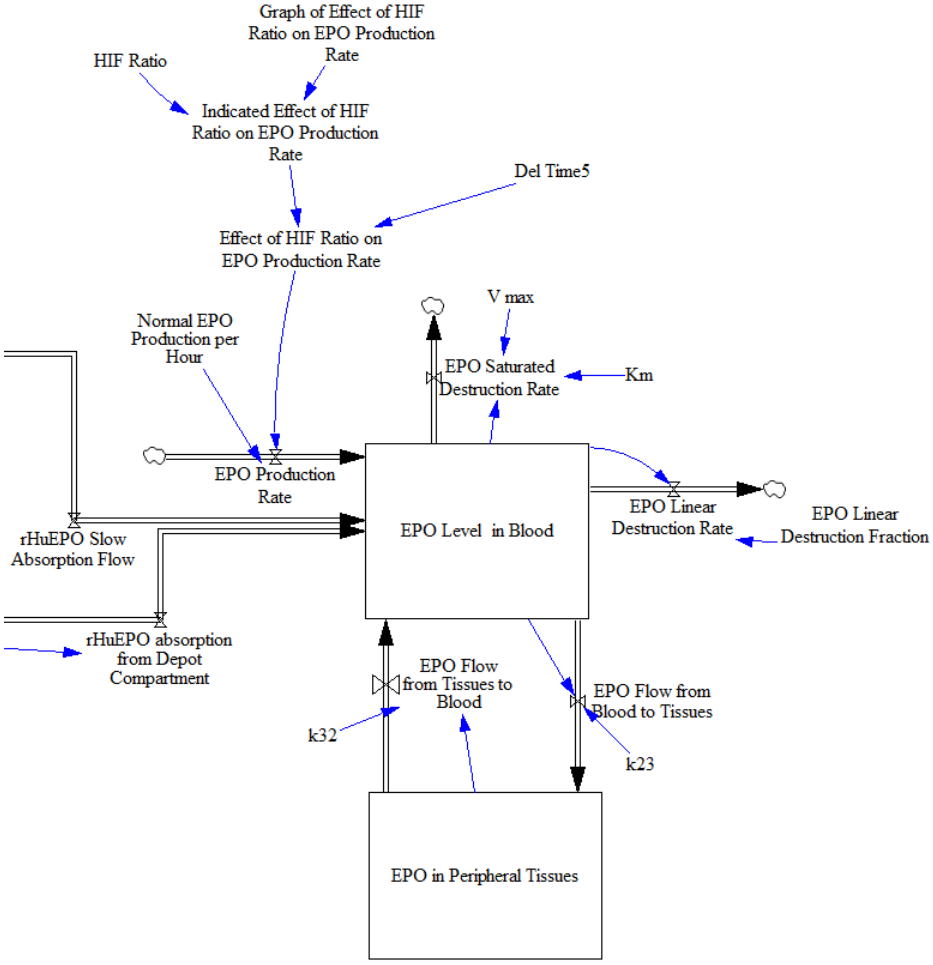


Figure 5.10. The diagram of EPO sector.

As discussed before, HIF is the factor that is responsible for facilitating EPO production in kidneys with a delay of around three hours [9]. The equation for *EPO Production Rate* is given below.

$$\begin{aligned} \textit{EPO Production Rate} = \textit{Normal EPO Production per Hour} \\ \times \textit{Effect of HIF Ratio on EPO Production Rate} \end{aligned} \quad (5.14)$$

The graphical function for the *Effect of HIF Ratio on EPO Production Rate* can be found in Figure 5.11. Increasing *HIF Ratio* promotes EPO production. However,

the number of EPO-producing cells in the body is limited. For this reason, the *Effect of HIF Ratio on EPO Production Rate* becomes constant after some HIF ratio level. On the other hand, the presence of HIF molecules is required for EPO production. Accordingly, when HIF level decreases, EPO production decreases as well and becomes zero when no HIF molecule is available.

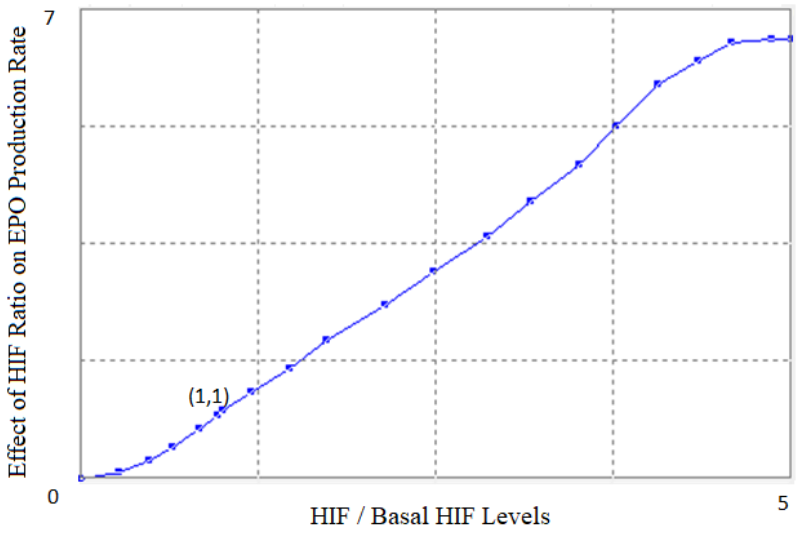


Figure 5.11. The effect of HIF ratio on EPO production.

The *EPO Production Rate* flow goes into the *EPO Level in Blood* stock. In human body, EPO hormone is not always in the blood stream. The hormone can sometimes pass into the tissues and flow back into the blood stream after a delay [17]. The *EPO in Peripheral Tissues* stock and the flows between it and *EPO Level in Blood* stock represent this phenomenon. Studies show that there exist two ways for EPO to be eliminated from the body. The first one is the *Linear Destruction Rate* flow which represents the clearance of EPO from the body by ways of urination etc. The second flow is called the *EPO Saturated Destruction Rate*, which represents the destruction of EPO after bonding with its target cells. This is called *Saturated Destruction* since the number of receptor cells for EPO in body is limited [3,10,17]. The equations for *Linear Destruction Rate* and *EPO Saturated Destruction Rate* are given below. The constant *Vmax* is the maximum elimination rate of the saturable destruction and K_m is the

EPO level at which the saturable destruction operates at half of its maximum [17].

$$\begin{aligned} \textit{EPO Linear Destruction Rate} &= \textit{EPO Level in Blood} \\ &\times \textit{EPO Linear Destruction Fraction} \end{aligned} \tag{5.15}$$

$$\textit{EPO Saturated Destruction Rate} = \frac{\textit{Vmax} \times \textit{EPO Level in Blood}}{K_m + \textit{EPO Level in Blood}} \tag{5.16}$$

The *EPO Level in Blood* stock is then converted to mU and divided by the *Blood Volume* to calculate the *EPO Concentration*.

5.2.5. rHuEPO-Injection Sector

The rHuEPO-injection sector represents the pathways of rHuEPO drug after its injection into the body. The injection is a *subcutaneous (under the skin) (SC)* injection. This injection method prevents the drug from rapidly flowing into the blood stream where it will be eliminated. The unit for the dosage of rHuEPO is IU (international units). The stock-flow diagram of this sector can be found in Figure 5.12.

We start with the variable *Dose*, which represents the injection dose of rHuEPO. Then, based on the *Dose*, the bioavailability of the drug F is calculated:

$$F = F_0 + \frac{E_{max} * \textit{Dose}}{ED_{50} + \textit{Dose}} \tag{5.17}$$

The constant F_0 is the minimum absolute bioavailability, E_{max} represents the maximum increase in bioavailability with *Dose* and finally the constant ED_{50} is the *Dose* that results in a 50% increase in F [17].

Around 1% of the dosage is assumed to be lost [17]. The body can absorb the drug in two different ways: fast and slow pathways. For the two pathways, the dosage

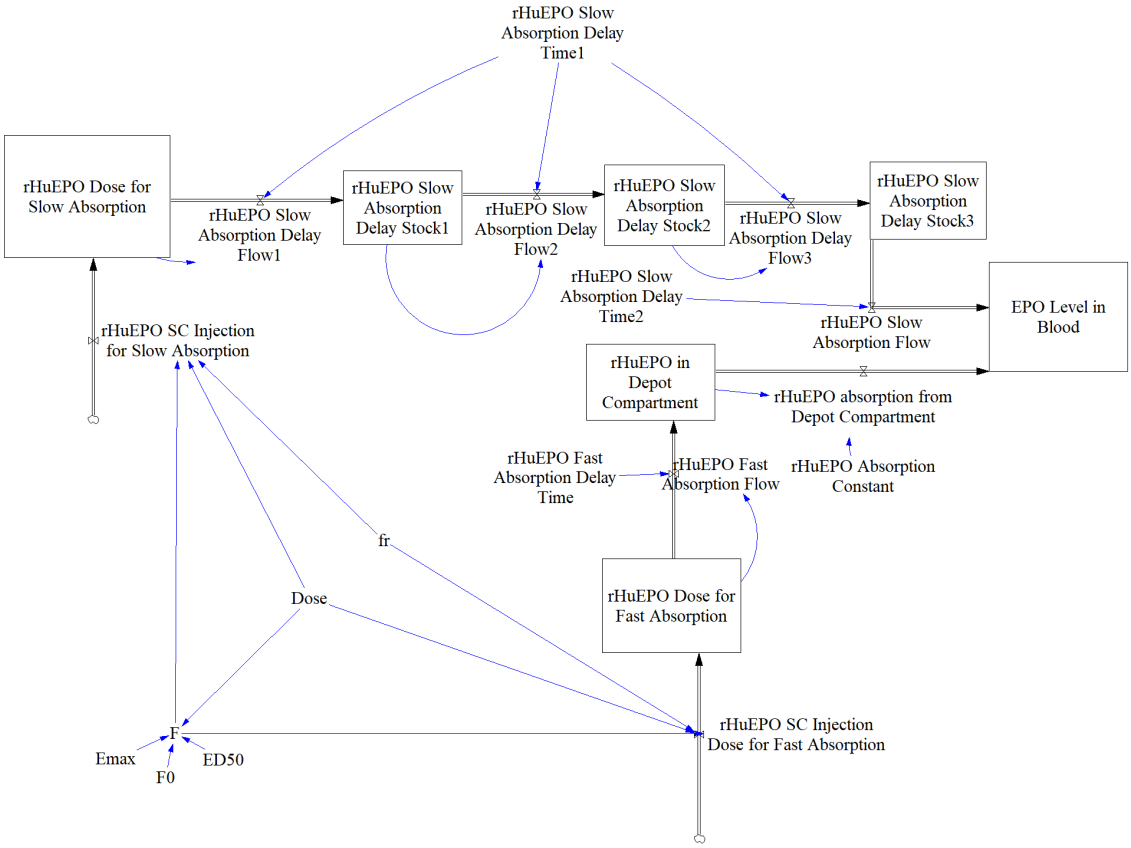


Figure 5.12. The diagram of rHuEPO-injection Sector.

is calculated by the formulas below:

$$rHuEPO\ SC\ Injection\ for\ Slow\ Absorption = Dose \times (F - 0.01) \times (1 - fr) \quad (5.18)$$

$$rHuEPO\ SC\ Injection\ for\ Fast\ Absorption = Dose \times (F - 0.01) \times fr \quad (5.19)$$

The constant fr represents the ratio of bioavailable dosage that goes into the fast absorption. These flows can be modified according to different dosage regimens which will be discussed in Chapter 7.

The fast-absorbed drug goes into the stock called *rHuEPO in Depot Compartment*. The drug is then absorbed and released into the blood stream. This is represented by the flow *rHuEPO Absorption from Depot Compartment* that flows into the *EPO Level in Blood* stock. The formula for this flow is given below.

$$rHuEPO\ Absorption\ from\ Depot\ Compartment = rHuEPO\ in\ Depot\ Compartment \times rHuEPO\ Absorption\ Constant \quad (5.20)$$

The slow absorbed drug is first subjected to a third order material delay. The general equation for a 1^{st} order material delay outflow is given below. After three cascaded 1^{st} order delays (meaning a third order delay), the drug flows into the *EPO Level in Blood* stock.

$$Delay\ Flow = \frac{Delay\ Stock}{Delay\ Time} \quad (5.21)$$

6. MODEL VALIDATION

In this chapter, model validation is carried out by several scenarios starting with an equilibrium run. Note that the structural validation of our model is followed through the entire model construction process by comparing the model sub-structures and their corresponding behaviors to the functional relationships and data presented by the studies that exist in the literature. We formulate and improve the model sub-structures as necessary so that we depict the relationships adequately and produce valid behaviors.

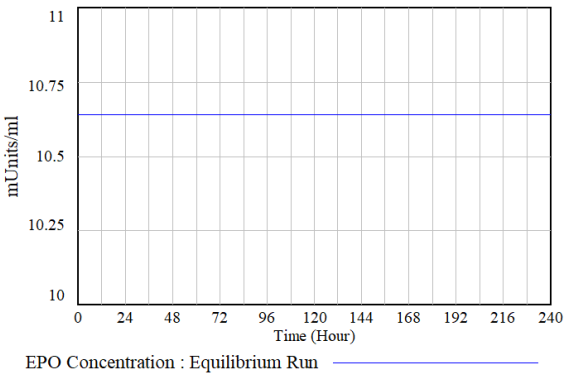
6.1. Equilibrium Run

In normal conditions, the human body maintains homeostasis. Because of this fact, the model is expected to stay in equilibrium without any disturbance. To verify this, an equilibrium run is made. The run is assumed to be taken at the sea level with an altitude of zero meters. Figure 6.1 shows the behavior of *EPO (erythropoietin) Concentration*, *Hematocrit* value, *Hemoglobin Concentration* and *Reticulocyte Count*.

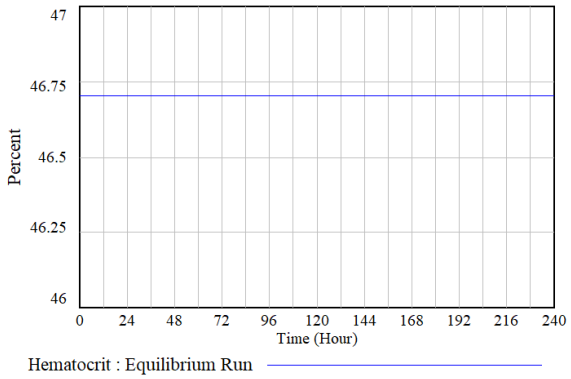
As expected, the variables remain constant. Equilibrium EPO concentration is 10.64 mU/mL. This concurs with the reported basal range of EPO concentration between 6 to 32 mU/mL [3]. The hematocrit value is 46.7% which is again between the normal male range of 40% to 54%. Hemoglobin concentration in equilibrium is 15.05 gr/dL. The normal range for Hemoglobin concentration is 14-18 gr/dL [25]. Finally, the equilibrium reticulocyte count is 0.983% and the normal range for reticulocyte count is between 0.5% and 2.5% [26]. The results confirm that our model is able to simulate a healthy male human whose body is in homeostasis.

6.2. Altitude Experiments

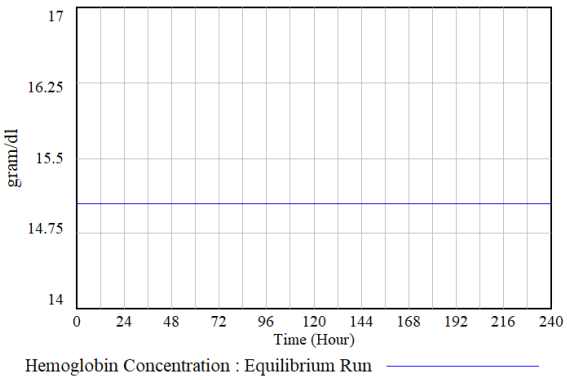
We conduct two altitude runs to find out if the model-generated dynamics behave similar to real data. First run includes staying at an altitude of 2800 m and the second



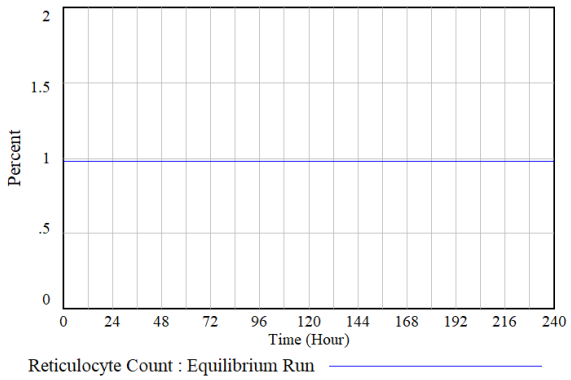
(a) *EPO Concentration*



(b) *Hematocrit*



(c) *Hemoglobin Concentration*



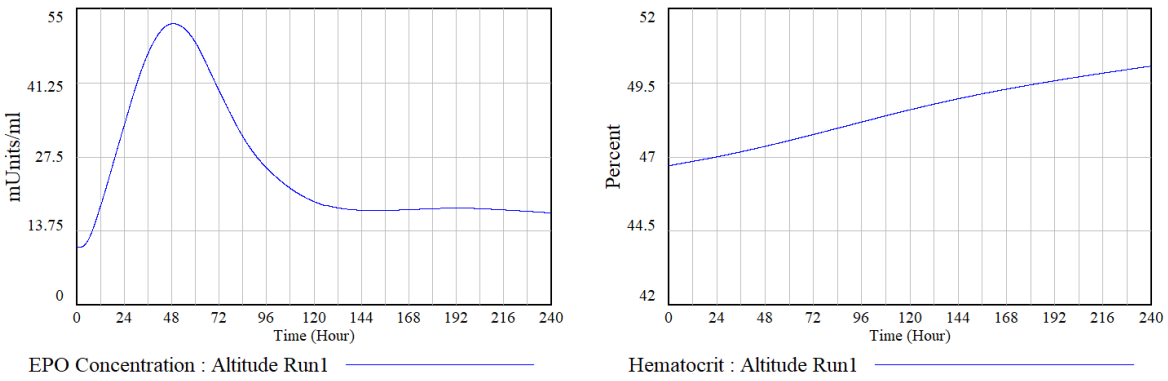
(d) *Reticulocyte Count*

Figure 6.1. Equilibrium run results of main variables.

run include staying at an altitude of 4340 m.

