Thesis Portfolio

Sugaway: Using Synthetic Biology to Treat Diabetes
(Technical Report)

The Growing War between the Pharmaceutical Industry and Diabetic Patients
(STS Research Paper)

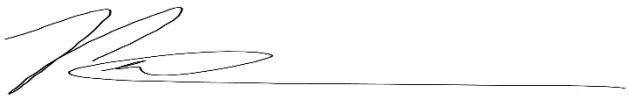
An Undergraduate Thesis

Presented to the Faculty of the School of Engineering and Applied Science
University of Virginia • Charlottesville, Virginia

In Fulfillment of the Requirements for the Degree
Bachelor of Science, School of Engineering

Promotto Islam
Spring, 2022

Department of Biomedical Engineering



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Sociotechnical Synthesis

Diabetes mellitus consists of a group of metabolic diseases which all share a common characteristic of inducing elevated blood glucose levels. This increase is often due to a dysregulation (type I diabetes) or dysfunction (type II diabetes) of native insulin, and current statistics suggest approximately 34.2 million individuals in the United States are affected by diabetes. The current standard for treatment is the self-administration of insulin, but this has become difficult for many diabetic patients as insulin prices are at an all-time high and are predicted to continue increasing. This situation calls for a deeper look into the insulin market and the consequent rising tensions between diabetic patients and pharmaceutical companies, as well as the viability of a new supplemental treatment which can be taken by diabetic patients to reduce their reliance on insulin.

My technical project aimed to find a new supplemental treatment for diabetic patients through a synthetic biology approach. Specifically, I designed a bacterium which can mimic the effects of insulin by increasing the conversion from glucose to glycogen. This was done by designing a novel plasmid, called pKID1, which contains four genes involved in glycogen synthesis. These genes are placed under the control of a rhamnose-inducible promoter, such that the genes in glycogen synthesis are only expressed in the presence of rhamnose. This plasmid was transformed into electrocompetent JM109 cells and the expression under rhamnose was verified by measuring the protein expression with a western blot. Functionality of the plasmid was then tested by measuring the intracellular glycogen concentration after a 6-hour culture in glucose-supplemented M9 media, which showed a significant increase in intracellular glycogen in pKID1 transformed cells compared to control cells. This design represents a prototype probiotic treatment, which would work by having a diabetic patient take probiotic pill prior to a

meal. The bacteria would then work in the small intestine and mimic the effects of insulin by uptaking extracellular glucose and converting it into glycogen. The potential of this prototype to be used as a probiotic offers a less expensive treatment method for diabetic patients by reducing their reliance on expensive insulin.

My STS research focuses on how insulin market came to the position where it is today and the relationship between diabetic patients and the pharmaceutical industry. I begin by discussing the history of insulin and the price trends which have brought the United States insulin market to where it is today. I then look into policy and legislation changes at both the federal and state level and the attempts made by the government to control the insulin market. To understand the relationship between diabetic patients and the pharmaceutical industry, I break down the insulin production pipeline and the advocates for each side of this relationship. Through this paper, I conclude that the conflict between diabetic patients and pharmaceutical companies is complex and that blame can not easily be placed on either side. This conflict arises from several different factors all leading to the rise in insulin prices and the decrease in insulin availability for diabetic patients. While there is no clear solution currently to this conflict, a combination of new legislation giving control to the insulin market combined with looking outwards at how other nations have controlled drug prices may give insight into a potential solution.

Working on both the technical and sociotechnical aspects of this project have given me important insight into how the technical skills and concepts I have learned have an impact on other individuals worldwide. Specifically, the design of a potential drug is only the first part of the drug design and manufacturing process. How the drug is actually accessible to individuals and their efficacy outside of the laboratory setting is a completely separate process which needs

to be considered during the research and development phase in order to maximize the benefits of the product to its intended consumers.

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On my honor as a University Student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments

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Dr. Keith Kozminski, Department of Biology

Introduction

Diabetes Mellitus are a group of metabolic disorders which all share a common characteristic of inducing elevated blood glucose levels. Blood glucose levels are primarily under the hormonal control of insulin and glucagon. Under hyperglycemic conditions, insulin is secreted by pancreatic β-cells and binds to the insulin receptor of other cells to trigger the uptake of extracellular glucose. Glucose is then used for various metabolic purposes, or stored as glycogen for long-term energy storage (Petersen & Shulman, 2018). Under hypoglycemic conditions, glucagon is secreted from pancreatic α-cells, which bind to the glucagon receptor and initiate the release of glucose through glycogenolysis and gluconeogenesis (Ojha et al., 2019). The most predominant forms of DM are type 1 diabetes (T1D) and type 2 diabetes (T2D), which effect roughly 2 million and 34 million Americans, respectively (CDC, 2022; *Statistics About Diabetes | ADA*, n.d.). T1D arises from an autoimmune response where T-cell and B-cell responses attack and destroy pancreatic β-cells, which leads to the lack of insulin production. T1D is influenced by both genetic and environmental factors, and is characterized by the presence of autoantibodies circulating the plasma (Los & Wilt, 2022). T2D occurs due to a growing insulin resistance in target cells, which prevents them from uptaking extracellular glucose. The mechanism behind insulin resistance is still unknown, but studies have shown that genetic factors and lifestyle choices can play a role in developing insulin resistance (Wilcox, 2005). If left unmanaged, diabetes can lead to several complications such as cardiovascular disease, diabetic neuropathy, and cancer (Wu et al., 2014).

