APPROVAL SHEET

Title of Thesis: A Simple Model for the Degradation of Cross-Sectional Area of a Skeletal Muscle Fiber Due to the Transcription Factor FOXO-1

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ABSTRACT

Title of Thesis: A Simple Model for the Degradation of Cross-Sectional Area of a Skeletal Muscle Fiber Due to the Transcription Factor FOXO-1

BreAsia Deal, Master, 2018

Thesis directed by: Doctor Bradford Peercy, Associate Professor
Department of Mathematics and
Statistics

We are investigating, through mathematical modeling and analysis, the signaling pathway of the FOXO-1 protein family transcription factors that involve the activation by Insulin Growth Factors (IGF) and Protein Kinase B (Akt). In doing so, it is important to know how the phosphorylation state of the FOXO-1 proteins causes the degeneration of muscular-skeletal fibers (Wimmer *et al.* 2014). In this paper, we will be modeling biochemical behaviors involving the FOXO-1 protein family and its effects in muscle atrophy. In studying muscle atrophy we consider how the nuclear to cytoplasm FOXO-1 ratio, the total FOXO-1, and the nuclear FOXO-1 effect the amount of degradation protein produced by FOXO-1 to breakdown the muscle volume, and the cross-sectional area of the muscle over the course of several days.

A Simple Model for the Degradation of Cross-Sectional Area of a Skeletal Muscle Fiber Due to the Transcription Factor FOXO-1

by

BreAsia Deal

Thesis submitted to the Faculty of the Graduate School
of the University of Maryland in partial fulfillment
of the requirements for the degree of
Master
2018

This thesis is dedication to all who have helped and encouraged me, I appreciate all of your love and support. To my mother, who knew my potential before I could see it, to my siblings, who I know will do more than I will, to a family that believed in me, and to the countless number of people that have touched my life.

"I will lift my eyes to the hills, from whence comes my help?"- Psalm 121:1

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BACKGROUND

We are investigating, through mathematical modeling and analysis, the signaling pathway of the FOXO-1 protein family transcription factors that are post-transcriptionally modified by Protein Kinase B (Akt) and the upstream external regulators such as Insulin Growth Factor (IGF). We focus on this system in skeletal muscle fibers. In doing so, it is important to know how the phosphorylation of the FOXO-1 proteins causes the degeneration of skeletal muscle fibers (Wimmer *et al.* 2014). In this paper, we will be modeling the behavior of biochemical interactions involving the FOXO-1 protein family and their effects on muscle atrophy. During muscle atrophy, FOXO-1 is phosphorylated in the cytoplasm and nucleus. When the FOXO-1 protein is phosphorylated in the nucleus, it travels through a nuclear pore, into the cytoplasm. This prevents the reduction of protein, which triggers the degeneration of the skeletal muscle. Furthermore, when FOXO-1 is phosphorylated in the cytoplasm it is prevented from entering the nucleus also preventing degeneration.

To connect phosphorylated FOXO-1 and unphosphorylated FOXO-1 protein to muscle atrophy (muscular mass decrease), we study the breakdown of the skeletal muscle by constructing two mathematical models for muscle atrophy as it relates to un/phosphorylated FOXO-1 protein. In creating our basic model, we focus on (a) the ratio of FOXO-1 protein

within the nucleus to the cytoplasm, (b) a generic degradation protein regulated by FOXO-1, and (c) the cross-sectional area of the skeletal muscle change over the course of several days. In our second model, we consider feedback from total FOXO-1 changing over the course of several days.

1.1 Nucleus to Cytoplasm FOXO-1 Ratio Calculations

For this paper, we consider a four state model and its two state reduction found in (Wimmer *et al.* 2014). We begin with a four state model of the phosphorylated and unphosphorylated FOXO-1 protein in the nucleus and cytoplasm. The four states are represented by (U_c) the unphosphorylated cytoplasmic FOXO-1, (P_c) the phosphorylated cytoplasmic FOXO-1, (U_n) the unphosphorylated nuclear FOXO-1, and (P_n) the phosphorylated nuclear FOXO-1. For the four state model uses rate constants: k_{P_c}, k_{U_c}, k_{P_n}, and k_{U_c}. We use a rapid phosphorylation assumption to reduce the four state model to the two state model shown below in equations (1.1-1.2). The two state model is presented here,

$$\frac{dN}{dt} = I - E, \tag{1.1}$$

$$\frac{dC}{dt} = \varepsilon(E - I), \tag{1.2}$$

where $N = U_n + P_n$ is the FOXO-1 in the nucleus, $C = U_c + P_c$ is the FOXO-1 in the cytoplasm, I is the rate of nuclear influx and E is the rate of nuclear efflux. In equation (1.2), the nuclear to cytoplasmic volume ratio is $\varepsilon = \frac{V_n}{V_c}$. We apply the nucleus to cytoplasmic volume ratio to our model of the cross-sectional area. When calculating the nucleus to cytoplasm volume ratio we take into consideration the difference between the nucleus and cytoplasm volume because the nuclear volume is small in comparison to the cytoplasmic volume. The nucleus to cytoplasm volume ratio is the volume of the nucleus divided by the

volume of the cytoplasm, this is taken into consideration because FOXO-1 is moving back and forth from the nucleus and cytoplasm. In the two state model shown in equations (1.3-1.4), there are new variables introduced from rapid phosporylation assumption in the four state model; (Wimmer *et al.* 2014) introduces $U_c = \left(\frac{k_{U_c}}{k_{U_c}+k_{P_c}}\right) C$ and $P_n = \left(\frac{k_{P_n}}{k_{P_n}+k_{U_n}}\right) N$ as the un/phosphorylated FOXO-1 in the cytoplasm and nucleus. Below, influx is proportional to the unphosphorylated cytoplasmic FOXO-1 and the efflux is proportional to the phosphorylated nuclear FOXO-1. We can rewrite the nuclear and cytoplasmic FOXO-1 equations as,

$$\frac{dN}{dt} = k_I U_c - k_E P_n, \tag{1.3}$$

$$\frac{dC}{dt} = \varepsilon(k_I P_n - k_E U_c). \tag{1.4}$$

Letting $k_I^{'} = k_I \left(\frac{k_{U_c}}{k_{U_c}-k_{P_c}}\right)$ and $k_E^{'} = k_E \left(\frac{k_{P_n}}{k_{P_n}-k_{U_n}}\right)$ where k_U and k_P are the de/phosphorylation rates with the c or n subscript denoting whether the reaction is in the cytoplasm or nucleus, respectively. With these assumptions we get the two state model,

$$\frac{dN}{dt} = k_I^{'} C - k_E^{'} N, \tag{1.5}$$

$$\frac{dC}{dt} = \varepsilon(k_E^{'} N - k_I^{'} C). \tag{1.6}$$

The presence of k_{Pn} and k_{Pc} is important because they depend on the level of Akt,

thus allowing us to interpret changes in $k_E^{'}$ and $k_I^{'}$ in terms of Akt. By conservation, $C_T = C + \varepsilon N$, where C_T is the total constant FOXO-1 in relation to the cytoplasm's volume. This allows us to consider a single equation for the nuclear FOXO-1,

$$\frac{dN}{dt} = k_I^{'} C_T - (k_E^{'} - \varepsilon k_I^{'}) N. \tag{1.7}$$

Therefore the FOXO-1 concentration in the nucleus is represented as:

$$N = C_T \left[\left(\frac{N_0}{C_T} - \frac{k_I'}{k_E' + \varepsilon k_I'} \right) e^{-(k_E' + \varepsilon k_I')t} + \frac{k_I'}{k_E' + \varepsilon k_I'} \right]. \tag{1.8}$$

In conclusion, the cytoplasm volume is much larger than the nuclear volume so we take the nuclear to cytoplasm volume ratio limit to be small, so that $\varepsilon \to 0$. Therefore, $C_T = C$ and now we can divide equation (1.8) by C to get the nucleus to cytoplasm FOXO-1 ratio. The nucleus to cytoplasm FOXO-1 ratio is

$$\frac{N}{C} = \left(\frac{N_0}{C_T} - \frac{k_I'}{k_E'} \right) e^{-(k_E')t} + \frac{k_I'}{k_E'} \tag{1.9}$$

.

1.2 Calculating the rate of change of the nuclear to cytoplasm FOXO-1 ratio

In modeling the degradation of the cross sectional area of a skeletal muscle, we will show the dynamics of the FOXO-1 proteins in the nucleus and the cross sectional area. In doing so, we must understand the process happening within the nucleus to produce degradation protein and degrade the muscle. Within the IGF pathway, the receptor of the Insulin Growth Factor activates Protein Kinase B, Akt, which leads to the phosphorylation of FOXO-1 protein. When a FOXO-1 protein is phosphorylated, it is unable to enter into the nucleus but, when the IGF pathway is blocked FOXO-1 is unphosphorylated allowing it to enter into the nucleus. This then leads to an increase in the degradation protein production that causes muscle atrophy.

