# NOVEL IN SILICO MODEL FOR INTERACTION OF ADVANCED GLYCATION END PRODUCTS AND COLLAGEN

By

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**Abstract**

Processed foods can contain large amounts of sugars, excessive consumption of which has been linked to numerous metabolic disorders. However, there is evidence that these dietary factors may more broadly influence aging-associated diseases, like glaucoma. Advanced glycation end-products (AGEs) are macromolecules created via the attachment of carbohydrate groups to proteins. They are produced endogenously, but can additionally be created exogenously from diet (dAGEs). One prominent role of AGEs within the extracellular matrix (ECM) is that AGEs crosslink and reduce turnover of the ECM. The blinding disease glaucoma affects over 70 million people throughout the world. While the etiology of glaucoma is complex, stiffening and increased crosslinking of the trabecular meshwork (TM) ECM is thought to be a major contributor. **The overarching hypothesis of this work is that increased AGE concentrations can lead to TM stiffening through crosslinking of long-lived proteins, like collagen**. More specifically, we developed an *in silico* model that uses serum AGE concentrations as inputs, models TM collagen turnover as dependent on crosslinking density, and correlates collagen and crosslink density to stiffness. Rate constants were approximated from literature regarding similar systems; varying serum AGE levels from control to unhealthy resulted in changes in collagen density consistent with glaucoma as well as increased stiffness. Using relevant rate contestants in the model provided meaningful data trends, which suggested the simplistic model may capture a key mechanism of TM stiffening. Increased AGE inputs resulted in increased ECM stiffening, from 150kPA to 220kPA, and increases in collagen production, crosslinking, and density, all consistent with glaucomatous pathology. In future work, this model will be expanded to include other factors of AGE biology and glaucoma pathology. Future work hopes to expand on the limitations of our early model.

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# Introduction

## America and Disease

There is increasing interest in "Lifestyle-related diseases" (LSRDs) which can include a wide variety of diseases like: infertility, Alzheimer's disease (AD), cardiovascular disease, non-alcoholic fatty liver disease (NAFLD), and atherosclerosis (Twarda-Clapa). Many of these diseases are highly associated with the western lifestyle itself that is characterized by: low physical activity, consumption of sugar as a food ingredient, and thermally processed foods making up most of their diet (Twarda-Clapa). Sugar has specifically been associated with the pathogenesis of *many* diseases related to metabolism (broadly described as "metabolic syndrome-related diseases") including diabetes mellitus, cardiovascular disease, and NAFLD. Other diseases include diabetic retinopathy, kidney diseases, and neuropathy, which are also thought to be impacted by a consequence of sugar in the diet. This is partly due to the fact that sugar, in combination with thermal processing like baking or broiling, can cause a reaction called a "non-enzymatic browning", also known as the Milliard reaction. This reaction is most commonly used to enhance the flavor, taste, and aroma of various foods in the western diet where foods with high sugar and protein content are often ingested in large quantities and at high frequencies. This has been implicated in a number of diseases. A good example of this is that hyperglycemia in diabetic patients is linked to the pathology of other diseases like: retinopathy, cardiomyopathy, nephropathy, and neuropathy, which can be grouped under the umbrella term of "diabetic complications". One promising line of inquiry for these phenomena would be investigating the build up of byproducts involved in glucose catabolism which are highly reactive and unstable, termed Advanced Glycation End-products (AGEs). These byproducts have further been shown to play a role in age-related neurodegenerative diseases, possibly through the

increasing of aggregated proteins, sometimes referred to as plaques, within the brain, like Lewy bodies in Parkinson's Disease (PD) or tau and amyloid proteins in AD (Chaudhuri et al.).

**What Are AGEs?**

AGEs can come from exogenous and endogenous sources, and represent a broad class of molecules. Importantly, AGEs have been investigated for their role in a number of age-related, inflammatory, and metabolic related diseases. AGEs have been shown to play a significant role in increasing oxidative stress and inflammation in the body and have been linked to the current crisis in the West of diabetes and cardiovascular disease. Of specific relevance to this work, AGEs are formed as a side effect of the thermal processing of highly palatable foods, typically filled with carbohydrates (Chaudhuri et al.). This category of AGEs is referred to as dietary AGEs (dAGEs). dAGEs are formed exogenously from the body due to this thermal processing common in preparation of processed foods. Evidence from rodent studies have shown that diets rich in AGEs are associated with diseases of the kidney and arteries. Further, it was shown in similar rodent studies that restricting the amount of dAGEs consumed increased lifespan and reduced the impact of diseases like diabetes and atherosclerosis. More specifically, AGEs have been shown in mouse models to directly increase triglyceride levels and induce premature development of insulin resistance. Likewise, dAGEs have had a proven influence in other mouse models for increasing the conditions of diabetic complications, which lead to increased pro-inflammatory markers in the blood (Chaudhuri et al.). Some AGEs have been linked to more severe health outcomes than others, including methylglyoxal (MGO), 3-deoxyglucose (3DG), and glyoxal (GO). These are referred to as the "alpha-dicarbonyl compounds" (α-DCs) and are elevated in cases of diabetes and are linked to chronic hyperglycemia; these AGEs are likely

more pathogenic due to their high reactivity and unstable nature (Chaudhuri et al.). Supporting the animal studies described above, studies in humans have similarly shown that reducing dAGE intake resulted in decreased markers of oxidative stress and inflammation (Uribarri).

Different types of AGEs, also referred to as “glycotoxins", exist and their classification ultimately depends on what pathway and starting reagents were used in their conception. **Figure 1** demonstrates AGE classification diversity and nomenclature (Twarda-Clapa). In essence, AGEs are a group of heterogeneous glycated proteins formed endogenously and exogenously through Hyens and Amadori rearrangements, thermal processing, cellular respiration pathways, and the polyol pathway. Their starting products can range from simple glucose and fructose which interacts with a simple amine or carbonyl group, although many more complex varieties exist. Due to difficulties quantifying and separating different AGE subtypes, research often focuses on broad categories. However, it is important to keep in mind the diversity of AGEs in regards to their molecular weights, their ability to crosslink, other aspects of their biochemistry, and their pathogenicity.