While there are no current cures for diabetes, both T1D and T2D can be managed with a combination of lifestyle changes and medication. Reducing carbohydrate intake and increased exercise has been shown to lower blood glucose levels, while medications such as Metformin or

different Sulfonylureas can be prescribed based on the severity of the diabetes (*Diabetes Management*, n.d.; Rajkumar, 2020). However, the primary and most potent treatment for diabetes is to begin insulin therapy by administering insulin directly into the bloodstream. However, insulin has been the prime target of price gouging by United States pharmaceutical companies, which has led to insulin prices increasing over 1000% in the past 20 years (Rajkumar, 2020). This has left many diabetic patients without access to affordable insulin, and has led to several deaths from individuals unable to use insulin or using expired insulin (Rajkumar, 2020).

Recent advances in the field of synthetic biology have opened up new avenues for the treatment of metabolic disorders. Several studies have shown the usage of synthetic gene circuits to either detect various biomarkers or to trigger particular cascades which lead to the transcription of a specific gene product, which can be fine-tuned to treat specific disorders (Teixeira, Ana P. & Fussenegger, Martin, 2016). For example, Bai et al. showed the usage of a closed-loop genetic circuit to detect liver failure and consequently secrete hepatic growth factor as a therapeutic protein in response to the liver failure (Bai et al., 2016). Similar work has been done with diabetes by both Ye et al. and Xie et al., and their work suggests that a synthetic biology approach may also be used to develop a less expensive insulin supplement which can reduce a diabetic patient's reliance on insulin injections (Xie, Mingqi et al., 2016; Ye et al., 2017). We aim to do this by designing a genetically modified bacterium, which will mimic the effects of insulin by up taking extracellular glucose and converting it into glycogen. This bacterium is a proof-of-concept prototype for a probiotic insulin supplement which could be taken prior to a meal as a way to reduce postprandial glycemic spikes.

Glycogen synthesis in Escherichia coli is well documented and are controlled by multiple genes, many which are present in the glgBXCAP operon. Of particular importance are the genes phosphoglucomutase (PGM), branching enzyme (glgB), ADP-glucose phosphorylase (glgC), and glycogen synthase (glgA). PGM catalyzes the movement of a phosphate group from the 1-carbon to the 6- carbon to produce glucose 6-phosphate. glgC catalyzes the addition of ADP from ATP, converting glucose 6-phosphate to ADP-glucose. The ADP-glucose is then used by glgA to form the backbone chain of α-(1,4) glucan chains. glgB will catalyze the formation of new α-(1,6) linkages to branch off the main α-(1,4) glucan chain (Almagro et al., 2015). We hypothesize that an increase in expression of key glycogen synthesis enzymes will consequently lead to an increase in intracellular glycogen concentration.

Results

A glycogen synthesis plasmid, named pKID1, was generated by placing genes PGM, glgB, glgC, and glgA under the influence of a rhamnose inducible promoter. This sequence was then cloned into plasmid backbone pD861 and transformed into JM109 cells for further analysis.

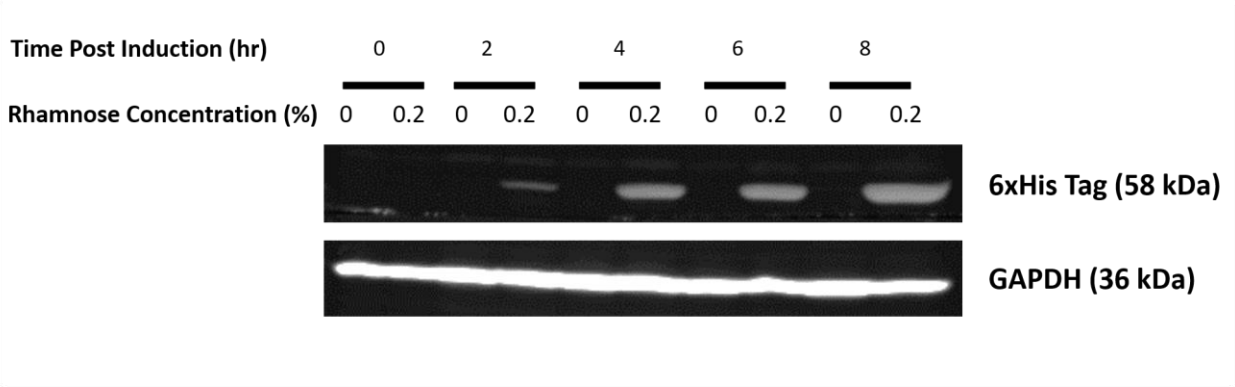
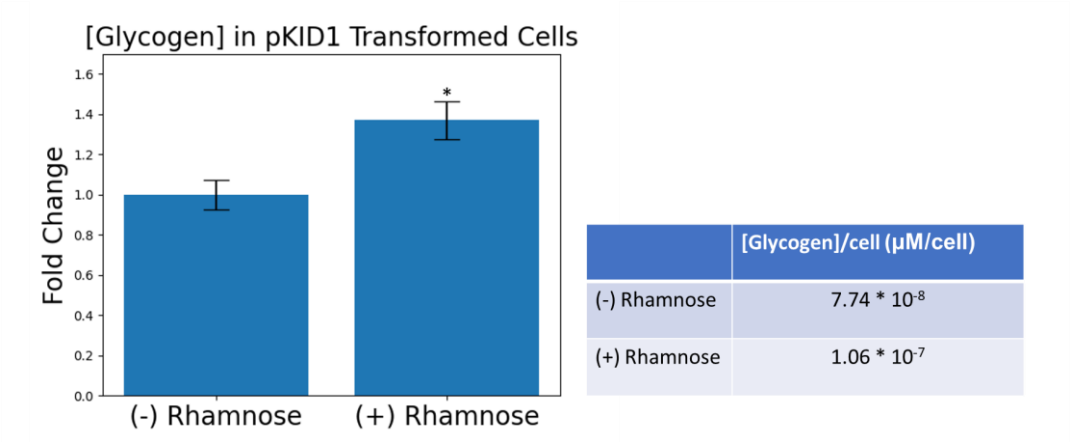