We begin by calculating the rate of nuclear to cytoplasm FOXO-1 ratio over time. We represent the nuclear to cytoplasm ratio as $r = (\frac{N}{C})$ and set $\varepsilon = 0$ from equation (1.9). The rate of nuclear to cytoplasm FOXO-1 ratio is,

$$\frac{dr}{dt} = k'_I - k'_E r. \tag{1.10}$$

We can rewrite the above equation as,

$$\frac{dr}{dt} = \frac{\frac{k'_I}{k'_E} - r}{\frac{1}{k'_E}}. \tag{1.11}$$

let $r_\infty = \frac{k'_I}{k'_E}$ and $\tau = \frac{1}{k'_E}$, then

$$\frac{dr}{dt} = \frac{r_\infty - r}{\tau}. \tag{1.12}$$

The solution compatible to equation (1.9) is:

$$r(t) = r_\infty + (r_0 - r_\infty)e^{\frac{-t}{\tau}}. \tag{1.13}$$

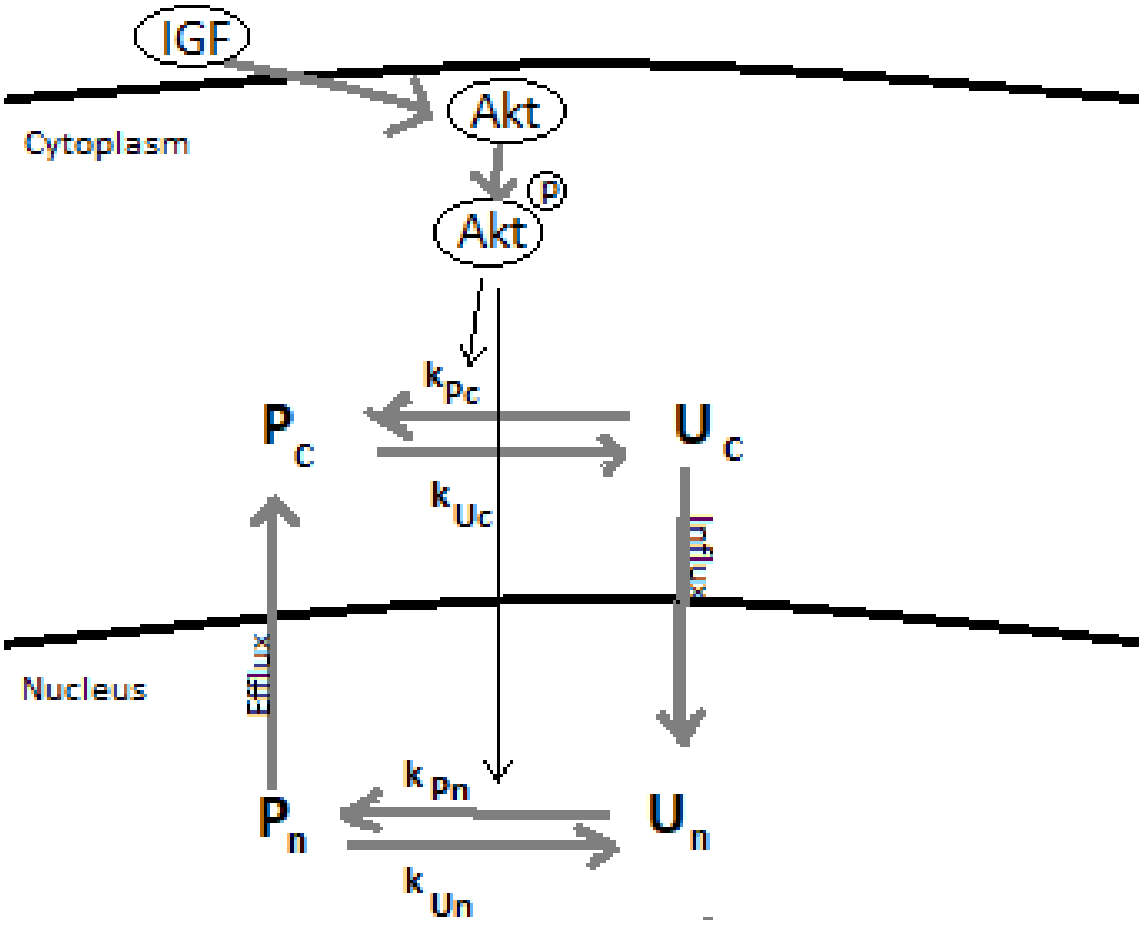

FIG. 1.1. The basic model of the IGF/Akt FOXO-1 pathway.

Chapter 2

METHODS

In this section, we develop our basic and dynamic FOXO-1 models. We go through the methods we used to model muscle atrophy using two different models.

2.1 Basic Model

In this section, we model the production of the degradation proteins. The degradation proteins already exist inside the muscle cell, but the phosphorylation of the FOXO-1 protein activates the free floating degradation proteins. It is this protein production that we model. The FOXO-1 proteins in the nucleus produces these degradation proteins. We assume that these degradation proteins (such as ubiquitin ligase (Smith & Shanley 2010)) contribute to the degradation of the the cross sectional area of a skeletal muscle fiber. The rate of the creation of the proteins connections over time is equivalent to the production rate of the degradation protein minus the degradation rate of the protein:

$$\frac{dp}{dt} = kr - \delta p. \tag{2.1}$$

Using equation (1.11), yields

$$p(t) = \frac{k}{\delta}(r_\infty + \tau(r_0 - r_\infty)e^{-\frac{t}{\tau}}) + Ae^{-\delta t}. \tag{2.2}$$

The initial condition of the degradation protein is the function $p(0) = 1$, yields the solution:

$$p(t) = e^{-\delta t} + \frac{k}{\delta}(1 - e^{-\delta t}) + \frac{k}{\delta}(\tau(r_0 - r_\infty))(-e^{-\frac{t}{\tau}} + e^{-\delta t}). \tag{2.3}$$

We have established equations for nuclear to cytoplasm FOXO-1 ratio, equation (1.13), and degradation protein, equation (2.1). We now establish rate equations for cross-sectional area. The rate at which the cross-sectional area degrades over time we take to be either proportional to degradation protein concentration or proportional to the product of degradation protein and cross-sectional area.

In the model below, the rate of degradation of the cross-sectional area over time is proportional to the concentration of degradation protein with rate constant, α, as seen by:

$$\frac{da}{dt} = -\alpha p(t). \tag{2.4}$$

We solve for the cross-sectional area by using the solution from the degradation protein equation (2.3) and get the solution

$$\begin{aligned} a(t) = & -\alpha[\frac{-1}{\delta}p_0 e^{-\delta t} + \frac{k}{\delta}(r_\infty(t + \frac{1}{\delta}e^{-\delta t})) + \frac{k}{\delta}(\tau(r_0 - r_\infty)) \\ & (\tau e^{-\frac{t}{\tau}} - \frac{1}{\delta}e^{-\delta t})] + B. \end{aligned} \tag{2.5}$$

Since the initial condition of the cross-sectional area is: $a(0) = 1$, the solution is

represented below by the cross-sectional area over time,

$$a(t) = 1 + \frac{\alpha}{\delta}p_0(1+e^{-\delta t}) + \frac{\alpha k}{\delta}(r_\infty(\frac{1}{\delta} - t - \frac{1}{\delta}e^{-\delta t}) + \frac{\alpha k}{\delta}(\tau(r_0 - r_\infty))(\tau - \frac{-1}{\delta} - \tau e^{-\frac{t}{\tau}} + \frac{1}{\delta}e^{-\delta t}). \tag{2.6}$$

The second mathematical model for the cross-sectional area when the rate of the cross-sectional area is proportional to the cross-sectional area is

$$\frac{da}{dt} = -\alpha p a, \tag{2.7}$$

with the general solution:

$$a(t) = Ce^{-\alpha[\frac{-1}{\delta}p_0 e^{-\delta t} + \frac{k}{\delta}(r_\infty(t+\frac{1}{\delta}e^{-\delta t}) + \frac{k}{\delta}(\tau(r_0 - r_\infty))(\tau e^{-\frac{t}{\tau}} - \frac{1}{\delta}e^{-\delta t})]}. \tag{2.8}$$

Since the initial condition of the cross-sectional area is: $a(0) = 1$, the solution for the cross-sectional area,

$$B = \frac{1}{e^{-\alpha[\frac{-1}{\delta}p_0 + \frac{k}{\delta^2}(r_\infty + \frac{k}{\delta}(\tau(r_0 - r_\infty))(\tau - \frac{1}{\delta})]}}$$
$$a(t) = Be^{-\alpha[\frac{-1}{\delta}p_0 e^{-\delta t} + \frac{k}{\delta}(r_\infty(t+\frac{1}{\delta}e^{-\delta t}) + \frac{k}{\delta}(\tau(r_0 - r_\infty))(\tau e^{-\frac{t}{\tau}} - \frac{1}{\delta}e^{-\delta t})]}.$$

After graphing the solutions to the two cases, for the cross sectional area, we have witnessed very little difference between the two. We utilize equation (2.4).

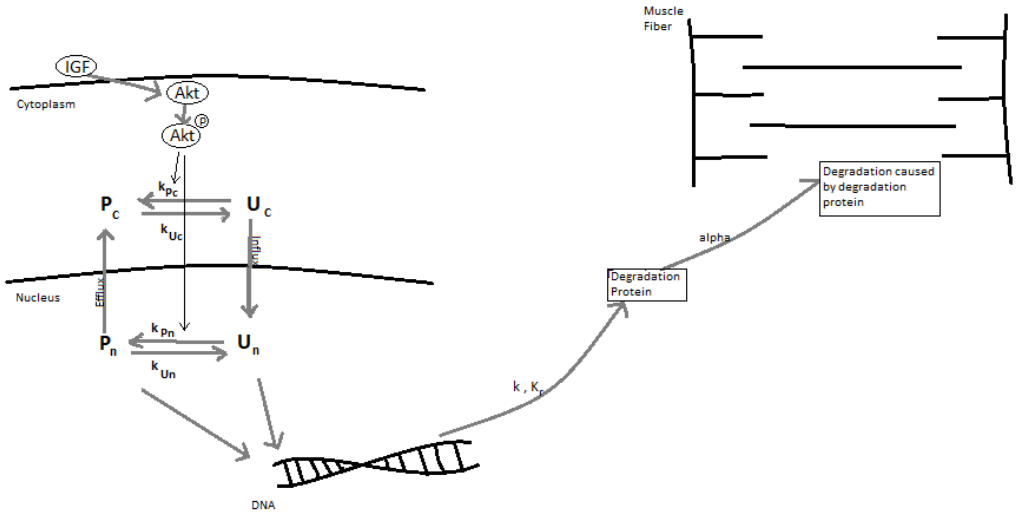

FIG. 2.1. The basic model of the IGF/Akt FOXO-1 pathway, the pathway of ubiquitin ligase proteins produced by the FOXO-1 protein, and the cross-sectional area degradation caused by the ubiquitin ligase.