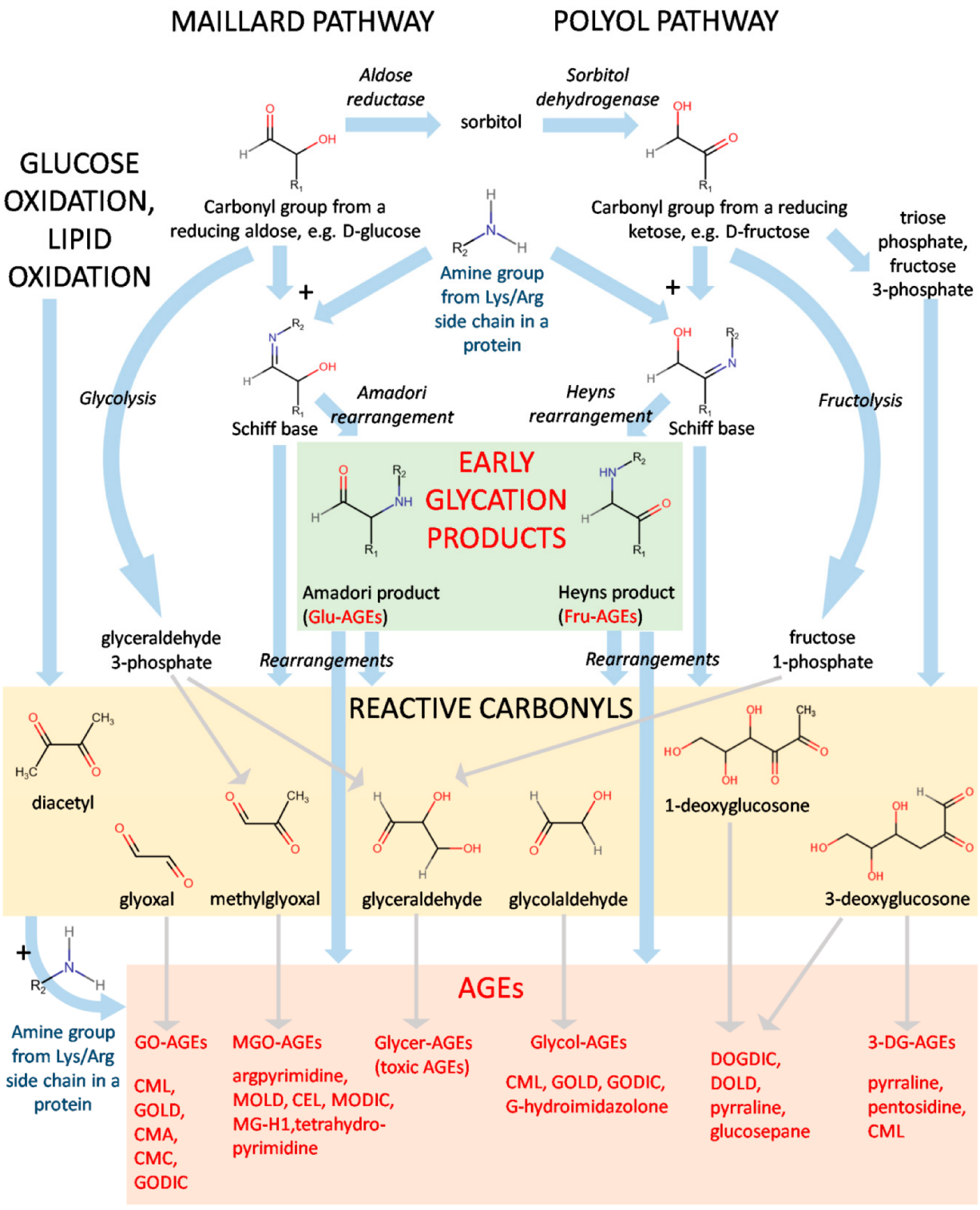


**Figure 1.** This figure represents the many pathways that AGEs may take and the type of end product variations which could arise from each mechanistic pathway **(Twarda-Clapa)**.

As mentioned above, many different types of AGEs exist, requiring different quantification methods, and consideration of the different mechanisms of action in health and disease. Current research conducts studies using different AGEs as the key biomarkers of AGE concentration levels in the body and it often focus on AGEs like: pentosidine, Methylglyoxal (MGO), Glycated Hemoglobin (HbA1c), and Carboxymethyllysine (CML). Recent research in levels of MGO and CML in food revealed different AGE subtypes present in different foods. For example, MGO is highest for thermally processed foods like broiled salmon, french fries, and fried egg, while CML is highest in fried bacon, whipped butter, sesame oil, and chicken skin. Interestingly, cheeses were high in both CML and MGO, possibly reflecting the impact of the thermal processing methods needed to make it in the first place (Uribarri).

**How AGEs Work**

There are two main mechanisms at which AGEs act: the first being their ability to bind to receptors on the body's own cells, and the second being its interaction at the extracellular matrix (ECM). This project specifically focuses on the impacts of ECM-AGE interaction, which we describe in more detail below. For discussion of the receptor mediated mechanisms, we direct the reader to a recent 2022 review in the *Cells* journal titled "Advanced Glycation End-Products (AGEs) Formation, Chemistry, Classification, Receptors, and Diseases Related to AGEs" (Twarda-Clapa). The ECM functions as a physical scaffolding of tissues and organs, although the ECM itself is a non-cellular component, it provides biochemical cues that are extremely important for tissue differentiation, homeostasis, and development. ECM is composed of water, proteins, and polysaccharides in a configuration dependent on resident cells that deposit and

remodel the ECM. The primary fibrous protein of ECM is collagen, which is important for cell adhesion, cell migration, and tensile strength. Importantly, the ECM is dynamic, that is, it is continuously remodeled by resident cells and the chemical environment. The ECM can change density over time through altered rates of deposition and degradation by the cells, as well as through chemical modification that changes the ECM structure. One of the most important modifications to the ECM is crosslinking. Crosslinking is chemical modification of ECM molecules, where adjacent ECM fibers are covalently linked together.