Fig. 1. Rhamnose induction leads to increased 6xHis expression on first open reading frame. Membrane was probed with an anti-6xHis antibody to determine expression of first open reading frame on pKID1. GAPDH was probed as a loading control to ensure equal protein loading in each well.

pKID1 produces a protein product under rhamnose induction

Protein expression from the plasmid was verified by inoculating pKID1-transformed bacteria in 0% rhamnose (control) or 0.2% rhamnose (rhamnose-induced samples) for 8 hours to determine the inducibility of pKID1. Protein extracts were separated and probed with an anti6xHis antibody to show stable protein expression after 4 hours of rhamnose induction (Figure 1). Equal protein loading was ensured by probing with GAPDH, which showed consistent protein loading across all samples. These results were verified across two separate biological replicates to ensure that pKID1 was able to express protein only in the presence of rhamnose.

pKID1 increases intracellular glycogen concentration under rhamnose induction



	[Glycogen]/cell (μM/cell)
(-) Rhamnose	$7.74 * 10^{-8}$
(+) Rhamnose	$1.06 * 10^{-7}$

Fig. 2. Rhamnose induction leads to an increased intracellular glycogen concentration. (Left) Intracellular glycogen concentration increased by 37% in the presence of rhamnose compared to uninduced cells (* p = 0.01 with a Student's t-test at α = 0.05). **(Right)** Intracellular glycogen concentration per cell shows a 37% increase in induced cells compared to uninduced cells.

pKID1-transformed cells were incubated in M9 media supplemented with 0.8% glucose and tryptone to validate the functionality of the protein product produced from the plasmid. Cell extracts were assayed for glycogen content, which showed that rhamnose-induced cells had a 37% increase in intracellular glycogen content compared to uninduced cells (Figure 2). These results were statistically significant with a Student's t-test (p = 0.01) at an α = 0.05. To further quantify

the effects of pKID1, glycogen concentration of an individual bacterium was calculated, which showed a 37% increase in intracellular content compared to uninduced cells. These results were verified with two independent technical replicates and cells transformed with the plasmid backbone (pD861) were used as a negative control.

Discussion

The growing inaccessibility to insulin treatment in the United States due to the rising cost of insulin and growing prevalence of diabetes has indicated the need for an alternative supplemental treatment. Our work here shows the use of a genetically modified bacterium as a biological device that can increase the intracellular concentration of glycogen. This biological device serves as a prototype for a less expensive probiotic treatment which can reduce the reliance on insulin treatment.

The first step in the creation of the biological device was the design of the glycogen synthesis plasmid pKID1. The plasmid was designed using the primary enzymes involved in glycogen synthesis: PGM, glgB, glgC, and glgA. The three genes glgB, glgC, and glgA were placed in their native sequential order as seen in the glgBXCAP operon. PGM was placed in front of these three genes and as the first open reading frame of the construct. These enzymes were all placed under a rhamnose inducible promotor to allow for selective transcription of gene products. This allowed for selective expression of construct, which would be intended as the bacterium should only activate in hyperglycemic conditions. This plasmid serves as one component of a larger synthetic gene network which would be used to generate the most therapeutic bacterium. Due to its modularity and presence of an inducible promoter, this plasmid design serves as a template for future constructs which can be added to the synthetic gene network. This includes

constructs which can increase the glucose uptake into the bacterium, or a kill-switch plasmid which will limit the growth of the bacterium.

Once pKID1 had been fully designed, it had to be ensured that it was able to produce a protein product and that the resulting proteins were functional. This was validated and supported by the results shown in Figure 1 and Figure 2, which showed that there is a protein product formed and that it leads to a statistically significant increase in intracellular glycogen concentration. This result suggests that the addition and activation of pKID1 by rhamnose induction increases the rate of glycogen synthesis through the increased expression of each enzyme involved in the glycogen synthesis pathway.

To further support these results, additional experiments need to be done to further characterize the effects of pKID1 on the glycogen synthesis pathway. Modeling flux through the glycogen synthesis pathway can reveal regulatory points and rate-limiting steps in the synthesis, which would be points of improvement to the pKID1 design to better optimize glycogen formation. This can be determined through computational modeling, and then verified *ex vivo* by measuring both protein expression and protein functionality. A current limitation to the pKID1 design is that only the first open reading frame is tagged with an epitope tag, which meant that the results from Figure 1 are limited to a protein product being produced only from the first reading frame. In future iterations of the pKID1 design, multiple epitope tags would be placed on all open reading frames to provide an easy way to check protein expression.

The results presented herein support the design of a novel biological device which has implications to be used as a diabetic therapeutic. As a therapeutic, the genetically modified bacteria will be packaged into a probiotic pill which a diabetic patient could take prior to a meal. This would consequently replace their typical insulin dosage. As the probiotic pill is ingested, the

bacteria in the probiotic will colonize the small intestine where other microorganisms are present as a part of the microbiota. Here, the bacteria will be able to mimic the effects of insulin by uptaking excess glucose from a digested meal and converting it into glycogen. A cheaper probiotic option for diabetic patients is more financially accessible and alternative option to give to diabetic patients who may not be comfortable with using needles for an insulin injection. This decreases their current reliance on expensive insulin therapies and would have large beneficial implications on the current diabetic therapeutic market.