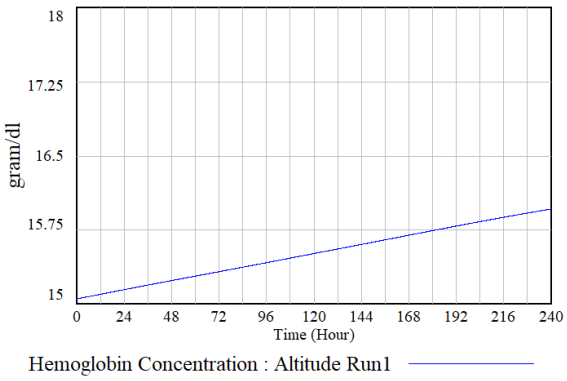
6.2.1. Stay at an Altitude of 2800 m

The model is initialized at an altitude of 2800 m and run for 240 hours. The behavior of *EPO Concentration*, *Hemoglobin Concentration* and *Hematocrit* values are observed for comparison with the real data. The model output for these variables and for *Reticulocyte Count* can be found in Figure 6.2.

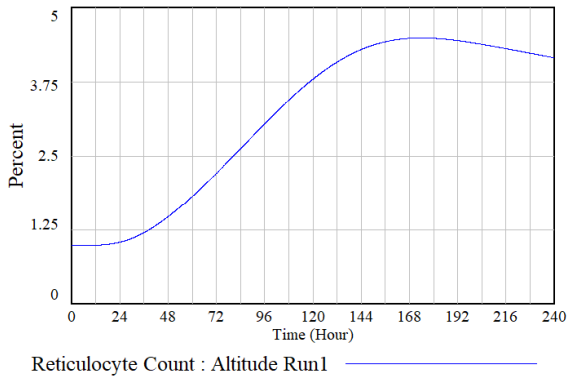


(a) *EPO Concentration*

(b) *Hematocrit*



(c) *Hemoglobin Concentration*

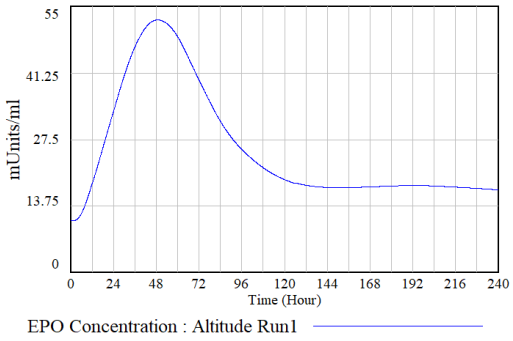


(d) *Reticulocyte Count*

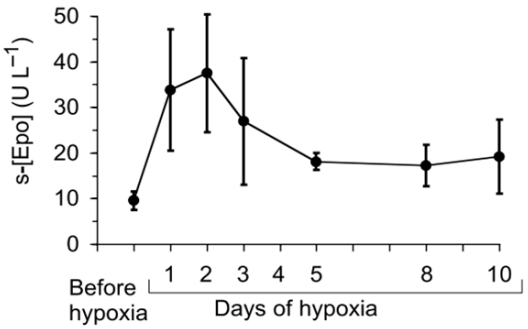
Figure 6.2. Model-generated dynamics for "Stay at an Altitude of 2800 m" scenario.

EPO Concentration reaches a peak around 48 hours and then decreases down to almost twice its normal value around 120 hours. This is consistent with the previous studies [2, 27]. Figure 6.3 shows the comparison of *EPO Concentration, Hemoglobin Concentration and Hematocrit* value of model-generated data and real data. The graphs for the real data are taken from Berglund *et al.* [27]. The unit for the *Hemoglobin Concentration* for the real data is reported in gr/L. The model-generated data is in gr/dL. This is the reason for the scale difference between two graphs. *EPO Concentration* is in the confidence intervals reported in real data for all points, except for day two where it is slightly above the upper bound. *Hemoglobin Concentration* is between all the reported confidence limits. *Hematocrit* starts slightly above the upper bound of the first confidence interval. Then, it is in the confidence intervals for the two other

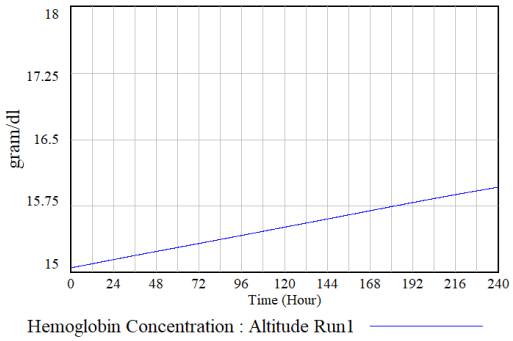
points given. According to the results, our model captures the real dynamics quite well. The model's purpose is dynamic pattern projection rather than point prediction, so we can say that the model is valid for this scenario.



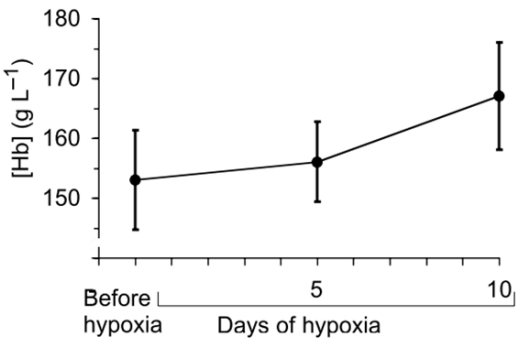
(a) *EPO Concentration*



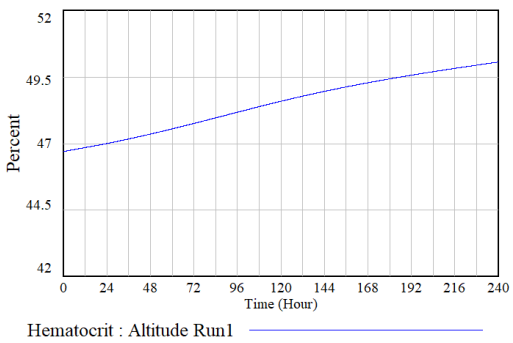
(b) *EPO Concentration* of [27]



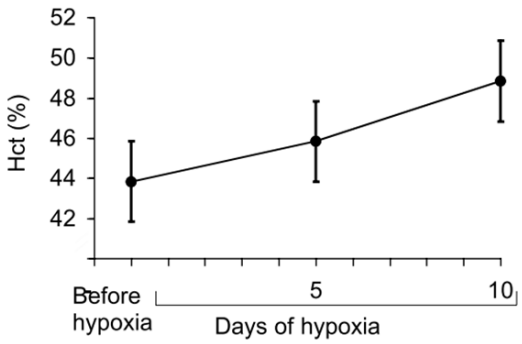
(c) *Hemoglobin Concentration* behavior



(d) *Hemoglobin Concentration* of [27]



(e) *Hematocrit* behavior

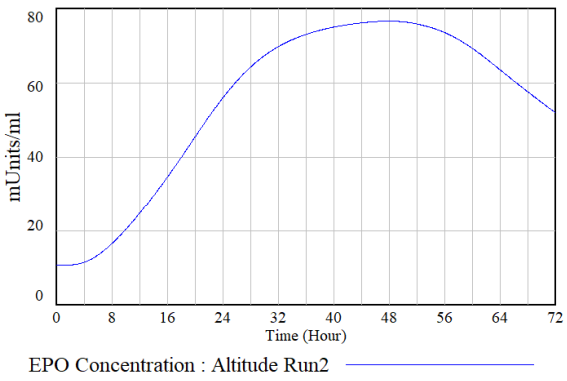


(f) *Hematocrit* of [27]

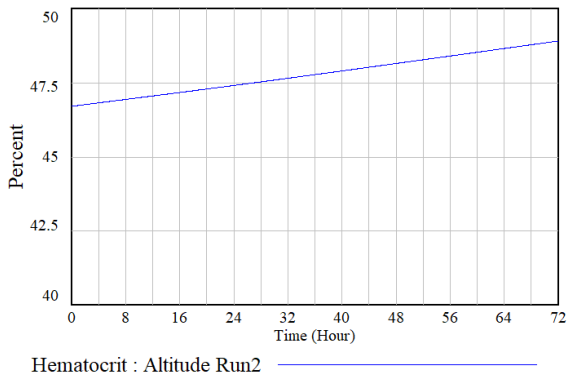
Figure 6.3. Model-generated dynamics versus real data of [27] for "Stay at an Altitude of 2800 m".

6.2.2. Stay at an Altitude of 4340 m

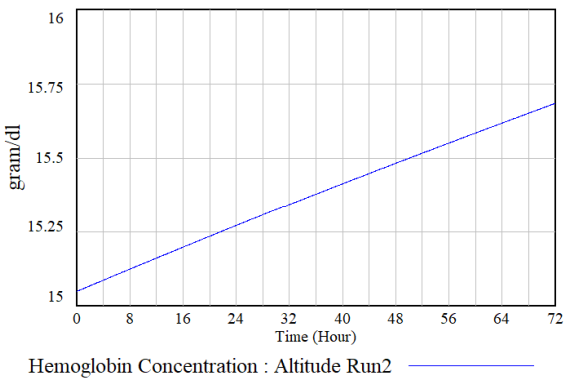
For this scenario, the altitude is changed to 4340 m and the model is run for 72 hours. The behavior of *EPO Concentration*, *Hemoglobin Concentration*, *Hematocrit* value and *Reticulocyte Count* can be found in Figure 6.4.



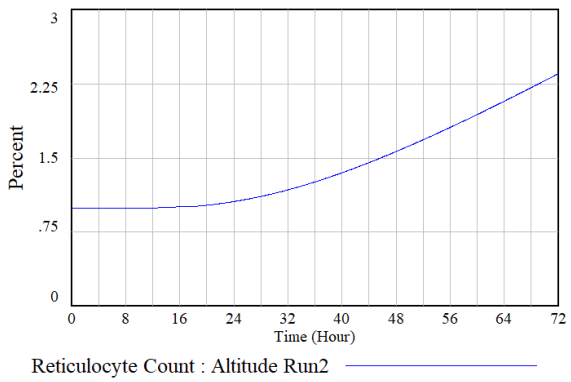
(a) *EPO Concentration*



(b) *Hematocrit*



(c) *Hemoglobin Concentration*

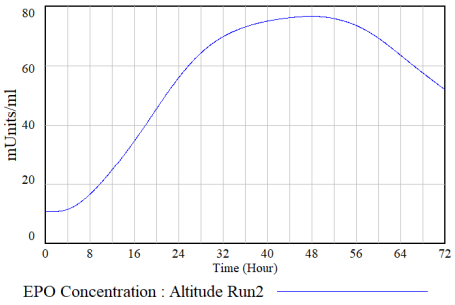


(d) *Reticulocyte Count*

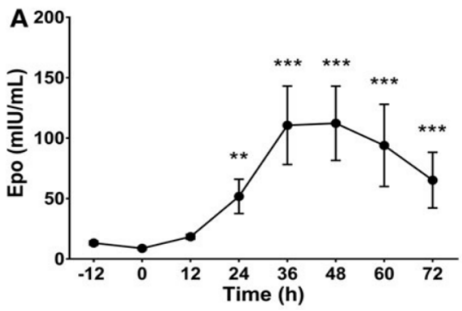
Figure 6.4. Model-generated dynamics for "Stay at an Altitude of 4340 m" scenario.

According to run results, *EPO Concentration* reaches its peak after around 48 hours. Then it starts falling down. The reason for this is the increased destruction of HIFs. Moreover, *EPO Concentration* reaches a higher peak than the previous scenario of 2800 m. This is logical since the oxygen content of air is less at 4340 m and this triggers a stronger EPO response. *Reticulocyte Count* starts to increase after around 24 hours. The comparison of the model-generated dynamics to the data taken from the

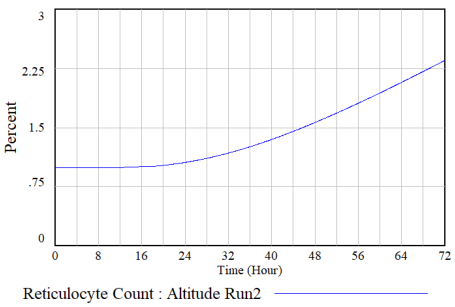
study of Vizcardo *et al.* [28] can be observed in Figure 6.5. *EPO Concentration* is in all the reported confidence intervals except for 36 hours where it slightly undershoots the confidence interval. *Reticulocyte Count* closely follows the reported intervals. *Hematocrit* slightly overshoots the intervals. However, its behavior closely follows the real data. Based on this validation scenario, we can say that our model generates reasonable values and valid dynamics compared to real data.



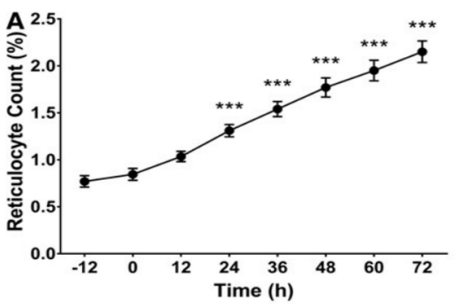
(a) *EPO Concentration* behavior



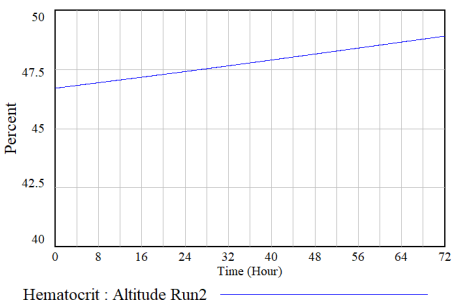
(b) *EPO Concentration* of [28]



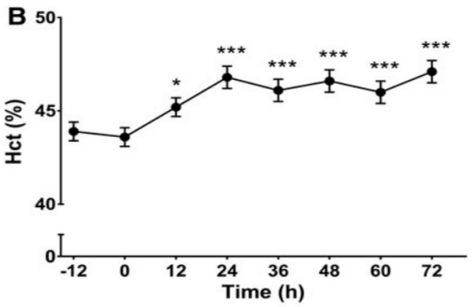
(c) *Reticulocyte Count* behavior



(d) *Reticulocyte Count* of [28]



(e) *Hematocrit* behavior

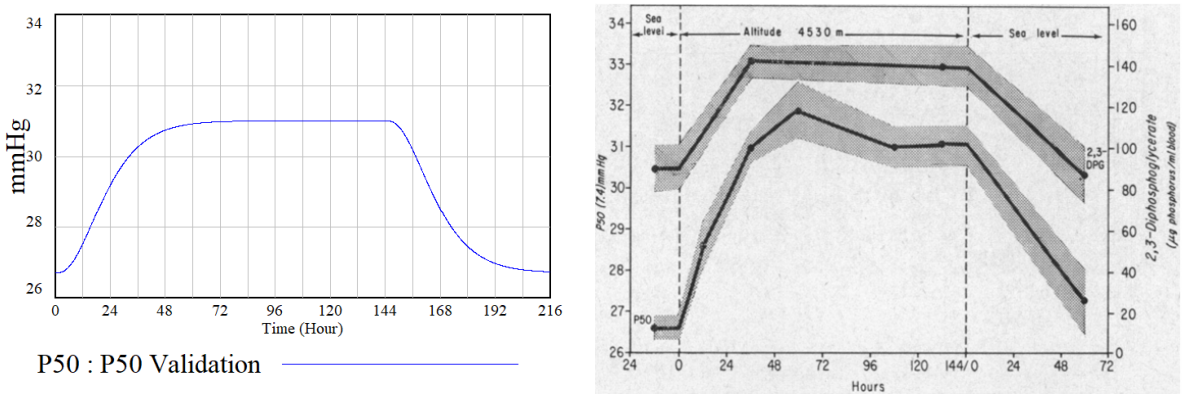


(f) *Hematocrit* of [28]

Figure 6.5. Model-generated dynamics versus real data of [28] for "Stay at an Altitude of 4340 m".

6.2.3. Tests with Changed P50 and Plasma Volume

As discussed before, the oxygen hemoglobin dissociation curve shifts with the changing altitude. P50 value increases as altitude increases. The model is started with an altitude of 4530 m. After 144 hours, the altitude is decreased to sea level. The behavior of P50 and its comparison with real data obtained in same condition [24] can be found in Figure 6.6.

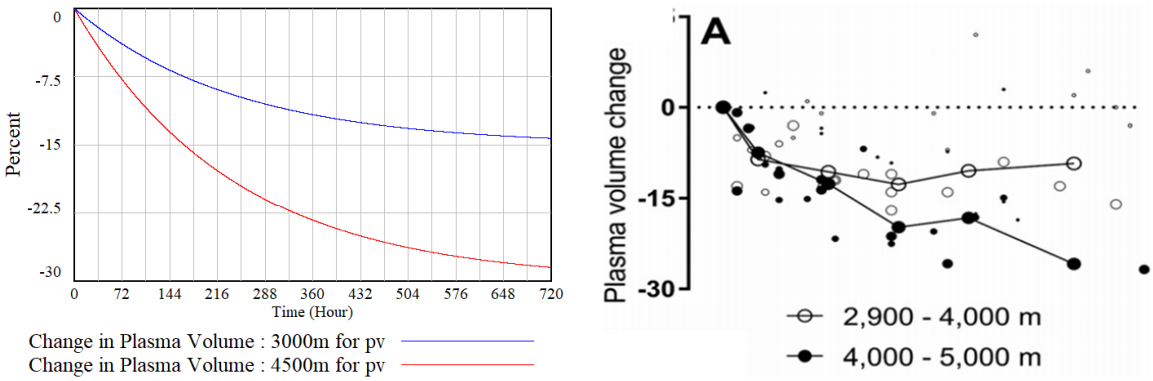


(a) *P50* (b) *P50* values of [24]

Figure 6.6. Model-generated dynamics versus real data [24] for "Changed P50".

The bottom curve in the real data graph shows the dynamics of P50. The model-generated P50 starts within the first confidence interval. It undershoots the given confidence intervals for 12 hours, 36 hours and 60 hours. Then, it is in the confidence intervals for the three other points given. According to this comparison, we can conclude that the model-generated P50 behaves similar to the real data.