AGEs can modify collagen, and other ECM proteins, through a process called "glycation" that chemically crosslinks collagen fibers together (Lu et al.; Frantz et al.). Collagen crosslinking is known to be increased in aging tissue and its accumulation contributes to stiffer and less resilient tissues that are mechanically weaker (Frantz et al.). The ECM is a major site for AGE accumulation, in part due to the long-lived proteins like collagen type I and III (Chaudhuri et al.; Abu-Hassan). Long-lived proteins are more susceptible to AGE accumulation due to the relatively irreversible nature of AGE modification and reduced turnover rate for the entire protein. Measurements of AGE Millard-like crosslinking in ECM were increased in the collagen of diabetic and aged individuals when compared to control individuals' collagen, indicating AGE's presence there (Sell and Monnier).

As mentioned above, it has been shown that the crosslinking of collagen is associated with increased ECM stiffness (Vincent et al.). Collagen crosslinking also increases the tensile strength of the ECM (Lu et al.). Crosslinking can be damaging to tissues and can be caused by: alkylating agents, free radicals, polyhalo derivatives, polyvalent metals and AGEs (Uribarri; Bjorksten; Chaudhuri et al.). The most common side chains of proteins which AGEs will crosslink seems to be lysine and arginine residues (Sell and Monnier; Chaudhuri et al.).

Additionally, AGE crosslinking extends the life span of the protein due to inhibition of peptide degradation. This inhibition even extends to other portions of the protein which aren't directly glycated (Twarda-Clapa).

**Glaucoma Pathology**

In the paper "Extracellular Matrix: The Driving Force of Mammalian Diseases", it is discussed how ultimately every disease's mechanism of action and development can be traced back to some abnormality of a component in the extracellular matrix (Iozzo and Gubbiotti). While overly simplified, this is demonstrated in primary open angle glaucoma (POAG), an eye disease driven by defects in the ECM of a specialized tissue called the trabecular meshwork (TM). More specifically, POAG is a highly prevalent neurodegenerative disease which affects over 65 million people worldwide. Defined as the progressive and irreversible loss of retinal ganglion cells (RGCs) due to damage in the optic nerve, POAG has a complex etiology with multiple upstream mechanisms. A core mechanism of POAG pathogenesis is the regulation of aqueous humor (AH), the fluid that hydrates and nourishes the internal structures of the eyes. The AH is generated from blood serum ultrafiltrate in the ciliary body and flows out through the anatomical region called the angle, at the junction of the cornea, the sclera, and the iris. In the angle, the TM is the outflow path for AH (Acott and Kelley; Abu-Hassan; Belmares). In glaucoma, the TM is dense and crosslinked, reducing the outflow facility of the eye. Indeed, changes in the TM directly correlate to increased RGC cell death and loss of visual field in glaucoma. Reduced outflow facility causes an increase in intraocular pressure (IOP), resulting in the damage to the optic nerve head (ONH). As pressure increases on the ONH, the axons of retinal ganglion cells are severed, leading to cell death and irreversible vision loss (*What Is*

*Glaucoma?*; Belmares). The TM ECM is primarily composed of type 1 and 2 collagens. **Figure 2** schematically lays out the anatomy of the trabecular meshwork.

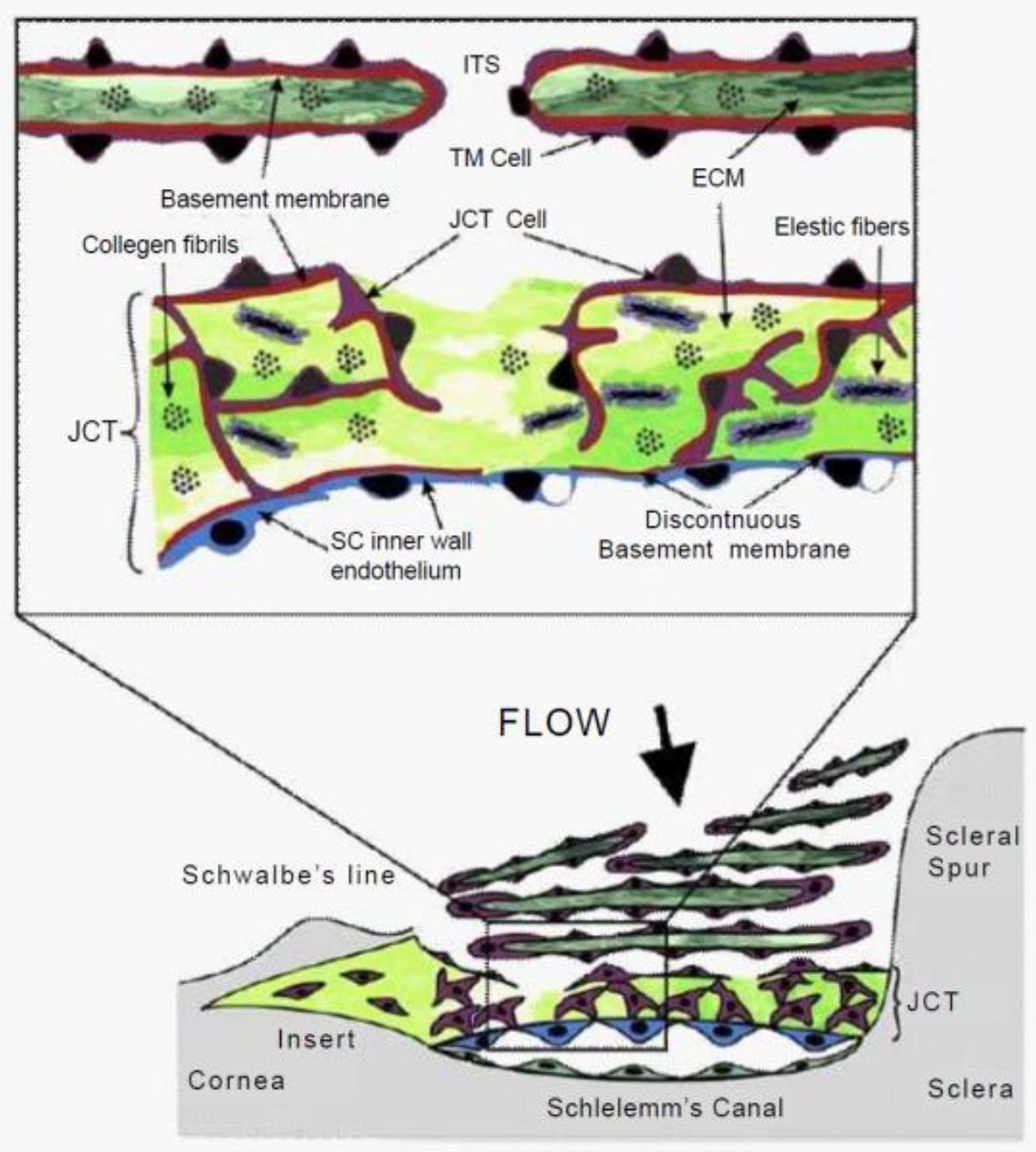


**Figure 2.** Schematic of the anatomic region called the angle, showing the location and anatomy of the TM and its outflow mechanics (Abu-Hassan; Acott and Kelley).