Materials and Methods:

Design and Generation of pKID1

Construct pKID1 was generated by sequentially placing genes PGM, glgB, glgC, and glgA under the influence of a rhamnose inducible promoter. This sequence was then cloned into plasmid backbone pD861 (provided by Dr. Steven Zeichner's laboratory at the University of Virginia) between restriction sites XbaI and NotI. Plasmid was amplified in electrocompetent DH5α cells and extracted with a Maxiprep kit (Qiagen).

Cells and Reagents

All further cell-based experiments after the initial pKID1 amplification process were done in chemically competent JM109 cells. Chemical transformations involved adding 100ng of plasmid to ice-cold JM109 cells for 5 minutes prior to a 1-minute pulse in 42°C water bath. Cells were returned to ice and incubated for 1 hour with S.O.C. media (ThermoFisher) prior to streaking on LB Broth + Kanamycin (50 μg/mL) plates. Successful transformants were verified by gel electrophoresis, where plasmids were extracted by a Miniprep kit (Qiagen) and separated on a 0.8% agarose gel.

Rhamnose Induction Assay

Cells were inoculated overnight in LB + Kanamycin (50 μg/mL) prior to rhamnose induction. Cells were consequently inoculated the next day at OD600 = 0.05 in either the presence of 0.2% L-Rhamnose (Sigma) or absence of L-Rhamnose for 8 hours. Samples were collected every two hours to determine the ideal induction period for protein expression.

Western Blotting

Protein was extracted from each sample from the rhamnose induction assay and electrophoretically separated by SDS-PAGE. Proteins were transferred to a 0.2 μm nitrocellulose membrane and blocked with a 5% BSA solution. The membrane was then probed with a primary mouse anti6xHis antibody and secondary goat anti-mouse conjugated antibody prior to being developed. Equal protein loading was ensured by probing the membrane for GAPDH as a loading control. Glycogen Concentration Assay Cells were inoculated overnight in LB + Kanamycin (50 μg/mL) prior to measuring intracellular glycogen. Cells were inoculated the next day at OD600 = 0.05 in media (M9 media + 0.8% glucose + 1% tryptone) in either the presence or absence of 0.2% rhamnose for 6 hours. Samples were then collected and prepared by resuspending bacterial pellets in PBS and heating to 95°C for 15 minutes. Silica beads were then added to the resuspended bacterial pellets and vortexed for 1 minute to disrupt the bacterial membrane. Cells were then spun at 10,000 RPM for 30 minutes at 4°C and the supernatants were collected for glycogen measurement. Glycogen measurement was done with Cell Biolabs, Inc. colorimetric Glycogen Assay kit (MET-5022) as per manufacturer's protocol.

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The Growing War between the Pharmaceutical Industry and Diabetic Patients

Scientific and Financial History of Insulin

With the initial discovery of insulin in 1923, the original scientists decided to sell their patent to the University of Toronto rather than keep a profit for themselves – this ensured that no company could monopolize the treatment of diabetes with insulin. The scientists sold their patent for $1.00 to the university, which was then licensed for other companies to use and improve the initial formulation of insulin. One of the original scientists, John J. Macleod cited this as "… a great service to humanity," as many diabetics now had access to a crucial medication they needed (Johnson, 2021). However, soon after insulin treatment became popular prices of insulin began to rise. From 1991 to 2001, the average list price of insulin per milliliter rose 2.9% annually. This rate of annual increase then rose to 9.5% from 2002-2012, and then again to 20.7% from 2012-2016 (Hayes, 2020). This continued increase in insulin prices has become a burden on many diabetic patients, with the annual expenses becoming close to $6,000 now and potentially doubling to $12,000 by 2024 (Hayes & Farmer, 2020).

Increasing insulin prices has left many diabetics in dire conditions where they must ration or used expired insulin to control their diabetes. Improperly using insulin can lead to potentially-fatal diabetic ketoacidosis, which led to several deaths occurring during 2017-2019 (RCA, 2021). Advocates have cited these deaths in their fight against the pharmaceutical giants that produce and price synthetic insulin in hopes to reduce the price gouging which is done against insulin. Their voices and protests have manifested in the form of legislations being introduced at both the federal and state levels.

Federal Legislation and Policy to Combat Increasing Insulin Prices

Several pieces of legislation have been introduced into congress over the past several years in an attempt to control insulin prices. During the Obama administration, an executive order was signed which aimed to increase transparency from drug companies to state when they are undergoing drug shortages (Exec. Order No. 13588, 2011). The Preserving Access to Life-Saving Medications Act was also introduced by the House of Representatives and the Senate, but neither body voted on the legislation. Under the Trump Administration, an agreement was made between the United States government and insulin manufacturers to reduce the insulin price for individuals with Medicare (Alonso-Zaldivar, 2020; Exec. Order No. 13937, 2020). This was, however, later rescinded under the Biden administration under the claim that this would only benefit a small proportion of all insulin users. Arguably the most important piece of legislation to be introduced in the past several years is the Affordable Insulin Now Act, which was passed by the House of Representatives in March 2022 (H.R.6833, 2022). This act was originally part of the larger Build Back Better plan initiated by the Biden administration, but was introduced into congress as an independent act once the Build Back Better plan failed to gain a majority vote in the senate (Smith, 2022). The Affordable Insulin Now Act aims to lower insulin prices for insured individuals to either $35 per month or to 25% of the insurance companies negotiated price. However, one of the largest complaints behind this bill is that it only affects individuals who are insured and leaves uninsured individuals paying with the same high prices for insulin. Furthermore, the bill does not try to regulate the price-gouging techniques utilized by pharmaceutical companies and rather only tries to control current insulin prices. Many other bills were also introduced within this time period, such as the Lower Costs, More Cures Act of 2019

and the Preserving Access to Life-Saving Medications Act of 2011, but these bills never gained enough support to be voted on and were consequently killed (Hayes *et al*., 2020; S. 296, 2011).