Overall, the equations that were used to create the basic model of the nuclear to cytoplasmic FOXO-1 protein ratio, the degradation proteins produced by the FOXO-1 protein, and the cross-sectional area are represented by,

$$\begin{aligned} \frac{dr}{dt} &= \frac{(r_\infty - r)}{\tau}, \\ \frac{dp}{dt} &= kr - \delta p, \\ \frac{da}{dt} &= -\alpha p(t). \end{aligned} \tag{2.9}$$

We then alter the p and a equations (2.1 and 2.4). In the protein equation, to boost the sensitivity of the ubiquitin ligase production in response to the addition of IGF we added a Hill function to the ubiquitin ligase production equation (2.1). We represent IGF in the

new equation (2.10) for degradation protein by the nuclear to cytoplasm ratio variable, r. The degradation protein equation is,

$$\frac{dp}{dt} = \frac{kr^n}{r^n + (K_r)^n} - \delta_p p. \tag{2.10}$$

To represent the small baseline area decay and production with ratio we added $\delta_a a$ and σ to the cross-sectional area equation (2.4),

$$\frac{da}{dt} = \sigma - \delta_a a - \alpha p(t). \tag{2.11}$$

Below in Table 1.1 are the parameters for the model in equation (2.10)-(2.12).

2.2 Testing the Basic Model Against Data

In testing our models of muscle atrophy, we partnered with Sarah Russell, a PhD student in Dr. Martin Schneider's lab at UMB, and considered her preliminary work in the regulation of FOXO-1 protein in skeletal muscle atrophy. From her research we estimated the volume of a skeletal muscle treated with IGF (call this IGF experiment) and not treated with IGF (call this Control experiment). The first data set, we identify as Data Set 1, has two different skeletal muscle volume measurements, a control and IGF experiment treated from day 0. The second set of data, we identify as Data Set 2, has two different skeletal muscle volume measurements as well. In Data Set 2, we estimated the volume of the control and the other was treated with IGF at day 5, instead of day 0. From the two data sets we fit parameters, δ_p and k, and set values to the other set of parameters from Table 1.1. We do this to apply values to the parameters to our equations for the the nuclear to cytoplasm FOXO-1 ratio, the degradation protein production, and the cross-sectional area so that we can graph and compare the degradation of the cross-sectional area for our model

Table 2.1. Table of parameters with aligned units.

Parameters Name	Unit	Values	Definition
k'_I	min^{-1}	0.119 without IGF and 0.024 with IGF	represents the influx of FOXO-1 proteins into the nucleus
k'_E	min^{-1}	0.014 without IGF and 0.109 with IGF	represents the efflux of FOXO-1 proteins leaving the nucleus into the cytoplasm
k	$\frac{protein}{day}$	3.233	production rate of ubiquitin ligase produced by FOXO-1 proteins
δ_p	day^{-1}	2.330	decay rate of the ubiquitin ligase produced by FOXO-1 proteins
α	$\frac{area}{proteinday}$	0.0309	decay rate of the cross sectional area of the present degradation proteins
n	no units	4	hill coefficient for $\frac{N}{C}$ dependent ubiquitin ligase production
K_r	FOXO-1	0.2629	sensitivity of ubiquitin ligase production to nuclear FOXO
σ	$\frac{area}{day}$	0.001	source parameter
δ_a	day^{-1}	0.001	the growth rate of existing cross sectional area

and the estimated data. To get values for k_I' and k_E' we used values from (Wimmer *et al.* 2014), for the Control experiment, $k_I' = 0.119$ and $k_E' = 0.014$, and for the IGF experiment $k_I' = 0.024$ and $k_E' = 0.109$. We estimated parameters K_r and α by solving a two equation and two variable system. We build the system of equations by using the $k_I^{'}$ and $k_E^{'}$ IGF and control values to get a steady state low and high for nuclear FOXO-1 values. Then using the same method to get a low and high for degradation protein values, and solved for αp_{hi} and αp_{lo} for the two equations and two unknowns K_r and α. Then we set values for n to be 4, and $\delta_a = 0.01$ and $\sigma = 0.01$ to be small baseline area decay and production with ratio of 1 for normalization. We use data set 1 to fit parameters δ_p and k using Matlab's lsqcurvefit program.

Chapter 3

RESULTS FOR MODELING CROSS-SECTIONAL AREA

3.1 Fitting the Cross-Sectional Area of Skeletal Muscles In MATLAB Using Nuclear to Cytoplasm FOXO-1 protein Ratio

We begin by finding the best fit parameters to Data Set 1. We use Data Set 1 to fit parameters δ_p and k. In the process of finding the best technique for the model equation, we use the two values for k_I' and k_E' for the IGF and control experiments. When k_I' is high and k_E' is low, FOXO-1 is phosphorylated leading to nuclear FOXO-1 entry and to muscle atrophy (this represents the Control) and when k_I' is low and k_E' is high then Akt is activated and FOXO-1 is more phosphorylated (this represents the application of IGF). Next in fitting the cross sectional area, we use the Data Set 1 and the k_I' and k_E' values to fit K and δ_p parameters to the cross-sectional area for the control experiment. To mimic the control experiment, we used $k_I' = 0.119$ and $k_E' = 0.014$, and for the IGF experiment we used $k_I' = 0.024$ and $k_E' = 0.109$ (Wimmer *et al.* 2014). We apply the list of parameters from Table 1 to the models that represent equations (2.9-2.11). After finding the the parameter values for δ_p and k,and applying the values for the other parameters for the model we graph Data Set 1 Control and IGF Data with our model best fit of the Control and IGF.

When graphing our model we set the initial conditions to $p_0 = 0$, $a_0 = 1$, and $r_0 = r_\infty$. Results for nucleus to cytoplasm FOXO-1 protein ratio (Figure 3.1), the degradation protein amount produced by FOXO-1 protein (Figure 3.2), and the two cross-sectional areas (Figure 3.3) are shown below.

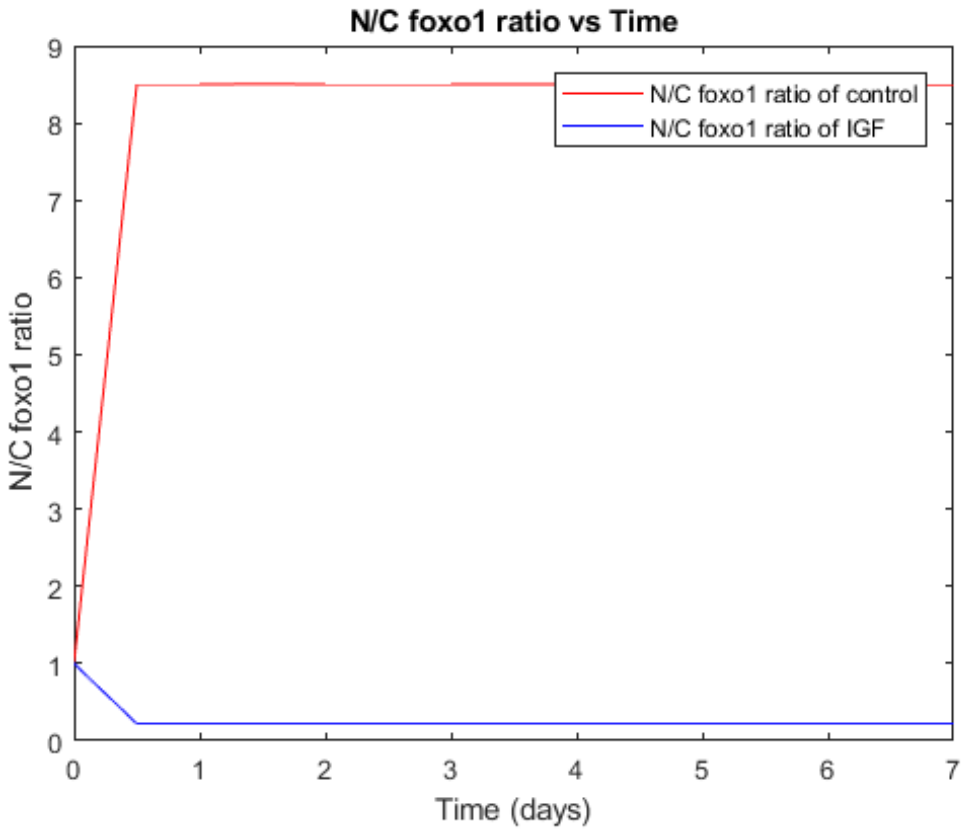

FIG. 3.1. The nuclear to cytoplasmic ratio over time for the control and IGF for Data Set 1. Model is sampled at every 0.5 days to compare with Data.

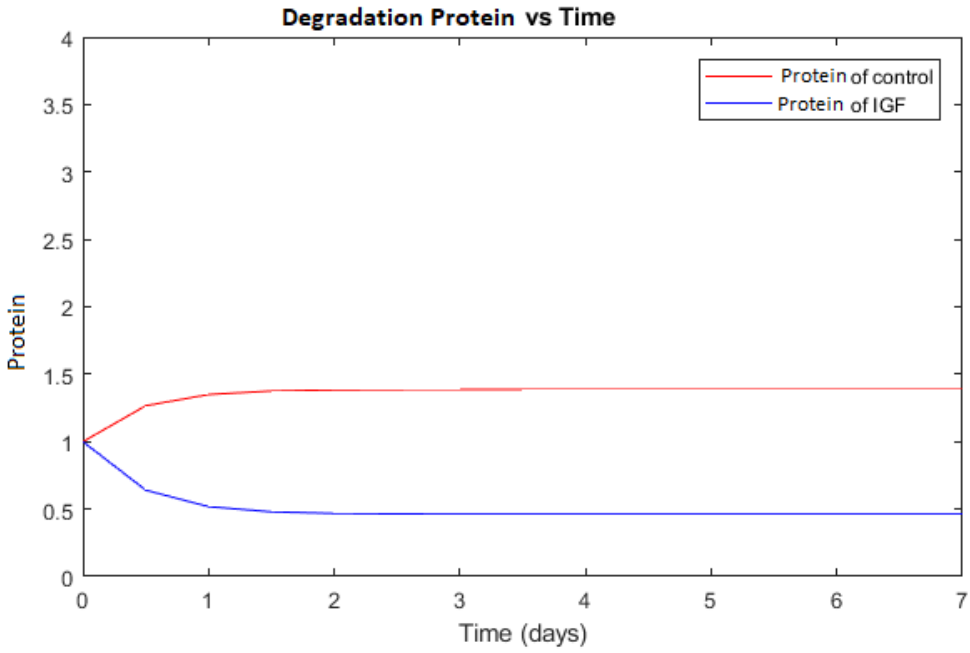

FIG. 3.2. Degradation protein production over time for the control and IGF Data Set 1. When the nuclear to cytoplasm FOXO-1 ratio increases the degradation protein follows suit and the same occurs when the nuclear to cytoplasm FOXO-1 ratio decreases. This happens because the production of degradation protein is controlled by the amount of FOXO-1 in the nucleus.