**Who Is at Risk?**

Risk factors for developing glaucoma include family history, other eye diseases like myopia, high IOP levels, abnormal optic nerve anatomy, age, and race. Some of the highest risk factors for glaucoma include: being over 60 years of age, using steroid medications like glucocorticoids, having a thin cornea or being of African, or Hispanic descent (*What Is Glaucoma?*; Belmares; Abu-Hassan). One debated risk factor for glaucoma is diabetes, with some studies showing increased risk, and others not observing an effect. One possibility is that diabetes does not directly increase risk for glaucoma, but a diabetes related factor does, such as increased serum AGE concentrations in patients with poorly controlled diabetes. Considering that the AH is primarily composed of blood serum ultrafiltrate, this leads directly to our hypothesis: **Increased serum AGE increases collagen density and crosslinking in the trabecular meshwork.** As a first step, we developed an *in silico* model which could model TM collagen density and crosslinking based on varying AGE inputs. The changing collagen density would further be used to estimate the elastic modulus, a measure of stiffness, of the TM ECM based on varying AGE inputs. This model was developed through careful review of the literature for estimates of biochemical rate constants, and the model predictions were compared to known values for collagen density and stiffness for both control TM and glaucomatous TM.

## Model Development

The initial creation of our model began with the consideration of relevant factors that may regulate AGE and the interaction with TM ECM. A core goal of the model is converting an AGE input to a prediction of stiffness in the TM ECM. We first identified the pathways involved

in AGE levels, including their excretion, absorption, detoxification, and other potential mechanisms. Despite the fact that dAGEs are consumed quite frequently, nearly 80% of them cannot be absorbed by human tissues and are excreted (Twarda-Clapa). In healthy individuals a third of AGEs are excreted in the urine, with a noticeable decrease in excretion all the way down to 5% among individuals with diabetes. The primary impact of decreased excretion is increased serum AGE levels. After considering the factors regulating AGE levels, we identified key components of ECM remodeling including collagen synthesis and degradation by resident cells. We further considered the impacts of external factors on these mechanisms, including oxidative stress and enzymatic mechanisms modifying the ECM. Finally, we identified regulators of ECM stiffness including collagen composition and crosslink density. ECM composition and stiffness are considered the primary outputs of our model, and allow comparison to published literature on TM ECM in healthy and glaucomatous eyes (Belmares; Last et al.). The full conceptual model, or "Complex Model", is shown in **Figure 3**.

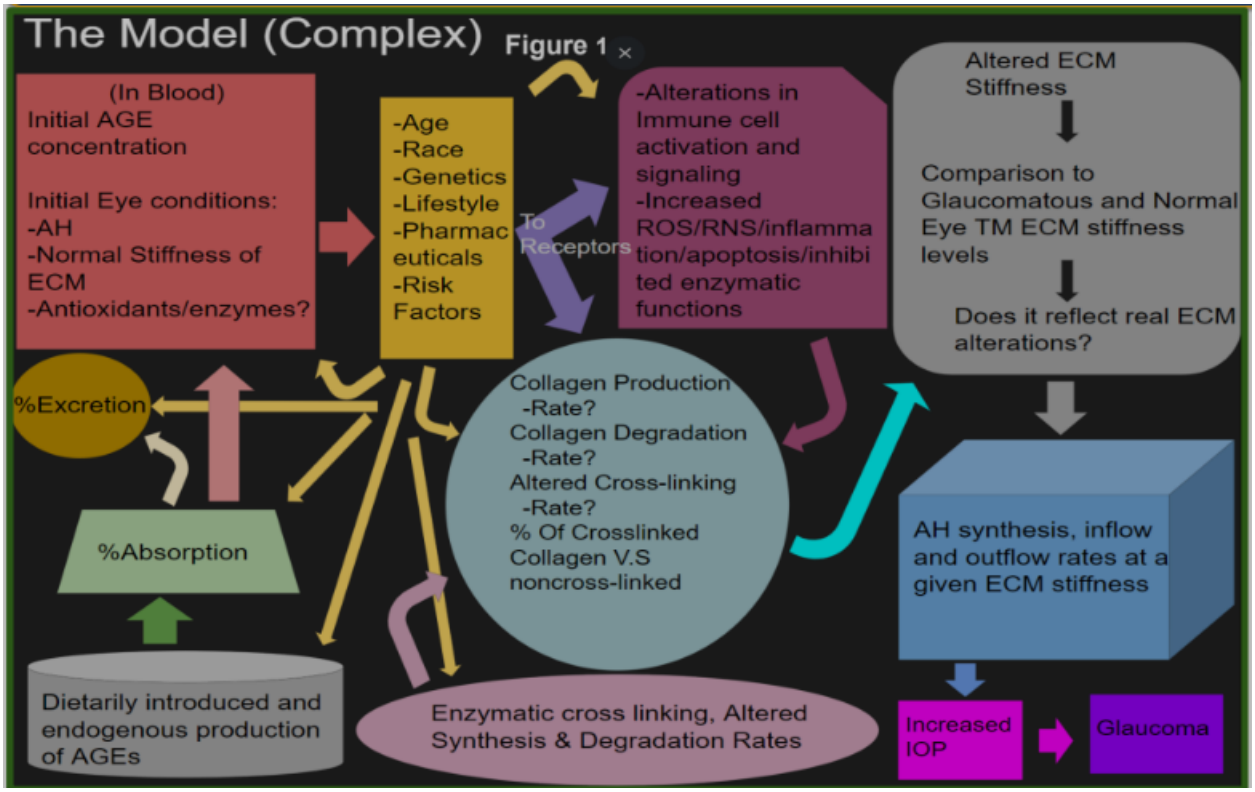


**Figure 3.** Demonstrates the factors which could be important in the hypothesized process of AGE leading to increased TM stiffness, based on our literature review.