Plasma volume is also affected by change in altitude. On ascent to higher altitude, we feel less thirsty and our urination frequency increases. These in turn lower our body water and, therefore, lower the plasma volume of blood [29]. To compare against available data, two runs are generated using our model: one at an altitude of 3000 m and one at an altitude of 4500 m. The time horizon of the available data is 30 days (720 hours). Then comparisons are made with the real data from Siebenmann *et al.* [29] in Figure 6.7. The dynamics fit reasonably well.



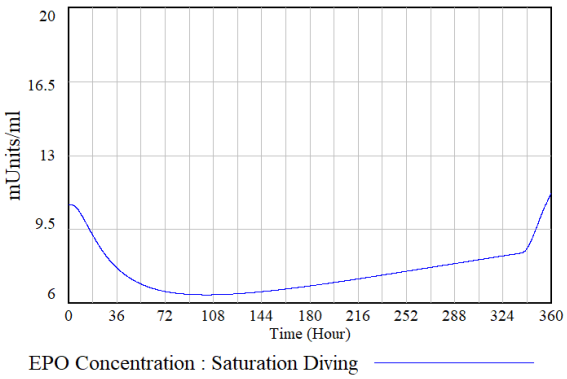
(a) *Percent Plasma Volume Change* (b) *Percent Plasma Volume Change* of [29]

Figure 6.7. Model-generated dynamics versus real data of [29] for "Percent Plasma Volume Change" test.

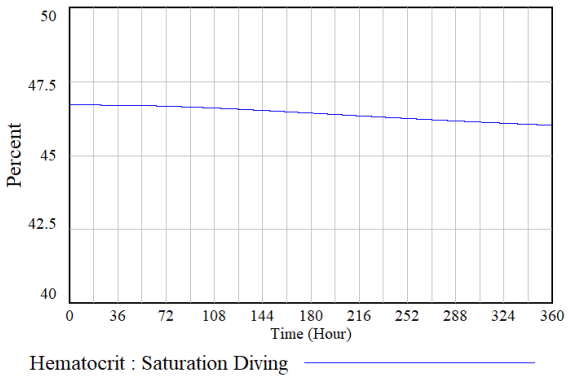
6.3. Saturation Diving Run

The study of Revelli *et al.* [12] is utilized for the validation of the excess oxygen cases (hyperoxia). In their study, divers were underwater for 14 continuous days. The oxygen tubes that they were breathing had 1.8 -2 atm of air. During the 14 days, their blood *EPO Concentrations*, *Reticulocyte Counts*, and *Hematocrit* levels all decreased. EPO level decreased to almost half of its normal value. The blood samples taken 24 hours after returning back to surface indicated that the EPO levels of the divers increased to levels above their normal values [12]. To simulate a similar case, the partial pressure of oxygen is doubled from the 1 atm case of 100 mmHg to 200 mmHg. We simulate our model for a total of 15 days: 14 continuous days of breathing air containing high partial pressure of oxygen, and one day of breathing air containing normal partial pressure of oxygen (resurfacing). The results can be found in Figure 6.8. According to our results, after diving, the *EPO Concentration* in blood is lowered. The cause for this is the high partial oxygen pressure of the oxygen tanks. The lowering of *EPO Concentration* has negative effect on reticulocyte production and therefore the *Reticulocyte Count* decreases. This in turn lowers the *Hematocrit* and *Hemoglobin Concentration*. After surfacing, the *EPO Concentration* increases to a level above its

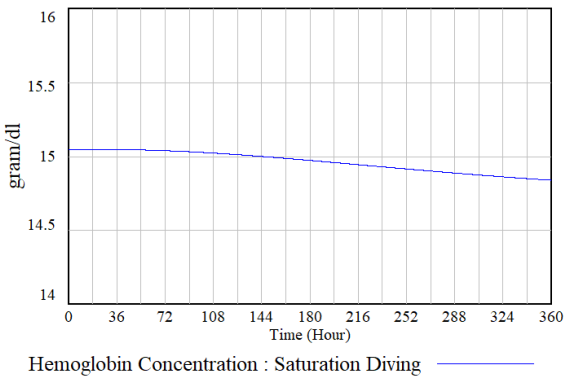
normal value since the body lost red blood cells for the duration of diving. This is in agreement with the real experiment [12]. Accordingly, we can say that our model gives expected behaviors for the excess oxygen test.



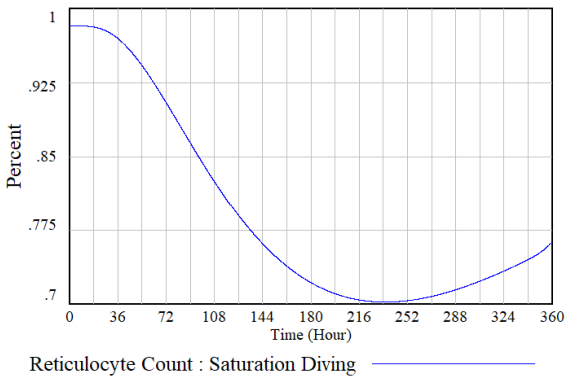
(a) *EPO Concentration*



(b) *Hematocrit*



(c) *Hemoglobin Concentration*



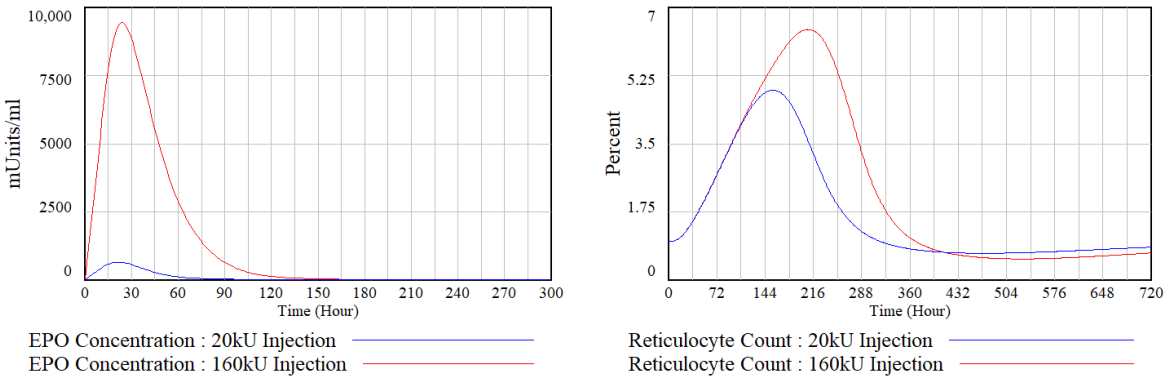
(d) *Reticulocyte Count*

Figure 6.8. Model-generated dynamics for "Saturation Diving" scenario.

6.4. rHuEPO Injection Runs

The validity tests for rHuEPO (recombinant human erythropoietin) injection are carried out by employing two different doses at sea level for which the real data are available: 20 kU (kilounits) and 160 kU. The behavior of *EPO Concentration* is compared with the real data from the study of Perez-Ruixo *et al.* [17]. In their study, rHuEPO was injected into the body by SC (subcutaneous) injection. The output of *EPO Concentration* and *Reticulocyte Count* for 20 kU and 160 kU runs can be found

in Figure 6.9.



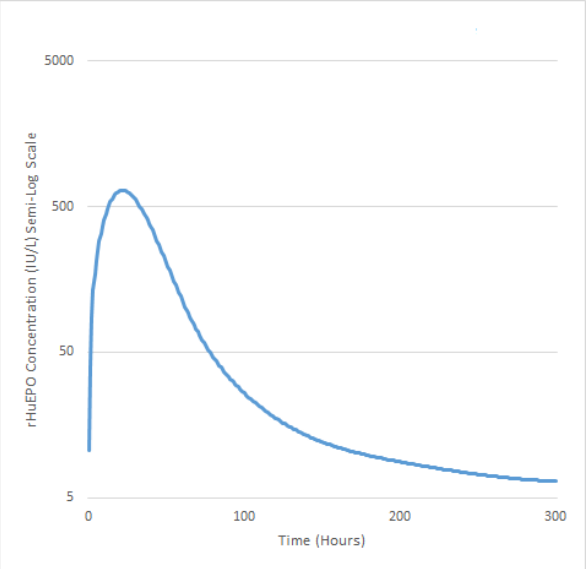
(a) *EPO Concentration* for 20 kU and 160 kU rHuEPO injection

(b) *Reticulocyte Count* for 20 kU and 160 kU rHuEPO injection

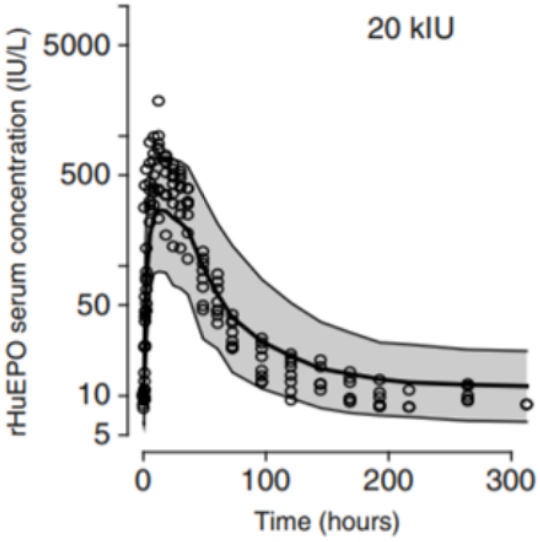
Figure 6.9. Model-generated dynamics for "20 kU rHuEPO Injection" and "160 kU rHuEPO Injection" tests.

As can be seen in the figure, *EPO Concentration* quickly reaches to a peak value at around 48 hours after injection. Then at around 144 hours, it almost returns to its basal value. The *Reticulocyte Count* reaches its peak much later at around 144 hours for 20 kU and at around 216 hours for 160 kU after injection. After their peak, they slowly return to their normal at around 360 hours after injection. The comparison with real data from [17] can be found in Figure 6.10 and Figure 6.11. Note that both the real data and model output for *EPO Concentration* are plotted in semi-log scale. Figures 6.10 and 6.11 show that the simulated *EPO Concentration* and *Reticulocyte Counts* are all within the reported confidence intervals. Their behaviors are similar to the behaviors depicted by real data.

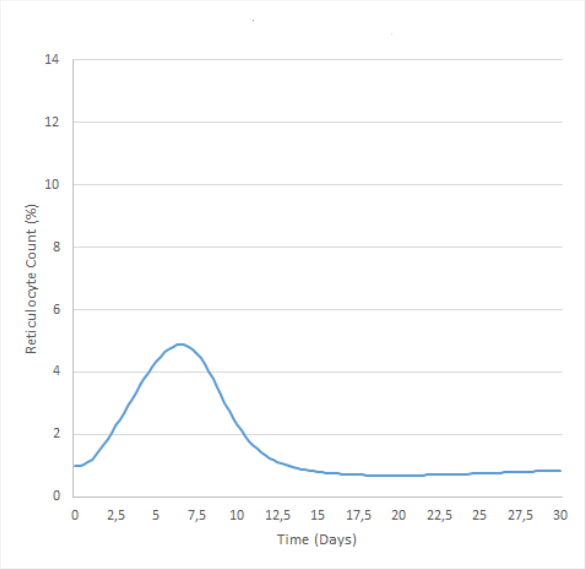
To summarize, based on various altitude and drug injection runs, we can conclude that our model is structurally and behaviorally valid.



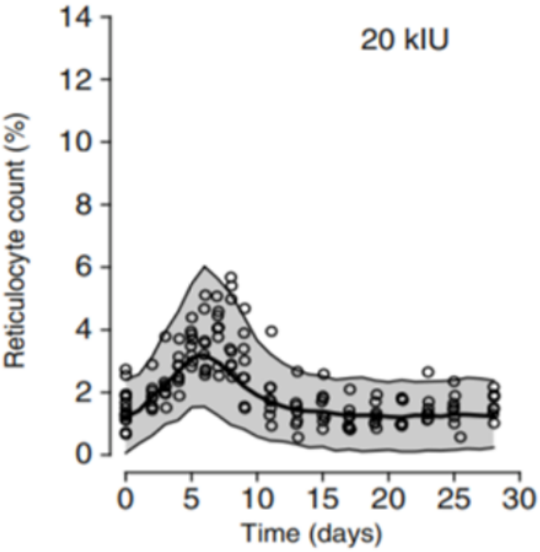
(a) *EPO Concentration* behavior (in semi-log scale)



(b) *EPO Concentration* of [17] (in semi-log scale)

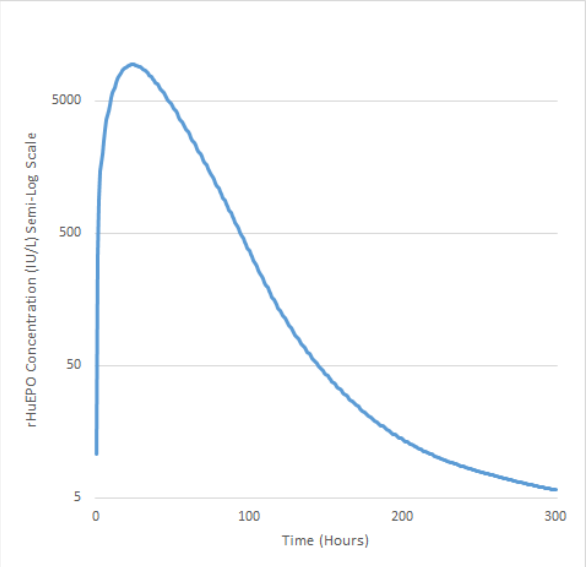


(c) *Reticulocyte Count* behavior

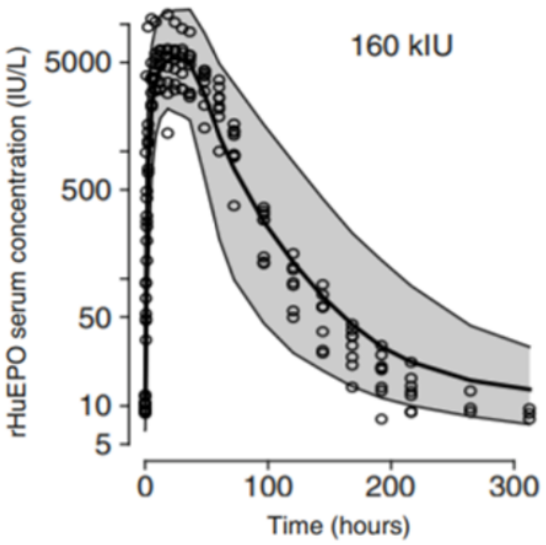


(d) *Reticulocyte Count* of [17]

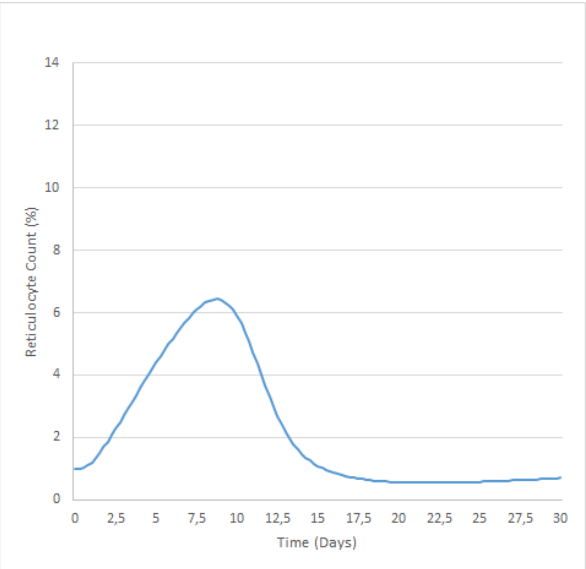
Figure 6.10. Model-generated dynamics versus real data of [17] for "20 kU rHuEPO Injection" case.



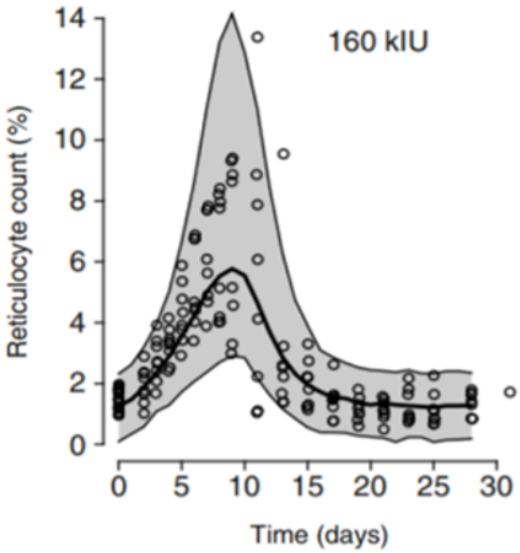
(a) *EPO Concentration* behavior (in semi-log scale)



(b) *EPO Concentration* of [17] (in semi-log scale)



(c) *Reticulocyte Count* behavior



(d) *Reticulocyte Count* of [17]

Figure 6.11. Model-generated dynamics versus real data of [17] for "160 kU rHuEPO Injection" case.

7. BASE MODEL DYNAMICS AND SCENARIO ANALYSIS

In this chapter, we run the model under two different disequilibrium conditions, one with EPO (erythropoietin) and one with hematocrit, to show the base dynamic behaviors of the model. Then, we experiment with a base scenario involving a single rHuEPO (recombinant human erythropoietin) shot to obtain the model dynamics as a basis for our next three scenarios. First of these three scenarios simulates an athlete (doper) who is on a continuous rHuEPO regimen. The second one involves an athlete who trains at an altitude and descends to sea level for a competition. We discuss the differences of altitude training and rHuEPO usage and how one can separate the two practices. The last scenario involves a doper who uses rHuEPO while altitude training, but then stops using rHuEPO when he descends to the sea level.

7.1. Base Dynamics with Initial Disequilibrium

7.1.1. Initial EPO Disequilibrium

The aim of this section is to explore the dynamics when *EPO Concentration* is initialized away from its equilibrium value. The equilibrium values for *EPO Concentration, Hematocrit* and *Reticulocyte Count* are given in Table 7.1.

Table 7.1. The equilibrium values for *EPO Concentration, Hematocrit* and *Reticulocyte Count*.