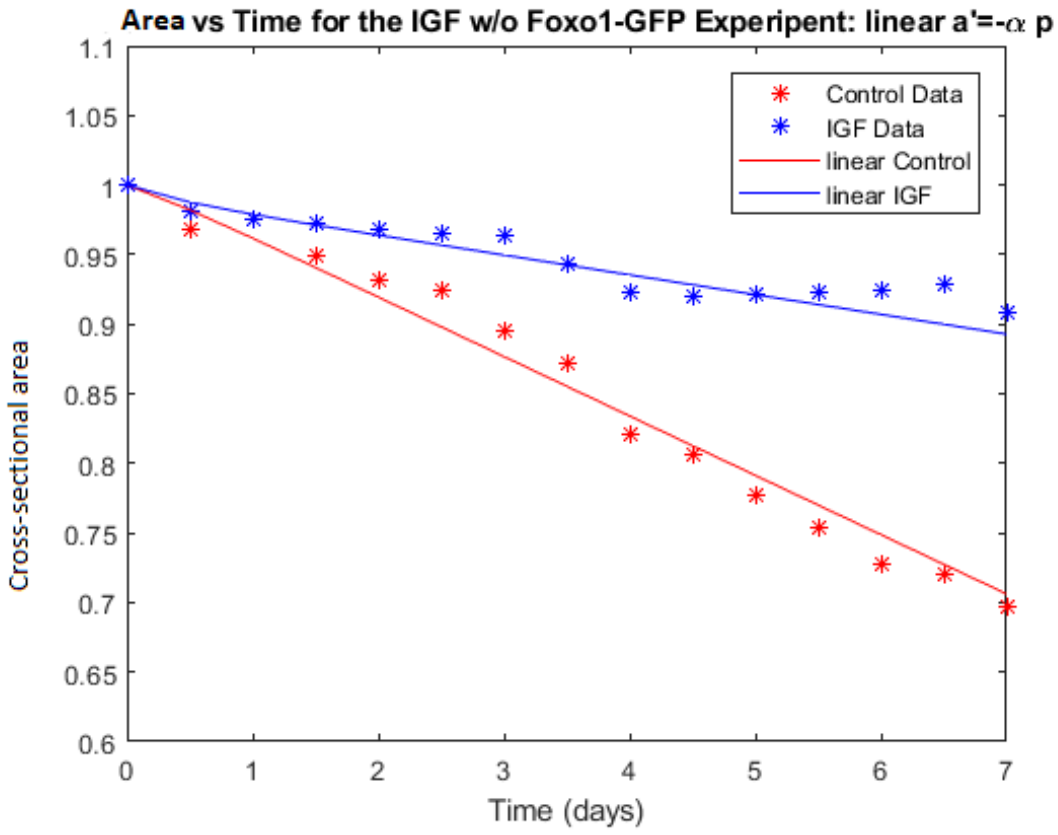

FIG. 3.3. The cross-sectional area of fiber over the span of seven days for Data Set 1. The difference between the model control (red line) and IGF (blue line) is the choice of $k_I^{'}$ and $k_E^{'}$ which are given in Table 1.1. Data Set 1 control is in red markers and IGF is in blue markers. Since the length of the muscle fiber is unchanged volume is proportional to cross-sectional area and also normalized to the initial area.

We now want to run the same procedures for Data Set 2, where the IGF is added at day 5 instead of day 0. Based on Data Set 2, we applied the set of parameter values we found during the first best fit model. Using the same method as before we plot Data Set 2 Control and IGF and then graph our model of best fit for the cross sectional area (Figure 3.4). When graphing our model we set the initial conditions to $p_0 = 0$, $a_0 = 1$, and $r_0 = r_\infty$. For the

control fit we use the same $k_I' = 0.119$ and $k_E' = 0.014$ (Wimmer *et al.* 2014) and for the IGF fit we use the same values from the control fit until day 5, at day 5 we used $k_I' = 0.024$ and $k_E' = 0.109$ (Wimmer *et al.* 2014). The results of the graphs for the two cross sectional areas are shown in Figure 3.4.

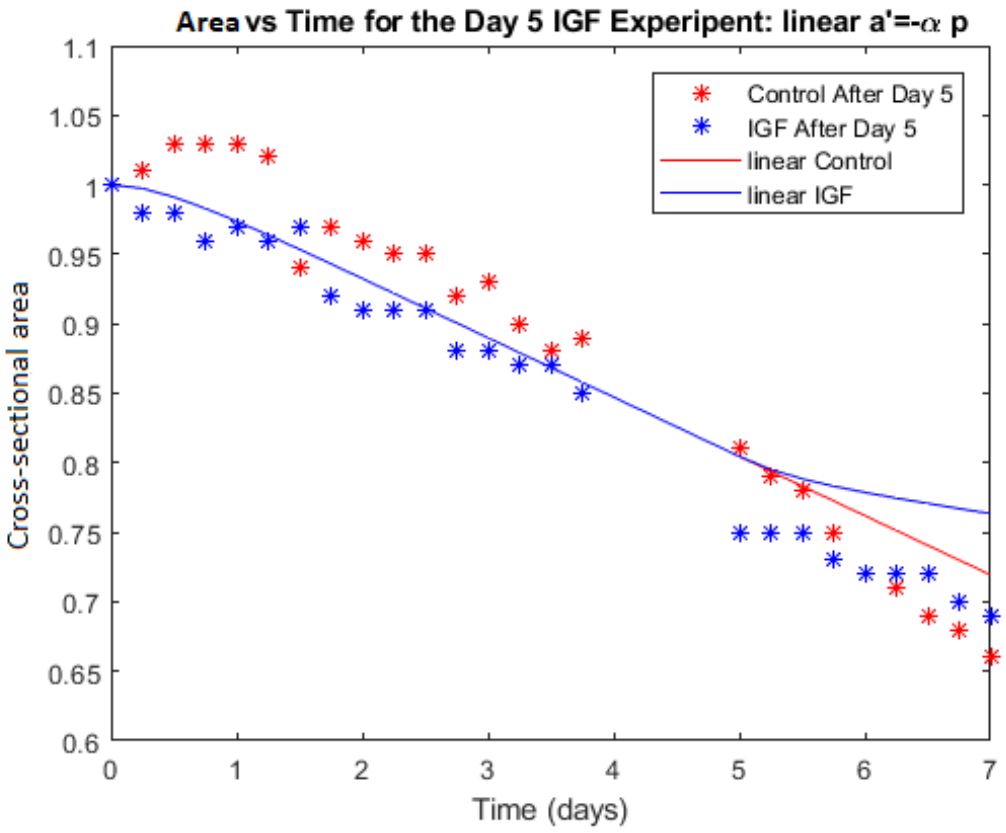

FIG. 3.4. The cross-sectional area of fiber over the span of seven days for Data Set 2 still using the same parameter values as Data Set 1.

3.1.1 Normalizing the IGF Data for Fitting

From the two graphs of the cross sectional area, the slopes of the IGF model in Figure 3.3 seem similar to the the slope of the data at day 5 in Figure 3.4. To be able to better compare the IGF slopes we normalize the data presented at day 5, of Data Set 2. In Figure 3.3 and 3.4 at day 0, the model of IGF and the control, all start at the same point. This is not true in Figure 3.4 of the IGF and Control with the IGF starting at day 5. At day 5, the Control, IGF, and the Data from Data Set 2 do not start at the same point. To normalize Data Set 2, we used model data sets at day 5 normalized both the control and IGF Data to this value. This allowed us to better compare the slope of the Data and model. It normalized the IGF to start at the Control value from Data Set 2. Then using the parameters we established earlier for the Control Data and IGF Data in Data Set 1, we graphed the model for the best fit Control Data and IGF Data and normalized the points after day 5 by shifting the Data to start at the same day 5 value as the control, this is shown in Figure 3.5. We see from the graph that after normalizing the IGF data in Data Set 1 and Data Set 2 that the slopes are similar.

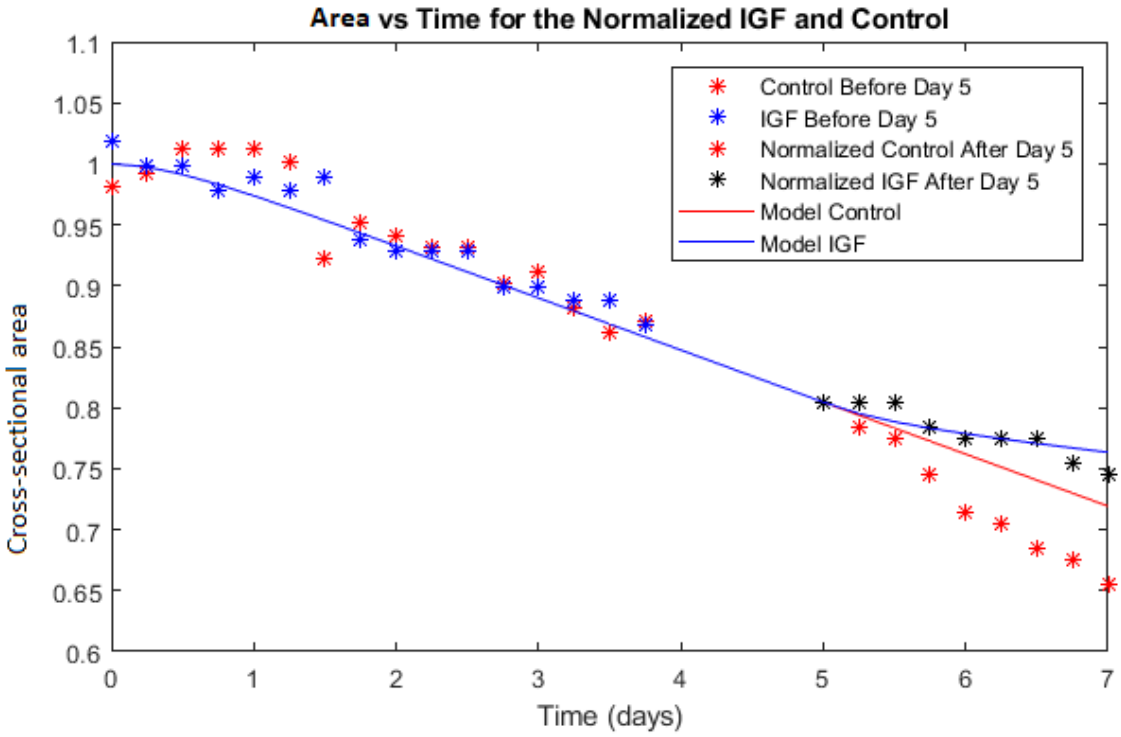

FIG. 3.5. The normalized cross-sectional area of fiber over the span of seven days for Data Set 2. Keeping the parameters, normalize the IGF Data in the Day 5 Set to the control by shifting the control and IGF Data points at day 5 by the difference between the control and IGF Data so that the Data at day 5 in the model and the given Data Set are normalized to the control point at day 5.