In order to develop a more tractable model, we identified the key theoretical aspects and focused on those. Those decisions are described below and the reduced model is shown in **Figure 4**. First, we removed complexity involved with AGE regulation and subtypes, and assumed the input to be represented by just an overall serum AGE level in the blood. Next, we further assumed that blood serum AGE would approximate AH serum AGE levels in the eye. For ECM regulation, we assumed that collagen is the only component, and exists in unmodified and crosslinked forms. Then, it was also assumed that collagen crosslinking rate depends on AGE concentration, and deposition of unmodified collagen by TM cells occurs at a fixed rate, and that degradation occurs at concentration dependent rates for unmodified and crosslinked forms, with

crosslinked degradation occurring slower. Finally, we assumed an empirical model of ECM stiffness dependent on collagen concentration (Licup).

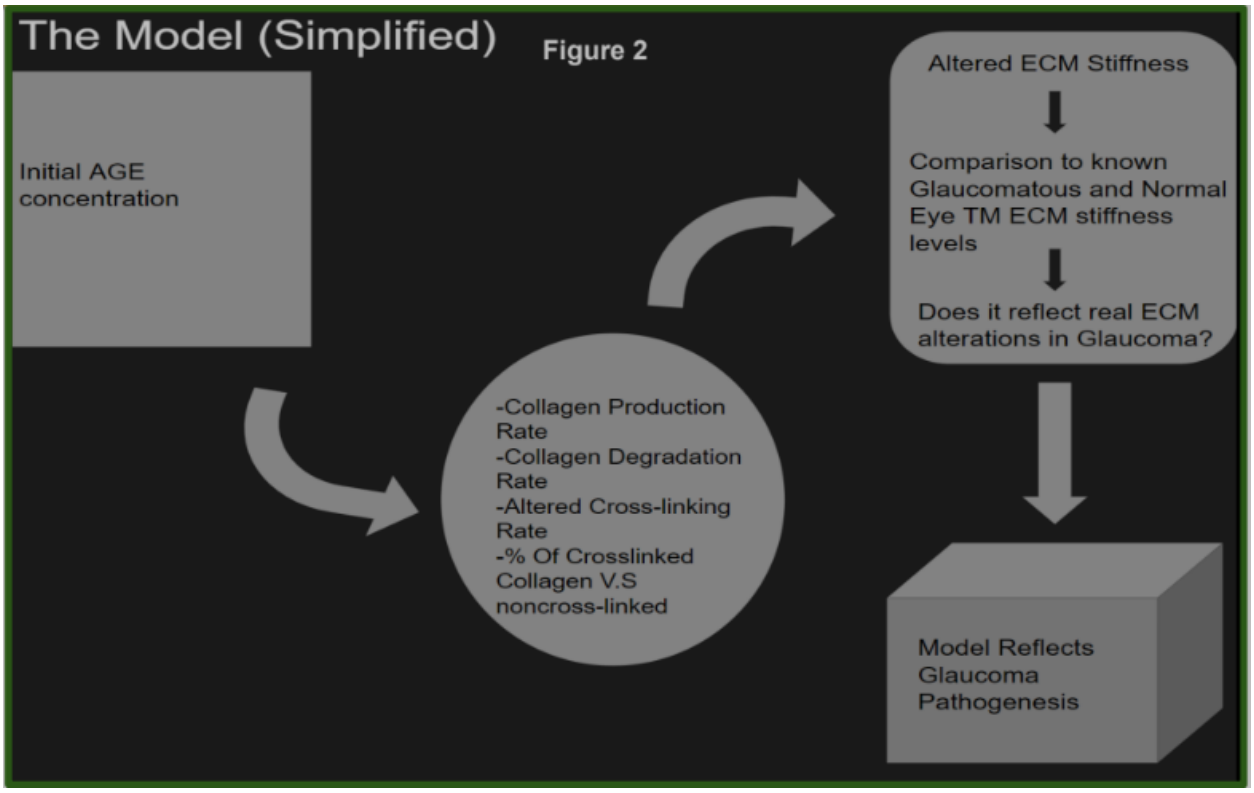


**Figure 4.** Represents the bare-bones model for AGE induced stiffness-based glaucoma pathology.

**Parameter Discovery**

In order to implement this conceptual model, we had to determine rate constants from literature sources and establish the system of equations. First, established a range of total AGEs which were present in the human body, for this we looked at research involving a comparison of normal and diabetic individuals to provide us with low, normal, and high values in relation to healthy versus diseased individuals (Indyk). Based on that data, we chose 0.26, 0.5244, and

1.0488 mg/ml of AGE concentration as a representative for low, average, and high AGE values in humans, which we termed $[AGE]_{serum}$. While these values do not include extrema, they approximate first quartile levels of control patients, median levels of control patients, and median levels in diseased patients.

Next we implemented the dynamic nature of collagen within the ECM. We had to consider the role of TM cells in collagen synthesis, or deposition, as well as collagen degradation. Given the limited TM cell specific data we had available, we recognized that TM cells operate similarly to fibroblasts and used fibroblast rates as an approximation. Fibroblast rates for unmodified collagen were 7.5 x $10^{-4}$ %/hr ($k_{c.dep}$) and 2.5x$10^{-3}$ %/hr ($k_{c.deg}$), respectively (Rouillard and Holmes; Stamer and Clark).

Next we implemented the impact of AGEs on the ECM via crosslinking activity. Crosslinking rates specifically for collagen are not available, but extensive work has been done looking at bovine serum albumin (Booth). Using empirical fits to bovine serum albumin glycation reactions in 0.5 M ribose, provided a collagen crosslinking ($k_{c.x\text{-}link}$) rate of .03 1/(day * [AGE] mg/mL). As crosslinked collagen is known to degrade slower, we assessed literature values for the differing rates of collagen degradation and found that crosslinking reduces degradation rates by approximately 60%, and therefore defined ($k_{c.x\text{-}deg}$) = 0.60*($k_{c.deg}$) (DeGroot).