	Equilibrium Value
EPO Concentration (in mU/ml)	10.64
Hematocrit (%)	46.7
Reticulocyte Count (%)	0.98

The normal levels of *EPO Concentration* range from 6 to 32 mU/mL [3]. To explore the effects of EPO disequilibrium, the *EPO Level in Blood* stock is initialized

so that the initial *EPO Concentration* is 50 mU/mL. The model is run for 1000 hours. The resulting dynamics for *EPO Concentration* and its comparison with the equilibrium level can be found in Figure 7.1.

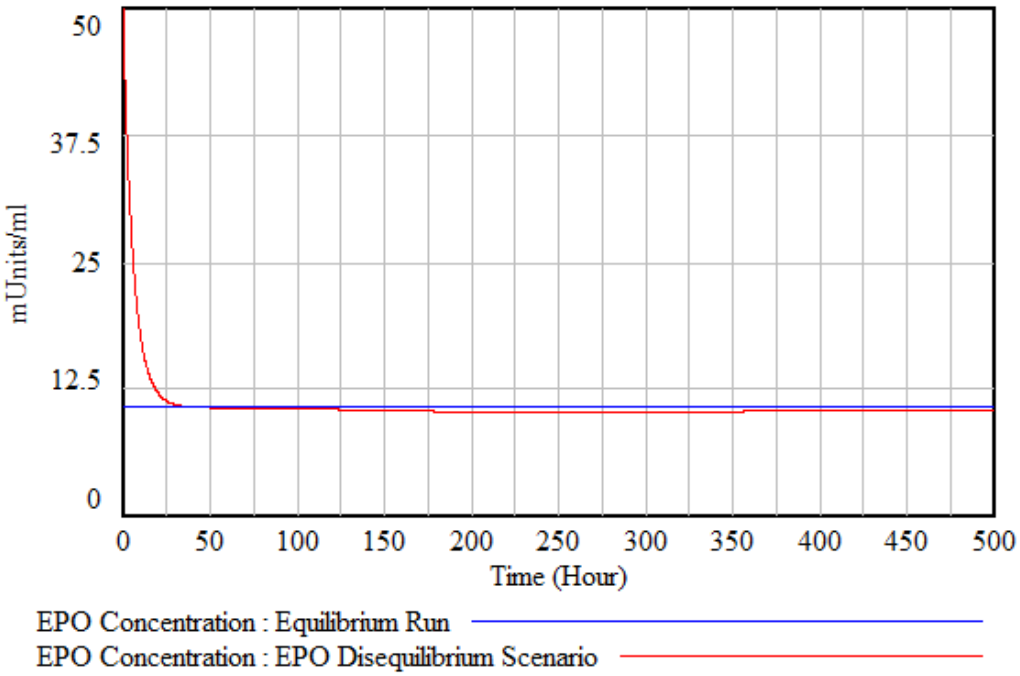
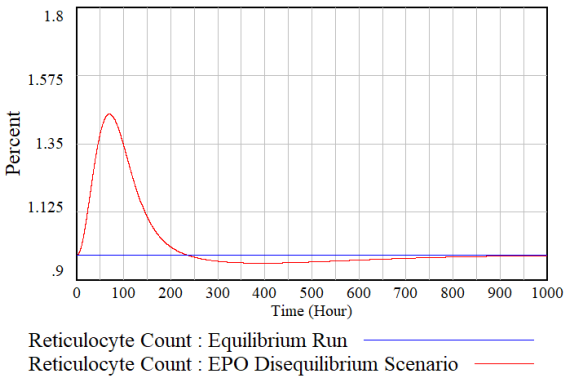


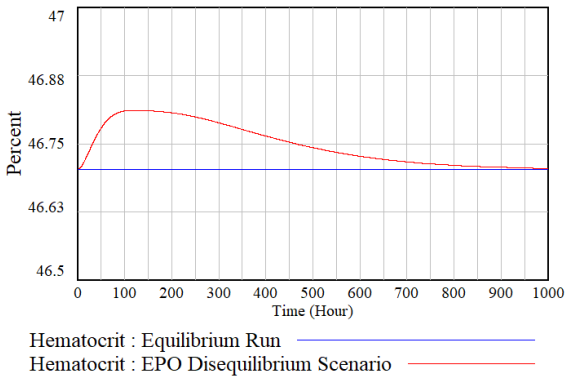
Figure 7.1. Dynamics of EPO concentration for EPO disequilibrium scenario and its equilibrium level.

EPO Concentration quickly decreases to its equilibrium value at around 48 hours. However, after around 110 hours, *EPO Concentration* starts decreasing below its equilibrium value. The reason for this decrease is the excess red blood cells that have been produced because of the initial higher than normal *EPO Concentration*. These excess red blood cells increase the oxygen level in blood. This suppresses EPO production in kidneys and, thus, lowers the *EPO Concentration* in blood. Figure 7.2 shows the delayed effects of *EPO Concentration* on *Hematocrit* and *Reticulocyte Count*. The initial high *EPO Concentration* facilitates the production and release of reticulocytes. For this reason, *Reticulocyte Count* increases, reaching a peak at around 75 hours. The initial increase in reticulocytes also increases *Hematocrit* since reticulocytes are also included in it. After reaching its peak, *Reticulocyte Count* decreases rapidly since *EPO*

Concentration is now normalized. However, *Hematocrit* does not experience a rapid decline, since the reticulocytes now mature into erythrocytes and stay in the blood stream for around 120 days. Because of this, *Hematocrit* stays at an increased level longer than *EPO Concentration* and *Reticulocyte Count*. Moreover, the increased *Hematocrit* suppresses *EPO Concentration*. The suppressed *EPO Concentration* in turn decreases the reticulocyte production and *Reticulocyte Count*. Practically speaking, the system returns back to its equilibrium at around 1000 hours without any oscillations.



(a) *Reticulocyte Count*



(b) *Hematocrit*

Figure 7.2. Dynamics of reticulocyte count (a) and hematocrit (b) for EPO disequilibrium scenario and their equilibrium levels.

7.1.2. Initial Hematocrit Disequilibrium

Hematocrit, as mentioned before, is the red blood cell percentage in human blood. Its normal upper and lower bound can change between genders. In our study, we model a healthy male athlete for whom the *Hematocrit* level can be between 40% to 54% and the assumed equilibrium level is 46.7%. For this run, *Erythrocytes in Blood* stock is initialized such that the initial *Hematocrit* is 52%. Our aim is to show how and when the disturbed *Hematocrit* level can return back to its equilibrium and how it affects the *EPO Concentration* and *Reticulocyte Count*. To see the complete dynamics, the model is run for 2000 hours. The model-generated dynamics for *Hematocrit* can be found in Figure 7.3.

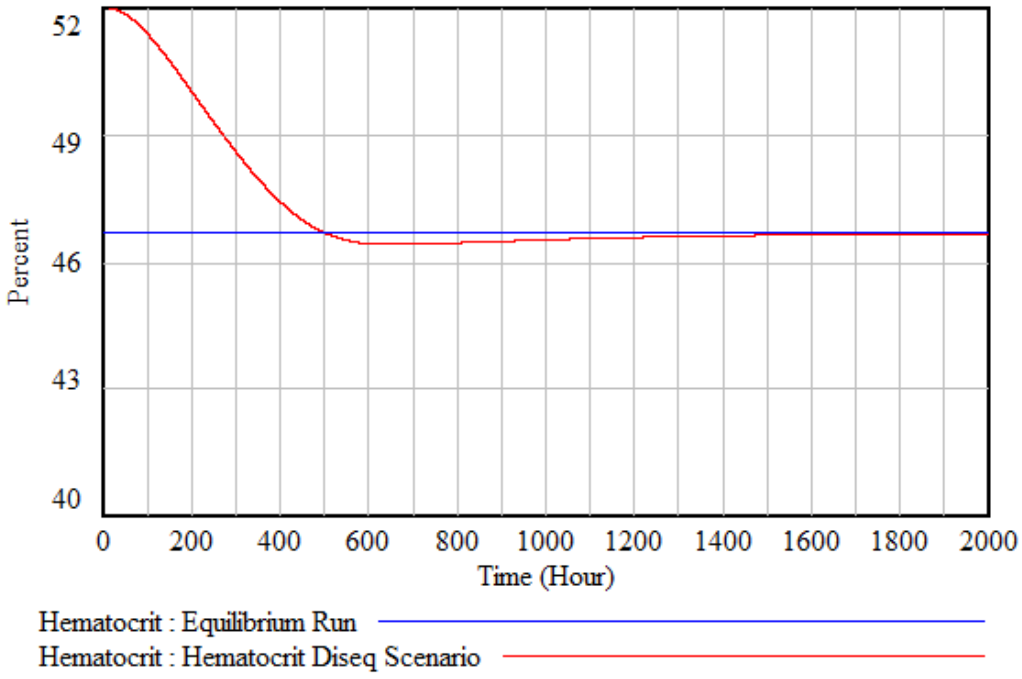
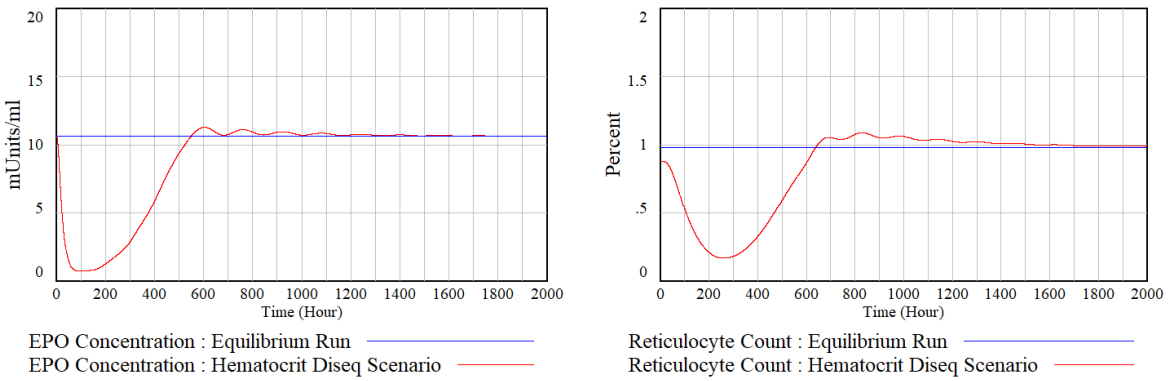


Figure 7.3. Dynamics of hematocrit for hematocrit disequilibrium scenario and its equilibrium level.

According to Figure 7.3, *Hematocrit* level decreases down from its initial level of 52%. Then, it undershoots its equilibrium level. The reason for this is the suppressed *EPO Concentration* due to the increased blood oxygen caused by high *Hematocrit*. After undershooting, *Hematocrit* slowly climbs back up to its equilibrium value at around 2000 hours. The effect of *Hematocrit* disequilibrium on *EPO Concentration* and *Reticulocyte Count* can be seen in Figure 7.4. Increased initial *Hematocrit* level suppresses *EPO Concentration* which in turn lowers *Reticulocyte Count*. Initial *Reticulocyte Count* value is already lower than equilibrium since its calculation involves *Erythrocytes in Blood* (see Equation 5.5). After *Hematocrit* undershoots its equilibrium level, *EPO Concentration* increases and oscillates around its equilibrium level. Practically speaking, the system returns to its equilibrium at around 2000 hours.



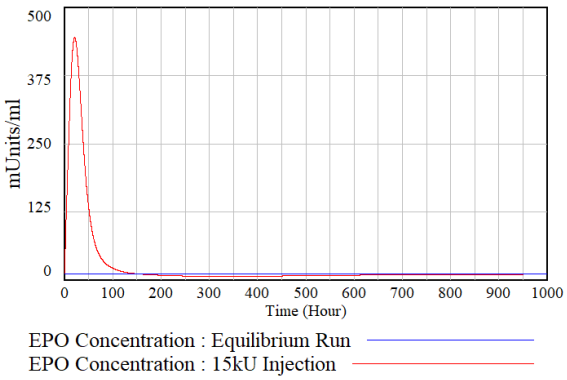
(a) *EPO Concentration* (b) *Reticulocyte Count*

Figure 7.4. Dynamics of EPO concentration (a) and reticulocyte count (b) for hematocrit disequilibrium scenario and their equilibrium levels.

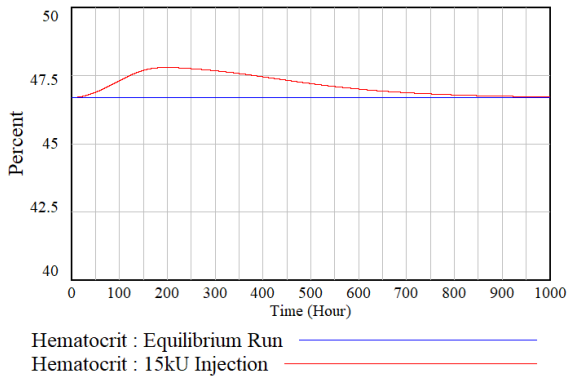
7.2. Base Scenario Dynamics as a Result of Single rHuEPO Shot

In this section, we discuss the dynamic effects of single rHuEPO shot on *EPO Concentration*, *Hematocrit*, *Reticulocyte Count* and *Hemoglobin Concentration*. By doing this, we hope to have a general understanding of the behaviors of blood values for a rHuEPO user. In our model, *EPO Concentration* can be increased either by natural EPO production or by rHuEPO injection. This scenario starts with an injection of 15 kU rHuEPO. The time horizon is 1000 hours. The resulting dynamics for *EPO Concentration*, *Hematocrit*, *Reticulocyte Count* and *Hemoglobin Concentration* can be found in Figure 7.5. After the shot, *EPO Concentration* rapidly increases, reaching a peak at around 48 hours. Then, it rapidly decreases down to its equilibrium value. An important phenomenon happens at around 150 hours, *EPO Concentration* drops below its equilibrium value. The reason for this is the increase in blood oxygen level caused by elevated *Hematocrit* level. The *EPO Concentration* then slowly returns to its equilibrium. *Reticulocyte Count* reaches a peak and decreases below its equilibrium level similar to *EPO Concentration*. *Hematocrit* level remains elevated for the duration of our run. Practically speaking, at around 1000 hours, system reaches its equilibrium. This scenario tells us two important things: (1) After an injection of rHuEPO, *EPO Concentration* and *Reticulocyte Count* first peak and then decrease below their equilib-

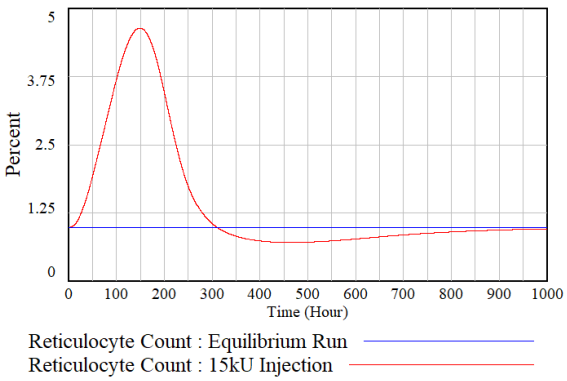
rium values; (2) after a single injection of rHuEPO, the system can take a long period of time before it returns to its equilibrium.



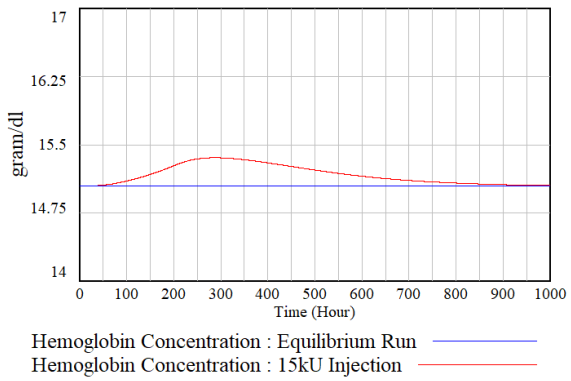
(a) *EPO Concentration*



(b) *Hematocrit*



(c) *Reticulocyte Count*



(d) *Hemoglobin Concentration*

Figure 7.5. Dynamics of EPO concentration (a), hematocrit (b), reticulocyte count (c) and hemoglobin concentration (d), under single rHuEPO shot.

7.3. Sensitivity Analysis with rHuEPO Use

rHuEPO regimen among athletes typically involves two phases. The first phase involves high dosages of rHuEPO to stimulate erythrocyte production and increase the athlete's oxygen carrying capacity. When the desired hematocrit level is reached, the second phase begins. In this phase, the dosage is lowered so that the desired hematocrit level is kept constant. In the following three sensitivity scenarios, the athlete injects a high dose of rHuEPO drug every week for around 21 days (500 hours). Then the

dosage is decreased. This regimen goes on for 14 more days and then the usage stops. We assume that there is a competition 35 days from the beginning of the run (840 hours). The last rHuEPO injection is done at 672 hours . The model is run for 1200 hours. We deal with three different dosage scenarios: (1) 2 kU then switchover to 0.2 kU, (2) 10 kU then switchover to 1 kU and (3) 25 kU then switchover to 3 kU.

Current testing practice against rHuEPO involves the creation of personal baselines for every athlete. This method is called the "Athlete's Biological Passport". Any change in the blood values of athletes is then compared to their own personal baselines to determine whether this change is natural or artificial (by rHuEPO usage). But even these personalized tests can be cheated by clever microdosing, by distorting the baseline values etc. [30]. In these personalized tests, WADA takes into consideration *Hematocrit*, *Hemoglobin Concentration*, *Reticulocyte Count* and *OFF-hr score* [31]. *OFF-hr Score* formula of WADA (World Anti-Doping Agency) is given as:

$$\textit{OFF-hr Score} = \textit{Hemoglobin (in gr/L)} - 60 \times \sqrt{\textit{Reticulocyte Count (\%)}} \qquad (7.1)$$

The main purpose of *OFF-hr Score* is to detect dopers who have recently stopped rHuEPO usage. After the cessation of rHuEPO usage, *Reticulocyte Count* drops below its equilibrium level and the *Hemoglobin Concentration* remains elevated (see Section 7.2). These in turn increase the *OFF-hr Score*. Therefore, increased *OFF-hr Score* can be an indicator for the recent cessation of rHuEPO usage.

As a reference, the normal medical limits for *EPO Concentration*, *Hematocrit*, *Hemoglobin Concentration* and *Reticulocyte Count* are given in Table 7.2 [3, 25, 26].