State Legislation and Policy to Combat Increasing Insulin Prices

Passing of state legislation is significantly easier than federal legislation as the disparities in political ideology between lawmakers tends to be smaller, and therefore there have been more successes seen in controlling insulin prices at the statewide level (Quorum, 2021). As of July 2021, 15 different states (Colorado, Connecticut, Delaware, Illinois, Maine, Minnesota, New Hampshire, New Mexico, New York, Texas, Utah, Vermont, Virginia, Washington, and West Virginia) have enacted policies to limit insulin copay prices (Kenney, 2021). In addition to this, New Mexico and Virginia have also passed laws which limit out-of-pocket insulin expenses for individuals who do not have insurance (Hayes *et al*., 2020). Currently, the most drastic legislation has been passed in Minnesota, where in 2020 the Alec Smith Insulin Affordability act was passed. This act allowed for individuals to receive an emergency supply of insulin at minimum cost regardless of insurance status (MBP, 2022). This law was a major success for diabetic patients, as more than 1,100 residents in Minnesota were able to gain access to insulin which they otherwise would not have had access to (Associated Press, 2022). Similar to the complaints with federal legislation, many argue that these laws do not support those who are uninsured and are primarily targeting the insurance companies instead of the manufacturers who are determining the list price of the drug. As a result, they argue that legislations such as these will only increase the premiums that insurance companies will charge to their patients.

While both legislations and policies passed at the statewide and federal level have been beneficial steps towards making diabetic treatment more accessible, they are far from solving the problem of rising insulin prices. Statewide legislations are only available in certain states, and

nationwide legislations have either failed to pass or will take several years before they take effect (H.R.5376, 2021). In ideal conditions, legislation and deaths should not be needed to control the accessibility to insulin. But that is not the case in the United States, and with studies suggesting that the situation may only get worse for diabetic patients, it is important to take a deeper look into this conflict between pharmaceutical companies and diabetic patients to understand the current prices of insulin treatment.

Monopolization and Innovation in Insulin Design

The insulin market is primarily controlled by 3 different companies - Novo Nordisk, Sanofi, and Eli Lilly (Rajkumar, 2020). Current United States patent law states that a patent will protect intellectual property for 20 years, allowing manufacturers to have some degree of monopolization on their product during this time period but not afterwards. However, companies such as Novo Nordisk and Eli Lilly have been able to keep their control over the market through patent evergreening, which is the process where several patents are claimed for the same product. (Kaplan & Beall, 2016). Since the initial discovery of insulin in 1921, there have been several modifications made to the formulation and synthesis of insulin which have increased the efficacy and shelf-life of insulin treatments. Each modification made by these companies had allowed for them to file for more patents and consequently extending their control over the insulin market. For example, Lantus, a long-acting insulin used by type I and type II diabetics made by Sanofi, has had 70 secondary patents filed while their initial patent expired in 2015 (Amin, 2018). The combination of number of patents along with broad intellectual property definitions has significantly extended their claim to intellectual properly, and has consequently given them the power to defend their intellectual property by filing lawsuits against anything that may infringe on their intellectual property.

A method to try and break the monopoly held by these three companies is through the production of biosimilars. For many other name brand medications such as Tylenol® and Advil®, their primary ingredient (acetaminophen and ibuprofen, respectively) can be identically reproduced with different inactive ingredients to produce cheaper generic variants. This is possible due to the nature of the key ingredient being a small-molecule which can be identically reproduced through a defined chemical process. However, the production of generic insulin is not possible because insulin is a much larger compound which is produced through the help of biological organisms. The usage of biological organisms in manufacturing insulin introduces heterogeneity which does not affect the efficacy of the drug, but does affect the primary structure of the drug. To account for this, the term "biosimilars" was assigned to medications (as opposed to the term "generics") influenced by biological manufacturing methods that prevent the creation of exact duplicates of a particular drug (Kim & Bindler, 2016). The FDA approval process for biosimilars is stringent, and to date there are only 35 approved biosimilar drugs in the United States (of which many have yet to come to market due to patent litigations) (FDA, 2022). Of these 35, only two (Semglee produced by Mylan and Biocon and Rezvoglar produced by Eli Lilly) are insulin biosimilars that have been approved by the FDA. Both Semglee and Rezvoglar compete with Sanofi's Lantus and are cheaper alternatives which were approved in 2021 and 2022, respectively (Briskin & Hopcroft, 2022; Yan, 2021). There are also other alternative insulin options which are either considered "follow-on" insulin or "authorized generic" (Lispro produced by Eli Lilly, Insulin Aspart by Novo Nordisk, Admelog by Sanofi, and Basaglar by Eli Lilly), but these are all produced by the same three pharmaceutical giants which control the insulin market in the United States (DiabetesMine, 2021).

One reason why it has been difficult for other companies to enter the insulin production space is because it is often much more expensive attempting to produce a biosimilar than making a novel drug (White & Goldman, 2019). Furthermore, patent evergreening done by Eli Lilly, Sanofi, and Novo Nordisk has made it even more difficult to produce a biosimilar product without infringing on the broad intellectual claims made by their patents. However, with the approval of Semglee, it brings hope of new manufacturers being able to enter this space and bring diversity to the insulin products available.

Insulin Production Pipeline

In the war between the pharmaceutical companies and diabetic patients, it is important to consider how the price of insulin treatments are determined. Most pharmaceutical companies will cite the ever-increasing price of insulin treatment as being justified by the research and development needed to continuously improve on current treatments (JP, 2018). However, a deeper analysis into insulin production and supply chain reveals that the insulin pipeline is complex and contains several different parts (Cefalu *et al.*, 2018).