Finally, we wished to correlate collagen density to ECM mechanics. Prior work has done extensive mechanical testing of collagen gels of varying density, specifically measuring the bulk modulus (K) (Licup). A power-law fit to the available data revealed $K = 5.385*[Col\%]^{3.362}$, which can be readily converted to the elastic modulus through the relation E = 3*K*(1-0.6) for collagen, where the 0.6 term is twice the Poisson's ratio; 0.3 is frequently used as a Poisson's ratio for collagen gels. E is a measure of the stiffness of a linearly elastic material and is defined

by the relative relationship between the stress and the strain. While collagen and tissue are known to not perfectly match the assumptions for a linear elastic material, for small stresses and strains this approximation is often used (Licup).

**Equations and Implementation**

The core of the model is represented by two equations, the first being the change in collagen concentration with respect to time d[Col]/dt, and the second being the change in crosslinked collagen with respect to time, d[X-Col]/dt. We can define $d[Col]/dt = k_{c.dep} - k_{c.deg}[Col] - k_{c.x\text{-}link}\ [Col][AGE]_{serum,}$ or the change in collagen subtracted to the rate of collagen crosslinking which is based on the initial collagen and AGE concentrations. The second equation was d[X-Col]/dt is represented by $k_{c.x\text{-}link}[Col][AGE]_{serum} - k_{c.deg}[X\text{-}Col]$ which stands for the rate of collagen crosslinking subtracted by the rate of crosslinked collagen degradation. This system was implemented in MATLAB 2022a using the variable order solver ode45 over a timespan of one year or three years for varying AGE input conditions, which can be found in the Appendix (Mathworks).

**Results**

As an initial test of the model, we compared the ECM behavior under constant “Low”, “Median”, and “High” AGE values threshold as seen in diabetic patients, shown in **Figure 5**. Regarding total collagen density (the sum of crosslinked and unmodified collagen), the steady state results of the model correlate well with literature data on TM in glaucoma. Normal eyes exhibited a density of collagen of about 24.84% while glaucomatous eyes had a collagen density in their ECM of 38.49% (Belmares). Turning to the model, we can see the total collagen amount

increase with increased AGE levels from approximately 32% to 37%. While the model output doesn't exactly match the experimental values, the approximate magnitude and range is promising for a simplified model with approximate constants. The model also shows stiffening with increasing AGE, ranging from approximately 150 kPa to 220 kPa. While the trend is directionally consistent with data on normal and glaucomatous TM, the range is different, where normal TM is <10 kPa and glaucomatous is 50-300 kPa (Raghunathan et al.; Morgan et al.).

As a further demonstration of the model, we implemented varying AGE levels to simulate lifestyle changes, shown in **Figure 6**. For the first simulated year the level is median, transitions to high in the second year to simulate poor diet, and finally transitioning to a low value in the third year. As expected, the stiffness of the ECM changed relatively directly in correspondence with the AGE input per year with different baselines and stiffnesses reflecting in the change in the initial AGE concentration. While this is an early demonstration of the simplified model, it demonstrates potential to utilize this model for studying the impacts of lifestyle changes.

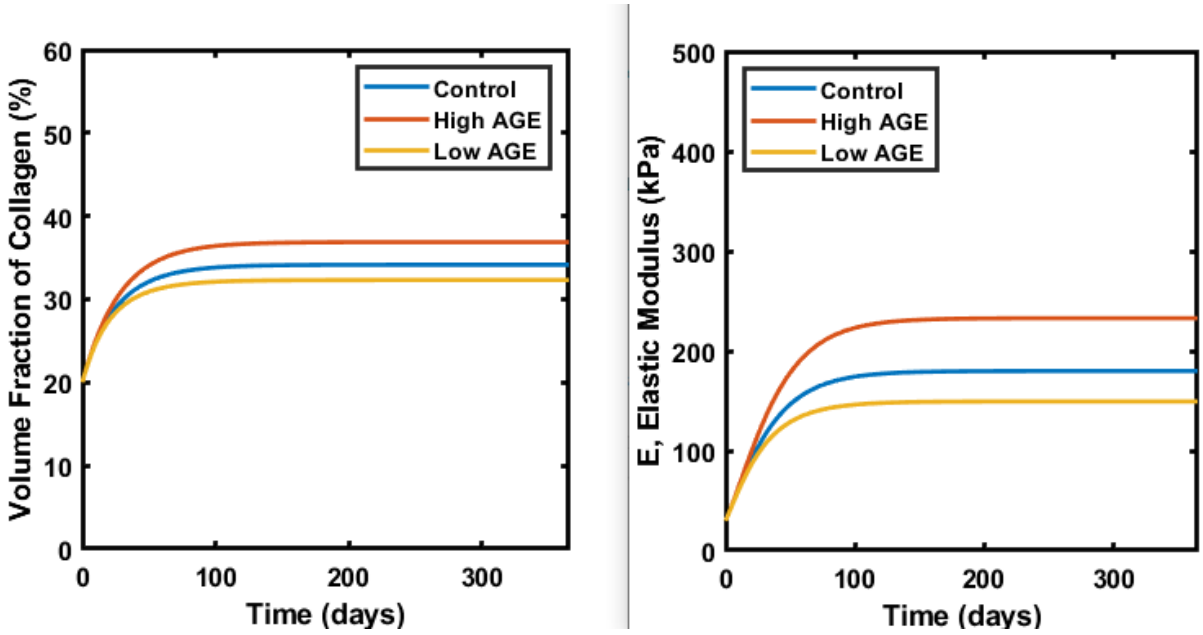


**Figure 5. Demonstration of the model outputs for both collagen density and elastic modulus. Higher AGE inputs yield an increase in collagen density and, therefore, ECM stiffness.**