3.2 Modeling the Cross Sectional Area of Skeletal Muscles With the Total FOXO-1 Dynamically Changing

There is evidence for FOXO-1 changing on the order of days (Wimmer *et al.* 2014). To account for this, we need to model the nuclear concentration of FOXO-1 protein, not the nuclear to cytoplasm ratio that was done in prior sections. We do this because the nuclear FOXO-1 protein is what will be the factor in determining muscle atrophy, since it

is when FOXO-1 protein is in the nucleus that causes muscle atrophy. Nuclear FOXO-1 protein promotes the transcription of genes for Ubiquitin ligase, which promotes muscle degradation. Since we are looking to model FOXO-1 protein in the nucleus, we look at the rate of synthesis and of breakdown of FOXO-1 protein. When we calculated the nuclear to cytoplasmic ratio, we are calculating the ratio between how much FOXO-1 protein is in the nucleus and how much is in the cytoplasm, but now we take the total FOXO-1 protein to be dynamic. The nuclear equation (1.7) is now, in the $\varepsilon = 0$ limit, $\frac{dN}{dt} = k_I^{'}F - k_E^{'}N$. The ratio equilibrates more quickly than other processes so we take $\frac{dN}{dt} \approx 0$ and $N \approx \frac{k_I^{'}}{k_E^{'}}F$ as shown in equation (3.1). In the degradation protein equation (2.14) as the nuclear FOXO-1 becomes a function of total FOXO-1, the sensitivity parameter K_r must be converted, hence we define $k_n = C_T K_r$. This becomes equation (3.2). Thus, if the total amount of FOXO-1 protein is less, C will be less, and then even for the same ratio, r, there will be a lower rate of muscle volume loss. Equation (3.2) represents the total amount of FOXO-1 protein in the nucleus, so we model the the amount of FOXO-1 protein in the nucleus over seven days. Then, FOXO-1 protein in the nucleus will determine the amount of degradation protein being created to aid in breaking down the muscle volume, because of this we will also be modeling degradation protein again, shown in equation 3.3. Then we are able to graph the cross sectional area of the muscle from the degradation protein production over the span of seven days, Figure 2.8. We took the equations for total FOXO-1 protein, nuclear FOXO-1 protein, degradation protein and the cross sectional area and solved them simultaneously in MATLAB and graphed them. The equations for total FOXO-1 protein, nuclear FOXO-1 protein, degradation protein and the cross sectional area are presented below:

$$N \approx \frac{k_I^{'}}{k_E^{'}} F = \gamma F, \tag{3.1}$$

$$\frac{dF}{dt} = \alpha_f - \delta_f - \delta_{fp} pF, \tag{3.2}$$

$$\frac{dp}{dt} = \frac{kN^n}{N^n + (k_n)^n} - \delta_p P \approx \frac{k(\gamma F)^n}{(\gamma F)^n + (k_n)^n} - \delta_p P. \tag{3.3}$$

where $\gamma = \frac{k_I^{'}}{k_E^{'}}$, α_f, and δ_f to be small baseline FOXO-1 decay and production with ratio of 1 for normalization, and δ_{fp} to be the decay rate of the total FOXO-1. The cross-sectional area equation remains the same as before:

$$\frac{da}{dt} = \sigma - \delta_a - \alpha p(t). \tag{3.4}$$

After solving the equations, we graph the results over the span of seven days time with Data Set 2. These results are shown below in Figures 3.6-3.8.

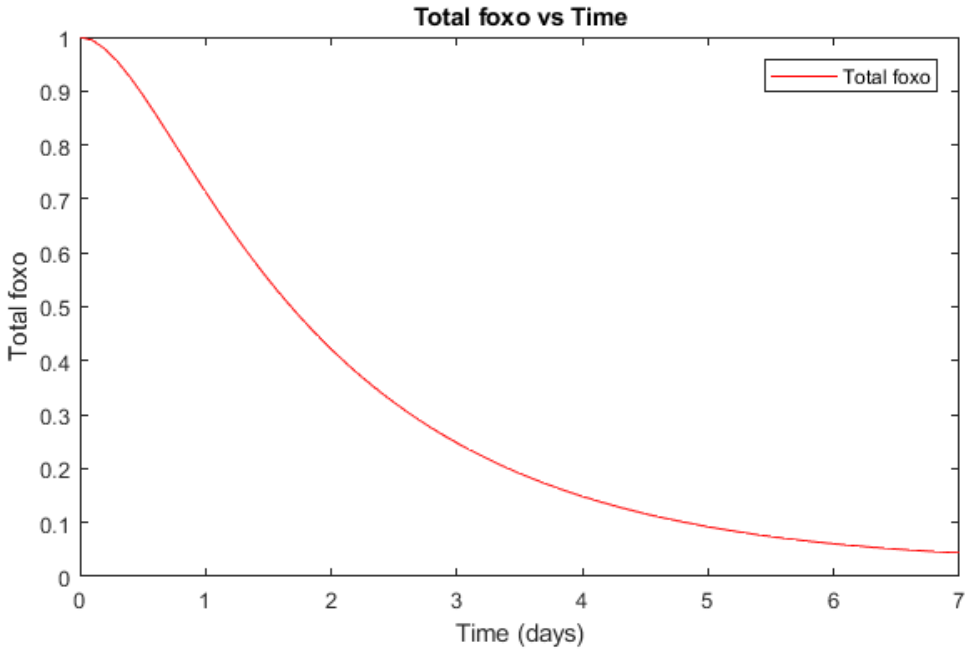

FIG. 3.6. The Total FOXO-1 over the span of seven days

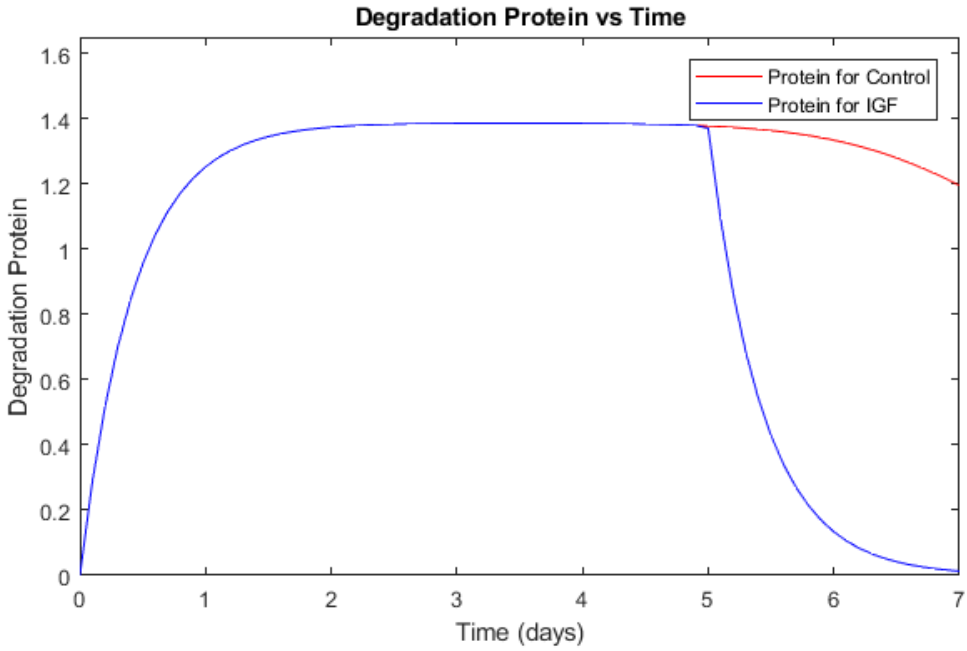

FIG. 3.7. Degradation protein over the span of seven days due to the total FOXO-1 in the nucleus.

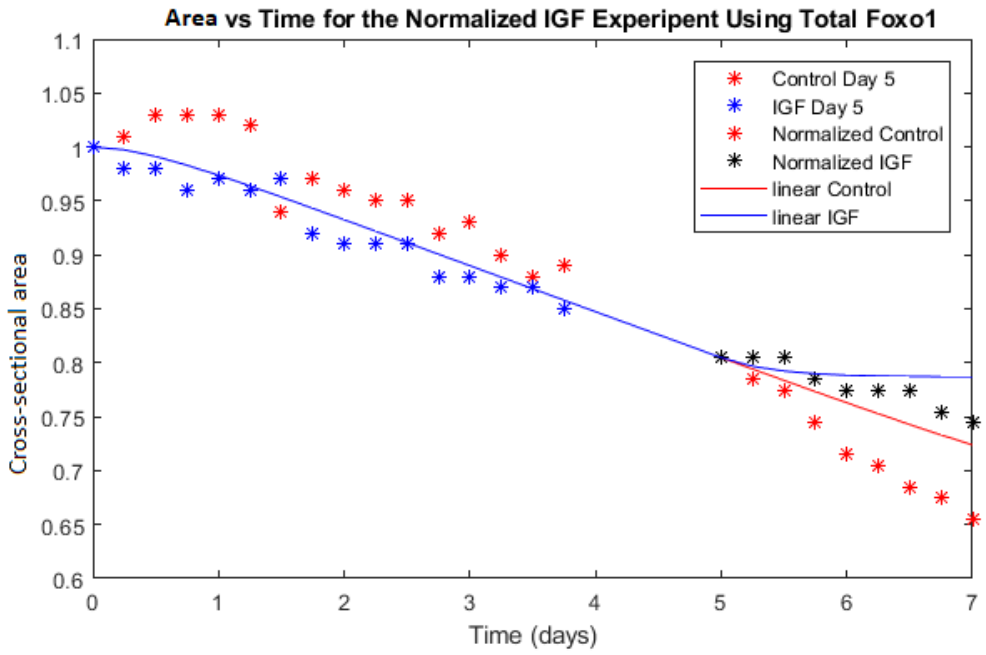

FIG. 3.8. The cross-sectional area of fiber over the span of seven days.The growth and decay is affected by the degradation protein production or decay. When degradation protein production is high the cross-sectional area is decaying (FOXO-1 unphosphorylated) and when the degradation protein production is decreasing the cross-sectional area decay rate decreases at a slower rate (IGF pathway is activated, hence FOXO-1 phosphorylated).