The movement of the physical drug follows a relatively linear path from the manufacturer to wholesaler to pharmacy to patient, but the movement of the associated money is much less clear. Manufacturers have a list price for their medication which is paid by wholesale distributers or directly by pharmacies. The wholesale distributer will then sell the medication to a pharmacy, but for a potential higher list price then the price paid to the manufacturer. The pharmacy will consequently charge a different price to the patient which will depend on a multitude of factors, the most important being the insurance coverage that the patient has.

The complexity in the insulin supply chain shows that varying fees and price changes can lead to a growing gap between the price that is listed by the manufacturer and the price paid by

the customer. Furthermore, while the manufacturer determines the list price based on their research and development costs, the blame for higher insulin prices cannot fall directly on the manufacturer due to each stakeholder in the supply chain playing a role in the final price paid by the patient.

Role of Insurance Companies and Pharmacy Benefit Managers in Insulin Pricing

Outside of the supply chain, the insurance companies and pharmacy benefit managers (PBM) play a central role in connecting the manufacturer, pharmacy, and patient. Patients pay a certain premium to an insurance company to gain the benefits from the plans that they offer. The PBM interconnects the pharmacy, insurance company, and manufacturer by taking and giving payments between all three parties – the PBM takes payment from the insurance company and will negotiate a price for the product with the pharmacy, while also receiving payment in the form of rebates from the manufacturer. Rebates paid by the manufacturer are a double-edged sword in that they help to lower the overall price paid by the patient, but it is in the PBM's interest to get the largest rebate possible from the manufacturer to maximize their own profits, which is only done by increasing the list price determined by the manufacturer (Rajkumar, 2020). The market is monopolized by 3 PBM's: Express Scripts, CVS Caremark, and OptumRX. However, these three PBM's are not independent as they all have some degree of influence from insurance companies – Express Scripts is part of Cigna, CVS Caremark is part of Aetna, and OptumRX is a subsidiary of United Healthcare (Beyond Type 1, 2022). Manufacturer's must compete with other manufacturer's by offering attractive rebate options to PBM's, otherwise they risk losing an outlet to sell their product. This has been a large culprit into why insulin price spikes have been universal and in unison over the past several years (Tribble, 2017).

Advocates for Diabetic Patients and Pharmaceutical Companies

For the diabetic patients, the two largest advocacy groups are the Diabetes Patient Advocacy Coalition (DPAC) and the Right Care Alliance (RCA). The DPAC was formed by other diabetic patients who believed their voice was not being heard when important legislation was being passed, and they aim to ensure quality and access of care to diabetic patients (DPAC-about, n.d). A few of their important advocacy points include better insurance coverage for all types of insulin treatments and stricter regulation of the rebate system which often will increase the final price of insulin paid by a patient (DPAC-advocacy, n.d). The RCA is a more generalized grass-roots coalition consisting of physicians, patients, and community members who aim to hold health care organizations accountable for putting patients over their own profits. One of their largest campaigns was their insulin campaign, where they have organized multiple protests to raise awareness for the deaths caused by insulin rationing (RCA, 2021). Some of the largest successes from advocacy groups such as DPAC and RCA have been lobbying for statewide and nationwide legislations, such as state-wise legislation for insulin price-caps in several different states and increased Medicare coverage through the Expanding Access to Diabetes Self-Management Training (DSMT) Act (Healthline Media, 2020).

For pharmaceutical companies, their primary voice is given through the Pharmaceutical Research and Manufacturers of America (PhRMA), a lobbying organization that claims to advocate for “public policies that encourage the discovery of important, new medications for patients by biopharmaceutical research companies” (PhRMA - about, n.d). There are several pharmaceutical companies which are members apart of PhRMA including the three insulin pharmaceutical giants Novo Nordisk Inc, Eli Lilly, and Sanofi. One of the primary ways that PhRMA does their lobbying is through campaign donations to legislatures in Congress. In 2021,

reports cited PhRMA spending over $117 million on lobbying and campaign donations (Aboulenein & O'donnell, 2021). The consequence of this is having many legislatures oppose legislation which may negative impact PhRMA's members. This was seen when looking at legislatures who opposed the Lower Drug Costs now act, where many of those who opposed had received close to $1 million in funding from PhRMA. PhRMA also attempted to prevent the Alec Smith Insulin Affordability Act from being passed in Minnesota, claiming that it was unconstitutional for the state to take private property from the manufacturer without pay. This lawsuit was eventually thrown out by a US District Court judge (MMA, 2021). While many of PhRMA's actions have shown their organization as being less patient-centric and more focused on the profits of their members, PhRMA does offer resources to introduce further transparency into the insulin supply chain and why insulin prices are continuously increasing (PhRMA – Diabetes and Insulin, n.d).

Outside of the advocacy groups mentioned here, there is another group of individuals who term themselves as "biohackers." Biohacking is a broad term given to freelance researchers who are finding novel ways to optimize and improve human body performance. Specifically, to diabetes, the Open Insulin Foundation was founded by a series of biohackers who aiming to formulate an open-source protocol on creating insulin treatments that could be used by anyone (OIP, n.d). Their research offers a more direct solution to the war between pharmaceutical companies and diabetic patients by making insulin more easily accessible to all without having to pass significant legislation. While there is no guarantee of this protocol working, researchers are hopeful since insulin production and synthesis are very well documented. If positive results are achieved, there is hope that the protocol could be adapted to treat other metabolic diseases and disorders.