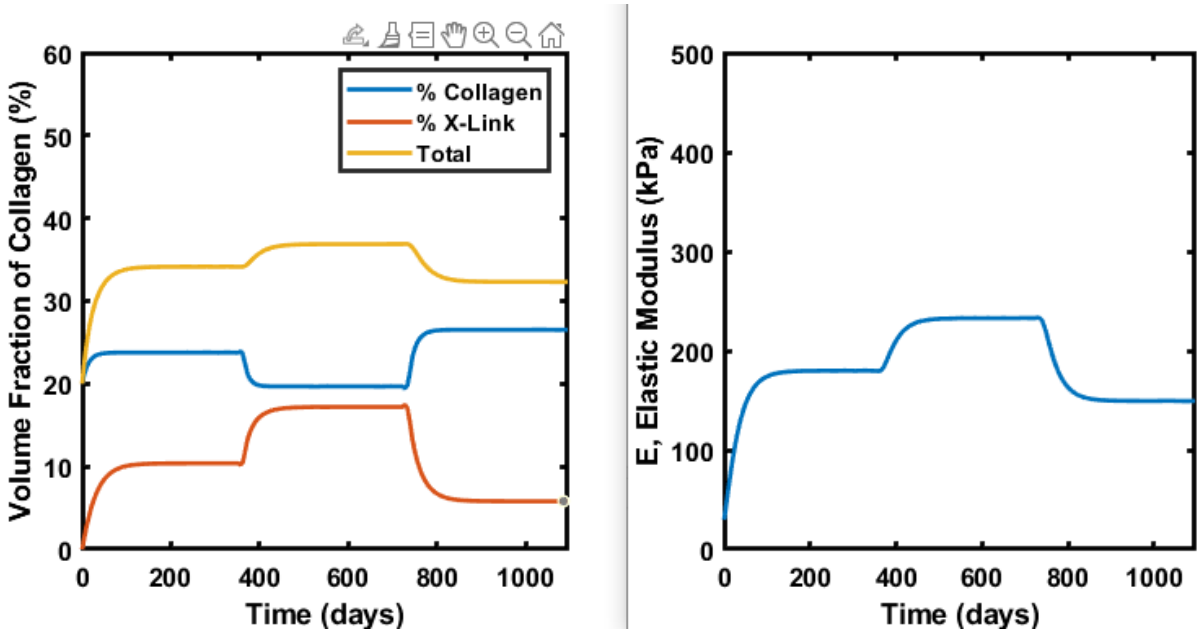


**Figure 6. Demonstration of the model outputs for multi-year studies with time-varying**

**inputs. Differential AGE inputs across time can have markedly noticeable effects on collagen density and ECM stiffness.**

## Discussion

Here we present a simplified model for TM collagen deposition, using rate constants estimated from literature sources. An important finding of this work was that increased AGE levels resulted in a model prediction of collagen density that approximately matches glaucomatous TM, while low or control AGE levels resulted in lower collagen densities more consistent with healthy TM. Further, stiffness was predicted to increase, although the model did not successfully predict the low TM stiffness expected in healthy TM, the order of magnitude of the model's output was consistent with prior experimental measurements of glaucomatous TM. While early work, this simplified model is consistent with our primary hypothesis, and AGE-mediated stiffening of the TM deserves further study both computationally and experimentally.

This model has a number of limitations. A primary limitation is that different AGE subtypes are known to have different biological effects, however the model aggregates all AGE subtypes into a single overall AGE level. Further, AGE levels are known to be regulated by the availability of antioxidants, like ascorbic acid. Ascorbic acid is especially relevant to glaucoma, as it is highly elevated in ocular tissues and AH, where it inhibits ocular inflammation and UV radiation damage (Chen; Uribarri; Brubaker et al.). Further, ascorbic acid can also function as an inhibitor of protein glycation as well (NOVOS; Sadowska-Bartosz and Bartosz). Ascorbic acid could be especially relevant in this context as levels are dramatically reduced in POAG patients, with POAG aqueous humor having 415 ± 17 μM compared to 720 ± 30 μM in controls

(Ferreira). Including the effects of ascorbic acid in reducing collagen crosslinking would reduce the overall levels of collagen and reduce stiffness in the model, this would be more pronounced in the low and control groups. Another important pathway that was not included in the model is the detoxification pathway of MGO, which occurs when MGO is converted into lactic acid via glyoxalase I and II via the glutathione-dependent glyoxalase pathway or via the glutathione-independent cytosolic enzyme pathway by glyoxalase III (Chaudhuri et al.).

Further, this model used a simplified understanding of the TM ECM. First, we neglected many of the protein components, including fibulin, fibronectin, and elastin; instead focusing solely on the dominant component of collagen. These other components are known to have impacts on overall ECM mechanics in other systems, however they are less poorly studied than collagen in the TM. Further, our model did not consider the changes in collagen mechanics once it's crosslinked and how that would affect the ECM's stiffness. Finally, the model assumed simplified versions of ECM deposition and degradation. These processes are both mediated by cells, and TM cells are known to change phenotype in POAG. While a full review of TM cell changes is outside the scope of this work there are two key points that relate to the model. One, increased expression of transforming growth factor β in POAG is known to increase ECM deposition and reduce degradation. Two, TM cell population decreases in aging, and this is accelerated in POAG. A reduced population would result in lower remodeling, longer lived collagen molecules, and increased crosslinking.

This model also poorly accounted for lifestyle factors. Regular physical activity has been correlated with lower AGE levels in active compared to non-active individuals (Drenth et al.). This finding has been shown in mice where it was found that mice which ran at a moderate intensity for ten weeks on the treadmill had significantly decreased serum AGE levels (Boor et

al.). In another human study, lifelong trained athletes had 21% lower amounts of AGE based crosslinks within their patellar tendons when compared to people their age who were untrained (Kim et al.; Hooshiar).

This model also did not account for pharmaceutical interventions that alter the impact of AGEs. One example is the diabetes treatment drug, Metformin, which functions by inhibiting AGE-induced inflammatory responses and can inhibit AGE-induced aggregation of glycated proteins (Zhou et al.). The way Metformin does this is through downregulating certain proinflammatory cytokines and upregulating expression of mRNA for anti-inflammatory cytokines. In conclusion, we have developed a novel *in silico* model linking AGE concentration to TM collagen density and stiffness, and compared it to known values.