From our equations and the graphs on total FOXO-1 protein found in Smith and Shanley (Smith & Shanley 2010), we hypothesize that there is a negative feedback loop on the nuclear FOXO-1 protein that causes ubiquitin ligase to be produced faster in the cytoplasm, causing for a faster degradation of FOXO-1 primarily protein in the cytoplasm. When the nuclear FOXO-1 protein increases, there is an increase in the rate of ubiquitin ligase synthesis in the cytoplasm, which then increases concentration of ubiquitin ligase in the cytoplasm. From this process, the rate coefficient for degradation of FOXO-1 protein increases in the cytoplasm, causing a decrease in cytoplasmic FOXO-1 protein, which de-

creases nuclear FOXO-1 protein. This negative feedback is important for moving forward in modeling the cross-sectional area because we will be observing the possibility of oscillations in the degradation protein and cross-sectional area graphs.

Chapter 4

OSCILLATIONS IN CROSS-SECTIONAL AREA

4.1 Finding Eigenvalues for Oscillatory Conditions

There seems to be an oscillatory nature from the IGF and Control data from data set 2 with the order of two days that is also seen in Smith and Stanley's total FOXO-1 protein graphs. If this is true then under specific conditions our model may be able to also get oscillations (or oscillatory decay) in our fitted models that would be able to better represent Data Set 2. We need to find the parameters that would create oscillations in the cross sectional area graph (Simone, Sandeep, & Jensen 2007). To test for oscillations, we use the equations for the total amount of FOXO-1 protein and the amount of degradation protein produced we create a Jacobian matrix (linear system) and linearize around the equilibrium point. We are only using the degradation protein and total FOXO-1 equations to test linearity because they create a feedback loop, both equations affect one another. From the matrix we are able to test different values for the parameters for k and δ_p to get oscillations in the degradation protein graph, this is important because if the total amount of degradation protein shows oscillations then it may also show up in the cross sectional area graph because the degradation protein feeds directly into the cross-sectional area equation. We used the Jacobian matrix to output the complex eigenvalues with the largest imaginary number. We need the parameters that would be able to produce complex eigenvalues with large imaginary parts

because the imaginary part determines the frequency of the oscillations, we are looking for oscillations that have a frequency of 3 days for Figure 3.8. We use these parameters to give matching oscillations to the data, we also want to make sure the set of parameters gives a good fit for the model and are biologically reasonable. Another way we test to make sure we are getting reasonable parameters for oscillations is by taking the trace of the Jacobian matrix versus the determinant of the matrix. We do this to test if the eigenvalues displaced from the trace versus determinant graph lie in the region of complex eigenvalues. Based on this graph we are able to graph where components of each eigenvalue lies on the graph and this will determine if the eigenvalues of the Jacobian will allow for oscillations (meaning they are complex eigenvalues). We use the k and δ_p values we found in the beginning to fit the Data Set 2 and use it to get a set of eigenvalues. We want to see a range of values for k and δ_p where there can be possible oscillations, so we start by applying a range of values to δ_p, applying those values to the Jacobian matrix, and calculating the eigenvalues, shown in Figure 4.2; we did the same for k this is shown in Figure 4.3. We use those results to graph the eigenvalues on the trace versus determinant graph shown in Figure 4.2 and 4.3. From the trace versus determinant graphs we have for k and δ_p values that would process higher frequency oscillations (complex numbers with larger imaginary parts). We found a percentage change using the higher frequency oscillatory values for k and δ_p and the default values for k and δ_p. Then add or subtract the percentage from the default values to get increase or decrease value that we use as a new point and then plot. The graphical representation can be found below in Figure 4.2 and 4.3, where the green points are all the values greater then the default value and the red points are all the points less than the default value.

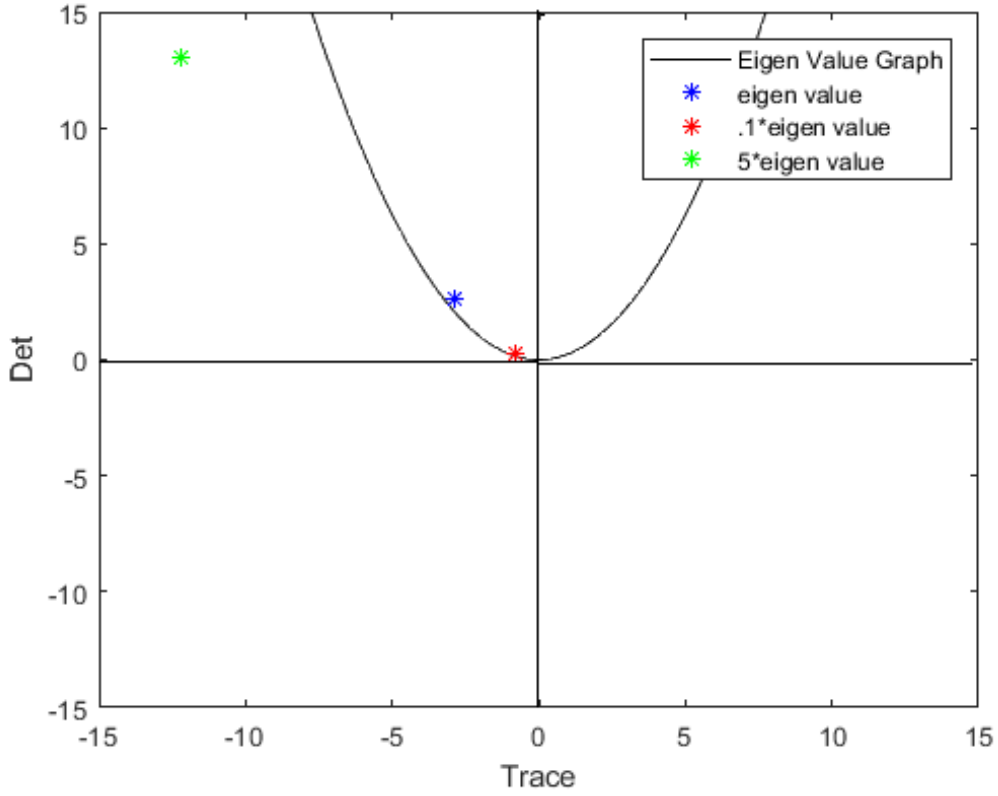

FIG. 4.1. Trace vs. Determinant graph: we look at three different eigenvalues, one is our default values for $k = 3.23$ and $\delta_p = 2.33$. The upper point is found from using five times our default k and δ_p. The lower point is found from using one tenth of our default k and δ_p.

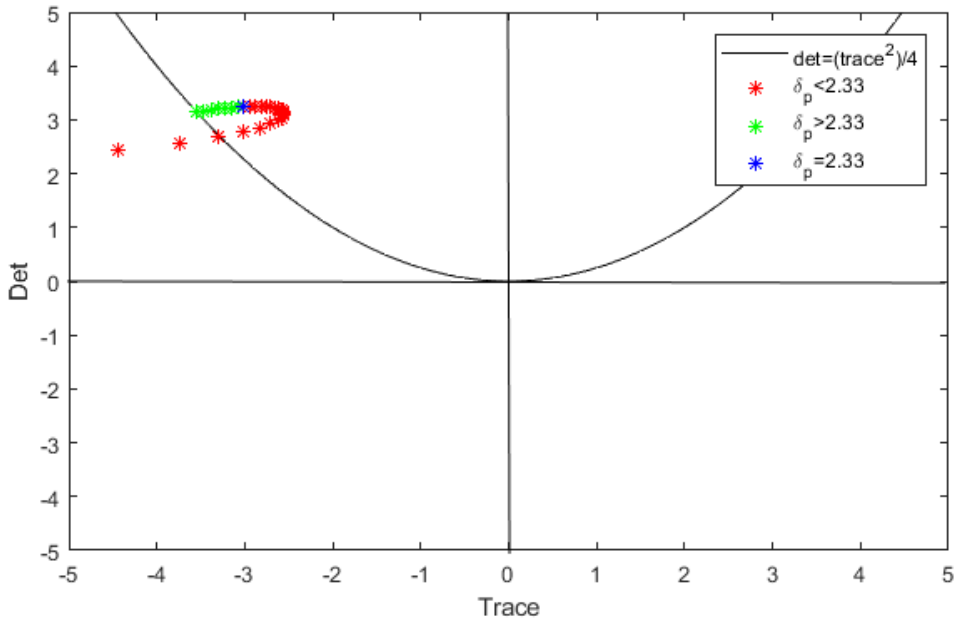

FIG. 4.2. Trace vs. Determinant when only δ_p is changing and k is kept at the default value 3.23. For $\delta_p > 2.33$ (green) the determinant is increasing and for $\delta_p < 2.33$ (red) the determinant is decreasing.