7.3.1. '2 kU of rHuEPO then Switchover to 0.2 kU' Sensitivity Scenario

In this scenario, we simulate an athlete who injects 2 kU of rHuEPO every week for 21 days. Then, switchover to 0.2 kU of rHuEPO happens. The competition, as mentioned before, is at 840 hours (35 days). The model-generated dynamics for *EPO*

Table 7.2. The normal medical limits for *EPO Concentration*, *Hematocrit*, *Reticulocyte Count* and *Hemoglobin Concentration*.

	Lower Bound	**Upper Bound**
EPO Concentration (mU/mL)	6	32
Hematocrit (%)	40	54
Reticulocyte Count (%)	0.5	2.5
Hemoglobin Concentration (gr/dL)	14	18

Concentration can be found in Figure 7.6.

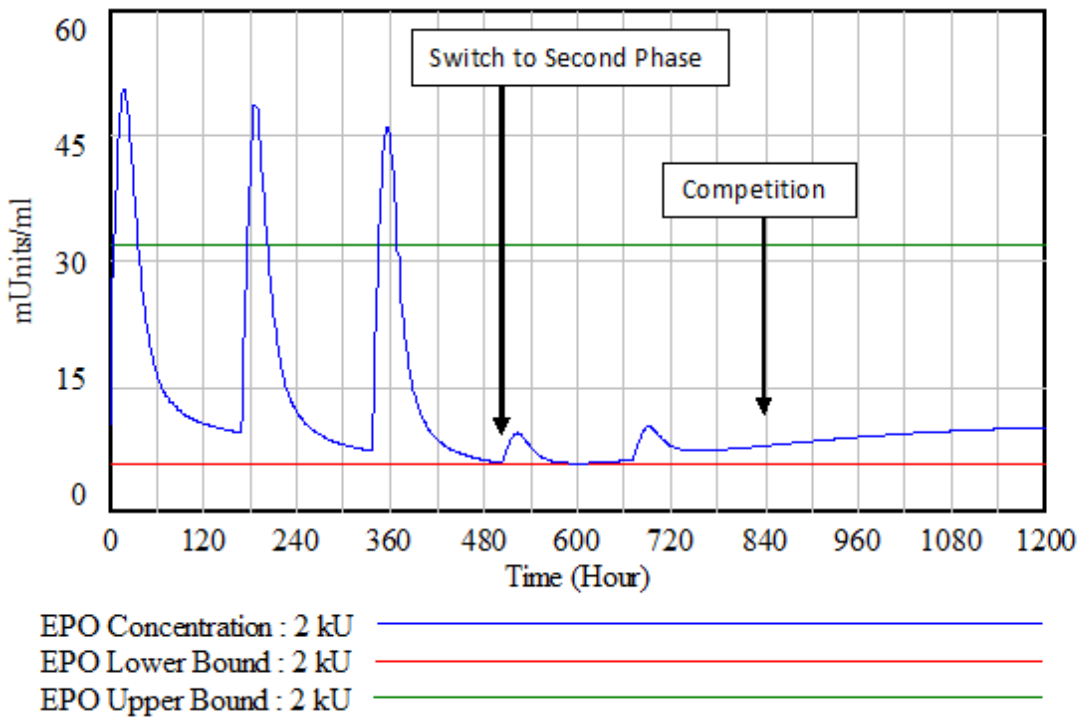
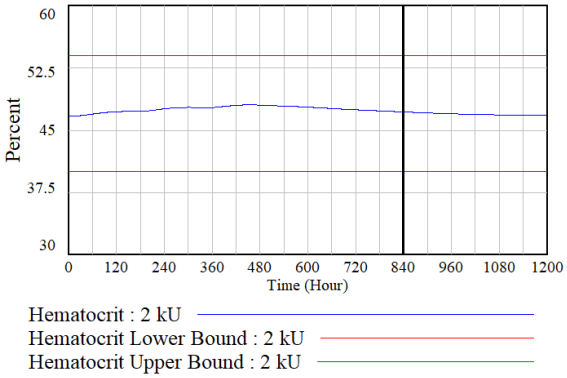


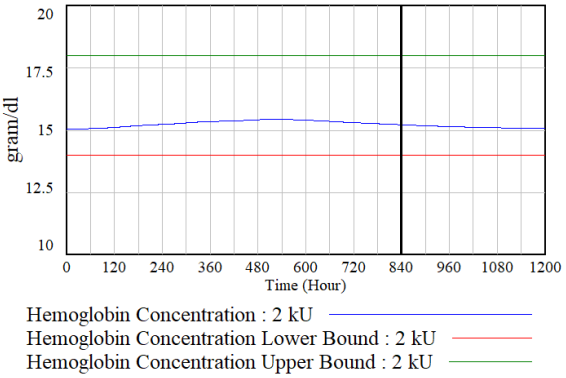
Figure 7.6. Dynamics of EPO concentration under '2 kU of rHuEPO then Switchover to 0.2 kU' scenario.

EPO Concentration peaks at beginning of every week when the injection is administered. Then, it rapidly declines to its normal level. With this strategy, rHuEPO does not accumulate in blood and makes the detection of the drug even harder. This regimen also ensures that, at the competition, the EPO level in blood is within the normal medical boundaries. However, this regimen does not have a high erythrocyte

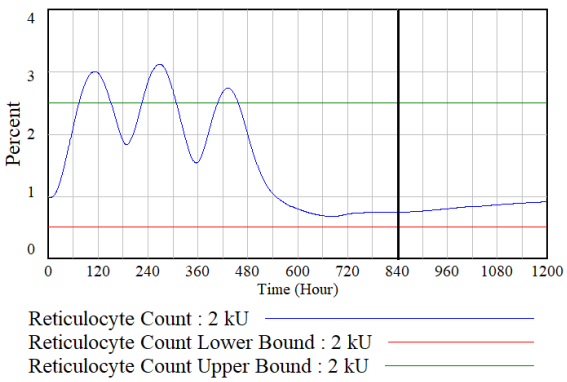
producing capability as can be seen from Figure 7.7.



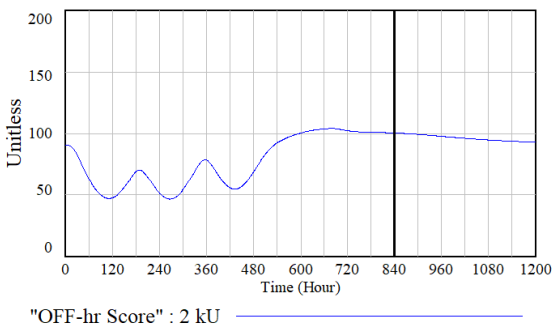
(a) *Hematocrit*



(b) *Hemoglobin Concentration*



(c) *Reticulocyte Count*



(d) *OFF-hr Score*

Figure 7.7. Dynamics of blood values under '2 kU of rHuEPO then Switchover to 0.2 kU' scenario (competition at 840 Hours).

Hematocrit increases to around 48% from 46.7%. This increase is not significant enough to boost the oxygen carrying capacity of the blood therefore it does not give the doper a competitive edge. *Reticulocyte Count* can cross its normal upper bound during the first phase of rHuEPO administration. However, after the second phase, *Reticulocyte Count* is always between its normal values. There is a slight increase in *OFF-hr Score* after the switch to second phase. This increase in *OFF-hr Score* is caused by the decrease in *Reticulocyte Count*. Although all values are in their normal boundaries, the dynamics above can provide useful information to develop strong tests to detect rHuEPO users, as will be explained below in the next more informative

scenario.

7.3.2. '10 kU of rHuEPO then Switchover to 1 kU' Sensitivity Scenario

For this scenario, the rHuEPO drug dosage is increased to 10 kU for the first phase and 1 kU for the second phase. The model-generated dynamics for *EPO Concentration* is given in Figure 7.8.

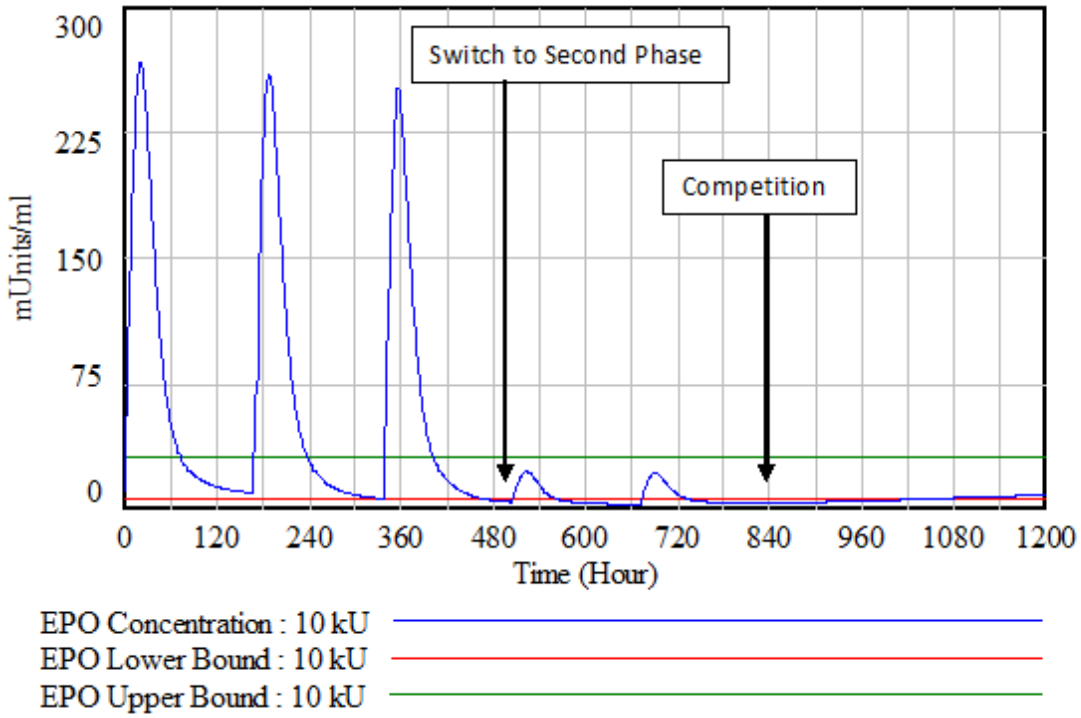
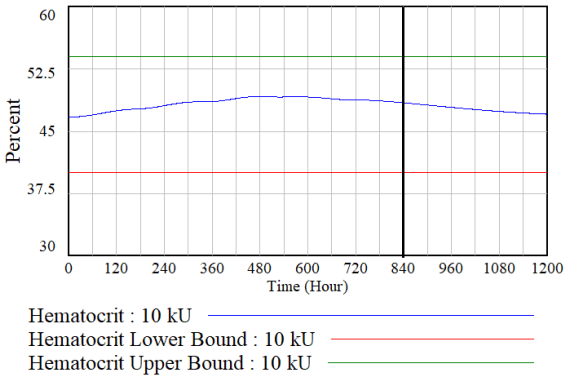


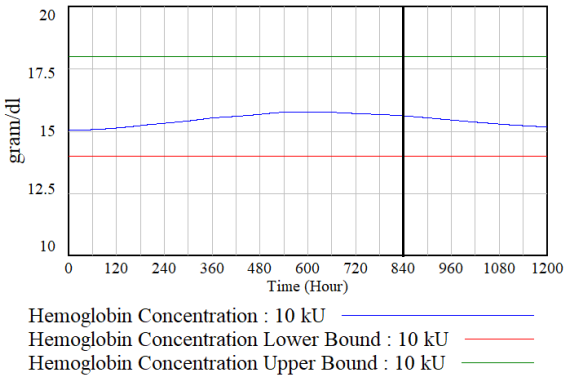
Figure 7.8. Dynamics of EPO concentration under '10 kU of rHuEPO then Switchover to 1 kU' scenario.

EPO Concentration peaks at beginning of every week when the injection is administered, as expected. Then, it rapidly declines to its normal levels. With this strategy, again, rHuEPO does not accumulate in blood and makes the detection of the drug even harder. Moreover, this strategy does not limit the erythrocyte producing capabilities of rHuEPO since as discussed before, this effect appears after a delay. Figure 7.9 clearly shows the erythrocyte producing capabilities of this regimen. This regimen also ensures that, at the competition, the EPO level in blood is within the

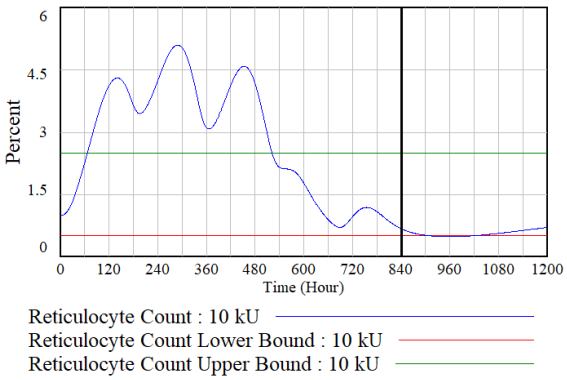
normal boundaries. After ending the rHuEPO usage, *EPO Concentration* in blood stays below its basal value for some time. The cause for this effect is the unnatural increase in blood oxygen level. The increased oxygen level destabilizes HIF values and cause them to be destroyed. This reduces the production of EPO and therefore the blood EPO level.



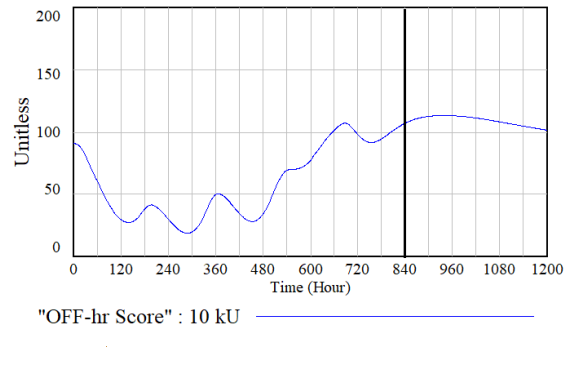
(a) *Hematocrit*



(b) *Hemoglobin Concentration*



(c) *Reticulocyte Count*



(d) *OFF-hr Score*

Figure 7.9. Dynamics of blood values under '10 kU of rHuEPO then Switchover to 1 kU' scenario (competition at 840 Hours).

In this scenario, *Hematocrit*, increases to around 50% from 46.7%. This is a remarkable increase that can give unfair advantage to athletes. The second phase of rHuEPO usage keeps the *Hematocrit* value at around the desired levels until the competition. *Hemoglobin Concentration* also follows the behavior of *Hematocrit*. *Reticulocyte Count* is higher than the normal upper bound value for the duration of the first phase

of rHuEPO administration. However, after the second phase, *Reticulocyte Count* drops inside the normal level boundaries, and after ending the regimen, it drops below its normal lower bound. This is consistent with the fact that the *EPO Concentration* is also lower than its normal lower bound. *OFF-hr Score* increases slightly after the cessation of drug usage. The dynamics above can provide useful information to develop strong tests to detect rHuEPO users. For instance, note that *Reticulocyte Count* displays a significant negative trend during the couple of weeks just before the competition. It continues to decline (mildly) during competition, and starts increasing about a week after the competition. These three different dynamics (trends) can be detected by blood samples taken n days before, during, and m days after competition and a related dynamic testing algorithm can be developed.

7.3.3. '25 kU of rHuEPO then Switchover to 3 kU' Sensitivity Scenario

In this scenario, rHuEPO dosage is increased to 25 kU then switchover to 3 kU. This is a relatively high dosage compared to previous two scenarios. The dynamics for *EPO Concentration* can be found in Figure 7.10.

EPO Concentration peaks with every injection. After switching to second phase (3 kU of rHuEPO), the peaks of *EPO Concentration* reach above its normal upper bound. Because of this, this regimen is troublesome for a rHuEPO user compared to the previous scenarios. Similar to the previous scenario, *EPO Concentration* drops below to its lower bound after the cessation of rHuEPO usage. Figure 7.11 shows if this regimen benefits the rHuEPO user more than the previous two scenarios.

Hematocrit increases to around 50% from 46.7% similar to the previous scenario even though the dosage is increased. The reason for this is that the duration of the first phase is same for the two scenarios. Therefore, we can say that the duration of the stimulation of red blood cell production is as important as the level of the stimulation. This fact makes this scenario unattractive for the rHuEPO user, since now the benefits of rHuEPO usage is the same as the previous scenario.