Looking Outside of the United States

Much of the conflict between diabetic patients and pharmaceutical companies has been isolated to within the United States. While other nations have also seen an increase in insulin prices, nowhere has it been as significant as in the United States. Studies have shown that compared to other high-income nations, insulin prices within the United States are eight times higher (Mulcahy *et al.*, 2020). Compared to the United States, many nations that have cheaper insulin prices also have governments which directly negotiate with manufacturers to determine their value. This combined with higher taxation rates allows for those nations to provide greater coverage for their citizens. Specifically in Germany, drugs are evaluated by an independent board to determine their value within the market. Once valued, they determine the maximum value that an individual will pay out-of-pocket for that drug. The maximum value is determined by the household income for that individual (either 1% or 2% of their income depending on the drug and condition) and is tightly regulated since many individuals in Germany are on public health insurance plans (Luthra, 2019). While the situation in Germany is not directly comparable to what is occurring in the United States, it gives insight into potential legislation and reform which could increase insulin accessibility without hindering manufacturer profits.

Conclusion

At the surface level, it appears that the conflict between pharmaceutical companies and diabetic patients can be simplified to high prices set by pharmaceutical companies which diabetic patients are the victim of. However, a deeper analysis reveals that the is not straight forward and is impacted by a variety of stakeholders in the insulin production pipeline. This machine has been allowed to become so large and complex due to the lack of federal regulation, which is what starkly distinguishes the conflict in the United States compared to other places around the

world. To counter this, pressure has been placed on legislatures from advocacy groups providing a voice to diabetic patients, and this has resulted in changes to both federal and state legislation. While the conflict is far from being resolved, the introduction of biosimilar insulin products and price-controlling regulations have already increased the accessibility to insulin for diabetic patients, and opens the door for further changes to be made that may potentially help resolve the conflict.

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Sugaway: Using Synthetic Biology to Treat Diabetes
(Technical Report)

The Growing War between the Pharmaceutical Industry and Diabetic Patients
(STS Research Paper)

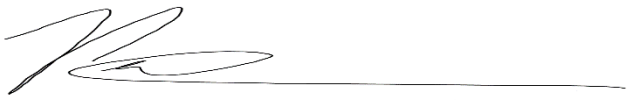
A Thesis Prospectus Submitted to the

Faculty of the School of Engineering and Applied Science
University of Virginia • Charlottesville, Virginia

In Fulfillment of the Requirements for the Degree
Bachelor of Science, School of Engineering

Promotto Islam
Spring, 2022

On my honor as a University Student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments



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Approved: _____ Date: _____

Dr. Keith Kozminski, Department of Biology

Approved: _____ Date: _____

Dr. Joshua Earle, Department of Engineering and Society

General Research Problem

How can the treatment and prevention of diabetes be improved?

According to the Centers for Disease Control and Prevention (CDC), diabetes mellitus affects 34.2 million individuals in the US (CDC, 2020); by 2060, cases may exceed 60 million (Lin et al., 2018). Administration of synthetic insulin is the primary treatment for diabetes, but since the discovery of synthetic insulin in 1978, its price has risen faster than its cost of manufacture (Belluz, 2019). High prices limit access to insulin, jeopardizing the health of many diabetics. (Rajkumar, 2020).

Through synthetic biology, novel biological systems can be engineered, including genetically engineered bacteria. Recent advances in synthetic biology have harnessed the power of preexisting biological systems in order to engineer novel biological systems. Such bacteria may be used to treat phenylketonuria, a metabolic disease that prevents the digestion of phenylalanine, an important amino acid. (Durrer et al., 2017). This suggests that genetically modified bacteria may also serve as a treatment for diabetes, specifically as an insulin supplement.

Alternatives to Insulin for Treating Diabetes

How can genetically modified bacteria be used to supplement insulin use in diabetic patients?

Faculty Advisor: Dr. Keith Kozminski (Department of Biology)

Project Type: Capstone Project

Diabetes mellitus consists of a group of metabolic diseases which all share a common characteristic of inducing high blood glucose levels (Kerner & Brückel, 2014). This increase is often due to a dysregulation (type I diabetes) or dysfunction (type 2 diabetes) of native insulin

(Guthrie & Guthrie, 2004). Glucose is a carbohydrate that is naturally ingested in most diets, and to control blood glucose levels insulin is secreted to induce the conversion of glucose into a long-term storage form called glycogen. However, in diabetics this balance is disturbed and chronically elevated blood glucose levels can cause serious complications such as cardiovascular disease, nerve damage, or kidney damage. After a clinical diagnosis of diabetes, individuals must make careful lifestyle changes and continue a strict medication regimen. However, this is not always effective or doable, which contributes to the growing prevalence of diabetes in the United States. One method in countering this is through the production of cheaper and more effective medication, and the field of synthetic biology offers promising methods to finding a better treatment for diabetes.

The goal of this capstone project is to genetically modify bacteria with plasmids in order to uptake excess extracellular glucose and to safely convert it into glycogen. There are no unusual constraints for this project.

Current guidelines set by the American Diabetes Association (ADA) state that the primary treatment for diabetes should be a treatment plan of insulin (ADA, 2021). Other glucose lowering medications are also considered such as Metformin or Pramlintide depending on the patient's condition. For severe cases of type I diabetes, surgical transplantation of the pancreas is an option, but will require lifelong immunosuppressant medication and could trigger further complications. Insulin treatment can be effectively administered either by an injection or by an insulin pump, but are expensive to own and are not an option that is available to everyone (The BMJ, 2017). The aforementioned medications have also been shown to have similar benefits to insulin treatment (Mercurio, 2017), but not all medications are easily accessible due to their price and many come with unfavorable side effects. Besides medication guidelines, there are also

lifestyle changes such as getting more exercise and dietary restrictions, but these are often not enough to help individuals with severe diabetes or a genetic basis for diabetes.