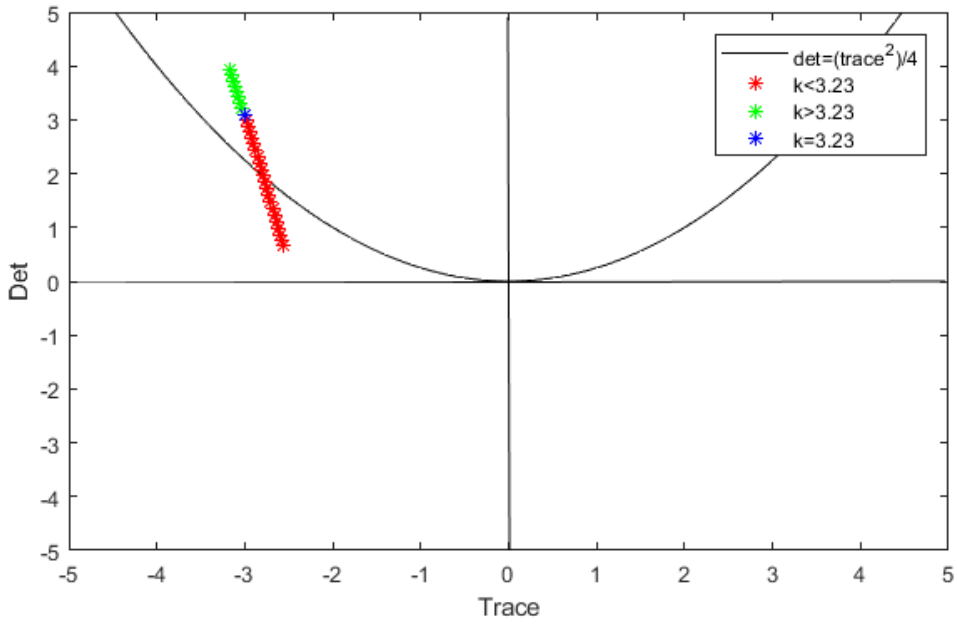

FIG. 4.3. Trace vs. Determinant when only$k$ is changing and δ_p is kept at the default value 2.33. For $k > 3.23$ (green) the determinant is increasing and for $k < 3.23$ (red) the determinant is decreasing.

After finding stable complex eigenvalues and the parameters associated with the eigenvalues, we use those parameters in the total FOXO-1 protein, degradation FOXO-1 protein, and cross sectional area equations to graph the cross sectional area model of IGF and the control with Data Set 2. Then using the method we used before to normalize the data at day 5 for Data Set 2, we do the same thing again to normalize this data. We are going to apply three sets of k and δ_p values from the above graphs to the total FOXO-1, degradation protein, and cross sectional area models to get oscillations in the cross sectional area graphs that resemble the data plotted from Data Set 2. The first set of k and δ_p values are a default values, $k = 3.23$ and $\delta_p = 2.33$, this is shown in Figures 4.1-4.3. To get the other set of values for k and δ_p, we found the eigenvalue from our range of k and δ_p values that

is complex and has the largest imaginary number. After finding the k and δ_p associated with that specific eigenvalue, we took the default δ_p minus the new δ_p and divided by the default, we then add or subtract that number from the default k and δ_p to get the new value for k and δ_p with the percent change.The second set of k and δ_p values are, $k = 3.23 - .48$ and $\delta_p = 2.33 - .48$, the results of the solving the model degradation protein are shown in figures 4.7-4.9. The last set of k and δ_p values are a default values, $k = 3.23 + .48$ and $\delta_p = 2.33 + .48$, the results of the solving the model degradation protein are shown Figures 4.9-4.11.

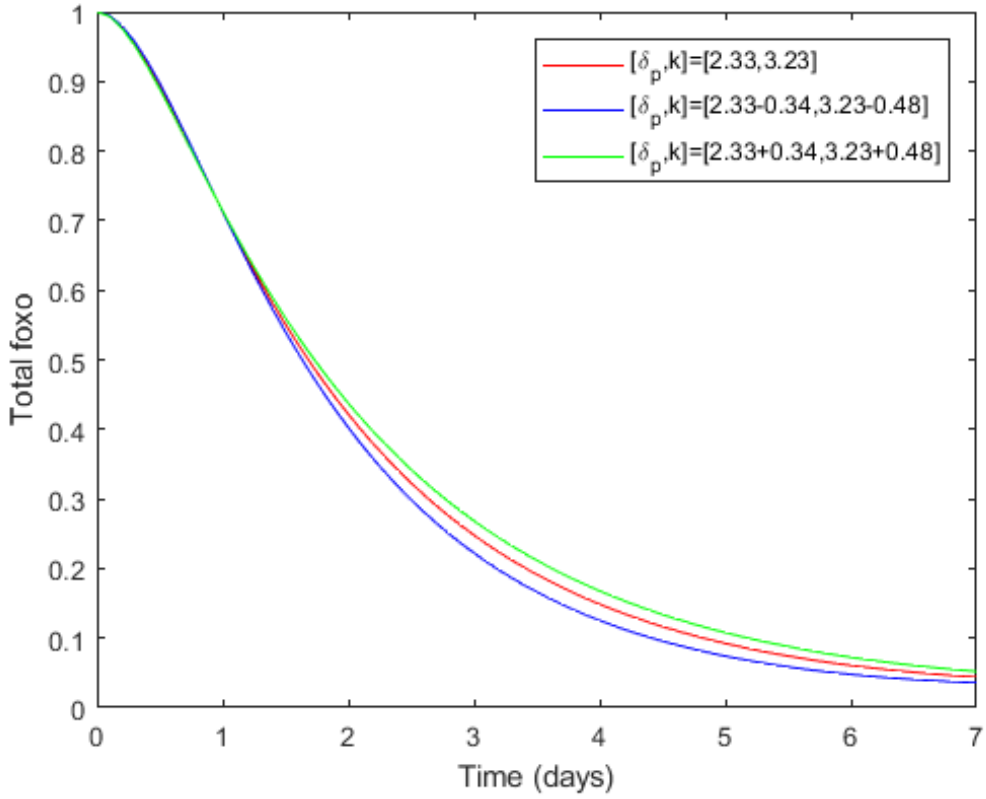

FIG. 4.4. Total FOXO-1 verse time for three set of k and δ_p values, first $k = 3.23$ and $\delta_p = 2.33$ (red), second $k = 3.23 - .34$ and $\delta_p = 2.33 - .48$ (blue), and third $k = 3.23 + .34$ and $\delta_p = 2.33 + .48$ (green). Since the total amount of FOXO-1 is decreasing then the nuclear FOXO-1 should be increasing, thus causing the degradation protein to increase to its steady state value.

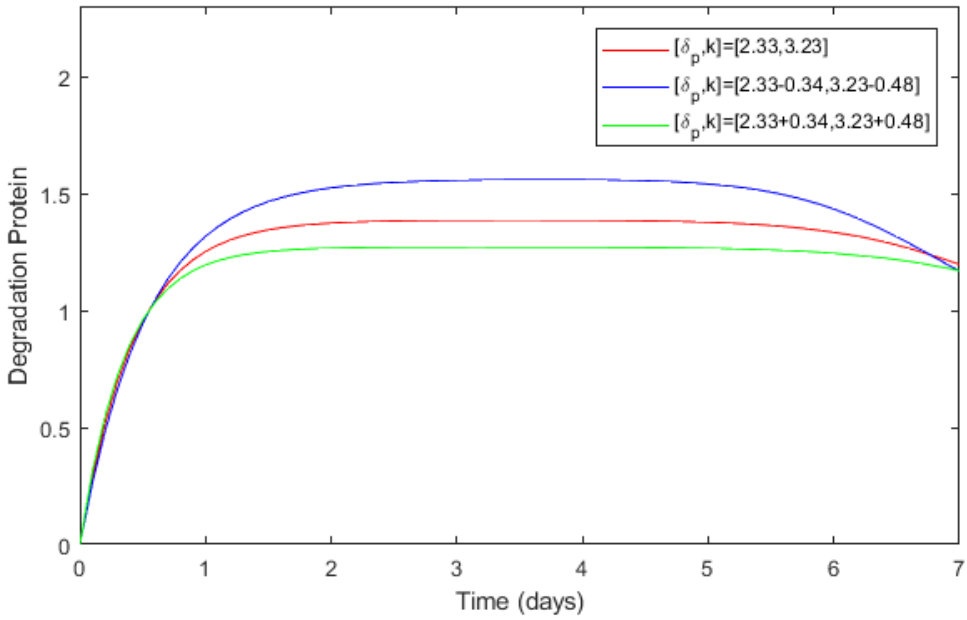

FIG. 4.5. Degradation protein verse time for three set of k and δ_p values, first $k = 3.23$ and $\delta_p = 2.33$ (red), second $k = 3.23 - .34$ and $\delta_p = 2.33 - .48$ (blue), and third $k = 3.23 + .34$ and $\delta_p = 2.33 + .48$ (green). Since the degradation protein is increases to its steady state value the, the cross sectional area is decreasing for large amount of degradation protein. When there is a lot of degradation protein the cross sectional area is decreasing and a decrease in degradation protein causes increase in the cross sectional area

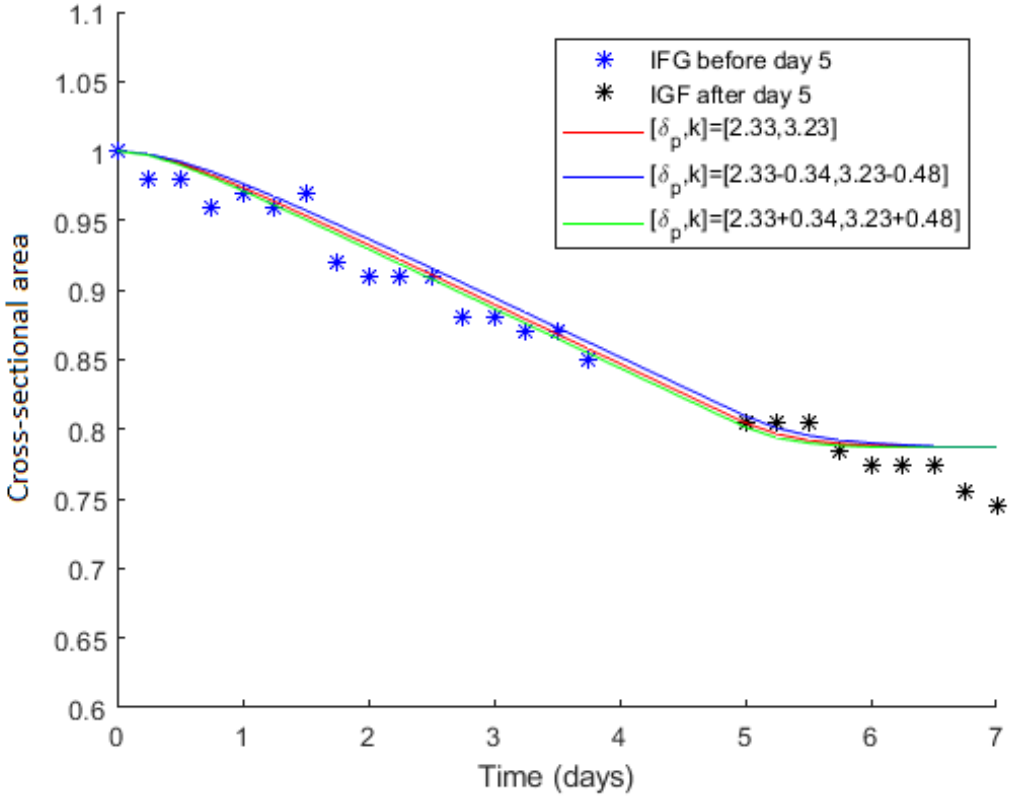

FIG. 4.6. Cross-sectional area verse time for three set of k and δ_p values, first $k = 3.23$ and $\delta_p = 2.33$ (red), second $k = 3.23 - .48$ and $\delta_p = 2.33 - 4.8$ (blue), and third $k = 3.23 + .48$ and $\delta_p = 2.33 + .48$ (green). The blue lines represent the IGF and the red lines represent the control.