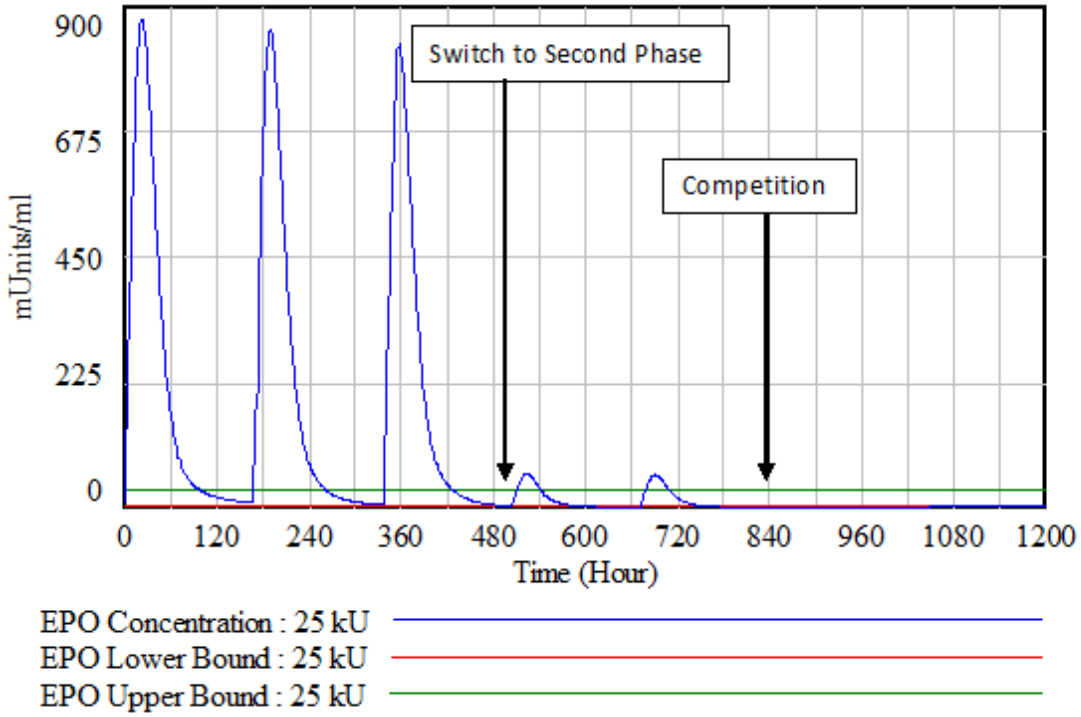
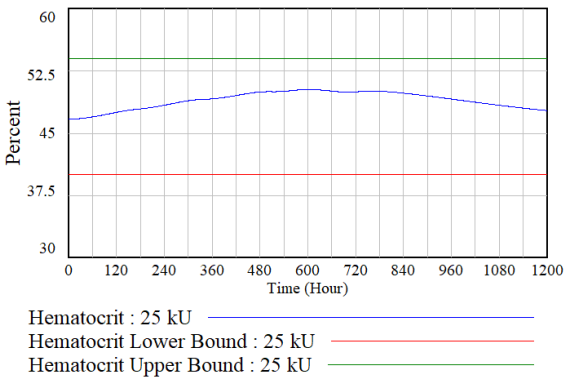


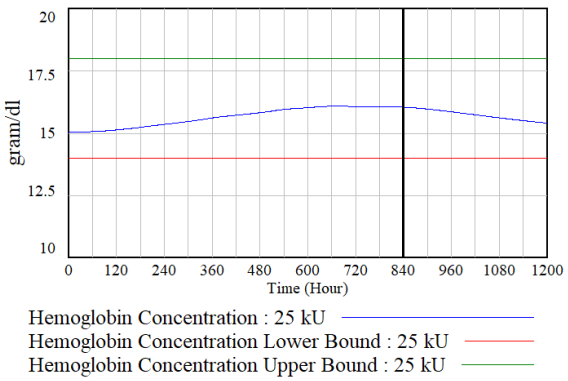
Figure 7.10. Dynamics of EPO concentration under '25 kU of rHuEPO then Switchover to 3 kU' scenario.

The first rHuEPO usage scenario (2 kU then switchover to 0.2 kU) is the hardest to detect compared to the other two scenarios. However, it offers almost no benefit for the oxygen capacity of the athlete. Our second rHuEPO usage scenario (10 kU then switchover to 1 kU) is also hard to detect and offers a significant boost for the oxygen capacity compared to the first scenario. Our last rHuEPO usage scenario (25 kU then switchover to 3 kU) is the easiest to detect in our scenarios. Also, it does not offer as high a boost for the oxygen capacity as expected. Its boost is almost similar to the 10 kU then switchover to 1 kU scenario. In all three of these scenarios, our model-generated dynamics suggest that the *Reticulocyte Count* is lower during the competition compared to during the first phase of rHuEPO administration, and it assumes a positive trend about a week after the competition. Based on these scenarios we conclude that, our model can be calibrated for different rHuEPO usage regimens to evaluate the dynamic behaviors of key blood values. The above 'cheating dynamics' can be detected by blood samples taken n days before, during, and m days after

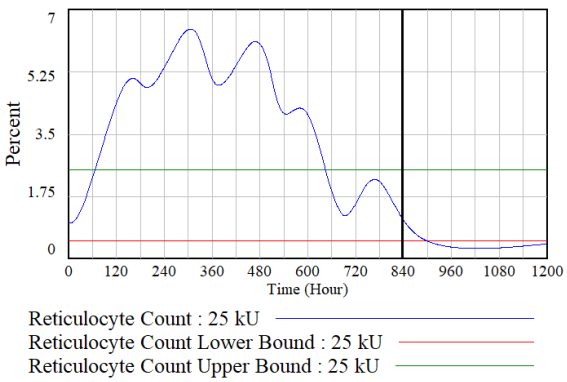
competition and a related dynamic testing algorithm can be developed. There exist more complex and hard to detect cheating regimens that can fool even the personalized tests of WADA. Our model can be personalized with the data from individual athletes and the dynamic patterns that are generated by our model can help design dynamic tests that can detect such cheating regimens.



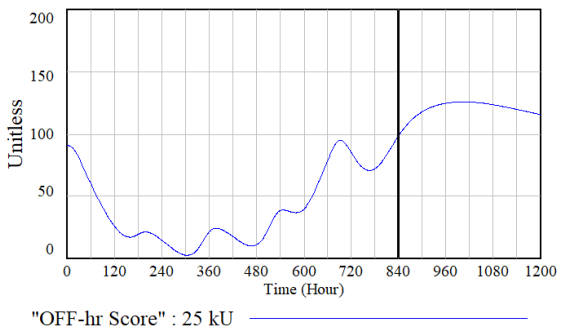
(a) *Hematocrit*



(b) *Hemoglobin Concentration*



(c) *Reticulocyte Count*



(d) *OFF-hr Score*

Figure 7.11. Dynamics of blood values under '25 kU of rHuEPO then Switchover to 3 kU' scenario (competition at 840 Hours).

7.4. Altitude Training Scenario

Altitude training is popular among athletes. It involves ascending to a higher altitude and training at that level. As we discussed before, this stimulates EPO production and eventually increases hematocrit levels and the oxygen carrying capacity of

the blood. This practice is not banned according to WADA. Athletes usually ascend to 2000-3000 m [32]. Our scenario analysis starts at an altitude of 2800 m. The athlete stays there for around 33 days (792 hours) and then descends to sea level, 48 hours before the competition which is held at 840 hours. The model is run for a total of 1200 hours. The results for *EPO Concentration* can be found in Figure 7.12.

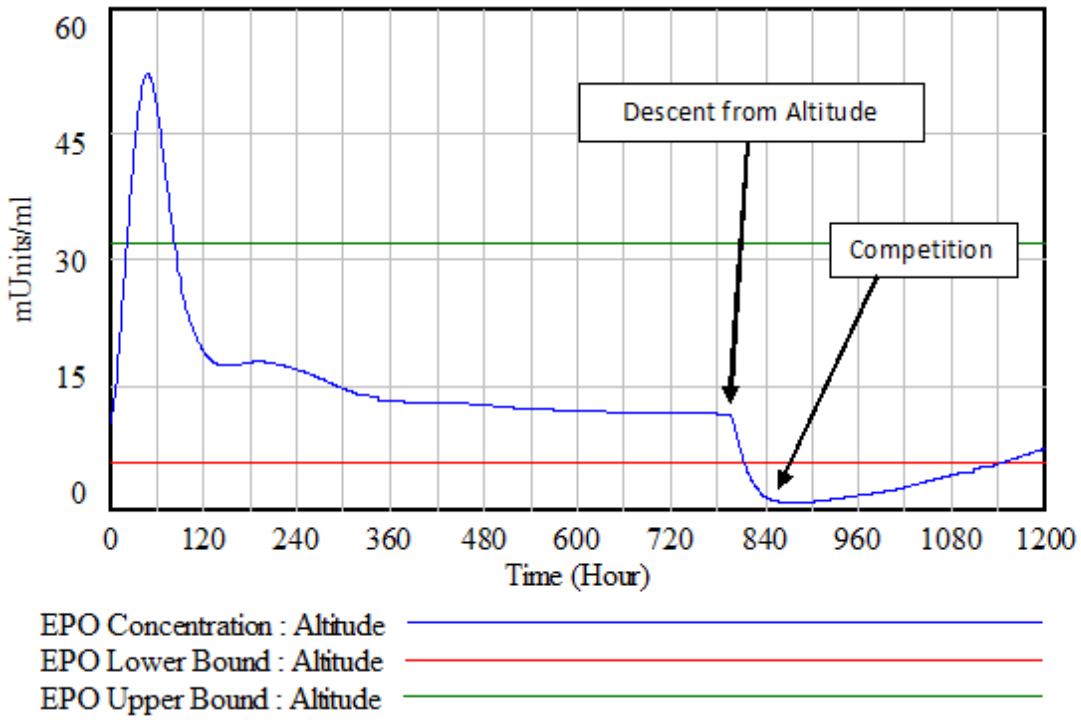
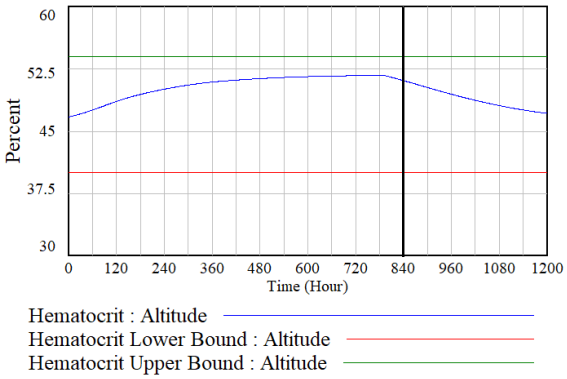


Figure 7.12. Dynamics of EPO concentration in regular altitude training scenario.

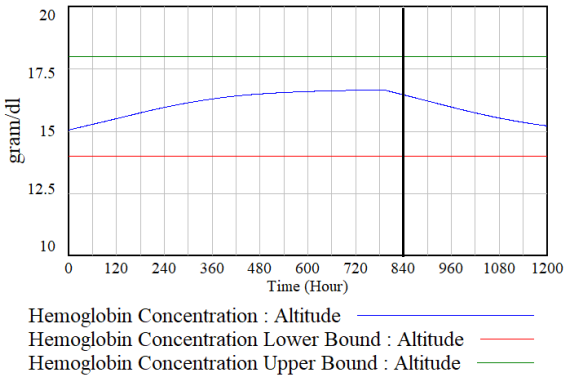
EPO Concentration peaks in 24 hours then stabilizes at a level that is twice its basal level. Later, it begins to decrease down to its basal level. After descending to sea level, EPO rapidly disappears from blood. The reason for this is the increased arterial oxygen content. During the competition, *EPO Concentration* is almost zero in blood. This is consistent with studies of descent from high altitude that suggest a decrease of EPO level to levels below their normal values [2, 33].

The blood values for this scenario can be found in in Figure 7.13. *Hematocrit* increases to around 51% during altitude training. After descent to sea level, it starts rapidly decreasing. It is around 50% at the competition time. *Hematocrit* normalizes

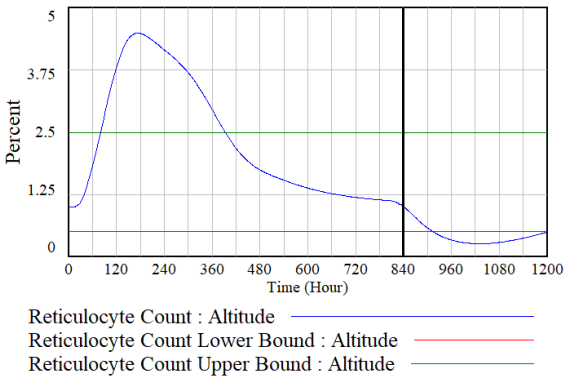
around 360 hours after descent. This behavior is consistent with the studies [34]. The athlete gains a major boost for his oxygen capacity. *Reticulocyte Count* decreases to below normal values after descent and recovers at around 400 hours after descent. This is expected since the EPO value is almost non-existent after the descent.



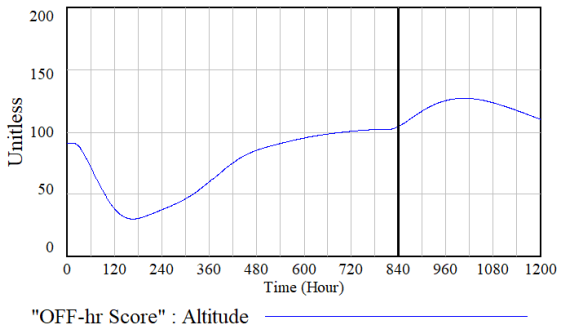
(a) *Hematocrit*



(b) *Hemoglobin Concentration*



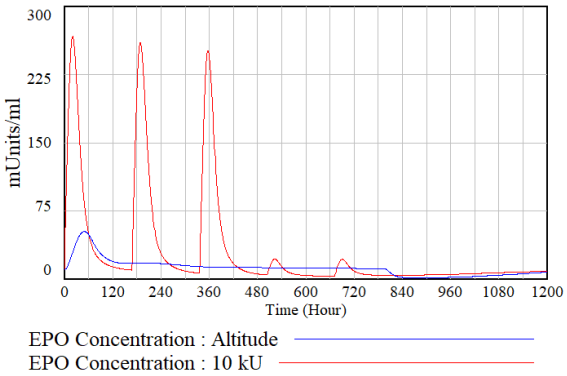
(c) *Reticulocyte Count*



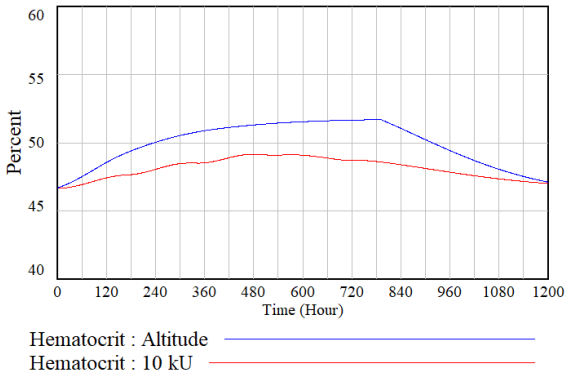
(d) *OFF-hr Score*

Figure 7.13. Dynamics of blood values in altitude training scenario (competition at 840 Hours).

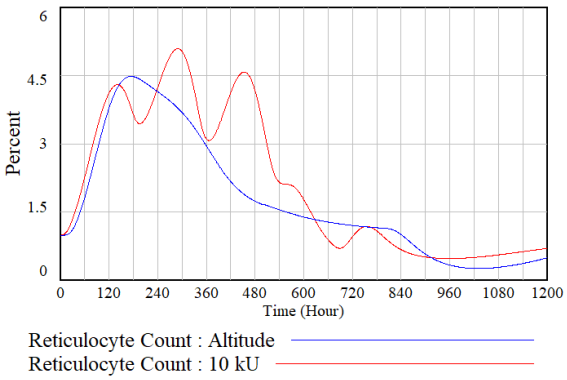
We also compare this scenario with the previous scenario of rHuEPO usage of 10 kU with switchover to 1 kU. The comparison graphs for *EPO Concentration*, *Hematocrit* value, *Reticulocyte Count* and *OFF-hr* score can be found in Figure 7.14. *EPO Concentration* differs drastically between two scenarios. Important thing to note is that, even though the *EPO Concentration* increases to higher peaks during rHuEPO usage, the continuous EPO existence during altitude training increases *Hematocrit*



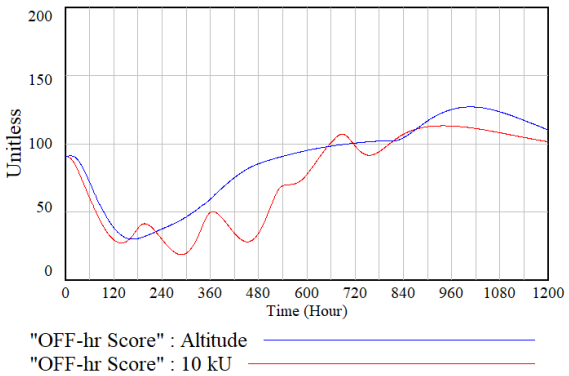
(a) *EPO Concentration*



(b) *Hematocrit*



(c) *Reticulocyte Count*



(d) *OFF-hr Score*

Figure 7.14. Altitude training and rHuEPO usage comparison (competition at 840 Hours).

higher than rHuEPO usage. Also, higher *EPO Concentration* peaks increase the maturation time of reticulocytes, thus, slowing their maturation to become erythrocytes. *EPO Concentration* stays suppressed longer in altitude training compared to rHuEPO usage. Our scenario comparison shows that, both altitude training and rHuEPO usage have similar blood value dynamics before the competition. A key difference is the oscillating *Reticulocyte Count* for rHuEPO usage. After the competition, *Reticulocyte Count* increases for rHuEPO users. However, for altitude trainers, *Reticulocyte Count* continues to decrease for some time before it eventually starts increasing. Our model can be calibrated (personalized) for individual athletes to check for the aforementioned dynamic patterns. These dynamic patterns can be compared with dynamic patterns

obtained from blood tests taken before, during, and after competition, to determine whether the athlete is an altitude trainer or a rHuEPO user.

7.5. Altitude Training with rHuEPO Usage Scenario

In this scenario, the possibility of masking rHuEPO usage with altitude training is simulated. Model is initialized with the same parameters as altitude training scenario with one difference. The athlete also takes weekly injections of 5 kU of rHuEPO for the duration of altitude stay. The competition is again at 840 hours. The athlete descends to sea level 48 hours before the competition and stops rHuEPO injection. Results for the *EPO Concentration* can be found in Figure 7.15.