The common factor in both the medical and lifestyle treatments described are their accessibility. Many medications are not easily accessible due to their costs, and lifestyle changes may not be feasible for individuals who live in food deserts and lack access to affordable, healthy, food. Therefore, there is a need for an effective and cheap treatment to diabetes which can be readily accessible by all diabetic patients.

To investigate the feasibility of an insulin supplement, bacteria will have to be genetically modified using plasmids. The initial plasmid will be a glycogen synthesis plasmid that will contain instructions to increase the metabolic conversion of glucose to glycogen. After transformation, expression of proteins will be verified by a Western Blot and protein functionality will be tested with a glycogen concentration assay. Once verified that the expressed proteins are working as expected, the sustainability and toxicity of transformed bacteria will be measured. This will ensure the bacteria can safely convert glucose to glycogen without causing harm to other surrounding microorganisms.

The proposed deliverable from this project is a genetically modified bacteria which mimics the functionality of synthetic insulin. This represents the basis for a probiotic which would be taken as an oral drug, which would be ingested and supplement the typical synthetic insulin taken by diabetics. This probiotic is cheaper and easier to administer compared to current treatments, and will reduce the dependency on synthetic insulin.

The Growing War between Pharmaceutical Industry and Diabetic Patients

In the United States, why do many diabetics distrust or resent pharmaceutical companies?

High prices and consequent access inequities have been matters of contention between diabetic patients and the pharmaceutical companies. From 2012 to 2018, synthetic insulin prices rose an average of 14 percent a year. For one diabetic, the annual expenses for insulin are now close to $6,000. About a quarter of diabetics cannot cover the costs of their insulin; more than half rely on public assistance for their medical expenses. If recent trends continue, the annual per-patient cost for insulin may double to $12,000 by 2024 (Hayes & Farmer, 2020). High prices have forced some diabetics to ration or used expired insulin, causing some deaths in the past 5 years (RCA, 2021). Advocates have cited these deaths in their fight against the pharmaceutical giants that produce and price synthetic insulin.

The insulin production pipeline is complex, incurring costs at each stage (fig. 1). The

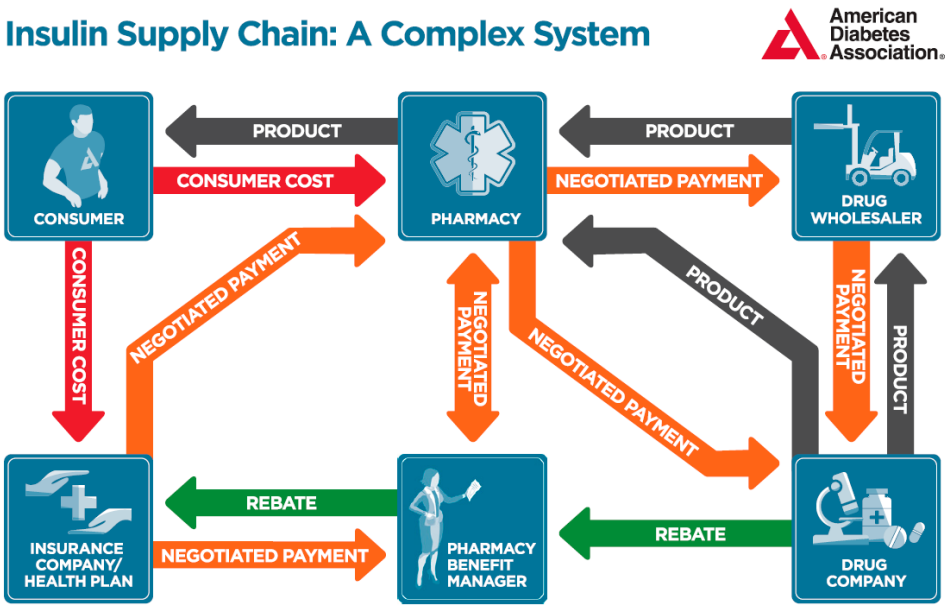

Figure 1. Schematic of Insulin Supply Chain (Cefalu et al., 2018)

complexities obscure cash flows, while negotiated payments and rebates elevate prices.

This complex system is unique to the United States, which has the most privatized healthcare sector among developed countries. No federal agency centrally establishes or regulates drug prices. Instead, multiple pharmaceutical companies and insurers negotiate prices. The system affords companies opportunities for price gouging, which has inflated prices for EpiPens, opioids, and insulin (Belluz, 2019). Pharmaceutical companies justify high prices by citing the costs of drug development and clinical trials, as stated specifically by Johnson and Johnson in their *2017 Janssen U.S. Transparency Report* (JP, 2018). Yet the costs of newer insulin variants may not outweigh the benefits that pharmaceutical companies advertise (Luo et al., 2019).

In this struggle, the advocacies that represent diabetic compete with the trade associations that represent pharmaceutical companies Insurers are also engaged. The Right Care Alliance (RCA, n.d.) claims it strives "to make health care institutions accountable to their communities" by prioritizing patient care. One of their campaigns is to increase the affordability of insulin treatment, and they have organized multiple protests since 2018 against pharmaceutical company price gouging. The Pharmaceutical Research and Manufacturers of America (PhRMA, n.d.) is a trade association representing pharmaceutical companies. It claims to promote "effective advocacy for public policies that encourage discovery of important, new medicines." In 2020, PhRMA sued the Minnesota Board of Pharmacy, claiming that a state law the board used to regulate insulin prices was unconstitutional (MMA, 2021). Some researchers who are committed to finding a cheaper way to produce insulin call themselves Biohackers. The Open Insulin Project is a group of volunteer researchers developing "an open-source (freely available) model for insulin production" (OIP, n.d.). Applying synthetic biology, OPI is genetically modifying yeast cells to produce human insulin, which they hope can be harvested for us by diabetics.

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