After observing the above figures, even though the k and δp values we chose are associated with complex eigenvalues, we are still not able to get oscillations in the cross sectional area. This is probably due to the damping effect of α. We tested for oscillation in another example, where we left k as the default value and found the complex eigenvalue with the largest imaginary part when we allow δ_p to change. We found that for $\delta_p = 1.2$ we not only get a complex eigenvalue with the largest imaginary part but also oscillations with

higher frequency in the degradation protein, Figure 4.8. In the cross-sectional area there is a dip in the model around day 5 but nothing before then. This is because the degradation protein does not drop until day 5 causing this a very small curve in the cross-sectional area in Figure 4.9.

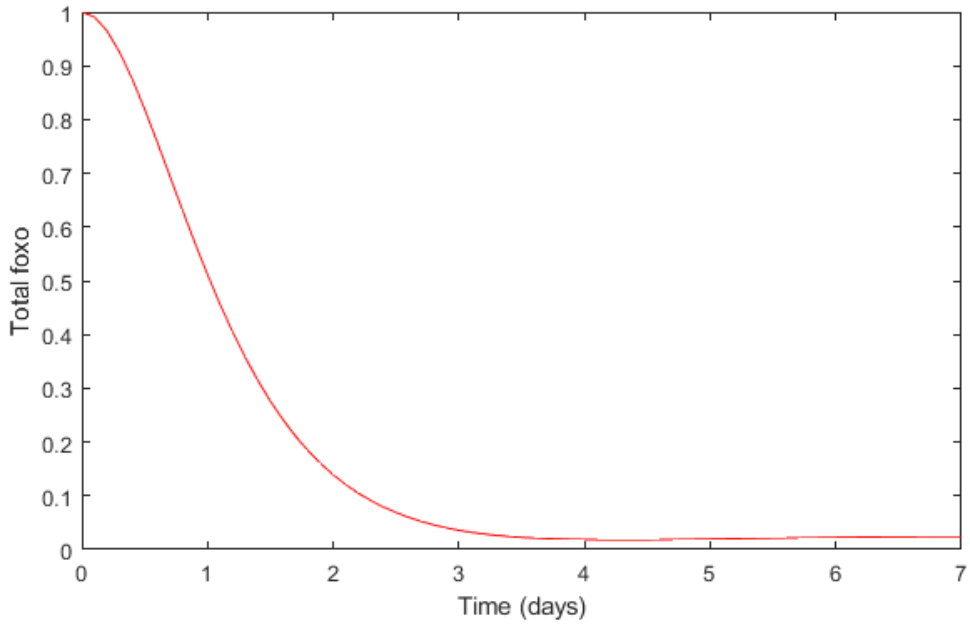

FIG. 4.7. Total FOXO-1 over a span of seven days for $\delta_p = 1.2$ and $k = 3.23$

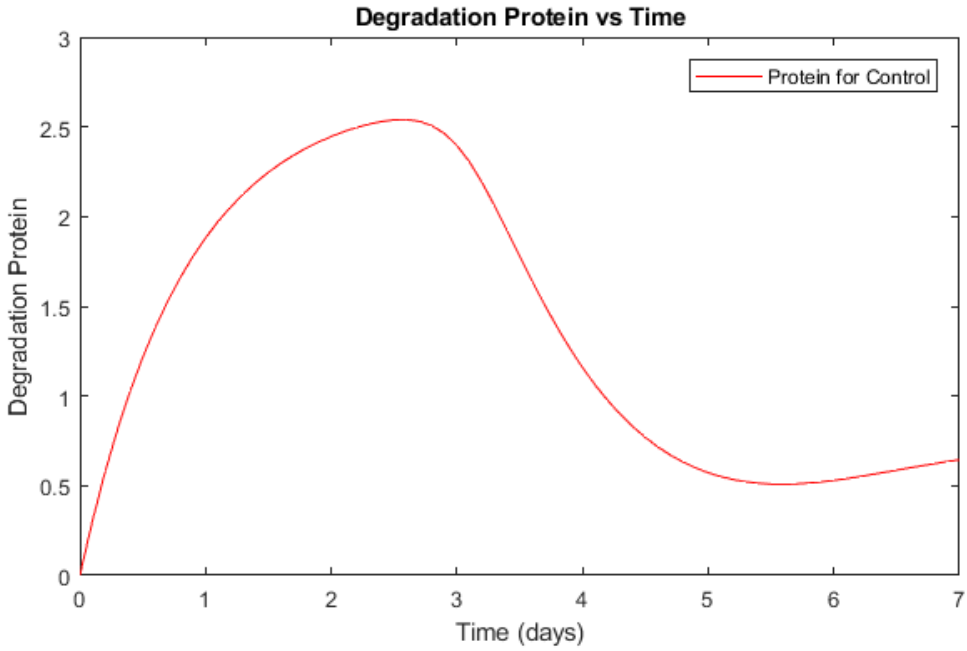

FIG. 4.8. Degradation protein over a span of seven days for $\delta_p = 1.2$ and $k = 3.23$. At day 5 the degradation protein show oscillatory behavior but is not able to build back enough degradation protein again to continue to oscillate

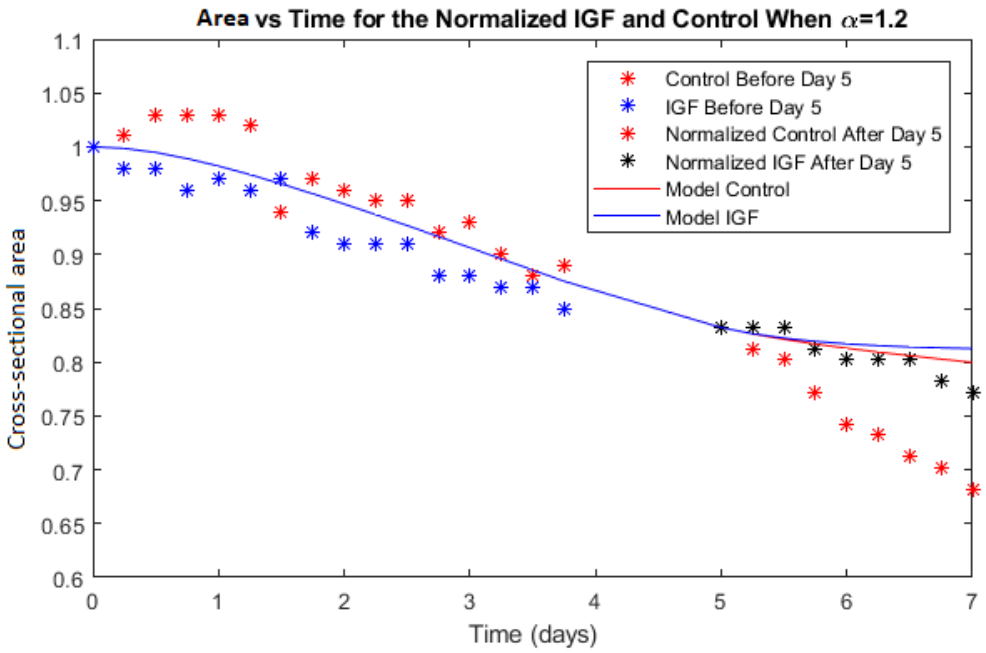

FIG. 4.9. Cross-sectional area over a span of seven days for $\delta_p = 1.2$ and $k = 3.23$. The cross-sectional area there is a dip in the model around day 5 but nothing before then, this is because the degradation protein does not drop until day 5 causing this a very small curve in the cross-sectional area..

Chapter 5

DISCUSSION

In this paper, we have used two different models to capture the degradation of a skeletal muscle due to FOXO-1. In our first instance, we used the nuclear to cytoplasm FOXO-1 ratio and in the other we used the total FOXO-1. Both of these models produced similar results and show no substantial difference between the two models. We were able to show a good fit to the data set 1 using the different control and IGF choices of $k_I^{'}$ and $k_E^{'}$ from (Wimmer *et al.* 2014). When we applied those same parameters to data set 2 we where not able to get the same goodness of fit because of natural variation. Further data will likely be necessary to figure out if the data is oscillating or how much is due to noise in the data measurements. We attempted to create oscillations in our model by finding complex eigenvalues from the Jacobian matrix using the total FOXO-1 and degradation protein production equations. Previous work coupling FOXO-1 to muscle atrophy has been limited. Most research ties to the disease muscular dystrophy, but there is little about the flow of IGF in connection to muscle atrophy. However, two articles show some examples of connecting IGF and muscle atrophy. One example is by Smith and Shanley, where they modeled behaviors of FOXO-1 with and without Akt activation; they observed how FOXO-1 interacts with other enzymes (Smith & Shanley 2010). Another example is by Zho et al., where they create a model for muscle atrophy during long periods under spaceflight conditions, then

they model what happens when exercise is added to these conditions (Kuo & Ehrlich 2015); however, this model does not take into account the intracellular FOXO-1 movement.

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