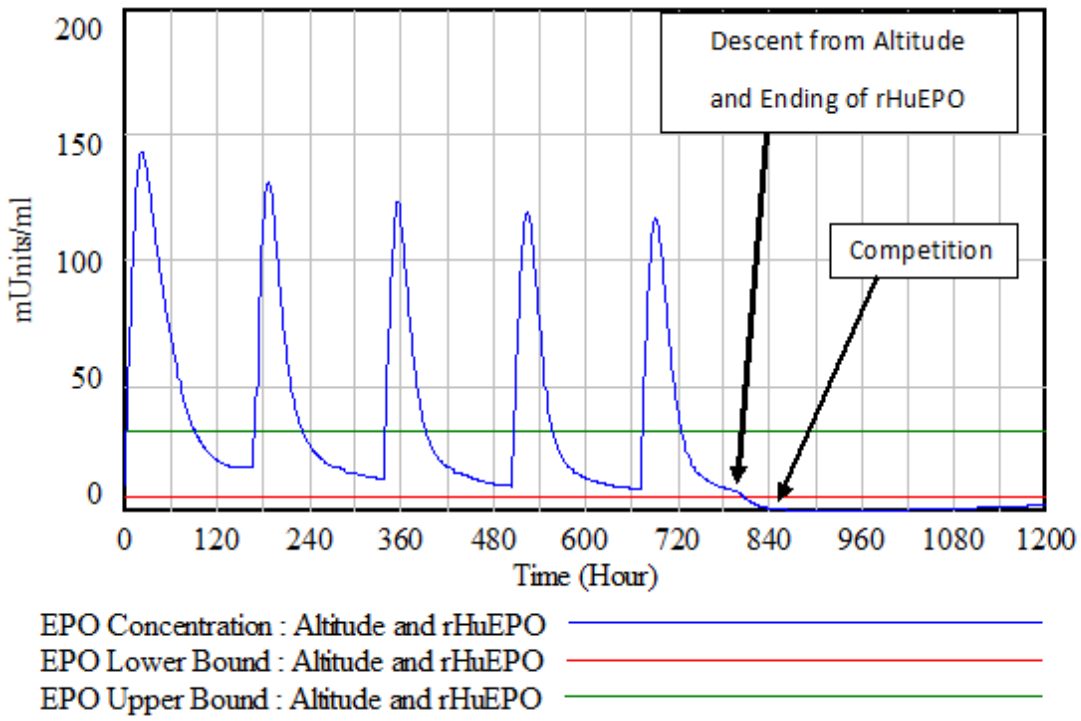
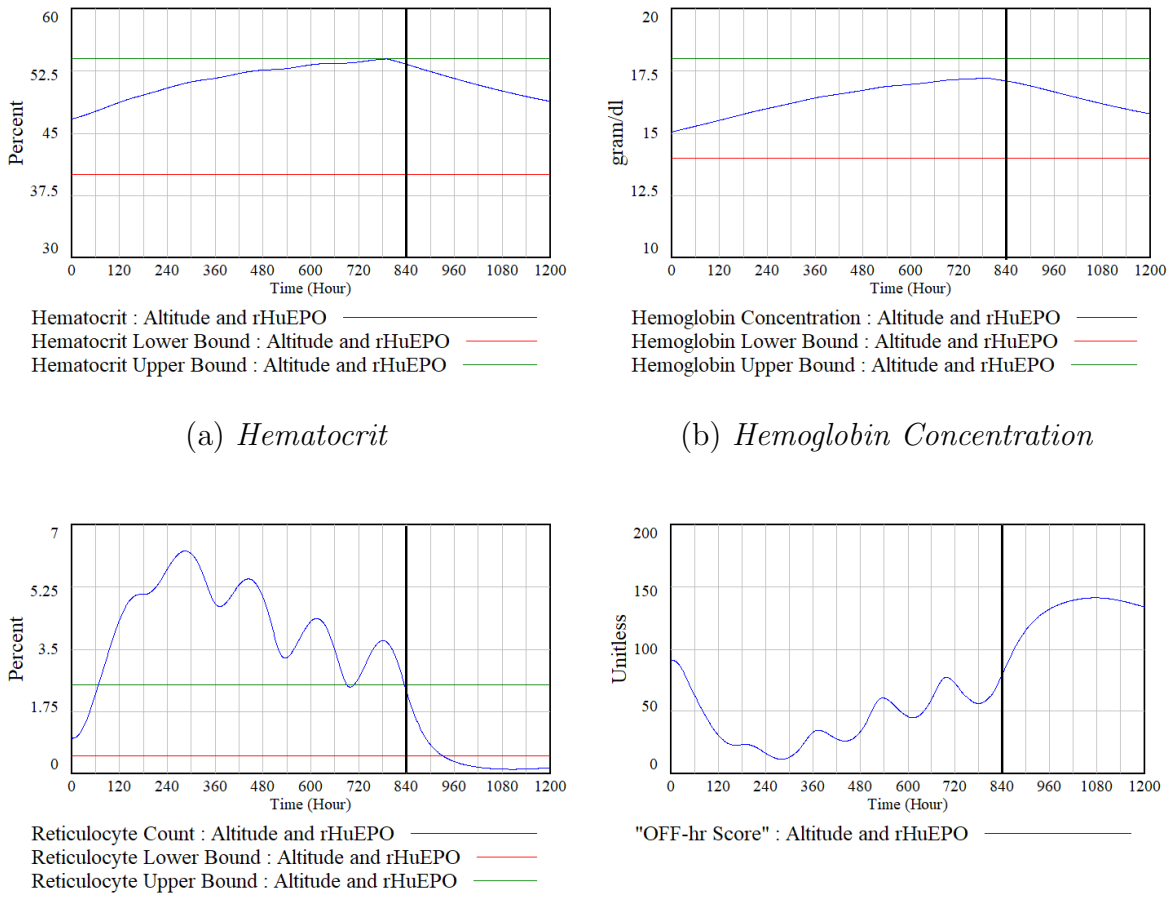


Figure 7.15. Dynamics of EPO concentration in altitude training with rHuEPO usage scenario.

The *EPO Concentration* after descent decreases to zero, since the body has excess red blood cells, similar to the previous scenario. However, this decrease lasts longer. The model-generated dynamics for the blood values can be found in Figure 7.16.



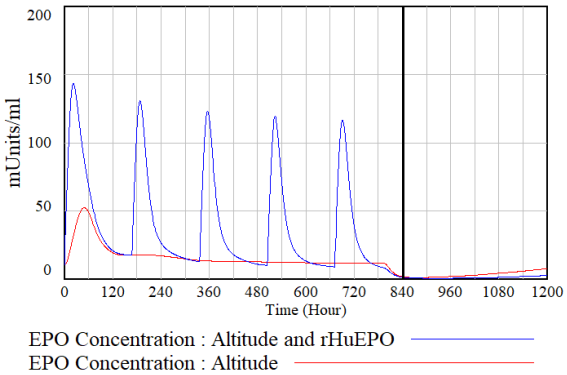
(a) *Hematocrit* (b) *Hemoglobin Concentration*

(c) *Reticulocyte Count* (d) *OFF-hr Score*

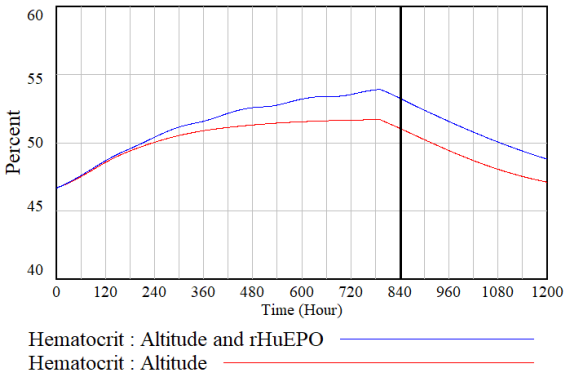
Figure 7.16. Dynamics of blood values under altitude training with rHuEPO usage scenario (competition at 840 Hours).

rHuEPO injection during altitude training has profound effects on the blood values on top of the effect of altitude training. Firstly, the *Hematocrit* increases quite significantly. It even comes close to its normal upper bound. *Reticulocyte Count* oscillates above its normal value for the duration of altitude stay and rHuEPO injection. The oscillations only die down after the descent to sea level and the cessation of drug use. During the competition, *Reticulocyte Count* decreases to below its normal bound and stays below its normal bound for the duration of the scenario. The reason for this is the almost non-existent *EPO Concentration*. Since both reticulocytes and erythrocytes have hemoglobin, the *Hemoglobin Concentration* of blood goes up. *OFF-hr score* oscillates near zero for the duration of altitude training and rHuEPO usage. After the

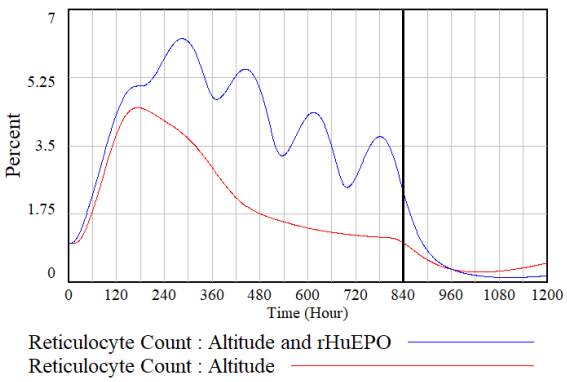
descent to sea level, the value for OFF-hr score goes up significantly. The reason for this behavior is the increase in the *Hemoglobin Concentration* and the decrease in the *Reticulocyte Count* (see Equation 7.1).



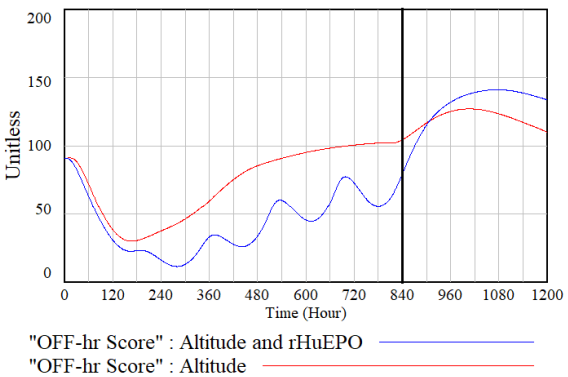
(a) *EPO Concentration*



(b) *Hematocrit*



(c) *Reticulocyte Count*



(d) *OFF-hr Score*

Figure 7.17. Comparison of altitude training with and without rHuEPO usage (competition at 840 Hours).

The comparison of this scenario with altitude training can be found in Figure 7.17. The general behaviors of the blood values are similar to altitude training. This fact makes rHuEPO usage during altitude training worthwhile for athletes since their blood oxygen capacity is boosted to higher levels and their blood values behave similarly compared to altitude training. There is one important difference between altitude training and altitude training with rHuEPO injection. The difference is that EPO level for altitude training with rHuEPO injection stays near zero longer than with

just-altitude training. This scenario is an example of how our model can be utilized to generate personalized dynamic patterns that can separate altitude trainers from rHuEPO users.

7.6. Discussion

We first conduct two disequilibrium scenarios, one for *EPO Concentration* and one for *Hematocrit*, and explore the model-generated dynamics. Based on these two scenarios, we conclude that our model can reach its equilibrium when disturbed, similar to human body trying to maintain homeostasis.

Single rHuEPO injection scenario is utilized to create a baseline for the dynamics of rHuEPO injection. This scenario starts with an injection of 15 kU of rHuEPO drug. After a single rHuEPO shot, *EPO Concentration* and *Reticulocyte Count* peak and rapidly decrease to below their equilibrium values. Also, based on this scenario, we conclude that the effects of a single rHuEPO injection on blood values can be detected long after the injection.

According to our rHuEPO usage scenarios, a clever rHuEPO user can avoid being caught and can also benefit from the effects of rHuEPO. Our model-generated dynamics show that the *Reticulocyte Count* is lower during the competition compared to the first phase of rHuEPO administration before the competition, and it starts increasing shortly after the competition. Also, *Reticulocyte Count* is in oscillation before the competition. Altitude trainers also have similar blood value dynamics before the competition compared to rHuEPO users. A key difference is that the *Reticulocyte Count* is not in oscillation for altitude trainers. Another key difference is that, for altitude trainers, *Reticulocyte Count* continues to decrease for about a week after the competition whereas for rHuEPO users it starts increasing a couple of days after competition.

Lastly, our final scenario involves a masking attempt of rHuEPO usage by altitude training. In this scenario, the hematocrit levels come close to the upper bound

of normal hematocrit levels during the competition. The blood values have similar dynamics between altitude training with and without rHuEPO usage. The behavior of *EPO Concentration* after the competition can be useful to identify athletes who try to mask rHuEPO usage with altitude training. For regular altitude trainers, *EPO Concentration* has an increasing trend between the blood tests during and after the competition. This is not the case for altitude training with rHuEPO usage where *EPO Concentration* stays close to zero between the two tests. Thus a dynamic testing procedure can be developed for the detection of rHuEPO users together with altitude training.

Based on the above scenario results, we show that our model can be calibrated for different rHuEPO regimens, for altitude training, and for altitude training together with rHuEPO usage. Our model can also be calibrated for individual athletes to develop strong personalized tests. The model can provide the dynamics of key blood values for a given athlete with and without rHuEPO usage. By taking blood samples before, during, and after competition, the real dynamics of these blood values can be estimated and a dynamic doping test can be developed by comparison against the model-generated dynamics.

8. CONCLUSION

In this study, a model is constructed to analyze the pharmacodynamics of a popular blood doping drug (rHuEPO-recombinant human erythropoietin) and its effects on various blood values. rHuEPO is a highly popular performance enhancing drug because of its short residence time in human body and the long-lasting effects of its usage on blood values and blood oxygen capacity. Altitude training -not considered doping by WADA (World Anti-Doping Agency)- is also highly utilized among athletes because of its similar effects on blood oxygen capacity. Our aim is to use our model to show the dynamic differences between altitude training and rHuEPO usage. Dynamic consequences of rHuEPO usage together with altitude training are also investigated.

Validation of our model is carried out by structure confirmation tests and by output behavior tests with real data. Two settings are used to validate the effects of altitude on EPO (erythropoietin) level and the effect of EPO level on reticulocyte production. The first setting involves altitude staying at 2800 m and the second setting involves staying at 4340 m. To check the validity of the changes in P50 (the partial pressure of oxygen when the blood is 50% saturated) and in plasma volume with altitude, two validation scenarios are employed. Another validation scenario is carried out to test the behavior of our model under excess oxygen levels (provided by oxygen tubes during diving). Finally, two rHuEPO dosage scenarios are utilized for the validation of the pharmacodynamics of rHuEPO and its effects on reticulocyte production. According to the results of our validation scenarios, our model creates dynamics that are consistent with the existing literature and data.

Using our model, we first run disequilibrium experiments to show how our model returns to equilibrium when initialized at disequilibrium. We then conduct a base scenario that displays the dynamics with rHuEPO usage. We next analyze three test scenarios. The first of these scenarios involves sensitivity analysis with a two-phase rHuEPO dosage regimen involving three different doses. According to the scenario,

the rHuEPO user may be able to avoid being caught with clever dosage regimens. We show that, for rHuEPO users, *Reticulocyte Count* is lower during the competition compared to during the first phase of rHuEPO administration prior to the competition. Second and third scenarios involve altitude training. The second scenario shows the behavior of EPO and various blood values during training at an altitude of 2800 m after descent to sea level. Our results indicate that, the behaviors of blood values for rHuEPO users and altitude trainers are similar up until the competition day. Based on our parameters, we show that, for altitude trainers, *Reticulocyte Count* continues to decrease after the competition before it eventually starts increasing. However, for rHuEPO users, *Reticulocyte Count* starts increasing almost immediately after competition. This dynamic pattern (trend) information can be used to design a strong anti-doping test involving the dynamics of key blood parameters. To this end, blood samples must be taken at least three times: n days before, during and m days after the competition. As our last scenario, the usage of rHuEPO together with altitude training is explored. *Hematocrit* increases close to the upper boundary for normal *Hematocrit* in this case. *EPO Concentration* also stays suppressed longer than regular altitude training. With these scenario analyses, we show how our model can be calibrated for various doping regimens and altitude training. With future research and practice, our model can be personalized with individual data of athletes. These simulated dynamics of key indicators can be compared to the real dynamics estimated by blood samples taken before, during and after competition. By this approach, it should be possible to develop dynamic, hard-to-cheat, strong personalized tests against rHuEPO use.

Our model can also be utilized for other scenarios involving different rHuEPO injection times and regimens. The model can be calibrated for mountain climbers and deep-sea divers to predict their blood values during long expeditions and dives and help them prepare. In future research, the model can be modified for other red blood cell stimulating drugs and their effects. The iron metabolism can be added to the model to see the limitations of red blood cell production and to find new markers for rHuEPO usage. Our model can also be modified to analyze the therapeutic effects of rHuEPO drug on anemic patients and to come up with personal optimal dosage regimens.