Future work will pursue this hypothesis through various improvements for the *in silico* models along with further experimental work. Specifically, improved versions of the model will target the impacts of ascorbic acid on AGE-mediated crosslinking, changes in cell-mediated ECM remodeling that are known to occur in glaucoma, and the impacts of crosslinking on ECM mechanics. Experimentally, *in vitro* and *in vivo* tests on the impact of AGEs on TM cell function and ECM deposition would be critical in developing more accurate rate constants for this model as well as validating the theoretical framework.

Additional future work will expand on this model and expand the focus to develop a model for AGEs impact on the pathology of LSRDs in general, rather than just focusing on the pathology of one disease (POAG). Other future directives would be to put our model through a sensitivity test to see if our model can also approximate other diseases which are similar enough to be tested by our model.

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**Appendix:** MATLAB Code for Generating Figure 6

```
clearvars %clears are variables from the workspace
close all %closes all figure windows
clc %clears command window
%Expected Endpoints:
%collagen concentration we can get from: Histological Investigation of Human
% Glaucomatous Eyes: Extracellular Fibrotic
%Changes and Galectin 3 Expression in the Trabecular
%Meshwork and Optic Nerve Head
%RICARDO BELMARES,1,2 URMIMALA RAYCHAUDHURI,1 SANDRA MAANSSON,1 AND ABBOT F.
      CLARK
%Control (n = 12) 29.88%
% Glaucomatous(n = 11) 38.49%
%we can get stiffness from Last et al.
%Control ~ 3 kPa and Glaucoma 50-300 (~75 kPa mean) kPa
%Hypothesis: higher AGE leads to more collagen and higher stiffness
%TIME
ts=[0 365]*3;  %Time in days
%initial conditions
Col = 0.2;%[Col]
XCol = 0;%[XlinkCol]
y0=[Col;XCol];
%Constants
%from [5]
%The dependence of AGEs concentration determined in the competition ELISA
% test on the occurrence of ischemic stroke; (n = 39; YES (0.9252 ± 0.0687
      mg/ml),
% NO (0.5244 ± 0.0517 mg/ml))
```

```
% from [7] they use 50 mM Ribose as AGE, which is 7.5 mg/mL
AGEi = 0.5244; %AGE level in mg/mL
%these deposition parameters reach steady state of ~30% if we set kc.xlink
%to 0, they are from [6]
%eg in the absence of crosslinking, we get steady state 30% collagen volume
kc.dep= (7.5 * 10^-4)*24;      %Rate of Collagen Synthesis/Deposition
kc.deg= (2.5 * 10^-3)*24;      %Rate of Collagen Degradation
kx.deg = kc.deg*0.60;     %Rate of x-linked collagen degradation
%if we set kc.dep and kc/x.deg to 0 and have an initial condition of 0.5 for
%collagen, we can vary the xlink parameter until we match the kinetics
      presented in [7]
%0.03 replicates results with AGEi input of 7.5 mg/mL
kc.xlink = 0.03; %units of fractionCol/(day*(mgAGE/mL))
[t,y] = ode45(@(t,y) myfun(t,y,AGEi, kc, kx), ts, y0);
y(:,1:2) = y(:,1:2)*100; % convert y from fraction to percent, eg 0-1 to
      0-100
%plot data
PS = [4 4]; %figure size in inches
figure('color','w','Units','inches','position',[1 1
      PS],'paperunits','inches','PaperPosition',[0 0 PS],'PaperSize',PS)
plot(t,[y(:,1:2) sum(y(:,1:2),2)],'LineWidth',2)
legend({'% Collagen','% X-Link','Total'},'location','northeast')
axis([0 365*3 0 60])
set(gca,'fontsize',12,'fontweight','bold','LineWidth',2,'XColor','k','ycolor'
      ,'k')
ylabel('Volume Fraction of Collagen (%)'); xlabel('Time (days)');
%From Collagen concentration we can predict stiffness, K, from the
%following: Stress controls the mechanics of collagen networks
```

```
%Albert James Licupa,1, Stefan Münsterb,c,1, Abhinav Sharmaa,1, Michael
      Sheinmana, Louise M. Jawerthd, Ben Fabryb,
%David A. Weitzc,d, and Fred C. MacKintosha,2
%but those values are in [col] mg/mL instead of %...a rough estimate of
%dense collagen in a tumor from "Diffusion and Convection in Collagen Gels:
      Implications for Transport in
%the Tumor Interstitium" is that 100% collagen area would be 50 mg/mL.
ColmgmL = sum(y(:,1:2),2)*50/100; %sum both types of collagen together and
      convert to mg/mL
%From Fig1A of Licupa, 3.6 mg/mL is 800 Pa, 2.4 is 200 Pa, 1.8 is 80 Pa, 0.9
      is 30 Pa and
%0.45 is 11 Pa
%K = [800 200 80 30 11]; D = [3.6 2.4 1.8 0.9 0.45];
%using a simple power law fit: 10.77*ColmgmL.^3.362; Rsquared
%is 0.9985
K = 10.77*ColmgmL.^3.362/1000; %divide by 1000 to get into kPa
%we can approximate the elastic modulus:
E = 3*K*(1-2*0.3);
%plot data
PS = [4 4]; %figure size in inches
figure('color','w','Units','inches','position',[2 2
      PS],'paperunits','inches','PaperPosition',[0 0 PS],'PaperSize',PS)
plot(t,E,'LineWidth',2)
axis([0 365*3 0 500])
set(gca,'fontsize',12,'fontweight','bold','LineWidth',2,'XColor','k','ycolor'
      ,'k')
ylabel('E, Elastic Modulus (kPa)'); xlabel('Time (days)');
function dy = myfun (t,y,AGEi, kc, kx)
%Function will double AGE input in Year 2 and then half it in Year 3
```

```
if t> 365 & t<=365*2
   AGEi = AGEi*2;
elseif t>365*2
   AGEi = AGEi*0.5;
end
dy =zeros(size(y));
dy(1) = kc.dep - kc.deg*(y(1)) - kc.xlink*(y(1))*AGEi; % d[Col]/dt
dy(2) = kc.xlink*(y(1))*AGEi - kx.deg*(y(2)); % d[X-Col]/dt
end
```