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APPENDIX A: MODEL EQUATIONS

Altitude=0

Units: meters

Altitude 2800=2800

Units: meters

Altitude Ratio=Altitude/Altitude 2800

Units: Dmnl

Altitude Ratio for Plasma Volume= Altitude/Threshold Altitude for Plasma Volume Change

Units: 1

Arterial Blood Oxygen Concentration Ratio= Oxygen Concentration of Arterial Blood/ Normal Oxygen Concentration of Arterial Blood

Units: 1

Basal HIF Levels=50

Units: Factors

Blood Oxygen Saturation at Arteries=((Partial Oxygen Pressure in Arteries/P50) ∧2.7)/(1+((Partial Oxygen Pressure in Arteries/P50) ∧2.7))

Units: Dmnl

Blood Volume=RBC Volume+Plasma Volume

Units: dl

Blood Volume in ml=Blood Volume*dl to ml Convertor

Units: ml

Change in Plasma Volume=((Plasma Volume-Normal Plasma Volume)/Normal Plasma Volume)*Percentage Conversion

Units: Percent

Corrected Reticulocytes=(Reticulocyte Count*Hematocrit)/Normal Hematocrit

Units: Percent

Del Time2=50

Units: Hour

Del Time3=24

Units: Hour

Del Time4=240

Units: Hour

Del Time5=3

Units: Hour

Del Time6=70

Units: Hour

dl to ml Convertor=100

Units: ml/dl

Dose=0

Units: Units

ED50=63200

Units: Units

Effect of Altitude on Partial Oxygen Pressure in Arteries=LOOKUP EXTRAPOLATE(Graph of Effect of Altitude on Partial Pressure of Oxygen in Arteries ,Altitude Ratio)

Units: Dmnl

Effect of Altitude on Plasma Volume= SMOOTHI(Indicated Effect of Altitude on Plasma Volume,Del Time4,1)

Units: Dmnl

Effect of Arterial Blood Oxygen Concentration Ratio on HIF Levels= LOOKUP EXTRAPOLATE(Graph of Effect of Arterial Blood Oxygen Concentration Ratio on HIF Levels ,Arterial Blood Oxygen Concentration Ratio)

Units: Dmnl

Effect of EPO Ratio on Reticulocyte Maturation Delay= SMOOTH3I(Indicated Effect of EPO Ratio on Reticulocyte Maturation Time,Del Time2 ,1)

Units: Dmnl

Effect of EPO Ratio on Reticulocyte Production= LOOKUP EXTRAPOLATE(Graph of Effect of EPO Ratio on Reticulocyte Production ,EPO Ratio)

Units: Dmnl

Effect of HIF Ratio on EPO Production Rate= SMOOTH3I(Indicated Effect of HIF Ratio on EPO Production Rate,Del Time5,1)

Units: Dmnl

Effect of HIF Ratio on HIF Destruction Rate= SMOOTH3I(Indicated Effect of HIF Ratio on HIF Destruction Rate,Del Time6,1)

Units: Dmnl

Emax= 0.649

Units: 1

EPO Concentration= EPO in mUnits/Blood Volume in ml

Units: mUnits/ml

EPO Flow from Blood to Tissues= EPO Level in Blood*k23

Units: Units/Hour

EPO Flow from Tissues to Blood= EPO in Peripheral Tissues*k32

Units: Units/Hour

EPO in mUnits= EPO Level in Blood*mUnits Convertor

Units: mUnits

EPO in Peripheral Tissues= INTEG (EPO Flow from Blood to Tissues-EPO Flow from Tissues to Blood, 2.1959)

Units: Units

EPO Level in Blood= INTEG (rHuEPO absorption from Depot Compartment+EPO Flow from Tissues to Blood+EPO Production Rate +rHuEPO Slow Absorption Flow-EPO Flow from Blood to Tissues-EPO Linear Destruction Rate -EPO Saturated Destruction Rate, 53.5)

Units: Units

EPO Linear Destruction Fraction= 0.0716

Units: 1/Hour

EPO Linear Destruction Rate= EPO Level in Blood*EPO Linear Destruction Fraction

Units: Units/Hour

EPO Production Rate= Normal EPO Production per Hour*Effect of HIF Ratio on EPO Production Rate

Units: Units/Hour

EPO Ratio= EPO Level in Blood/Normal EPO Level

Units: Dmnl

EPO Saturated Destruction Rate= (V max*EPO Level in Blood)/(Km+EPO Level in Blood)

Units: Units/Hour

Erythrocyte Lifetime= 2880

Units: Hour

Erythrocytes in Blood= INTEG (Reticulocyte Maturation Rate-Hemolysis, 2.5e+13)

Units: cells

F= F0+((Emax*Dose)/(ED50+Dose))

Units: 1

F0= 0.62

Units: 1

fr= 0.6

Units: 1

Graph of Effect of Altitude on Partial Pressure of Oxygen in Arteries([(0,0)-(4,2)],(0, 1.695),(0.00357,1.695),(0.0357,1.6627),(1,1),(1.21412,0.882562),(1.59059,0.768683), (1.97647,0.683274),(2.37176,0.604982),(2.76706,0.533808),(3.16,0.47),(3.36941, 0.434164),(3.66118,0.384342),(3.85882,0.3843))

Units: Dmnl

Graph of Effect of Altitude on Plasma Volume([(0,0)-(8,1)],(0,1),(1,1),(1.5,0.85),(2.25, 0.7),(2.96471,0.601424),(3.95294 ,0.548043),(4.91765,0.512456),(5.90588,0.505338),(7, 0.5),(8,0.5))

Units: 1

Graph of Effect of Arterial Blood Oxygen Concentration Ratio on HIF Levels([(0.8,0)-(1.4,7)],(0.8,7),(0.825896,7),(0.85259,6.80556),(0.881275,6.14444),(0.900398,5.28889), (0.912351,4.74444),(0.92,4.25979),(0.926588,3.91667),(0.933865,3.52778),(0.938645, 3.25),(0.943426,3.05556),(0.952471,2.63889),(0.960159 ,2.22222),(0.969721,1.80556), (0.981673,1.44444),(0.988471,1.17438),(1,1),(1.02709 ,0.694445),(1.06056,0.444445), (1.08685,0.305555),(1.11076,0.194445),(1.15618 ,0.111111),(1.3,0),(1.31,0))

Units: Dmnl

Graph of Effect of EPO on Reticulocyte Maturation Time([(1,0)-(8.167,6)],(0,1),(1,1),(1.60471,2.41993),(2.2,3.30961),(3.10588,4.27046),(4.24471,4.7331),(5.53882,5.01779),(6.85176,5.08897),(8.16706,5.089))

Units: Dmnl

Graph of Effect of EPO Ratio on Reticulocyte Production([(0,0)-(3.5,3)],(0,0),(0.063745,0.0666667),(0.135458,0.144444),(0.219124,0.244444),(0.320717,0.35),(0.432271,0.466667),(0.571713,0.616667),(0.752988,0.783333),(0.864542,0.9),(1,1),(1.11554,1.1),(1.29681,1.26667),(1.47809,1.43333),(1.71514 ,1.61667),(1.85458,1.75),(2.0498,1.88333),(2.23108,2),(2.41235,2.06667),(2.59363 ,2.15),(2.84118,2.2),(3.5,2.2))

Units: Dmnl

Graph of Effect of HIF Ratio on EPO Production Rate([(0,0)-(5,7)],(0,0),(0.278884,0.0833335),(0.486056,0.255556),(0.657371,0.472222),(0.836653,0.738889),(0.968127,0.944444),(1,1),(1.21176,1.29537),(1.4741,1.63333),(1.73307,2.04444),(2.15139,2.56667),(2.49004,3.07222),(2.86853,3.6),(3.16471 ,4.13523),(3.50598,4.66667),(3.76494,5.25),(4.06375,5.87222),(4.34263,6.22222),(4.58167,6.49444),(4.86056,6.53333),(5,6.533))

Units: Dmnl

Graph of Effect of HIF Ratio on HIF Destruction Rate([(1,0)-(5,5)],(0,1),(1,1),(1.1753,1.52778),(1.36653,1.91667),(1.55777,2.22222),(1.82869,2.55556),(2.08367,2.80556),(2.49801,3.16667),(2.89641,3.5),(3.34263 ,3.88889),(3.80478,4.27778),(4.21912,4.61111),(4.56972,4.86111),(5,5))

Units: Dmnl

Hematocrit= (RBC Volume/Blood Volume)*Percentage Conversion

Units: Percent

Hemoglobin Concentration= Total Blood Hemoglobin/Blood Volume

Units: gram/dl

Hemoglobin Mass per Erythrocyte= 3e-11

Units: gram/cells

Hemoglobin mass per Reticulocyte= 2.7e-11

Units: gram/cells

Hemolysis= Erythrocytes in Blood/Erythrocyte Lifetime

Units: cells/Hour

HIF= INTEG (HIF Production Rate-HIF Destruction Rate, 50)

Units: Factors

HIF Destruction Delay= 25

Units: Hour

HIF Destruction Rate= (HIF/HIF Destruction Delay)*Effect of HIF Ratio on HIF Destruction Rate

Units: Factors/Hour

HIF Normal Production Rate= 2

Units: Factors/Hour

HIF Production Rate= HIF Normal Production Rate*Effect of Arterial Blood Oxygen Concentration Ratio on HIF Levels

Units: Factors/Hour

HIF Ratio= HIF/Basal HIF Levels

Units: 1

Hours to Days Convertor= 1/24

Units: Day/Hour

Indicated Effect of Altitude on Plasma Volume= LOOKUP EXTRAPOLATE(Graph of Effect of Altitude on Plasma Volume,Altitude Ratio for Plasma Volume)

Units: Dmnl

Indicated Effect of EPO Ratio on Reticulocyte Maturation Time= LOOKUP EXTRAPOLATE(Graph of Effect of EPO on Reticulocyte Maturation Time, EPO Ratio)

Units: Dmnl

Indicated Effect of HIF Ratio on EPO Production Rate= LOOKUP EXTRAPOLATE(Graph of Effect of HIF Ratio on EPO Production Rate,HIF Ratio)

Units: Dmnl

Indicated Effect of HIF Ratio on HIF Destruction Rate= LOOKUP EXTRAPOLATE(Graph of Effect of HIF Ratio on HIF Destruction Rate,HIF Ratio)

Units: Dmnl

Indicated P50= Intercept+Slope*Altitude

Units: mmHg

Intercept= 26.7

Units: mmHg

k23= 0.011

Units: 1/Hour

k32= 0.268

Units: 1/Hour

Km= 394*5

Units: Units

mUnits Convertor= 1000

Units: mUnits/Units

Normal EPO Level= 53.5

Units: Units

Normal EPO Production per Hour= 9.4093

Units: Units/Hour

Normal Hematocrit= 46.7

Units: Percent

Normal Oxygen Concentration of Arterial Blood= 19.61

Units: ml/dl

Normal Plasma Volume= 26.8

Units: dl

Normal Reticulocyte Maturation Delay= 28.57

Units: Hour

Normal Reticulocyte Production per Hour= 8.68e+09

Units: cells/Hour

Oxygen Concentration of Arterial Blood= Oxygen Content of Arterial Blood/Blood Volume

Units: ml/dl

Oxygen Content of Arterial Blood= Oxygen Content per One Gram of Hemoglobin* Total Blood Hemoglobin*Blood Oxygen Saturation at Arteries

Units: ml

Oxygen Content per One Gram of Hemoglobin= 1.34

Units: ml/gram

P50= SMOOTH3I(Indicated P50,Del Time3,P50 Initial)

Units: mmHg

P50 Initial= 26.7

Units: mmHg

Partial Oxygen Pressure= IF THEN ELSE(Time<336, 200 , 100)

Units: mmHg

Partial Oxygen Pressure in Arteries= Partial Oxygen Pressure of 59*Effect of Altitude on Partial Oxygen Pressure in Arteries

Units: mmHg

Partial Oxygen Pressure of 59= 59

Units: mmHg

Percentage Conversion= 100

Units: Percent

Plasma Volume= Normal Plasma Volume*Effect of Altitude on Plasma Volume

Units: dl

RBC Volume= Erythrocytes in Blood*Volume Per Erythrocyte+Volume per Reticulocyte*Reticulocytes in Blood

Units: dl

"Ret. Maturation in Days"= Reticulocyte Maturation Delay*Hours to Days Convertor

Units: Day

Reticulocyte Count= (Reticulocytes in Blood/(Erythrocytes in Blood+Reticulocytes in Blood))*Percentage Conversion

Units: Percent

Reticulocyte Maturation Delay= Normal Reticulocyte Maturation Delay*Effect of EPO Ratio on Reticulocyte Maturation Delay

Units: Hour

Reticulocyte Maturation Rate= Reticulocytes in Blood/Reticulocyte Maturation Delay

Units: cells/Hour

Reticulocyte Production Rate= Normal Reticulocyte Production per Hour*Effect of EPO Ratio on Reticulocyte Production

Units: cells/Hour

Reticulocyte Release Delay= 75.58

Units: Hour

Reticulocyte Release Rate= Reticulocytes in Bone Marrow/Reticulocyte Release Delay

Units: cells/Hour

Reticulocytes in Blood= INTEG (Reticulocyte Release Rate-Reticulocyte Maturation Rate, 2.48e+11)

Units: cells

Reticulocytes in Bone Marrow= INTEG (Reticulocyte Production Rate-Reticulocyte Release Rate, 6.56e+11)

Units: cells

rHuEPO Absorption Constant= 0.034

Units: 1/Hour

rHuEPO absorption from Depot Compartment= rHuEPO in Depot Compartment*

rHuEPO Absorption Constant

Units: Units/Hour

rHuEPO Dose for Fast Absorption= INTEG (rHuEPO SC Injection Dose for Fast Absorption-rHuEPO Fast Absorption Flow, 0)

Units: Units

rHuEPO Dose for Slow Absorption= INTEG (rHuEPO SC Injection for Slow Absorption-rHuEPO Slow Absorption Delay Flow1,0)

Units: Units

rHuEPO Fast Absorption Delay Time= 0.5

Units: Hour

rHuEPO Fast Absorption Flow= rHuEPO Dose for Fast Absorption/rHuEPO Fast Absorption Delay Time

Units: Units/Hour

rHuEPO in Depot Compartment= INTEG (rHuEPO Fast Absorption Flow-rHuEPO absorption from Depot Compartment, 0)

Units: Units

rHuEPO SC Injection Dose for Fast Absorption= IF THEN ELSE(Time=0, (Dose*(F-

0.01)*(fr))/TIME STEP , 0)

Units: Units/Hour

rHuEPO SC Injection for Slow Absorption= IF THEN ELSE(Time=0, (Dose*(F-0.01)*(1-fr))/(TIME STEP) , 0)

Units: Units/Hour

rHuEPO Slow Absorption Delay Flow1= rHuEPO Dose for Slow Absorption/rHuEPO Slow Absorption Delay Time1

Units: Units/Hour

rHuEPO Slow Absorption Delay Flow2= rHuEPO Slow Absorption Delay Stock1 /rHuEPO Slow Absorption Delay Time1

Units: Units/Hour

rHuEPO Slow Absorption Delay Flow3= rHuEPO Slow Absorption Delay Stock2 /rHuEPO Slow Absorption Delay Time1

Units: Units/Hour

rHuEPO Slow Absorption Delay Stock1= INTEG (rHuEPO Slow Absorption Delay Flow1-rHuEPO Slow Absorption Delay Flow2, 0)

Units: Units

rHuEPO Slow Absorption Delay Stock2= INTEG (rHuEPO Slow Absorption Delay Flow2-rHuEPO Slow Absorption Delay Flow3, 0)

Units: Units

rHuEPO Slow Absorption Delay Stock3= INTEG (rHuEPO Slow Absorption Delay Flow3-rHuEPO Slow Absorption Flow, 0)

Units: Units

rHuEPO Slow Absorption Delay Time1= 4

Units: Hour

rHuEPO Slow Absorption Delay Time2= 9

Units: Hour

rHuEPO Slow Absorption Flow= rHuEPO Slow Absorption Delay Stock3/rHuEPO Slow Absorption Delay Time2

Units: Units/Hour

Slope= 0.00095

Units: mmHg/meters

Threshold Altitude for Plasma Volume Change= 2000

Units: meters

TIME STEP = 0.0625 Units: Hour [0,?]

The time step for the simulation.

Total Blood Hemoglobin= Erythrocytes in Blood*Hemoglobin Mass per Erythrocyte+

Reticulocytes in Blood *Hemoglobin mass per Reticulocyte

Units: gram

V max= 211

Units: Units/Hour

Volume Per Erythrocyte= 9.28e-13

Units: dl/cells

Volume per Reticulocyte= 1.15e-12

Units: dl/cells

Stay at an Altitude of 2800 m:

Altitude=2800

Units: meters

Stay at an Altitude of 4340 m:

Altitude=4340

Units: meters

P50:

Altitude=4530

Units: meters

Plasma Volume 3000 m:

Altitude=3000

Units: meters

Plasma Volume 4500 m:

Altitude=3000

Units: meters

Saturation Diving:

Blood Oxygen Saturation at Arteries=((Partial Oxygen Pressure/P50) ∧2.7)/(1+((Partial

Oxygen Pressure/P50) ∧2.7))

Units: Dmnl

Partial Oxygen Pressure= 200

Units: mmHg

20 kU Injection:

Dose=20000

Units: Units

160 kU Injection:

Dose=160000

Units: Units

EPO Disequilibrium:

EPO Level in Blood= INTEG (rHuEPO absorption from Depot Compartment+EPO Flow from Tissues to Blood+EPO Production Rate +rHuEPO Slow Absorption Flow-EPO Flow from Blood to Tissues-EPO Linear Destruction Rate -EPO Saturated Destruction Rate, 251.45)

Units: Units

Hematocrit Disequilibrium:

Erythrocytes in Blood= INTEG (Reticulocyte Maturation Rate-Hemolysis, 2.7889e+13)

Units: cells

Blood Volume=23.49+Plasma Volume

Units: dl

Base Model Dynamics as a Resut of Single rHuEPO Shot:

Dose=15000

Units: Units

2 kU of rHuEPO then Switchover to 0.2 kU:

Dose=IF THEN ELSE(Time<500,2000,200)

Units: Units

rHuEPO SC Injection Dose for Fast Absorption= IF THEN ELSE(Time<840, (IF THEN ELSE(MODULO(Time, 168)=0, (Dose*(F-0.01) *(fr))/(TIME STEP) , 0)),0)

Units: Units/Hour

rHuEPO SC Injection for Slow Absorption= IF THEN ELSE(Time<840, (IF THEN

ELSE(MODULO(Time, 168)=0, (Dose*(F-0.01) *(1-fr))/(TIME STEP) , 0)),0)

Units: Units/Hour

10 kU of rHuEPO then Switchover to 1 kU:

Dose=IF THEN ELSE(Time<500,10000,1000)

Units: Units

rHuEPO SC Injection Dose for Fast Absorption= IF THEN ELSE(Time<840, (IF THEN ELSE(MODULO(Time, 168)=0, (Dose*(F-0.01) *(fr))/(TIME STEP) , 0)),0)

Units: Units/Hour

rHuEPO SC Injection for Slow Absorption= IF THEN ELSE(Time<840, (IF THEN ELSE(MODULO(Time, 168)=0, (Dose*(F-0.01) *(1-fr))/(TIME STEP) , 0)),0)

Units: Units/Hour

25 kU of rHuEPO then Switchover to 3 kU:

Dose=IF THEN ELSE(Time<500,25000,3000)

Units: Units

rHuEPO SC Injection Dose for Fast Absorption= IF THEN ELSE(Time<840, (IF THEN ELSE(MODULO(Time, 168)=0, (Dose*(F-0.01) *(fr))/(TIME STEP) , 0)),0)

Units: Units/Hour

rHuEPO SC Injection for Slow Absorption= IF THEN ELSE(Time<840, (IF THEN ELSE(MODULO(Time, 168)=0, (Dose*(F-0.01) *(1-fr))/(TIME STEP) , 0)),0)

Units: Units/Hour

Altitude Training:

Altitude= IF THEN ELSE(Time<792, 2800 , 0)

Units: meters

Altitude Training with rHuEPO Usage:

Dose=5000

Units: Units

rHuEPO SC Injection for Slow Absorption= IF THEN ELSE(Time<792, (IF THEN ELSE(MODULO(Time, 168)=0, (Dose*(F-0.01)* (1-fr))/(TIME STEP) , 0)),0)

Units: Units/Hour

rHuEPO SC Injection Dose for Fast Absorption= IF THEN ELSE(Time<792, (IF THEN ELSE(MODULO(Time, 168)=0, (Dose*(F-0.01)* (fr))/(TIME STEP) , 0)),0)

Units: Units/Hour

Altitude= IF THEN ELSE(Time<792, 2800 , 0)

Units: meters